NEW GOLD RAINY RIVER MINE
APPENDIX M
WILDLIFE REPORT

PHASE 3 EEM INTERPRETIVE REPORT FOR RAINY RIVER MINE

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PHASE 3 EEM INTERPRETIVE REPORT FOR RAINY RIVER MINE

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EXECUTIVE SUMMARY

The Rainy River Mine (RRM) is owned by New Gold Inc. (New Gold). The mine is located approximately 65 km northwest of Fort Frances from Highway 600 within the Township of Chapple and the District of Rainy River. The RRM is within the Pinewood River watershed, which flows past the site eventually draining into the Rainy River approximately 40 km downstream. At present, operations at RRM are comprised of open pit and underground mining with ore processed at the Rainy River Mill, located on site. Production commenced in 2017 with an average milled rate of approximately 24,000 t/day in 2023.

The mine became subject to Section 7 of the Metal and Diamond Mining Effluent Regulations (MDMER) in September 2015. Section 7 of the MDMER states, among other things, that "the owner or operator of a mine shall conduct environmental effects monitoring studies of the potential effects of effluent on the fish population, on fish tissue and on the benthic invertebrate community in accordance with the requirements and within the periods set out in Schedule 5". Consistent with this provision, the RRM submitted its first EEM Study Design Report to Environment Canada in September 2016 and subsequently its first EEM Interpretive Report in March 2018. Since, the Phase 2 IR was submitted in March 2021.

Overview of the Phase 3 EEM Program

RRM had several discharge points during the construction phase of the buildout process. Since the end of construction discharge has been limited to three discharge points. Those being, the Water Discharge Pipeline (EDL1), the Loslo Creek discharge (EDL2), and Sediment Pond 2 (SED2). All three discharges report to the Pinewood River. The effluent discharge quality and quantity at the EDL1 and EDL2/Sediment Pond 2 as well as the Pinewood River flows at these locations were used to determine the potential impact on the aquatic environment.

Phase 3 was the first EEM that discharge from EDL1 was investigated as part of the EEM biological component. EDL2 was assessed during the Phase 1 and Phase 2 EEMs. Phase 3 followed a control-impact design with an area located downstream of the EDL1 discharge and one appropriately selected reference areas on another watershed (i.e., La Vallée River). Similarly, EDL2 was studied in the same locations as Phase 1 and 2. This study followed a standard assessment of effluent quality, water and sediment analysis, and an assessment of MDMER prescribed benthic and fish community endpoints.

Acute and Sublethal Toxicity of Treated Mine Effluent

Acute toxicity was assessed on Rainbow Trout (*Oncorhynchus mykiss*) and *Daphnia magna*. Since the release of treated water or runoff from the temporary construction discharges ceased near the end of Phase 1, 100% effluent has not caused more 10% toxicity in a single sample.

Sublethal toxicity measured survival, growth, and reproduction of Fathead Minnow (*Pimephales promelas*), water flea (*Ceriodaphnia dubia*), duckweed (*Lemna minor*) and the green alga

Raphidocelis subcapitata. Fathead minnow survival and growth was not affected since Phase 1. Water flea survival and reproduction were consistently high since Phase 1. Duckweed endpoints were highly variable, ranging from 0.23% v/v to >97% v/v. Apart from two tests from EDL1, the growth of green algae has been constantly high through all tests.

Sediment Characteristics and Water Quality

Sediment was assessed for physical composition (particle size, texture) and chemical characteristics. Both EDL2 and EDL1 were dominated in small-particle sizes, including clay, silt, and fine sand. Particle size was generally uniform between the exposure and reference areas, with minor statistical differences. Total organic carbon (TOC) in sediment was above the guideline thresholds (> 1%) in all areas, suggesting the area is naturally high in TOC. There was no indications that differences were associated with proximity to the mine wastewater.

Sediments were also analyzed for a suite of 24 metals and compared to provincial guidelines. No elements exceeded the severe effect limit (SEL). Some areas were above the lowest effect limit (LEL), including one out of five sites sampled for Ni in STUC-REF (Ni = 19 µg/L) and one out of five sites sampled for Fe in PIN-EXP (Fe = 13,000 µg/L). The minimal differences suggest that outfall does not significantly change the constituent concentrations of sediments.

Generally, there was little variability comparing the conventional water parameters between the reference and exposure areas. There was, however, elevated conductivity in the exposure areas compared to some reference areas. In May 2023, conductivity in PINR-EXP, downstream of EDL2, was, on average, 3.2x higher than the reference areas. Similarly, conductivity was 1.9x higher than the reference areas in September 2023. In PINR-EXP2, downstream of EDL1, conductivity was 1.7x higher than the reference in May, but there was no difference in September.

When examining the water chemistry, the only parameter that was elevated beyond the CCME water quality guidelines was Fe, in all sites but PINR-EXP. This suggests that the area is naturally elevated in Fe. When comparing the relative differences between sites, antimony was >10% elevated compared to the reference areas. No other measured parameters appear elevated.

Benthic Invertebrates

A total of 41 benthic families were identified in the study area. In EDL2, the density ranged from 2,181 to 36,282 organisms/m^2. In EDL1, density ranged from 1,894 to 7,175 organisms/m^2. The dominant taxon in both areas was of the family Chironomidae. By proportion, the most common taxa were from the Phylum Annelida, the Family Chironomidae, and other representatives of the Order Diptera.

Some of the measured endpoints of density, Simpson's Diversity, and Simpson's Evenness were significantly different between the reference and exposure areas at both EDL1 and EDL2. Density was significantly lower in PINR-EXP compared to STUC-REF and density was significantly higher in PINR-REF2 compared to PINR-EXP2. The difference in EDL1 was above the critical effect size (CES) of ± 2 reference area standard deviations (Ref SDs). Diversity was significantly higher in

PINR-EXP2 compared to LVR2-REF. Evenness was significantly lower in PINR-EXP compared to LVR-REF. The differences for diversity and evenness were below the CES. Taxa richness was not significantly different in any measured sites.

The Bray-Curtis Index was significantly different between the exposure and reference sites for all comparisons. This is the third EEM indicating a difference, confirming the effect, and requiring an investigation of cause (IOC). An investigation of cause will most likely involve univariate and multivariate desktop investigations relating communities to their respective water chemistry, sediment chemistry, and landscape catchment features as nMDS analysis suggests little overlap in the communities.

Fish

A total of 16 unique fish species were caught in the reference areas and exposures areas in May 2023. The dominant fish per area varied, but commonly it was Brassy Minnow (*Hybognathus hankinsoni*), Brook Stickleback (*Culaea inconstans*), and Central Mudminnow (*Umbra limi*). A small proportion of fish had health defects, with no distinct pattern related to mine effluent exposure.

The sentinel species were Central Mudminnow and Brook Stickleback. There was insufficient sample size for male and female Brook Stickleback at STUC-REF and female Central Mudminnow at EDL1 for statistical analyses. The fish were compared for survival, energy storage, and energy use. There were several statistical differences, but only a limited number of differences exceeded the CES. There was a significant difference between Central Mudminnow age between PINR-EXP and STUC-REF, with a +100% magnitude of difference (MOD). This trend is likely due to their short lifespan, and not related to mine effluent.

For energy storage, female Brook Stickleback body weight at body length (i.e., condition) had an MOD of +10.4%. Also, smaller female Central Mudminnow liver weight at body weight had an MOD +52% compared to STUC-REF. For female Brook Stickleback, comparing PINR-EXP2 to LVR2-REF, the MOD was 40.5% for small fish; comparing PINR-EXP2 to PINR-REF2, the MOD was 50.9% for small fish and 26.9% for large fish – all three exceeded their respective ±25% CES.

For energy use, large female Central Mudminnow gonad weight at body weight at PINR-EXP had a MOD of +36% compared to STUC-REF. Also, female Central Mudminnow at Age-2 had a MOD of 80.1% for body weight at age when comparing PINR-EXP and LVR-REF. Female Brook Stickleback gonad weight at body weight had an MOD of 32% when PINR-EXP2 to LVR2-REF were compared. Male Central Mudminnow body weight at Age 1, had a statistical difference between PINR-EXP2 and PINR-REF2, with an MOD of -52%.

Temporally, there were a number of confirmed effects comparing PINR-EXP to LVR-REF. Namely, female Brook Stickleback had confirmed effects for higher body weight at length compared to LVR-REF exceeding the 10% CES. Larger female Brook Stickleback (i.e., through the ANCOVA min-max procedure) had higher gonad weight at body weight compared to LVR-REF exceeding the 25% CES. Male Brook Stickleback from PINR-EXP had confirmed effects for lower body

weight at Age-1 when compared to LVR-REF but this difference was below the 25% CES. Larger female Central Mudminnow at PINR-EXP had confirmed effects for higher gonad weight at body weight compared to LVR-REF exceeding the 25% CES.

There were also a number of confirmed effects comparing PINR-EXP to STUC-REF. Namely, female Central Mudminnow had confirmed effects for lower body weight at length compared to STUC-REF but below the 10% CES. Male PINR-EXP Central Mudminnow had confirmed effects for lower gonad weight at body weight compared to STUC-REF exceeding the 25% CES in Phase 2 and below the 25% CES in Phase 3

Conclusions and Recommendations for Future Monitoring

Based on the combined results, there is a confirmed effect to the benthic community based on consecutive differences in the Bray-Curtis Index. The next phase will require an IOC to determine the driver of this difference. Similarly, there are a number of confirmed effects comparing both Brook Stickleback and Central Mudminnow at EDL2 with its respective reference areas. Normally, the next phase would require an IOC to determine the driver of those differences. However, EDL2 is no longer the primary discharge at New Gold and next steps in this instance will be discussed with Environment and Climate Change Canada (ECCC) in time for the study design preparation for Phase 4.

Regardless of the results of the EDL2 comparisons the next monitoring study and interpretative report should be submitted in 36 months as determined by following the decision tree in the Metal Mining Effluent Regulations technical guidance (EC, 2012). The next interpretative report will be submitted by March 17th 2027. All aspects of the Phase 4 study will be outlined in a study design report that will be submitted to ECCC for review at least six months prior to any proposed field program.

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1.0 Environmental Effect Monitoring Framework

The Assessment of the Aquatic Effects of Mining in Canada (AQUAMIN, 1996) provided recommendations regarding the review of and amendments to the Metal Mining Liquid Effluent Regulations (now called the Metal and Diamond Mining Effluent Regulations [MDMER]; Government of Canada, 2024) and the design of a national Environmental Effects Monitoring (EEM) program for the metal mining sector. EEM is a requirement of the MDMER under the authority of the Fisheries Act. The regulation requires all metal mines and mills in Canada, to which the regulations apply, conduct EEM according to the guidelines provided in the MDMER, as well as appropriate technical guidance (TGD; EC, 2012).

EEM is a scientific monitoring approach that can be used to help assess the potential effects in aquatic ecosystems caused by industrial effluents, as well as the effectiveness of environmental protection measures. The objective of the metal and diamond mining EEM program is to evaluate the effects of mine effluent on fish, fish habitat, and the beneficial use of fisheries resources. This information may be used to contribute to an assessment of the adequacy of the MDMER to protect the environment. A nationally consistent EEM program, along with social and economic information, can be used to assess the effectiveness of pollution prevention and control technologies, practices, programs, and indicate where there is a local, regional, or national need for enhanced protection.

1.1 EEM at the Rainy River Mine

The Rainy River Mine (RRM) is located west of the community of Fort Frances, Ontario, in Chapple Township, Rainy River District (**Figure 1-1**). The property became subject to Section 7 of the subsequently amended Metal Mining Effluent Regulations (MDMER) on 17 September 2015. Section 7 of the MDMER states, among other things, that "*the owner or operator of a mine shall conduct environmental effects monitoring studies of the potential effects of effluent on the fish population, on fish tissue, and on the benthic invertebrate community in accordance with the requirements and within the periods set out in Schedule 5*". Consistent with this provision, RRM submitted its first Study Design Report to Environment Canada in September 2016 (Minnow 2016) and subsequently its first EEM Interpretive Report was submitted in March 2018 (Minnow 2018). The Second EEM Study Design and Interpretive Reports were submitted in November 2019 and March 2021, respectively with an addendum for the Phase 2 fish survey submitted in September 2021 due to COVID-19 related delays. The Phase 3 Study Design was submitted March 2023.

The Initial EEM (Phase 1) study included benthic invertebrate and fish collections (as well as supporting environmental information in one exposure area on Pinewood River and one reference area on Sturgeon Creek. There was a significant effect on the exposure area benthic invertebrate community for the Bray-Curtis Index, although these community-based differences were likely the result of habitat differences between the two sampling areas (Minnow, 2018). A number of statistical differences were reported in Phase 1 for both sexes and both sentinel fish species (i.e., Brook Stickleback and Central Mudminnow) endpoints. These included differences

in female Brook Stickleback with respect to relative gonad size, relative liver size, and body condition all of which also exceeded their respective CES.

The Phase 2 study followed a similar design with the addition of a second reference area on La Vallée River. There were six significant differences in the benthic community endpoints and five differences in the fish community endpoints. There was a single confirmed effect on Bray -Curtis Index for the benthic community over Phases 1 and 2, however this was interpreted to be due to habitat differences between the exposure area and the original Sturgeon Creek reference area. There were no confirmed effects between the exposure area and the Sturgeon Creek reference area or the La Vallée reference areas for either fish sentinel species. Taking into consideration that the final discharges and water management practices under operational conditions were only finalized in 2020 and there is only one Phase of data from the newly established La Vallée River reference area, Ecometrix recommended that the Phase 3 study should repeat the Phase 2 design.

New Gold has four Final Discharge Points (FDPs) according to the MDMER. According to the TGD (EC, 2012) the biological component of the EEM conducted as required by Schedule 5 of the MDMER is to assess the discharge with the highest potential of an adverse affect on the environment.

The Phase 3 Study Design report indicated that EDL1 should now be considered the primary discharge with the highest likelihood of potential impact and therefore subject to the biological monitoring components of the EEM program. This was based on loadings, duration of discharge, effluent mixing, and the sensitivity of the habitat as outlined in the TGD.

In the preparation of the Phase 3 Study Design report an assessment of which final discharge point should be considered the primary discharge was undertaken (Section 4.2). Details of the assessment that resulted in this change is provided in the Phase 3 Study Design. The results of the previous EEMs conducted on the EDL2 discharge are provided in this report for context but the Phase 3 Design is presented to provide the greatest level of simplicity and repeatability whilst meeting the requirements of the MDMER. It is New Gold's view that this will provide the most reasonable approach to assessing potential impacts to the environment over the longer-term operation of the mine.

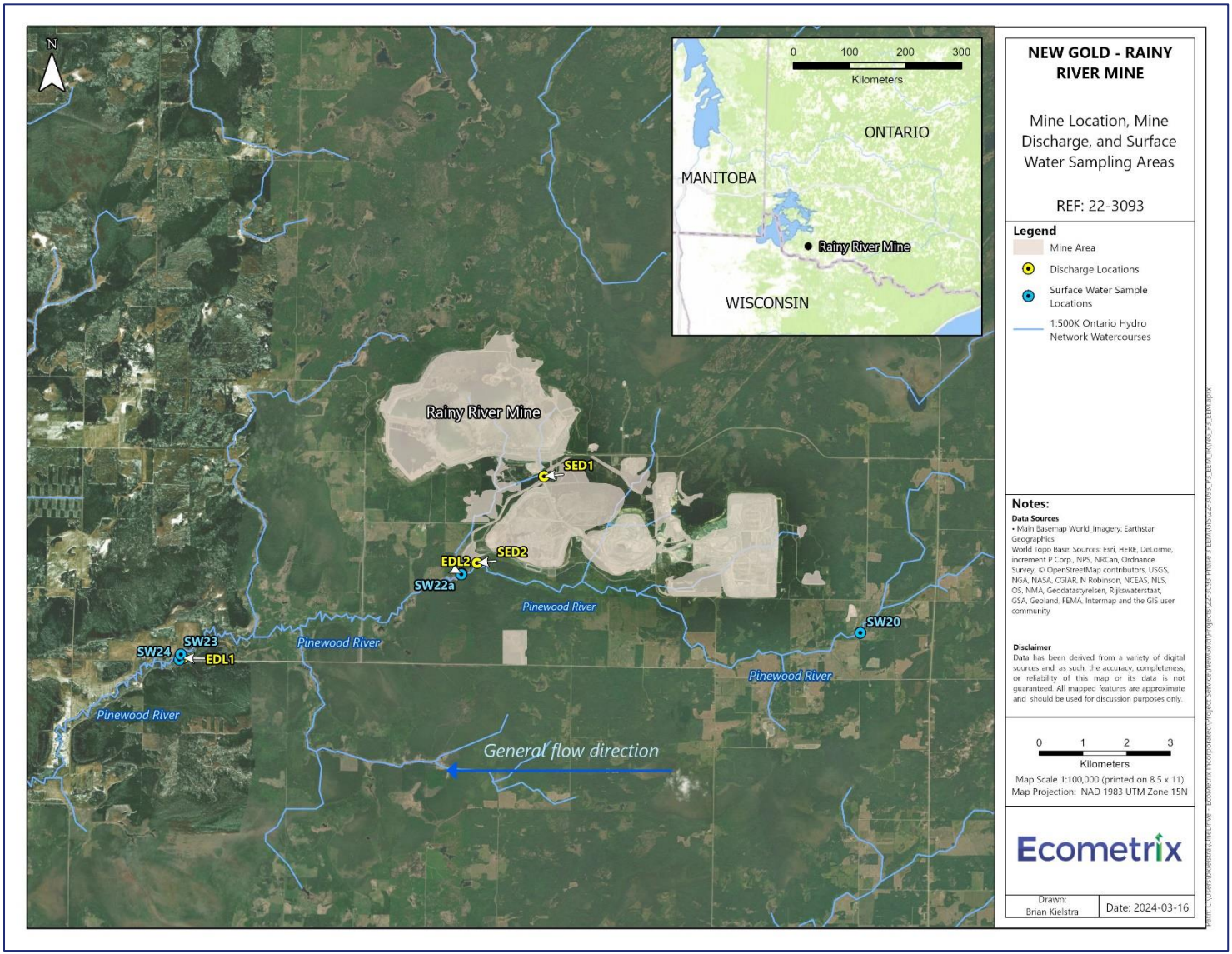

Figure 1-1: Regional location of the Rainy River Mine.

1.2 Report Format

Following this introductory section, the remainder of the report is organized as follows. In **Section 2.0**, the site is characterized with respect to current mine operations; mine effluent quantity and quality including effluent acute and sublethal toxicity, mine wastewater dispersion in the Pinewood River, the Pinewood River receiver water chemistry, and an overview of the ecological aspects of the study area is provided. In **Section 3.0**, a summary of the results of the Phase 1 and Phase 2 EEM studies is provided. In **Section 4.0**, supporting environmental information for the current EEM study. In **Section 5.0**, the benthic invertebrate community survey from the current EEM study. In **Section 6.0**, the fish survey from the current EEM study. In **Section 7.0**, the results of study components completed to date are evaluated together with the context of other relevant information (e.g., mine effluent quality data, effluent toxicity results, and previous EEM study results) to provide a holistic weight-of-evidence based assessment of the biological conditions downstream of the mine effluent discharges. In **Section 8.0**, recommendations for future EEM monitoring programs at the RRM are provided. In **Section 9.0** the references consulted in the preparation of this report are given.

Notes on QA/QC measures implemented during this study are embedded in appropriate sections of the report. Appendices provided with this report contain raw data and other information collected during the completion of the study.

2.0 Site Characterization

2.1 Rainy River Mine

Detailed site characterization information for the RRM and effluent receiving environment were provided by Minnow (2016, 2018) and are summarized herein.

RRM is currently owned and operated by New Gold. It is a gold mine originally approved for 21,000 tonnes per day, with both open pit and underground facilities with an anticipated mine life of around 16 years (AMEC, 2014). The mine came into commercial production in September 2017. Aside from the MDMER the mine is also subject to an Environmental Compliance Approval (ECA) Number 2290-CAVKGN from the Ontario Ministry of Environment, Climate and Parks (MECP) that has an updated allowable throughput of up to 32,400 tonnes per day with a quarterly average throughput of up to 27,000 tonnes per day. Ore generated at the mine is processed and refined at the on-site milling facility.

Table 2-1: Summary of exploration, development, and ownership changes for the Rainy River Mine

Year	Activity
1967	First record of exploration
2005	Property purchased by Rainy River Resources Ltd.
2008	Rainy River Resources Ltd. commences baseline data collection
2013	New Gold Inc. purchases RRM
2014	Environmental Assessment submitted (AMEC, 2014)
2015	Site construction begins
2015	Mine becomes subject to the MDMER
2015	Original ECAs Number 5781-9VJQ2J and 5178-9TUPD9 approved
2017	Mine commissioned September 2017
2018	Temporary Discharges removed
2019	EDL1 Water Pipeline discharge commissioned
2020	EDL2 Loslo Creek discharge commissioned
2020	Amended ECA Number 7004-BC7KQ5 approved
2022	Amended ECA Number 2290-CAVKGN approved

2.1.1 Location

RRM is located approximately 65 km northwest of the town of Fort Frances, Ontario, in Chapple Township, Rainy River District approximately 6 km west of Highway 71. The location of the RRM is shown in **Figure 1-1**.

2.1.2 Site Layout

Key mine-related infrastructure on the site includes an open pit, underground mine portal, waste rock stockpiles, rock crushing facilities, ore storage facilities, a processing plant, a Tailings Management Area (TMA), watercourse diversions, site drainage works, a fuel tank farm, explosives manufacturing facilities, and explosives storage facilities (Minnow 2018, **Figure 2-1**).

2.1.3 Discharge Locations

During the construction phase of the project there were a number of temporary discharge points. However, consistent with provincial approvals, discharge from these locations ceased as of May 2018. After that time all water was directed to appropriate collection facilitates through constructed infrastructure until September 2019.

As described in a memorandum to ECCC provided on 07 May 2020, the RRM now has a total of four discharge locations according to their current provincial permit (**Figure 2-1**):

1. At the confluence of Loslo Creek with the Pinewood River (also referred to as EDL2);
2. Near SW24, the former location of the water intake pipe (also referred to as Water Discharge Pipeline and EDL1);
3. Sediment Pond 1 and,
4. Sediment Pond 2.

Discharges 1 and 2 listed above were equipped with diffusers in December 2019 and August 2020 and will be the consistent discharge locations used for the life of the mine. Mine planning predicted that both sediment pond discharges would be needed in the early part of the mine life to manage water levels through direct discharge to the West Creek diversion (i.e., Sediment Pond 1) and to the Pinewood River (i.e., Sediment Pond 2), with water collected in these ponds requiring pump-back and treatment as the mine-life progresses. However, based on operational constraints Sediment Pond 1 does not normally discharge to the environment and moving forward water from this location will be pumped to Sediment Pond 2 for subsequent discharge. Early predictions indicated that as the mine life progressed EDL2 would be used as the primary discharge location. However, discharge limits at EDL2 are bounded by flows in the Pinewood River (ECA Number 2290-CAVKGN) and therefore discharge has been expected to be more frequent at the EDL1 which is located further downstream in the Pinewood River catchment.

According to the Metal Mining TGD Section 2.2.2.1 (EC, 2012), if multiple discharges are used the discharge anticipated to have the largest potential adverse effect on the environment requires assessment through EEM biological components.

An assessment of discharge volumes, loadings, site water management practices and provincial permit requirements indicated that during Phase 1 and Phase 2 the EDL2 Loslo Creek Discharge was the primary discharge. However, further assessment of recent and expected future loadings, as part of the development of the Phase 3 EEM indicated EDL1 should be considered the primary discharge going forward. This is based on the flow regime in the Pinewood River and its influence on operational requirements for water management.

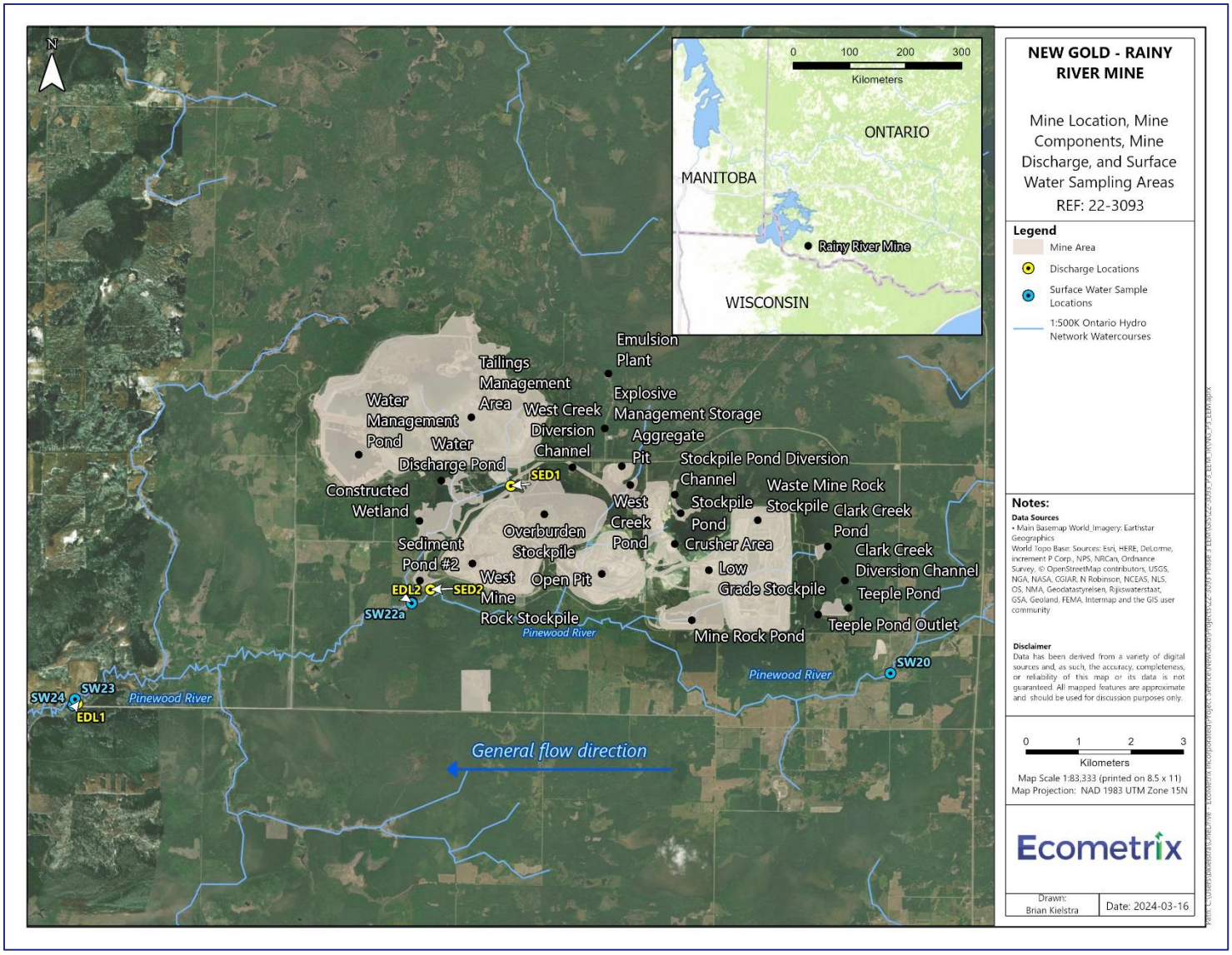

Figure 2-1: Layout of Rainy River Mine

2.1.4 Operations

Ore is produced from an open pit. The two-lobed open pit will cover a surface area of approximately 200 ha when completely mined and mining will occur to a depth of 400 m. The current milled target rate of production from the open pit is approximately 24,000 tonnes per day. Currently, the underground workings are under development and will continue to a depth of 800 m allowing access to higher grade ore than the open pit. The underground workings will be mined using both long-hole and open stoping methods as well as the overhand cut and fill methods.

Rock is transported from the open pit and eventually the underground to the primary crusher. Ore is crushed and transported to the mill by conveyor. Processing consists of a grinding and crushing circuit with a SAG mill, pebble crushing and a ball mill (Minnow, 2016). Other steps in the process also include a gravity separation circuit, cyanide leaching with carbon-in-pulp (CIP) gold adsorption circuit and the carbon stripping and electrowinning circuit. An induction furnace is then used for doré bar production. The chemicals required for a year of milling at RRP were provided in Table 2.1 of the Phase 1 EEM Study Design (Minnow, 2016).

Waste rock generated from mining operations is stored in clean waste rock stockpiles, whereas low grade ore is stockpiled on site and will be milled during the latter part of the mine life cycle. Overall, the mine design includes storage of approximately 80 Mt of overburden and 350 to 400 Mt of mine rock.

Tailings treatment occurs within the processing plant with SO_2/Air treatment to precipitate heavy metals and destroy cyanide and metallo-cyanide complexes (Minnow, 2016).

Tailings deposition commenced in the TMA Cell 1 in September 2017 followed by TMA Cell 2 in April 2018 and TMA Cell 3 in May 2019.

2.1.5 Water Management

Now that the mine is in production a number of sumps, ditches and drains collect water in the open pit and the underground to maintain them in working condition. Water is directed to the mine rock pond for eventual re-use in the processing plant with surplus being directed through the Water Treatment Train (process water) and subsequently the WMP or to the Sediment Pond 1 or 2 discharges (surface water, seepage, and groundwater collections).

As mentioned, process water is treated by the Water Treatment Train. The Water Treatment Train was commissioned in September 2019 and consists of a lime treatment plant, nitrification cells, biochemical reactors and a compliance sump. A pump back system from the compliance sump (Outflow Basin) to the WMP allows for further treatment of water that may not meet provincial or federal discharge criteria.

Effluent discharge to the Pinewood River also occurs under the authority of a provincial ECA (Number 2290-CAVKGN). The primary discharge according to the MDMER, and therefore the new discharge proposed to be assessed by the Phase 3 EEM, is through Water Discharge

Pipeline (EDL1) 10 km west of the site. This pipeline is designed to convey up to 33,000 m^3/day to the Pinewood River. However, the amount of discharge at this location is limited to a 1:1 ratio with the receiver after full mixing of the effluent. If flows in the Pinewood River exceed 10,000 m^3/day discharge can occur through the Loslo Creek discharge (EDL2) and or EDL1. These discharges combined with the discharge from Sediment Pond 2 can also only equal a 1:1 ratio with the river flows. EDL1 and EDL2 can discharge approximately 45,000 m^3 per day combined, Sediment Pond 2 can discharge a maximum of 20,000 m^3 per day so if the flow in the Pinewood River allows it RRM can discharge a maximum of approximately 65,000 m^3 per day.

2.2 Mine Effluent Quantity and Quality

2.2.1 Effluent Discharge Volume

Discharge volumes for each location from 2020 through 2023 to December 31st are summarized in **Table 2-2**. New Gold manages discharge volumes in accordance with provincial requirements for flow in the Pinewood River and as such the volume of effluent discharged from the EDL1, EDL2 and Sediment Pond 2 locations is greatly influenced by variation in precipitation, both rain and snow, on short and long temporal periods (i.e., month to month and year to year).

Table 2-2: Annual discharge total at the RRM (m^3), 2020 to 2023.

Final Discharge Point	2020	2021	2022	2023
Sediment Pond 1	162	0	0	0
Sediment Pond 2	262,570	665,491	1,062,771	790,915
EDL1 (Water Discharge Pipeline)	1,137,571	1,267,716	2,242,667	2,209,135
EDL2	248,013	80,295	842,244	1,683,860

2.2.2 Effluent Characteristics

New Gold provided updated effluent chemistry for Schedule 4 effluent parameters from 2020 to the end of 2023 that are summarized in

Table 2-3. For parameter concentrations that were below the detection limits, the detection limit value was used to calculate the summary statistics. **Appendix A** contains the detailed Schedule 4 results as well as the parameters required as part of Schedule 5 (4) of the MDMER.

Discharge from the Sediment Pond 1 began in September 2019 followed by Sediment Pond 2 and the Water Discharge Pipeline in October 2019. EDL2 discharge began in September 2020. Sediment Pond 1 will not be used as a final discharge for the remainder of the mine life.

2.2.2.1 Arsenic

Arsenic levels have not exceeded MDMER values since monitoring began in 2015. From 2020 to 2023, effluent arsenic concentrations have ranged from 0.001 to 0.005 mg/L.

2.2.2.2 Copper

Copper concentrations have not exceeded the MDMER values since monitoring began in 2015. Copper concentrations in 2020 to 2023 currently range from 0.0008 to 0.0052 mg/L.

2.2.2.3 Cyanide

From 2020 to 2023 cyanide has typically been one to two orders of magnitude less that the discharge limit of 1.0 mg/L.

2.2.2.4 Lead

Lead concentrations have been at or near the detection limit since 2016 and have never exceeded MDMER limits.

2.2.2.5 Nickel

Nickel concentrations from 2020 to 2023 were well below the MDMER criteria (1.0 mg/L) and the annual average concentration ranged from 0.0008 to 0.0082 mg/L.

2.2.2.6 Zinc

Zinc concentrations have been consistently low since sampling began and have not exceeded the MDMER guidelines. Annual average zinc concentrations for 2020 to 2023 ranged from 0.0005 mg/L to 0.484 mg/L.

2.2.2.7 TSS

From 2020 to 2023, TSS levels have ranged from 0.5 mg/L to 18.0 mg/L. TSS was the only parameter that has exceeded the MDMER a grab sample concentration limit.

2.2.2.8 Radium-226

Radium-226 has ranged from less than, to slightly greater, than the method detection limit (0.01 Bq/L). This is below the MDMER limit.

2.2.2.9 Un-ionized Ammonia

Un-ionized ammonia was added to the list of deleterious substances in Schedule 4 of the MDMER and has been required to be monitored in final effluent since June 1, 2021. Un-ionized ammonia has ranged from less than the detection limit of 0.001 mg/L to 0.081 mg/L and no values have exceeded the MDMER grab limit of 1.0 mg/L.

2.2.2.10 pH

The pH values in the effluent have always remained within acceptable range as defined in the MDMER (6.0 to 9.5).

Table 2-3: Summary of prescribed deleterious substances in Rainy River Mine effluent, 2020 to 2023.

Year	Final Discharge Point	Input Description	Parameter	Arsenic	Copper	Cyanide	Lead	Nickel	Zinc	TSS	Rd-226	Un-ionized Ammonia	pH	Effluent Volume
			MDMER Grab Limit	**0.6**	**0.6**	**1**	**0.2**	**1**	**1**	**30**	**1.11**	**1**	**6.0-9.5**	
			MDMER Monthly Limit	**0.3**	**0.3**	**0.5**	**0.1**	**0.5**	**0.5**	**15**	**0.37**	**0.5**	**6.0-9.5**	
			Units	**mg/L**	**mg/L**		**mg/L**	**mg/L**	**mg/L**	**mg/L**	**Bq/L**	**mg/L**	**pH units**	**m^3/day**
2020	EDL2	Sediment Pond 1	N	1	1	1	1	1	1	1	-	1	1	1
			Mean	0.0027	0.0041	0.0090	0.00004	0.0021	0.210	0.5	-	0.00259	7.82	162
			Minimum	0.0027	0.0041	0.0090	0.00004	0.0021	0.210	0.5	-	0.00259	7.82	162
			Maximum	0.0027	0.0041	0.0090	0.00004	0.0021	0.210	0.5	-	0.00259	7.82	162
		Sediment Pond 2	N	14	14	14	14	14	14	14	13	10	10	87
			Mean	0.0020	0.0024	0.0011	0.00004	0.0013	0.004	2.0	0.01	0.01000	8.20	3,018
			Minimum	0.0015	0.0020	0.0002	0.00001	0.0011	0.001	1.0	0.01	0.01000	6.94	74
			Maximum	0.0023	0.0029	0.0020	0.00012	0.0016	0.012	3.0	0.01	0.01000	8.64	6,702
		Loslo Creek Discharge	N	9	9	14	9	9	9	14	6	6	15	29
			Mean	0.0030	0.0047	0.0007	0.00035	0.0017	0.006	2.2	0.01	0.00583	7.72	8,552
			Minimum	0.0018	0.0017	0.0004	0.00016	0.0010	0.001	0.5	0.01	0.00100	7.23	1,623
			Maximum	0.0049	0.0083	0.0016	0.00115	0.0020	0.016	10.0	0.01	0.01000	8.10	11,535
	EDL1	Water Discharge Pipeline	N	17	17	32	17	17	17	31	12	16	19	67
			Mean	0.0023	0.0099	0.0018	0.00013	0.0019	0.002	2.1	0.01	0.02169	8.04	16,979
			Minimum	0.0018	0.0053	0.0002	0.00003	0.0013	0.001	0.5	0.01	0.00100	7.56	177
			Maximum	0.0039	0.0203	0.0148	0.00026	0.0035	0.005	4.5	0.01	0.03800	8.48	24,938
2021	EDL2	Sediment Pond 2	N	11	11	11	11	11	11	11	13	5	18	101
			Mean	0.0021	0.0019	0.0015	0.00006	0.0012	0.002	4.6	0.01	0.00900	8.20	6,132
			Minimum	0.0018	0.0015	0.0006	0.00001	0.0008	0.001	1.5	0.01	0.00200	6.95	52
			Maximum	0.0024	0.0024	0.0036	0.00016	0.0017	0.005	8.0	0.01	0.02800	8.91	16,590
		Loslo Creek Discharge	N	1	1	4	1	1	1	4	1	1	6	9
			Mean	0.0012	0.0015	0.0017	0.00023	0.0013	0.001	1.6	0.01	0.00300	7.85	8,168
			Minimum	0.0012	0.0015	0.0006	0.00023	0.0013	0.001	0.5	0.01	0.00300	7.45	216
			Maximum	0.0012	0.0015	0.0042	0.00023	0.0013	0.001	2.5	0.01	0.00300	8.08	12,936
	EDL1	Water Discharge Pipeline	N	8	8	18	8	8	8	18	10	7	27	59
			Mean	0.0029	0.0058	0.0012	0.00017	0.0015	0.002	3.5	0.01	0.00900	8.13	20,948
			Minimum	0.0016	0.0024	0.0004	0.00002	0.0010	0.001	1.0	0.01	0.00500	7.67	1,501
			Maximum	0.0052	0.0146	0.0030	0.00034	0.0019	0.004	9.5	0.01	0.01500	8.60	24,879
2022	EDL2	Sediment Pond 2	N	20	20	20	20	20	20	19	17	19	22	136
			Mean	0.0016	0.0017	0.0011	0.00008	0.0011	0.008	4.3	0.01	0.00837	8.06	9,150

Year	Final Discharge Point	Input Description	Parameter	Arsenic	Copper	Cyanide	Lead	Nickel	Zinc	TSS	Rd-226	Un-ionized Ammonia	pH	Effluent Volume
			MDMER Grab Limit	**0.6**	**0.6**	**1**	**0.2**	**1**	**1**	**30**	**1.11**	**1**	**6.0-9.5**	
			MDMER Monthly Limit	**0.3**	**0.3**	**0.5**	**0.1**	**0.5**	**0.5**	**15**	**0.37**	**0.5**	**6.0-9.5**	
			Units	**mg/L**	**mg/L**		**mg/L**	**mg/L**	**mg/L**	**mg/L**	**Bq/L**	**mg/L**	**pH units**	**m^3/day**
			Minimum	0.0011	0.0014	0.0002	0.00001	0.0009	0.001	1.0	0.00	0.00100	7.40	1,238
			Maximum	0.0024	0.0022	0.0042	0.00024	0.0015	0.063	16.5	0.01	0.02500	9.19	18,000
		Loslo Creek Discharge	N	13	13	28	13	13	13	28	11	13	31	70
			Mean	0.0020	0.0038	0.0010	0.00009	0.0027	0.059	5.4	0.01	0.01123	7.65	14,029
			Minimum	0.0011	0.0007	0.0002	0.00002	0.0009	0.000	0.5	0.01	0.00200	6.92	2,952
			Maximum	0.0048	0.0074	0.0020	0.00022	0.0082	0.484	18.0	0.01	0.05600	8.68	22,815
	EDL1	Water Discharge Pipeline	N	18	18	38	18	18	18	38	15	18	55	129
			Mean	0.0016	0.0048	0.0010	0.00013	0.0022	0.016	4.9	0.01	0.01250	7.83	20,299
			Minimum	0.0011	0.0024	0.0002	0.00001	0.0010	0.001	1.0	0.01	0.00200	6.77	740
			Maximum	0.0032	0.0163	0.0030	0.00050	0.0050	0.105	13.5	0.04	0.08100	8.86	25,732
2023	EDL2	Sediment Pond 2	N	15	15	15	15	15	15	15	14	15	14	68
			Mean	0.0017	0.0025	0.0016	0.00012	0.0012	0.008	4.8	0.01	0.00231	8.09	11,631
			Minimum	0.0014	0.0011	0.0004	0.00005	0.0008	0.003	3.0	0.01	0.00100	7.66	1,600
			Maximum	0.0022	0.0058	0.0020	0.00042	0.0034	0.024	12.0	0.01	0.01000	8.61	23,712
		Loslo Creek Discharge	N	14	14	33	14	14	14	33	13	13	32	84
			Mean	0.0011	0.0052	0.0017	0.00140	0.0034	0.013	3.3	0.01	0.01329	7.76	20,093
			Minimum	0.0008	0.0018	0.0004	0.00003	0.0027	0.004	0.5	0.01	0.00200	7.14	256
			Maximum	0.0013	0.0281	0.0027	0.00907	0.0050	0.030	5.5	0.01	0.05820	8.33	24,812
	EDL1	Water Discharge Pipeline	N	15	15	35	15	15	15	35	14	14	35	93
			Mean	0.0012	0.0028	0.0020	0.00008	0.0034	0.009	3.5	0.01	0.01552	7.81	23,826
			Minimum	0.0010	0.0019	0.0002	0.00001	0.0026	0.003	1.0	0.01	0.00200	7.28	749
			Maximum	0.0013	0.0050	0.0141	0.00050	0.0050	0.030	7.5	0.01	0.04720	8.39	30,216

2.2.3 Mine Effluent Acute Toxicity

Acute lethality of mine effluent is defined as 100% effluent that causes more that 50% mortality in the test species. Mine effluents have not been acutely lethal to either Rainbow Trout (*Oncorhynchus mykiss*) or *Daphnia magna* according to the MDMER definition since the release of treated water or runoff from the temporary construction discharges ceased near the end of Phase 1. The summary of acute toxicity tests from 2020 to date are in **Appendix A**.

2.2.4 Mine Effluent Sublethal Toxicity

2.2.4.1 Test Species

Sublethal testing using Fathead Minnow (*Pimephales promelas*), water flea (*Ceriodaphnia dubia*), duckweed (*Lemna minor*) and the green alga, *Raphidocelis subcapitata* are summarized in **Table 2-4**. All testing was completed according to the appropriate published protocols. According to Schedule 5 6 (3) of the MDMER the most sensitive species was to have been chosen according to discharge data from 2016 until the end of 2018. However, at that time construction was still underway at the mine and the effluent quality did not reflect that of the full treatment that is used now. Review of the 2019 to 2020 data indicate that the most sensitive species according to its geometric mean over that period is *C. dubia*. Therefore, moving forward New Gold will conduct toxicity testing on *C. dubia* using effluent from each final discharge in each quarter of discharge.

Table 2-4: Summary of sublethal toxicity results, RRM Phase 1 through 3 EEMs.

Date	Fathead Minnow		Water Flea		Duckweed		Algae
	P. promelas		*C. dubia*		*L. minor*		*R. subcapitata*
	Survival	Growth	Survival	Reproduction	Frond Count	Frond Weight	Growth
	$LC50_a$	$IC25_b$	$LC50_a$	$IC25_b$	$IC25_b$	$IC25_b$	$IC25_b$
EEM Phase 1 – FDP2 (Sump 3, 6)							
25-Jul-16	> 100	37.1 (22.5 – 53.7)	91.7 (30 -DNC)[c]	71.4 (43.6 – 100)	55 (33.1 – 80.0)	76.2 (38 – 100)	90.9
28-Aug-17	> 100	> 100	> 100	> 100	63.7 (50.8 – 75.3)	> 97	90.9
GeoMean	**> 100**	**> 61**	**> 96**	**> 84**	**> 59**	**> 86**	**> 91**
EEM Phase 1 – FDP3 (Sump 4)							
27-Sep-16	> 100	> 100	> 100	>100	95.3 (26.4 – 96.7)	>97	> 90.9
20-Jun-17	Indeterminate[d]	Indeterminate[e]	> 100	> 100	> 97	> 97	> 90.9
GeoMean	**> 100**	**> 100**	**> 100**	**> 100**	**> 96**	**> 97**	**> 91**
EEM Phase 2 – EDL1							
28-Oct-19	> 100	>100	> 100	>100	>97	>97	>90.91
EEM Phase 2 – EDL2							
23-Sep-19[f]	> 100	>100	> 100	>100	0.23	>97	>90.91
21-Oct-20	> 100	>100	> 100	>100	>97	>97	>90.91
EEM Phase 3 – EDL2							
30-Jun-22	>100	>100	>100	0.32	39.99	0.27	>90.91
09-Nov-22	>100	>100	>100	>100	>97	>97	>90.91
05-May-23	>100	>100	>100	88	54.06	>97	>90.91
06-Nov-23	>100	>100	>100	2.1	6.9	>97	>91
GeoMean	**> 100**	**>100**	**> 100**	8.77	34.68	22.28	**>90.91**
EEM Phase 3 – SP2							
04-Oct-21	> 100	>100	> 100	<0.137	>97	>97	>90.91
27-Apr-22	>100	>100	>100	>100	>97	>97	>90.91
28-Sep-22	>100	>100	>100	10.58	>97	>97	>90.91
09-Nov-22	>100	>100	>100	>100	>97	>97	>90.91
05-May-23	>100	79.59	>100	>100	>97	>97	>90.91
28-Nov-23	>100	40.33	>100	27.17	>97	>97	>90.91
GeoMean	**>100**	82.75	**>100**	18.45	**>97**	**>97**	**>90.91**
EEM Phase 3 – EDL1							
27-Apr-22	>100	>100	>100	50.69	79.25	>97	79.31
28-Serp-22	>100	>100	>100	>100	10.9	>97	37.95

Date	Fathead Minnow		Water Flea		Duckweed		Algae
	P. promelas		*C. dubia*		*L. minor*		*R. subcapitata*
	Survival	Growth	Survival	Reproduction	Frond Count	Frond Weight	Growth
	$LC50_a$	$IC25_b$	$LC50_a$	$IC25_b$	$IC25_b$	$IC25_b$	$IC25_b$
09-Nov-22	>100	>100	>100	>100	87.6	>97	>90.91
05-May-23	>100	>100	>100	>100	8.29	7.03	90.91
06-Nov-23	>100	>100	-	-	7.2	>97	>91
GeoMean	**>100**	**>100**	**>100**	**84.38**	**21.43**	**57.39**	**74.30**

Notes:
a – Effluent concentration causing 50% mortality among exposed organisms.
b – Effluent concentration at which a 25% inhibition/reduction in an endpoint was observed among effluent- exposed organisms relative to the control group.
c – Range could not be calculated
d – A statistically reasonable estimate could not be calculated. However, according to Equal Variance t two-sample Test (CETIS), there was no significant reduction in growth between the control and the 100% test concentration.
e – A statistically reasonable estimate could not be calculated. However, according to the Fisher Exact Test (CETIS), mortality in the 100% effluent concentration (0%) was not significantly higher than in control (10%).
f – EDL2 was not commissioned until 2020, this round of tests was conducted on Sediment Pond 1 discharge.

2.2.4.2 Test Results

Fathead Minnow survival and growth has generally not been affected by exposure to treated mine wastewater with one exception in the early portion of Phase 1. Since Phase 1 all sublethal tests on Fathead Minnow have resulted in no effect.

Generally, *C. dubia* survival and reproduction were consistently high. Survival was unaffected in Phase 3. However, the geomean (GM) of reproduction was 8.77% v/v in EDL2, 18.45% v/v in SP2, and 83.48% v/v in EDL1. This is a decrease from previous phases.

For *L. minor*, the IC25 for frond count was variable in the past at both FDP3 and FDP2, ranging from an average of 55% in July 2016 to >97% in June 2017. Frond count tests since Phase 1 had ranged from IC25 of 0.23 % v/v to unaffected with results of >97% v/v. The GM for frond count was >97% v/v in SP2, but 34.68% v/v in EDL2 and 21.43% v/v in EDL1. Similarly, frond weight was >97% v/v in SP2, but 22.28% v/v in EDL2 and 57.39% v/v in EDL1. This is a decrease from previous phases.

With the exception of two tests from EDL1, the growth of green algae (*R. subcapitata*) has been constant through all exposures to treated mine wastewater. The Phase 3 test results from the EDL1 discharge ranged from 37.95% to >91% v/v whereas all other previous tests have resulted in an IC25 of >90.1% v/v. The GM IC25 for Phase 3 is 79.2 % v/v.

2.3 Mine Wastewater Dispersion in Pinewood River

Schedule 5 Division 1 10 (a) (i) of the MDMER stipulates that as part of the First Study Design that the site characterization submitted should include a description of effluent mixing in the receiving environment for each permitted discharge and that this description

should include an estimate of the effluent concentration at distances of 100 and 250 m downstream of the applicable discharges. A formal plume delineation study of the effluent dispersion in the Pinewood River in the exposure area downstream of the EDL2 or the EDL1 discharge areas have not been completed to date. The reasoning in Phase 1 and 2 was that until the mine determined the final locations of their consolidated discharges following construction that a plume delineation would not be valid. Additionally, at the time of the Phase 1 study discharges were not sufficient to be used as a tracer to determine the extent of the plume. Calculations based on effluent discharge rates and the Pinewood River and Water Survey of Canada flow rates estimate that the effluent concentration would be approximately 14% at 250 m downstream of the EDL2 discharge location, which was extrapolated to assume that the percent effluent at both 100 m and 250 m was greater than 1% and therefore, triggered both fish and benthic surveys.

Under their provincial permit, the mine is permitted to discharge at a maximum ratio of 1:1 once the flow in the river reaches 10,000 m3/ day. A flow rate of 10,000 m3/day represents the worst-case low flow condition. There is no appreciable dilution in the first 250 m downstream of the discharge diffuser and therefore the worse case scenario is that the effluent concentration is 50%, exceeding 1% at the 100 and 250 m distances to necessitating both fish and benthic surveys.

Upon finalization of discharge locations, a study was completed by Ecometrix at EDL1 and EDL2 to determine whether effluent concentration at 250 m likely exceeded 1% coincident with a rigorous mixing study completed by Knight Piesold Ltd (2024). Briefly, the mixing study used Rhodamine WT as a conservative tracer to estimate effluent mixing in up to a 60 m zone from each discharge location. After steady state conditions were established and Rhodamine WT was identified as having dispersed to 250 m downstream, Ecometrix staff used a portable water quality meter affixed with a calibrated Rhodamine WT sensor to measure concentrations at and in between 75 m, 100 m, 125 m, 225 m, 250 m, and 275 m downstream locations. Data from the mixing study and field measurements of Rhodamine WT supported estimating whether effluent exceeded 1% at 100 m and 250 m.

2.3.1 Mixing Study Methods and Results Summary

The mixing study was completed by Knight Piesold Ltd injecting a constant amount of Rhodamine WT dye into the effluent discharge pipelines, while monitoring Rhodamine WT dye concentrations at various transects downstream from the discharges (EDL1 and EDL2).

Knight Piesold Ltd. placed YSI EXO sondes across the river at three transects downstream of the diffusers – at each transect one sonde was placed at channel centre and one sonde was placed at each channel bank. The three transect locations were selected based on visual observations of mixing conditions but were up to 60 m away from the discharge locations.

The Rhodamine WT dye was injected into the effluent discharge pipeline at a constant rate and YSI EXO sonde readings were monitored until it was determined that a steady state condition in the river had been established. This steady state condition was maintained for

at least 30 minutes, or long enough to ensure that the concentration of Rhodamine in the river was not notably varying in time, and that both Knight Piesold Ltd and Ecometrix had collected sufficient data to meet their own study objectives. The injection was then stopped, and the recordings continued as dye concentrations decreased. The mixing study is attached in **Appendix A**.

2.3.2 Ecometrix Sampling and Analysis

After steady state conditions were met and the Rhodamine WT was identified as having dispersed to 250 m downstream, Ecometrix staff used a portable water quality meter affixed with a calibrated Rhodamine WT sensor to measure concentrations at and in between 75 m, 100 m, 125 m, 225 m, 250 m, 275 m downstream locations by traversing curvilinear patterns from upstream to downstream. Further details are provided below.

Note that Knight Piesold Ltd's mixing study reported study times as if operating in the Pacific Time zone. Hereafter, times are reported in the Central Time zone.

At EDL1, Ecometrix sampled from 11:44 – 12:23 on November 8 during steady state conditions. The GPS malfunctioned on the water quality meter so manual points were taken and aligned with sensor readings. To be conservative, only those measurements that were aligned the spatial points were used for this analysis. A total of 94 points were taken at a mean depth of 0.27 m during this sampling event.

At EDL2, Ecometrix sampled from 12:57 – 13:10 on November 9 during steady state conditions. For this sampling event, the water quality meter GPS was functioning. A total of 397 points were taken at a mean depth of 0.53.

At both locations, a GIS was used to construct 5 m circular buffers surrounding the 75 m, 100 m, 125 m, 225 m, and 275 m sampling locations. Sampling points intersecting this buffer were used to calculate summary statistics for those locations. The 5 m circular buffer was chosen to capture points across the stream width.

2.3.3 Results

Table 2-5presents the concentrations of Rhodamine WT moving at downstream distances from the discharge locations. For the Knight Piesold Ltd. values, a grand mean and grand standard deviation was calculated from the mean and standard deviation values reported for the three sondes per transect. Effluent was reported as fully mixed at 60 m by Knight Piesold Ltd.. At EDL1, the ambient-to-effluent discharge ratio was reported as ~5:1 during the study (19% effluent) with an updated mixing length of 38 m. At EDL2, the ambient-to-effluent discharge ratio was reported as ~2:1 during the study (49% effluent) with an updated mixing length of 35 m.

Concentrations taken at 60 m were similar to those taken by Ecometrix at 75 m. The proportion of effluent at downstream locations was calculated as the ratio of the mean

concentration taken from sampling points in the 5 m buffer to the concentration reported at 60 m.

Results show no appreciable dilution of effluent at 100 m or 250 m with all values >1% effluent indicating the need for fish and benthic biological studies.

Table 2-5: Results of mixing study and field investigation for determining effluent concentration >1%.

Discharge	Distance	Rhodamine (µg/L) Mean	Rhodamine (µg/L) SD	% Effluent	Comment
EDL2	15 m	88.67	4.65	--	
	25 m	90.33	3.20	--	
	60 m	88.67	2.77	19.00	Fully mixed; ~5:1 ambient:effluent
	75 m	83.72	0.94	17.94	N = 22
	100 m	83.44	1.25	17.88	N = 50
	125 m	83.95	0.06	17.99	N = 12
	225 m	80.88	0.02	17.33	N = 7
	250 m	79.01	1.16	16.93	N = 80
	275 m	78.92	0.05	16.91	N = 18
EDL1	20 m	133.00	6.56	--	
	40 m	130.33	6.03	--	
	60 m	134.00	3.35	49.00	Fully mixed; ~2:1 ambient:effluent
	75 m	128.44	0.00	46.97	N = 1
	100 m	120.85	0.20	42.36	N = 4
	125 m	129.25	0.22	40.85	N = 4
	225 m	125.21	1.19	38.18	N = 3
	250 m	122.61	6.86	34.93	N = 4
	275 m	125.72	0.21	32.77	N = 2

2.4 Receiving Water Chemistry

A summary of the water characteristics for receiving water in the Pinewood River can be found in **Table 2-6** and **Table 2-7**. Complete data provided by New Gold from January 2020 – December 2023 inclusive is available in **Appendix A**.

Currently the RRM routinely samples a variety of surface water stations within the mine site and adjacent areas. Most of these stations are sampled as part of the Provincial ECA and the frequency of this sampling is more than that required as part of the MDMER. Data from four of these stations are used for both federal EEM and provincial ECA compliance purposes and characterize the exposure and reference areas upstream and downstream of both the

EDL1 and EDL2 discharges (**Figure 2-1**). Surface water station SW20, located off Heatwole Road, is a reference area water quality monitoring station for the EDL2 discharge. SW22A, located downstream of the mine effluent discharges (i.e., Loslo Creek and Sediment Pond 2) is the exposure area location. These two stations continue to be monitored at least four times per year according to the MDMER requirements and data collected there through from 2020 to 2023 are summarized in **Table 2-6**. New Gold also samples water downstream in the Pinewood River near the EDL1 discharge. Station SW23 represents the "reference" area upstream of the EDL1 discharge and SW24 represents the exposure water quality downstream of the discharge. It should be noted that SW23 water quality will capture the discharges at EDL2 and Sediment Pond 2 that enter the receiver approximately 10 km upstream.

Comparing SW20 to SW22A, there does not appear to be major differences in water quality. In both the reference and exposure area, there were exceedances outside of the PWQO and CCME guidelines for ammonia, pH, phosphorus, aluminum, chromium, iron, and mercury. Only copper and zinc had PWQO and/or CCME exceedances in the exposure area but not the reference area.

Similar trends were observed when comparing SW23 to SW24. In both the reference and exposure area, there were exceedances outside of the PWQO and CCME guidelines for ammonia, pH, phosphorus, total suspended solids, aluminum, chromium, copper, iron, and mercury. Interestingly, there was an exceedance for zinc in the reference area but not the exposure area.

Table 2-6: Summary of receiving water chemistry to SW20 and SW22A from 2020 to 2023.

					SW20 (upstream)									SW22A (downstream)							
Parameter	Units	ECA	PWQO	CCME	N ≠ ECA	N ≠ PWQO	N ≠ CCME	N	Mean	SD	Min	Median	Max	N ≠ ECA	N ≠ PWQO	N ≠ CCME	N	Mean	SD	Min	Median	Max
Alkalinity, Total as CaCO3	mg/L	--	--	--	0	0	0	47	125.2	46.3	2.0	133.0	185.0	0	0	0	47	169.6	42.2	59.6	176.0	253.0
Ammonia, Total as N	mg/L	--	--	0.35	0	0	0	48	0.030	0.035	0.000	0.020	0.166	0	0	1	49	0.062	0.111	0.000	0.030	0.700
Ammonia, Un-ionized as N	mg/L	--	0.02	0.019	0	1	1	37	0.009	0.005	0.000	0.010	0.028	0	0	0	40	0.007	0.004	0.000	0.010	0.010
Conductivity	uS/cm	--	--	--	0	0	0	47	291.9	111.0	0.2	318.0	453.0	0	0	0	47	470.8	215.1	167.0	410.0	1030.0
Dissolved Oxygen	mg/L	--	--	--	0	0	0	36	7.0	5.8	0.7	4.8	26.9	0	0	0	37	5.9	4.1	0.0	5.1	16.2
Field pH	--	--	6.5-8.5	6.5-9	0	11	11	39	7.1	0.6	5.1	7.2	8.2	0	8	8	39	7.3	0.7	5.1	7.3	9.3
Hardness as CaCO3	mg/L	--	--	--	0	0	0	47	136.6	48.0	0.5	146.0	186.0	0	0	0	47	213.8	66.3	90.2	202.0	390.0
Chloride	mg/L	--	--	--	0	0	0	48	19.4	12.9	0.1	18.2	53.0	0	0	0	50	15.9	7.6	4.1	14.2	34.6
Cyanide, Total	mg/L	--	--	--	0	0	0	48	0.001	0.001	0.000	0.001	0.003	0	0	0	50	0.001	0.001	0.000	0.001	0.004
Nitrate as N	mg/L	--	--	13	0	0	0	47	0.028	0.027	0.002	0.020	0.128	0	0	0	47	0.257	0.562	0.002	0.020	2.370
Phosphorus	mg/L	--	0.03	--	0	27	0	46	0.040	0.024	0.005	0.038	0.106	0	35	0	46	0.081	0.066	0.010	0.069	0.324
Total Suspended Solids	mg/L	30	--	--	0	0	0	47	4.9	2.9	0.5	4.0	14.0	0	0	0	47	6.0	4.2	0.5	4.5	20.0
Aluminum	mg/L	--	--	0.1	0	0	33	47	0.1639	0.1602	0.0004	0.1360	1.0600	0	0	30	47	0.16561	0.14448	0.02940	0.12800	0.76700
Arsenic	mg/L	0.01	0.1	0.05	0	0	0	47	0.0011	0.0006	0.0000	0.0009	0.0031	0	0	0	47	0.00153	0.00075	0.00061	0.00129	0.00404
Cadmium	mg/L	--	0.0005	0.00021	0	0	0	47	0.0000	0.0000	0.0000	0.0000	0.0000	0	0	0	47	0.00001	0.00001	0.00000	0.00001	0.00003
Chromium	mg/L	--	0.001	0.001	0	2	2	47	0.0006	0.0004	0.0001	0.0005	0.0025	0	3	3	47	0.00055	0.00025	0.00020	0.00050	0.00148
Cobalt	mg/L	--	--	0.009	0	0	0	47	0.0004	0.0002	0.0001	0.0003	0.0010	0	0	0	47	0.00060	0.00054	0.00013	0.00038	0.00263
Copper	mg/L	0.008	0.005	0.0031	0	0	0	47	0.0009	0.0005	0.0002	0.0008	0.0030	1	1	2	47	0.00136	0.00124	0.00026	0.00104	0.00838
Iron	mg/L	--	0.3	0.3	0	40	40	47	0.6577	0.3962	0.0020	0.5430	1.6900	0	32	32	47	0.72730	0.76629	0.14000	0.44600	3.87000
Lead	mg/L	0.008	0.005	0.0047	0	0	0	47	0.0001	0.0001	0.0000	0.0001	0.0007	0	0	0	47	0.00016	0.00017	0.00003	0.00010	0.00088
Mercury	mg/L	--	--	0.000026	0	0	24	47	0.0000	0.0000	0.0000	0.0000	0.0000	0	0	21	47	0.00002	0.00001	0.00001	0.00001	0.00003
Molybdenum	mg/L	--	0.04	0.073	0	0	0	47	0.0003	0.0002	0.0001	0.0003	0.0010	0	0	0	47	0.00136	0.00182	0.00014	0.00055	0.00716
Nickel	mg/L	--	0.025	0.12	0	0	0	47	0.0014	0.0004	0.0001	0.0014	0.0030	0	0	0	47	0.00172	0.00033	0.00114	0.00170	0.00246
Selenium	mg/L	--	0.1	0.001	0	0	0	47	0.0002	0.0001	0.0001	0.0001	0.0003	0	0	0	47	0.00022	0.00007	0.00014	0.00020	0.00048
Thallium	mg/L	--	0.0003	0.0008	0	0	0	47	0.0002	0.0001	0.0000	0.0003	0.0003	0	0	0	47	0.00013	0.00015	0.00000	0.00001	0.00030
Uranium	mg/L	--	0.005	0.015	0	0	0	47	0.0006	0.0010	0.0000	0.0004	0.0050	0	0	0	47	0.00092	0.00043	0.00027	0.00086	0.00224
Zinc	mg/L	0.09	0.02	--	0	0	0	47	0.0039	0.0031	0.0005	0.0030	0.0170	0	1	0	47	0.00487	0.00452	0.00050	0.00300	0.02300

Note: Variables with exceedances are highlighted.

Table 2-7: Summary of receiving water chemistry to SW23 and SW24 from 2020 2023

Parameter	Units	ECA	PWQO	CCME	SW23 (upstream)									SW24 (downstream)							
					N ≠ ECA	N ≠ PWQO	N ≠ CCME	N	Mean	SD	Min	Median	Max	N ≠ ECA	N ≠ PWQO	N ≠ CCME	N	Mean	SD	Min	Median	Max
Alkalinity, Total as CaCO3	mg/L	--	--	--	0	0	0	41	156.9	72.7	47.6	151.0	512.0	0	0	0	42	146.7	44.3	50.4	142.0	236.0
Ammonia, Total as N	mg/L	--	--	0.35	0	0	1	43	0.062	0.083	0.000	0.038	0.372	0	0	2	42	0.0928	0.1500	0.0000	0.0450	0.7580
Ammonia, Un-ionized as N	mg/L	--	0.02	0.019	0	0	0	37	0.007	0.004	0.000	0.010	0.010	0	0	0	37	0.0072	0.0041	0.0001	0.0100	0.0102
Conductivity	uS/cm	--	--	--	0	0	0	42	352.4	133.0	129.0	326.5	697.0	0	0	0	42	427.1	242.6	1.0	373.0	1010.0
Dissolved Oxygen	mg/L	--	--	--	0	0	0	35	5.5	3.7	0.0	5.0	13.6	0	0	0	34	5.7	3.5	0.0	5.4	12.6
Field pH	--	--	6.5-8.5	6.5-9	0	13	12	37	7.3	0.5	6.7	7.2	8.7	0	13	12	36	7.2	0.5	6.3	7.3	8.6
Hardness as CaCO3	mg/L	--	--	--	0	0	0	43	170.6	54.4	0.5	174.0	270.0	0	0	0	42	194.6	66.5	61.9	182.5	346.0
Chloride	mg/L	--	--	--	0	0	0	43	8.0	5.0	2.4	6.6	22.1	0	0	0	43	9.2	6.6	2.0	6.1	30.7
Cyanide, Total	mg/L	--	--	--	0	0	0	43	0.001	0.001	0.000	0.001	0.003	0	0	0	42	0.001	0.001	0.000	0.001	0.003
Nitrate as N	mg/L	--	--	13	0	0	0	42	0.144	0.229	0.004	0.041	0.884	0	0	0	42	0.375	0.638	0.002	0.050	2.310
Phosphorus	mg/L	--	0.03	--	0	35	0	42	0.075	0.041	0.015	0.068	0.196	0	30	0	41	0.068	0.042	0.010	0.065	0.170
Total Suspended Solids	mg/L	30	--	--	1	0	0	42	11.5	7.0	3.0	10.8	43.5	1	0	0	42	10.4	7.0	3.0	8.8	39.5
Aluminum	mg/L	--	--	0.1	0	0	43	43	0.44284	0.20134	0.13000	0.44800	1.20000	0	0	42	42	0.43290	0.26167	0.11200	0.33400	1.17000
Arsenic	mg/L	0.01	0.1	0.05	0	0	0	43	0.00195	0.00133	0.00061	0.00136	0.00669	0	0	0	42	0.00195	0.00135	0.00069	0.00141	0.00707
Cadmium	mg/L	--	0.0005	0.00021	0	0	0	43	0.00002	0.00001	0.00001	0.00001	0.00004	0	0	0	42	0.00002	0.00001	0.00001	0.00002	0.00004
Chromium	mg/L	--	0.001	0.001	0	24	24	43	0.00108	0.00048	0.00040	0.00106	0.00293	0	20	20	42	0.00112	0.00066	0.00032	0.00099	0.00384
Cobalt	mg/L	--	--	0.009	0	0	0	43	0.00073	0.00042	0.00019	0.00064	0.00219	0	0	0	42	0.00080	0.00039	0.00020	0.00080	0.00247
Copper	mg/L	0.008	0.005	0.0031	0	1	1	43	0.00181	0.00081	0.00080	0.00156	0.00568	2	3	6	42	0.00233	0.00181	0.00084	0.00164	0.01020
Iron	mg/L	--	0.3	0.3	0	43	43	43	1.19288	0.63399	0.34300	1.08000	2.99000	0	41	41	42	1.10910	0.66620	0.26100	1.09000	3.70000
Lead	mg/L	0.008	0.005	0.0047	0	0	0	43	0.00040	0.00022	0.00011	0.00037	0.00113	0	0	0	42	0.00039	0.00024	0.00009	0.00033	0.00103
Mercury	mg/L	--	--	0.000026	0	0	18	43	0.00002	0.00001	0.00001	0.00001	0.00005	0	0	20	42	0.00002	0.00001	0.00001	0.00001	0.00005
Molybdenum	mg/L	--	0.04	0.073	0	0	0	43	0.00085	0.00087	0.00022	0.00059	0.00371	0	0	0	42	0.00159	0.00197	0.00019	0.00066	0.00653
Nickel	mg/L	--	0.025	0.12	0	0	0	43	0.00245	0.00069	0.00118	0.00244	0.00436	0	0	0	42	0.00248	0.00077	0.00116	0.00242	0.00411
Selenium	mg/L	--	0.1	0.001	0	0	0	43	0.00021	0.00006	0.00005	0.00019	0.00034	0	0	0	41	0.00024	0.00008	0.00005	0.00023	0.00041
Thallium	mg/L	--	0.0003	0.0008	0	0	0	43	0.00008	0.00013	0.00000	0.00001	0.00030	0	0	0	42	0.00008	0.00013	0.00000	0.00001	0.00030
Uranium	mg/L	--	0.005	0.015	0	0	0	43	0.00075	0.00031	0.00019	0.00070	0.00142	0	0	0	42	0.00089	0.00039	0.00039	0.00082	0.00198
Zinc	mg/L	0.09	0.02	--	0	1	0	43	0.00496	0.00461	0.00100	0.00400	0.02700	0	0	0	42	0.00445	0.00223	0.00050	0.00400	0.01060

Note: Variables with exceedances are highlighted.

2.5 Ecological Aspects of the Study Area

A more fulsome regional setting and ecological site characterization of the study area was provided previous baseline study reports (AMEC, 2013b; AMEC, 2014; KCB, 2011) and in the Phase 1 EEM Study Design (Minnow, 2016) with a brief summary provided below.

2.5.1 Topography

The mine site is surrounded by rural agricultural property with gently undulating terrain and areas of forest. The natural elevation of the site ranges from 350 to 410 masl. Regional soils are predominantly glacial tills, and lacustrine soils, including gleysol, humo-ferric podzol, grey luvisol, rockland soils and humisol (AMEC, 2014; KCB, 2011) whereas as on- site soils range from clay loam to sandy clay loam near the surface and clay at depth (KCB, 2011). The regional area is referred to as the Rainy River Greenstone Belt of the Wabigoon Subprovince and predominantly metamorphic grade greenschist to lower- amphibolite facies. The site is over intermediate-felsic mineralization (AMEC, 2013b; AMEC, 2014; KCB, 2011).

2.5.2 Vegetation

Uncleared areas near the exposure and reference areas on the Pinewood River are comprised of a forested riparian vegetation of poplar, spruce, larch, elm, and White Birch. However, some reaches of these areas have been historically cleared up to the channel edge for agricultural and pasturing and as a result regeneration of riparian vegetation is more dominated by sedge, alder, and willow, with mixed forest available within the valley at a greater distance from the channel margin (AMEC, 2013a). Similar conditions exist near both the reference areas although the amount of forest cover is higher compared to the exposure areas.

2.5.3 Aquatic Resources

2.5.3.1 Benthic Invertebrates

The baseline studies associated with the RRM development sampled at a variety of locations from upstream of the mine to the confluence with Rainy River (AMEC 2013a, Minnow 2016). The benthic invertebrate community described as part of the baseline could be classified as tolerant, likely owing to the low gradient, low and variable nature of the Pinewood River (AMEC, 2013a & 2014). As is consistent with a tolerant community, the midge family Chironomidae were the dominant taxa present. Other taxa that were relatively abundant included biting midges, phantom midges, worms, amphipods, mud snails, ramshorn snails, and bivalve molluscs (AMEC, 2014). The variability of the flow regime both seasonally and spatially within the Pinewood River resulted in highly variable benthic community endpoints may be due to microhabitat differences (AMEC 2012 & 2013a).

2.5.3.2 Fisheries

Multiple types of gear were used to sample the fish community in the Pinewood River system (AMEC, 2012; AMEC, 2013a & b; AMEC, 2014; KCB, 2011; Minnow, 2016). Most large-

bodied fish are confined to the lower reaches downstream of the mine site as a result of depth and size of the watercourse. Many of the fish within the watershed are small-bodied and have a larger distribution in the watercourse owing to their ability to inhabit smaller reaches of the Pinewood River and associated tributaries. The catch per unit effort associated with previous studies varied widely but overall was normally low at most locations for most species. This may be the result of the types of gear and seasonality of the sampling but may also be the result of the Pinewood River's variable flow regime and consequently low abundance of fish (Minnow 2016)

Lake Sturgeon are the only species listed under the Provincial the Committee on the Status of Species at Risk in Ontario (COSSARO) legislation. No Lake Sturgeon were identified near the RRM site but rather were observed in the lower reaches of the Pinewood River, (AMEC 2012, 2013a, 2014). Sturgeon Creek is known to provide some spawning habitat which is periodically used. Lake Sturgeon were not captured or reported as part of the Phase 1 or Phase 2 EEM programs in any study areas. The La Vallée River at the location of the both reference areas have diverse small-bodied fish communities and are actively used by local bait fishermen to supply anglers.

2.5.4 Resource Utilization

The recreational activities within the regional setting of the mine include hunting, trapping, and snowmobile and ATV use. The mine is located within a low-density rural area with the primary agriculture limited to cattle and associated feed crops. Logging and more intensive agriculture were historically more prevalent.

Generally, the Pinewood River does not support any substantial fishing for either bait fish or sportfish in the general vicinity of the mine. Most fishing is limited to the lower reaches (Minnow 2016)

2.5.5 Habitat Description of the EEM Study Areas

The Pinewood River is the receiver of the effluent from all permitted discharges (Sediment Pond 1, Sediment Pond 2, Loslo Creek (EDL2) and the Water Pipeline Discharge (EDL1). The Pinewood River originates from Pinewood Lake approximately 24.2 km upstream of SW22A and flows south to Rainy River which flows west into the southernmost point of Lake of the Woods. The main stem of the Pinewood River is 75 km long and drains a watershed of approximately 576 km^2. The flow conditions in the Pinewood River are highly variable and change seasonally based on spring freshet, summer drought conditions, and the effects of alterations from wildlife (i.e., beaver dams). Overall, the river is shallow and meandering with a low gradient (average <0.1% KCB, 2011). The substrates are predominantly fines in the clay silt fraction with detritus and lower proportions of sand, gravel and boulder in some areas (AMEC, 2014). Wetted width varies from 1.5 m to 40 m proceeding downstream (AMEC, 2012; KCB, 2011). Baseline studies indicated the wetted width near the RRM was around 10 m with a depth range of 0.3 to 1 m (AMEC, 2012; KCB, 2011).

Downstream of the mine site various habitat types are present that could provide spawning for species such as Walleye (*Stizostedion vitreum*), Northern Pike (*Esox lucius*) and Yellow Perch (*Perca flavescens*). These are generally associated with wider channel width and coarser substrates with Walleye and harder substrate spawning fish habitat being confined to below McCallum Creek whereas vegetation spawners such as pike and perch have a large proportion of habitat and the extent of this is generally upstream of McCallum Creek. Upstream of the mine site progressing towards Pinewood Lake the receiver is ponded and small with substantial evidence of beaver activities both current and historical.

2.5.5.1 Pinewood River Areas

During Phase 1, a single exposure area (PINR-EXP) was located on the Pinewood River downstream of its confluence with Loslo Creek. In Phase 2 a similar area was sampled on the Pinewood River. This exposure location is situated downstream of the discharge structure commissioned in September 2020 and is situated to assess the potential effects of the combined discharges of Loslo Creek, Sediment Pond 1 and Sediment Pond 2 on the Pinewood River (Figure 2-2: Rainy River Mine Phase 1–3 EEM Areas.**Figure 2-2**).

Sampling areas were also established upstream (SW23 – PINR-REF2) and downstream (SW24 – PINR-EXP2) of the Water Discharge Pipeline outfall that was also commissioned in 2020. These areas were previously sampled to meet the requirements of New Gold's provincial operating permit, however now form part of the assessment for EEM with the change of the primary discharge to EDL1.

The EDL2 exposure area used in Phase 1 and 2 and the current study is situated in a reach that was characterized by AMEC (2013) as habitat Type 1 and/or 2. These habitat types primarily consist of relatively deep and wide channels (10 to 50 m wide) mostly comprised of flat morphology with some pools (up to > 2 m deep). Generally, these habitat types are characterized by relatively narrow flood prone widths and a variable composition of riparian vegetation. Although both habitat types have some similarity in channel dimension, substrate and cover availability, Type 1 was characterized by AMEC (2013a) as having a narrower floodplain with moderate entrenchment and forested riparian vegetation extending close to the channel edge. Type 1 aquatic vegetation was dominated by Richardson's Pondweed (*Potamogeton richardsonii*) and Coontail (*Ceratophyllum demersum*).

Type 2 habitat is characterized by a slightly wider floodplain (maximum 50 m) dominated by sedge, Alder and Willow, with mixed forest available within the valley at a greater distance from the channel margins. Aquatic vegetation in Type 2 reaches is dominated by Yellow Pond-lily (*Nuphar luteum*), Broad-leaved Arrowhead (*Sagittaria latifolia*), Tapegrass (*Vallisneria spirallis*) and Coontail.

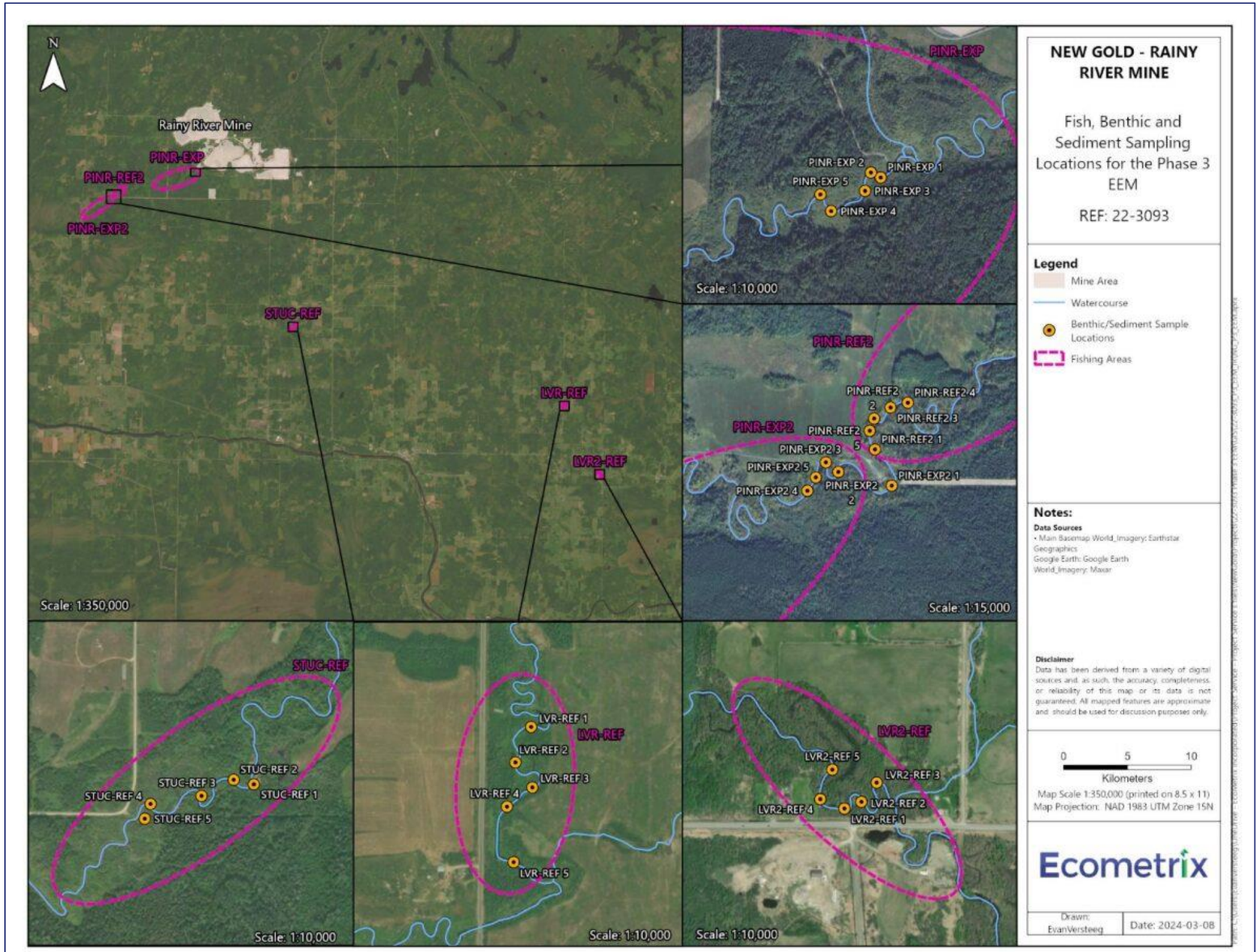

Figure 2-2: Rainy River Mine Phase 1–3 EEM Areas.

Substrate throughout both habitat types is relatively uniform and dominated by silt/muck, sand, clay and detritus mixed with some presence of larger substrate particles (gravel, boulder). PINR-EXP was described as having silt and clay dominated substrates with some sand with relatively high TOC (compared to the Sturgeon Creek reference area).

Mixed forest species associated with both habitat types are Black Spruce (*Picea mariana*), Tamarack (*Larix laricina*), Balsam Poplar (*Populus balsamifera*), American Elm (*Ulmus Americana*) and White Birch (*Betula papyrifera*).

The EDL1 exposure area for the Phase 3 program is approximately 10 km downstream of the mine site. At this location the Pinewood River watershed is approximately 213 km^2. This is more than double the watershed area at the site. The habitat in the Pinewood River near the discharge was referred to as Type 1 habitat as described above. During the baseline study this location was assessed and contains 20-55% instream cover in the form of woody debris, various species of aquatic vegetation (i.e., emergent, submergent, overhanding) AMEC 2012). Bankfull width ranges from 5 to 10 m and steam morphology is approximately 80% flats and 20% pools with depths ranging from 0.25 to 1.75 m. The Pinewood River is bordered by New Gold owned grassland on one side with a mixed forest providing riparian cover and canopy over above 50% of the area.

2.5.5.2 Phase 1–3 Reference Areas

Sturgeon Creek

Sturgeon Creek was chosen as a suitable reference to PINR-EXP (i.e., EDL2) from a shortlist of nine potential sites based on stream morphology, length, mean annual flow and many more (Minnow, 2016). The primary criteria used to select the Sturgeon Creek reach in Phase 1 were watershed area, agricultural land use and stream morphology. Sturgeon Creek is a fourth order stream located southeast of the RRM. It is approximately 97 km long and has a watershed area of around 128 km^2 (Minnow, 2016).

Results of the Phase 1 field program indicated that Sturgeon Creek had similar depth and velocity to that of the Pinewood exposure area. Dissolved oxygen and pH were also similar, whereas the exposure area did have slightly colder temperatures and higher conductance than the selected reference area. The Sturgeon Creek reference area is slightly smaller than the Pinewood River exposure area and contained only seven fish species compared to eleven captured in the exposure during Phase 1 (**Figure 2-2**).

La Vallée River

The Phase 1 EEM reported that some of the statistically significant results that were noted in the benthic community may have been the result of subtle habitat differences between the Pinewood River exposure and the Sturgeon Creek reference areas. One habitat difference that was noted quantitatively was substrate type. Total organic carbon (TOC) and silt were significantly higher in concentration and proportion, respectively, when compared to the reference area. As a result, it was recommended that a second reference area be established as

part of the Phase 2 EEM program (Ecometrix, 2019). To this end, field reconnaissance and test sampling was undertaken in July 2020 (Ecometrix, 2020).

Each of the eight remaining short-listed reference areas from the Phase 1 Study Design (Minnow 2016) were first visited for preliminary observation of suitability. This suitability was based on water depth, morphology, riparian vegetation, and observation of barriers. These visual observations resulted in one location on each of the Kishkakoesis River, Sturgeon Creek, and La Vallée River being prioritized for further investigation via targeted biological collections. Targeted collections included benthos and sediments (TOC, grain size) and a preliminary determination of the local fish community.

Location 23 on the La Vallée River (hereafter, LVR-REF) was chosen as the secondary reference location. This was based on the combined results from the benthic, sediment and fish collections as well as the observed habitat. The stream order, depth, and width of the watercourse as well as the surrounding riparian and upland habitat were similar to that of the exposure area. Additionally, the benthic invertebrate community in the test samples from the La Vallée River were dominated by oligochaetes and chironomids similar to the exposure area; and finally, the fish community included both of the sentinel species used in Phase 1 and thereby allowed comparability for the Phase 2 fish component. Substrate distribution in all of the areas sampled during the reconnaissance trip were slightly different that the exposure area although all dominated by fine particles. Attempts during the benthic field program were made to match sediments more closely between all sampling areas.

The reference area for EDL1 is located downstream of LVR-REF with similar catchment area to EDL1 as well as having similar riparian characteristics and catchment compositions as the upstream LVR-REF.

2.5.6 Identification of Confounding Factors

With the exception of runoff associated with current agricultural practices, namely cattle grazing and livestock feed production the study area is not impacted by other anthropogenic sources. Livestock based agricultural impacts include cattle in the stream altering habitat, leading to increased erosion, and also contributing to the nutrient loadings through livestock waste. During the selection of the reference locations the percent agricultural land cover was one factor used during the selection process as a way to control for this confounding factor.

Further to the aforementioned confounding factors, the presence of multiple discharges used by the mine can cause between and within EEM phase confounding effects. Each Phase will have different volumes of discharge from different locations based on precipitation and water management requirements.

Now that the discharge locations for the remainder of the mine life have been located confounding factors will be reduced. However, at present direct comparisons of the Phase 1 and 2 results to the Phase 3 results should be considered solely a reflection of exposure to mine effluent. The study herein also assesses potential effects based on the EDL1 discharge in

isolation. However, it must be noted that the area upstream from EDL1 includes the potential effects of the EDL2, and Sediment Pond 1 and 2 discharges upstream. Multiple discharges and water management practices based on the local precipitation require a determination of the primary discharge in each EEM phase. In Phase 2 it was reported that using the EDL2 area would remain consistent. However, assessment of 2020–2023 flow data indicates that this was not the case since EDL1 discharge rate exceeded EDL2 discharge. Changing the exposure area phase after phase does not provide the most simplistic way to determine effect but, is in keeping with the regulation that requires assessment of the discharge with the higher potential effect.

The additional confounding factor related to the multiple discharges is that reference areas cannot be established on the same watershed. EDL2, assessed as part of Phase 1 and 2 is located along the Pinewood River at a transition point in habitat types and therefore potential effects cannot be assessed using an upstream and downstream, reference vs. exposure design. Similarly, EDL1 is downstream of EDL2 and the site upstream of EDL1 is not a true reference because it is subject to the potential effects from the discharge upstream, that is EDL2. For both discharges, reference areas are also located on other watersheds. This inherently introduces watershed differences in both biotic and abiotic factors between the exposure and reference area comparisons confounding interpretation of what differences are related to those between watershed natural differences

3.0 Summary of Previous EEM Results

3.1 Phase 1 EEM

3.1.1 Overview

EEM Phase 1 at RRM employed a control-impact design that included sampling water, sediment, benthic invertebrates and fish from a single exposure area (PINR-EXP1) and a single reference area (STUC-Ref) (Minnow 2018). The results of the field programs were interpreted along with other relevant data, including acute and sublethal toxicity test results, effluent characterization, and data from routine surface water quality monitoring, to assess the potential effects, if any, resulting from the discharge of mine effluent in the Pinewood River. The results of the previous EEM program are summarized below.

3.1.2 Supporting Environmental Information

3.1.2.1 Water Quality

In Phase 1 water quality was similar between the reference and exposure areas, with a few exceptions; the exposure area had elevated conductivity, hardness, calcium, potassium and sodium compared to the Sturgeon Creek reference area. Iron, nitrate and selenium all exceeded their respective guidelines for the protection of aquatic life. However, iron and selenium exceeded their guidelines in both the reference and exposure areas indicating elevated concentrations from sources other than the mine.

3.1.2.2 Sediment Quality

The grain size analysis of Phase 1 sediment indicated that the exposure area sediment contained significantly more total organic carbon (TOC) and silt than the reference area sediments, whereas the opposite was true for the clay fraction.

Chromium and nickel concentrations in both sampling areas exceeded their respective Provincial Sediment Quality Guideline (PSQG) Lowest Effect Levels (LEL). Manganese and phosphorus also exceeded their PSQG in the exposure area sediments. In the case of these metals the exposure concentrations were also elevated compared to reference concentrations. TOC was above the PSQG LEL in both areas. Total Kjeldahl nitrogen (TKN) in the exposure area was the only parameter to exceed the Severe Effects Level (SEL). It should be noted that TKN was also greater than the PSQG LEL at the reference area.

3.1.3 Benthic Invertebrate Community (BIC)

In Phase 1, five replicate benthic invertebrate samples were collected in both the reference and exposure areas. Generally, the benthic communities within the two areas were similar. There were no statistical differences between the areas in terms of invertebrate abundance and diversity. The only EEM effect endpoint that differed between the areas was the Bray- Curtis Index, indicating a moderate level of community structure difference between the areas. This difference is likely the result of subtle habitat differences not effluent exposure (Minnow 2018)

3.1.4 Fish

The Phase 1 lethal fish survey targeted Brook Stickleback and Central Mudminnow. The fish species assemblage was similar between the two area although overall the number of species captured in the reference was lower (i.e., seven vs. 11 fish species collected).

Female Brook Stickleback and Central Mudminnow from the exposure area were significantly different than their reference area counterparts with respect to relative gonad size, relative liver size, and body condition. For Brook Stickleback all these differences exceeded the applicable CES whereas for Central Mudminnow only relative gonad size exceeded the applicable CES.

Similarly, exposure area males from both sentinel species had significantly larger relative liver size and body condition compared to their reference area counterparts and the MODexceeded the CES on all accounts.

Overall, it was suggested that the pattern of differences in the fish endpoints indicated more food availability within the exposure area compared to the reference area (Minnow 2018).

3.1.5 Fish Usability

In Phase 1 mercury concentrations in the RRM effluent did not require an assessment of fish usability in the Pinewood River. That is, the mean annual concentration of mercury was < 0.1 ug/L in RRMs effluent. However, as a requirement of provincial approvals, the RRM undertakes a mercury monitoring program which assesses body burden levels in small- bodied fish and large-bodied fish, the results of which are presented under separate cover.

At the time of the Phase 1 EEM selenium was not part of the fish usability component of the program.

3.1.6 Conclusions

The results of the water samples collected during the Phase 1 field studies indicated that the effluent being discharged to the Pinewood River is detectable based on a limited number of parameters. There was also elevated concentrations of manganese and phosphorus in the exposure area sediments when compared to the reference area and were above their LELs. From a biological standpoint only one of the four benthic community endpoints was significantly different between the two sampling areas and this may be the result of small habitat differences. With respect to fish there were a number of significant differences in both sexes of both sentinel species. Additionally, the majority of these noted differences were above their respective CES. However, it was suggested that the differences noted were indicative more resource/food rather than impairment due to effluent exposure.

As this was the first Phase for the EEM, subsequent periodic monitoring in a similar fashion was required to determine any confirmed effects (i.e., statistical significance in the same direction).

3.2 EEM Phase 2

3.2.1 Overview

The Phase 2 EEM study followed the same general design as the Phase 1 assessment with one major addition. As noted, Phase 1 indicated that differences between the EDL1 exposure areas and the Sturgeon Creek reference area were likely related to habitat differences. Therefore, after discussion with ECCC a reference area reconnaissance study was conducted which resulted in the addition of a second reference area within the La Vallée River.

3.2.2 Supporting Environmental Variables

Overall, there were elevated concentrations of most parameters in the exposure area sample compared to the reference areas. Many of these elevated parameters are normal indicators of a mine discharge. Some of the parameters that were higher in the exposure area than the reference areas may be due to the difference in watercourses as the results are not the same as during the more consistent effluent and receiver water EEM monitoring within the Pinewood River (i.e., upstream and downstream of the discharge). The exceedance of the cobalt PWQO in the exposure area appeared anomalous as this value is two orders of magnitude higher than the mean value reported during the other EEM monitoring on the Pinewood River in Phase 2. Furthermore, mine effluent has never exceeded MDMER criteria for any parameter with the exception of TSS and this has not occurred since 2017.

Generally, if the sediment LEL was exceeded in the exposure area it was also exceeded in the reference area. Sediment chemistry is highly variable and up to 50% variance is possible in close proximity within a waterbody. Therefore, an increase in the sediment concentration in the exposure area was considered potentially meaningful if the relative percent increase between the median values was greater than 25%. It should also be noted that because the reference and exposure areas are in different watersheds there are inherent differences in the sediment chemistry that are not the result of the mine discharge. However, the aforementioned criterion was used to flag potential parameters of interest. As such; median arsenic, boron, calcium, copper, magnesium, manganese, potassium, sodium, strontium, thallium, titanium and zirconium were identified as elevated greater than 25% in the exposure area compared to the STUC reference area and median cadmium, calcium, copper, magnesium, strontium, titanium and zirconium were 25% greater in the exposure area compared to the LVR reference area.

3.2.3 Benthic Invertebrates Community

The key findings associated with the Phase 2 benthic invertebrate community survey are:

- A total of 32 benthic families were identified in the study area of which 24 were identified in the LVR reference area, 25 in the STUC reference area and 26 in the exposure area. Seventeen of the 32 taxa were found in both reference areas and the exposure area.

- Invertebrate density ranged from approximately 3,086 to 37,486 organisms/m^2 across the study area. Taxa that were generally prominent across the study area (present at 60% of study stations in each area) include the nematodes, naidid oligochaetes, ostracods, elmid beetles, caenid mayflies, biting midge larvae (Ceratopogonidae), phantom midge larvae (Chaoboridae), midge larvae (Chironomidae), mud snails from the taxonomic family Hydrobiidae and sphaeriid (fingernail) clams.

- The dominant taxa in the exposure area were chironomids (25.1%), whereas oligochaetes were dominant in both LVR reference (60.4%) and STUC reference (32.6%) areas. EPT taxa (orders Ephemeroptera, Plecoptera, and Trichoptera) were proportionally more abundant in the exposure area (13.7%), followed by the STUC reference (7.3%) and the LVR reference (2.7%).

- No statistical difference in total benthic invertebrate density was found between the exposure area and either of the reference areas.

- No statistical difference in taxa richness was detected between the exposure area and the LVR reference area. Taxa richness was significantly higher in the exposure area, than in the STUC reference area ($p=0.055$), however this difference did not exceed the CES of ±2 ref SDs.

- A significant difference was detected between the LVR reference and exposure area in terms of Simpson's Evenness (EXP>REF), but the magnitude of difference was relatively small and less than the CES.

- Bray Curtis dissimilarity index was significantly different between the exposure area and both the LVR reference area ($p=0.007$) and the STUC reference area ($p=0.007$). Re-analysis of the Phase 1 data using the dbRDA method also found a statistically significant difference between the exposure area and the STUC reference area ($p=0.02$).

- The proportion of silt in the sediment was significantly higher in the exposure area than in the LVR reference area. This difference may be an influential factor in the differences observed in Simpson's Evenness and Diversity across the exposure and LVR reference areas, as significant linear relationships were identified between proportion of silt in sediment and these two endpoints across these sites.

- Some differences in endpoint values were noted between Phases 1 and 2, but no pattern reflecting a potential confirmed effect (i.e., statistical difference in same direction with same endpoint from phase to phase) was identified for effect endpoints of density, richness or evenness.

- The Bray-Curtis index was significantly different between STUC and EXP in each of Phase 1 and Phase 2. However, further analysis indicated that the dissimilarity observed between replicate sampling stations in the STUC area may be as great as that between the STUC versus EXP stations. This further demonstrates that the STUC reference area

may not provide the most comparable control for this ongoing study and the secondary LVR reference area provides important natural context.

3.2.4 Fish Community

Overall, the majority of fish collected and subsequently retained for further processing (Brook Stickleback and Central Mudminnow) appeared to be in good physical health.

While some statistical differences were noted, no EEM effect endpoint was statistically different in the same direction between the exposure area and both reference areas. ECCC correspondence indicates that the MDMER does not discriminate between reference areas and therefore and IOC study should be implemented in Phase 3. If both reference areas had been sampled in both Phase 1 and 2 it would be appropriate to proceed to and IOC study. However, proceeding to an IOC study based on one Phase of data from the La Vallée River is not necessarily supported scientifically given that there were no confirmed effects based on Sturgeon Creek Reference area comparisons and limited statistically significant differences between the exposure area and Sturgeon Creek overall. Furthermore, Phase 2 contained two sentinel species, and with the exception of relative gonad weight there is no consistency in significant effects between the two species as would be expected if the mine discharge was the cause. Overall, there is a lack of corroborating information to indicate that a confirmed effect was identified.

3.2.5 Fish Usability

As per the study design (Ecometrix, 2019), fish usability studies were not required for inclusion in the Phase 2 EEM.

3.2.6 Conclusions

For benthic invertebrates, the Bray-Curtis Index was found to be significantly different between the Sturgeon Creek reference and the exposure area in both Phase 1 and Phase 2. This is a confirmed effect. However, further statistical analysis indicated that the Sturgeon Creek reference area is not likely the most appropriate reference area for the Pinewood River with respect to the benthic invertebrate community. Accordingly, we recommended that the mine remain in periodic monitoring to obtain a second phase of benthic invertebrate community data from the newly established La Vallée River reference area. Similarly, we recommended that a second Phase of data be collected for fish at the newly established La Vallée River reference location.

4.0 Supporting Environmental Information

4.1 Sediment Characterization

Sediment was collected alongside benthic samples and identified in **Table 4-1**. The particle size of the sites is summarized in **Table 4-2**. Detailed lab results are provided in **Appendix B** Sediment was collected from September 19 to September 22, 2023. The locations of sampling sites are within a few metres of the sites in previous phases (i.e., sites associated with EDL2 discharge) to support temporal comparisons.

Table 4-1: Sediment sampling stations near Rainy River Mine, 2023.

Associated Exposure Site	Type	Site	Station	Latitude	Longitude
EDL2 (PINR-EXP)	Reference	STUC-REF	1	48.72189	-93.95711
			2	48.72197	-93.95772
			3	48.72164	-93.95870
			4	48.72147	-93.96024
			5	48.72117	-93.96041
	Reference	LVR-REF	1	48.66936	-93.66928
			2	48.66864	-93.66975
			3	48.66814	-93.66922
			4	48.66775	-93.66999
			5	48.66664	-93.66978
	Exposure	PINR-EXP	1	48.82947	-94.06442
			2	48.82956	-94.06472
			3	48.82919	-94.06489
			4	48.82878	-94.06591
			5	48.82911	-94.06625
EDL1 (PINR-EXP2)	Reference	LVR2-REF	1	48.61974	-93.63166
			2	48.61988	-93.63115
			3	48.62027	-93.63069
			4	48.61992	-93.63240
			5	48.62052	-93.63204
	Reference	PINR-REF2	1	48.81200	-94.15099
			2	48.81293	-94.15105
			3	48.81327	-94.15031
			4	48.81342	-94.14952
			5	48.81151	-94.25255
	Exposure	PINR-EXP2	1	48.81091	-94.15020
			2	48.81130	-94.15265
			3	48.81158	-94.15323
			4	48.81072	-94.15405
			5	48.81113	-94.15368

4.1.1 Methods

Each sediment sample was collected using three Petite Ponar grabs. The top 5 cm of each grab was transferred to a clean holding container and homogenized using a stainless-steel spoon. Samples were then transferred into labelled 500 mL wide-mouthed bottles. Following collection, samples were placed in a cooler with ice until they were shipped to the analytical laboratory. Sediment samples were shipped to Bureau Veritas in Mississauga, Ontario, with appropriately completed chain-of-custody forms for subsequent analysis. Bureau Veritas Laboratories are fully certified and accredited by several agencies (e.g., MDDELCC, SCC, CALA, AIHA, NELAP, NVLAP). Sediment samples were analyzed for TOC, grain size, and total metals using acid extractable atomic spectroscopy.

Potential elevation concentrations are identified using Ontario's PSQG. The PSQG defines two effect levels. The Lowest Effect Level (LEL) indicates a concentration that can be tolerated by most sediment-dwelling organisms. Sediments meeting the LEL are considered clean-to-marginally polluted. The Severe Effect Level (SEL) indicates a concentration that is expected to be detrimental to most sediment-dwelling organisms. Sediments exceeding the SEL are considered heavily contaminated.

4.1.2 Statistical Approaches

Sediment characteristics (e.g., TOC and percent sand, silt, and clay) of the reference and exposure sites were compared statistically. To select the appropriate analysis, the distribution of the data was first examined using a Shapiro-Wilk test. If the data were parametrically distributed, a Barlett's test was used to assess the variance in the residuals. If the data was nonparametrically distributed, a Levene's test was used. When data were normally distributed and have homogenous variance, comparisons of endpoint data were made using a single factor analysis of variance (ANOVA). When the data deviated from a normal and homogenous distribution, transformations (e.g., $\log_{10}$, square root) were applied in attempt to satisfy the assumptions of an ANOVA. If data transformation could not achieve a normal distribution or homogenous variance, then a Kruskal-Wallis test was used to compare samples. A Kruskal-Wallis test is a non-parametric alternative to an ANOVA that is effective in low-sample analyses. If there was a significant difference identified between the groups, a Tukey's HSD post-hoc test was used to determine which sites were different. All statistical procedures were carried out using the R v.4.3.2 (R Core Team 2023), supplemented with the packages 'vegan' v.2.6-4 (Oksanen et al. 2022) and 'agricolae' v.1.3-5 (de Mendiburu 2021).

The concentrations of elements below the detection limit were replaced with the detection limit for statistical analyses.

R v.4.3.2 base packages, package *tidyverse* v. 2.0.0 and related packages, and an Ecometrix in-house EEM companion package *emsR* v. 5.0.0.

4.1.3 Results

4.1.3.1 Sediment Composition and Texture

The sediments measured in the EDL1 and EDL2 reference and exposure areas were generally similar in particle size and texture. In EDL2, small particle sizes were dominant, including clay, silt, and fine sand (**Figure 4-1**, **Table 4-2**, **Figure 4-2**). In the reference sites, clay was dominant (41% of the composition of LVR-REF and 44% of the composition of STUC-REF). In the exposure site, fine sand was dominant (36%). The differences are also statistically significant (**Figure 4-2**, **Table 4-2**, **Table 4-3**). These differences may impact the benthic community composition and must be considered when interpreting those results. EDL1 was also dominated by small particle sizes, including clay, silt, and fine sand (**Figure 4-1**,**Table 4-2**)
). In the reference sites, clay was dominant (36% of the composition of PINR-REF2 and 24% of the composition of LVR-REF2). In the exposure site, clay was 46% of the composition. Despite clay being dominant, the percentages are also statistically significant (**Figure 4-3, Table 4-3**). These differences may impact the benthic community composition and must be considered when interpreting those results.

There was no statistical difference in TOC between the EDL2 ($p = 0.207$; ANOVA) or EDL1 ($p= 0.191$; ANOVA) where compared to their corresponding reference areas (**Figure 4-2**, **Figure 4-3**; **Table 4-3**). Importantly, the average TOC in both the reference and exposure area were above the LEL of 1% established by the Province of Ontario. However, all areas are above the LEL threshold, the elevated TOC appear to be natural to the area and not related to mine effluent.

Overall, there are differences in the texture and sediment composition of areas in Rainy River. However, the differences do not appear to be related to proximity to mine discharge (**Figure 4-1**). The lack of extreme outliers implies that the reference and exposure areas have high levels of consistency within sites. The differences in sediment composition may influence the benthic communities.

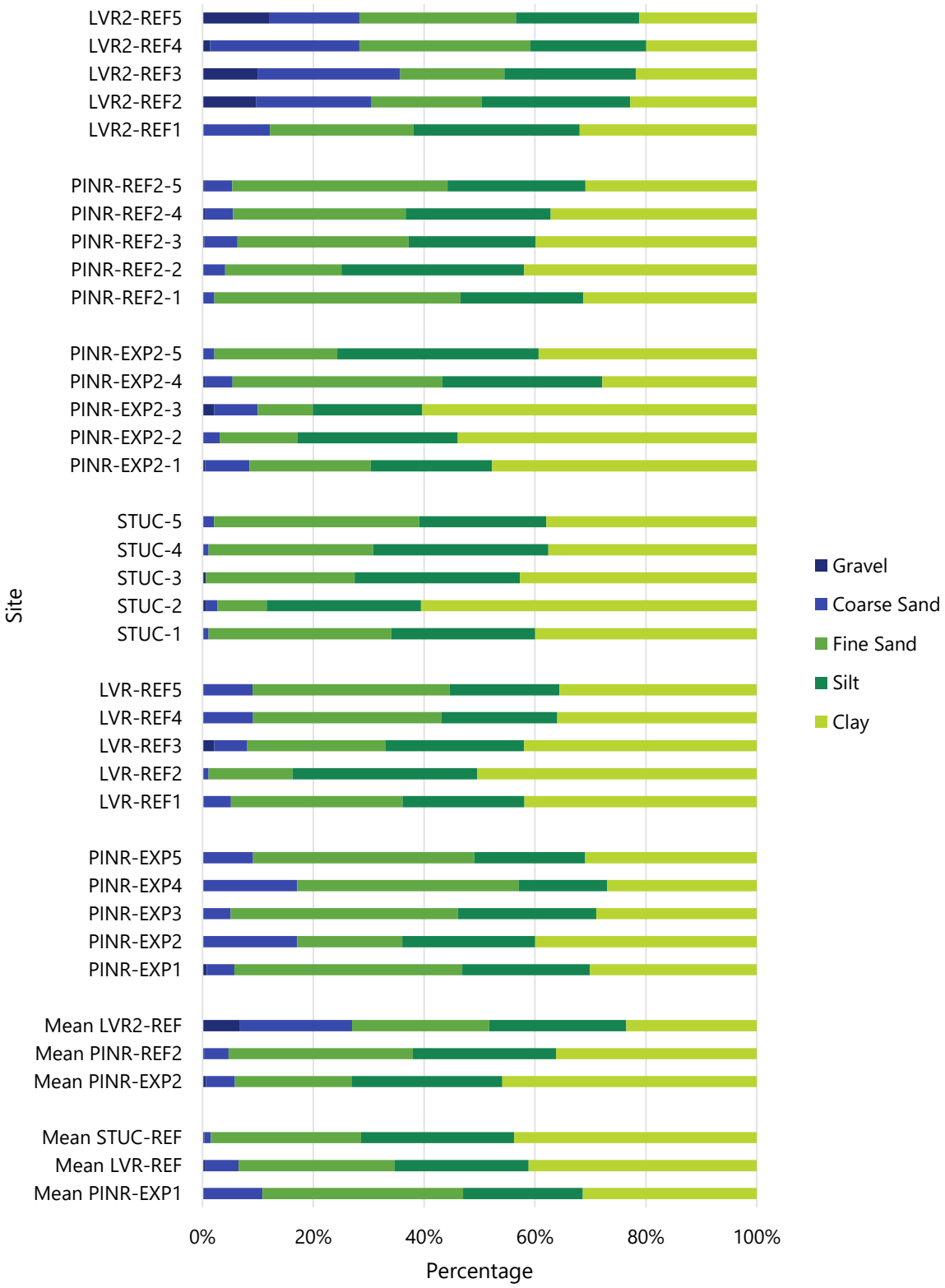

Figure 4-1: Particle size distribution of sediments for Phase 3 EEM sites, 2023.

Table 4-2: Summary of the particle size and TOC distributions for Phase 3 EEM sites, 2023.

Associated Exposure Site	Type	Site	Texture	Mean	Median	Min	Max	SD	SE
EDL2 (PINR-EXP)	Reference	STUC-REF	Clay (< 0.0039 mm)	0.3	0.1	0.1	0.7	0.3	0.1
			Silt (0.062-0.0039 mm)	1.2	1.0	0.0	2.0	0.8	0.4
			Fine Sand (0.25-0.062 mm)	27.2	30.0	9.0	37.0	10.8	4.8
			Coarse Sand (2.0-0.25 mm)	27.8	28.0	23.0	32.0	3.5	1.6
			Gravel (>2.0 mm)	44.0	40.0	38.0	61.0	9.7	4.3
			TOC (mg/kg)	32400	24000	23000	67000	19360	8658
	Reference	LVR-REF	Clay (< 0.0039 mm)	0.5	0.1	0.1	2.1	0.9	0.4
			Silt (0.062-0.0039 mm)	6.0	6.0	1.0	9.0	3.3	1.5
			Fine Sand (0.25-0.062 mm)	28.2	31.0	15.0	36.0	8.5	3.8
			Coarse Sand (2.0-0.25 mm)	24.2	22.0	20.0	33.0	5.3	2.4
			Gravel (>2.0 mm)	41.2	42.0	36.0	50.0	5.8	2.6
			TOC (mg/kg)	28360	21000	8800	58000	19487	8715
	Exposure	PINR-EXP	Clay (< 0.0039 mm)	0.2	0.1	0.1	0.7	0.3	0.1
			Silt (0.062-0.0039 mm)	10.6	9.0	5.0	17.0	6.1	2.7
			Fine Sand (0.25-0.062 mm)	36.2	40.0	19.0	41.0	9.6	4.3
			Coarse Sand (2.0-0.25 mm)	21.6	23.0	16.0	25.0	3.6	1.6
			Gravel (>2.0 mm)	31.4	30.0	27.0	40.0	5.0	2.2
			TOC (mg/kg)	14200	15000	11000	17000	2588	1158
EDL1 (PINR-EXP2)	Reference	LVR2-REF	Clay (< 0.0039 mm)	6.7	9.7	0.2	12.0	5.5	2.4
			Silt (0.062-0.0039 mm)	20.4	21.0	12.0	27.0	6.4	2.9
			Fine Sand (0.25-0.062 mm)	24.8	26.0	19.0	31.0	5.2	2.3
			Coarse Sand (2.0-0.25 mm)	24.8	24.0	21.0	30.0	3.7	1.7
			Gravel (>2.0 mm)	23.6	22.0	20.0	32.0	4.8	2.2
			TOC (mg/kg)	10780	10000	8300	14000	2589	1158
	Reference	PINR-REF2	Clay (< 0.0039 mm)	0.3	0.3	0.1	0.5	0.2	0.1
			Silt (0.062-0.0039 mm)	4.4	5.0	2.0	6.0	1.5	0.7

Associated Exposure Site	Type	Site	Texture	Mean	Median	Min	Max	SD	SE
			Fine Sand (0.25-0.062 mm)	33.2	31.0	21.0	44.0	8.8	3.9
			Coarse Sand (2.0-0.25 mm)	25.8	25.0	22.0	33.0	4.3	1.9
			Gravel (>2.0 mm)	36.2	37.0	31.0	42.0	5.1	2.3
			TOC (mg/kg)	12400	11000	9000	18000	3435	1536
	Exposure	PINR-EXP2	Clay (< 0.0039 mm)	0.6	0.4	0.1	2.1	0.8	0.4
			Silt (0.062-0.0039 mm)	5.2	5.0	2.0	8.0	2.8	1.2
			Fine Sand (0.25-0.062 mm)	21.2	22.0	10.0	38.0	10.7	4.8
			Coarse Sand (2.0-0.25 mm)	27.2	29.0	20.0	36.0	6.4	2.9
			Gravel (>2.0 mm)	46.0	48.0	28.0	61.0	12.9	5.8
			TOC (mg/kg)	15400	16000	10000	22000	4980	2227

Table 4-3: Summary Statistics of the Particle Size Comparison of Sediments from Rainy River, 2023.

Metric	Three-group comparison			Pairwise comparisons			
	p-value	Significant difference?	Test	Area (I)	Area (J)	p-value	Significant difference?
EDL2							
% Clay	0.040	Yes	KW	LVR-REF	PINR-EXP	0.117	No
				LVR-REF	STUC-REF	0.812	No
				PINR-EXP	STUC-REF	0.040	Yes
% Silt	0.105	No	ANOVA	-	-	-	-
% Fine Sand	0.107	No	KW	-	-	-	-
% Coarse Sand	0.010	Yes	ANOVA	LVR-REF	PINR-EXP	0.208	No
				LVR-REF	STUC-REF	0.185	No
				PINR-EXP	STUC-REF	0.008	Yes
% Gravel	0.841	No	KW	-	-	-	-
TOC (mg/kg)	0.207	No	ANOVA	-	-	-	-
EDL1							
% Clay	0.004	Yes	ANOVA	LVR2-REF	PINR-EXP2	0.003	Yes
				LVR2-REF	PINR-EXP2	0.087	Yes
				PINR-EXP2	PINR-REF2	0.202	No
% Silt	0.748	No	ANOVA	-	-	-	-
% Fine Sand	0.115	No	ANOVA	-	-	-	-
% Coarse Sand	<0.001	Yes	ANOVA	LVR2-REF	PINR-EXP2	<0.001	Yes
				LVR2-REF	PINR-EXP2	<0.001	Yes
				PINR-EXP2	PINR-REF2	0.950	No
				LVR2-REF	PINR-EXP2	0.029	Yes
% Gravel	0.077	Yes	KW	LVR2-REF	PINR-EXP2	0.021	Yes
				PINR-EXP2	PINR-REF2	0.983	No
TOC (mg/kg)	0.191	No	ANOVA	-	-	-	-

Note: KW = Kruskal Wallis test. Statistically significant differences are highlighted.

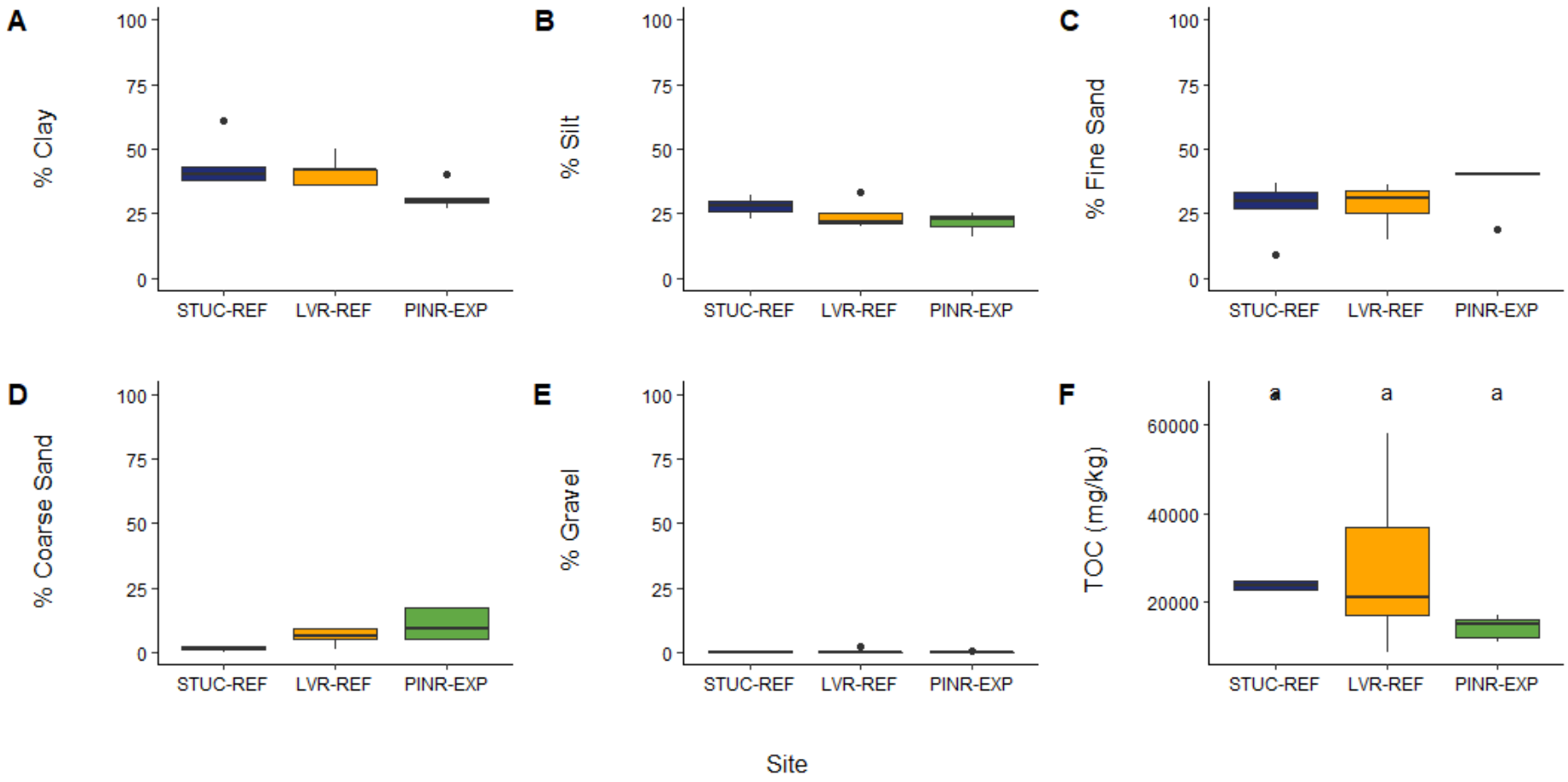

Figure 4-2: Box and Whisker Comparison of Sedimentary Characteristics for EDL2, 2023.

Notes: Top and bottom of the box are the third and first quantiles, respectively; the bold midline is the median; the whiskers at the top and bottom are the maximum and minimum, respectively; the closed points are outliers. Lettering (A, B) indicate a significant statistical difference ($p < 0.10$).

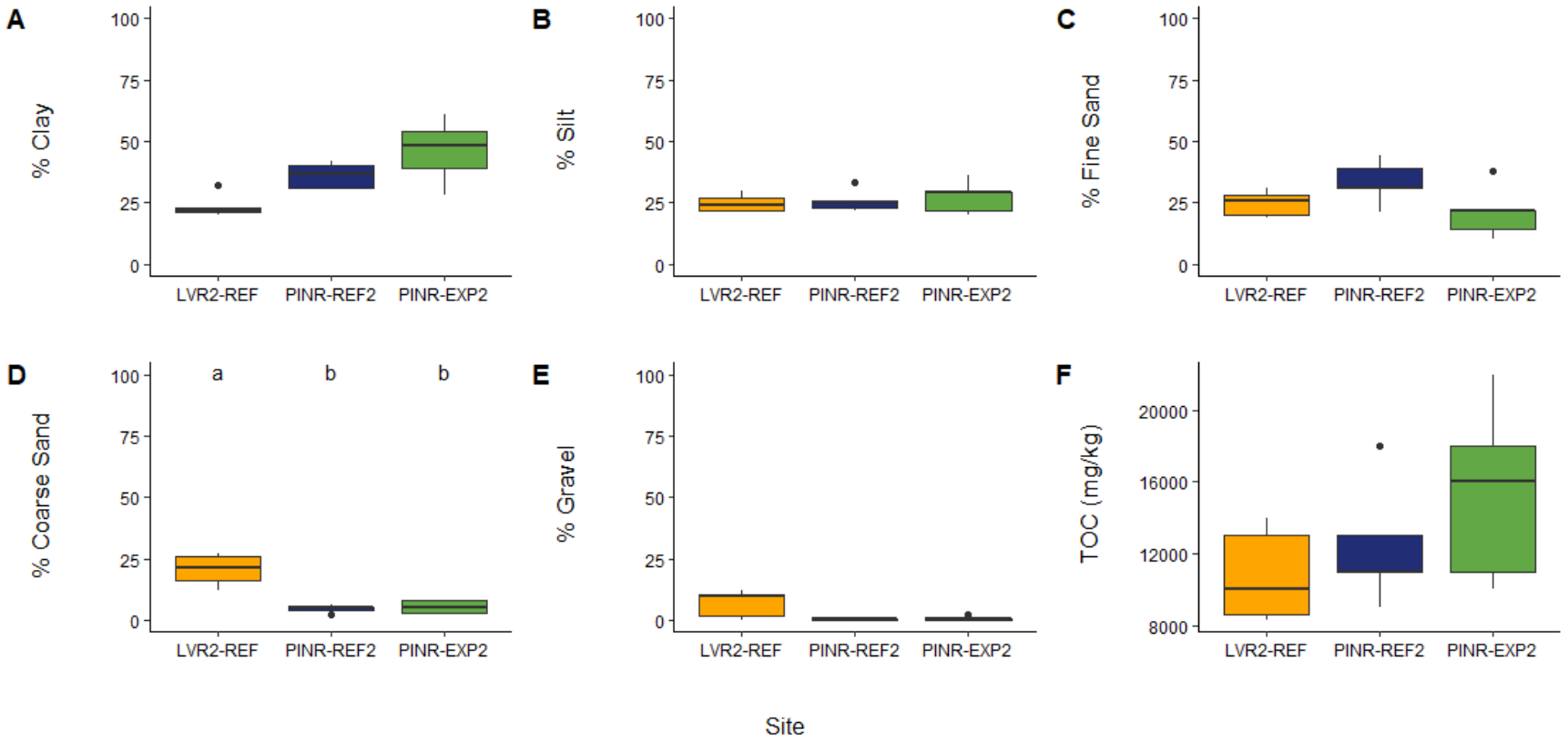

Figure 4-3: Box and Whisker Comparison of Sedimentary Characteristics for EDL1, 2023.

Notes: Top and bottom of the box are the third and first quantiles, respectively; the bold midline is the median; the whiskers at the top and bottom are the maximum and minimum, respectively; the closed points are outliers. Lettering (A, B) indicate a significant statistical difference ($p < 0.10$).

4.1.3.2 Sediment Chemistry

A suite of 24 elements were measured in the sediments and compared to provincial guidelines (**Table 4-4**; **Table 4-5**). No elements exceeded the SEL, but some were above the LEL. For example, in one of five stations Ni was above the LEL in STUC-REF (Ni = 19 µg/L) and Fe in PINR-EXP (Fe = 13,000 µg/L).

Table 4-4: Summary for Sediment Chemistry for Sites Associated with EDL2 Discharge, 2023

Parameter	Unit	PSQG		LVR-REF						STUC-REF						PINR-EXP					
		LEL	SEL	Mean	Median	Min.	Max.	SD	SE	Mean	Median	Min.	Max.	SD	SE	Mean	Median	Min.	Max.	SD	SE
Aluminum	µg/L	-	-	9,400	8,600	7,100	13,000	2,543	1,137	10,540	11,000	8,400	13,000	1,774	793	6,840	6,700	5,300	8,900	1,428	638
Arsenic	µg/L	6	33	1.6	1.3	1.1	2.6	0.6	0.3	1.72	1.5	1.2	3.1	0.8	0.3	1.3	1.2	1.1	1.7	0.3	0.1
Barium	µg/L	-	-	54.4	53	38	74	15	7	67.8	65	54	88	12	6	40	39	33	47	7	3
Beryllium	µg/L	-	-	0.392	0.33	0.29	0.56	0.11	0.05	0.444	0.43	0.35	0.58	0.09	0.04	0.29	0.29	0.23	0.38	0.06	0.03
Boron	µg/L	-	-	7.54	6.6	5.9	10	2	1	7.12	6.8	6	8.9	1.2	0.5	6	5	5	7	1	0
Cadmium	µg/L	0.6	10	0.146	0.12	0.1	0.22	0.06	0.02	0.236	0.2	0.18	0.4	0.1	0.0	0.12	0.11	0.10	0.14	0.02	0.01
Calcium	µg/L	-	-	5,820	5,900	4,500	6,700	807	361	6,080	6,000	5,300	7,100	736	329	6,400	6,200	4,900	8,600	1,358	607
Chromium	µg/L	26	110	19.8	21	15	25	5	2	22.4	23	18	28	4	2	15	15	13	20	3	1
Cobalt	µg/L	-	-	6.62	6	5.3	8.5	1.4	0.6	8.22	7.9	7.5	10	1	0	6.0	5.6	5.4	7.1	0.7	0.3
Copper	µg/L	16	110	8.92	9.4	5.7	12	3	1	9.74	9	7.4	14	2	1	8	8	6	12	3	1
Iron	µg/L	20,000	40,000	12,600	12,000	10,000	17,000	2,966	1,327	14,000	14,000	11,000	18,000	2,550	1,140	9,980	9,800	8,200	13,000	1,836	821
Lead	µg/L	31	250	6.8	7.6	4.4	8.2	1.6	0.7	6.82	6.6	5.5	9.1	1.4	0.6	4.5	4.3	3.7	5.8	0.9	0.4
Magnesium	µg/L	-	-	4,420	4,300	3,400	5,100	712	318	4,620	4,500	4,000	5,400	522	233	4,360	4,000	3,600	5,900	907	406
Manganese	µg/L	460	1,100	226	240	150	280	52	23	292	310	210	370	63	28	254	220	200	330	61	27
Mercury	µg/L	0.2	2	0.05	0.05	0.05	0.05	0.00	0.00	0.05	0.05	0.05	0.05	0.00	0.00	0.05	0.05	0.05	0.05	0.00	0.00
Nickel	µg/L	16	75	13.18	13	9.9	17	3	1	14.6	14	12	19	3	1	11	11	9	14	2	1
Phosphorus	µg/L	-	-	454	480	340	540	77	34	486	460	430	570	58	26	392	390	330	450	43	19
Potassium	µg/L	-	-	1,276	1,200	880	1,700	325	146	1,320	1,300	1,100	1,600	192	86	924	910	700	1,200	186	83
Sodium	µg/L	-	-	96.6	77	59	150	37	17	99.2	98	82	130	19	8	82	77	65	97	14	6
Strontium	µg/L	-	-	18.4	17	14	24	4	2	20.6	21	17	25	3	1	17	17	14	23	4	2
Thallium	µg/L	-	-	0.1232	0.13	0.078	0.17	0.04	0.02	0.144	0.14	0.12	0.18	0.02	0.01	0.09	0.09	0.07	0.12	0.02	0.01
Uranium	µg/L	-	-	1.224	1.3	0.75	1.7	0.4	0.2	1.52	1.4	1.2	2.4	0.5	0.2	0.8	0.8	0.6	0.9	0.1	0.1
Vanadium	µg/L	-	-	28.6	26	22	39	8	3	30.2	30	24	39	6	3	20	19	15	26	4	2
Zinc	µg/L	120	820	44.6	47	33	59	11	5	65	61	51	91	15	7	42	39	35	53	7	3
TOC	mg/kg	10,000	100,000	28,360	21,000	8,800	58,000	19,487	8,715	32,400	24,000	23,000	67,000	19,360	8,658	14,200	15,000	11,000	17,000	2,588	1,158

Notes:
PSQG = Ontario's Provincial Sediment Quality Guidelines.
LEL = Lowest Effect Level; SEL = Severe Effect Level
SD = Standard Deviation; SE = Standard Error
TOC = Total Organic Carbon.
Blue highlighted measurements exceed the LEL limits. No samples exceeded SEL limits.

Table 4-5: Summary for Sediment Chemistry for Sites Associated with EDL1 Discharge, 2023

Parameter	Unit	PSQG		LVR2-REF						PINR-REF2						PINR-EXP2					
		LEL	SEL	Mean	Median	Min.	Max.	SD	SE	Mean	Median	Min.	Max.	SD	SE	Mean	Median	Min.	Max.	SD	SE
Aluminum	µg/L	-	-	7,340	7,600	6,300	7,900	658	294	7,900	7,700	6,200	10,000	1,386	620	8,880	9,800	6,700	11,000	1,954	874
Arsenic	µg/L	6	33	1.4	1.4	1.2	1.5	0.1	0.1	1.8	1.8	1.3	2.5	0.4	0.2	2.2	2.5	1.5	3.2	0.7	0.3
Barium	µg/L	-	-	46.2	48.0	41.0	49.0	3.3	1.5	48.2	46.0	37.0	64.0	9.9	4.4	56	56	38.0	84.0	17.4	7.8
Beryllium	µg/L	-	-	0.3	0.3	0.3	0.4	0.0	0.0	0.3	0.3	0.3	0.5	0.1	0.0	0	0	0.3	0.5	0.1	0.0
Boron	µg/L	-	-	6.8	6.8	5.5	7.9	0.9	0.4	7.1	6.5	5.7	10.0	1.7	0.8	8	9	5.2	9.6	1.9	0.8
Cadmium	µg/L	0.6	10	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0	0	0.1	0.2	0.0	0.0
Calcium	µg/L	-	-	12,000	12,000	11,000	14,000	1,225	548	18,060	18,000	5,300	34,000	10,323	4,617	18,460	21,000	7,300	24,000	6,489	2,902
Chromium	µg/L	26	110	16.6	17.0	15.0	18.0	1.5	0.7	17.8	17	13	24	4	2	20	22	15.0	25.0	4.6	2.1
Cobalt	µg/L	-	-	6.6	6.9	5.6	7.3	0.7	0.3	6.76	6.8	5.5	8.3	1.0	0.5	7	8	6.0	8.8	1.1	0.5
Copper	µg/L	16	110	7.4	6.9	6.3	9.8	1.4	0.6	8.96	8.8	5.7	13	3	1	12	13	7.5	16.0	3.6	1.6
Iron	µg/L	20,000	40,000	10,680	11,000	9,600	12,000	986	441	12,080	12,000	9,400	15,000	1,983	887	13,400	15,000	10,000	17,000	3,209	1,435
Lead	µg/L	31	250	6.1	6.0	4.9	7.9	1.2	0.5	5.14	4.8	4.7	6.2	0.6	0.3	6	7	4.5	7.7	1.5	0.7
Magnesium	µg/L	-	-	7,640	7,800	6,800	8,200	555	248	11,100	11,000	3,700	19,000	5,470	2,446	11,700	13,000	4,500	15,000	4,177	1,868
Manganese	µg/L	460	1,100	196	200	170	220	21	9	258	260	160	350	68	30	356	390	200	450	96	43
Mercury	µg/L	0.2	2	0.05	0.05	0.05	0.05	0.00	0.00	0.05	0.05	0.05	0.05	0.00	0.00	0.05	0.05	0.05	0.05	0.0	0.0
Nickel	µg/L	16	75	12	12	10	13	2	1	15	14	9.7	20	4	2	17	19	11.0	22.0	4.6	2.1
Phosphorus	µg/L	-	-	492	490	480	500	8	4	414	410	360	460	36	16	448	450	410	490	33	15
Potassium	µg/L	-	-	1,074	1,100	970	1,100	58	26	1,302	1,200	910	1,900	365	163	1,580	1,800	1,000	2,100	460	206
Sodium	µg/L	-	-	83	84	72	93	10	4	111.6	110	80	160	30	13	138	140	110	170	24	11
Strontium	µg/L	-	-	17	18	16	18	1	0	20.8	20	14	30	6	3	26	28	19	31	5	2
Thallium	µg/L	-	-	0.11	0.11	0.09	0.12	0.01	0.01	0.132	0.12	0.12	0.17	0.02	0.01	0.14	0.16	0.10	0.19	0.04	0.02
Uranium	µg/L	-	-	0.83	0.83	0.79	0.89	0.04	0.02	0.832	0.8	0.75	0.98	0.10	0.04	1.02	1.10	0.82	1.20	0.17	0.07
Vanadium	µg/L	-	-	24.0	25.0	22.0	26.0	1.9	0.8	24.8	23.0	21.0	33.0	4.7	2.1	28	30	19.0	35.0	6.8	3.0
Zinc	µg/L	120	820	41.4	43.0	36.0	46.0	3.9	1.7	37.6	37.0	33.0	44.0	4.0	1.8	48	49	36.0	54.0	7.4	3.3
TOC	mg/kg	10,000	100,000	10,780	10,000	8,300	14,000	2,589	1,158	12,400	11,000	9,000	18,000	3,435	1,536	15,400	16,000	10,000	22,000	4,980	2,227

Notes:
PSQG = Ontario's Provincial Sediment Quality Guidelines.
LEL = Lowest Effect Level; SEL = Severe Effect Level
SD = Standard Deviation; SE = Standard Error
TOC = Total Organic Carbon.
Blue highlighted measurements exceed the LEL limits. No samples exceeded SEL limits.

4.2 Water Quality

4.2.1 Methods

Surface water grab samples were collected in the exposure and each of the reference areas in May and September, 2023. Water in each season was collected in similar locations. Temperature, conductivity, pH, and dissolved oxygen were measured *in-situ* concurrent with fish and benthic collections. Samples were collected from the middle of the reach for the analysis of nutrients (ammonia, nitrate, nitrite), alkalinity, hardness, TSS, sulphate, pH, conductivity, total cyanide, selected metals (Al, As, Cd, Cu, Fe, Hg, Mo, Ni, Pb, Tl, Zn) and radium-226. Appropriate preservatives were added where necessary following sample collection.

4.2.2 Results

4.2.2.1 Data Quality

The water quality meter was calibrated daily as required by Ecometrix Standard Operating Procedures (SOPs). It is expected therefore that these instruments provided accurate measures of the desired parameters.

For those analytes that required laboratory analyses the quality assurance/quality control (QA/QC) program included checks on the integrity of both field sampling and laboratory analyses. One duplicate sample was analyzed to assess potential issues with the field collections. Laboratory QA/QC comprised the analysis of method blanks, concentration standards and run duplicates. The QA/QC results for theses samples were as follows:

- all analytes were below detection levels in analyzed method blank;
- matrix spike recovery was in the order of ≥ 91% and ≤ 109% in all cases; and
- the reproducibility of analysis results from run duplicates was high and met internal laboratory standards.

See **Appendix B** for laboratory reports and Certificates of Analysis (COA).

4.2.2.2 Conventional Parameters

Generally, there was little variability comparing the water quality between the reference and exposure areas (**Table 4-6**). The primary difference was consistently elevated conductivity in the exposure areas. In May, conductivity in PINR-EXP was on average 3.2x higher than the corresponding reference areas. Similarly, conductivity was 1.9x higher than the reference areas in September, on average. In PINR-EXP2, conductivity was 1.7x higher than the LVR-REF area in May. The PINR-EXP2 was not elevated in conductivity in September.

Table 4-6: Average Conventional Water Parameter Measurements from Rainy River, 2023

EDL2						
Parameter	**May**			**September**		
	STUC	**LVR**	**PINR EXP**	**STUC**	**LVR**	**PINR EXP**
Field Temperature (°C)	12.6	12.2	11.0	15.6	15.6	14.9
Conductivity µS/cm	144	100	373	306	295	566
pH	8	7.54	7.98	7.55	7.58	7.53
DO mg/L	8.9	7.2	8.7	3.5	4.45	5.68
DO %	83.8	67.2	78.9	365	46.2	583
EDL1						
Parameter	**May**			**September**		
	LVR 2	**PINR-REF2**	**PINR-EXP2**	**LVR 2**	**PINR-REF2**	**PINR-EXP2**
Field Temperature (°C)	11.8	9.0	8.8	15.1	14.8	14.7
Conductivity µS/cm	120	213	267	443	337	349
pH	7.45	7.34	7.34	7.39	7.28	7.49
DO mg/L	9.48	9.26	9.36	2.54	3.92	5.05
DO %	87.6	80.1	80.8	26.5	39.9	51.5

4.2.2.3 Water Chemistry

Surface water quality data collected in May and September is presented in **Table 4-7**. The relative percent difference in the exposure areas compared to their corresponding reference areas is provided in **Table 4-8**. The only parameter that was elevated beyond the CCME water quality guidelines was Fe, in all sites but PINR-EXP. This suggests that the area is naturally elevated in Fe. When comparing the relative differences between sites, antimony was >10% higher in both exposure areas compared to their corresponding reference areas in May. However, this result was not evident in September. No other measured parameters appear elevated.

Table 4-7: Water Chemistry Results from Rainy River, Measured in May and September 2023

Parameter	Units	CCME	PWQO	EDL2						EDL1					
				May			September			May			September		
				Reference	Reference	Exposure	Reference	Reference	Exposure	Reference	Reference	Exposure	Reference	Reference	Exposure
				STUC-REF	LVR-REF	PINR-EXP	STUC-REF	LVR-REF	PINR-EXP	LVR2-REF	PINR-REF2	PINR-EXP2	LVR2-REF	PINR-REF2	PINR-EXP2
Total Phosphorus	mg/L	-	-	0.046	0.038	0.03	0.08	0.13	0.098	0.047	0.035	0.034	0.47	0.11	0.1
Total Ammonia	mg/L	-	-	<0.050	<0.050	0.069	<0.050	0.075	0.069	<0.050	<0.050	0.11	<0.050	<0.050	<0.050
TSS	mg/L	-	-	175	95	270	<10	28	<10	145	250	360	11	12	15
Hardness ($CaCO_3$)	mg/L	-	-	90	66	160	170	160	290	80	130	200	200	190	190
Alkalinity	mg/L	-	-	71	52	88	150	140	240	65	84	110	160	170	170
Ag	mg/L	-	-	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010
Al	mg/L	-	-	0.237	0.25	0.101	0.0909	0.428	0.0889	0.432	0.271	0.282	0.0877	0.747	0.789
As	mg/L	0.005	0.1	0.000751	0.000664	0.000905	0.00138	0.00251	0.0013	0.000803	0.000893	0.000856	0.00392	0.00332	0.00322
B	mg/L	1.5	-	0.014	0.014	0.036	0.016	0.018	0.023	0.015	0.022	0.027	0.013	0.018	0.019
Ba	mg/L	-	-	0.0148	0.0129	0.0221	0.011	0.0242	0.0216	0.0169	0.0186	0.0192	0.0225	0.0249	0.0258
Be	mg/L	-	-	0.000025	0.000023	0.000012	0.000023	0.000034	0.000012	0.000034	0.000024	0.000023	0.000023	0.00005	0.000046
Bi	mg/L	-	-	<0.000010	<0.000010	<0.000010	<0.000010	0.000011	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	0.000013	0.000011
Ca	mg/L	-	-	19.4	14.9	50.7	30.9	29.3	53.9	18.8	33.3	38.2	34.5	36.3	37.6
Cd	mg/L	-	0.0002	0.0000164	0.0000088	0.0000088	0.000007	0.000011	0.000007	0.0000165	0.0000083	0.0000138	0.000008	0.000011	0.000014
Co	mg/L	-	-	0.000211	0.000173	0.000397	0.000329	0.000736	0.00021	0.000367	0.000361	0.000425	0.00104	0.000888	0.000856
Cr	mg/L	0.001	0.0089	0.00062	0.00067	0.00035	0.00028	0.00084	0.00033	0.00096	0.00094	0.00072	0.00028	0.00134	0.00135
Cu	mg/L	-	-	0.00124	0.00095	0.00126	0.00107	0.00151	0.00099	0.00142	0.00139	0.00146	0.00075	0.0048	0.00214
Fe	mg/L	0.3	0.3	0.422	0.455	0.275	0.396	1.34	0.24	0.704	0.487	0.44	1.95	1.57	1.47
K	mg/L	-	-	1.62	2	8.54	1.07	1.61	2.81	2.25	4.72	6.59	2.67	2.24	2.65
Li	mg/L	-	-	0.00374	0.00311	0.00729	0.00455	0.00459	0.00805	0.00375	0.00505	0.00553	0.00464	0.00495	0.00521
Mg	mg/L	-	-	8.9	6.95	14.9	13.8	12.3	21.5	8.82	11.1	11.5	14.4	13.5	13.7
Mn	mg/L	-	-	0.0159	0.0113	0.0382	0.0943	0.287	0.136	0.0288	0.0385	0.0396	1.08	0.701	0.553
Mo	mg/L	0.073	-	0.000598	0.000569	0.00262	0.000263	0.000686	0.000342	0.000608	0.00165	0.00195	0.00034	0.000601	0.000678
Na	mg/L	-	-	4.37	2.6	21.5	4.16	7.1	6.92	3.26	11.1	15.6	13.1	3.5	4.16
Ni	mg/L	-	0.025	0.00167	0.00149	0.00171	0.00193	0.00226	0.00107	0.0019	0.00288	0.00209	0.00172	0.00309	0.00321
P	mg/L	-	-	0.046	0.038	0.034	0.0788	0.144	0.0904	0.047	0.035	0.03	0.276	0.108	0.113
Pb	mg/L	-	-	0.000155	0.000147	0.000092	0.000165	0.000771	0.000093	0.000292	0.000212	0.000196	0.000289	0.000928	0.000964
S	mg/L	-	-	<3.0	<3.0	36.4	<3.0	<3.0	5.4	3.2	18.7	27.5	<3.0	<3.0	<3.0
Sb	mg/L	-	-	0.000052	0.000048	0.00202	0.000063	0.000094	0.000069	0.000079	0.00097	0.00149	0.000066	0.00015	0.000246
Se	mg/L	0.001	0.1	0.000152	0.00016	0.000222	0.000168	0.000189	0.000132	0.000161	0.000179	0.000208	0.000164	0.000214	0.000224
Si	mg/L	-	-	2.39	2.09	1.45	2.39	2.48	3.28	2.73	2.22	2.14	2.04	5.49	5.91
Sn	mg/L	-	-	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020
Sr	mg/L	-	-	0.048	0.0357	0.215	0.0717	0.0662	0.133	0.0453	0.113	0.144	0.0761	0.0893	0.0945
Ti	mg/L	-	-	0.0082	0.0071	0.0038	0.003	0.013	0.003	0.0131	0.011	0.0091	0.0043	0.0258	0.0319
Tl	mg/L	-	-	0.0000046	0.0000059	0.0000048	0.000003	0.000008	0.000002	0.0000096	0.0000061	0.0000062	0.000002	0.000011	0.000011
U	mg/L	-	-	0.00065	0.000323	0.000971	0.000498	0.000703	0.00111	0.000551	0.000588	0.000661	0.000347	0.000684	0.000822
V	mg/L	-	0.006	0.00124	0.00116	0.00068	0.00088	0.00222	0.00069	0.0018	0.00117	0.00106	0.00113	0.00257	0.00276
Zn	mg/L	-	0.03	0.0067	0.0024	0.0039	0.0045	0.0044	0.0015	0.0038	0.0033	0.0038	0.0015	0.0042	0.0038
Zr	mg/L	-		0.00045	0.00041	0.00023	0.00029	0.00052	0.0002	0.00058	0.00038	0.00035	0.00046	0.00112	0.00118
Ra-226	Bq/L	-	-	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010

Table 4-8: Relative Percent Difference in Water Quality Parameters

Parameter	EDL2				EDL1			
	May		September		May		September	
	PINR-EXP:STUC	PINR-EXP:LVR	PINR-EXP:STUC	PINR-EXP:LVR	PINR-EXP2:LVR2	PINR-EXP2:PINR-REF2	PINR-EXP2:LVR2	PINR-EXP2:PINR-REF2
TP	-0.35	-0.35	0.23	-0.25	-0.28	-0.28	-0.79	-0.09
Ammonia	N/A	N/A	N/A	-0.08	N/A	N/A	N/A	N/A
TSS	0.54	0.54	N/A	N/A	1.48	1.48	0.36	0.25
Hardness	0.78	0.78	0.71	0.81	1.50	1.50	-0.05	0.00
Alkalinity	0.24	0.24	0.60	0.71	0.69	0.69	0.06	0.00
Ag	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Al	-0.57	-0.57	-0.02	-0.79	-0.35	-0.35	8.00	0.06
As	0.21	0.21	-0.06	-0.48	0.07	0.07	-0.18	-0.03
B	1.57	1.57	0.44	0.28	0.80	0.80	0.46	0.06
Ba	0.49	0.49	0.96	-0.11	0.14	0.14	0.15	0.04
Be	-0.52	-0.52	-0.48	-0.65	-0.32	-0.32	1.00	-0.08
Bi	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-0.15
Ca	1.61	1.61	0.74	0.84	1.03	1.03	0.09	0.04
Cd	-0.46	-0.46	0.00	-0.36	-0.16	-0.16	0.75	0.27
Co	0.88	0.88	-0.36	-0.71	0.16	0.16	-0.18	-0.04
Cr	-0.44	-0.44	0.18	-0.61	-0.25	-0.25	3.82	0.01
Cu	0.02	0.02	-0.07	-0.34	0.03	0.03	1.85	-0.55
Fe	-0.35	-0.35	-0.39	-0.82	-0.38	-0.38	-0.25	-0.06
K	4.27	4.27	1.63	0.75	1.93	1.93	-0.01	0.18
Li	0.95	0.95	0.77	0.75	0.47	0.47	0.12	0.05
Mg	0.67	0.67	0.56	0.75	0.30	0.30	-0.05	0.01
Mn	1.40	1.40	0.44	-0.53	0.38	0.38	-0.49	-0.21
Mo	3.38	3.38	0.30	-0.50	2.21	2.21	0.99	0.13
Na	3.92	3.92	0.66	-0.03	3.79	3.79	-0.68	0.19
Ni	0.02	0.02	-0.45	-0.53	0.10	0.10	0.87	0.04
P	-0.26	-0.26	0.15	-0.37	-0.36	-0.36	-0.59	0.05
Pb	-0.41	-0.41	-0.44	-0.88	-0.33	-0.33	2.34	0.04
S	N/A	N/A	N/A	N/A	7.59	7.59	N/A	N/A
Sb	37.85	37.85	0.10	-0.27	17.86	17.86	2.73	0.64
Se	0.46	0.46	-0.21	-0.30	0.29	0.29	0.37	0.05
Si	-0.39	-0.39	0.37	0.32	-0.22	-0.22	1.90	0.08
Sn	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Sr	3.48	3.48	0.85	1.01	2.18	2.18	0.24	0.06
Ti	-0.54	-0.54	0.00	-0.77	-0.31	-0.31	6.42	0.24
Tl	0.04	0.04	-0.33	-0.75	-0.35	-0.35	4.50	0.00
U	0.49	0.49	1.23	0.58	0.20	0.20	1.37	0.20
V	-0.45	-0.45	-0.22	-0.69	-0.41	-0.41	1.44	0.07
Zn	-0.42	-0.42	-0.67	-0.66	0.00	0.00	1.53	-0.10
Zr	-0.49	-0.49	-0.31	-0.62	-0.40	-0.40	1.57	0.05
Ra-226	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Note: Values with below detection limits were omitted from calculation, marked with N/A. Relative percentage differences > 10% are highlighted.

4.3 Additional Habitat Measures

4.3.1 Methods

The depth of Ponar measurements, Secchi depth, and flow was measured at each benthic station. Depth was measured using a portable depth sounder and confirmed manually with a weighted line. Secchi depth was measured with a Secchi disk. Flow was measured using a flow metre pointed in the upstream of the flow.

4.3.2 Results

The depth of measurements in EDL2 ranged from 0.46 to 0.71 m, and Secchi depth from 0.75 to 0.91 m (**Table 4-9**). The flow rate was consistent, ranging from 0.0250 to 0.0513 m/s. At EDL1, the depth of measurements ranged from 0.58 to 0.78 m and Secchi from 0.41 to 0.51 m. Flow ranged from 0.0027 to 0.0061 m/s.

Table 4-9: Secchi, Flow, and Depth of Sites

Discharge	Area	Secchi (m)	Flow (m/s)	Mean Depth Ponar Benthics (m)	Mean Depth Ponar Sediment (m)
EDL2	STUC-REF	0.84	0.0513	0.57	0.56
	LVR-REF	0.75	0.0250	0.69	0.71
	PINR-REF	0.91	0.0293	0.49	0.46
EDL1	LVR2-REF	0.51	0.0061	0.60	0.58
	PINR-REF2	0.41	0.0027	0.72	0.78
	PINR-EXP2	0.48	0.0027	0.71	0.67

5.0 Benthic Invertebrate Community Survey

5.1 Objective

An assessment of the benthic invertebrate community in the receiving environment serves as a basis to delineate the extent and magnitude of fish habitat degradation, if any, due to physical and chemical contamination potentially associated with mine operations. It also provides a basis for an evaluation of the aquatic food resources available for fish communities in the receiving environment. "Effects" were assessed based on a series of "EEM effect endpoints" that describe key attributes of the benthic invertebrate community structure.

5.2 Study Design and Methods

5.2.1 Overview

The Phase 3 benthic invertebrate community survey followed a control/impact design and was executed in a manner generally consistent with the previous phases of study for the mine. Benthic invertebrates were collected with a petite Ponar grab from areas receiving mine effluent discharge (exposure) and reference areas not influenced by the mine. The benthic invertebrate sampling program was conducted between the 19th and 22nd of September 2023. This timeframe is similar to Phases 1 and 2, which were conducted in September 2017 and 2020, respectively.

5.2.2 Sampling Areas

Benthic invertebrates were collected from several different areas during Phase 3 to address comments and recommended actions from the past two phases. Sediment samples were collected concurrently with benthic samples (**Table 4-1**). The most upstream exposure area (PINR-EXP), was located just downstream of the mine and established to capture the potential effect of the combined Loslo Creek (EDL2), Sediment Pond 1 and Sediment Pond 2 discharges on the Pinewood River. The Reference areas for the EDL2 discharge were Sturgeon Creek (STUC-REF) and La Vallée River (LVR-REF).

During Phase 3, the previous sample areas were maintained, and three additional sites were added to evaluate effects of the EDL1 discharge. The first area sampled was upstream of the discharge (PINR-REF2) to capture potential effects of the upstream EDL2, Sediment Pond 1, and Sediment Pond 2 discharges. The second sample area sampled was the Exposure area downstream of EDL1 (PINR-EXP2), and the third area was a new reference location in the La Vallée River (LVR2-REF). A new reference in the LVR was chosen as neither of the other two reference areas used for EDL2 had comparable size and habitat to the EDL1 discharge area.

In total, 6 areas were sampled during the Phase 3 benthic invertebrate community survey (PINR-EXP1, PINR-EXP2, PINR-REF2, STUC-REF, LVR-REF, and LVR2-REF2). These areas are divided into two categories for the analysis of potential effects: areas associated with the EDL2 discharge are all the sample areas from Phases 1 and 2 (PINR-EXP1, STUC-REF, LVR-REF), while areas associated with the EDL1 discharge are PINR-EXP2, PINR-REF2, and LVR2-REF.

5.2.3 Sample Collection

Benthic invertebrate samples were collected with a series of Petite Ponar grabs (individual sampling area = 0.023 m^2) in triplicate (total sampling area = 0.069 m^2). Samples from individual grabs were consolidated and sieved in the field using a 500 µm mesh and subsequently transferred into labeled containers and preserved with formalin to achieve a 10% buffered concentration.

To minimize sample variability within and among stations, habitat variables such as substrate and water depth were standardized to the highest degree possible. Samples were collected from five stations within each sampling area. Five stations per area is sufficient to detect a difference equal to ± for α (i.e., the likelihood of a Type I error) = 0.1 and power (i.e., 1 – ß; where ß is the likelihood of a Type II error) =0.9.

5.2.4 Sample Processing and Analysis

When the invertebrate samples arrived at the Ecometrix office (Mississauga, Ontario) following the field program, each was logged and crosschecked with field data sheets to ensure that all the samples were received and that all the samples were labeled correctly. Samples were also checked to ensure that they had been properly preserved in the field.

Detailed taxonomic identifications were completed by Zaranko Environmental Assessment Services (ZEAS), Nobleton, Ontario. When the samples arrived at ZEAS, they were checked for proper labeling and preservation, and stained to ease removal of the invertebrates. Before further processing, the samples were washed free of formalin. Samples were sorted with the aid of a dissecting microscope at ten times magnification. All benthic fauna were identified to the lowest practical level.

Invertebrate recovery checks (i.e., the number of animals remaining in previously sorted sample fractions) were completed on two benthic samples. Recovery in these sample was 95.7%. Recovery standards as set for the EEM program by Environment Canada indicate that 90% recovery is acceptable (EC, 2012).

All benthic samples were subsampled prior to sorting, A list of the sample fractions that were sorted are provided in **Appendix C**.

5.2.5 Data Management, Data Summary and Statistical Analyses

5.2.5.1 Data Management

Data received from the taxonomist was imported directly into a database format to minimize transcription errors. Non-benthic and unidentified taxa were removed.

5.2.5.2 Data Summary

Data were summarized and several benthic invertebrate community metrics were calculated using embedded Microsoft Excel™ spreadsheet functions. These metrics included those defined

as "EEM effect parameters" (EC, 2012), as well as several others that may help to reveal patterns of invertebrate community structure:

- total invertebrate density[1];
- invertebrate taxa richness[1];
- Simpson's Evenness (E)[1];
- Bray-Curtis Dissimilarity Index (B-C)[1];
- Simpson's Diversity (D);
- Density of major taxonomic groups (sum of individuals present within each group); and,
- Relative density of major invertebrate taxonomic groups (proportion of individuals present within each group relative to total).

A description and calculation approach for each metric is described below.

Total invertebrate density is the total number of individuals of all taxonomic categories collected at a station expressed per unit area (in this case organisms/m^2).

Invertebrate richness (family richness), also known as taxa richness, is the total number of unique taxonomic categories (generally to the family-level) collected at a sampling station.

Evenness (E) is an expression of species representation within a given sample. In some cases, disturbance or stress can create conditions whereby relatively few taxa are favoured (e.g., only species with extreme tolerance to a disturbance), leading to those taxa becoming disproportionately abundant in a location. Evenness is bound by 0 and 1, where an evenness of 1 would indicate that the sample's taxa are of equal abundance. Evenness is calculated following Smith and Wilson (1996):

$$E = 1/\sum_{i=1}^{S}(p_i)^2 / S$$

where: E = Evenness
p_i = the proportion of the i^{th} taxon (family) at the station
S = the total number of taxa (families) at the station

[1] Total invertebrate abundance, richness, E and B-C are "EEM effect parameters". According to EEM technical guidance, during the Initial, Periodic and Focused phases of the monitoring program, an effect on the benthic invertebrate community is said to occur if there are statistical differences between reference and exposure areas in any of the effect parameters.

Bray-Curtis Dissimilarity Index (B-C Index) is an expression of species composition between two sites, based on unique species. The B-C index ranges from 0 to 1, where 0 indicates that the two sites are occupied by identical species, and 1 indicates that there are no species in common. B-C index is measured as the percentage of difference between sites, following Legendre and Legendre (1983):

$$BC_{ij} = \sum_{i=1}^{n} |y_{i1} - y_{i2}| \Big/ \sum_{i=1}^{n} (y_{i1} + y_{i2})$$

where: BC_{ij} = Bray-Curtis Dissimilarity index between sites 1 and 2
Y_{i1} = Species counted at site 1
Y_{i2} = Species counted at site 2
n = Total number of taxa present at the two sites

Analysis of B-C index outputs followed a distance-based redundancy analysis (dbRDA) using an approach provided by Environment Canada. The B-C Index is calculated between all possible comparisons for each replicate station and each taxon (family level or higher) within those stations with up to 999 permutations.

The B-C index was visualized using non-metric multidimensional scaling (nMDS). This non-parametric approach provides a 2-dimensional representation of the differences in between and within site communities. Higher levels of overlap indicate higher similarity. Here, the ability of the nMDS to collapse the data in a meaningful manner is expressed as "stress", where stress < 0.2 is considered valid (Clarke 1993).

Simpson's Diversity (D) is a measure of the benthic community diversity that downweighs rare taxa in place of common taxa (Krebs, 1994). This measure can inform site diversity while considering species evenness. Simpson's Diversity ranges from 0 to 1, where 0 indicates low diversity and 1 indicates high diversity. Simpson's Diversity is calculated as:

$$D = 1 - \sum_{i=1}^{S} (p_i)^2$$

where: s = the total number of taxa (family) at the station
p_i = the proportion of the i^{th} taxon (family) at the station

The density of the major taxonomic groups identified in the samples was calculated as the sum of individuals present within each group; whereas the relative density of these same taxonomic groups was calculated as the proportion of individuals present within each group relative to the total abundance in the sample.

To ensure a high level of analytical quality, all calculations were checked by an independent scientist.

5.2.5.3 Statistical Analyses

The benthic invertebrate data were analyzed in a manner consistent with the overall objective of identifying potential spatial differences (i.e., among sampling area differences) in benthic community structure (as represented by community endpoint data) within the study area. To this end, statistical analysis followed the MDMER TGD (EC, 2012). All statistical procedures were carried out using R Statistical Software (RStudio v. 2023.09.1+494).

Prior to statistical analysis, the Phase 3 endpoint data were tested to determine whether they met the assumptions of normality (normal probability plot of residuals) and homoscedasticity (variance plot of residuals). In the case where these assumptions were met, among-area comparisons of the Phase 3 benthic macroinvertebrate data were made via single factor ANOVA. Where the assumptions were not met, the data were transformed (as appropriate) to meet the assumptions prior to ANOVA. Non-parametric analysis was performed as an alternative to ANOVA when assumptions were not met and could not be met via data transformation. The Kruskal-Wallis test, a non-parametric equivalent of a one-way ANOVA, was used on data for this purpose.

In the case of dbRDA, the recommendation by Borcard (2014) to test for homogeneity and dispersion of group variance using the Betadisper test was undertaken. However, the EC function for Bray-Curtis applies a natural log transformation to the data, so if overdispersion is present, it is inappropriate to try and correct this using other transformations. Any overdispersion in the data is noted in the results. Analysis of dissimilarity was conducted using taxon density (individuals per m^2).

For any test that resulted in a statistical difference, the direction and magnitude of the difference was noted. Direction provides an indication of whether the exposure means were larger or smaller than the reference means. The magnitude of the difference was calculated relative to the standard deviation for a given endpoint based on the reference area according to the following equation:

$$MoD = \frac{exposure\ area\ mean - reference\ area\ mean}{reference\ area\ standard\ deviation}$$

The magnitude of the difference was compared to the critical effect size (CES; 2 within-Reference area standard deviations (RefSD) for each "EEM effect endpoints" except for Bray-Curtis). The CES is a threshold above which an effect may be indicative of a higher risk to the environment (EC, 2012).

If a significant difference between areas was not detected for a benthic invertebrate community metric, then the minimum effect size that could be detected was calculated using the mean square error generated from the ANOVA as an estimate of variability, with alpha and beta equal to 0.10. The minimum detectable effect size was based on the minimum number of reference area standard deviations, according to the following equation:

$$MDES\ (\delta) = \frac{(t_{\alpha(2)} + t_{\beta})MSE^{1/2} \times \left(\frac{2}{n}\right)^{1/2}}{SD}$$

where: δ = Minimum detectable effect size (between reference and exposure area)
$t_{\alpha(2)}$ = Two-tailed critical value of the Student's t-test statistic (given Type I error probability α)
t_{β} = One-tailed critical value of Type II error probability β as taken from Student's tables with 2(n-1) degrees of freedom
MSE = Mean square error from the ANOVA model
n = Average number of samples (between reference and exposure area)
SD = Standard deviation of the reference mean

Significant differences in the groups produced by the nMDS of Bray-Curtis Dissimilarity Index data was determined using an analysis of similarities (ANOSIM). An ANOSIM uses a matrix of ranked data to determine if the similarities *between* groups is greater than the similarity *within* groups. The purpose of an ANOSIM is to examine if the difference *between* the areas (control vs. exposure) are greater than the difference *within* an area. The hypotheses tested are as follows:

H1: there are no differences among sites within each area

H2: there are no differences between control and exposure area

The ANOSIM provides an R statistic for interpretation along with a p-value. If R = 1, then all replicate stations within an area are more like each other than they are to any replicate station from different areas (i.e., no overlap in an nMDS space). If the R value approaches 0, the similarities between and within areas are the same (e.g., complete overlap in an nMDS space).

Differences among groups of data were considered statistically significant at $\alpha < 0.10$ (i.e., a 10% probability of committing a Type I error or rejecting a true null hypothesis). Consistent with Environment Canada guidance, no correction of α (e.g., Bonferroni correction) was made, as might typically be done when multiple statistical tests are completed on the same data set, to compensate for the multiplicative nature of probability. In this application, fixing α at 0.10 rather than correcting for multiple tests errs on the side of being protective of the environment.

All statistical results were reviewed by a senior statistician. This process included a review of input parameters, confirming that appropriate analyses were used, and a review of the analysis outputs.

5.3 Results

5.3.1 EEM Phase 3 Survey Data

Raw benthic invertebrate community data are provided in **Appendix C**. Invertebrate community data as absolute densities are summarized by sampling area in **Table 5-1** for EDL2 and

Table 5-2 for EDL1. Similarly, invertebrate relative densities are summarized in **Table 5-3** for EDL2 and **Table 5-4** for EDL1. Differences in presence/absence of major taxonomic families is presented in **Table 5-5**. Summary statistics for benthic invertebrate community endpoint data are provided in **Table 5-6**. A summary of the results of the statistical comparisons of the Phase 3 invertebrate community endpoint data are provided in **Table 5-7** for EDL2 and **Table 5-8** for EDL1.

5.3.1.1 General Taxonomic Characteristics

A total of 41 families (or higher taxonomic classification) of benthic invertebrates were identified in the study areas, of which 7 were identified in the two exposure and four reference areas (**Table 5-1**;

Table 5-2). *Limnodrilus udekemianus* (an oligochaete worm in the Naididae family) was the only taxon prominent across all six study areas (present at 60% of the stations within each study area).

Taxa that were generally prominent across the EDL2 sample areas includes nematodes, naidid oligochaetes (F. Naididae), biting and non-biting midges (F. Ceratopogonidae and Chironomidae) and sphaeriid clams. These taxa were also the most abundant within all the EDL2 sampling areas (**Table 5-1**).

Taxa that were generally prominent across the EDL1 sample areas includes naidid oligochaetes, non-biting midges, and fingernail clams. These taxa were also among the five most abundant taxa within all the EDL1 sampling areas, along with riffle beetles (F. Elmidae), and biting midges (F. Ceratopogonidae) (**Table 5-2**)

Table 5-1: Absolute Densities of Major Taxonomic Families in the EDL2 Sites at the Rainy River Mine, Phase 3 EEM

Taxa	PINR-EXP (EDL2)			STUC-REF			LVR-REF		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
P. Nematoda	114.8	459.3	0.0	3421.5	11366.7	459.3	298.5	688.9	0.0
F. Enchytraeidae	0.0	0.0	0.0	0.0	0.0	0.0	45.9	229.6	0.0
F. Naididae	1957.6	4592.6	574.1	5350.4	13318.5	1607.4	4569.6	12400.0	574.1
F. Glossiphoniidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Hygrobatidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O. Harpacticoida	0.0	0.0	0.0	275.6	1377.8	0.0	45.9	229.6	0.0
Cl. Ostracoda	23.0	57.4	0.0	734.8	1377.8	0.0	160.7	688.9	0.0
F. Hyalellidae	11.5	57.4	0.0	344.4	688.9	0.0	137.8	459.3	0.0
F. Chrysomelidae	0.0	0.0	0.0	0.0	0.0	0.0	45.9	229.6	0.0
F. Dytiscidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Elmidae	361.7	746.3	86.1	68.9	229.6	0.0	0.0	0.0	0.0
F. Haliplidae	0.0	0.0	0.0	91.9	459.3	0.0	0.0	0.0	0.0
F. Hydrophilidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Baetidae	0.0	0.0	0.0	45.9	229.6	0.0	0.0	0.0	0.0
F. Caenidae	74.6	229.6	0.0	91.9	229.6	0.0	780.7	1837.0	0.0
F. Ephemeridae	152.1	387.5	0.0	5.7	14.4	0.0	0.0	0.0	0.0
F. Heptageniidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Leptophlebiidae	0.0	0.0	0.0	23.0	114.8	0.0	0.0	0.0	0.0
F. Sialidae	34.4	114.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Coenagrionidae	0.0	0.0	0.0	45.9	229.6	0.0	91.9	459.3	0.0
F. Corduliidae	0.0	0.0	0.0	2.9	14.4	0.0	0.0	0.0	0.0
F. Libellulidae	0.0	0.0	0.0	2.9	14.4	0.0	11.5	57.4	0.0
F. Dipseudopsidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Leptoceridae	0.0	0.0	0.0	183.7	918.5	0.0	0.0	0.0	0.0
F. Limnephilidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Phryganeidae	0.0	0.0	0.0	0.0	0.0	0.0	91.9	229.6	0.0
F. Polycentropodidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Ceratopogonidae	970.2	1550.0	229.6	2709.6	5511.1	459.3	780.7	2985.2	0.0
F. Chaoboridae	0.0	0.0	0.0	183.7	459.3	0.0	91.9	229.6	0.0
F. Chironomidae	993.2	1722.2	459.3	8105.9	11711.1	2640.7	3421.5	8725.9	344.4
F. Culicidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Tabanidae	0.0	0.0	0.0	0.0	0.0	0.0	45.9	229.6	0.0
F. Limoniidae	0.0	0.0	0.0	0.0	0.0	0.0	45.9	229.6	0.0
F. Ancylidae	5.7	28.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Hydrobiidae	80.4	287.0	0.0	0.0	0.0	0.0	643.0	2066.7	114.8
F. Physidae	0.0	0.0	0.0	137.8	688.9	0.0	0.0	0.0	0.0
F. Planorbidae	0.0	0.0	0.0	390.4	918.5	0.0	711.9	1377.8	0.0

Taxa	PINR-EXP (EDL2)			STUC-REF			LVR-REF		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
F. Valvatidae	5.7	28.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Viviparidae	0.0	0.0	0.0	137.8	688.9	0.0	0.0	0.0	0.0
F. Sphaeriidae	106.2	244.0	43.1	2399.6	5051.9	918.5	416.2	932.9	0.0
F. Unionidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 5-2: Absolute Densities of Major Taxonomic Families in the EDL1 Sites at the Rainy River Mine, Phase 3 EEM

Taxa	PINR-EXP2 (EDL1)			LVR2-REF			PINR-REF2		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
P. Nematoda	1217.0	4133.3	0.0	11.5	57.4	0.0	14.4	57.4	0.0
F. Enchytraeidae	0.0	0.0	0.0	11.5	57.4	0.0	2.9	14.4	0.0
F. Naididae	1366.3	2296.3	459.3	459.3	688.9	229.6	714.7	918.5	459.3
F. Glossiphoniidae	23.0	114.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Hygrobatidae	11.5	57.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O. Harpacticoida	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cl. Ostracoda	275.6	918.5	0.0	11.5	57.4	0.0	0.0	0.0	0.0
F. Hyalellidae	344.4	688.9	0.0	11.5	57.4	0.0	20.1	57.4	0.0
F. Chrysomelidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Dytiscidae	0.0	0.0	0.0	11.5	57.4	0.0	0.0	0.0	0.0
F. Elmidae	1400.7	2066.7	688.9	23.0	114.8	0.0	192.3	459.3	100.5
F. Haliplidae	45.9	229.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Hydrophilidae	0.0	0.0	0.0	0.0	0.0	0.0	2.9	14.4	0.0
F. Baetidae	103.3	459.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Caenidae	459.3	918.5	114.8	11.5	57.4	0.0	111.9	272.7	0.0
F. Ephemeridae	23.0	43.1	0.0	0.0	0.0	0.0	269.8	918.5	0.0
F. Heptageniidae	0.0	0.0	0.0	0.0	0.0	0.0	23.0	57.4	0.0
F. Leptophlebiidae	0.0	0.0	0.0	23.0	114.8	0.0	77.5	215.3	0.0
F. Sialidae	0.0	0.0	0.0	0.0	0.0	0.0	14.4	57.4	0.0
F. Coenagrionidae	91.9	459.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Corduliidae	0.0	0.0	0.0	11.5	57.4	0.0	0.0	0.0	0.0
F. Libellulidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Dipseudopsidae	0.0	0.0	0.0	0.0	0.0	0.0	8.6	43.1	0.0
F. Leptoceridae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Limnephilidae	0.0	0.0	0.0	11.5	57.4	0.0	0.0	0.0	0.0
F. Phryganeidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Polycentropodidae	0.0	0.0	0.0	0.0	0.0	0.0	11.5	57.4	0.0
F. Ceratopogonidae	3628.2	7807.4	1607.4	103.3	229.6	0.0	433.4	918.5	57.4
F. Chaoboridae	160.7	459.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Chironomidae	1917.4	2870.4	746.3	2675.2	5740.8	1664.8	562.6	918.5	172.2
F. Culicidae	0.0	0.0	0.0	23.0	114.8	0.0	0.0	0.0	0.0
F. Tabanidae	23.0	114.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Limoniidae	45.9	229.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Ancylidae	218.1	918.5	0.0	11.5	57.4	0.0	261.2	631.5	0.0
F. Hydrobiidae	757.8	2296.3	0.0	103.3	229.6	0.0	45.9	172.2	0.0
F. Physidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Planorbidae	252.6	918.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Taxa	PINR-EXP2 (EDL1)			LVR2-REF			PINR-REF2		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
F. Valvatidae	103.3	459.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Viviparidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. Sphaeriidae	904.2	1492.6	301.4	212.4	344.4	0.0	149.3	401.9	0.0
F. Unionidae	2.9	14.4	0.0	0.0	0.0	0.0	5.7	14.4	0.0

5.3.1.2 Percent Composition

5.3.1.2.1 Areas Associated with the EDL2 Discharge

Generally, the exposure (PINR-EXP) and two reference areas (STUC-REF and LVR-REF) were comprised of similar taxa except for beetles (O. Coleoptera), which were much more prevalent in the exposure area than either of the reference areas. The dominant taxa in the exposure area were non-chironomid dipterans, whereas chironomids were dominant in the STUC reference area, and Annelids in the LVR reference area (**Figure 5-1**). EPT Taxa (orders Ephemeroptera, Plecoptera, and Trichoptera) were most abundant in the LVR reference area at 6.51%, followed by the exposure area at 4.46%, and the STUC reference area at 1.94%. The LVR reference area possessed substantially more gastropods (15.4%) than either the STUC reference (2.8%) or exposure (7.4%) areas.

The percent composition of invertebrate taxa was generally conserved to a few groups across the reference and exposure areas. In the LVR-REF reference area, the dominant groups were from Naididae (32.7%), and Chironomidae (26.4%). The dominant groups in the STUC-REF reference area were similar, with Naididae (19.0%), Chironomidae (34.9%), and to a lesser extent Nematoda (13.6%) and Ceratopogonidae (11.6%). Likewise, the PINR-EXP exposure area was dominated by Naididae (36.3%), Chironomidae (22.5%), and Ceratopogonidae (21.3%). Notably, the exposure area had the smallest proportion of Chironomids in the EDL2 areas but had the highest proportion of Naididae.

Variability between sampling stations was low in the EDL2 reference and exposure areas. The largest variation found was with Elmidae, which comprised 24.7% of the relative abundance of PINR-EXP Site 1, but the other four sites had an average of 10.4%.

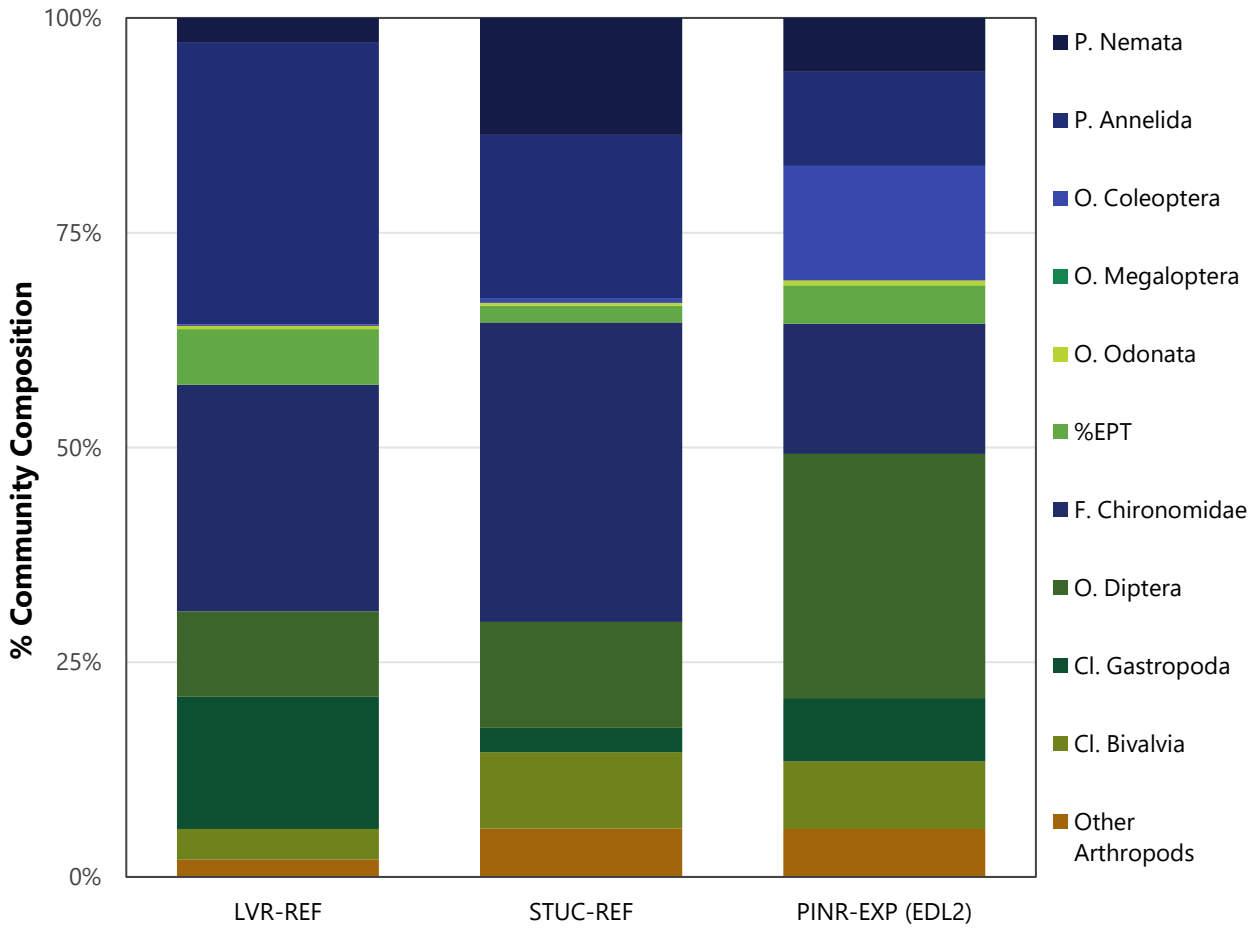

Figure 5-1: Percent Composition of Major Benthic Invertebrate Taxonomic Groups Associated with the EDL2 Discharge, Rainy River Mine, Phase 3 EEM

5.3.1.2.2 Areas associated with the EDL1 Discharge

Generally, the exposure (PINR-EXP2) and two reference areas (LVR2-REF and PINR-REF2) were comprised of similar taxa. The dominant taxa in the PINR-EXP2 exposure area and PINR-REF2 reference area were Annelids, whereas chironomids comprised nearly 75% of the LVR2-REF reference area (**Figure 5-2**). EPT Taxa were most abundant in the PINR-REF2 reference area at 16.9%, followed by the exposure area at 4.8%, and the LVR-REF2 reference area at 1.3%. The PINR-REF2 reference area possessed substantially more gastropods (10.6%) than either the LVR2-REF reference (2.9%) or PINR-EXP2 exposure (2.6%) areas.

The percent composition of invertebrate taxa was generally conserved to a few groups across the reference and exposure areas. In the LVR2-REF reference area, the dominant groups were from Naididae (14.3%), and Chironomidae (70.9%). The dominant groups in the PINR-REF2 reference area were slightly different, with Naididae (25.1%), Chironomidae (18.0%), and Ceratopogonidae (14.2%). The PINR-EXP2 exposure area was dominated by Naididae (10.7%), Elmidae (13.1%), Ceratopogonidae (26.8%), and Chironomidae (15.2%).

Variability between sampling stations was low in the EDL1 reference and exposure areas. The largest variations were both found in samples from the PINR-REF2 area. Ancylidae, for example, comprised 25.9% of Station 5, while only comprising 12.1% and 7.5% of two other stations and absent from two others. Similarly, Sphaeriidae made up 21.2% of Station 1, with the next most abundant station at 4.1%. Like the EDL2 areas however, sample stations were generally homogeneous in their composition in EDL1 areas.

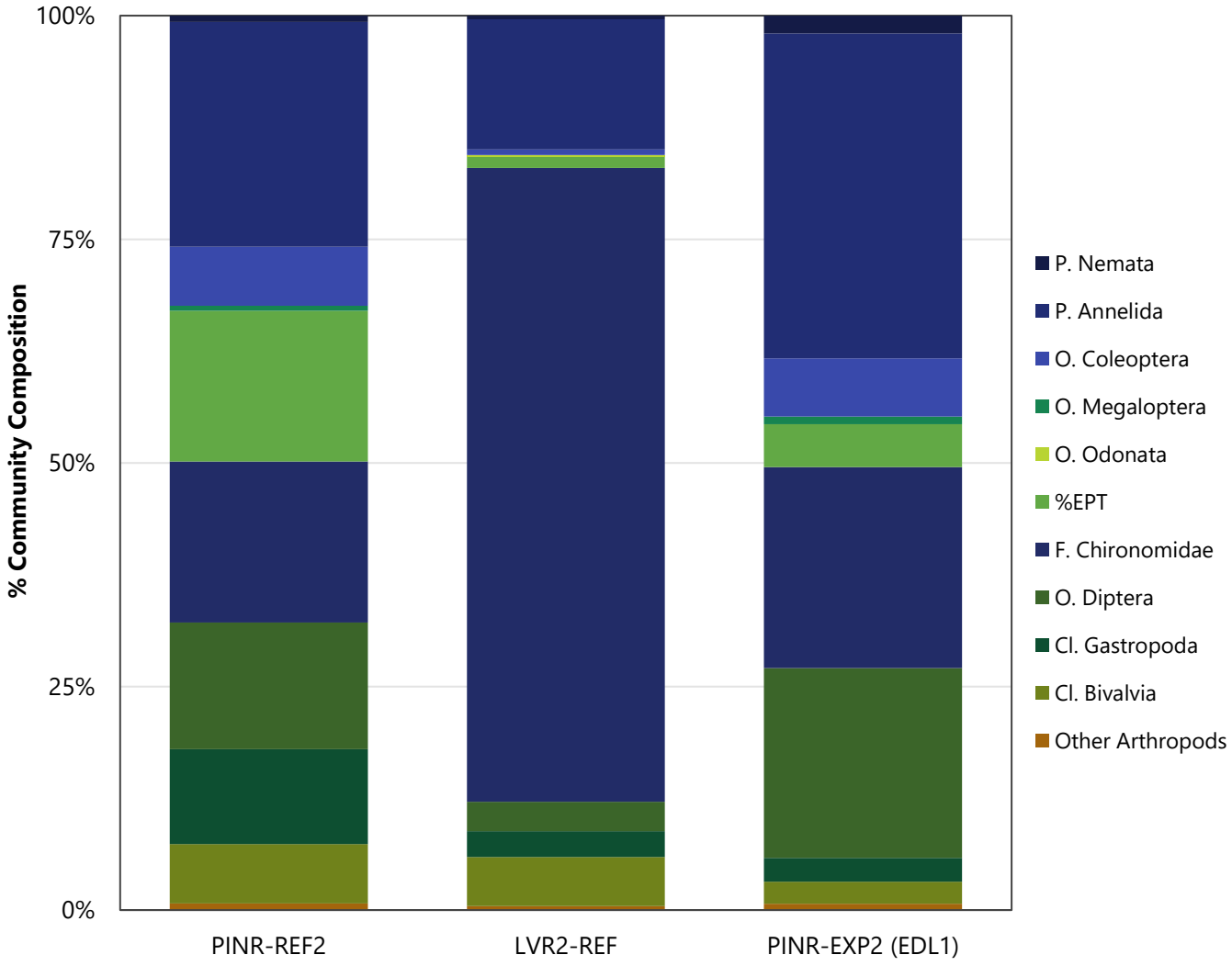

Figure 5-2: Percent Composition of Major Benthic Invertebrate Taxonomic Groups Associated with the EDL1 Discharge, Rainy River Mine, Phase 3 EEM

Table 5-3: Relative Abundance of the Benthic Invertebrate Groups found in Areas Associated with the EDL2 Discharge, Rainy River Mine, Phase 3 EEM

Taxa	STUC-REF			LVR-REF			PINR-EXP (EDL2)		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
P. Nematoda	13.6%	44.0%	3.0%	2.8%	5.9%	0.0%	2.0%	6.7%	0.0%
F. Enchytraeidae	0.0%	0.0%	0.0%	0.2%	0.8%	0.0%	0.0%	0.0%	0.0%
F. Naididae	19.0%	36.7%	8.7%	32.7%	47.4%	19.2%	36.3%	66.5%	17.2%
F. Glossiphoniidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Hygrobatidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
O. Harpacticoida	0.8%	3.8%	0.0%	0.2%	0.8%	0.0%	0.0%	0.0%	0.0%
Cl. Ostracoda	3.0%	5.3%	0.0%	1.3%	3.8%	0.0%	0.5%	1.7%	0.0%
F. Hyalellidae	1.8%	4.5%	0.0%	0.5%	1.8%	0.0%	0.2%	0.9%	0.0%
F. Chrysomelidae	0.0%	0.0%	0.0%	0.2%	0.8%	0.0%	0.0%	0.0%	0.0%
F. Dytiscidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Elmidae	0.3%	1.1%	0.0%	0.0%	0.0%	0.0%	6.5%	11.5%	2.1%
F. Haliplidae	0.3%	1.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Hydrophilidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Baetidae	0.2%	1.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Caenidae	0.4%	1.1%	0.0%	6.2%	14.7%	0.0%	1.3%	3.3%	0.0%
F. Ephemeridae	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	3.5%	6.9%	0.0%
F. Heptageniidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Leptophlebiidae	0.1%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Sialidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.9%	3.4%	0.0%
F. Coenagrionidae	0.3%	1.5%	0.0%	0.3%	1.6%	0.0%	0.0%	0.0%	0.0%
F. Corduliidae	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Libellulidae	0.0%	0.1%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%
F. Dipseudopsidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Leptoceridae	1.2%	6.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Limnephilidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Phryganeidae	0.0%	0.0%	0.0%	0.3%	0.9%	0.0%	0.0%	0.0%	0.0%
F. Polycentropodidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Ceratopogonidae	11.6%	26.0%	2.5%	6.6%	15.8%	0.0%	21.3%	37.9%	3.3%
F. Chaoboridae	0.8%	1.5%	0.0%	3.0%	5.9%	0.0%	0.0%	0.0%	0.0%
F. Chironomidae	34.9%	59.0%	10.2%	26.4%	34.6%	15.8%	22.5%	35.2%	6.7%
F. Culicidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Tabanidae	0.0%	0.0%	0.0%	0.2%	0.8%	0.0%	0.0%	0.0%	0.0%
F. Limoniidae	0.0%	0.0%	0.0%	0.2%	0.8%	0.0%	0.0%	0.0%	0.0%
F. Ancylidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	1.2%	0.0%
F. Hydrobiidae	0.0%	0.0%	0.0%	7.3%	15.8%	1.6%	2.2%	8.6%	0.0%
F. Physidae	0.6%	3.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Planorbidae	1.5%	3.2%	0.0%	8.1%	19.2%	0.0%	0.0%	0.0%	0.0%

F. Valvatidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	1.2%	0.0%
F. Viviparidae	0.6%	3.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Sphaeriidae	8.9%	13.9%	5.6%	3.6%	11.8%	0.0%	2.5%	5.5%	0.8%
F. Unionidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 5-4: Relative Abundance of the Benthic Invertebrate Groups found in Areas Associated with the EDL1 Discharge, Rainy River Mine, Phase 3 EEM

Taxa	LVR2-REF			PINR-REF2			PINR-EXP2 (EDL1)		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
P. Nematoda	0.4%	2.1%	0.0%	0.7%	3.0%	0.0%	6.3%	15.5%	0.0%
F. Enchytraeidae	0.3%	1.4%	0.0%	0.1%	0.4%	0.0%	0.0%	0.0%	0.0%
F. Naididae	14.3%	20.8%	4.8%	25.1%	30.6%	16.4%	10.7%	17.7%	4.9%
F. Glossiphoniidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	1.2%	0.0%
F. Hygrobatidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.7%	0.0%
O. Harpacticoida	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Cl. Ostracoda	0.2%	0.8%	0.0%	0.0%	0.0%	0.0%	1.6%	3.4%	0.0%
F. Hyalellidae	0.3%	1.4%	0.0%	0.7%	2.4%	0.0%	3.8%	9.3%	0.0%
F. Chrysomelidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Dytiscidae	0.3%	1.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Elmidae	0.3%	1.6%	0.0%	6.5%	14.2%	3.1%	13.1%	24.7%	5.2%
F. Haliplidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	1.4%	0.0%
F. Hydrophilidae	0.0%	0.0%	0.0%	0.1%	0.4%	0.0%	0.0%	0.0%	0.0%
F. Baetidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.5%	1.7%	0.0%
F. Caenidae	0.3%	1.4%	0.0%	4.0%	8.5%	0.0%	3.7%	6.2%	1.2%
F. Ephemeridae	0.0%	0.0%	0.0%	8.3%	23.9%	0.0%	0.3%	0.5%	0.0%
F. Heptageniidae	0.0%	0.0%	0.0%	1.1%	3.0%	0.0%	0.0%	0.0%	0.0%
F. Leptophlebiidae	0.6%	2.9%	0.0%	2.8%	7.1%	0.0%	0.0%	0.0%	0.0%
F. Sialidae	0.0%	0.0%	0.0%	0.6%	2.4%	0.0%	0.0%	0.0%	0.0%
F. Coenagrionidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.6%	2.8%	0.0%
F. Corduliidae	0.2%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Libellulidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Dipseudopsidae	0.0%	0.0%	0.0%	0.3%	1.3%	0.0%	0.0%	0.0%	0.0%
F. Leptoceridae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Limnephilidae	0.4%	2.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Phryganeidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Polycentropodidae	0.0%	0.0%	0.0%	0.5%	2.4%	0.0%	0.0%	0.0%	0.0%
F. Ceratopogonidae	2.7%	6.2%	0.0%	14.2%	28.4%	2.4%	26.8%	32.9%	21.9%
F. Chaoboridae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.1%	3.6%	0.0%
F. Chironomidae	70.9%	88.2%	60.1%	18.0%	28.4%	9.1%	15.2%	20.7%	10.3%
F. Culicidae	0.6%	2.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Tabanidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	1.2%	0.0%
F. Limoniidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	1.4%	0.0%
F. Ancylidae	0.2%	0.8%	0.0%	9.1%	25.9%	0.0%	1.6%	5.7%	0.0%
F. Hydrobiidae	2.7%	8.3%	0.0%	1.5%	4.5%	0.0%	4.1%	8.6%	0.0%
F. Physidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Planorbidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.2%	3.4%	0.0%

Taxa	LVR2-REF			PINR-REF2			PINR-EXP2 (EDL1)		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
F. Valvatidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.5%	1.7%	0.0%
F. Viviparidae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
F. Sphaeriidae	5.5%	10.9%	0.0%	6.5%	21.2%	0.0%	7.8%	15.8%	3.6%
F. Unionidae	0.0%	0.0%	0.0%	0.2%	0.4%	0.0%	0.0%	0.1%	0.0%

5.3.1.3 Presence and Absence of Major Taxonomic Families

Seven of the 41 taxonomic groups identified in samples collected across sites associated with the EDL2 and EDL1 discharges were found in all the exposure and reference areas (**Table 5-5**).

At stations associated with the EDL2 discharge location, there were 31 taxonomic groups identified, eight of which were ubiquitous among the exposure and both reference areas (Nemtoda, Naididae, Ostracoda, Hyalellidae, Caenidae, Ceratopogonidae, Chironomidae, and Sphaeriidae). There were an additional 3 groups that were only found in the PINR-EXP exposure area (Sialidae, Ancylidae, and Valvatidae), and 17 groups that were found only in either the LVR-REF and STUC-REF reference areas (**Table 5-5**).

At stations associated with the EDL1 discharge location, there were 35 taxonomic groups identified, ten of which were ubiquitous among the exposure and both reference areas (Nemata, Naididae, Hyalellidae, Elmidae, Caenidae, Ceratopogonidae, Chironomidae, Ancylidae, Hydrobiidae, and Sphaeriidae). There were an additional 10 groups which were only found in the PINR-EXP2 exposure area and 12 groups that were found only in the either the LVR2-REF and PINR-REF2 reference areas.

Table 5-5: Presence and Absence of the Major Benthic Invertebrate Taxonomic Families, Rainy River Mine, Phase 3 EEM

Taxa	LVR-REF	STUC-REF	PINR-EXP (EDL2)	PINR-REF2	LVR2-REF	PINR-EXP2 (EDL1)
P. Nemata	Y	Y	Y	Y	Y	Y
F. Enchytraeidae	Y	N	N	Y	Y	N
F. Naididae	Y	Y	Y	Y	Y	Y
F. Glossiphoniidae	N	N	N	N	N	Y
F. Hygrobatidae	N	N	N	N	N	Y
O. Harpacticoida	Y	Y	N	N	N	N
Cl. Ostracoda	Y	Y	Y	N	Y	Y
F. Hyalellidae	Y	Y	Y	Y	Y	Y
F. Chrysomelidae	Y	N	N	N	N	N
F. Dytiscidae	N	N	N	N	Y	N
F. Elmidae	N	Y	Y	Y	Y	Y
F. Haliplidae	N	Y	N	N	N	Y
F. Hydrophilidae	N	N	N	Y	N	N

Taxa	LVR-REF	STUC-REF	PINR-EXP (EDL2)	PINR-REF2	LVR2-REF	PINR-EXP2 (EDL1)
F. Baetidae	N	Y	N	N	N	Y
F. Caenidae	Y	Y	Y	Y	Y	Y
F. Ephemeridae	N	Y	Y	Y	N	Y
F. Heptageniidae	N	N	N	Y	N	N
F. Leptophlebiidae	N	Y	N	Y	Y	N
F. Sialidae	N	N	Y	Y	N	N
F. Coenagrionidae	Y	Y	N	N	N	Y
F. Corduliidae	N	Y	N	N	Y	N
F. Libellulidae	Y	Y	N	N	N	N
F. Dipseudopsidae	N	N	N	Y	N	N
F. Leptoceridae	N	Y	N	N	N	N
F. Limnephilidae	N	N	N	N	Y	N
F. Phryganeidae	Y	N	N	N	N	N
F. Polycentropodidae	N	N	N	Y	N	N
F. Ceratopogonidae	Y	Y	Y	Y	Y	Y
F. Chaoboridae	Y	Y	N	N	N	Y
F. Chironomidae	Y	Y	Y	Y	Y	Y
F. Culicidae	N	N	N	N	Y	N
F. Tabanidae	Y	N	N	N	N	Y
F. Limoniidae	Y	N	N	N	N	Y
F. Ancylidae	N	N	Y	Y	Y	Y
F. Hydrobiidae	Y	N	Y	Y	Y	Y
F. Physidae	N	Y	N	N	N	N
F. Planorbidae	Y	Y	N	N	N	Y
F. Valvatidae	N	N	Y	N	N	Y
F. Viviparidae	N	Y	N	N	N	N
F. Sphaeriidae	Y	Y	Y	Y	Y	Y
F. Unionidae	N	N	N	Y	N	Y

Taxa found in all six sites
Taxa found in reference areas only
Taxa found in exposure areas only

5.3.1.4 Taxa Diversity

5.3.1.4.1 Areas Associated with the EDL2 Discharge

The greatest numbers of taxa were found within the taxonomic family Chironomidae. There was a total of 30 chironomid genera identified. The PINR-EXP exposure area contained 21 different genera, whereas the LVR-REF and STUC-REF reference areas had 18 and 15 genera of chironomids, respectively. Despite this, there were no midge genera that would be considered

ubiquitous (found in 60% of the samples in both reference and exposure areas) across stations associated with EDL2 discharge.

Only four mayfly genera were identified in the study area. Of these, the genus *Caenis* was the most abundant and widely distributed. Four caddisfly taxa were also identified across the study area. However, these were generally uncommon and were primarily found in the reference areas.

Nine snail and six clam taxa were identified. Snails in the genus *Gyraulus,* from the family Planorbidae were widely distributed across the exposure and reference areas. Sphaeriid clams were found in all exposure stations and, and in 75% of the reference stations.

5.3.1.4.2 Areas Associated with the EDL1 Discharge

The greatest numbers of taxa in sample areas associated with the EDL1 discharge were also found within the taxonomic family Chironomidae. There was a total of 21 chironomid genera identified. The PINR-EXP2 exposure area contained 10 different genera, and the LVR2-REF and PINR-REF2 reference areas had 10 and 13 genera of chironomids, respectively. The most ubiquitous midge genera (found in 60% of the samples in both reference and exposure areas) were *Procladius* from the subfamily Tanypodinae.

Four mayfly genera were identified in the study area. Of these, the mayfly *Caenis* was the most abundant and widely distributed. Three caddisfly taxa were also identified across the study area. However, these were generally uncommon and were only found in the reference areas. Notably, the LVR2-REF reference area had few mayfly and caddisfly in terms of both species richness and density.

Four snail and five clam taxa were identified. *Probythinella emarginatafrom,* in the family Hydrobiidae, and *Ferrissia*, in the family Ancylidae, were widely distributed across the exposure and reference areas. Sphaeriid clams were found in all exposure stations, and in 75% of the reference stations.

5.3.1.5 Benthic Invertebrate Community Endpoint Data

Benthic invertebrate community endpoint data are summarized in **Table 5-6**, illustrated in **Figure 5-3** and **Figure 5-4,** and discussed by sampling area below.

Benthic invertebrate density at the LVR reference area ranged from 2,181 to 28.072 organisms/m^2 and was on average 12,437 organisms/m^2. Mean taxa richness was 20.8, with richness at individual sampling stations ranging from 9 to 32. The mean Simpson's Diversity (D) value was 0.90 with scores at individual sampling stations ranging from 0.80 to 0.94. Evenness ranged from 0.40 to 0.77 with an average of 0.59.

Benthic invertebrate density in the STUC reference area ranged from 15,170 to 36,282 organisms/m^2 and was on average 24,754 organisms/m^2. Mean taxa richness was 23.2, with richness at individual sampling stations ranging from 21 to 26. The mean diversity value was

0.87 with scores at individual sampling stations ranging from 0.77 to 0.935. Evenness ranged from 0.17 to 0.615 with an average of 0.39.

Benthic invertebrate density in the exposure area ranged from 6,171 to 26,651 organisms/m^2 and was on average 13,376 organisms/m^2. Mean taxa richness was 24.2, with richness ranging from 20 to 29. The mean diversity value was 0.89 with scores ranging from 0.87 to 0.93 for individual stations. Evenness ranged from 0.28 to 0.48 and was on average 0.40.

Table 5-6: Summary Statistics of the Benthic Invertebrate Community Endpoint Data from Areas Associated with the EDL2 and EDL1 Discharge Locations, Rainy River Mine Phase 3 EEM

Associated Discharge	Site	Endpoint	Mean	Median	Max	Min	SD	SE
EDL2 (PINR -EXP)	STUC-REF	Density (organisms/m^2)	24,754	25,259	36,282	15,170	7,728	3,456
		Family Richness	23.2	23.0	26.0	21.0	1.9	0.9
		Simpson's Diversity	0.87	0.898	0.935	0.77	0.068	0.036
		Simpson's Evenness	0.399	0.41	0.61	0.17	0.16	0.07
	LVR-REF	Density (organisms/m^2)	12,437	3,904	28,072	2,181	12,949	5,791
		Family Richness	20.8	18.0	32.0	9.0	9.2	4.1
		Simpson's Diversity	0.90	0.92	0.94	0.80	0.06	0.03
		Simpson's Evenness	0.59	0.58	0.77	0.40	0.14	0.06
	PINR-EXP	Density (organisms/m^2)	13,376	9,444	26,651	6,171	8,317	3,720
		Family Richness	24.2	23.0	29.0	20.0	4.1	1.8
		Simpson's Diversity	0.89	0.89	0.93	0.87	0.02	0.01
		Simpson's Evenness	0.40	0.42	0.48	0.28	0.08	0.03
EDL1 (PINR -EXP2)	LVR2-REF	Density (organisms/m^2)	3,726	2,770	7,176	1,952	2,060	921
		Family Richness	6.4	7.0	9.0	2.0	3.0	1.3
		Simpson's Diversity	0.63	0.70	0.75	0.31	0.18	0.08
		Simpson's Evenness	0.29	0.30	0.42	0.16	0.11	0.05
	PINR-REF2	Density (organisms/m^2)	2,922	3,200	3,846	1,894	761	340
		Family Richness	10.6	10.0	16.0	5.0	4.2	1.9
		Simpson's Diversity	0.87	0.88	0.91	0.79	0.05	0.02
		Simpson's Evenness	0.53	0.55	0.68	0.37	0.15	0.07
	PINR-EXP2	Density (organisms/m^2)	4,891	5,382	6,903	2,368	1,975	883
		Family Richness	8.8	9.0	11.0	7.0	1.8	0.8
		Simpson's Diversity	0.85	0.85	0.91	0.79	0.04	0.02
		Simpson's Evenness	0.43	0.36	0.67	0.35	0.14	0.06

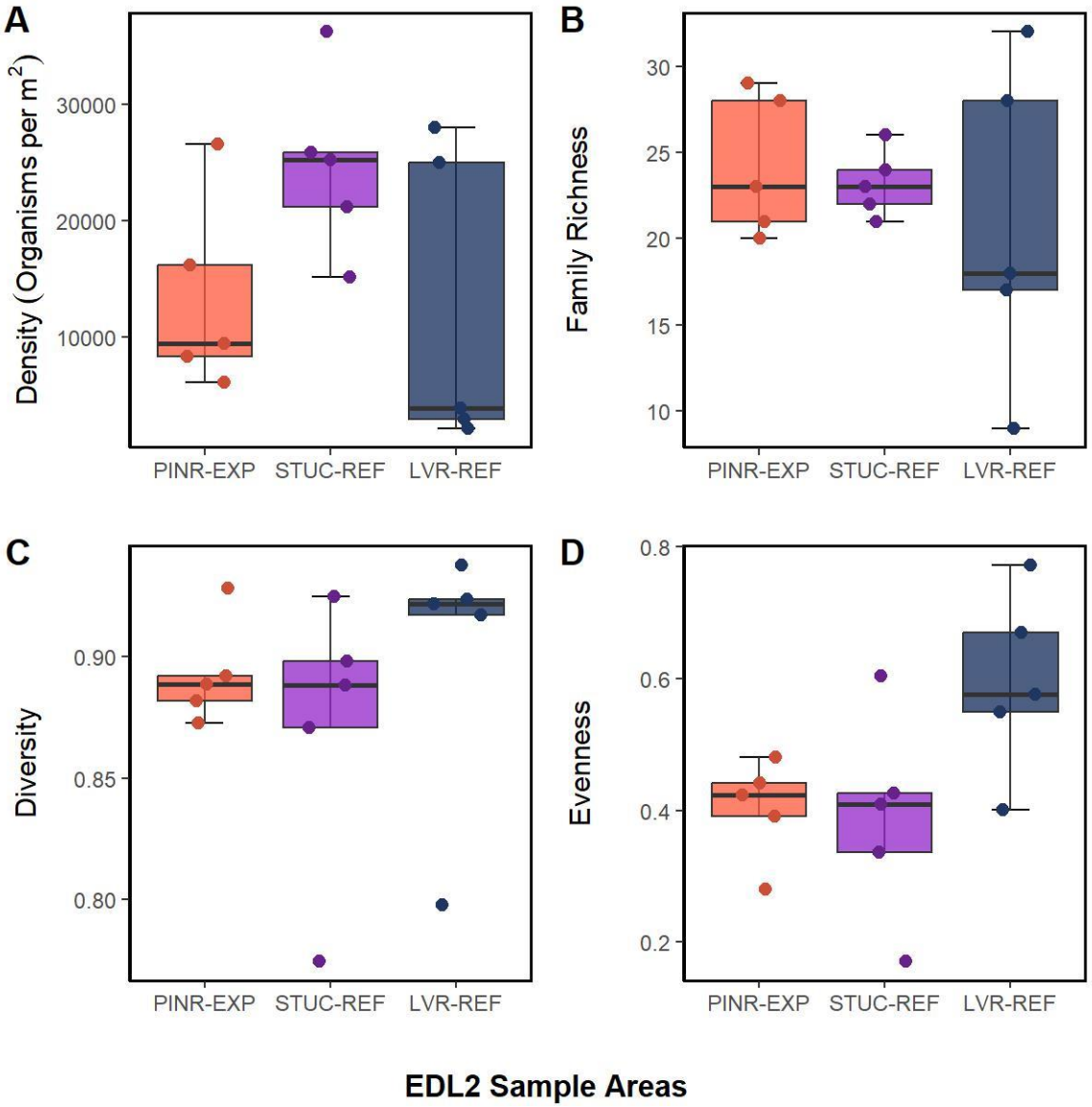

Figure 5-3: Benthic invertebrate density (A), family richness (B), Simpson's diversity (C), and Simpson's evenness (D) box and whisker plots for EDL2 study areas, Rainy River Mine Phase 3 EEM

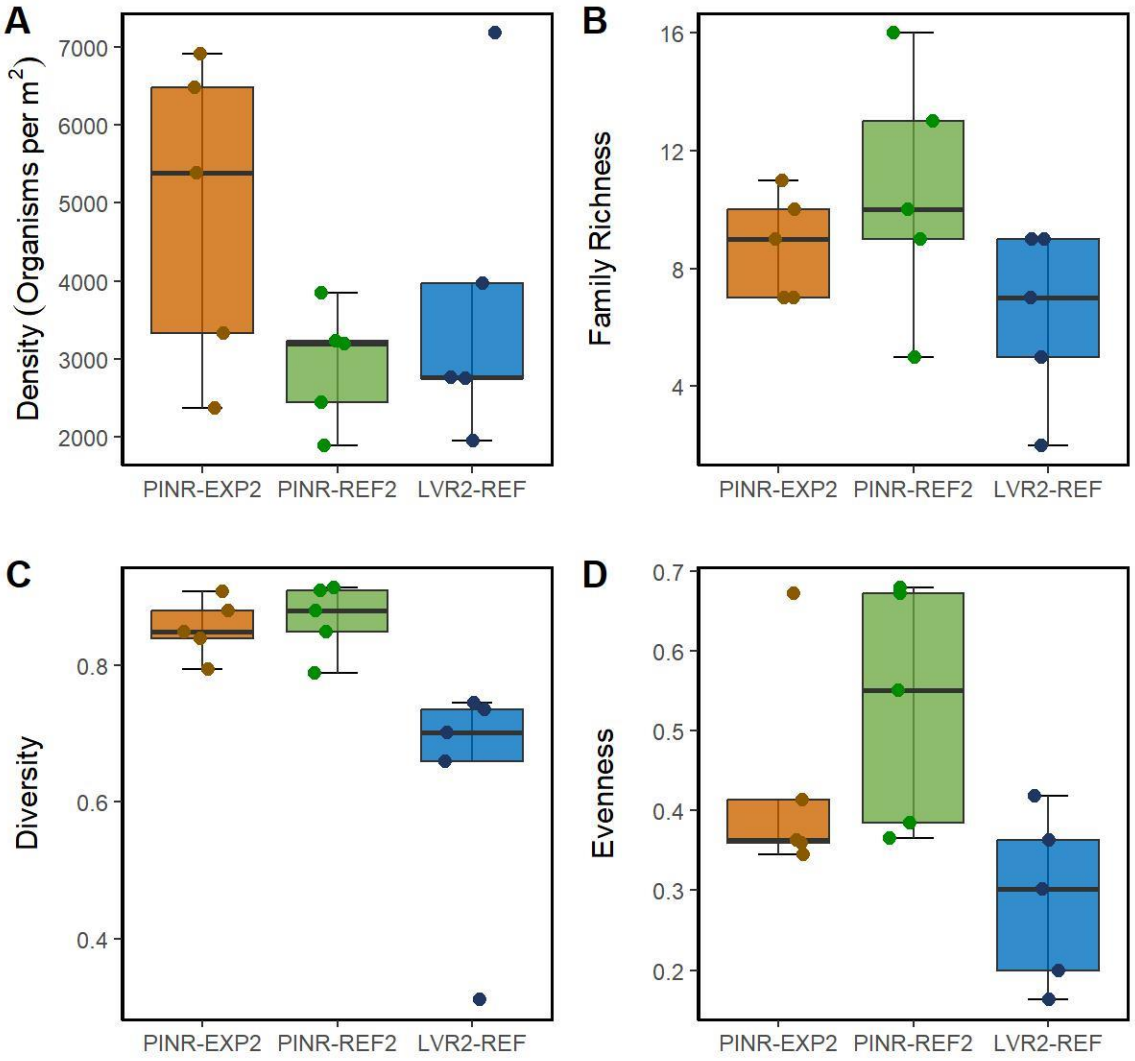

Figure 5-4: Benthic invertebrate density (A), family richness (B), Simpson's diversity (C), and Simpson's evenness (D) box and whisker plots for EDL1 study areas, Rainy River Mine Phase 3 EEM

5.3.1.6 Statistical Comparisons of EEM Phase 3 Effect Endpoints

A summary of the results of the statistical comparison of EEM Phase 3 endpoint data is provided in **Table 5-7** for EDL2 and **Table 5-8**. Graphical depictions of the endpoint results are provided in **Figure 5-3** and **Figure 5-4**. nMDS depictions of the benthic communities are provided in **Figure 5-5** to **Figure 5-8**.

5.3.1.6.1 Invertebrate Density

Density was significantly lower in PINR-EXP compared to STUC-REF (p = 0.055), with a magnitude of difference of 1.47 RefSD. Similarly, density was significantly higher in PINR-REF2

compared to PINR-EXP2 (p = 0.071), with a magnitude of difference of 2.589 RefSDs. The difference in EDL1 was above the CES of ± 2 RefSDs.

5.3.1.6.2 Taxa Richness

Taxa richness was not significantly different in any of the measured sites.

5.3.1.6.3 Simpson's Diversity

Diversity was significantly higher in PINR-EXP2 compared to LVR2-REF (p = 0.009), with a magnitude of difference of 0.81 RefSD. The difference is below the CES of ± 2 RefSDs.

5.3.1.6.4 Simpson's Evenness

Evenness was significantly lower in PINR-EXP compared to LVR-REF (p = 0.028), with a magnitude of difference of 1.37 RefSD. The difference is below the CES of ± 2 RefSDs.

5.3.1.6.5 Bray-Curtis Index

There were significant differences in the Bray-Curtis Index when comparing STUC-REF to PINR-EXP (p = 0.006), LVR-REF to PINR-EXP (0.005), and LVR2-REF to PINR-EXP2 (p = 0.001). The nMDS plots of these communities indicate that the groups of species have high amounts of variability across areas and little species overlap.

Table 5-7: Summary of Benthic Invertebrate Endpoint Statistics from Areas Associated with the EDL2 Discharge Location, Rainy River Mine, Phase 3 EEM

Comparison	Endpoint	Area	n	Model	Mean Ref.	Mean Exp.	*p* value	Significant Difference Between Areas?	Magnitude of Difference (# of ref. SDs)	Minimum Detectable Effect Size (# of ref. SDs)
STUC-REF vs PINR-EXP	Density (organisms/m^2)	STUC-REF	5	ANOVA	24,754.1	13,376.0	0.055	Yes	1.472	-
		PINR-EXP	5							
	Family Richness	STUC-REF	5	ANOVA	23.2	24.2	0.634	No	-	3.849
		PINR-EXP	5							
	Simpson's Diversity	STUC-REF	5	ANOVA	0.871	0.893	0.458	No	-	1.747
		PINR-EXP	5							
	Simpson's Diversity	STUC-REF	5	KW	0.888	0.889	0.602	No	-	NA
		PINR-EXP	5							
	Simpson's Evenness	STUC-REF	5	ANOVA	0.389	0.403	0.864	No	-	1.821
		PINR-EXP	5							
	Bray-Curtis Distance	STUC-REF	5	dbRDA	NA	NA	0.006	Yes	NA	-
		PINR-EXP	5							
LVR-REF vs PINR-EXP	Density (organisms/m^2)	LVR-REF	5	ANOVA	12,437.3	13,376.0	0.895	No	-	1.948
		PINR-EXP	5							
	Density (organisms/m^2)	LVR-REF	5	KW	3,903.7	9,443.5	0.465	No	-	NA
		PINR-EXP	5							
	Family Richness	LVR-REF	5	ANOVA	20.8	24.2	0.472	No	-	1.793
		PINR-EXP	5							
	Simpson's Diversity	LVR-REF	5	ANOVA	0.900	0.893	0.804	No	-	1.747
		PINR-EXP	5							
	Simpson's Diversity	LVR-REF	5	KW	0.922	0.889	0.347	No	-	NA
		PINR-EXP	5							
	Simpson's Evenness	LVR-REF	5	ANOVA	0.594	0.403	0.028	Yes	1.367	-
		PINR-EXP	5							
	Bray-Curtis Distance	LVR-REF	5	dbRDA	NA	NA	0.005	Yes	NA	-
		PINR-EXP	5							

Indicates that the data did not meet the ANOVA assumption of Normality
Highlights a significant difference ($p<0.1$) between the Reference and Exposure areas

Table 5-8: Summary of Benthic Invertebrate Endpoint Statistics from Areas Associated with the EDL1 Discharge Location, Rainy River Mine, Phase 3 EEM

Comparison	Endpoint	Area	N	Model	Mean Ref.	Mean Exp.	*p* value	Significant Difference Between Areas?	Magnitude of Difference (# of ref. SDs)	Minimum Detectable Effect Size (# of ref. SDs)
LVR2-REF vs PINR-EXP2	Density (organisms/m^2)	LVR2-REF	5	ANOVA	3,725.7	4,891.1	0.388	No	-	2.271
		PINR-EXP2	5							
	Family Richness	LVR2-REF	5	ANOVA	6.4	8.8	0.160	No	-	1.914
		PINR-EXP2	5							
	Simpson's Diversity	LVR2-REF	5	ANOVA	0.630	0.853	0.028	Yes	1.228	-
		PINR-EXP2	5							
	Simpson's Diversity	LVR2-REF	5	Kruskal Wallis	0.701	0.849	0.009	Yes	0.813	-
		PINR-EXP2	5							
	Simpson's Evenness	LVR2-REF	5	ANOVA	0.289	0.431	0.107	No	-	2.657
		PINR-EXP2	5							
	Bray-Curtis Distance	LVR2-REF	5	dbRDA	NA	NA	0.001	Yes	NA	-
		PINR-EXP2	5							
PINR-REF2 vs PINR-EXP2	Density (organisms/m^2)	PINR-REF2	5	ANOVA	2,922.0	4,891.1	0.071	Yes	2.589	-
		PINR-EXP2	5							
	Family Richness	PINR-REF2	5	ANOVA	10.6	8.8	0.400	No	-	1.784
		PINR-EXP2	5							
	Simpson's Diversity	PINR-REF2	5	ANOVA	0.867	0.853	0.655	No	-	2.135
		PINR-EXP2	5							
	Simpson's Evenness	PINR-REF2	5	ANOVA	0.531	0.431	0.306	No	-	2.216
		PINR-EXP2	5							
	Bray-Curtis Distance	PINR-REF2	5	dbRDA	NA	NA	0.513	No	-	NA
		PINR-EXP2	5							

Indicates that the data did not meet the ANOVA assumption of Normality
Highlights a significant difference ($p<0.1$) between the Reference and Exposure areas

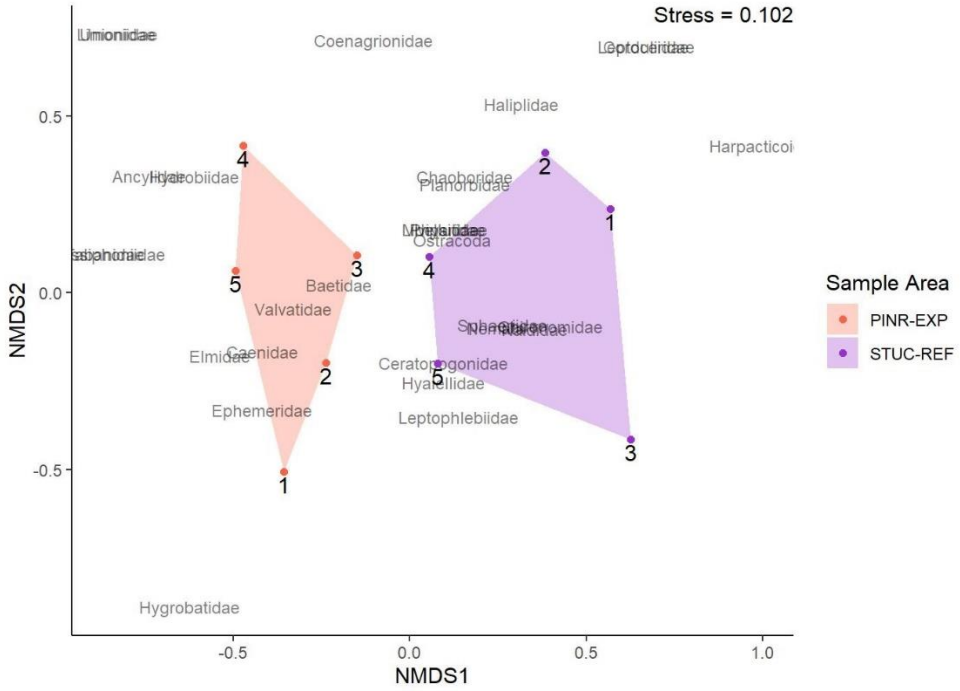

Figure 5-5: nMDS graph depicting the PINR-EXP and STUC-REF Benthic Invertebrate Communities, Rainy River Mine, Phase 3 EEM

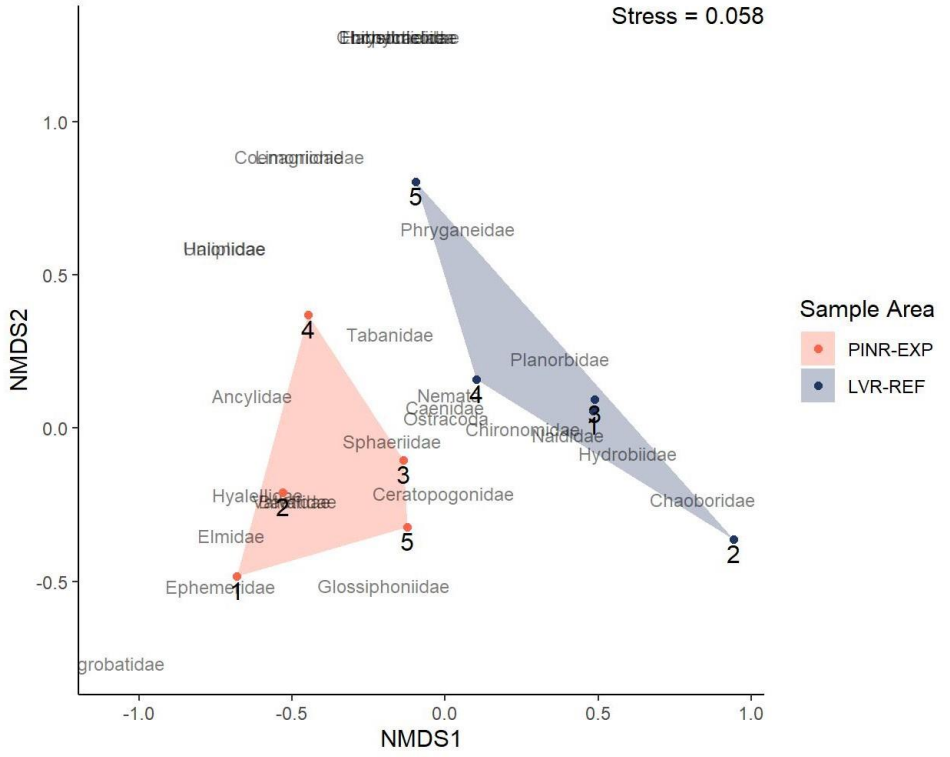

Figure 5-6: nMDS graph depicting the PINR-EXP and LVR-REF Benthic Invertebrate Communities, Rainy River Mine, Phase 3 EEM

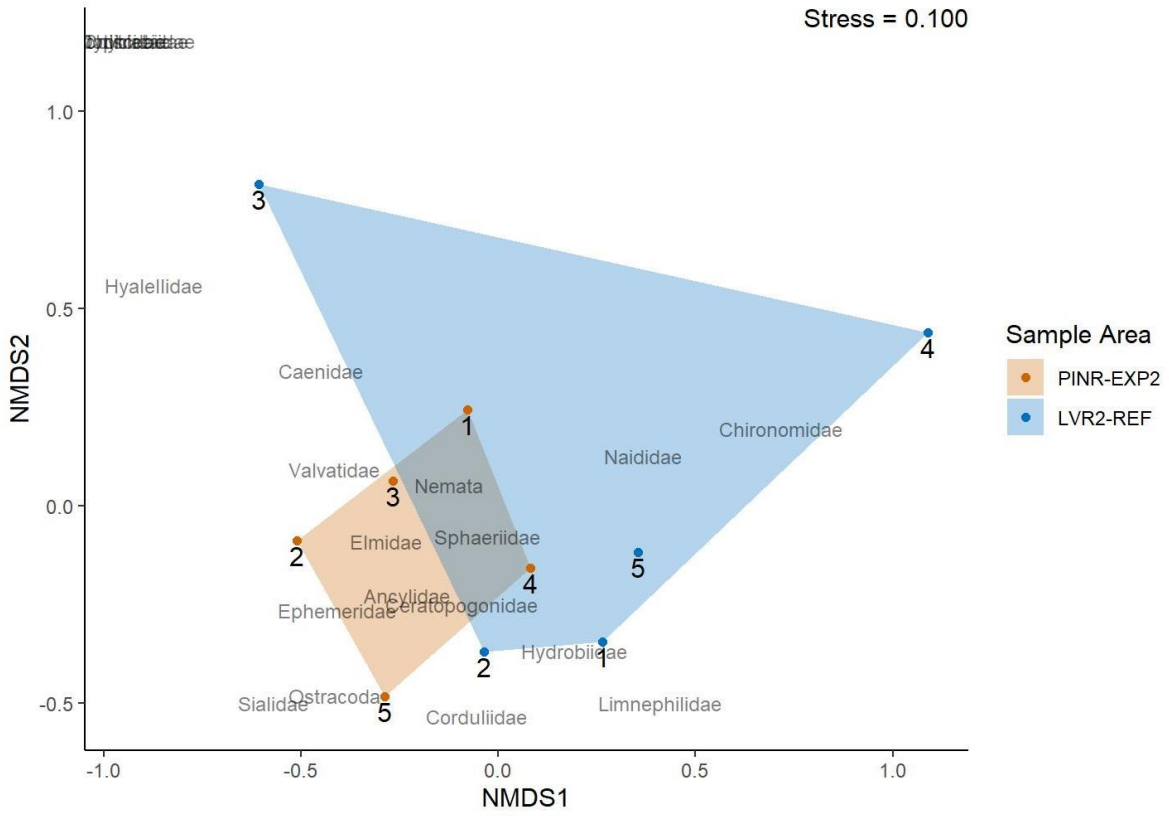

Figure 5-7: nMDS graph depicting the PINR-EXP2 and LVR2-REF Benthic Invertebrate Communities, Rainy River Mine, Phase 3 EEM

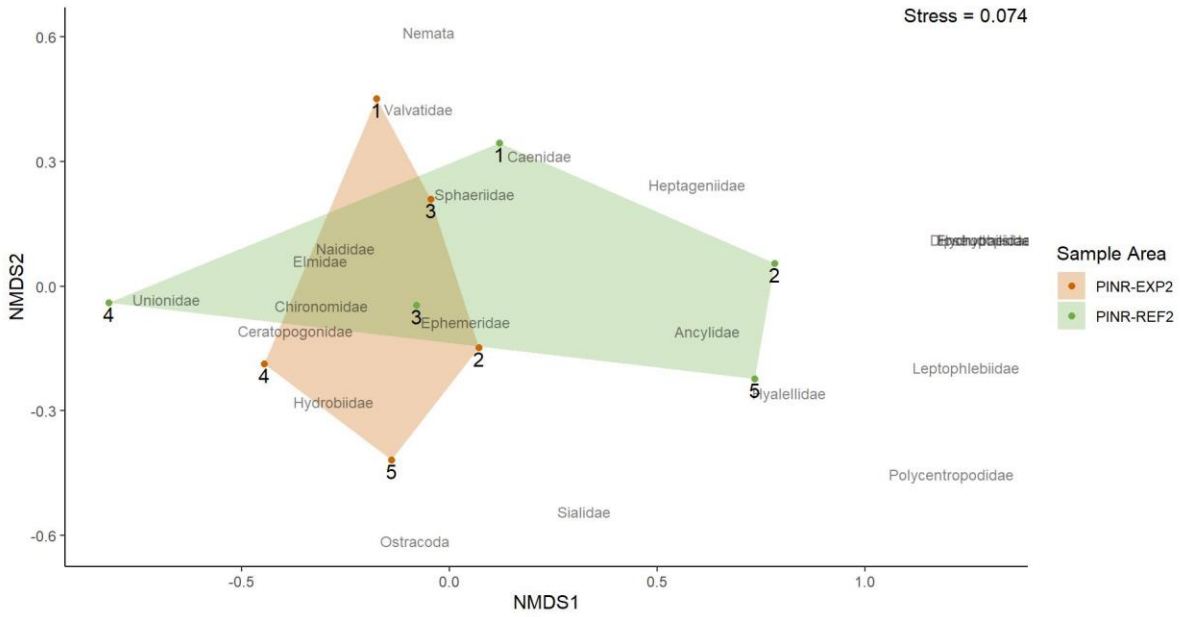

Figure 5-8: nMDS graph depicting the PINR-EXP2 and PINR-REF2 Benthic Invertebrate Communities, Rainy River Mine, Phase 3 EEM

5.3.2 Temporal Trends in Invertebrate Community Structure

A comparison of the mean values for each benthic endpoint from Phase 1 to 3 are presented in **Figure 5-9** and **Table 5-9**. The trends are visualized in

Table 5-10. From Phase 2 to 3, density decreased in the exposure site (PINR-EXP) compared to STU-REF by a magnitude of 1.47 RefSD. Previously, density was unaffected. Similarly, the evenness declined in PINR-EXP compared to LVR-REF by a magnitude of 1.37 RefSD. Previously, evenness was significantly higher in PINR-EXP. There were no other notable trends in the remaining effect endpoints.

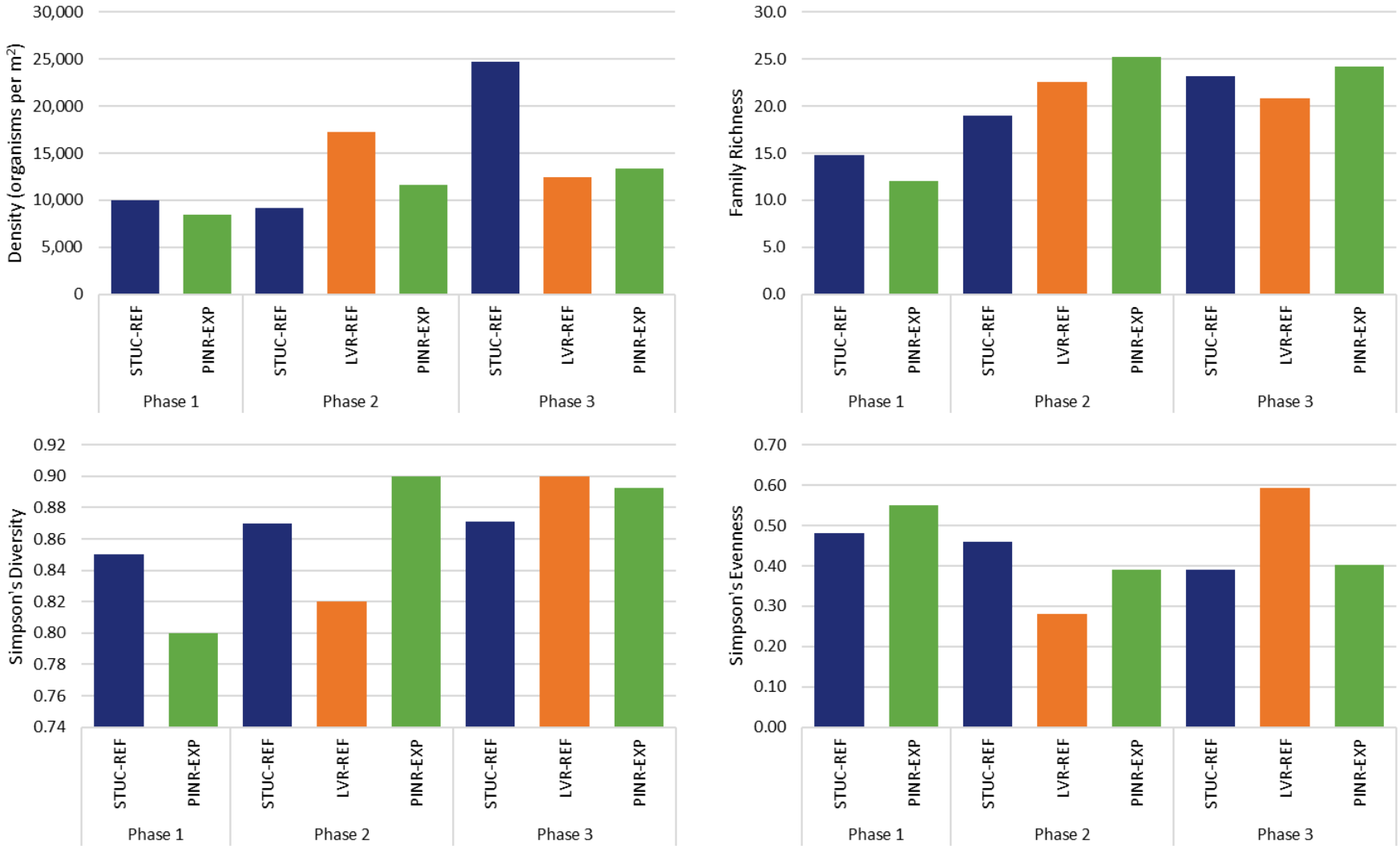

Figure 5-9: Temporal Comparison of Mean Benthic Invertebrate Community Endpoint Values from EEM Phases 1-3 at the Rainy River Mine

Table 5-9: Summary of Mean Benthic Invertebrate Community Endpoint Values from EEM Phases 1-3 at the Rainy River Mine

Metric	Phase 1		Phase 2			Phase 3		
	STUC-REF	PINR-EXP	STUC-REF	LVR-REF	PINR-EXP	STUC-REF	LVR-REF	PINR-EXP
Density	9,950	8,445	9,203	17,249	11,657	24,754	12,437	13,376
LPL Richness	14.8	12.0	19.0	22.6	25.2	23.2	20.8	24.2
Simpson's Diversity	0.85	0.80	0.87	0.82	0.90	0.87	0.90	0.89
Evenness	0.48	0.55	0.46	0.28	0.39	0.39	0.59	0.40

Table 5-10: Temporal Comparisons of Benthic Community Endpoint Statistical Results from EEM Phases 1-3 at the Rainy River Mine

Effects Endpoint	Phase 1	Phase 2	Phase 3
STU-REF Reference vs PINR-EXP Exposure			
Density	0	0	↓ (1.47)
Richness	0	↑	0
Simpson's Diversity	0	0	0
Simpson's Evenness	0	0	0
Bray-Curtis Index	Yes	Yes	Yes
LVR-REF Reference vs PINR-EXP Exposure			
Density	-	0	0
Richness	-	0	0
Simpson's Diversity	-	↑	0
Simpson's Evenness	-	↑	↓ (1.37)
Bray-Curtis Index	-	Yes	Yes

Note: The values in brackets indicate the magnitude of difference. (RefSD).

5.4 Summary of Key Findings

The key findings associated with the Phase 3 benthic invertebrate community survey are:

- A total of 41 benthic families were identified in the study area. Seven families were identified in the two exposure and four reference areas. *Limnodrilus udekemianus* the only taxon prominent across all six study areas (present at 60% of the stations within each study area).

- In EDL2, the density ranged from 2,181 to 36,282 organisms/m^2. In EDL1, density ranged from 1,894 to 7,175 organisms/m^2. The dominant taxon was of the family Chironomidae. By proportion, the most common taxa were from the Phylum Annelida, Family Chironomidae, and other member of the Order Diptera.

- Density was significantly lower in PINR-EXP compared to STUC-REF ($p = 0.055$), with a magnitude of difference of 1.47 RefSD. Similarly, density was significantly higher in PINR-REF2 compared to PINR-EXP2 ($p = 0.071$), with a magnitude of difference of 2.589 RefSD. The difference in EDL1 was above the CES of ± 2 RefSDs.

- Taxa richness was not significantly different in any of the measured sites.

- Diversity was significantly higher in PINR-EXP2 compared to LVR2-REF ($p = 0.009$), with a magnitude of difference of 0.81 SD. The difference is below the CES of ± 2 RefSDs.

- Evenness was significantly lower in PINR-EXP compared to LVR2-REF ($p = 0.028$), with a magnitude of difference of 1.37 RefSD. The difference is below the CES of ± 2 RefSDs.

- There were significant differences in the Bray-Curtis Index when comparing STUC-REF to PINR-EXP ($p = 0.006$), LVR-REF to PINR-EXP (0.005), and LVR2-REF to PINR-EXP2 ($p = 0.001$).

- There are no consistent trends in benthic endpoint data from Phase 1 to 3 other than Bray-Curtis Index. Based on the nMDS plots of these communities, there appears to be high amounts of variability across areas and little species overlap. As suggested by the results of Phase 2, STUC may not be a strong reference area for the EDL2 discharge. Continued study of both LVR areas as a reference will help develop trends in benthic community.

6.0 Fish Survey

6.1 Objectives

The data collected in the fish survey provides the basis to determine if there are significant differences in fish growth, energy storage, and reproduction capabilities between sentinel species in the reference areas and the areas exposed to mining effluent. The fish survey does not attempt to provide an absolute or direct assessment of the health of the fish population. Instead, the objective of the survey is to use the results of the assessment of the sentinel fish species as a reflection of the overall aquatic environment.

6.2 Study Design and Methods

6.2.1 Overview

The fish survey followed a control/impact design, where fish are compared between exposure areas and reference areas. Brook Stickleback and Central Mudminnow were targeted in collections as they were successfully caught in high numbers in previous phases. For both species, at least 20 mature male and 20 mature female fish were targeted with the intent to measure the full suite of health related and reproductive endpoints. In addition to adult fish collected, up to 20 immature fish of each species were targeted for a subset of endpoints.

As outlined previously, PINR-EXP is the exposure area downstream of the EDL2 discharge location and the corresponding reference areas were on Sturgeon Creek (STUC-REF) and the La Vallée River (LVR-REF). The exposure area downstream of EDL1is PINR-EXP2 with an associated areaupstream-of-EDL1 and downstream-of-EDL2, PINR-REF2, and a similarly sized catchment area reference area on the La Vallée River (LVR-REF2). The sampling design and mapped areas are in **Table 6-1** and **Figure 2-2**.

Table 6-1: Sampling design for fish survey, 2023.

Associated Exposure Site	Type	Site
EDL2 (PINR-EXP)	Reference	STUC-REF
	Reference	LVR-REF
	Exposure	PINR-EXP
EDL1 (PINR-EXP2)	Reference	LVR2-REF
	Reference	PINR-REF2
	Exposure	PINR-EXP2

6.2.2 Fish Collection

Fish from the reference and exposure areas were collected between May 1st and 9th, 2023. This timeframe was ideal from a life history perspective to consider important effect indicators, such as growth, reproduction, condition, and survival. Also, the timing was consistent with Phase 2 thus allowing for temporal comparisons.

Sampling was initiated with minnow traps as this was the most efficient fish capture method used in the Phase 1 and 2 EEM. However, as the study proceeded it was evident that catches of sentinel species in some areas would need to be supplemented with other methods for capture. Therefore, backpack electrofishing effort and a seine net were employed to increase capture numbers. Non-target fish species were identified, counted, and released in the same location in waterbody from which they were collected.

For minnow traps, fishing effort was recorded as fish caught per trap hour. For electrofishing, fishing effort was recorded as fish per minute. For seine, fishing effort was recorded in fish per square metre.

6.2.3 Fish Processing

Fish processing was completed within a few hours of the time of capture. The measurements included sex, length, body weight, gonad weight, and liver weight. Abnormalities, such as internal and external parasites, tumors, or scarring, were also noted. This information provided a qualitative measure of fish health.

Fish were measured on a standard measuring board, to the nearest millimetre. Whole fish were weighed to the nearest 0.001 g using a portable electronic balance, providing a precision of ±1%. Balance accuracy was assessed each day using standard weights and the balance was re-calibrated, as necessary. Liver and gonad were extracted using forceps and weighed to the nearest 0.001 g using an electronic balance. Ovaries were also collected from females for subsequent estimation of egg weight and fecundity. Ovaries were placed in labeled vials and preserved to a level of 10% formalin for subsequent egg counts.

Otoliths were collected to age the fish. Following extraction of liver and ovaries the remaining body was placed in a labelled bag for shipment to the age determination laboratory.

6.2.4 Laboratory Processing

Aging structures (otoliths) were processed at AAE Tech Services Inc. Processing was completed by one technician, and QA/QC as performed on 20% of the otoliths by an independent technician to ensurethat the EEM QA/QC requirements of ± one year were met.

Fecundity and egg counts were processed at ZEAS Inc. The process, in brief, is as follows. Prior to laboratory processing, the ovaries were gently rinsed in a 180 μm sieve to remove formalin. The weight of each ovarian sample, which was initially determined in the field, was re-weighed to the nearest 0.001 g using an electronic balance to obtain a preserved mass. Generally, the mass of preserved eggs is slightly greater than that of unpreserved eggs due to fluid uptake. When possible, three subsamples from each ovarian sample were weighed and the number of eggs within each of the three subsamples were counted with the aid of a dissecting microscope under 8x magnification. Once counted, the eggs were re-preserved and archived. Fecundity was determined by dividing the total laboratory (preserved) weight by the subsample weight multiplied by the subsample egg number. For each female, the estimates were averaged to determine the total

fecundity. Fresh egg weight was determined by dividing the total weight of the unpreserved ovaries (measured in the field) by the estimated fecundity (i.e., number of eggs per female).

6.2.5 Data Management, Summary, and Analyses

Catch data was summarized to generate comparable measures of fishing effort and success (i.e., catch-per-unit-effort [CPUE]) between the sampling areas. Descriptive statistics (n, mean, median, maximum, minimum, standard error, standard deviation) were determined for key fish survey endpoints.

The statistical procedures were consistent with the EC TGD (EC, 2012). Specific effect endpoints used for the fish survey included those recommended by the TGD, which may be broadly grouped into the following response categories: survival, energy use and energy storage. The specific endpoints used to assess these response categories, as well as the appropriate statistical approaches, are summarized in **Table 6-2**. As some variability associated with individual physiological/morphological endpoints may be dependent upon other characteristics (covariates), it is necessary to compare exposure and reference fish according to the covariate combinations outlined in **Table 6-2**. "Effect" endpoints are those that are used to establish whether the is a mine-related effect (i.e., statistically significant differences between exposure and reference areas). "Support" endpoints are for informational purposes and significant differences between exposure and reference areas are not necessarily used to designate an effect.

As noted, effect endpoints may be broadly grouped into the three response categories: survival, energy use and energy storage. *Survival* is a measure of the effect of the difference in mean age of all fish among areas (separated by species). A healthy population should exhibit variability in age. *Energy storage* is a measure of the current condition of the fish population. A healthy fish will demonstrate a greater body weight relative to length and have a liver weight that is proportional to its body size. Natural and anthropogenic stressors from the environment can affect the condition of a fish population. *Energy use* is a measure of the ability of the fish population to utilize resources in their environment to grow and reproduce.

The calculated fish measures included the condition factor (K), gonadosomatic index (GSI), and liversomatic index (LSI). These measures are calculated as:

$$K = [\text{body weight} / (\text{fork length}^3)]*100$$

$$GSI = 100*(\text{gonad weight} / \text{body weight})$$

$$LSI = 100*(\text{liver weight} / \text{body weight}),$$

where weights are measured in grams and fork length is measured in centimetres.

6.2.6 Statistical Approaches

All statistical processing was performed using *R* v.4.2.1 base packages, package *tidyverse* v. 2.0.0 and related packages, and an Ecometrix in-house EEM companion package *emsR* v. 5.0.0.

An analysis of variance (ANOVA) was used to determine if there were statistically significant differences in fish age, weight, and length from reference and exposure areas. Assumptions of the ANOVA were assessed using a test of normality of residuals per area (Shapiro-Wilk test) and homogeneity of variance between areas (Levene's test). Outliers were also identified using Cook's distance and Studentized residual tests. Considering the normality and homogeneity of variance assessment, the outlier assessment, and the evaluation of residuals using approaches in statistical tests (e.g., Quinn and Keough 2002), observations were removed to meet the assumptions of ANOVA. When observations were removed, statistical comparisons were performed and reported with and without the observations to assess the effect of the outliers on the results. If assumptions of ANOVA were still not met, non-parametric alternatives such as the Kruskal-Wallis test was employed.

If a statistically significant difference was identified, then the magnitude of difference was also calculated. Direction (positive or negative) provides an indication of whether the exposure area mean is larger or smaller than the reference mean. Magnitude is the percent difference between two areas and is calculated by comparing the means between reference and exposed areas according to the following equation:

$$[(\text{exposed mean}) - (\text{reference mean}))/ \text{reference mean}] \times 100$$

In the event of multiple reference or exposure areas, MDMER guidelines specify an ANOVA or ANCOVA to be run comparing one reference and exposure area at a time. This design was implemented during the Phase 2 EEM, however these pairwise comparisons do not always allow for a statistical analysis of where all study areas lie relative to each other. To help separate the relationships between exposure areas and their associated reference areas, they were compared in the same statistical test (ANOVA or ANCOVA tests), and any significant differences were followed up with a post-hoc Tukey Test. In the event of a significant result from an ANOVA or ANCOVA that compares more than two groups, the Tukey Test uses the studentized range distribution to determine where these differences lie through the comparison of all possible pairs of means. In the Phase 3 Study Design, Phase 2 EEM endpoints were re-evaluated with this approach and are summarized in **Section 6.3.5** related to temporal comparisons.

An analysis of covariance (ANCOVA) was used to assess body weight at age, length at age, gonad weight at body weight, body weight at fork length, and liver weight at body weight. If, during the ANCOVA procedure, slopes were not equal between areas, further outliers were identified using Cook's methodology to determine if exclusion of Cook's outliers results in equal slopes between areas. The ANCOVA tests for homogeneity of slopes between the dependent variable and covariate for each area. If a significant interaction between area and covariate was found (i.e., slopes are different), the difference between areas was estimated using the min-max method, as prescribed by the TGD (EC, 2012). The min-max procedure determines the MOD between the minimum and maximum values of covariate overlap. These MODs are calculated using the following equation:

$$[(\text{exposed value} - \text{reference value})/\text{reference value}] \times 100$$

In cases where the ANCOVA results in a non-significant interaction between areas, an ANCOVA is performed with the interaction term removed to determine if there is a difference between the estimated marginal means of each area. The marginal means are calculated at the mean value of the covariate across areas. The magnitude of the difference between areas is calculated using the following formula:

[(exposed marginal mean – reference marginal mean)/reference marginal mean] x 100

Again, Tukey tests were employed to make individual comparisons amongst sites in the event that there was an overall statistical difference between areas.

Table 6-2: Fish endpoint and statistical analyses completed as part of the Phase 3 EEM.

Response category	Endpoint	Dependent Variable (Y)	Covariate (X)	Statistical test	Effect or Support
Survival	Age	--	--	ANOVA	Effect
Energy Storage	Condition	Body weight	Length	ANCOVA	Effect
	Relative liver size	Liver weight	Body weight	ANCOVA	Effect
	Relative egg size	Mean egg weight	Body weight	ANCOVA	Support
Energy Use	Total body weight	--	--	ANOVA	Support
	Length	--	--	ANOVA	Support
	Size-at-age	Body weight	Age	ANCOVA	Effect
		Length	Age	ANCOVA	Support
	Relative gonad size	Gonad weight	Body weight	ANCOVA	Effect
	Relative fecundity	Number of eggs per female	Body weight	ANCOVA	Support

6.2.7 Power Analysis

A priori power analyses for the Brook Stickleback and Central Mudminnow analyses were used to identify the sample sizes required to detect differences of a specific magnitude (i.e., 25% for relative liver weight, relative gonad size, and 10% for condition) between the reference area and exposure area with respect to individual parameters. The power analysis was used to determine the samples sizes required to detect differences of these magnitudes in subsequent surveys. *A priori* power analysis is based on the measurement of variability in the sample populations and assumes that the variability measured in the sample population is representative of the entire population. Estimates of the sample size required is provided for critical effect sizes at 5%, 10%, 20%, 50%, and 100%. Power analyses were conducted using a power level of 0.9 (1-β), following:

$$n = 2\,(t_{\alpha}(2) + t_{\beta})_2\,(SD/\delta)_2$$

where:

n is the number of samples
SD is the sample standard deviation (or MSE from ANOVA or ANCOVA model)
δ is the specified effect size (between reference and exposure area)
$t_{\alpha(2)}$ (two tailed test) and t_{β} (one tailed test) are critical values of the Student's t-test statistic (given Type I error probability α and Type II error probability β as taken from Student's tables with 2(n-1) degrees of freedom).

6.2.8 Fish Usability

As per the study design (Ecometrix, 2023), no fish usability studies were required for the Phase 3 EEM.

6.3 Results

6.3.1 Fish collections

A total of 16 unique fish species were caught in the reference areas and exposures areas in May 2023 (**Table 6-3** and **Table 6-4**).

For sites relevant to the EDL2 discharge exposure, PINR-EXP had seven (7) species, STUC-REF had four (4) species, and LVR-REF had 10 species. For minnow traps, PINR-EXP CPUE was 0.27 fish/trap hour and was dominated by Brassy Minnow (29%), Brown Bullhead (*Ameiurus nebulosus*; 29%), Brook Stickleback (27%), and Central Mudminnow (14%). Catch in STUC-REF was much lower (0.04 fish/trap hour) and was dominated by Central Mudminnow (92%). Catch in LVR-REF was the highest (9.84 fish/trap hour) dominated by Brook Stickleback (48%) and Brassy Minnow (31%) followed to a lesser extent by Northern Redbelly Dace (*Chrosomus eos*; 7%) and Creek Chub (*Semotilus atromaculatus*; 7%). At PINR-EXP, 1,325 trap hours were used that collected the majority of community fish and 1,418 electrofishing seconds were employed to target sentinel species. At STUC-REF, 2,186 trap hours were used to collect fish and, at LVR-REF, 710 trap hours and 1,431 electrofishing seconds were used to collect community fish and sentinel species, respectively.

For sites relevant to the EDL1, PINR-EXP2 had 12 species, PINR-REF2 had 10 species, and LVR2-REF had seven (7) species. For minnow traps, PINR-EXP2 CPUE as 0.06 fish/trap hour and was dominated by Brook Stickleback (47%), Central Mudminnow (22%), and to a lesser extent by Brassy Minnow (9.4%). Catch in PINR-REF2 was higher (0.19 fish/trap hour) and was dominated by Brassy Minnow (40%) followed by Brook Stickleback (27%) and Central Mudminnow (22%). Catch in LVR-REF2 was the highest (0.57 fish/trap hour) and dominated by Brook Stickleback (94%) with Central Mudminnow only making up 2% of the total catch. At PINR-EXP2, 2,507 trap hours, 2,601 electrofishing seconds, and one seine net haul (225 m^2) were used to collect fish with the latter two types of gear being used to target sentinel species. At PINR-REF2, 1,708 trap hours were used to collect fish. At LVR2-REF, 1,182 trap hours and 200 seconds of electrofishing effort were used.

Table 6-3: Summary of fish caught at sites relevant to EDL2 PINR-EXP exposure area, May 2023.

Gear	Fish species	Exposure (PINR-EXP)			Reference (STUC-REF)			Reference (LVR-REF)[a]		
		No. captured	CPUE (fish/trap hour)	% Total	No. captured	CPUE (fish/trap hour)	% Total	No. captured	CPUE (fish/trap hour)	% Total
Minnow Traps	Blacksided Darter (*Percina maculate*)	0	0.0000	0.00%	0	0.0000	0.00%	0	0.0000	0.00%
	Brassy Minnow (*Hybognathus hankinsoni*)	105	0.0792	29.01%	0	0.0000	0.00%	2188	3.0777	31.27%
	Brook Stickleback (*Culaea inconstans*)	**99**	**0.0747**	**27.35%**	**2**	**0.0009**	**2.25%**	**3366**	**4.7347**	**48.11%**
	Brown Bullhead (*Ameiurus nebulosus*)	105	0.0792	29.01%	3	0.0014	3.37%	0	0.0000	0.00%
	Central Mudminnow (Umbra limi)	**50**	**0.0377**	**13.81%**	**82**	**0.0375**	**92.13%**	**91**	**0.1280**	**1.30%**
	Common Shiner (*Luxilus cornutus*)	1	0.0008	0.28%	0	0.0000	0.00%	44	0.0619	0.63%
	Creek Chub (*Semotilus atromaculatus*)	1	0.0008	0.28%	0	0.0000	0.00%	466	0.6555	6.66%
	Fathead Minnow (*Pimephales promelas*)	0	0.0000	0.00%	0	0.0000	0.00%	124	0.1744	1.77%
	Finescale Dace (*Chrosomus neogaeus*)	0	0.0000	0.00%	0	0.0000	0.00%	0	0.0000	0.00%
	Golden Shiner (*Notemigonus crysoleucas*)	0	0.0000	0.00%	0	0.0000	0.00%	11	0.0155	0.16%
	Johnny Darter (*Etheostoma nigrum*)	0	0.0000	0.00%	0	0.0000	0.00%	0	0.0000	0.00%
	Northern Pike (*Esox lucius*)	1	0.0008	0.28%	2	0.0009	2.25%	0	0.0000	0.00%
	Northern Redbelly Dace (*Chrosomus eos*)	0	0.0000	0.00%	0	0.0000	0.00%	490	0.6893	7.00%
	Pearl Dace (*Margariscus margarita*)	0	0.0000	0.00%	0	0.0000	0.00%	173	0.2433	2.47%
	Rock Bass (*Ambloplites rupestris*)	0	0.0000	0.00%	0	0.0000	0.00%	0	0.0000	0.00%
	Undifferentiated Cyprinid YOY	0	0.0000	0.00%	0	0.0000	0.00%	0	0.0000	0.00%
	White Sucker (*Catostomus commersonii*)	0	0.0000	0.00%	0	0.0000	0.00%	44	0.0619	0.63%
	Total	**362**	**0.2731**	**100%**	**89**	**0.0407**	**100.00%**	**6997**	**9.8422**	**100.00%**
		No. captured	**CPUE (fish/min)**	**% Total**	**No. captured**	**CPUE (fish/min)**	**% Total**	**No. captured**	**CPUE (fish/min)**	**% Total**
Electrofishing	**Central Mudminnow (*Umbra limi*)**	**10**	**0.4231**	**90.91%**	--	--	--	**20**	**0.8386**	**100.00%**
	Brook Stickleback (*Culaea inconstans*)	**1**	**0.0423**	**9.09%**	--	--	--	**0**	**0**	**0.00%**
	Total	**11**	**0.4654**	**100%**	--	--	--	**20**	**0.8386**	**100.00%**

Notes:
Sentinel species highlighted in **bold green** font.
a. At LVR-REF, proportionalized catches based on previous efforts for some efforts due to high sample size and targeting only Brook Stickleback and Central Mudminnow.

Table 6-4: Summary of fish caught at sites relevant to PINR-EXP2 exposure area, May 2023.

Gear	Fish species	Exposure (PINR-EXP2)			Reference (PINR-REF2)			Reference (LVR2)		
		No. captured	CPUE (fish/trap hour)	% Total	No. captured	CPUE (fish/trap hour)	% Total	No. captured	CPUE (fish/trap hour)	% Total
Minnow Traps	Blacksided Darter (*Percina maculate*)	0	0.0000	0.00%	0	0.0000	0.00%	0	0.0000	0.00%
	Brassy Minnow (*Hybognathus hankinsoni*)	13	0.0052	9.42%	137	0.0768	39.48%	20	0.0169	2.98%
	Brook Stickleback (*Culaea inconstans*)	**65**	**0.0259**	**47.10%**	**92**	**0.0516**	**26.51%**	**628**	**0.5313**	**93.59%**
	Brown Bullhead (*Ameiurus nebulosus*)	2	0.0008	1.45%	2	0.0011	0.58%	1	0.0008	0.15%
	Central Mudminnow (*Umbra limi*)	**30**	**0.0120**	**21.74%**	**77**	**0.0431**	**22.19%**	**13**	**0.0110**	**1.94%**
	Common Shiner (*Luxilus cornutus*)	8	0.0032	5.80%	0	0.0000	0.00%	0	0.0000	0.00%
	Creek Chub (*Semotilus atromaculatus*)	1	0.0004	0.72%	15	0.0084	4.32%	4	0.0034	0.60%
	Fathead Minnow (*Pimephales promelas*)	3	0.0012	2.17%	7	0.0039	2.02%	0	0.0000	0.00%
	Finescale Dace (*Chrosomus neogaeus*)	3	0.0012	2.17%	1	0.0006	0.29%	0	0.0000	0.00%
	Golden Shiner (*Notemigonus crysoleucas*)	0	0.0000	0.00%	0	0.0000	0.00%	0	0.0000	0.00%
	Johnny Darter (*Etheostoma nigrum*)	3	0.0012	2.17%	0	0.0000	0.00%	0	0.0000	0.00%
	Northern Pike (*Esox lucius*)	0	0.0000	0.00%	0	0.0000	0.00%	2	0.0017	0.30%
	Northern Redbelly Dace (*Chrosomus eos*)	6	0.0024	4.35%	6	0.0034	1.73%	3	0.0025	0.45%
	Pearl Dace (*Margariscus margarita*)	0	0.0000	0.00%	7	0.0039	2.02%	0	0.0000	0.00%
	Rock Bass (*Ambloplites rupestris*)	1	0.0004	0.72%	0	0.0000	0.00%	0	0.0000	0.00%
	Undifferentiated Cyprinid YOY	0	0.0000	0.00%	0	0.0000	0.00%	0	0.0000	0.00%
	White Sucker (*Catostomus commersonii*)	3	0.0012	2.17%	3	0.0017	0.86%	0	0.0000	0.00%
	TOTAL	**138**	**0.0550**	**100%**	**347**	**0.1945**	**100.00%**	**671**	**0.5677**	**100.00%**
		No. captured	**CPUE (fish/min)**	**% Total**	**No. captured**	**CPUE (fish/min)**	**% Total**	**No. captured**	**CPUE (fish/min)**	**% Total**
Electrofishing	**Brook Stickleback (*Culaea inconstans*)**	**0**	**0.0000**	**0.00%**	**--**	**--**	**--**	**3**	**0.9**	**42.86%**
	Central Mudminnow (*Umbra limi*)	**18**	**0.4152**	**56.25%**	**--**	**--**	**--**	**2**	**0.6**	**28.57%**
	Common Shiner (*Luxilus cornutus*)	5	0.1153	15.63%	--	--	--	1	0.3	14.29%
	Creek Chub (*Semotilus atromaculatus*)	3	0.0692	9.38%	--	--	--	0	0	0.00%
	Northern Pike (*Esox lucius*)	0	0.0000	0.00%	--	--	--	1	0.3	14.29%
	White Sucker (*Catostomus commersonii*)	6	0.1384	18.75%	--	--	--	**0**	**0**	0.00%
	TOTAL	**32**	**0.7382**	**100.00%**	**--**	**--**	**--**	**7**	**2.1**	**100.00%**
		No. captured	**CPUE (fish/m2)**	**% Total**	**No. captured**	**CPUE (fish/m2)**	**% Total**	**No. captured**	**CPUE (fish/m2)**	**% Total**
Seine	Brassy Minnow (*Hybognathus hankinsoni*)	20	5.3333	7.14%	--	--	--	--	--	--
	Central Mudminnow (*Umbra limi*)	**2**	**0.5333**	**0.71%**	**--**	**--**	**--**	**--**	**--**	**--**
	Common Shiner (*Luxilus cornutus*)	182	48.5333	65.00%	--	--	--	--	--	--
	Creek Chub (*Semotilus atromaculatus*)	56	14.9333	20.00%	--	--	--	--	--	--
	Fathead Minnow (*Pimephales promelas*)	16	4.2667	5.71%	--	--	--	--	--	--
	Pearl Dace (*Margariscus margarita*)	3	0.8000	1.07%	--	--	--	--	--	--
	White Sucker (*Catostomus commersonii*)	1	0.2667	0.36%	--	--	--	--	--	--
	TOTAL	**280**	**74.6667**	**100.00%**	**--**	**--**	**--**	**--**	**--**	**--**

Notes:
Sentinel species highlighted in **bold green** font.

6.3.2 General Indicators of Fish Health

Nematodes, liver cysts, and parasites ordered by rank of occurrence were identified in a low proportion of fish in exposure and reference areas. Seven (13%) and four (9%) of Brook Stickleback and no Central Mudminnow had nematodes in PINR-EXP and PINR-EXP2, respectively, in exposure areas. In reference areas, five Brook Stickleback (8%) and one Central Mudminnow (2%) at LVR; one (2%) Brook Stickleback and no Central Mudminnow at LVR2; no Brook Stickleback and one Central Mudminnow (2%) at PINR-EXP2; and one of two Brook Stickleback and no Central Mudminnow at STUC had nematodes.

In exposure areas, three Brook Stickleback (6%) and no Central Mudminnow had liver cysts in PINR-EXP whereas no liver cysts were found in the downstream PINR-EXP2 in either species. In reference areas, one Brook Stickleback (2%) had a liver cyst in LVR and one Central Mudminnow (2%) had a liver cyst in PINR-REF2 upstream of PINR-EXP2.

In a single reference area, LVR, seven Brook Stickleback were identified as having spotted parasites on the exterior of their body.

Overall, variability in abnormalities suggests no distinct pattern related to mine outfall. A detailed list of the health abnormalities are provided in the fish meristic data in **Appendix D**.

6.3.3 QA/QC

6.3.3.1 Fish Aging

Otoliths were aged by one technician and QA/QC as performed on 20% of the samples by an independent technician. Aging was 99% consistent (105/106) between technicians and no issues were identified.

6.3.3.2 Fecundity

The results of the QA/QC analysis for fecundity are detailed in **Appendix D**. Total fecundity was recounted for twelve fish, six of each species. The overall range in percent difference between fecundity count and re-counts ranged from 0% to 23%, with a mean difference of 4.1%. This exceeds the precision of 1% according to technical guidance (EC, 2012) but is very common for small-bodied fish with difficult to discern eggs.

6.3.4 Statistical Analyses

Summaries of the statistical comparisons of EEM endpoint data are provided in **Table 6-9** to **Table 6-15**. Generally, the statistical results are well supported through large sample sizes that provided robust parametric analyses and supported by power analyses (**Appendix D**). There was insufficient sample size for Brook Stickleback at STUC-REF for both male (0 fish caught) and female (2 fish caught) to undertake statistical analysis using this species. There was also insufficient sample size for female Central Mudminnow statistical analysis at sites related to the EDL1 discharge.

6.3.4.1 Immature Fish

Immature fish were identified in several ways. First, using lab bench discrimination of gonads – if gonads were undifferentiated then the fish were considered immature. Second, using plots of gonad weight as a function of body weight including a 1% slope representing the GSI = 1 – fish below this line were visually evaluated against the variation in other datapoints.

For Brook Stickleback, no fish were identified as immature while taking fish measurements. Two female fish were below the GSI =1 line but did not stand out as a distinct group per the technical guidance and were not excluded (EC, 2012). For male Brook Stickleback, all but one fish were below the GSI = 1 line. There was no grouping evident in the plot of gonad weight against body weight. Brook Stickleback are relatively short-lived (2-3 years), generally range from 3.8-6.8 cm total length as adults, and reach sexual maturity at Age 1 (Eakins 2023). All Brook Stickleback collected were > 4 cm length. One Age 0 fish with GSI <1 was excluded from statistical analysis but not summary statistics.

For Central Mudminnow, three fish were identified as immature while taking fish measurements. Four females were identified as "could be spent" and three were identified as "spent". The immature fish were removed from statistical analysis except for summary statistics where applicable but the "could be spent" and "spent" individuals were left in all statistical analyses except for gonad at body weight, mean egg weight, and total fecundity.

6.3.4.2 Summary statistics

Summary statistics of fish measurements for Brook Stickleback and Central Mudminnow are provided in **Table 6-5** through **Table 6-8**.

6.3.4.3 Survival

For sites associated with the EDL2 discharge, there were significantly different fish ages between PINR-EXP and LVR-REF for female and male Brook Stickleback as well as female and male Central Mudminnow (Kruskal-Wallis $p < 0.10$). However, the findings were equivocal likely because the species is short-lived.

For Brook Stickleback, median age was 1 at both PINR-EXP and LRV-REF for females and males which would indicate a 0% magnitude of difference (MOD) despite finding a significant difference (note STUC-REF could not be compared due to low sample size). Considering Section 3.4.1.1 of the TGD (EC, 2012), length can be considered a reasonable surrogate for Age in short-lived species. Using this approach, there were again significant differences between PINR-EXP and LVR-REF for both female and male for length (ANOVA $p < 0.1$) but the MODs were -6% at PINR-EXP relative to LVR-REF for female and -9% for male – both below the CES of ±25%.

For Central Mudminnow, ages at PINR-EXP, LVR-REF, and STUC-REF could be compared. For females and males, there was a significant Area effect (Kruskal-Wallis $p < 0.1$) indicating that at least one site was statistically different. For females, only reference sites were different when using individual Kruskal-Wallis tests and age data; however, there was a significant difference

between PINR-EXP and LVR-REF using length data with a magnitude of difference of +15.5% (below the CES of ±25%). In contrast, there was a significant difference between PINR-EXP and STUC-REF using age data with a +100% MOD based on median ages (from median Age 2 at PINR-EXP to median Age 1 at STUC-REF) but there was no statistical difference using length data.

For sites associated with the EDL1 discharge, there were no significant differences identified for male for female Brook Stickleback and no significant difference identified for male Central Mudminnow. Comparisons were not possible for female Central Mudminnow due to low sample size at LVR2-REF (2 individuals), PINR-REF2 (12 individuals), and PINR-EXP2 (2 individuals).

6.3.4.4 Energy Storage

For Brook Stickleback at sites associated with the EDL2 discharge statistically significant differences in body weight at body length (condition) were determined for female and male fish (ANCOVA $p < 0.1$) from PINR-EXP and LVR-REF. Only female Brook Stickleback exceeded the CES of ±10% (+10.4%). No statistical differences were determined for liver weight at body weight for females. However, a statistical difference was determined for males but the MOD (-12% relative to LVR-REF) did not exceed the CES of ±25%.

For Central Mudminnow at sites associated with the EDL2 discharge, all three sites could be compared to determine if at least one area was different. For both females and males, Area effects were determined for body weight at body length indicating at least one site was statistically different ($p < 0.1$). Based on Tukey tests, a statistical difference was found comparing females at PINR-EXP with LVR-REF but the MOD (-7.8%) did not exceed the CES of ±10%. Similarly, a statistical difference was found comparing males at PINR-EXP with STUC-REF but the MOD (+7.4%) did not exceed the CES of ±10%. With respect to liver weight at body weight, a significant Area interaction was found for females such that smaller fish at PINR-EXP had a MOD of +52% compared to STUC-REF exceeding the CES of ±25% whereas the MOD was only 13% compared to LVR-REF. For males, a significant difference was found when comparing PINR-EXP to STUC-REF but its MOD (-18.8%) did not exceed the ±25%).

For Brook Stickleback at sites associated with EDL1 (LVR2-REF, PINR-REF2, and PINR-EXP2), all three sites could be compared to determine if at least one area was different. An Area effect was determined for body weight at length for females but not males. Based on Tukey tests, a statistical difference was found comparing females at PINR-EXP2 with PINR-REF2 but its MOD (7.7%) did not exceed ±10%. For liver weight at body weight, Area effects were determined for both females and males. For females, comparing PINR-EXP2 to LVR2-REF, the MOD was 40.5% for small fish; comparing PINR-EXP2 to PINR-REF2, the MOD was 50.9% for small fish and 26.9% for large fish – all three exceeded their respective ±25% CES. For males, only LVR2-REF and PINR-REF2 were statistically different.

For male Central Mudminnow at sites associated with EDL1, there was no statistical differences observed between sites for body weight at length. For liver weight at body weight, there was not enough statistical power to interpret a result.

6.3.4.5 Energy Use

For female Brook Stickleback at sites associated with the EDL2 discharge (LVR-REF, STUC-REF, and PINR-EXP), a comparison of gonad weight at body weight determined a significant interaction where the MOD was +183% for larger fish at PINR-EXP compared to LVR-REF. There was no statistical difference in male gonads at body weight. For body weight at age analysis, only Age-1 fish could be compared using ANOVA due to small sample sizes in other age classes across areas. For females, there was no statistical difference in body weight at Age-1, however, there was a statistical difference for males but the MOD (-12%) did not exceed the CES of ±25%.

For Central Mudminnow at sites associated with the EDL2 discharge, all three sites could be compared to determine if at least one area was different. For female gonad weight at body weight, a significant Area interaction was found such that larger fish at PINR-EXP had a MOD of +36% compared to STUC-REF that exceeded the ±25%. CES whereas the MOD was -5.5% for smaller fish. The MOD did not exceed the ±25% CES when comparing to LVR-REF. For males, there was a significant Area effect using ANCOVA and individual statistical differences using Tukey tests comparing PINR-EXP to LVR-REF and STUC-REF; the MOD compared to LVR-REF was -22% and the MOD compared to STUC-REF was -18%, both below the CES of ±25%. For body weight at age analysis, Age-1 and Age-2 fish were compared separately using ANOVA as there were few fish and limited site covariate overlap if including older fish. For females at Age-1, there was not enough statistical power to determine a result. For females at Age-2, a significant Area effect was found, and Tukey tests revealed significant differences between PINR-EXP and LVR-REF with a MOD of 80.1%, over the ±25% CES. For males, there were no statistical difference at Age-1 or Age-2.

For Brook Stickleback at sites associated with the EDL1 discharge, all three sites could be compared to determine if at least one area was different. For female gonad weight at body weight, Area and individual statistical differences were determined using ANCOVA and Tukey tests; the MODs were above the ±25% CES comparing PINR-EXP2 to LVR2-REF (MOD = 32%) but below when comparing to PINR-REF2 (MOD = 23%). There was no statistical difference for male gonad weight at body weight. For female body weight at Age-1, based on Tukey tests there was a statistical difference comparing PINR-EXP2 to LVR2-REF with an MOD (12.6%) not exceeding the ±25% CES. There was no statistical difference for male body weight at Age-1.

For male Central Mudminnow at sites associated with EDL1, there not enough statistical power to determine the gonad weight at body weight endpoint. For body weight at Age-1, there was a statistical difference between PINR-EXP2 and PINR-REF2 with an MOD of -52% exceeding the ±25% CES.

Table 6-5 Summary statistics for Brook Stickleback at sites associated with EDL2 discharge.

Area	Statistic	Female										Male							
		Age (years)	Body Weight (g)	Total Length (cm)	Condition	Liver Weight (g)	LSI	Gonad Weight	GSI	Mean Egg Weight (g)	Total Fecundity (#)	Age (years)	Body Weight (g)	Total Length (cm)	Condition	Liver Weight (g)	LSI	Gonad Weight (g)	GSI
LVR-REF	N	35	35	35	35	35	35	35	35	35	35	25	25	25	25	25	25	25	25
	Mean	1.3	1.354	5.431	0.831	0.082	5.973	0.063	4.795	0.0000530	1226.3	1.3	1.821	5.864	0.889	0.073	3.968	0.009	0.475
	SD	0.5	0.402	0.635	0.088	0.035	1.644	0.035	2.816	0.0000281	335.6	0.5	0.413	0.397	0.074	0.023	0.777	0.003	0.098
	SE	0.1	0.068	0.107	0.015	0.006	0.278	0.006	0.476	0.0000048	56.7	0.1	0.083	0.079	0.015	0.005	0.155	0.001	0.020
	Min	1.0	0.684	4.300	0.675	0.029	3.229	0.007	0.432	0.0000050	618.0	1.0	1.092	5.100	0.777	0.031	2.775	0.004	0.308
	Median	1.0	1.266	5.300	0.815	0.081	5.888	0.054	4.240	0.0000500	1248.0	1.0	1.815	5.900	0.869	0.069	3.904	0.009	0.481
	Max	3.0	2.199	6.800	1.059	0.180	9.790	0.189	17.292	0.0001600	2164.0	2.0	2.459	6.500	1.032	0.126	5.371	0.014	0.673
STUC-REF	N	2	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0	0	0
	Mean	1.0	0.711	4.400	0.844	0.045	6.181	0.061	8.109	0.0001550	554.0	--	--	--	--	--	--	--	--
	SD	0.0	0.122	0.141	0.224	0.020	1.727	0.043	4.680	0.0001626	301.2	--	--	--	--	--	--	--	--
	SE	0.0	0.086	0.100	0.158	0.014	1.221	0.031	3.309	0.0001150	213.0	--	--	--	--	--	--	--	--
	Min	1.0	0.625	4.300	0.686	0.031	4.960	0.030	4.800	0.0000400	341.0	--	--	--	--	--	--	--	--
	Median	1.0	0.711	4.400	0.844	0.045	6.181	0.061	8.109	0.0001550	554.0	--	--	--	--	--	--	--	--
	Max	1.0	0.797	4.500	1.002	0.059	7.403	0.091	11.418	0.0002700	767.0	--	--	--	--	--	--	--	--
PINR-EXP	N	28	28	28	28	28	28	28	28	28	28	25	25	25	25	25	25	25	25
	Mean	1.1	1.291	5.114	0.950	0.084	6.419	0.136	9.707	0.0001093	1207.8	1.1	1.462	5.312	0.968	0.050	3.392	0.007	0.488
	SD	0.3	0.387	0.524	0.148	0.038	1.733	0.120	6.233	0.0000818	305.8	0.3	0.345	0.426	0.146	0.019	0.923	0.003	0.156
	SE	0.0	0.073	0.099	0.028	0.007	0.328	0.023	1.178	0.0000155	57.8	0.1	0.069	0.085	0.029	0.004	0.185	0.001	0.031
	Min	1.0	0.728	4.300	0.663	0.025	3.205	0.025	3.281	0.0000200	671.0	1.0	0.870	4.500	0.787	0.019	1.850	0.003	0.277
	Median	1.0	1.259	5.100	0.972	0.076	6.490	0.082	7.827	0.0000800	1194.5	1.0	1.444	5.300	0.954	0.047	3.222	0.007	0.467
	Max	2.0	2.327	6.200	1.248	0.190	9.215	0.541	23.249	0.0003700	1727.0	2.0	2.321	6.300	1.500	0.092	4.906	0.014	1.028

Table 6-6 Summary statistics for Central Mudminnow at sites associated with EDL2 discharge.

Area	Statistic	Female										Male							
		Age (years)	Body Weight (g)	Total Length (cm)	Condition	Liver Weight (g)	LSI	Gonad Weight	GSI	Mean Egg Weight (g)	Total Fecundity (#)	Age (years)	Body Weight (g)	Total Length (cm)	Condition	Liver Weight (g)	LSI	Gonad Weight (g)	GSI
LVR-REF	N	20	20	20	20	20	20	20	20	20	20	35	35	35	35	35	35	35	35
	Mean	2.3	8.748	9.025	1.113	0.261	3.102	1.380	15.267	0.0016640	829.1	2.0	4.990	7.649	1.034	0.134	2.736	0.122	2.319
	SD	0.9	3.962	1.305	0.118	0.098	0.468	0.711	3.333	0.0004134	422.7	0.8	2.285	1.250	0.091	0.145	3.112	0.077	0.876
	SE	0.2	0.886	0.292	0.026	0.022	0.105	0.159	0.745	0.0000924	94.5	0.1	0.386	0.211	0.015	0.025	0.526	0.013	0.148
	Min	1.0	3.303	6.900	0.951	0.118	2.194	0.180	5.450	0.0005400	292.0	1.0	1.143	4.900	0.866	0.019	0.821	0.012	0.840
	Median	2.5	7.485	8.800	1.080	0.243	3.150	1.274	15.369	0.0016700	760.5	2.0	5.001	7.900	1.031	0.104	2.279	0.128	2.321
	Max	3.0	15.996	11.100	1.380	0.411	4.162	2.833	19.610	0.0023900	1595.0	4.0	10.313	10.400	1.367	0.904	20.383	0.323	3.730
STUC-REF	N	16	16	16	16	16	16	16	16	14	14	37	37	37	37	37	37	36	36
	Mean	1.7	10.136	9.506	1.093	0.296	2.833	1.785	15.901	0.0018743	1098.1	1.4	5.947	8.095	0.991	0.108	1.858	0.185	2.652
	SD	0.5	5.375	1.367	0.088	0.193	0.481	1.657	7.020	0.0004028	878.3	0.6	3.382	1.681	0.062	0.077	0.748	0.316	3.078
	SE	0.1	1.344	0.342	0.022	0.048	0.120	0.414	1.755	0.0001076	234.7	0.1	0.556	0.276	0.010	0.013	0.123	0.053	0.513
	Min	1.0	4.241	7.300	0.934	0.092	1.876	0.143	1.503	0.0011900	303.0	1.0	1.249	5.000	0.815	0.022	1.065	0.009	0.352
	Median	2.0	9.299	9.550	1.099	0.273	2.914	1.241	16.523	0.0019000	798.5	1.0	5.434	8.300	0.994	0.092	1.740	0.111	1.605
	Max	2.0	24.904	12.800	1.218	0.926	3.718	5.784	27.513	0.0024500	2960.0	3.0	14.292	11.100	1.140	0.375	5.638	1.910	16.083
PINR-EXP	N	13	13	13	13	13	13	13	13	4	4	35	35	35	35	35	35	35	35
	Mean	1.8	12.228	10.423	1.052	0.318	2.796	1.158	11.908	0.0019525	1224.3	1.9	6.411	7.774	1.070	0.116	1.871	0.140	1.720
	SD	0.6	3.593	1.063	0.102	0.113	1.194	1.415	17.871	0.0002446	501.0	0.5	4.862	2.303	0.062	0.090	0.385	0.163	1.132
	SE	0.2	0.996	0.295	0.028	0.031	0.331	0.393	4.956	0.0001223	250.5	0.1	0.822	0.389	0.010	0.015	0.065	0.028	0.191
	Min	1.0	6.048	8.400	0.844	0.106	0.655	0.139	0.995	0.0017000	561.0	1.0	0.927	4.600	0.952	0.016	1.032	0.006	0.546
	Median	2.0	12.586	10.600	1.023	0.291	2.608	0.220	1.624	0.0019550	1319.0	2.0	6.667	8.500	1.063	0.100	1.857	0.067	1.115
	Max	3.0	18.096	12.100	1.249	0.542	5.589	3.827	63.277	0.0022000	1698.0	4.0	16.282	11.100	1.223	0.312	3.132	0.619	3.962

Table 6-7 Summary statistics for Brook Stickleback at sites associated with EDL1 discharge.

Site	Statistic	Female										Male							
		Age (years)	Body Weight (g)	Total Length (cm)	Condition	Liver Weight (g)	LSI	Gonad Weight	GSI	Mean Egg Weight (g)	Total Fecundity (#)	Age (years)	Body Weight (g)	Total Length (cm)	Condition	Liver Weight (g)	LSI	Gonad Weight (g)	GSI
LVR2-REF	N	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	Mean	1.1	1.019	4.972	0.824	0.058	5.581	0.043	4.148	0.0000548	777.2	1.1	1.131	5.096	0.842	0.046	4.040	0.006	0.502
	SD	0.3	0.190	0.270	0.071	0.022	1.037	0.011	0.495	0.0000082	186.4	0.4	0.272	0.438	0.082	0.016	0.770	0.002	0.111
	SE	0.1	0.038	0.054	0.014	0.004	0.207	0.002	0.099	0.0000016	37.3	0.1	0.054	0.088	0.016	0.003	0.154	0.000	0.022
	Min	1.0	0.769	4.600	0.682	0.036	4.152	0.029	3.159	0.0000400	584.0	0.0	0.511	4.000	0.660	0.017	2.740	0.002	0.334
	Median	1.0	0.964	4.900	0.819	0.054	5.412	0.039	4.161	0.0000500	724.0	1.0	1.100	5.100	0.850	0.046	3.843	0.005	0.506
	Max	2.0	1.675	5.600	0.954	0.134	8.000	0.081	5.155	0.0000700	1328.0	2.0	1.590	5.900	0.992	0.076	6.079	0.010	0.745
PINR-REF2	N	26	26	26	26	26	26	26	26	25	25	25	25	25	25	25	25	25	25
	Mean	1.0	0.966	4.908	0.811	0.045	4.694	0.043	4.503	0.0000770	927.4	1.0	1.202	5.168	0.857	0.045	3.815	0.006	0.506
	SD	0.0	0.166	0.299	0.051	0.014	1.100	0.012	1.398	0.0000979	179.4	0.0	0.296	0.421	0.067	0.016	1.320	0.002	0.137
	SE	0.0	0.033	0.059	0.010	0.003	0.216	0.002	0.274	0.0000196	35.9	0.0	0.059	0.084	0.013	0.003	0.264	0.000	0.027
	Min	1.0	0.619	4.300	0.728	0.028	3.258	0.006	0.589	0.0000400	632.0	1.0	0.705	4.400	0.757	0.025	2.554	0.001	0.107
	Median	1.0	0.940	4.900	0.802	0.042	4.537	0.046	4.502	0.0000500	936.0	1.0	1.134	5.100	0.853	0.041	3.505	0.006	0.503
	Max	1.0	1.285	5.500	0.966	0.076	7.766	0.071	9.416	0.0004000	1298.0	1.0	1.807	5.900	1.086	0.090	9.385	0.010	0.864
PINR-EXP2	N	19	19	19	19	19	19	19	19	19	19	25	25	25	25	25	25	25	25
	Mean	1.2	1.033	4.874	0.886	0.061	5.677	0.064	6.085	0.0000900	790.9	1.0	1.201	5.100	0.892	0.046	3.802	0.006	0.460
	SD	0.5	0.249	0.429	0.142	0.029	2.330	0.041	3.846	0.0000886	237.9	0.2	0.313	0.486	0.084	0.017	0.844	0.002	0.177
	SE	0.1	0.057	0.098	0.033	0.007	0.535	0.009	0.882	0.0000203	54.6	0.0	0.063	0.097	0.017	0.003	0.169	0.000	0.035
	Min	1.0	0.617	4.000	0.669	0.009	1.438	0.021	3.404	0.0000300	309.0	1.0	0.712	4.100	0.738	0.016	2.247	0.001	0.115
	Median	1.0	1.085	4.800	0.898	0.059	5.689	0.053	4.634	0.0000600	800.0	1.0	1.198	5.100	0.894	0.045	3.644	0.005	0.482
	Max	3.0	1.386	5.500	1.214	0.117	10.783	0.192	19.896	0.0004400	1304.0	2.0	1.838	5.900	1.093	0.078	5.423	0.012	0.737

Table 6-8 Summary statistics for Central Mudminnow at sites associated with EDL1 discharge

Site	Statistic	Female										Male							
		Age (years)	Body Weight (g)	Total Length (cm)	Condition	Liver Weight (g)	LSI	Gonad Weight	GSI	Mean Egg Weight (g)	Total Fecundity (#)	Age (years)	Body Weight (g)	Total Length (cm)	Condition	Liver Weight (g)	LSI	Gonad Weight (g)	GSI
LVR2-REF	N	2	2	2	2	2	2	2	2	2	2	7	7	7	7	7	7	7	7
	Mean	2.0	14.412	11.150	1.039	0.429	2.988	2.604	17.949	0.0014300	1900.0	1.7	5.838	7.871	0.954	0.130	2.186	0.147	2.412
	SD	0.0	0.814	0.071	0.039	0.031	0.385	0.758	4.246	0.0006505	332.3	1.1	5.395	2.056	0.102	0.130	0.320	0.134	1.018
	SE	0.0	0.576	0.050	0.028	0.022	0.272	0.536	3.002	0.0004600	235.0	0.4	2.039	0.777	0.038	0.049	0.121	0.050	0.385
	Min	2.0	13.836	11.100	1.012	0.407	2.716	2.068	14.947	0.0009700	1665.0	1.0	2.003	6.100	0.836	0.038	1.829	0.030	1.325
	Median	2.0	14.412	11.150	1.039	0.429	2.988	2.604	17.949	0.0014300	1900.0	1.0	2.792	6.700	0.912	0.072	2.097	0.061	2.375
	Max	2.0	14.987	11.200	1.067	0.451	3.260	3.140	20.951	0.0018900	2135.0	4.0	16.709	11.400	1.128	0.403	2.579	0.339	4.039
PINR-REF2	N	12	12	12	12	12	12	12	12	12	12	28	28	28	28	28	28	28	28
	Mean	1.6	9.586	9.483	1.019	0.277	2.845	1.634	15.864	0.0015483	1016.8	1.8	6.200	8.232	1.019	0.127	2.019	0.165	2.460
	SD	0.7	5.363	1.594	0.082	0.181	0.604	1.071	3.931	0.0003971	566.3	0.7	2.860	1.394	0.074	0.070	0.487	0.103	0.715
	SE	0.2	1.548	0.460	0.024	0.052	0.174	0.309	1.135	0.0001146	163.5	0.1	0.540	0.263	0.014	0.013	0.092	0.020	0.135
	Min	1.0	3.411	7.100	0.895	0.097	1.898	0.214	6.274	0.0008900	241.0	1.0	1.328	5.200	0.798	0.022	1.201	0.013	0.831
	Median	1.5	8.670	9.300	1.027	0.226	2.898	1.612	16.618	0.0015150	1107.0	2.0	6.371	8.500	1.021	0.118	2.013	0.153	2.468
	Max	3.0	21.536	12.300	1.157	0.694	3.850	3.810	21.191	0.0024700	1912.0	3.0	12.693	10.600	1.129	0.347	3.375	0.395	4.229
PINR-EXP2	N	3	3	3	3	3	3	3	3	3	3	15	15	15	15	15	15	15	15
	Mean	2.0	10.229	9.467	1.109	0.281	2.860	1.967	18.783	0.0016833	1155.7	1.5	3.527	6.807	0.994	0.077	2.164	0.085	2.129
	SD	0.0	5.986	1.779	0.107	0.151	0.489	1.201	2.590	0.0003968	704.5	0.6	2.251	1.541	0.084	0.050	0.486	0.079	1.144
	SE	0.0	3.456	1.027	0.062	0.087	0.282	0.693	1.496	0.0002291	406.7	0.2	0.581	0.398	0.022	0.013	0.126	0.020	0.295
	Min	2.0	4.901	7.900	0.994	0.167	2.467	0.787	16.058	0.0013100	600.0	1.0	1.154	4.700	0.851	0.019	1.479	0.010	0.817
	Median	2.0	9.079	9.100	1.128	0.224	2.706	1.926	19.077	0.0016400	919.0	1.0	2.796	6.900	0.974	0.074	2.125	0.062	2.074
	Max	2.0	16.706	11.400	1.205	0.452	3.407	3.187	21.214	0.0021000	1948.0	3.0	8.520	9.200	1.112	0.161	2.929	0.302	4.112

Table 6-9: Statistical comparisons of EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF and STUC-REF) for female Brook Stickleback.

Effect Indicator	Comparisons			Sample Size		Model	Mean Squared Error	Slopes Equal? (p-value < 0.1)		Areas Difference? (p-value < 0.1)		Covariate value (model scale)	Median, Mean, Adjusted Mean, or Predicted Value		Magnitude of Difference (%)	Minimum Detectable Effect Size (%)	
	Model #	Parameter	Covariate	LVR	PINR-EXP			p-value	Yes/No	p-value	Yes/No		LVR-REF	PINR-EXP	PINR-EXP - LVR	Increase	Decrease
Survival	1	Rank of Age (years)	--	35	28	KW	--	--	--	0.089	Yes	--	1.000	1.000	0	--	--
Energy Storage	2	Body Weight (g)	Total Length (cm)	35	28	ANCOVA	0.0246	0.726	Yes	0.002	Yes	5.290	1.267	1.399	10.4	9.4	-9.4
	3	Log_{10} Liver Weight (g)	Log_{10} Body Weight (g)	35	28	ANCOVA	0.0137	0.991	Yes	0.227	No	0.104	0.073	0.079	8.7	22.7	-18.5
	4	Log_{10} Mean Egg Weight (g)	Log_{10} Body Weight (g) min	35	28	ANCOVA	0.0641	0.017	No	--	--	-0.138	0.0000470	0.0000445	-5.3	55.5	-35.7
			Log_{10} Body Weight (g) max									0.342	0.0000468	0.0001745	272.9		
	5	Log_{10} Mean Egg Weight (g)	Log_{10} Body Weight (g) min	34	24	ANCOVA	0.0369	0.034	No	--	--	-0.138	0.0000455	0.0000469	3.2	41.9	-29.5
			Log_{10} Body Weight (g) max									0.282	0.0000536	0.0001389	159.3		
Energy Use	6	Body Weight (g)	--	35	28	ANOVA	0.1563	--	--	0.527	No	--	1.354	1.291	-4.7	22.1	-22.1
	7	Total Length (cm)	--	35	28	ANOVA	0.3464	--	--	0.038	Yes	--	5.431	5.114	-5.8	8.2	-8.2
	8	Body Weight (g); Age-1	--	26	24	ANOVA	0.0109	--	--	0.196	No	--	1.147	1.254	9.4	22.9	-18.6
	9	Total Length (cm); Age-1	--	27	26	ANOVA	0.2961	--	--	0.478	No	--	5.222	5.115	-2.0	8.7	-8.7
	10	Log10 Gonad Weight (g)	Log_{10} Body Weight (g) min	35	28	ANCOVA	0.0566	0.017	No	--	--	-0.138	0.037	0.037	1.7	51.4	-34.0
			Log_{10} Body Weight (g) max									0.342	0.079	0.291	268.0		
	11	Log10 Gonad Weight (g)	Log_{10} Body Weight (g) min	34	25	ANCOVA	0.0342	0.008	No	--	--	-0.138	0.036	0.034	-4.7	39.6	-28.4
			Log_{10} Body Weight (g) max									0.282	0.082	0.232	183.1		
	12	Total Fecundity (#)	Body Weight (g)	35	28	ANCOVA	54803.6239	0.965	Yes	0.767	No	1.326	1210.192	1227.939	1.5	14.7	-14.7

Notes:
Indicates significant effect endpoint for final model per parameter
Indicates magnitude of difference > CES for final model per parameter
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions
Companion table with post-hoc power analysis available in Appendix D.
1. Non-parametric employed due to not meeting parametric assumptions
2. Parallel model run because R2 for parallel model is also > 0.8 and is less than 0.02 (i.e. 2 percentage points) less than R2 for interaction model.
6. Model violated ANCOVA assumptions. Non-parametric approach considered but this obscured an obvious interaction in the data. Removed Cook's outliers PINEXP-BSB-31, PINEXP-BSB-32, PINEXP-BSB-47, PINEXP-BSB-51, LVR-BSB-29 in attempt to better meet ANCOVA assumptions.
8 and 9. Could only examine for Age-1 fish. Statistical comparisons could not be performed due to limited sample size for Age-2 and Age-3 fish across sites.
10 and 11. Model 10 includes outliers and Model 11 (final model) does not. Model 11 violated ANCOVA assumptions. Non-parametric approach considered but this obscured an obvious interaction in the data. Removed Cook's outliers PINEXP-BSB-32, PINEXP-BSB-47, PINEXP-BSB-51, and LVR-BSB-29 in attempt to better meet ANCOVA assumptions.

Table 6-10: Statistical comparisons of EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF and STUC-REF) for male Brook Stickleback.

Effect Indicator	Comparisons			Sample Size		Model	Mean Squared Error	Slopes Equal? (p-value < 0.1)		Area Difference? (p-value < 0.1)		Covariate value (model scale)	Median, Mean, Adjusted Mean, or Predicted Value		Magnitude of Difference (%)		Minimum Detectable Effect Size (%)	
	Model #	Parameter	Covariate	LVR	PINR-EXP			*p*	Yes/No	*P*	Yes/No		LVR	PINR-EXP	PINR-EXP to LVR	PINR-EXP to STUC	Increase	Decrease
Survival	1	Rank of Age	--	25	25	KW	--	--	--	0.036	Yes	--	1.000	1.000	0	--	--	--
Energy Storage	2	Body Weight	Total Length	25	25	ANCOVA	0.0024	0.066	No	0.116	No	0.746	1.541	1.639	6.4	--	10.1	-9.2
	3	Body Weight	Total Length	25	22	ANCOVA	0.0012	0.091	No	0.045	Yes	0.750	1.571	1.662	5.8	--	7.3	-6.8
	4	Liver Weight	Body Weight	25	25	ANCOVA	0.0002	0.694	Yes	0.082	Yes	1.642	0.065	0.057	-11.9	--	18.3	-18.3
Energy Use	5	Body Weight	--	25	25	ANOVA	0.1450	--	--	0.002	Yes	--	1.821	1.462	-19.7	--	17.9	-17.9
	6	Total Length	--	25	25	ANOVA	0.1693	--	--	<0.001	Yes	--	5.864	5.312	-9.4	--	6.0	-6.0
	7	Body Weight; Age-1	--	17	23	ANOVA	0.0995	--	--	0.064	Yes	--	1.621	1.428	-11.9	--	18.8	-18.8
	8	Total Length; Age-1	--	17	23	ANOVA	0.1277	--	--	0.001	Yes	--	5.671	5.278	-6.9	--	6.1	-6.1
	9	Log10 Gonad Weight	Log_{10} Body Weight	25	25	ANCOVA	0.0122	0.376	Yes	0.868	No	0.201	0.007	0.007	1.3	--	24.4	-19.6

Notes:
Indicates significant effect endpoint for final model per parameter
Indicates magnitude of difference > CES for final model per parameter
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions
Companion table with post-hoc power analysis available in Appendix D.
1. Non-parametric employed due to not meeting parametric assumptions
2 and 3. Model 10 includes outliers and Model 11 (final model) does not.
3. Removed Cook's outliers PINEXP-BSB-3, PINEXP-BSB-16, PINEXP-BSB-43 to better meet ANCOVA assumptions. Parallel model run because R2 for parallel model is also > 0.8 and is less than 0.02 (i.e. 2 percentage points) less than R2 for interaction model.
7 and 8. Could only examine for Age-1 fish. Statistical comparisons could not be performed due to limited sample size for Age-2 and Age-3 fish across sites.

Table 6-11: Statistical comparisons of EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF and STUC-REF) for female Central Mudminnow.

Effect Indicator	Comparisons			Sample Size			Model	Mean Squared Error	Slopes Equal? (p < 0.1)		Area Difference? (p-value < 0.1)		Covariate Value	Median, Mean, Adjusted Mean, or Predicted Value			Magnitude of Difference (%)		Individual Comparisons (e.g., Tukey HSD p-value or KW)			Minimum Detectable Effect Size (%)[f]	
	Model #	Parameter	Covariate	LVR-REF	STUC-REF	PINR-EXP			p	Yes /No	p	Yes /No		LVR	STUC	PINR-EXP	PINR-EXP to LVR-REF	PINR-EXP to STUC-REF	PINR-EXP to LVR-REF	PINR-EXP to STUC-REF	LVR-REF to STUC-REF	Increase	Decrease
Survival	1	Rank of Age	--	20	16	13	KW	--	--	--	0.055	Yes	--	2.500	2.000	2.000	-20.0	0.0	0.115	0.447	0.0265	--	--
Energy Storage	2	Log_{10} Body Weight	Log_{10} Total Length	20	16	13	ANCOVA	0.0017	0.645	Yes	0.095	Yes	0.976	9.457	9.219	8.717	-7.8	-5.4	0.0788	0.2842	0.7043	10.7	-9.6
	3	Log_{10} Liver Weight	Log_{10} Body Weight	14	11	11	ANCOVA	0.0060	0.026	No	--	--	0.795 (min)	0.217	0.162	0.246	13.3	52.1	--	--	--	25.9	-20.6
													1.102 (max)	0.350	0.414	0.338	-3.7	-18.4					
	4	Mean Egg Weight	Body Weight	20	14	4	ANCOVA	0.0000	0.464	Yes	0.311	No	9.589	0.00168	0.00187	0.00192	14.7	3.1	--	--	--	29.7	-29.7
Energy Use	5	Log_{10} Body Weight	--	20	16	13	ANOVA	0.0358	--	--	0.051	Yes	--	7.900	9.102	11.681	47.9	28.3	0.0396	0.2842	0.5991	60.1	-37.6
	6	Total Length	--	20	16	13	ANOVA	1.6076	--	--	0.013	Yes	--	9.025	9.506	10.423	15.5	9.6	0.0092	0.1396	0.4992	15.2	-15.2
	7	Body Weight; Age-1	--	5	5	3	ANOVA	8.3482	--	--	0.139	No	--	5.311	8.365	9.553	79.9	14.2	--	--	--	142.5	-142.5
	8	Log_{10} Body Weight; Age-2	--	5	11	9	ANOVA	0.0347	--	--	0.069	Yes	--	6.802	9.783	12.249	80.1	25.2	0.0551	0.4831	0.2768	99.6	-49.9
	9	Total Length; Age-1	--	5	5	3	ANOVA	1.0311	--	--	0.083	Yes	--	7.840	8.960	9.633	22.9	7.5	0.0842	0.6468	0.2363	33.9	-33.9
	10	Total Length; Age-2	--	5	11	9	ANOVA	1.7345	--	--	0.033	Yes	--	8.560	9.755	10.633	24.2	9.0	0.0257	0.3155	0.2327	24.8	-24.8
	11	Gonad Weight	Body Weight	20	14	4	ANCOVA	0.0757	<0.001	No	--	--	6.979 (min)	1.075	1.122	1.015	-5.5	-9.5	--	--	--	32.0	-32.0
													14.011 (max)	2.288	3.089	3.102	35.6	0.4					
	12	Total Fecundity	Body Weight	9	7	4	ANCOVA	83970.6931	0.115	Yes	0.832	No	10.345	995.915	1062.733	1093.407	9.8	2.9	--	--	--	54.4	-54.4

Notes:
Indicates significant effect endpoint for final model per parameter.
Indicates magnitude of difference > CES for final model per parameter.
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions.
Companion table with post-hoc power analysis available in Appendix D.
1. Non-parametric employed due to not meeting parametric assumptions.
3. Used covariate overlap range to meet parametric ANCOVA assumptions.
7-10. Could only examine for Age-1 and Age-2 fish. Statistical comparisons could not be performed due to limited sample size for other aged fish across sites.

Table 6-12: Statistical comparisons of EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF and STUC-REF) for male Central Mudminnow.

Effect Indicator	Comparisons			Sample Size			Model	Mean Squared Error	Slopes Equal? (p < 0.1)		Area Difference? (p-value < 0.1)		Covariate Value	Median, Mean, Adjusted Mean, or Predicted Value			Magnitude of Difference (%)		Individual Comparisons (e.g., Tukey HSD p-value or KW)			Minimum Detectable Effect Size (%)[f]	
	Model #	Parameter	Covariate	LVR-REF	STUC-REF	PINR-EXP			p	Yes /No	p	Yes /No		LVR	STUC	PINR-EXP	PINR-EXP to LVR-REF	PINR-EXP to STUC-REF	PINR-EXP to LVR-REF	PINR-EXP to STUC-REF	LVR-REF to STUC-REF	Increase	Decrease
Survival	1	Rank of Age	--	35	37	35	KW	--	--	--	<0.001	Yes	--	2.000	1.000	2.000	0.0	100.0	0.818	<0.001	0.0020	--	--
Energy Storage	2	Rank of Body Weight	Rank of Total Length	35	37	35	Non-parametric ANCOVA	24.6191	0.614	Yes	0.002	Yes	54.000	55.066	51.632	55.438	0.7	7.4	0.948	0.004	0.012	6.4	-6.4
	3	Log10 Liver Weight	Log10 Body Weight	33	33	23	ANCOVA	0.0242	0.495	Yes	0.004	Yes	0.649	0.103	0.078	0.080	-22.2	2.9	0.032	0.953	0.006	32.4	-24.4
	4	Log10 Liver Weight	Log10 Body Weight	30	32	23	ANCOVA	0.0078	0.169	Yes	<0.001	Yes	0.656	0.100	0.076	0.081	-18.8	6.7	0.001	0.473	<0.0001	17.7	-15.1
Energy Use	5	Rank of Body Weight	--	35	37	35	KW	--	--	--	0.513	No	--	5.001	5.434	6.667	33.3	22.7	--	--	--	--	--
	6	Rank of Total Length	--	35	37	35	KW	--	--	--	0.327	No	--	7.900	8.300	8.500	7.6	2.4	--	--	--	--	--
	7	Rank of Body Weight; Age-1	--	10	24	5	KW	--	--	--	0.473	No	--	3.575	5.181	4.893	36.9	-5.6	--	--	--	--	--
	8	Rank of Body Weight; Age-2	--	17	10	29	KW	--	--	--	0.330	No	--	4.447	6.080	6.667	49.9	9.7	--	--	--	--	--
	9	Rank of Total Length; Age-1	--	10	24	5	KW	--	--	--	0.310	No	--	7.200	8.100	7.900	9.7	-2.5	--	--	--	--	--
	10	Rank of Total Length; Age-2	--	17	10	29	KW	--	--	--	0.289	No	--	7.600	8.400	8.500	11.8	1.2	--	--	--	--	--
	11	Rank of Gonad Weight	Rank of Body Weight	35	37	35	Non-parametric ANCOVA	403.9333	0.345	Yes	0.021	Yes	54.000	59.349	56.228	46.296	-22.0	-17.7	0.0219	0.0957	0.789	24.0	-24.0

Notes:
Indicates significant effect endpoint for final model per parameter.
Indicates magnitude of difference > CES for final model per parameter.
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions.
Companion table with post-hoc power analysis available in Appendix D.
1 and 6-11. Non-parametric employed due to not meeting parametric assumptions.
3 and 4. Model 3 includes outliers and Model 4 (final model) does not. Removed Cook's outliers STUC-CMM-15, LVR-CMM-50, LVR-CMM-51, and LVR-CMM-53 to better meet ANCOVA assumptions.
3. Used covariate overlap range to meet parametric ANCOVA assumptions.
7-10. Could only examine for Age-1 and Age-2 fish. Statistical comparisons could not be performed due to limited sample size for other aged fish across sites.

Table 6-13: Statistical comparisons of EDL1 discharge exposure (PINR-EXP2) to reference (LVR2-REF and PINR-REF2) for female Brook Stickleback.

Effect Indicator	Comparisons			Sample Size			Model	Mean Squared Error	Slopes Equal? (p < 0.1)		Area Difference? (p-value < 0.1)		Covariate Value	Median, Mean, Adjusted Mean, or Predicted Value			Magnitude of Difference (%)		Tukey HSD/ Individual Comparisons (e.g., Tukey HSD p-value or KW)			Minimum Detectable Effect Size (%)[f]	
	Model #	Parameter	Covariate	LVR2-REF	PINR-REF2	PINR-EXP2			p	Yes /No	p	Yes /No		LVR2-REF	PINR-REF2	PINR-EXP2	PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	LVR2-REF to PINR-REF2	Increase	Decrease
Survival	1	Rank of Age	--	25	26	19	KW	--	--	--	0.207	No	--	1.000	1.000	1.000	0.0	15.8	--	--	--	--	--
Energy Storage	2	Log10 Body Weight	Log10 Total Length	25	26	19	ANCOVA	0.002	0.703	Yes	0.058	Yes	0.691	0.976	0.958	1.031	5.6	7.7	0.1981	0.0498	0.7762	0.1981	0.0498
	3	Log10 Liver Weight	Log10 Body Weight	25	26	19	ANCOVA	0.015	0.0378	No	--	--	-0.114	0.035	0.034	0.029	-18.6	-16.8	--	--	--	--	--
													0.109	0.083	0.061	0.088	4.8	44.2					
	4	Log10 Liver Weight	Log10 Body Weight	25	26	15	ANCOVA	0.008	0.0606	No	--	--	-0.076	0.041	0.038	0.057	40.5	50.9	--	--	--	--	--
													0.109	0.083	0.061	0.077	-7.7	26.9					
	5	Mean Egg Weight (g)	Body Weight	25	26	19	ANCOVA	344.536	0.296	Yes	0.006	Yes	35.500	0.000054	0.000048	0.000069	33.0	67.0	--	--	--	--	--
	6	Log10 Mean Egg Weight	Log10 Body Weight	25	22	17	ANCOVA	0.010	0.405	Yes	<0.001	Yes	0.001	0.000054	0.000048	0.000069	27.8	44.3	0.00294	<0.001	0.16791	0.00294	<0.001
Energy Use	7	Log10 Body Weight	--	25	26	19	ANOVA	0.008	--	--	0.571	No	--	1.004	0.952	1.002	-0.3	5.2	--	--	--	--	--
	8	Log10 Body Weight	--	24	25	16	ANOVA	0.004	--	--	0.036	Yes	--	0.983	0.968	1.094	11.3	13.0	0.1981	0.0373	0.9339	0.1981	0.0373
	9	Log10 Total Length	--	25	26	19	ANOVA	0.001	--	--	0.543	No	--	4.965	4.899	4.855	-2.2	-0.9	--	--	--	--	--
	10	Log10 Total Length	--	25	26	16	ANOVA	0.001	--	--	0.718	No	--	4.965	4.899	4.914	-1.0	0.3	--	--	--	--	--
	11	Body Weight; Age-1	--	22	26	17	ANOVA	0.041	--	--	0.623	No	--	1.021	0.966	1.006	-1.4	4.2	--	--	--	--	--
	12	Body Weight; Age-1	--	21	26	14	ANOVA	0.027	--	--	0.079	Yes	--	0.990	0.966	1.088	10.0	12.6	0.1943	0.0692	0.8738	--	--
	13	Total Length; Age-1	--	22	26	17	ANOVA	0.112	--	--	0.374	No	--	4.982	4.908	4.829	-3.1	-1.6	--	--	--	--	--
	14	Log10 Gonad Weight	Log10 Body Weight	22	23	12	ANCOVA	0.027	0.871	Yes	0.030	Yes	-0.007	0.040	0.040	0.056	39.5	40.7	0.048	0.0362	0.9966	0.048	0.0362
	15	Log10 Gonad Weight	Log10 Body Weight	22	22	9	ANCOVA	0.006	0.612	Yes	0.002	Yes	-0.007	0.040	0.043	0.053	32.4	22.7	0.0012	0.02	0.36636	0.0012	0.02
	16	Total Fecundity (#)	Body Weight	25	26	19	ANCOVA	18134.349	0.797	Yes	<0.001	Yes	1.003	765.342	956.079	768.710	0.4	-19.6	0.9966	<0.0001	<0.0001	0.9966	<0.0001

Notes:

Indicates significant effect endpoint for final model per parameter.

Indicates magnitude of difference > CES for final model per parameter.

Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions

Companion table with post-hoc power analysis available in Appendix D

3 and 4. Model 3 is Model 4 including outliers. In Model 4, PINR-EXP2 - BSB-19, PINR-EXP2 - BSB-37, PINR-EXP2 - BSB-40, and PINR-EXP2 - BSB-41 Cook's outliers removed to better meen ANCOVA assumptions.

5 and 6. Model 5 is Model 6 including outliers. In Model 6, PINR-EXP2 - BSB-33, PINR-EXP2 - BSB-41, PINR-REF1-BSB-7, PINR-REF1-BSB-8, and PINR-REF1-BSB-45 Cook's outliers removed to better meet ANCOVA assumptions.

7 and 8. Model 7 is Model 8 including outliers. PINR-EXP2 - BSB-33, PINR-EXP2 - BSB-41, PINR-REF1-BSB-7, PINR-REF1-BSB-8, and PINR-REF1-BSB-45 Cook's outliers removed to better meet ANOVA assumptions.

9 and 10. Model 9 is Model 10 including outliers. PINR-EXP2 - BSB-10, PINR-EXP2 - BSB-33, and PINR-EXP2 - BSB-37 Cook's outliers removed to better meet ANOVA assumptions.

11 and 12. Age-1 only as limited sample size across all sites to examine using all ages or Ages 2 and 3 separately. Cook's outliers PINR-EXP2 - BSB-37, PINR-EXP2 - BSB-40, PINR-EXP2 - BSB-41, and LVR2-BSB-41 removed to better meet ANOVA assumptions.

14 and 15. Model 14 is Model 15 including outliers. PINR-EXP2 - BSB-19, PINR-EXP2 - BSB-33, PINR-EXP2 - BSB-36, and PINR-REF1-BSB-27 Cook's outliers removed to better meet ANCOVA assumptions.

Table 6-14: Statistical comparisons of EDL1 discharge exposure (PINR-EXP2) to reference (LVR2-REF and PINR-REF2) for male Brook Stickleback.

Effect Indicator	Comparisons			Sample Size			Model	Mean Squared Error	Slopes Equal? (p < 0.1)		Area Difference? (p-value < 0.1)		Covariate Value	Median, Mean, Adjusted Mean, or Predicted Value			Magnitude of Difference (%)		Individual Comparisons (e.g., Tukey HSD p-value or KW)			Minimum Detectable Effect Size (%)[f]	
	Model #	Parameter	Covariate	LVR2-REF	PINR-REF2	PINR-EXP2			p	Yes /No	p	Yes /No		LVR2-REF	PINR-REF2	PINR-EXP2	PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	LVR2-REF to PINR-REF2	Increase	Decrease
Survival	1	Rank of Age	NA	24	25	25	KW	--	--	--	0.147	No	--	1.000	1.000	1.000	0.0	0.0	--	--	--	--	--
Energy Storage	2	Log10 Body Weight	Log10 Total Length	24	25	25	ANCOVA	0.001	0.525	Yes	0.0999	Yes	0.709	1.127	1.149	1.188	5.5	3.4	0.0868	0.3556	0.7149	7.7	-7.2
	3	Log10 Body Weight	Log10 Total Length	23	25	24	ANCOVA	0.001	0.575	Yes	0.271	No	0.710	1.145	1.155	1.187	3.7	2.8	0.273	0.452	0.926	7.2	-6.7
	4	Log10 Liver Weight	Log10 Body Weight	24	25	25	ANCOVA	0.010	0.306	Yes	0.313	No	0.062	0.046	0.042	0.043	-7.7	1.2	--	--	--	21.4	-17.6
	5	Log10 Liver Weight	Log10 Body Weight	24	24	23	ANCOVA	0.006	0.541	Yes	0.0504	Yes	0.069	0.047	0.041	0.044	-7.4	5.7	0.996	0.452	<0.0001	17.6	-15.0
Energy Use	6	Body Weight	--	24	25	25	ANOVA	0.082	--	NA	0.819	No	--	1.157	1.202	1.201	3.9	-0.1	--	--	--	21.4	-21.4
	7	Total Length	--	24	25	25	ANOVA	0.187	--	NA	0.855	No	--	5.142	5.168	5.100	-0.8	-1.3	--	--	--	7.3	-7.3
	8	Body Weight; Age-1	--	21	25	24	ANOVA	0.078	--	NA	0.957	No	--	1.195	1.202	1.179	-1.3	-1.9	--	--	--	20.8	-20.8
	9	Total Length; Age-1	--	21	25	24	ANOVA	0.179	--	NA	0.526	No	--	5.210	5.168	5.071	-2.7	-1.9	--	--	--	7.2	-7.2
	10	Log10 Gonad Weight	Log10 Body Weight	24	25	25	ANCOVA	0.029	0.812	Yes	0.245	No	0.062	0.006	0.006	0.005	-16.3	-13.6	--	--	--	40.4	-28.8
	11	Log10 Gonad Weight	Log10 Body Weight	24	24	22	ANCOVA	0.011	0.6	Yes	0.738	No	0.067	0.006	0.006	0.006	-0.8	-5.0	--	--	--	24.0	-19.3

Notes:
Indicates significant effect endpoint for final model per parameter.
Indicates magnitude of difference > CES for final model per parameter.
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions.
Companion table with post-hoc power analysis available in Appendix D.
2 and 3. Model 2 is Model 3 including outliers. PINR-EXP2 -BSB-34 and LVR2-BSB-21 Cook's outliers removed to better meet ANCOVA assumptions.
4 and 5. Model 4 is Model 5 including outliers. PINR-EXP2-BSB-4, PINR-EXP2-BSB-22, and PINR-REF2-BSB-23 Cook's outliers removed to better meet ANCOVA assumptions.
8 and 9. Could only examine Age-1 fish. Statistical comparisons could not be performed including Age-2+ fish due to limited sample size across sites.
10 and 11. Model 10 is Model 11 including outliers. PINR-EXP-2 - BSB-17 PINR-EXP2 - BSB-20 PINR-EXP2 - BSB-22 PINR-REF2-BSB-39 Cook's outliers removed to better meet ANCOVA assumptions.

Table 6-15: Statistical comparisons of EDL1 discharge exposure (PINR-EXP2) to reference (LVR2-REF and PINR-REF2) for male Central Mudminnow.

Effect Indicator	Comparisons			Sample Size			Model	Mean Squared Error	Slopes Equal? (p < 0.1)		Area Difference? (p-value < 0.1)		Covariate Value	Median, Mean, Adjusted Mean, or Predicted Value			Magnitude of Difference (%)		Individual Comparisons (e.g., Tukey HSD p-value or KW)			Minimum Detectable Effect Size (%)[f]	
	Model #	Parameter	Covariate	LVR2-REF	PINR-REF2	PINR-EXP2			p	Yes /No	p	Yes /No		LVR2-REF	PINR-REF2	PINR-EXP2	PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	LVR2-REF to PINR-REF2	Increase	Decrease
Survival	1	Rank of Age	--	7	28	15	KW	--	--	--	0.305	No	--	1.000	2.000	1.000	0.0	-50.0	--	--	--	--	--
Energy Storage	2	Log10 Body Weight	Log10 Total Length	7	28	15	ANCOVA	0.001	0.00346	No	0.196	No	0.879	4.122	4.398	4.337	3.6	-1.4	--	--	--	9.3	-8.5
	3	Log10 Liver Weight	Log10 Body Weight	7	28	15	ANCOVA	0.010	0.963	Yes	0.326	No	0.637	0.094	0.084	0.093	-0.6	10.9	--	--	--	27.7	-21.7
Energy Use	4	Rank of Body Weight	--	7	28	15	KW	--	--	--	0.013	Yes	--	2.792	6.371	2.796	0.1	-56.1	0.418	0.002	0.364	--	--
	5	Total Length	--	7	28	15	ANOVA	2.364	--	--	0.021	Yes	--	7.871	8.232	6.807	-9.3	-17.3	--	--	--	20.9	-20.9
	6	Log10 Body Weight; Age-1	--	4	10	9	ANOVA	0.038	--	--	0.006	Yes	--	2.309	3.995	1.914	-17.1	-52.1	0.960	0.004	0.046	113.7	-53.2
	7	Body Weight; Age-2	--	--	14	5	ANOVA	6.455	--	--	0.561	No	--	--	6.529	5.743	--	-12.0	--	--	--	113.69	-53.20
	8	Total Length; Age-1	--	4	10	9	ANOVA	0.847	--	--	0.004	Yes	--	6.350	7.440	5.833	0.6	-21.6	--	--	--	24.7	-24.7
	9	Rank of Total Length; Age-2	--	--	14	5	KW	--	--	--	0.404	No	--	--	8.750	8.000	--	-8.6	--	--	--	--	--
	10	Log10 Gonad Weight	Log10 Body Weight	7	28	15	ANCOVA	0.026	0.426	Yes	0.941	No	0.637	0.098	0.094	0.092	-5.8	-1.8	--	--	--	48.3	-32.6

Notes:
Indicates significant effect endpoint for final model per parameter.
Indicates magnitude of difference > CES for final model per parameter.
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions.
Companion table with post-hoc power analysis available in Appendix D.
4. Violation of ANOVA assumptions so non-parametric applied with individual comparisons being made using KW model.
7 and 9. Could only compare PINR-EXP2 to PINR-REF2 due to low sample size in LVR2-REF.

6.3.5 Effect Summary Table and Effect Temporal Comparisons

A comparison of fish effect endpoints for the EDL1 associated sites separated by sentinel species are provided in **Table 6-16** and **Table 6-17**. The Phase 3 EEM found the following:

- Female Brook Stickleback body weight at length was higher at PINR-EXP2 compared to PINR-REF2 but did not the 10% CES.
- Female Brook Stickleback liver weight at body weight was higher at PINR-EXP2 compared to LVR2-REF (smaller fish MOD = 41%) and compared to PINR-REF2 (smaller fish MOD = 51% and larger fish MOD = 27%) and tend to exceed the 25% CES.
- Female Brook Stickleback gonad weight at body weight was higher in PINR-EXP2 fish compared to LVR2-REF and PINR-REF2 and exceeded the 25% CES at LVR2-REF.
- PINR-EXP2 female Brook Stickleback body weight at Age-1 was higher compared to LVR2-REF but did not exceed the 25% CES.

There were a number of confirmed effects comparing PINR-EXP to both STUC-REF and LVR-REF. Confirmed effects above or equal to the CES occur: 1) when similar effects above or equal to the CES are observed in two consecutive phases, or 2) when similar effects occur in two consecutive phases with effects above or equal to CES in one phase and below CES in the other phase. The Phase 3 EEM found the following:

- PINR-EXP female Brook Stickleback had confirmed effects for higher body weight at length compared to LVR-REF exceeding the 10% CES.
- Larger female Brook Stickleback (i.e., through the ANCOVA min-max procedure) had higher gonad weight at body weight when comparing PINR-EXP to LVR-REF exceeding the 25% CES.
- PINR-EXP male Brook Stickleback had confirmed effects for lower body weight at Age-1 when compared to LVR-REF but the MOD was below the 25% CES.
- Female Central Mudminnow had confirmed effects for lower body weight at length when comparing PINR-EXP to their STUC-REF counterparts but the MOD was below the 10% CES.
- Larger female PINR-EXP Central Mudminnow had confirmed effects for higher gonad weight at body weight compared to LVR-REF exceeding the 25% CES.
- Male PINR-EXP Central Mudminnow had confirmed effects for lower gonad weight at body weight compared to STUC-REF exceeding the 25% CES in Phase 2 and below the 25% CES in Phase 3

Further details can be found in **Table 6-18** and **Table 6-19**

Table 6-16:Effect summary table comparing Brook Stickleback at PINR-EXP2 (EDL1 discharge) with associated reference sites.

Sex	Response	Parameter	Covariate	CES	Cycle 3	
					LVR2-REF	PINR-REF2
Female	Survival	Age	None	±25%	0 Age 0 Length	0 Age 0 Length
	Energy Storage	Body Weight	Total Length	±10%	0	↑ (8%)
		Liver Weight	Body Weight	±25%	↑ (41%) min ↓ (6%) max	↑ (51%) min ↑ (27%) max
	Energy Use (Reproduction)	Gonad Weight	Body Weight	±25%	↑ (32%)	↑ (23%)
	Energy Use (Growth)	Body Weight	Age 1	±25%	↑ (13%)	0
Male	Survival	Age	None	±25%	0 Age 0 Length	0 Age 0 Length
	Energy Storage	Body Weight	Total Length	±10%	0	0
		Liver Weight	Body Weight	±25%	0	0
	Energy Use (Reproduction)	Gonad Weight	Body Weight	±25%	0	0
	Energy Use (Growth)	Body Weight	Age 1	±25%	0	0

Notes:
-- indicates no statistical test was undertaken due to EEM-specific reasoning.
0 indicates no statistically significant effect.
↑ and ↓ indicates the direction of the magnitude of difference.
(%) percentages indicate the magnitude of difference of exposure relative to the specific reference site.

Table 6-17:Effect summary table comparing Central Mudminnow at PINR-EXP2 (EDL1 discharge) with associated reference sites.

Sex	Response	Parameter	Covariate	CES	Cycle 3	
					LVR2-REF	PINR-REF2
Female	Survival	Age	None	±25%	--	--
	Energy Storage	Body Weight	Total Length	±10%	--	--
		Liver Weight	Body Weight	±25%	--	--
	Energy Use (Reproduction)	Gonad Weight	Body Weight	±25%	--	--
	Energy Use (Growth)	Body Weight	Age	±25%	--	--
Male	Survival	Age	None	±25%	0 Age ↓ (9%) Length	0 Age ↓ (17%) Length
	Energy Storage	Body Weight	Total Length	±10%	0	0
		Liver Weight	Body Weight	±25%	MOD <MDES	MOD < MDES
	Energy Use (Reproduction)	Gonad Weight	Body Weight	±25%	MOD < MDES	MOD < MDES
	Energy Use (Growth)	Body Weight	Age 1	±25%	--	--

Notes:
-- indicates no statistical test was undertaken due to EEM-specific reasoning.
0 indicates no statistically significant effect.
↑ and ↓ indicates the direction of the magnitude of difference.
(%) percentages indicate the magnitude of difference of exposure relative to the specific reference site.

Table 6-18: Temporal comparison and interpretation of response endpoints across EEM phases for Brook Stickleback relevant to EDL2 discharge (PINR-EXP)

Sex	Response	Parameter	Covariate	CES	Cycle 1	Cycle 2		Cycle 3		Interpretation
					STUC-REF	STUC-REF	LVR-REF	STUC-REF	LVR-REF	
Female	Survival	Age	None	±25%	0	0	↑	--	(0%) Age ↓ (6%) Length	No confirmed effect
	Energy Storage	Body Weight	Total Length	±10%	↑ (15%)	0	↑ (14%)	--	↑ (10.4%)	Confirmed effect > CES
		Liver Weight	Body Weight	±25%	↑ (144%)	0	↑ (44%)	--	0	No effect
	Energy Use (Reproduction)	Gonad Weight	Body Weight	±25%	↑ (190%)	0	↑ (72%)	--	↓ (8%) ↑ (183%)	Confirmed effect >CES for larger fish
	Energy Use (Growth)	Body Weight	Age-1	±25%	--	0	0	--	↓ (8%)	No confirmed effect
			Age-2	±25%		↓ (19%)	0	--	--	--
			Age-3	±25%		--	--	--	--	--
Male	Survival	Age	None	±25%	0	0	0	--	(0%) Age ↓ (9%) Length	No confirmed effect
	Energy Storage	Body Weight	Total Length	±10%	↑ (15%)	0	0	--	↑ (5.8%)	No confirmed effect
		Liver Weight	Body Weight	±25%	↑ (22)	0	0	--	↓ (11.9%)	No confirmed effect
	Energy Use (Reproduction)	Gonad Weight	Body Weight	±25%	0	0	↓ (35%)	--	0	No effect
	Energy Use (Growth)	Body Weight	Age 1	±25%	--	0	↓ (20%)	--	↓ (12%)	Confirmed effect < CES
			Age 2	±25%		↓ (17%)	0	--	--	--
			Age 3	±25%		--	--	--	--	--

Notes:
-- indicates no statistical test was undertaken due to EEM-specific reasoning.
0 indicates no statistically significant effect.
↑ and ↓ indicates the direction of the magnitude of difference.
(%) percentages indicate the magnitude of difference of exposure relative to the specific reference site.

Table 6-19: Temporal comparison and interpretation of response endpoints across EEM phases for Central Mudminnow at sites relevant to the EDL2 discharge.

Sex	Response	Parameter	Covariate	CES	Cycle 1	Cycle 2		Cycle 3		Interpretation
					STUC-REF	STUC-REF	LVR-REF	STUC-REF	LVR-REF	
Female	Survival	Age	None	±25%	0	↑ (9%)	0	0 Age 0 Length	0 Age 0 Length	No effect
	Energy Storage	Body Weight	Total Length	±10%	↑ (10%)	↓ (7%)	0	↓ (5%)	↓ (8%)	Confirmed effect at STUC-REF < CES
		Liver Weight	Body Weight	±25%	↓ (14%)	0	0	↑ (52%) ↓ (18%)	↑ (13%) ↓ (4%)	No confirmed effect
	Energy Use (Reproduction)	Gonad Weight	Body Weight	±25%	↑ (37%)	0	↑ (58%)	↓ (9.5%) ↑ (0.4%)	↓ (6%) ↑ (36%)	Confirmed effect at LVR-REF > CES for larger fish
	Energy Use (Growth)	Body Weight	Age-1	±25%	↑ (53%)	--	--	MOD < MDES	0	No effect
			Age-2	±25%	↓ (11%)	0	0	↑ (25%)	↑ (80%)	No confirmed effect
			Age-3	±25%	--	0	0	--	--	--
Male	Survival	Age	None	±25%	0	0	0	↑(100) 0 Length	(0%) Age 0 Length	No confirmed effect
	Energy Storage	Body Weight	Total Length	±10%	↑ (55%)	0	0	↑ (7%)	↑ (1%)	No confirmed effect
		Liver Weight	Body Weight	±25%	↑ (55%)	0	0	↑ (7%)	↓ (19%)	No confirmed effect
	Energy Use (Reproduction)	Gonad Weight	Body Weight	±25%	0	↓ (44%)	0	↓ (18%)	↓ (22%)	Confirmed effect at STUC-REF < CES
	Energy Use (Growth)	Body Weight	Age 1	±25%	0	--	--	0	0	No effect
			Age 2	±25%	0	↓ (33%)	0	0	0	No effect
			Age 3	±25%	--	↓ (49%)	0	--	--	No effect

Notes:
-- indicates no statistical test was undertaken due to EEM-specific reasoning.
0 indicates no statistically significant effect.
↑ and ↓ indicates the direction of the magnitude of difference.
(%) percentages indicate the magnitude of difference of exposure relative to the specific reference site.

6.4 Summary of Key Findings

The key findings associated with the May 2023 fish survey near RRM are as follows:

- A total of 16 unique fish species were caught in the reference areas and exposures areas in May 2023. The dominant fish per area varied, but commonly it was Brassy Minnow , Brook Stickleback , and Central Mudminnow . A small proportion of fish had health defects, with no distinct pattern related to mine outfall.

- There was insufficient sample size for male and female Brook Stickleback at STUC-REF and female Central Mudminnow at EDL1 associated sites for statistical analyses. The fish were compared for survival, energy storage, and energy use. There were several statistical differences, but only differences that exceeded the critical effect size (CES) will be described in this summary. There was a significant difference between Central Mudminnow age between PINR-EXP and STUC, with a +100% magnitude of difference (MOD). This trend is likely due to their short lifespan, and not associated with mine outfall.

- For energy storage, female Brook Stickleback body weight at body length had an MOD of +10.4%. Also, a group of female Central Mudminnow liver weight at body weight had an MOD +52% compared to STUC-REF. For female Brook Stickleback, comparing PINR-EXP2 to LVR2-REF, the MOD was 40.5% for small fish; comparing PINR-EXP2 to PINR-REF2, the MOD was 50.9% for small fish and 26.9% for large fish – all three exceeded their respective ±25% CES.

- For energy use, large female Central Mudminnow gonad weight at body weight at PINR-EXP had a MOD of +36% compared to STUC-REF. Also, female Central Mudminnow at age 2 had a MOD of 80.1% for body weight at age when comparing PINR-EXP and LVR-REF. Female Brook Stickleback gonad weight at body weight had an MOD of 32% when PINR-EXP2 to LVR2-REF. Male Central Mudminnow body weight at Age 1, had a statistical difference between PINR-EXP2 and PINR-REF2, with an MOD of -52%.

- Temporally, there were a number of confirmed effects comparing PINR-EXP to LVR-REF. Namely, female Brook Stickleback had confirmed effects for higher body weight at length compared to LVR-REF exceeding the 10% CES. Larger female Brook Stickleback (i.e., through the ANCOVA min-max procedure) had higher gonad weight at body weight compared to LVR-REF exceeding the 25% CES. Male Brook Stickleback had confirmed effects for lower body weight at Age 1 when compared to LVR-REF but below the 25% CES. Larger female Central Mudminnow had confirmed effects for higher gonad weight at body weight compared to LVR-REF exceeding the 25% CES.

- There were also a number of confirmed effects comparing PINR-EXP to STUC-REF. Namely, female Central Mudminnow had confirmed effects for lower body weight at length compared to STUC-REF but below the 10% CES.. Male Central Mudminnow had

confirmed effects for lower gonad weight at body weight compared to STUC-REF exceeding the 25% CES in Phase 2 and below the 25% CES in Phase 3

7.0 Summary of Study Components

The Phase 3 EEM was the first EEM in which discharge from EDL1 was investigated as part of the EEM biological component. Since Phase 1, a reference area was added in Phase 2 (LVR-REF) and in Phase 3 a new exposure area downstream of EDL1 (PINR-EXP2) and two associated reference areas have been added (PINR-REF2 and LVR2-REF) and studied. Adding new locations and shifting discharge location priorities complicates interpretation but there are several trends that can be integrated herein.

Laboratory-based chronic toxicity testing with treated effluent suggests that adverse effects on aquatic biota would not be expected in the Pinewood River. Acute toxicity was assessed on Rainbow Trout (*Oncorhynchus mykiss*) and *Daphnia magna*. Since sampling began, 100% effluent has not caused more than 10% toxicity in a single sample. Sublethal toxicity measured survival, growth, and reproduction of Fathead Minnow , water flea , duckweed and the green alga . Fathead minnow survival and growth was not affected since Phase 1. Water flea survival and reproduction were consistently high since Phase 1. Duckweed endpoints were highly variable, ranging from 0.23% v/v to >97% v/v. Apart from two tests from EDL1, the growth of green algae has been constantly high through all tests.

Sediment analysis reveals both EDL2 and EDL1 are dominated by small particle sizes including clay, silt, and fine sand. Particle sizes were generally uniform between exposure and reference areas. Chemical analysis reveals total organic carbon was above guideline thresholds in all areas. Although some areas were above lowest effect levels (e.g., Ni in STUC-REF and Fe in PINR-EXP) the uniform distribution of substrates and minimal differences in chemistry suggest that outfall does not significantly change the structure or constituent concentrations in sediment.

Generally, there was little variability comparing the conventional water parameters between the reference and exposure areas. There was, however, elevated conductivity in the exposure areas compared to some reference areas. In May, conductivity in PINR-EXP was on average 3.2x higher than the reference areas. Similarly, conductivity was 1.9x higher than the reference areas in September. In PINR-EXP2, conductivity was 1.7x higher than the reference in May, but there was no difference in September.

When examining the chemistry composition of water chemistry, the only parameter that was elevated beyond the CCME water quality guidelines was Fe, in all sites but PINR-EXP. This suggests that the area is naturally elevated in Fe. When comparing the relative differences between sites, antimony was >10% elevated compared to the reference areas. No other measured parameters appear elevated.

From the benthic invertebrate survey, a total of 41 benthic families were identified in the study area. Community composition varied widely as indicated by the consistent and confirmed Bray-Curtis index. The lack of similarity is particularly noticeable in those areas relating to EDL2 where there is effectively no community overlap. An investigation of cause that examines univariate and multivariate correlations with community composition will aid in determining whether community differences are related to effluent exposure or other environmental factors. Other

measured endpoints of density, diversity, and evenness were also significantly different between reference and exposure areas. For example, density was lower in PINR-EXP compared to STUC-REF and lower in PINR-EXP2 compared with PINR-REF2.

From the fish survey, 16 unique fish species were caught in the reference areas and exposure areas. The areas are generally dominated by similar species of Brassy Minnow, Brook Stickleback, and Central Mudminnow with small proportional representation of other small-bodied fish. A few fish had health defects with no distinct pattern related to mine outfall.

Survival, energy storage, and energy use were examined for the Central Mudminnow and Brook Stickleback. Statistical differences did not appear to be strongly driven by outliers. For survival, any mean age differences appeared to be mostly related to short lifespan. As recommended by the TGD (EC, 2012), length appears a more appropriate measure assuming the sacrificed fish are a random sample from the population.

For energy storage, most endpoints that were significantly different had a positive magnitude of difference compared to reference sites. For example, female Brook Stickleback body weight at length had an MOD above its CES of 10%. Central Mudminnow liver weight had an MOD of 52% compared to STUC-REF. For female Brook Stickleback, comparing PINR-EXP2 to LVR2-REF, the MOD was 40.5% for small fish; comparing PINR-EXP2 to PINR-REF2, the MOD was 50.9% for small fish and 26.9% for large fish – all three exceeded their respective ±25% CES.

Similarly, for energy use, any significant differences tended to have positive MODs. For example, large female Central Mudminnow gonad weight at body weight at PINR-EXP had a MOD of +36% compared to STUC-REF. Also, female Central Mudminnow at Age-2 had a MOD of 80.1% for body weight at age when comparing PINR-EXP and LVR-REF. In contrast, male Central Mudminnow body weight at Age-1, did have a statistical difference between PINR-EXP2 and PINR-REF2, with an MOD of -52%.

Temporally, there were a number of confirmed effects comparing PINR-EXP to LVR-REF. Female Brook Stickleback had confirmed effects for higher body weight at length, larger female Brook Stickleback had higher gonad weight at body weight, male Brook Stickleback had confirmed effects for lower body weight at Age-1, and larger female Central Mudminnow had confirmed effects for higher gonad weight at body weight. Exceedance of the CES varied by endpoint.

There were also a number of confirmed effects comparing PINR-EXP to STUC-REF. Female Central Mudminnow had confirmed effects for lower body weight at length and male Central Mudminnow had confirmed effects for lower gonad weight at body weight Phase 3. Again, the exceedance of the CES varied by endpoint..

8.0 Recommendations for Future Monitoring

Based on the combined results, there is a confirmed effects on the benthic community based on consecutive differences in the Bray-Curtis Index for the EDL2 discharge. Similarly, there are a number of confirmed effects comparing both Brook Stickleback and Central Mudminnow at EDL2 associated sites with their respective reference areas. Considering that EDL2 is no longer considered the primary discharge for assessment during the biological component of the EEM program, next steps in this instance will be discussed with ECCC leading up to the preparation of the Phase 4 Study Design. In the very least a desktop exercise using univariate statistics can be used to investigate the benthic community endpoints and various environmental parameters. With respect to the fish many of the differences are in a positive biological direction and any IOC would need to be commensurate with the risk to the environment given it is the secondary discharges .

This is the first official EEM in which the potential effect of the EDL1 discharge has been assessed as the primary discharge. Therefore Phase 4 should continue with a repeat of Phase 3 to determine if there are any confirmed effects. According to the decisions trees and this path the next interpretive report is due in 36 months (i.e., March 2027).

All decisions on the Phase 4 design will consider the appropriate technical guidance available at that time, as well as the site-specific conditions at the mine and will be outlined in a study design report that will be submitted to ECCC for review at least six months prior to any proposed field program

9.0 References

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10.0 Appendices

Appendix A – Site Characterization

Appendix B – Supporting Environmental Information

Appendix C – Benthic Invertebrate Survey

Appendix D – Fish Survey

Appendix A Site Characterization

Table A-1: Raw data subset of mine effluent chemistry provided by New Gold related to Schedule 4 deleterious substances, 2020–2023.

Note: In the few cases of duplicates, we used the first instance for generating summary statistics.

Site	Sample Date	Ammonia, Un-ionized as N	Arsenic	Copper	Cyanide, Total	Field pH	Lead	Nickel	Radium 226	Total Suspended Solids	Zinc
EDL1	09-Oct-19	0.01	0.00153	0.00188	0.0044	7.55	0.00003	0.00152	0.01	3.5	0.0044
	15-Oct-19	0.007	0.00182	0.00276	0.0032	7.99	0.000085	0.0017	0.01	1.5	0.0076
	16-Oct-19		0.00172	0.00256	0.002	8.27	0.0001	0.00148		3.5	0.0074
	17-Oct-19		0.00165	0.00244	0.0006	8.16	0.000065	0.0013		1.5	0.007
	21-Oct-19	0.018	0.00179	0.00332	0.0004	8.29	0.000095	0.00148	0.01	2	0.0084
	22-Oct-19		0.00177	0.00336	0.002	7.33	0.00006	0.00148		1.5	0.0052
	23-Oct-19		0.00189	0.00396	0.0018	7.23	0.00004	0.00156		1	0.004
	28-Oct-19	0.002	0.0021	0.0051	0.0012	7.35	0.00009	0.0016	0.01	1	0.007
	29-Oct-19		0.002	0.0051	0.0012	8.39	0.00007	0.0017		1	0.0065
	30-Oct-19		0.0019	0.0049	0.0014	7.81	0.00009	0.0016		1	0.007
	04-Nov-19	0.004	0.00241	0.00734	0.002	7.77	0.000135	0.00194		2	0.0068
	06-Nov-19		0.00233	0.00706	0.0018	8.06	0.00011	0.00184		1.5	0.0064
	11-Nov-19	0.001	0.00254	0.00804	0.0026	7.67	0.0001	0.002	0.01	1.5	0.0088
	12-Nov-19		0.00249	0.0076	0.0024	8.33	0.000095	0.00186		1	0.008
	13-Nov-19		0.00245	0.0076	0.0022	7.12	0.00011	0.00192		2	0.0128
	16-Nov-19	0.004	0.00325	0.00872	0.0026	8	0.000865	0.0028	0.01	18	0.022
	29-Apr-20	0.032	0.00385	0.0053	0.0066		0.000175	0.00346	0.01	2	0.0035
	06-May-20	0.032	0.0032	0.00638	0.0018		0.000135	0.00258	0.01	2	0.004
	13-May-20	0.03	0.00273	0.00766	0.001		0.000185	0.00234	0.01	3	0.004
	20-May-20		0.00243	0.00922	0.001		0.000145	0.00228	0.01	1	0.0045
	27-May-20		0.00247	0.0123	0.001		0.00014	0.0022	0.01	3	0.0034
	07-Sep-20	0.003	0.00238	0.0113	0.001		0.00004	0.0013	0.01	2.5	0.0025
	14-Oct-20	0.016	0.00264	0.0203	0.0148	8.48	0.00004	0.00142	0.01	2	0.003
	20-Oct-20	0.001	0.00226	0.0141	0.0008	8.25	0.00003	0.00126	0.01	2	0.0005
	21-Oct-20	0.01	0.00229	0.0143	0.001	7.98	0.00004	0.00128	0.01	3	0.001
	28-Oct-20	0.01	0.00229	0.0144	0.0002	7.77	0.00003	0.00132	0.01	1.5	0.002

Site	Sample Date	Ammonia, Un-ionized as N	Arsenic	Copper	Cyanide, Total	Field pH	Lead	Nickel	Radium 226	Total Suspended Solids	Zinc
	04-Nov-20	0.01	0.00227	0.0137	0.001	8.21	0.00009	0.00134	0.01	3	0.0015
	05-Nov-20		0.00184	0.00542	0.0008	8.08	0.00024	0.00194		3	0.0005
	06-Nov-20		0.0018	0.00574	0.0014	7.64	0.00026	0.002		1	0.001
	07-Nov-20		0.00176	0.00618		7.81	0.00019	0.00194			0.0015
	08-Nov-20		0.00189	0.00686		7.84	0.00019	0.002			0.0015
	09-Nov-20		0.00187	0.00684	0.0006	7.98	0.00016	0.00194		1	0.0015
	11-Nov-20	0.01	0.0019	0.0082	0.0002	7.56	0.00013	0.0018	0.01	3	0.0015
	26-May-21		0.00185	0.0146	0.001	8.56	0.00017	0.00162	0.01	5.5	0.004
	29-May-21	0.012	0.00356	0.00424	0.0012	8.38	0.00024	0.00178	0.01	1	0.0015
	30-May-21	0.005	0.00284	0.00438	0.0012	8.25	0.00015	0.00172	0.01	2	0.001
	31-May-21	0.006	0.00524	0.00384	0.0014	7.85	0.00034	0.00194	0.01	1	0.002
	02-Jun-21	0.009	0.00458	0.00614	0.003	8.18	0.00026	0.00178	0.012	3	0.004
	27-Sep-21	0.005	0.00164	0.0024	0.0012	7.83	0.00016	0.0011	0.01	9.5	0.0035
	20-Oct-21	0.015	0.00176	0.00548	0.001	8	0.00004	0.00106	0.01	1.5	0.002
	25-Oct-21	0.011	0.00171	0.0055	0.0004	7.89	0.00002	0.001	0.01	3	0.0015
	13-Apr-22	0.004	0.00201	0.0101	0.0002	7.95	0.00029	0.00184	0.0062	10.5	0.0055
	20-Apr-22	0.002	0.00166	0.0163	0.003	7.44	0.00014	0.00234		2.5	0.0115
	27-Apr-22	0.005	0.0016	0.00665	0.0006	8.02	0.00004	0.00188	0.0063	3	0.0044
	04-May-22	0.01	0.00142	0.00625	0.0008	7.33	0.0005	0.00112	0.0084	2	0.0014
	11-May-22	0.002	0.0013	0.00475	0.0004	7.68	0.00016	0.00152	0.0064	7	0.0034
	25-May-22	0.01	0.00127	0.0043	0.0004	7.64	0.0001	0.00168	0.0084	2	0.0034
	01-Jun-22	0.002	0.00129	0.00445	0.0016	7.78	0.0002	0.00176	0.0074	13.5	0.0034
	08-Jun-22	0.004	0.00137	0.00394	0.002	7.91	0.00005	0.00132	0.0071	4.5	0.0015
	15-Jun-22	0.002	0.00318	0.00258	0.0008	7.44	0.00003	0.00096	0.0064	7	0.0025
	29-Jun-22	0.021	0.00242	0.00236	0.0008	8.37	0.00001	0.00132	0.036	3.5	0.001
	07-Jul-22	0.004	0.00179	0.00264	0.0006	7.89	0.00005	0.001	0.0077	4	0.0005
	03-Aug-22	0.004	0.00167	0.00314	0.0008	7.19	0.00001	0.0012	0.0082	3.5	0.0005
	21-Sep-22	0.023	0.00159	0.00256	0.002	7.86	0.00005	0.0029	0.01	3	0.044

Site	Sample Date	Ammonia, Un-ionized as N	Arsenic	Copper	Cyanide, Total	Field pH	Lead	Nickel	Radium 226	Total Suspended Solids	Zinc
	21-Oct-22	0.027	0.00135	0.00242	0.0006	8.18	0.00005	0.00232	0.01	1.5	0.0178
	26-Oct-22	0.081	0.00142	0.00276	0.0012	8.74	0.00005	0.00267		4.5	0.018
	02-Nov-22	0.004	0.00121	0.0025	0.0012	7.5	0.00005	0.00486	0.01	3	0.105
	09-Nov-22	0.011	0.00127	0.00375	0.001	7.85	0.00008	0.004	0.01	2	0.03
	16-Nov-22	0.009	0.0011	0.005	0.0008	7.78	0.0005	0.005		2	0.03
	17-Apr-23	0.003	0.00115	0.00286	0.001	7.39	0.00003	0.00388	0.005	3	0.0165
	24-Apr-23	0.002	0.00103	0.00266	0.0008	7.28	0.00002	0.00332	0.005	4	0.015
	01-May-23	0.003	0.00113	0.00288	0.001	7.39	0.00001	0.00362	0.005	4	0.016
	08-May-23	0.002	0.00098	0.00266	0.0008	7.47	0.00005	0.00302	0.005	1.5	0.0135
	15-May-23	0.0049	0.00098	0.00274	0.002	7.79	0.00005	0.00298	0.005	3	0.011
	22-May-23		0.00101	0.00301	0.0024		0.00005	0.00283	0.005	3	0.0085
	29-May-23	0.0065	0.0011	0.005	0.002	8.36	0.0005	0.005	0.005	5.5	0.03
	05-Jun-23	0.0246	0.00114	0.00313	0.002	8.18	0.00005	0.0029	0.006	4.1	0.0034
	12-Jun-23	0.0434	0.00121	0.00285	0.002	8.14	0.00005	0.00257	0.006	4.7	0.003
	20-Jun-23	0.0314	0.00133	0.00392	0.002	7.74	0.00005	0.00286	0.005	3	0.0052
	03-Nov-23	0.0085	0.00121	0.00185	0.002	7.5	0.00005	0.00321	0.005	4.5	0.003
	06-Nov-23	0.0079	0.00128	0.00194	0.0025	7.53	0.00005	0.00337	0.005	4.7	0.003
	08-Nov-23	0.026	0.00132	0.00243	0.002		0.00005	0.00389		4.9	0.0035
	13-Nov-23	0.0472	0.00122	0.00218	0.002		0.0001	0.00368	0.006	3	0.006
	20-Nov-23	0.0069	0.00121	0.00196	0.0024	7.53	0.00005	0.00356	0.009	3.2	0.003
EDL2	15-Oct-20	0.002	0.00489	0.00246	0.001	7.89	0.00115	0.00114	0.01	10	0.016
	20-Oct-20	0.001	0.00484	0.00166	0.0016	7.84	0.00031	0.00104	0.01	1	0.0045
	21-Oct-20	0.002	0.00467	0.00176	0.0008	7.92	0.00019	0.00104	0.01	1	0.002
	28-Oct-20	0.01	0.00316	0.00344	0.0004	7.94	0.00039	0.00176	0.01	2	0.0045
	04-Nov-20	0.01	0.00188	0.00532	0.0012	7.26	0.00029	0.00202	0.01	0.5	0.006
	06-Nov-20		0.00176	0.00572	0.0004	7.66	0.00026	0.00204		3	0.008
	08-Nov-20		0.00186	0.00678		7.72	0.0002	0.00196			0.001
	09-Nov-20		0.00186	0.00688	0.0004	7.27	0.00016	0.00204		3	0.001

Site	Sample Date	Ammonia, Un-ionized as N	Arsenic	Copper	Cyanide, Total	Field pH	Lead	Nickel	Radium 226	Total Suspended Solids	Zinc
	11-Nov-20	0.01	0.00183	0.00832	0.0006	7.23	0.00019	0.00186	0.01	1.5	0.015
	11-Nov-20	0.01	0.00183	0.0082	0.0002	7.23	0.00019	0.00188	0.01	2	0.0115
	20-Oct-21	0.003	0.00123	0.00154	0.0006	7.81	0.00023	0.00128	0.01	0.5	0.001
	20-Apr-22	0.002	0.00196	0.00598	0.0004	7.74	0.00003	0.00148		1.5	0.003
	27-Apr-22	0.002	0.0018	0.0062	0.0006	7.44	0.00004	0.00218	0.0053	3	0.017
	04-May-22	0.01	0.00152	0.0057	0.002		0.00002	0.00106	0.0094	3	0.0004
	11-May-22	0.002	0.00125	0.0053	0.0006	7.54	0.00014	0.00144	0.0069	3.5	0.003
	18-May-22	0.002	0.00133	0.00425	0.0014	7.71	0.00012	0.0015	0.01	5.5	0.0032
	25-May-22	0.01	0.0048	0.00175	0.0004	6.99	0.00022	0.00172	0.0075	4	0.007
	01-Jun-22	0.01	0.00355	0.0007	0.0006	7.62	0.00008	0.0012	0.0077	6.5	0.008
	08-Jun-22	0.01	0.00373	0.00094	0.0002	7.05	0.00008	0.00088	0.0058	8	0.0005
	29-Jul-22	0.01	0.00127	0.00188	0.0012	6.92	0.00011	0.00816	0.0092	8	0.484
	21-Oct-22	0.017	0.00135	0.00294	0.0008	7.96	0.000073	0.00268	0.01	2	0.0314
	26-Oct-22	0.056	0.00145	0.00743	0.0012	8.51	0.000133	0.00292		4.5	0.0384
	02-Nov-22	0.005	0.00122	0.00356	0.0018	7.53	0.0001	0.00588	0.01	2	0.143
	09-Nov-22	0.01	0.00108	0.00235	0.0006	7.82	0.00004	0.00396	0.01	3	0.0274
	17-Apr-23	0.002	0.00119	0.00318	0.002	7.23	0.00006	0.00418	0.005	0.5	0.0175
	24-Apr-23	0.002	0.00102	0.00602	0.0008	7.38	0.00017	0.00332	0.005	2.5	0.023
	01-May-23	0.002	0.00113	0.00308	0.001	7.33	0.00003	0.00368	0.006	4.5	0.017
	08-May-23	0.007	0.00098	0.00274	0.0008	7.86	0.00007	0.00296	0.005	4	0.015
	15-May-23	0.0044	0.00099	0.0032	0.002	7.72	0.00007	0.00289	0.005	3	0.0182
	22-May-23		0.0011	0.00339	0.002		0.000061	0.00288	0.005	4.3	0.0104
	29-May-23	0.0067	0.00112	0.005	0.002	8.33	0.0005	0.005	0.005	4.7	0.03
	05-Jun-23	0.0271	0.0012	0.00357	0.002	8.19	0.00005	0.00307	0.005	3.1	0.0042
	12-Jun-23	0.0582	0.00125	0.00308	0.0022	8.23	0.000055	0.00269	0.005	3.3	0.004
	03-Nov-23	0.01	0.00127	0.00436	0.002	7.58	0.00819	0.00341	0.005	5.3	0.0097
	06-Nov-23	0.012	0.00124	0.00193	0.002	7.66	0.00005	0.00352	0.005	4.7	0.0036
	07-Nov-23	0.0285	0.00126	0.0281	0.002		0.00907	0.00355		3.1	0.0161

Site	Sample Date	Ammonia, Un-ionized as N	Arsenic	Copper	Cyanide, Total	Field pH	Lead	Nickel	Radium 226	Total Suspended Solids	Zinc
	13-Nov-23	0.005	0.00123	0.00245	0.002		0.000558	0.0038	0.005	3	0.0066
	13-Nov-23	0.0048	0.00119	0.0025	0.002	7.4	0.000535	0.00377	0.005	3.7	0.0068
	13-Nov-23		0.0001	0.0005	0.002		0.00005	0.0005		3	0.003
	20-Nov-23	0.008	0.00116	0.00293	0.0027	7.61	0.000777	0.00349	0.005	3	0.0063
SP2	07-Oct-19	0.001	0.00144	0.00262	0.0004	8.58	0.000075	0.00146	0.01	5	0.0018
	15-Oct-19	0.01	0.0015	0.0025	0.0004	8.05	0.000165	0.00158	0.01	3.5	0.0016
	21-Oct-19	0.001	0.00157	0.00222	0.0004	8.21	0.0001	0.00156	0.01	4	0.0016
	28-Oct-19	0.01	0.0015	0.0022	0.0004	8.03	0.00011	0.0014	0.01	3	0.002
	04-Nov-19	0.01	0.00149	0.0021	0.0004	8.64	0.00011	0.00144	0.01	1.5	0.0016
	24-Jun-20		0.00153	0.00235	0.0004		0.00002	0.00128	0.01	1.5	0.0014
	30-Jun-20		0.00157	0.0024	0.0014		0.00004	0.00154	0.01	3	0.0028
	08-Jul-20		0.00173	0.00285	0.0018		0.00006	0.00158	0.01	3	0.0102
	15-Jul-20		0.00203	0.00258	0.0008		0.00004	0.00162	0.01	1.5	0.0075
	28-Sep-20		0.00229	0.00262	0.002		0.00012	0.00152	0.01	3	0.0115
	28-Sep-20	0.01	0.00237	0.00254	0.002	6.94	0.00011	0.00132	0.01	2	0.0125
	07-Oct-20	0.01	0.00223	0.0023	0.0006	8.38	0.00001	0.00126	0.01	3	0.003
	13-Oct-20	0.01	0.00227	0.0023	0.002		0.00001	0.00124		1	0.003
	20-Oct-20	0.01	0.00205	0.0022	0.0006	8.64	0.00003	0.00124	0.01	1	0.003
	21-Oct-20	0.01	0.00202	0.00228	0.0004	8.23	0.00003	0.00122	0.01	1	0.003
	28-Oct-20	0.01	0.0021	0.00226	0.002	8.28	0.00002	0.00138	0.01	1.5	0.001
	04-Nov-20	0.01	0.00186	0.00212	0.002	8.17	0.00002	0.00134	0.01	1.5	0.001
	11-Nov-20	0.01	0.00197	0.00254	0.0004	8.23	0.00005	0.00124	0.01	1	0.01
	18-Nov-20	0.01	0.0019	0.00202	0.0002	8.34	0.00003	0.00112	0.01	3	0.001
	25-Nov-20	0.01	0.00207	0.00242	0.0002	8.34	0.00004	0.0014	0.01	3	0.002
	14-Apr-21		0.00202	0.00195	0.0016		0.00012	0.00162	0.01	7	0.0026
	15-Apr-21		0.00202	0.00195	0.0018	7.99	0.00016	0.00166	0.01	8	0.0046
	21-Apr-21		0.00192	0.00165	0.0016	8.27	0.00006	0.00128	0.01	6.5	0.0014
	28-Apr-21		0.00198	0.00178	0.001	6.95	0.000045	0.00148	0.01	3.5	0.0015

Site	Sample Date	Ammonia, Un-ionized as N	Arsenic	Copper	Cyanide, Total	Field pH	Lead	Nickel	Radium 226	Total Suspended Solids	Zinc
	05-May-21		0.00177	0.00196	0.0006	8.91	0.00004	0.00124	0.01	5.5	0.001
	26-May-21		0.00208	0.0024	0.0008	8.42	0.00001	0.00132	0.01	4	0.001
	02-Jun-21	0.007	0.0022	0.00242	0.002	8.87	0.00005	0.0013	0.01	3	0.002
	22-Sep-21	0.028	0.00238	0.00192	0.0036	8.35	0.00009	0.00116	0.01	6.5	0.002
	13-Oct-21	0.005	0.00229	0.00166	0.001	8.09	0.00005	0.00076	0.01	1.5	0.003
	20-Oct-21	0.003	0.00222	0.00166	0.0014	8.02	0.00003	0.001	0.01	2.5	0.001
	25-Oct-21	0.002	0.00207	0.00154	0.0012	8.11	0.00002	0.00088	0.01	3	0.001
	13-Apr-22	0.01	0.00118	0.00166	0.0012	7.4	0.00013	0.0012	0.0066		0.003
	27-Apr-22	0.001	0.00108	0.00165	0.0042	7.56	0.00024	0.00142	0.0063	6.5	0.003
	04-May-22	0.01	0.00108	0.00145	0.0016	7.51	0.0002	0.0011	0.008	6	0.0026
	11-May-22	0.01	0.00169	0.0016	0.0006	7.45	0.00006	0.00124	0.0064	3	0.0018
	18-May-22	0.01	0.00125	0.0017	0.0004	7.85	0.00008	0.0011	0.01	3.5	0.0016
	25-May-22	0.01	0.00134	0.0016	0.0016	7.83	0.00012	0.0012	0.0067	5	0.0144
	01-Jun-22	0.01	0.00134	0.0017	0.0012	7.82	0.00016	0.00154	0.0061	16.5	0.0632
	08-Jun-22	0.002	0.00145	0.0017	0.0006	8.06	0.00004	0.00128	0.0056	5.5	0.0255
	15-Jun-22	0.001	0.00143	0.00166	0.0006	8.26	0.00005	0.00092	0.0049	7	0.0155
	22-Jun-22	0.01	0.00144	0.00176	0.0004	8.38	0.00005	0.00116	0.011	4.5	0.01
	29-Jun-22	0.007	0.00158	0.0019	0.0006	8.99	0.00001	0.00128	0.013	4.5	0.0055
	07-Jul-22	0.025	0.00147	0.00192	0.0002	9.19	0.00005	0.00104	0.012	2.5	0.0035
	28-Jul-22		0.00203	0.00176	0.002	7.75	0.00005	0.00094	0.006	3	0.002
	03-Aug-22	0.01	0.00211	0.00136	0.0004	8.15	0.00005	0.0009	0.0064	2	0.001
	21-Sep-22	0.001	0.00244	0.00218	0.002	8.2	0.00005	0.00138	0.01	2	0.003
	19-Oct-22	0.01	0.002	0.0015	0.002	7.55	0.00005	0.00096		2.5	0.0018
	26-Oct-22	0.001	0.00198	0.00169	0.0004	9.07	0.00005	0.00102		3	0.003
	02-Nov-22	0.01	0.00196	0.00165	0.001	8.03	0.00005	0.00102	0.01	1	0.003
	09-Nov-22	0.011	0.0018	0.00155	0.0008	7.97	0.00004	0.0009	0.01	2.5	0.0016
	16-Nov-22	0.01	0.00185	0.00159	0.0002	7.91	0.00005	0.00086		2	0.003
	14-Apr-23	0.001	0.00136	0.00226	0.0008	7.94	0.00042	0.00146	0.005	12	0.0055

Site	Sample Date	Ammonia, Un-ionized as N	Arsenic	Copper	Cyanide, Total	Field pH	Lead	Nickel	Radium 226	Total Suspended Solids	Zinc
	17-Apr-23	0.002	0.00153	0.00244	0.0012	7.93	0.00037	0.00342	0.005	4.5	0.009
	24-Apr-23	0.001	0.00154	0.00164	0.0004	7.66	0.00013	0.00112	0.005	6	0.0035
	01-May-23	0.01	0.00159	0.00162	0.0006	7.9	0.00011	0.00112	0.005	5	0.0065
	08-May-23	0.01	0.00144	0.00148	0.0006	8.53	0.00008	0.00104	0.005	4.5	0.0035
	15-May-23	0.0015	0.00151	0.00199	0.002	8.61	0.00012	0.00107	0.005	6.6	0.0052
	05-Jun-23	0.0011	0.00158	0.00209	0.002	8.43	0.000112	0.00112	0.005	3.1	0.0039
	20-Sep-23	0.001	0.00219	0.00407	0.002		0.00005	0.00097		4.6	0.003
	20-Oct-23	0.001	0.00204	0.00578	0.002	7.79	0.000058	0.00081	0.005	3.5	0.0144
	22-Oct-23	0.001	0.002	0.00529	0.002	8.26	0.000131	0.00082	0.005	4.3	0.0236
	24-Oct-23	0.001	0.00197	0.00199	0.002	8.04	0.00005	0.00101	0.005	3	0.0132
	30-Oct-23	0.001	0.00192	0.00165	0.002	8.14	0.00005	0.00085	0.005	4.7	0.0152
	05-Nov-23	0.001	0.00175	0.00181	0.002		0.00005	0.00079	0.007	3	0.003
	13-Nov-23	0.001	0.00184	0.00112	0.002		0.00005	0.00083	0.006	3.5	0.003
	20-Nov-23	0.001	0.00182	0.0017	0.002	7.79	0.00005	0.00102	0.005	3.6	0.003

Table A-2: Subset of mine effluent chemistry provided by New Gold related to Schedule 5 (4) effluent characterization, 2020–2023.

Note: In the few cases of duplicates, we used the first instance for generating summary statistics.

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Cadmium	Chloride	Chromium	Cobalt	Conductivity	Field Temp	Hardness as CaCO3	Iron	Manganese	Mercury	Molybdenum	Nitrate as N	Phosphorus	Selenium	Sulfate as SO4	Thallium	Uranium
EDL1	09-Oct-19	213	0.0764	0.00001	36.2	0.0004	0.000531	762	10.34	321	0.102	0.0336	0.00003	0.00375	3.98	0.026	0.000215	142	5.2E-06	0.00195
	15-Oct-19	212	0.0824	0.000017	114	0.00066	0.000653	788	9.5	320	0.117	0.0347	0.00003	0.00508	15.4	0.0225	0.000635	988	1.16E-05	0.00226
	16-Oct-19		0.0762	0.000015		0.00022	0.000608		9.78		0.098	0.0296		0.00506		0.01	0.00068		1.08E-05	0.00218
	17-Oct-19		0.0652	0.000013		0.0002	0.000577		5.97		0.089	0.0266		0.00497		0.01	0.000765		8.2E-06	0.00213
	21-Oct-19	186	0.0438	0.000013	18.1	0.0002	0.000788	795	8.8	331	0.0655	0.0253	0.00003	0.00521	2.91	0.017	0.00083	231	1.32E-05	0.00223
	22-Oct-19		0.0446	0.000016		0.0002	0.000796		10.75		0.071	0.0337		0.0055		0.01	0.000755		1.32E-05	0.00236
	23-Oct-19		0.052	0.000015		0.00018	0.00102		5.85		0.075	0.0266		0.0061		0.015	0.00068		1.38E-05	0.00232
	28-Oct-19	154	0.079	0.000015	14.7	0.0003	0.00112	954	4.01	376	0.08	0.0138	0.00003	0.00622	3.29	0.016	0.0008	311	0.000012	0.00217
	29-Oct-19		0.0745	0.00001		0.0003	0.00111		7.1		0.07	0.0115		0.00624			0.0008		0.000012	0.00189
	30-Oct-19		0.0605	0.000015		0.0007	0.00108		1.51		0.06	0.0124		0.00626			0.0008		0.000018	0.00188
	04-Nov-19	157	0.0574	0.000015	12.2	0.00012	0.00108	892	1.89	343	0.0735	0.011	0.00003	0.00665	4.2	0.0135	0.00084	277	1.34E-05	0.00259
	06-Nov-19		0.0436	0.000012		0.00016	0.00103		1.02		0.0595	0.0113		0.0065		0.05	0.00077		1.32E-05	0.00235
	11-Nov-19	171	0.0488	0.000019	13.3	0.00082	0.00113	967	0.6	379	0.0595	0.0217	0.00003	0.00721	5.44	0.012	0.000645	311	0.000007	0.00247
	12-Nov-19		0.0484	0.000017		0.0009	0.00118		5.97		0.06	0.0391		0.00702		0.01	0.000675		7.4E-06	0.00238
	13-Nov-19		0.0586	0.000022		0.00038	0.00126		0		0.096	0.121		0.00686		0.015	0.00057		9.6E-06	0.00241
	16-Nov-19	189	0.622	0.000035	17.4	0.00132	0.0017	1080	0.78	420	0.883	0.163	0.00003	0.00784	6.21	0.055	0.000765		1.02E-05	0.00256
	29-Apr-20	158	0.0758	0.000012	13.1	0.00024	0.00142	942		376	0.089	0.0787	0.00003	0.00653	4.09	0.0221	0.0005	279	0.00003	0.00411
	06-May-20	159	0.0564	0.000013	12.2	0.00022	0.00151	998		396	0.058	0.0564	0.00003	0.00724	3.6	0.0893	0.00054	341	0.00003	0.00277
	13-May-20	151	0.0918	0.000012	13.3	0.00028	0.00154	1040		387	0.08	0.0304	0.00002	0.00741	4.54	0.0203	0.00056	386	0.000027	0.00271
	20-May-20	146	0.0844	0.000016	10.7	0.00046	0.00179	1150		454	0.057	0.0144	0.00001	0.00825	3.86	0.0117	0.00053	330	0.000027	0.00261
	27-May-20	134	0.0584	0.00002	17.6	0.00016	0.00215	1330		487	0.035	0.0119	0.00003	0.00943	7.37	0.0103	0.000644	548	0.000024	0.00225
	07-Sep-20	127	0.0244	0.000015	20.7	0.00012	0.00177	1570		504	0.044	0.0148	0.000005	0.0123	4.82	0.009	0.000595	548	0.000015	0.00212
	14-Oct-20	120	0.0198	0.000017	27.9	0.0001	0.00177	1670	7.91	487	0.0455	0.0132	0.00003	0.0122	5.09	0.009	0.00056	668	0.00001	0.0022
	20-Oct-20	125	0.0144	0.000013	26.4	0.00016	0.00163	1490	4.61	473	0.022	0.006	0.00003	0.012	5.03	0.008	0.0006	677	0.00001	0.00239
	21-Oct-20	125	0.015	0.000016	24	0.0001	0.00162	1470	6.15	474	0.0305	0.0056	0.00003	0.0121	4.67	0.0075	0.00055	626	0.00001	0.00239
	28-Oct-20	124	0.0198	0.000019	26.5	0.00038	0.00164	1390	6.12	474	0.093	0.0054	0.00003	0.0122	5.34	0.0055	0.000555	677	0.000015	0.00241
	04-Nov-20	124	0.0524	0.000017	24	0.0003	0.00171	1570	2.91	482	0.163	0.01	0.00003	0.0124	4.84	0.011	0.000665	665	0.000015	0.00255

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Cadmium	Chloride	Chromium	Cobalt	Conductivity	Field Temp	Hardness as CaCO3	Iron	Manganese	Mercury	Molybdenum	Nitrate as N	Phosphorus	Selenium	Sulfate as SO4	Thallium	Uranium
	05-Nov-20		0.0226	0.000008		0.00016	0.00168		7.8		0.0505	0.013		0.0142		0.05	0.00035		0.00002	0.0028
	06-Nov-20		0.0212	0.000031		0.0001	0.00166		5.71		0.0415	0.0108		0.0141		0.05	0.000345		0.00002	0.0028
	07-Nov-20		0.0234	0.000014		0.00018	0.0017		4.92		0.0355	0.0082		0.0135		0.05	0.000335		0.000025	0.00265
	08-Nov-20		0.0278	0.000017		0.00016	0.00169		9.03		0.051	0.0088		0.0131		0.05	0.000345		0.000025	0.00269
	09-Nov-20		0.024	0.000014		0.00016	0.00169		9.51		0.033	0.0078		0.0132		0.05	0.000315		0.000025	0.00256
	11-Nov-20	126	0.0276	0.000013	28.4	0.00046	0.00168	1570	7.31	504	0.467	0.0074	0.00003	0.0129	4.73	0.0075	0.000345	685	0.000025	0.00254
	26-May-21	127	0.122	0.000013	24	0.00034	0.00122	1490	14.46	496	0.169	0.0316	0.00003	0.0123	3.4	0.013	0.00049	623	0.00001	0.00269
	29-May-21	125	0.0112	0.000013	25	0.00024	0.00123	1360	14.56	459	0.131	0.075	0.00003	0.0139	1.1	0.0145	0.000465	590	0.00002	0.00354
	30-May-21	112	0.0098	0.00001	24.6	0.00016	0.00122	1360	14.51	445	0.067	0.0582	0.00003	0.0141	1.05	0.0115	0.000425	588	0.000025	0.00335
	31-May-21	150	0.0118	0.000013	24.5	0.00014	0.00143	1390	14.66	483	0.262	0.126	0.00003	0.013	0.63	0.0205	0.000515	586	0.00003	0.00361
	02-Jun-21	149	0.0354	0.000014	24.4	0.0003	0.00123	1450	16.78	450	1.01	0.0824	0.00003	0.0125	0.75	0.02	0.00047	618	0.000015	0.00322
	27-Sep-21	116	0.0118	0.000005	37.5	0.00018	0.000955	1750	17.4	515	0.0925	0.0248	0.00003	0.0152	1.72	0.008	0.000235	746	0.000015	0.00228
	20-Oct-21	108	0.027	0.000008	39.2	0.00014	0.00105	1740	10.6	520	0.047	0.022	0.00003	0.0133	2.71	0.012	0.00039	770	0.00001	0.00203
	25-Oct-21	111	0.0168	0.000011	38.3	0.00014	0.00105	1750	10	519	0.0285	0.0156	0.00003	0.0136	2.07	0.014	0.00046	773	0.00001	0.00196
	13-Apr-22	121	0.146	0.000038	40.8	0.00036	0.00177	1880	6.09	549	0.299	0.207	0.00003	0.0133	2	0.021	0.00042	797	0.0003	0.00228
	20-Apr-22	133	0.0094	0.000011	42.9	0.00008	0.00109	1920	6.49	596	0.041	0.0174	0.000005	0.0144	2.01	0.0085	0.0005	811	0.000005	0.00232
	27-Apr-22	127	0.0358	1.88E-05	41.6	0.00016	0.00111	1890	6.46	605	0.026	0.0151	0.000005	0.0132	2.18	0.0085	0.000414	801	0.000016	0.0024
	04-May-22	127	0.0434	9.8E-06	41.8	0.005	0.00103	1830	7.13	560	0.052	0.0281	0.000005	0.0132	1.71	0.01	0.00041	801	0.000018	0.00245
	11-May-22	108	0.332	0.000016	30.2	0.00068	0.000806	1310	9.11	438	0.291	0.0641	0.000005	0.0091	1.62	0.017	0.000328	554	0.000011	0.00202
	25-May-22	126	0.0576	0.000014	29.7	0.00046	0.000794	1300	12.94	429	0.087	0.023	0.000005	0.00966	1.85	0.01	0.000298	503	0.000021	0.00216
	01-Jun-22	1120	0.245	2.08E-05	28.2	0.00068	0.000898	1270	14.72	441	0.321	0.0346	0.000005	0.00976	1.99	0.0165	0.000228	479	0.000011	0.00207
	08-Jun-22	122	0.0534	0.00001	45.4	0.00024	0.00079	1310	16.06	389	0.081	0.0278	0.000005	0.0102	1.76	0.011	0.0004	500	0.00001	0.00193
	15-Jun-22	141	0.0274	0.000014	31.8	0.0002	0.000795	1320	18.48	409	0.104	0.0902	0.000005	0.00705	0.888	0.049	0.00032	481	0.0003	0.00163
	29-Jun-22	153	0.0272	0.000003	35.2	0.0002	0.000835	1270	17.8	400	0.149	0.031	0.000005	0.0083	0.996	0.079	0.00032	511	0.000005	0.00169
	07-Jul-22	153	0.0122	0.000003	35.2	0.0002	0.000965	1270	20.79	404	0.104	0.036	0.000005	0.00806	0.99	0.075	0.00034	516	0.0003	0.00142
	03-Aug-22	103	0.0138	0.000006	36.5	0.0001	0.00162	1400	20.9	381	0.026	0.0308	0.000005	0.0134	1.86	0.0155	0.00047	561	0.00001	0.00163
	21-Sep-22	121	0.0174	0.000032	45.2	0.00022	0.00183	1480	16.15	498	0.0995	0.0424	0.000005	0.0127	2.03	0.0145	0.000545	622	0.000005	0.00268
	21-Oct-22	118	0.0169	1.73E-05	41.7	0.00014	0.00187	1490	8.72	460	0.028	0.00861	0.000005	0.0124	2.27	0.009	0.000519	603	0.00001	0.00228
	26-Oct-22	118	0.0194	2.21E-05	42.5	0.00033	0.00199	1480	5.57	477	0.028	0.00758	0.000005	0.0138	2.66	0.012	0.000632	629	0.00001	0.0025

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Cadmium	Chloride	Chromium	Cobalt	Conductivity	Field Temp	Hardness as CaCO3	Iron	Manganese	Mercury	Molybdenum	Nitrate as N	Phosphorus	Selenium	Sulfate as SO4	Thallium	Uranium
	02-Nov-22	137	0.0142	4.69E-05	42.7	0.00028	0.00185	1540	10.17	565	0.292	0.0431	0.000005	0.0128	3.05	0.017	0.000784	632	0.00001	0.00445
	09-Nov-22	122	0.0298	2.28E-05	42.8	0.00076	0.00207	1510	6.88	523	0.273	0.0279	0.000005	0.0135	2.38	0.012	0.000736	596	0.00001	0.00279
	16-Nov-22	121	0.05	0.00005	48.4	0.005	0.0019	1500	6.85	515	0.44	0.0346	0.000005	0.0129	2.63	0.0125	0.00064	607	0.0001	0.00282
	17-Apr-23	161	0.0342	0.000026	47.3	0.00026	0.00205	1650	2.6	562	0.0365	0.0266	0.000005	0.0142	3.08	0.05	0.000815	666	0.000015	0.00292
	24-Apr-23	141	0.0188	0.000023	46	0.00016	0.00185	1640	4.44	585	0.0645	0.0262	0.000005	0.0126	3.2	0.05	0.00066	667	0.000015	0.00267
	01-May-23	224	0.0166	0.000027	47.2	0.00016	0.00194	1600	5.83	535	0.0235	0.0326	0.000005	0.0142	3.03	0.05	0.00079	668	0.000015	0.00263
	08-May-23	124	0.031	0.000022	41.3	0.00028	0.00163	1380	7.03	523	0.0485	0.0782	0.000005	0.0112	2.63	0.05	0.000645	583	0.00001	0.0025
	15-May-23	137	0.0295	1.92E-05	40.2	0.0005	0.00148	1360	11.68	459	0.044	0.0549	0.000005	0.0114	2.64	0.05	0.000618	566	0.000014	0.00246
	22-May-23	123	0.0335	1.88E-05	39.6	0.0005	0.00149	1330		440	0.043	0.046	0.000005	0.0116	2.49	0.05	0.000642	550	0.000013	0.00251
	29-May-23	124	0.0412	0.00005	36.6	0.005	0.00144	1300	17.21	507	0.101	0.0327	0.000005	0.0116	2.36	0.5	0.000594	517	0.0001	0.00258
	05-Jun-23	122	0.024	0.000013		0.0005	0.00164	1270	21.46	500	0.037	0.0461	0.000005	0.0125	2.15	0.0137	0.00072		0.000014	0.00233
	12-Jun-23	119	0.0356	1.15E-05	40	0.0005	0.00179	1390	20.58	484	0.055	0.0375	0.000005	0.0144	2.28	0.05	0.000827	545	0.000011	0.00215
	20-Jun-23	118	0.0283	1.27E-05		0.0005	0.0021	1440	22.12	445	0.083	0.0526	0.000005	0.0161	2.56	0.0212	0.000961		0.000014	0.0024
	03-Nov-23	117	0.0092	1.05E-05	55.7	0.0005	0.00288	1730	5.05	569	0.022	0.0123	0.000005	0.0161	2.31	0.05	0.000905	759	0.00001	0.00158
	06-Nov-23	117	0.0111	1.16E-05	57.3	0.0005	0.00286	1750	4.15	544	0.023	0.0113	0.000005	0.0164	2.37	0.05	0.000862	781	0.00001	0.00154
	08-Nov-23	120	0.0273	1.31E-05	52.8	0.00096	0.00294	1730		539	0.327	0.0214	0.000005	0.0165	2.24	0.05	0.000905	714	0.00001	0.00152
	13-Nov-23	119	0.0137	1.03E-05	55.2	0.001	0.00304	1650		565	0.022	0.00898	0.000005	0.0155	2.52	0.1	0.00087	758	0.00002	0.00154
	20-Nov-23	120	0.0175	8.3E-06	54.2	0.0005	0.0029	1680	2.97	545	0.039	0.00857	0.000005	0.0163	2.36	0.05	0.000798	739	0.00001	0.00159
EDL2	15-Oct-20	157	0.113	0.000008	26.8	0.00038	0.00137	1500	8.08	495	0.335	0.0374	0.00003	0.00762	0.56	0.022	0.00028	665	0.0003	0.00223
	20-Oct-20	163	0.0114	0.000004	28.2	0.00018	0.00134	1540	3.78	475	0.209	0.0564	0.00003	0.00847	0.676	0.0105	0.000235	656	0.0003	0.00239
	21-Oct-20	159	0.011	0.000003	22.8	0.00012	0.00134	1460	4.62	477	0.185	0.0564	0.00003	0.00881	0.726	0.012	0.000265	550	0.0003	0.00235
	28-Oct-20	139	0.0112	0.000005	27.8	0.0001	0.00167	1380	0.82	484	0.767	0.041	0.00003	0.0133	3.29	0.0065	0.00029	697	0.00001	0.00278
	04-Nov-20	126	0.0184	0.000008	23.9	0.00014	0.00176	1580	0.93	483	0.0545	0.015	0.00003	0.0149	4.06	0.0085	0.000345	657	0.00002	0.00305
	06-Nov-20		0.0226	0.000011		0.00006	0.00168		4.59		0.044	0.011		0.0141		0.05	0.0003		0.000025	0.00275
	08-Nov-20		0.0264	0.000013		0.00016	0.00171		7.24		0.041	0.0082		0.0136		0.05	0.000405		0.000025	0.00265
	09-Nov-20		0.0236	0.000012		0.00012	0.00167		7.37		0.0335	0.0076		0.0132		0.05	0.00034		0.000025	0.00264
	11-Nov-20	128	0.0224	0.000018	28.5	0.00012	0.00168	1580	1.25	500	0.038	0.0072	0.00003	0.0129	4.69	0.008	0.00042	685	0.000025	0.00253
	11-Nov-20	127	0.025	0.000018	28.6	0.00016	0.00169	1580	1.25	498	0.037	0.007	0.00003	0.0129	4.67	0.008	0.00028	689	0.000025	0.00259
	20-Oct-21	118	0.0076	0.000006	39.2	0.00006	0.00098	1730	9.6	525	0.124	0.0246	0.00003	0.0149	0.834	0.009	0.000245	760	0.00001	0.00226

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Cadmium	Chloride	Chromium	Cobalt	Conductivity	Field Temp	Hardness as CaCO3	Iron	Manganese	Mercury	Molybdenum	Nitrate as N	Phosphorus	Selenium	Sulfate as SO4	Thallium	Uranium
	20-Apr-22	131	0.0062	0.00001	43.3	0.00008	0.00102	1880	3.69	599	0.088	0.0362	0.000005	0.0138	1.85	0.0095	0.000425	786	0.00001	0.00227
	27-Apr-22	124	0.0304	2.06E-05	46.5	0.00016	0.00133	1860	7.52	537	0.719	0.057	0.000005	0.0124	2.05	0.009	0.000322	782	0.000017	0.00236
	04-May-22	125	0.0274	1.84E-05	42	0.005	0.00101	1820		525	0.153	0.0352	0.000005	0.0127	1.58	0.011	0.000474	782	0.000016	0.00239
	11-May-22	118	0.146	1.46E-05	36.6	0.0003	0.00104	1500	10.99	510	0.307	0.0784	0.000005	0.0098	1.66	0.0145	0.00032	642	0.000008	0.0023
	18-May-22	111	0.103	1.52E-05	29.5	0.00032	0.000834	1320	12.67	415	0.27	0.0508	0.000005	0.00926	1.54	0.014	0.000344	508	0.000009	0.00196
	25-May-22	192	0.0198	1.26E-05	31.8	0.00032	0.00132	1340	13.23	456	0.475	0.242	0.000005	0.00726	0.2	0.119	0.000322	495	0.00001	0.00178
	01-Jun-22	150	0.0468	6.4E-06	30	0.0003	0.000948	1300	13.63	437	0.368	0.106	0.000005	0.00692	0.1	0.0665	0.000214	483	0.000002	0.00176
	08-Jun-22	196	0.0326	0.000001	34.5	0.00024	0.00073	1350	15.88	411	0.44	0.088	0.000005	0.00427	0.1	0.104	0.00024	479	0.0003	0.0014
	29-Jul-22	180	0.008	0.000325	9.15	0.00064	0.00395	1750	18.33	734	1.71	1.12	0.000005	0.0109	0.134	0.0855	0.000565	137	0.0003	0.00738
	21-Oct-22	119	0.0237	2.69E-05	41.1	0.00022	0.00184	1490	9.48	479	0.083	0.0173	0.000005	0.0122	2.2	0.0135	0.00051	606	0.00001	0.00269
	26-Oct-22	122	0.023	0.00045	42.4	0.00035	0.00198	1490	7.15	487	0.131	0.0172	0.000005	0.0135	2.69	0.0195	0.000656	637	0.00001	0.00282
	02-Nov-22	145	0.0645	6.69E-05	42.7	0.00038	0.00188	1560	11.45	602	0.485	0.0888	0.000005	0.0124	2.7	0.0175	0.000755	639	0.00001	0.00525
	09-Nov-22	156	0.0298	1.78E-05	5.02	0.00052	0.00195	602	5.81	542	0.219	0.0226	0.000005	0.0131	0.81	0.009	0.00062	161	0.00001	0.00295
	17-Apr-23	157	0.0324	0.000024	47.6	0.0005	0.00203	1670	2.8	551	0.0435	0.027	0.000005	0.0144	3.09	0.05	0.00074	667	0.000015	0.00293
	24-Apr-23	137	0.0138	0.000025	46.2	0.00034	0.00213	1640	3.65	577	0.063	0.0274	0.000005	0.0127	3.04	0.05	0.00069	658	0.000015	0.00274
	01-May-23	218	0.0176	0.000028	47.8	0.00022	0.00197	1610	4.68	546	0.033	0.034	0.000005	0.0145	3.05	0.01	0.000725	661	0.000015	0.00259
	08-May-23	124	0.0412	0.000029	40.5	0.00028	0.00163	1400	7.02	533	0.089	0.082	0.000005	0.0112	2.59	0.05	0.00058	543	0.00001	0.00254
	15-May-23	138	0.0271	2.23E-05	40.6	0.0005	0.00149	1370	11.94	457	0.117	0.0604	0.000005	0.0112	2.64	0.05	0.000612	568	0.000012	0.00253
	22-May-23	123	0.0498	2.03E-05	39	0.0005	0.00149	1330		445	0.113	0.0521	0.000005	0.0118	2.46	0.05	0.000598	544	0.000015	0.00257
	29-May-23	130	0.03	0.00005	39.7	0.005	0.00142	1310	17.18	486	0.1	0.0365	0.000005	0.0113	2.6	0.5	0.000594	522	0.0001	0.00257
	05-Jun-23	124	0.0389	1.57E-05		0.0005	0.00172	1290	21.19	506	0.049	0.0495	0.000005	0.0129	2.1	0.0103	0.000801		0.000016	0.00236
	12-Jun-23	118	0.0299	1.45E-05	39.8	0.0005	0.00181	1390	21.35	491	0.094	0.0398	0.000005	0.0149	2.25	0.05	0.000876	547	0.00001	0.00207
	03-Nov-23	120	0.0202	1.26E-05	54	0.0005	0.00293	1730	5.36	554	0.047	0.0182	0.000005	0.0166	2.16	0.05	0.000895	735	0.00001	0.0016
	06-Nov-23	117	0.0128	1.36E-05	55.4	0.0005	0.00291	1740	5.28	548	0.026	0.0113	0.000005	0.0165	2.31	0.05	0.000856	756	0.00001	0.00157
	07-Nov-23	116	0.0164	1.01E-05	52.9	0.0005	0.00286	1730		564	0.029	0.0119	0.000005	0.0162	2.19	0.05	0.000873	704	0.00001	0.00159
	13-Nov-23	118	0.0154	1.03E-05	56.2	0.001	0.00304	1680		576	0.02	0.0124	0.000005	0.0155	2.49	0.1	0.000844	772	0.00002	0.00161
	13-Nov-23	118	0.0127	1.03E-05	55.8	0.001	0.00299	1670	2.98	561	0.02	0.0108	0.000005	0.0152	2.53	0.1	0.000772	765	0.00002	0.0016
	13-Nov-23	2	0.0229	0.000005	0.1	0.0005	0.0001	3.6		0.59	0.01	0.0001	0.000005	0.00005	0.02	0.05	0.00005	0.3	0.00001	0.00001
	20-Nov-23	119	0.0184	8.4E-06	55.6	0.0005	0.00288	1670	2.77	544	0.022	0.0094	0.000005	0.0158	2.4	0.05	0.000864	752	0.00001	0.00162

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Cadmium	Chloride	Chromium	Cobalt	Conductivity	Field Temp	Hardness as CaCO3	Iron	Manganese	Mercury	Molybdenum	Nitrate as N	Phosphorus	Selenium	Sulfate as SO4	Thallium	Uranium
SP2	07-Oct-19	178	0.168	0.000006	4.19	0.00026	0.000152	366	9.2	185	0.158	0.00706	0.00003	0.00366	0.075	0.014	0.000545	47.9	0.0003	0.00147
	15-Oct-19	159	0.188	0.000008	4.01	0.00042	0.00019	400	7.14	191	0.217	0.0103	0.00003	0.00329	0.07	0.0215	0.00049	55.1	0.000003	0.00146
	21-Oct-19	164	0.164	0.000006	3.71	0.00036	0.000168	403	8.11	199	0.191	0.00836	0.00003	0.00286	0.108	0.021	0.00058	59.7	4.2E-06	0.00156
	28-Oct-19	166	0.188	0.000005	3.97	0.0004	0.00016	439	4.29	221	0.2	0.0072	0.00003	0.00292	0.174	0.017	0.0006	71	0.000004	0.00153
	04-Nov-19	167	0.137	0.000008	3.99	0.00028	0.000139	452	1.26	216	0.137	0.00622	0.00003	0.00315	0.326	0.0155	0.00074	72.2	0.000001	0.00179
	24-Jun-20	181	0.0486	4.4E-06	4.47	0.00056	0.000092	460		243	0.023	0.00678	0.00003	0.00282	0.02	0.0087	0.000512	77.3	0.000004	0.00165
	30-Jun-20	188	0.0326	4.4E-06	4.5	0.00102	0.00009	468		241	0.017	0.00452	0.00003	0.00301	0.02	0.0139	0.000532	78	0.000004	0.00168
	08-Jul-20	185	0.0402	4.8E-06	6.01	0.00106	0.000094	468		240	0.024	0.00454	0.00003	0.00306	0.03	0.0132	0.000484	78.6	0.000005	0.0015
	15-Jul-20	183	0.0562	0.000004	4.89	0.0002	0.0001	462		229	0.049	0.0074	0.000005	0.00323	0.042	0.0135	0.000485	76	0.0003	0.00163
	28-Sep-20	157	0.081	0.000006	6.56	0.00024	0.00013	498		222	0.102	0.005	0.000005	0.00375	0.96	0.01	0.00045	82.1	0.0003	0.0017
	28-Sep-20	164	0.0828	0.000004	6.73	0.00026	0.00013	482	13.26	220	0.103	0.0046	0.000005	0.00363	0.098	0.009	0.000515	101	0.0003	0.00176
	07-Oct-20	154	0.0344	0.000004	5.97	0.00018	0.00009	477	12.26	232	0.027	0.0026	0.00003	0.00362	0.02	0.0065	0.000425	101	0.0003	0.00164
	13-Oct-20	150	0.0322	0.000004	6.25	0.0002	0.00008	476		216	0.021	0.0026	0.00003	0.00355	0.01	0.01	0.00042	109	0.0003	0.00173
	20-Oct-20	149	0.0482	0.000003	6.02	0.00014	0.000095	470	3.57	213	0.049	0.003	0.00003	0.00348	0.02	0.009	0.000415	102	0.0003	0.00175
	21-Oct-20	148	0.0468	0.000004	5.66	0.00018	0.00008	468	3.04	212	0.0475	0.003	0.00003	0.00345	0.02	0.008	0.000445	97.9	0.0003	0.00167
	28-Oct-20	148	0.0534	0.000005	5.73	0.0002	0.000095	402	0.95	217	0.048	0.0032	0.00003	0.00353	0.008	0.0075	0.000405	95.6	0.0003	0.0017
	04-Nov-20	171	0.0444	0.000002	6.41	0.00018	0.000105	529	8.08	243	0.0545	0.0068	0.00003	0.00338	0.04	0.0105	0.00042	121	0.0003	0.00188
	11-Nov-20	151	0.0458	0.000002	5.34	0.00016	0.000085	474	5.77	230	0.0475	0.0032	0.00003	0.00366	0.002	0.008	0.00036	94.7	0.0003	0.00179
	18-Nov-20	149	0.0482	0.000002	5.75	0.00012	0.00007	480	6.39	238	0.0485	0.0032	0.00003	0.00354	0.0185	0.008	0.000315	105	0.0003	0.00173
	25-Nov-20	158	0.0724	0.000003	6.08	0.00026	0.00009	514	6.39	247	0.083	0.0114	0.00003	0.00434	0.0165	0.0085	0.000405	112	0.0003	0.00206
	14-Apr-21	156	0.245	6.6E-06	4.37	0.00056	0.000202	488		242	0.262	0.0245	0.00003	0.00369	0.148	0.0132	0.00035	93.8	0.000006	0.00208
	15-Apr-21	156	0.22	7.4E-06	4.1	0.0005	0.00019	489	7.3	239	0.238	0.0246	0.00003	0.00363	0.144	0.0119	0.00034	95.8	0.000005	0.0021
	21-Apr-21	161	0.0816	0.000007	5.52	0.00024	0.000148	539	6.2	290	0.085	0.0181	0.00003	0.00357	0.412	0.0148	0.000488	123	0.000006	0.00215
	28-Apr-21	162	0.0596	0.000006	6.84	0.00046	0.00014	554	8.3	317	0.055	0.0159	0.00003	0.00402	0.54	0.0124	0.000565	154	0.000006	0.00277
	05-May-21	168	0.0458	0.000006	6.85	0.00016	0.000138	574	10.85	300	0.046	0.0137	0.00003	0.00366	0.384	0.0183	0.000565	134	0.000004	0.00241
	26-May-21	179	0.0526	0.00001	6.01	0.00018	0.00013	573	15.8	280	0.056	0.017	0.00003	0.00393	0.15	0.0165	0.00045	143	0.0003	0.00286
	02-Jun-21	183	0.0222	0.000004	6.44	0.00018	0.000115	603	18.42	277	0.0305	0.0162	0.00003	0.00386	0.1	0.016	0.000505	148	0.0003	0.00276
	22-Sep-21	138	0.168	0.000017	7.41	0.0004	0.00032	610	16.3	243	0.207	0.0132	0.00003	0.00366	4.2	0.0285	0.00052	162	0.0003	0.00197
	13-Oct-21	144	0.0036	0.000003	8.09	0.00006	0.000315	639	16.6	275	0.005	0.002	0.00003	0.00398	5.08	0.015	0.000455	165	0.000005	0.00199

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Cadmium	Chloride	Chromium	Cobalt	Conductivity	Field Temp	Hardness as CaCO3	Iron	Manganese	Mercury	Molybdenum	Nitrate as N	Phosphorus	Selenium	Sulfate as SO4	Thallium	Uranium
	20-Oct-21	156	0.0272	0.000004	8.51	0.00012	0.000395	666	10.3	288	0.037	0.006	0.00003	0.00445	6.61	0.024	0.000495	170	0.0003	0.00213
	25-Oct-21	161	0.019	0.000005	8.59	0.00014	0.00042	682	8.5	285	0.0255	0.005	0.00003	0.0047	7.22	0.013	0.000585	174	0.0003	0.00217
	13-Apr-22		0.23	0.00001		0.00048	0.000315		7.49	207	0.279	0.062	0.00003	0.00265		0.019	0.000395		0.0003	0.00223
	27-Apr-22	126	0.56	1.54E-05	3.53	0.001	0.000368	470	5.64	202	0.486	0.0452	0.000005	0.00275	3.32	0.021	0.000524	98.8	0.000009	0.00217
	04-May-22	135	0.245	0.000014	3.96	0.0003	0.000336	514	5.99	222	0.268	0.0364	0.000005	0.00319	3.51	0.012	0.000584	120	0.000007	0.00242
	11-May-22	202	0.081	6.2E-06	9.4	0.00024	0.000414	734	10.08	359	0.088	0.0195	0.000005	0.00522	6.47	0.015	0.000654	205	0.000006	0.00335
	18-May-22	156	0.157	8.2E-06	5.55	0.00038	0.000314	624	13.06	275	0.144	0.00804	0.000005	0.00465	4.67	0.0135	0.000798	148	0.000006	0.00312
	25-May-22	210	0.176	0.000051	5.8	0.0004	0.000378	618	13.81	289	0.213	0.0165	0.000005	0.00487	4.6	0.016	0.000806	147	0.000006	0.00315
	01-Jun-22	1100	0.189	0.000207	4.96	0.0005	0.00037	1	14.57	261	0.235	0.0252	0.000005	0.00376	3.34	0.018	0.000614	127	0.000006	0.00248
	08-Jun-22	199	0.0438	0.000087	5.4	0.0003	0.000225	573	16.68	250	0.074	0.011	0.000005	0.00398	2.89	0.0145	0.00059	131	0.0003	0.00231
	15-Jun-22	148	0.0168	0.000058	4.92	0.00018	0.000205	590	19.43	255	0.0235	0.0084	0.000005	0.00403	2.73	0.0105	0.000685	131	0.0003	0.00228
	22-Jun-22	10.6	0.0374	0.000052	5.17	0.00022	0.00019	554	21.28	249	0.0445	0.0054	0.000005	0.00409	2.42	0.0125	0.000615	134	0.0003	0.00251
	29-Jun-22	158	0.057	0.000021	5.1	0.00028	0.000195	557	19.01	256	0.0615	0.0068	0.000005	0.00437	2.27	0.015	0.0006	143	0.0003	0.0025
	07-Jul-22	178	0.0416	0.000013	5	0.00022	0.000185	553	21.98	248	0.0475	0.0064	0.000005	0.00444	1.99	0.012	0.00051	149	0.0003	0.00253
	28-Jul-22	134	0.0368	0.000017	4.22	0.00012	0.000155	600	21.81	233	0.039	0.0058	0.000005	0.00485	1.27	0.006	0.00051	150	0.0003	0.00251
	03-Aug-22	132	0.0284	0.000003	4.63	0.00014	0.00015	573	21.9	241	0.028	0.005	0.000005	0.0051	1.26	0.01	0.00059	158	0.0003	0.0025
	21-Sep-22	146	0.0168	0.00001	4.66	0.00028	0.000115	558	16.14	288	0.016	0.005	0.000005	0.00454	0.448	0.008	0.00042	140	0.0003	0.00195
	19-Oct-22	158	0.0288	0.000008	4.54	0.00024	0.000092	573	5.55	256	0.02	0.00278	0.000005	0.00417	0.352	0.015	0.000368	144	0.000002	0.00197
	26-Oct-22	154	0.035	8.4E-06	4.76	0.00027	0.0001	568	7.02	237	0.031	0.00321	0.000005	0.00436	0.498	0.0115	0.000404	155	0.00001	0.00208
	02-Nov-22	156	0.0368	5.1E-06	4.65	0.0003	0.0001	587	7.2	257	0.031	0.00256	0.000005	0.00461	0.472	0.0525	0.000408	154	0.00001	0.00208
	09-Nov-22	122	0.0424	6.4E-06	43.3	0.00026	0.000126	1510	3.96	256	0.042	0.00394	0.000005	0.00468	2.62	0.014	0.00035	608	0.000003	0.00224
	16-Nov-22	156	0.0333	9.3E-06	5.3	0.0005	0.00014	615	2.08	272	0.034	0.0041	0.000005	0.0048	1.13	0.0115	0.000404	169	0.00001	0.0023
	14-Apr-23	110	0.476	0.000037	2.99	0.00104	0.000385	391	2.89	176	0.52	0.101	0.000005	0.00296	1.18	0.01	0.000425	89.1	0.00001	0.00171
	17-Apr-23	164	0.483	0.000028	4.46	0.00102	0.00036	510	1.58	233	0.398	0.0822	0.000005	0.00338	1.42	0.02	0.00044	122	0.000005	0.00217
	24-Apr-23	196	0.12	0.000015	5.34	0.00034	0.00023	579	3.48	285	0.177	0.106	0.000005	0.0034	1.23	0.05	0.000425	136	0.0003	0.00215
	01-May-23	359	0.0866	0.00001	6.05	0.00032	0.000235	606	4.14	280	0.092	0.103	0.000005	0.00358	1.25	0.04	0.000415	150	0.0003	0.00221
	08-May-23	222	0.032	0.000009	8.31	0.00022	0.000185	623	10.1	331	0.047	0.0392	0.000005	0.00321	1.15	0.02	0.000395	172	0.000005	0.00231
	15-May-23	254	0.0375	5.8E-06	6.75	0.0005	0.00016	652	17.64	300	0.039	0.021	0.000005	0.00346	1.19	0.05	0.000419	177	0.00001	0.00232
	05-Jun-23	201	0.0412	7.3E-06		0.0005	0.00017	636	25.26	335	0.038	0.0108	0.000005	0.00431	1.36	0.0149	0.000451		0.00001	0.00266

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Cadmium	Chloride	Chromium	Cobalt	Conductivity	Field Temp	Hardness as CaCO3	Iron	Manganese	Mercury	Molybdenum	Nitrate as N	Phosphorus	Selenium	Sulfate as SO4	Thallium	Uranium
	20-Sep-23	135	0.011	0.000005	7.83	0.0005	0.00012	741		348	0.024	0.0195	0.000005	0.00473	0.336	0.05	0.000379	245	0.00001	0.0024
	20-Oct-23	140	0.0203	0.000005	8.1	0.0005	0.00011	755	11.07	317	0.046	0.0318	0.000005	0.00446	0.049	0.05	0.00036	268	0.00001	0.00221
	22-Oct-23	141	0.0204	0.000005	8.22	0.0005	0.00011	763	11.14	314	0.042	0.0317	0.000005	0.00444	0.048	0.05	0.000366	268	0.00001	0.00222
	24-Oct-23	144	0.0318	0.000005	7.95	0.0005	0.00012	761	10.51	304	0.034	0.0311	0.000005	0.0039	0.054	0.05	0.000335	258	0.00001	0.00215
	30-Oct-23	144	0.0194	0.000005	8.18	0.00126	0.00014	758	4.87	332	0.032	0.0319	0.000005	0.0049	0.56	0.05	0.000315	266	0.00001	0.00237
	05-Nov-23	151	0.0134	6.3E-06	8.53	0.0005	0.00013	799		339	0.026	0.0289	0.000005	0.00491	0.8	0.05	0.000297	282	0.00001	0.00266
	13-Nov-23	149	0.0142	0.000005	8.53	0.0005	0.00013	768		357	0.023	0.0289	0.000005	0.00476	0.796	0.05	0.000336	283	0.00001	0.00255
	20-Nov-23	150	0.0124	0.000005	8.28	0.0005	0.00013	765	4.17	330	0.018	0.0293	0.000005	0.00491	0.748	0.05	0.000301	277	0.00001	0.0026

Table A-3: Subset of surface water chemistry provided by New Gold, 2020–2023.

Note: In the few cases of duplicates, we used the first instance for generating summary statistics.

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Ammonia, Total as N	Ammonia, Un-ionized as N	Arsenic	Cadmium	Chloride	Chromium	Cobalt	Field Conductivity	Copper	Cyanide, Total	Field Dissolved Oxygen	Field pH	Hardness as CaCO3	Iron	Lead	Mercury	Molybdenum	Nickel	Nitrate as N	Phosphorus	Selenium	Thallium	Total Suspended Solids	Uranium	Zinc
SW20	09-Jan-20	158	0.166	0.04	0.01	0.00092	0.000019	9.08	0.00056	0.000649		0.00074	0.0008		7.62	158	1.4	0.000175	0.00003	0.000272	0.00168	0.128	0.06	0.000215	2.6E-06	4.5	0.000515	0.006
	05-Feb-20	171	0.098	0.066	0.01	0.00088	0.000018	9.28	0.00036	0.000747		0.00064	0.0006			178	1.37	0.00013	0.00003	0.000198	0.0016	0.043	0.045	0.00016	2.2E-06	3	0.000675	0.0036
	10-Mar-20	156	0.154	0.106	0.01	0.00085	0.000011	8.32	0.0005	0.00072		0.00098	0.0006			179	1.28	0.00019	0.00003	0.000192	0.00148	0.057	0.055	0.00013	0.0003	6	0.000535	0.012
	08-Apr-20	62	1.06	0.03	0.01	0.00065	0.00001	5.23	0.00164	0.00045		0.0017	0.0002			67.9	1.09	0.00049	0.000005	0.00033	0.0019	0.068	0.046	0.0001	0.000008	12.5	0.00027	0.0075
	12-May-20	107	0.164	0.02	0.01	0.00087	0.00001	19.8	0.00044	0.000202		0.0011	0.0008			123	0.431	0.000135	0.000005	0.000422	0.00152	0.02	0.022	0.00017	0.000004	4.5	0.000507	0.0035
	16-Jun-20	112	0.118	0.0003		0.00131	1.02E-05	19.1	0.00044	0.00027		0.00105	0.0008			125	0.548	0.00014	0.00003	0.0004	0.00176	0.018	0.0418	0.000212	0.000004	4	0.000339	0.0034
	07-Jul-20	156	0.0388	0.000139		0.00305	0.000004	19.4	0.00036	0.00073		0.00035	0.001			166	1.05	0.00008	0.00003	0.000405	0.0019	0.008	0.106	0.000252	0.000001	7	0.000284	0.0008
	11-Aug-20	158	0.145	0.028	0.01	0.00183	0.00002	19.5	0.00046	0.00036		0.00152	0.0002			149	0.725	0.00015	0.00003	0.00021	0.0014	0.02	0.07	0.000225	0.0003	5.5	0.000208	0.002
	15-Sep-20	179	0.0266	0.012	0.01	0.00094	0.000002	37.1	0.00024	0.000185		0.00032	0.0004			185	0.2	0.00003	0.00003	0.00023	0.00118	0.02	0.02	0.00012	0.0003	1.5	0.000466	0.0035
	15-Sep-20										407			1.4	7.13													
	14-Oct-20	179	0.1	0.012	0.01	0.00086	0.000001	32.2	0.00038	0.00019	389	0.00034	0.0006		6.81	186	0.284	0.00005	0.00003	0.000278	0.00124	0.006	0.015	0.000115	0.0003	2.5	0.000584	0.003
	04-Nov-20	137	0.0986	0.02	0.01	0.00069	0.000005	42.1	0.00042	0.0002	351	0.00058	0.0004		7.39	162	0.379	0.00007	0.00003	0.000302	0.00132	0.01	0.01	0.000125	0.0003	2.5	0.00123	0.0005
	10-Nov-20	133	0.0988	0.008	0.01	0.00068	0.000004	44.4	0.00034	0.000165	3960	0.0006	0.002	2.83	7.14	162	0.344	0.00008	0.00003	0.000304	0.00124	0.01	0.02	0.00009	0.0003	1.5	0.000787	0.0015
	15-Dec-20	157	0.142	4.60E-05		0.00087	0.00001	15.6	0.0005	0.000295	338	0.0008	0.0012		7.19	164	0.865	0.00014	0.00003	0.000218	0.0016	0.058	0.035	0.00013	0.000005	3.5	0.00049	0.005
	13-Jan-21	177	0.132	0.000157	0.000157	0.0009	0.000016	9.24	0.00066	0.000465	197.5	0.00068	0.001	3.12	7.01	172	1.08	0.00016	0.00003	0.000202	0.00166	0.038	0.04	0.000125	0.0003	4	0.000513	0.0055
	24-Mar-21										255			26.9	6.93													
	21-Apr-21	85.8	0.174	7.70E-05		0.000605	5.6E-06	18.6	0.00046	0.000124	236	0.00115	0.001	8.95	7.46	117	0.264	0.00008	0.00003	0.000455	0.00122	0.02	0.012	0.000118	0.000004	0.5	0.000414	0.0028
	11-May-21	122	0.132	0.096		0.00087	0.000006	26.7	0.00054	0.00022		0.00098	0.0008			146	0.366	0.00008	0.00003	0.000442	0.00152	0.02	0.025	0.000175	0.0003	3	0.000561	0.0015
	11-May-21	122	0.14	0.038	0.01	0.00085	0.000009	28	0.00048	0.000205	333	0.001	0.001	9.6	7.57	146	0.372	0.00009	0.00003	0.000408	0.00156	0.02	0.025	0.000205	0.0003	3.5	0.000546	0.0015
	08-Jun-21	175	0.0156	0.016	0.01	0.00145	0.000004	34.8	0.00022	0.000195	431	0.00038	0.0006	3.02	7.58	184	0.247	0.00005	0.00003	0.000264	0.00144	0.02	0.035	0.000215	0.0003	4	0.000415	0.002
	13-Jul-21	177	0.125	0.03	0.01	0.00238	0.000001	38.2	0.00028	0.000525	127	0.00054	0.0012	2.8	7.57	185	0.538	0.00004	0.00003	0.000308	0.0015	0.008	0.055	0.00023	0.0003	7.5	0.000714	0.002
	14-Sep-21	169	0.043	0.03	0.01	0.00173	0.000017	39.4	0.00018	0.00027	346.6	0.00034	0.001	0.72	7.33	185	1.12	0.00005	0.00003	0.0005	0.00094	0.006	0.055	0.000105	0.0003	4	0.000606	0.0015
	12-Oct-21	96.4	0.242	0.016	0.01	0.00111	0.000013	28.3	0.00074	0.000205	460	0.0016	0.0028	6	7.66	120	0.597	0.00013	0.00003	0.000566	0.0017	0.016	0.03	0.00022	0.0003	3	0.000295	0.005
	09-Nov-21	90.4	0.119	0.018	0.01	0.00076	0.000007	16.4	0.00048	0.00013	150.4	0.00106	0.001	10.07	7.3	112	0.368	0.00007	0.00003	0.000312	0.00142	0.004	0.01	0.00022	0.0003	2	0.00037	0.004
	14-Dec-21	104	0.254	0.032	0.01	0.00074	0.000009	10.8	0.0007	0.0002	263	0.00076	0.0006	12.64	5.05	130	0.543	0.00012	0.00003	0.000236	0.00142	0.04	0.01	0.000145	0.0003	3	0.00036	0.0055
	11-Jan-22	110	0.176	0.036	0.01	0.00064	0.000013	4.14	0.0007	0.00019	248	0.00078	0.0006	13.64	6.66	120	0.491	0.00015	0.00003	0.000142	0.00102	0.042	0.025	0.000135	0.0003	3.5	0.000279	0.0055
	08-Feb-22	134	0.2	0.088	0.01	0.00074	0.000028	5.25	0.00078	0.000455	293	0.00162	0.0004	13.61	5.96	140	0.847	0.00031	0.00003	0.000148	0.0013	0.046	0.025	0.000125	0.0003	11	0.00035	0.0115
	08-Mar-22	120	0.259	0.13	0.01	0.00064	0.000009	3.94	0.00076	0.000405	244	0.0009	0.0004	9.45	6.53	125	0.846	0.00023	0.00003	0.000154	0.00124	0.074	0.045	0.000105	0.0003	11.5	0.000318	0.006
	05-Apr-22										0.277			9.37	7.21													
	03-May-22	44.4	0.436	0.004	0.01	0.000535	1.24E-05	4.81	0.00056	0.000194	108	0.0012	0.0008	9.75	6.21	49.6	0.466	0.00026	0.000005	0.00035	0.00118	0.018	0.02	0.000116	0.000008	5.5	0.000187	0.0048
	07-Jun-22	83.4	0.231	0.006	0.01	0.00097	0.000006	7.27	0.00072	0.00021	174	0.00092	0.001	6.71	7.03	91.2	0.48	0.00015	0.000005	0.00035	0.00162	0.02	0.015	0.00019	0.0003	3	0.000219	0.0045
	05-Jul-22	128	0.104	0.026		0.00138	0.000004	13.7	0.0005	0.00027	255	0.00058	0.001	10.68	6.63	123	0.495	0.00009	0.000005	0.000348	0.00134	0.002	0.035	0.00014	0.0003	5	0.000228	0.004
	09-Aug-22	2	0.0028	0.005		0.00001	0.000017	0.1	0.00052	0.0005		0.001	0.0002			0.51	0.002	0.00005	0.000005	0.001	0.00016	0.004	0.05	0.00005	0.0003	3	0.005	0.0005
	09-Aug-22	122	0.397	0.028	0.028	0.00222	0.000009	11.1	0.00252	0.0007	264	0.00088	0.0014	2.25	6.82	125	1.23	0.00032	0.000005	0.000302	0.00298	0.004	0.07	0.000275	0.0003	14	0.000274	0.0045
	06-Sep-22	137	0.096	8.30E-05		0.00177	0.000001	17.8	0.00042	0.00044	323	0.00018	0.0012	2.25	6.96	157	0.677	0.00007	0.000005	0.00025	0.00158	0.02	0.0611	0.000195	0.0003	2.5	0.000247	0.0005
	04-Oct-22	2	0.0004	0.005		0.0001	0.000005	0.1	0.00016	0.0001		0.0005	0.002			0.5	0.01	0.00005	0.000005	0.00005	0.0005	0.002	0.006	0.00005	0.00001	3	0.00001	0.003
	04-Oct-22	141	0.0622	0.008	0.01	0.001	0.000005	20.1	0.00046	0.000244	306	0.0004	0.0006	1.35	7.64	156	0.342	0.00008	0.000005	0.00018	0.00122	0.004	0.04	0.00015	0.000002	6	0.000303	0.0012

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Ammonia, Total as N	Ammonia, Un-ionized as N	Arsenic	Cadmium	Chloride	Chromium	Cobalt	Field Conductivity	Copper	Cyanide, Total	Field Dissolved Oxygen	Field pH	Hardness as CaCO3	Iron	Lead	Mercury	Molybdenum	Nickel	Nitrate as N	Phosphorus	Selenium	Thallium	Total Suspended Solids	Uranium	Zinc
	11-Nov-22	127	0.129	0.022	0.01	0.00067	0.000008	30.1	0.00078	0.000244	358	0.0006	0.0008	8.45	6.64	144	0.479	0.00012	0.000005	0.00026	0.00132	0.008	0.022	0.00013	0.000003	5	0.000486	0.0024
	10-Dec-22										381			2.93	6.58													
	07-Jan-23	2	0.005	0.005		0.001	0.000017	0.19	0.00014	0.0005		0.001	0.002			0.51	0.02	0.00005	0.000005	0.001	0.00008	0.02	0.005	0.00005	0.0003	3	0.005	0.001
	07-Jan-23	130	0.147	0.024	0.01	0.00067	0.000014	12.6	0.00034	0.00031	262	0.00052	0.0008	3.05	8.11	139	0.714	0.00015	0.000005	0.00015	0.00116	0.024	0.035	0.00005	0.0003	3.5	0.000346	0.0025
	07-Feb-23	154	0.206	0.07	0.01	0.00092	0.000033	15.2	0.00058	0.000785	183	0.00062	0.0014	2.15	7.42	159	1.37	0.00072	0.000005	0.000122	0.0015	0.03	0.055	0.0001	0.0003	8	0.000424	0.017
	07-Mar-23	171	0.178	0.166	0.01	0.000985	1.58E-05	16.5	0.00086	0.000976	343	0.0007	0.0006	2.96	7.68	185	1.69	0.00042	0.000005	0.000205	0.00142	0.104	0.08	0.000126	0.000004	8.5	0.000576	0.007
	02-May-23	77.4	0.254	0.016	0.01	0.00053	0.000009	13.1	0.00072	0.00014	195	0.00106	0.0018	10.7	7.36	85.9	0.345	0.00015	0.000005	0.000392	0.00122	0.008	0.01	0.00012	0.00001	5	0.000298	0.003
	06-Jun-23	106	0.122	0.0387	0.001	0.00201	9.5E-06	15.2	0.00054	0.00051	494	0.00086	0.002	3.51	7.16	118	0.827	0.000124	5.7E-06	0.000441	0.00195	0.02	0.086	0.000254	0.00001	3	0.000273	0.003
	08-Jul-23	117	0.223	0.0327	0.001	0.00176	0.000006	21	0.00057	0.00058	301	0.00127	0.002	5.22	7.25	131	0.914	0.000149	0.000005	0.000235	0.00179	0.02		0.000267	0.00001	7.2	0.000218	0.003
	05-Aug-23	144	0.11	0.0186	0.001	0.00188	0.000008	22.7	0.00052	0.0005	364	0.0008	0.002	3.18	7.29	158	0.667	0.000104	0.000005	0.000136	0.00162	0.02	0.075	0.000239	0.00001	6.4	0.000246	0.003
	04-Sep-23	171	0.136	0.017	0.001	0.00175	6.2E-06	23.6	0.0005	0.00044	397	0.00299	0.002	2.74	7.23	175	0.849	0.000165	0.000005	0.000135	0.00165	0.02	0.076	0.000183	0.00001	8.2	0.000447	0.003
	04-Oct-23	185	0.0936	0.0266	0.001	0.00151	0.000005	22.4	0.00056	0.00038	391	0.00051	0.002	2.97	7.31	179	0.512	0.000082	0.000005	0.000101	0.0015	0.02	0.065	0.000153	0.00001	5.3	0.00032	0.003
	13-Nov-23	139	0.192	0.0144	0.001	0.00072	6.2E-06	53	0.00053	0.00021	447	0.00107	0.002	8.61	8.15	174	0.421	0.000106	0.000005	0.000328	0.00131	0.09	0.05	0.00012	0.00001	3.5	0.000823	0.003
	09-Dec-23	152	0.159	0.0376	0.001	0.00085	5.9E-06	43	0.00064	0.00036	513	0.00114	0.002	4.44	7.42	180	0.758	0.000161	0.000005	0.000257	0.00192	0.02	0.05	0.000153	0.00001	3.7	0.000589	0.004
SW22A	09-Jan-20	188	0.306	0.062	0.01	0.00101	0.000024	10.8	0.00082	0.000648		0.00148	0.0008		7.29	204	1.23	0.00024	0.00003	0.000444	0.0021	0.06	0.08	0.000205	4.6E-06	9.5	0.00111	0.0104
	05-Feb-20	189	0.193	0.07	0.01	0.00103	0.000014	9.76	0.00058	0.000599		0.00116	0.0006			198	1.25	0.000215	0.00003	0.000318	0.002	0.054	0.08	0.00016	3.4E-06	6	0.00127	0.0086
	11-Mar-20	205	0.276	0.088	0.01	0.00097	0.000013	14.4	0.00066	0.00064		0.00144	0.0004			229	1.18	0.00024	0.00003	0.000476	0.00214	0.117	0.08	0.00019	0.0003	8.5	0.0016	0.023
	09-Apr-20	82.4	0.603	0.03	0.01	0.00068	0.000011	4.69	0.00102	0.000316		0.00166	0.0004			94.6	0.729	0.00031	0.000005	0.000552	0.00162	0.088	0.052	0.000145	0.000001	9	0.000395	0.004
	13-May-20	135	0.241	0.02	0.01	0.00097	0.00001	8.82	0.00058	0.000348		0.00144	0.0008			149	0.562	0.00023	0.000005	0.00057	0.0017	0.004	0.032	0.00018	0.000004	9.5	0.000909	0.004
	17-Jun-20	150	0.0848	0.00071		0.00162	0.000007	13.9	0.00032	0.000226		0.00095	0.0012			166	0.373	0.00018	0.00003	0.00054	0.00178	0.016	0.073	0.000222	0.000003	5	0.000495	0.0026
	10-Jul-20	184	0.133	0.00103		0.00212	0.00001	11.6	0.00038	0.000235		0.0008	0.0008			210	0.303	0.00009	0.00003	0.00125	0.00158	0.006	0.05	0.000245	0.0003	3	0.000548	0.0015
	11-Aug-20	181	0.459	0.042	0.01	0.00404	0.000019	11.8	0.00096	0.000565		0.00188	0.0004			180	0.721	0.00043	0.000005	0.000804	0.00246	0.004	0.205	0.00026	0.00001	20	0.000731	0.0055
	15-Sep-20	241	0.0294	0.014	0.01	0.00148	0.000002	12.6	0.00026	0.00017		0.00026	0.0004			224	0.168	0.00003	0.00003	0.000352	0.00124	0.02	0.07	0.00015	0.0003	4	0.000434	0.001
	15-Sep-20										435				7.05													
	19-Oct-20	184	0.0752	0.068	0.01	0.00258	0.000003	23.3	0.00024	0.000615		0.00096	0.0004			330	0.222	0.00008	0.00003	0.00342	0.00114	0.16	0.05	0.000205	0.0003	2.5	0.00168	0.002
	04-Nov-20	165	0.053	0.034	0.01	0.00129	0.000006	24	0.00022	0.000895	1000	0.00264	0.0004	6.23	7.26	342	0.151	0.00015	0.00003	0.00667	0.0017	1.88	0.015	0.000285	0.00001	1.5	0.00224	0.0005
	10-Nov-20	152	0.0498	0.01	0.01	0.00132	0.000011	27.3	0.0002	0.00084	933	0.00368	0.0002	6.03	7.46	330	0.141	0.0001	0.00003	0.00588	0.00156	2.17	0.02	0.000195	0.00001	0.5	0.00182	0.0015
	16-Dec-20	235	0.119	5.60E-05		0.00122	0.000016	33.7	0.00042	0.00078	1190	0.00078	0.0012		7.38	278	0.988	0.00013	0.00003	0.000364	0.00202	0.046	0.075	0.00019	0.0003	4.5	0.00112	0.0035
	12-Jan-21	245	0.214	5.20E-05	0.000052	0.00177	0.000011	28.7	0.00072	0.00214	605	0.0007	0.0008	0	6.88	277	2.77	0.00024	0.00003	0.000214	0.00208	0.022	0.23	0.00018	0.0003	13.5	0.000835	0.005
	24-Mar-21										277			6.54	6.85													
	21-Apr-21	117	0.105	0.016		0.0007	5.8E-06	14.1	0.00036	0.00015		0.0012	0.0012			165	0.222	0.00006	0.00003	0.000845	0.00118	0.002	0.014	0.000172	0.000003	2.5	0.000894	0.0016
	21-Apr-21	117	0.115	7.60E-05		0.000725	5.4E-06	13.3	0.00034	0.000146	300.2	0.0012	0.001	11.83	7.42	164	0.225	0.00008	0.00003	0.000875	0.00118	0.02	0.018	0.000158	0.000004	2.5	0.000884	0.0014
	12-May-21	163	0.0792	0.01	0.01	0.00107	0.000007	10.7	0.00034	0.000175	432	0.00106	0.001	6.95	8.11	206	0.198	0.00004	0.00003	0.00147	0.00136	0.02	0.015	0.00027	0.0003	3	0.00136	0.001
	08-Jun-21	216	0.135	0.036	0.01	0.00295	0.000009	10.5	0.00042	0.00045	853	0.00054	0.001	4.69	7.33	240	0.39	0.00008	0.000005	0.00127	0.00174	0.006	0.135	0.000265	0.0003	8.5	0.00107	0.0015
	15-Sep-21	253	0.161	0.046	0.01	0.00163	0.000002	23.1	0.00042	0.000265	994	0.00062	0.0006	2.39	7.71	390	0.383	0.00009	0.00003	0.000878	0.00184	0.018	0.075	0.000145	0.0003	8.5	0.00177	0.002
	13-Oct-21	129	0.061	0.024	0.01	0.00131	0.000007	17.6	0.00036	0.00018	577	0.00106	0.0022	4.5	7.33	165	0.325	0.00003	0.00003	0.0011	0.00158	0.22	0.04	0.00026	0.0003	2.5	0.000444	0.002
	09-Nov-21	131	0.065	0.036	0.01	0.00122	0.000013	12	0.00032	0.00026	268.8	0.00126	0.0008	10.13	7.27	188	0.246	0.00004	0.00003	0.00174	0.00124	2.37	0.01	0.00033	0.0003	1	0.00109	0.002
	14-Dec-21	140	0.122	0.028	0.01	0.00103	0.000011	13	0.00052	0.000355	363	0.00112	0.001	9.38	5.06	183	0.67	0.00007	0.00003	0.000366	0.0019	0.018	0.025	0.00021	0.0003	16	0.000602	0.017
	11-Jan-22	176	0.128	0.036	0.01	0.00117	0.000022	14.3	0.0007	0.00092	412	0.00094	0.001	16.16	6.33	196	1.26	0.00017	0.00003	0.000208	0.002	0.01	0.1	0.000235	0.0003	4	0.000612	0.009
	16-Feb-22	186	0.133	0.06	0.01	0.00108	0.000014	9.79	0.00056	0.00101	396	0.00066	0.0012	9.71	8.03	195	1.5	0.00014	0.00003	0.00014	0.00166	0.028	0.11	0.000185	0.0003	5	0.000593	0.0055
	08-Mar-22	199	0.159	0.102	0.01	0.00101	0.000013	9.25	0.00056	0.000855	394	0.00076	0.0014	0	7.09	202	1.26	0.00018	0.00003	0.00024	0.00186	0.02	0.11	0.00017	0.0003	3.5	0.000885	0.0135

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Ammonia, Total as N	Ammonia, Un-ionized as N	Arsenic	Cadmium	Chloride	Chromium	Cobalt	Field Conductivity	Copper	Cyanide, Total	Field Dissolved Oxygen	Field pH	Hardness as CaCO3	Iron	Lead	Mercury	Molybdenum	Nickel	Nitrate as N	Phosphorus	Selenium	Thallium	Total Suspended Solids	Uranium	Zinc
	05-Apr-22										280			10.9	7.19													
	04-May-22	59.6	0.453	0.024	0.01	0.00061	9.2E-06	5.32	0.00058	0.00024	206	0.00165	0.0004	6.47	6.93	90.2	0.446	0.0002	0.000005	0.00109	0.0012	0.14	0.03	0.000178	0.000007	3.5	0.000425	0.0024
	07-Jun-22	123	0.101	0.008	0.01	0.00172	0.000012	4.11	0.0005	0.000325	430	0.00144	0.0008	5.6	7.06	180	0.444	0.00012	0.000005	0.00136	0.0016	0.024	0.06	0.00025	0.0003	3.5	0.000796	0.007
	12-Jul-22	141	0.142	0.018		0.00206	0.000001	9.55	0.00056	0.00033	276	0.00088	0.0008	5.07	7.01	134	0.809	0.00008	0.000005	0.000438	0.0016	0.033	0.11	0.000225	0.0003	5.5	0.000338	0.003
	10-Aug-22	148	0.275	0.032	0.01	0.00197	0.000009	8.64	0.00122	0.00038	306	0.00104	0.0012	4.34	6.68	150	0.673	0.0002	0.000005	0.000508	0.00206	0.01	0.055	0.000255	0.00002	8	0.000527	0.0035
	07-Sep-22	178	0.216	0.00032		0.00198	0.000017	9.19	0.0006	0.000305	373	0.00068	0.001	3.04	7.39	197	0.503	0.0001	0.000005	0.00052	0.00162	0.004	0.0741	0.00029	0.0003	6	0.000695	0.0015
	04-Oct-22	184	0.0674	0.01	0.01	0.00108	0.000004	8.62	0.0004	0.00013	812	0.0006	0.002	2.03	7.64	217	0.158	0.00004	0.000005	0.00127	0.0012	0.028	0.03	0.000176	0.000002	2	0.00101	0.001
	11-Nov-22	160	0.0728	0.012	0.01	0.00101	5.6E-06	12.5	0.00056	0.000178	450	0.0007	0.002	11.26	7.01	194	0.264	0.00006	0.000005	0.00154	0.0012	0.24	0.028	0.000192	0.000002	3	0.00115	0.0022
	11-Dec-22	207	0.141	0.03	0.004	0.00099	0.000013	19.8	0.00052	0.000735	436	0.00064	0.0004	3.81	9.31	201	0.9	0.00015	0.000005	0.0003	0.00164	0.034	0.065	0.00014	0.0003	3.5	0.000727	0.0065
	09-Jan-23										403			0	8.14													
	10-Feb-23	211	0.157	0.116	0.01	0.00148	3.36E-05	15	0.00064	0.00208	301	0.00105	0.0012	2.23	5.61	217	3.02	0.00088	0.000005	0.000235	0.00194	0.018	0.232	0.000178	0.000003	11	0.000862	0.013
	08-Mar-23	238	0.174	0.22	0.01	0.00176	1.14E-05	16.9	0.00076	0.00263	513	0.00085	0.0006	0	7.55	246	3.87	0.0002	0.000005	0.00023	0.00192	0.016	0.324	0.000182	0.000003	14.5	0.000786	0.009
	11-Apr-23	122	0.767	0.112	0.01	0.00085	3.28E-05	8.33	0.00148	0.000636	247	0.00215	0.001	11.23	7.33	116	1.23	0.00084	0.000005	0.000615	0.00172	0.324	0.114	0.00016	0.00001	13	0.00078	0.0086
	02-May-23	113	0.133	0.154	0.001	0.00082	0.000013	18.1	0.0004	0.00056	641	0.00154	0.0006	13.42	7.67	243	0.262	0.00011	0.000005	0.00364	0.00176	0.786	0.015	0.000285	0.0003	8.5	0.00129	0.006
	06-Jun-23	126	0.0939	0.258	0.0025	0.00226	1.68E-05	22.3	0.0005	0.00097	1425	0.00229	0.002	6.09	7.35	256	0.759	0.000093	0.000005	0.00492	0.00228	0.829	0.126	0.000483	0.00001	6.6	0.00106	0.0041
	08-Jul-23	141	0.0929	0.0405	0.001	0.00216	0.000005	9.2	0.0005	0.0004	310	0.0005	0.002	4.72	7.28	147	0.468	0.000073	0.000005	0.000439	0.00174	0.02		0.000262	0.00001	3.8	0.000267	0.003
	05-Aug-23	187	0.0732	0.0137	0.001	0.00186	8.1E-06	18	0.0005	0.00021	452	0.00092	0.0036	3.71	7.38	211	0.198	0.000065	0.000005	0.000552	0.00153	0.02	0.068	0.000202	0.00001	4	0.000738	0.003
	05-Aug-23	188	0.0784	0.0102	0.001	0.00177	0.000006	17.9	0.0005	0.00019	457	0.00086	0.002	5.35	7.71	211	0.211	0.000055	0.000005	0.000559	0.00151	0.02	0.069	0.000198	0.00001	3.8	0.000719	0.003
	21-Aug-23	185	0.0934		0.001	0.00233	0.000005		0.0005	0.00027		0.00081				185	0.289	0.000081	0.000005	0.000356	0.00168	0.02	0.0864	0.000231	0.00001	5.2	0.000563	0.003
	06-Sep-23	175	0.18	0.0296	0.001	0.00365	0.000005	14.8	0.00084	0.00034	404	0.00093	0.002	2.5	7.33	187	0.605	0.000118	0.000005	0.000377	0.00204	0.02	0.198	0.000222	0.00001	5.9	0.000495	0.003
	04-Oct-23	210	0.0878	0.0098	0.001	0.00236	0.000005	24.6	0.0005	0.00028	510	0.00838	0.002	1.29	7.14	238	0.336	0.00019	0.000005	0.000505	0.0017	0.02	0.135	0.000169	0.00001	3.9	0.0011	0.007
	07-Nov-23	167	0.129	0.7	0.003	0.00098	1.21E-05	32.8	0.0005	0.00124		0.00277	0.002			334	0.322	0.00008	0.000005	0.00599	0.00224	0.87	0.05	0.000375	0.00001	6.3	0.00137	0.0036
	12-Nov-23	154	0.0537	0.0869	0.001	0.00107	7.1E-06	34.6	0.0005	0.0013	1074	0.00221	0.002	11.08	7.63	370	0.14	0.00005	0.000005	0.00716	0.00216	1.19	0.05	0.000424	0.00001	4	0.00143	0.003
	11-Dec-23	188	0.103	0.023	0.001	0.00095	5.9E-06	30.8	0.0005	0.00053	884	0.00081	0.002	5.04	7.35	220	0.809	0.000098	0.000005	0.000382	0.00166	0.02	0.054	0.000162	0.00001	3.4	0.000931	0.003
SW23	09-Jan-20	174	0.431	0.052	0.01	0.00106	0.000018	5.55	0.00108	0.000723		0.00156	0.0008		6.81	174	1.51	0.000365	0.00003	0.000332	0.00236	0.053	0.08	0.00022	0.000007	11.5	0.000885	0.005
	04-Feb-20	172	0.322	0.064	0.01	0.00103	0.000017	5.76	0.00086	0.000668		0.00142	0.001			182	1.48	0.00037	0.00003	0.000236	0.00218	0.089	0.06	0.000165	0.000006	10.5	0.00101	0.0046
	10-Mar-20	195	0.632	0.114	0.01	0.00114	0.000014	6.76	0.0012	0.000875		0.00182	0.0004			219	1.97	0.0005	0.00003	0.000274	0.0027	0.171	0.09	0.000175	0.000005	16.5	0.00131	0.008
	07-Apr-20	81.2	0.546	0.04	0.01	0.00072	0.000008	3.9	0.00104	0.000446		0.00156	0.0004			89.7	0.749	0.00036	0.00003	0.000404	0.00168	0.132	0.05	0.000145	0.000002	18.5	0.000391	0.004
	12-May-20	91	0.542	0.028	0.01	0.00103	0.000017	2.82	0.00108	0.000472		0.0019	0.0008			104	0.89	0.000355	0.000005	0.000324	0.00206	0.012	0.032	0.000155	0.00001	13	0.000422	0.0165
	17-Jun-20	105	0.494	0.00052		0.0019	2.32E-05	4.09	0.00112	0.00052		0.00195	0.0018			125	1.03	0.00042	0.00001	0.00045	0.00246	0.034	0.0625	0.000222	0.000011	14.5	0.000401	0.0034
	07-Jul-20	153	0.49	0.00085		0.00394	2.62E-05	2.44	0.00106	0.000802		0.0019	0.001			165	1.65	0.00054	0.00003	0.000765	0.00294	0.012	0.114	0.00028	0.000011	12	0.000596	0.0028
	11-Aug-20	195	0.784	0.054	0.01	0.00312	0.000034	3.92	0.00148	0.00083		0.00276	0.0022			184	1.43	0.00077	0.000005	0.000716	0.0034	0.02	0.105	0.000255	0.000015	10	0.000987	0.004
	15-Sep-20	143	0.535	0.022	0.01	0.00253	0.00001	2.45	0.00116	0.00044		0.00178	0.0004			143	1.05	0.00038	0.000005	0.000388	0.00256	0.004	0.085	0.00018	0.000005	4	0.000456	0.0015
	15-Sep-20										217				6.95													
	14-Oct-20	125	0.332	0.02	0.01	0.00177	0.00001	2.68	0.00086	0.00028	244	0.00114	0.0006	1.25	7.28	128	0.682	0.00022	0.00003	0.000262	0.00198	0.046	0.055	0.00016	0.0003	5	0.000458	0.002
	04-Nov-20	145	0.13	0.016	0.01	0.00112	0.000008	16.5	0.0004	0.000495	654	0.00138	0.0004		7.73	245	0.343	0.00013	0.00003	0.00314	0.00152	0.824	0.015	0.00017	0.0003	3	0.00142	0.001
	10-Nov-20	131	0.328	0.008	0.01	0.0012	0.000012	16.5	0.00074	0.00061	557	0.00192	0.002	0	6.87	225	0.657	0.00025	0.00003	0.00287	0.00184	0.884	0.03	0.000185	0.000005	6.5	0.00129	0.0025
	16-Dec-20	198	0.324	4.00E-05		0.00122	0.000014	13.4	0.00088	0.000585	458	0.00152	0.0012	7.21	6.92	228	1.03	0.00028	0.00003	0.00045	0.00234	0.036	0.035	0.00013	0.00001	4.5	0.00105	0.027
	12-Jan-21	217	0.362	8.50E-05	0.000085	0.00148	0.000018	14.8	0.00094	0.00124	475	0.00134	0.0008	0	6.86	234	1.71	0.00032	0.00003	0.000326	0.00258	0.038	0.07	0.000165	0.0003	9.5	0.000892	0.005
	23-Mar-21										242			3.77	7.03													
	20-Apr-21	83.6	0.253	6.80E-05		0.00075	1.12E-05	7.31	0.0005	0.000226	198.7	0.0013	0.001	10.67	7.38	115	0.402	0.00016	0.00003	0.00045	0.00118	0.088	0.022	0.00014	0.000004	6	0.000475	0.0018

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Ammonia, Total as N	Ammonia, Un-ionized as N	Arsenic	Cadmium	Chloride	Chromium	Cobalt	Field Conductivity	Copper	Cyanide, Total	Field Dissolved Oxygen	Field pH	Hardness as CaCO3	Iron	Lead	Mercury	Molybdenum	Nickel	Nitrate as N	Phosphorus	Selenium	Thallium	Total Suspended Solids	Uranium	Zinc
	11-May-21	107	0.455	0.012	0.01	0.00121	0.000013	5.03	0.001	0.00048	259	0.002	0.0012	6.03	7.81	133	0.792	0.00039	0.000005	0.000576	0.0022	0.02	0.025	0.00015	0.00001	11	0.00072	0.003
	08-Jun-21	167	0.658	0.04	0.01	0.00347	0.000024	5.24	0.00132	0.00102	538	0.00226	0.0006	1.6	7.29	177	1.08	0.00072	0.000005	0.0008	0.00336	0.02	0.09	0.0003	0.0003	18	0.00101	0.0045
	13-Jul-21	220	0.753	0.018	0.003	0.00669	0.000014	5.84	0.0014	0.00113	484	0.00192	0.001	3.42	8.74	212	1.33	0.00081	0.00003	0.00098	0.00396	0.012	0.125	0.00034	0.000005	9.5	0.00118	0.0035
	13-Oct-21	126	0.259	0.024	0.01	0.00148	0.000012	15.6	0.00074	0.00031	241.7	0.00132	0.003	5.99	7.24	162	0.623	0.0002	0.00003	0.000808	0.0019	0.098	0.055	0.000225	0.000005	8	0.000454	0.0025
	09-Nov-21	98.8	0.159	0.022	0.01	0.00106	0.000014	6.62	0.00058	0.00022	156.7	0.0015	0.001	11.55	7.28	136	0.405	0.00011	0.00003	0.000748	0.00154	0.656	0.015	0.00021	0.0003	3	0.000552	0.0015
	11-Jan-22	170	0.448	0.05	0.01	0.00128	0.000024	9.32	0.00114	0.000735	373	0.00158	0.001	0	6.82	194	1.45	0.00032	0.00003	0.000268	0.00262	0.036	0.085	0.000175	0.000005	9	0.000719	0.0045
	08-Feb-22	194	0.542	0.03	0.01	0.00136	0.000026	9	0.00152	0.00106	393	0.00176	0.0008	7.52	6.73	203	1.93	0.00044	0.00003	0.000232	0.00292	0.044	0.1	0.000195	0.00001	11.5	0.000832	0.0055
	08-Mar-22	197	0.263	0.146	0.01	0.00124	0.00002	6.76	0.00092	0.00088	445	0.00132	0.0008	13.6	6.93	198	1.85	0.00041	0.00003	0.000224	0.00244	0.076	0.105	0.000205	0.0003	8	0.000899	0.0045
	05-Apr-22										285			12.14	6.68													
	03-May-22	47.6	0.374	0.012	0.01	0.00061	1.44E-05	2.86	0.0005	0.00019	123	0.003	0.0006	7.37	6.65	60.9	0.425	0.00018	0.000005	0.00046	0.0025	0.052	0.028	0.000126	0.000007	4	0.000193	0.0026
	07-Jun-22	84	0.276	0.014	0.01	0.00124	0.000017	10.1	0.00074	0.000305	226	0.0014	0.001	5.04	6.82	116	0.565	0.00021	0.000005	0.000646	0.0017	0.134	0.04	0.00025	0.0003	5.5	0.000408	0.005
	05-Jul-22	125	0.238	0.04		0.00242	0.000009	3.7	0.00072	0.000515	239	0.00124	0.0012	6.12	7.34	134	1.07	0.00025	0.000005	0.000586	0.00212	0.004	0.065	0.000205	0.0003	7	0.000423	0.0025
	09-Aug-22		0.424	0.03	0.01	0.00302	0.000012	3.34	0.00106	0.000635	287	0.00138	0.0012	5.03	6.87	149	1.46	0.00042	0.000005	0.000614	0.00266	0.014	0.095	0.000255	0.0003	43.5	0.000474	0.003
	06-Sep-22	156	0.458	0.0039		0.00354	0.000005	2.41	0.00112	0.000605	284	0.00152	0.0016	4.71	8.35	178	1.25	0.00041	0.000005	0.000598	0.00284	0.006	0.102	0.00018	0.0003	7	0.000516	0.002
	04-Oct-22	168	0.366	0.024	0.01	0.00161	0.000011	5.68	0.00096	0.000418	330	0.0012	0.0014	4.15	6.8	170	0.729	0.0003	0.000005	0.000415	0.00208	0.02	0.06	0.000182	0.000007	9.5	0.000572	0.0032
	12-Nov-22	144	0.17	0.082	0.01	0.00084	0.000007	12.5	0.00055	0.00046	576	0.00098	0.0022	12.47	6.99	233	0.358	0.000122	0.000005	0.00281	0.00159	0.444	0.05	0.000221	0.00001	8	0.00139	0.0035
	08-Jan-23	219	0.18	0.052	0.01	0.00108	0.000006	11.5	0.00048	0.00063	265	0.0008	0.0008	3.09	7.75	200	1.25	0.00025	0.000005	0.000268	0.00198	0.026	0.08	0.00005	0.00001	12.5	0.000681	0.0035
	07-Feb-23	203	0.516	0.11	0.002	0.00141	0.000027	9.18	0.00128	0.00126	392	0.0013	0.0012	3.31	8.36	0.51	2.18	0.00051	0.000005	0.001	0.00258	0.036	0.11	0.000145	0.0003	12.5	0.000675	0.005
	07-Mar-23	233	0.48	0.2	0.01	0.00177	4.16E-05	10.6	0.0013	0.00202	282	0.00145	0.0008	2.72	7.45	243	2.73	0.00054	0.00002	0.000245	0.00294	0.036	0.13	0.000192	0.000008	19.5	0.000848	0.0084
	04-Apr-23	512	0.461	0.342	0.01	0.00172	2.36E-05	12.7	0.00118	0.00219	461	0.0012	0.001	4.5	7.08	248	2.99	0.00046	0.000005	0.000265	0.00264	0.09	0.196	0.000176	0.000009	18.5	0.000841	0.0046
	02-May-23	83.2	0.307	0.038	0.01	0.00085	0.000014	7.55	0.00072	0.000395	323	0.00158	0.0006	9.43	7.44	137	0.549	0.00024	0.000005	0.00151	0.0016	0.352	0.02	0.000175	0.000005	14.5	0.0006	0.0035
	06-Jun-23	98.5	0.594	0.0833	0.001	0.0023	2.62E-05	7.3	0.00138	0.00084	654	0.00258	0.002	5.07	7.14	155	1.22	0.000489	7.6E-06	0.00151	0.00273	0.205	0.106	0.00031	0.000011	23.7	0.000643	0.0048
	09-Jul-23	116	0.643	0.0877	0.001	0.00335	1.91E-05	3.43	0.00156	0.00089	236	0.00207	0.002	5.04	7.45	134	1.92	0.000713	0.000005	0.000465	0.00337	0.021		0.000311	0.000011	13.6	0.000459	0.0042
	06-Aug-23	151	1.2	0.03	0.001	0.00406	2.51E-05	4.08	0.00251	0.00119	335	0.0031	0.002	3.95	7.08	160	1.96	0.00113	0.000005	0.000756	0.00436	0.02	0.149	0.000323	0.000018	15.7	0.000698	0.0057
	05-Sep-23		0.611	0.0457	0.001	0.00448	1.67E-05		0.0013	0.00091	333	0.00228	0.002	2.28	7.47	170	1.2	0.00072	0.00005	0.000683	0.0034		0.125	0.000277	0.00001		0.000735	0.0036
	03-Oct-23	179	0.678	0.0588	0.001	0.00459	1.47E-05	3.85	0.00148	0.00089	335	0.00298	0.002	0.29	7.5	174	1.4	0.000688	0.000005	0.000674	0.00353	0.02	0.128	0.00027	0.00002	12.7	0.000701	0.006
	08-Nov-23	134	0.302	0.239	0.001	0.00104	1.51E-05	17.6	0.00293	0.00077		0.00198	0.002			246	0.604	0.000238	0.000005	0.00273	0.00182	0.431	0.05	0.000242	0.00001	11.7	0.00108	0.0044
	12-Nov-23	139	0.262	0.372	0.0034	0.00095	1.03E-05	22.1	0.00064	0.00088	726	0.00126	0.002	8.1	8	270	0.42	0.000152	0.000005	0.00371	0.00184	0.668	0.05	0.000255	0.00001	7.9	0.0012	0.003
	08-Dec-23	153	0.46	0.0569	0.001	0.00105	4.35E-05	15.1	0.00106	0.00054	468	0.00568	0.002	9.48	7.28	180	0.971	0.000347	0.000005	0.000649	0.00235	0.05	0.05	0.000162	0.00001	11.5	0.000876	0.0161
SW24	09-Jan-20	174	0.453	0.05	0.01	0.00104	0.000018	5.48	0.00106	0.000734		0.00152	0.0008		7.06	170	1.48	0.00037	0.00003	0.000248	0.00234	0.048	0.065	0.000195	6.8E-06	10.5	0.00089	0.0058
	04-Feb-20	176	0.313	0.068	0.01	0.00102	0.000015	5.86	0.00076	0.000632		0.0015	0.0008			180	1.39	0.00035	0.000005	0.000238	0.00216	0.088	0.07	0.00017	5.4E-06	9.5	0.00105	0.0044
	10-Mar-20	196	0.622	0.12	0.01	0.00108	0.00001	7.4	0.0012	0.000865		0.0016	0.0008			216	1.93	0.00051	0.00003	0.000302	0.00262	0.191	0.085	0.00018	0.000005	22	0.00131	0.0075
	07-Apr-20	82.2	0.707	0.04	0.01	0.00071	0.000009	3.85	0.00126	0.000482		0.00166	0.0004			87.4	0.911	0.00039	0.000005	0.000366	0.0018	0.12	0.06	0.00014	0.000002	19	0.00039	0.004
	12-May-20	106	0.422	0.288	0.005	0.00139	0.000017	4.8	0.00088	0.000704		0.00318	0.0008			168	0.693	0.00031	0.00001	0.00181	0.00214	0.86	0.024		0.00001	11.5	0.000966	0.0035
	17-Jun-20	104	0.576	0.00055		0.0019	2.82E-05	3.97	0.00124	0.0006		0.0023	0.0014			116	1.14	0.00052	0.00003	0.0004	0.00268	0.018	0.067	0.000238	0.000011	19	0.000404	0.0044
	07-Jul-20	142	0.448	0.00055		0.00377	0.000029	2.3	0.00098	0.00078		0.0022	0.001			155	1.55	0.00064	0.00003	0.000695	0.00286	0.012	0.132	0.00027	0.000008	15	0.000565	0.0038
	11-Aug-20	178	0.367	0.06	0.01	0.00242	0.000034	6.11	0.00384	0.00083		0.00332	0.0006			230	0.865	0.00041	0.00003	0.00172	0.00378	0.036	0.06	0.00022	0.000005	4	0.00122	0.0025
	15-Sep-20	144	0.519	0.016	0.01	0.00253	0.000015	2.01	0.00106	0.00045		0.00186	0.0006			139	1.12	0.00042	0.00003	0.000414	0.00252	0.02	0.095	0.00023	0.000015	3.5	0.000469	0.0055
	15-Sep-20										214				7.05													
	14-Oct-20	116	0.227	0.136	0.01	0.00205	0.000017	11.7	0.00056	0.000865	815	0.00868	0.0034	2.43	7.28	261	0.435	0.00024	0.00003	0.00472	0.00176	2.31	0.03	0.000345	0.0003	4	0.00123	0.0015
	04-Nov-20	138	0.112	0.024	0.01	0.00144	0.000011	19.3	0.00032	0.000825	889	0.00508	0.0006		7.8	313	0.261	0.00011	0.00003	0.00579	0.00138	2.22	0.015	0.00026	0.000015	3.5	0.00169	0.0005

Site	Sample Date	Alkalinity, Total as CaCO3	Aluminum	Ammonia, Total as N	Ammonia, Un-ionized as N	Arsenic	Cadmium	Chloride	Chromium	Cobalt	Field Conductivity	Copper	Cyanide, Total	Field Dissolved Oxygen	Field pH	Hardness as CaCO3	Iron	Lead	Mercury	Molybdenum	Nickel	Nitrate as N	Phosphorus	Selenium	Thallium	Total Suspended Solids	Uranium	Zinc
	10-Nov-20	127	0.218	0.014	0.01	0.00142	0.000014	19.4	0.0014	0.00093	782	0.00372	0.0004	1.97	6.32	296	0.453	0.00023	0.00003	0.00544	0.00186	2.04	0.02	0.00026	0.00001	6.5	0.00166	0.0025
	16-Dec-20	192	0.292	4.00E-05		0.00117	0.000015	15.9	0.00078	0.000565	466	0.00144	0.001	5.23	6.91	224	0.991	0.00024	0.00003	0.000444	0.00232	0.054	0.035	0.000195	0.00001	3.5	0.00101	0.01
	12-Jan-21	216	0.38	8.30E-05	0.000083	0.00147	0.00002	14.2	0.001	0.00124	477	0.0015	0.0008	11.54	6.83	236	1.77	0.00034	0.00003	0.000306	0.00264	0.026	0.07	0.000165	0.00001	10.5	0.00092	0.0045
	23-Mar-21										237			3.2	6.74													
	20-Apr-21	82.6	0.292	5.60E-05		0.00078	1.06E-05	5.17	0.0006	0.000248	196.6	0.0013	0.001	11.1	7.29	113	0.436	0.00018	0.000005	0.00045	0.00124	0.046	0.02	0.000144	0.000006	6.5	0.000486	0.0018
	11-May-21	109	0.46	0.012	0.01	0.00114	0.000015	5.22	0.00104	0.00047	265	0.00192	0.001	6.41	7.79	131	0.78	0.00032	0.00003	0.000574	0.00206	0.002	0.03	0.00017	0.00001	8.5	0.000714	0.0025
	08-Jun-21	167	1.15	0.054	0.01	0.00384	0.000026	5.35	0.00206	0.00121	536	0.00274	0.0006	1.22	7.14	179	1.46	0.0009	0.000005	0.000818	0.0038	0.002	0.105	0.00028	0.000005	15	0.00109	0.006
	13-Jul-21	224	0.297	0.018	0.002	0.00707	0.000006	5.44	0.0005	0.000855	447	0.00158	0.0012	4.05	8.55	214	0.774	0.00044	0.00003	0.0012	0.00366	0.01	0.125	0.00032	0.0003	4	0.00136	0.003
	13-Oct-21	125	0.256	0.032	0.01	0.00147	0.000018	15.5	0.0007	0.00033	255.7	0.00132	0.0032	5.55	7.29	161	0.619	0.00021	0.00003	0.000946	0.00182	0.096	0.05	0.00029	0.0003	7.5	0.000454	0.004
	09-Nov-21	100	0.139	0.148	0.01	0.00123	0.000008	13.2	0.00044	0.0004	368.3	0.00232	0.001	12.29	7.39	213	0.344	0.00009	0.00003	0.00318	0.00134	0.954	0.01	0.000265	0.0003	3	0.000904	0.0015
	11-Jan-22	171	0.322	0.046	0.01	0.0013	0.00002	9.58	0.0012	0.00068	360	0.0019	0.0008	0	6.71	191	1.33	0.00029	0.00003	0.000242	0.00246	0.044	0.07	0.000175	0.000005	9.5	0.000734	0.005
	08-Feb-22	196	0.346	0.046	0.01	0.00129	0.00002	8.96	0.00106	0.00099	404	0.00156	0.0008	5.69	6.63	208	1.68	0.00043	0.00003	0.000244	0.00278	0.046	0.075	0.000195	0.000005	13	0.000817	0.0055
	08-Mar-22	196	0.27	0.138	0.01	0.00123	0.000017	6.67	0.0009	0.000855	418	0.00132	0.0008	4.65	6.83	201	1.81	0.00036	0.00003	0.00021	0.0025	0.052	0.105	0.00019	0.0003	7.5	0.000854	0.004
	05-Apr-22										286			10.56	6.69													
	03-May-22	50.4	0.377	0.016	0.01	0.00069	1.32E-05	2.91	0.00042	0.000202	127	0.0015	0.0008	7.14	6.86	61.9	0.419	0.00022	0.000005	0.000475	0.00116	0.076	0.03	0.00013	0.000008	4	0.000509	0.0044
	07-Jun-22	86.8	0.272	0.024	0.01	0.0013	0.000016	5.27	0.0007	0.00033	303	0.0016	0.001	5.71	6.84	134	0.589	0.00021	0.000005	0.00126	0.00168	0.428	0.03	0.000265	0.0003	6.5	0.000539	0.0035
	05-Jul-22	126	0.238	0.044		0.00235	0.000008	4.03	0.0008	0.00052	261	0.00134	0.0012	5.6	7.29	139	1.07	0.00027	0.000005	0.000652	0.00218	0.01	0.075	0.00023	0.0003	6	0.000447	0.0025
	10-Aug-22	142	0.286	0.026	0.01	0.00297	0.00001	3.59	0.00094	0.00055	306	0.00134	0.0012	4.28	6.8	149	1.3	0.00031	0.000005	0.00067	0.0026	0.024	0.085	0.000315	0.0003	9	0.000465	0.003
	06-Sep-22										283			3.35	8.03													
	04-Oct-22	167	0.318	0.012	0.01	0.00164	1.26E-05	5.8	0.00082	0.000422	340	0.0012	0.0008	3.9	6.59	170	0.685	0.00028	0.000005	0.00045	0.00204	0.004	0.046	0.00018	0.000007	9.5	0.000569	0.004
	12-Nov-22	139	0.156	0.35	0.01	0.00091	1.81E-05	23.7	0.0006	0.00097	920	0.00138	0.0012	10.8	6.97	337	0.529	0.000116	0.000005	0.00577	0.00256	1.08	0.05	0.000345	0.00001	7	0.00198	0.0106
	08-Jan-23	221	0.205	0.056	0.01	0.00113	0.00001	11.8	0.00064	0.000645	370	0.00084	0.0008	2.97	7.66	201	1.3	0.00024	0.000005	0.000274	0.00208	0.074	0.08	0.00005	0.0003	7.5	0.000674	0.0035
	07-Feb-23	202	0.445	0.098	0.001	0.00139	0.00002	9.58	0.00174	0.0012	390	0.00118	0.0012	1.93	8.27	200	2.12	0.00047	0.000005	0.00019	0.00252	0.038	0.1	0.000175	0.0003	12	0.000679	0.006
	28-Feb-23	131	0.213	0.018	0.002	0.00178	1.90E-05	12	0.00062	0.00094		0.0102	0.002			336	0.417	0.00022	3.00E-05	0.00653	0.00204	1.62	0.025	0.00041	1.00E-05	8	0.0017	0.0025
	07-Mar-23	236	1.17	0.2	0.01	0.00202	4.22E-05	11.1	0.00268	0.00247	418	0.00235	0.0008	2.96	7.39	241	3.7	0.00098	0.000005	0.00026	0.00406	0.038	0.17	0.0002	0.00002	39.5	0.000906	0.01
	02-May-23	88	0.302	0.086	0.01	0.00086	0.000015	10.5	0.00084	0.000525	439	0.00162	0.0008	8.85	7.42	171	0.555	0.00024	0.000005	0.00242	0.00174	0.562	0.01	0.000205	0.000005	14	0.000815	0.004
	06-Jun-23	99.8	0.562	0.127	0.001	0.0022	3.03E-05	10.3	0.00132	0.00093	860	0.00204	0.002	5.5	7.24	188	1.15	0.000443	7.2E-06	0.00244	0.00274	0.406	0.094	0.000367	0.000011	21.9	0.000795	0.0044
	09-Jul-23	106	0.851	0.0462	0.001	0.00323	2.61E-05	2.98	0.00182	0.00096	216	0.00211	0.0024	6.51	7.35	117	2.01	0.00084	0.000005	0.000464	0.00351	0.044		0.000334	0.000013	13.2	0.000448	0.0056
	06-Aug-23	151	1.07	0.0239	0.001	0.00405	2.61E-05	4.11	0.00216	0.00107	413	0.00294	0.0022	4.78	7.3	157	1.75	0.00103	5.5E-06	0.000837	0.00411	0.02	0.136	0.000318	0.000016	10.3	0.000714	0.0054
	05-Sep-23	165	0.838	0.0377	0.001	0.005	1.47E-05	3.9	0.00176	0.00103	946	0.00249	0.002	3.16	7.45	169	1.55	0.000863	0.00005	0.000756	0.00387	0.02	0.167	0.000334	0.000012	5.7	0.000786	0.0043
	03-Oct-23	174	0.61	0.0263	0.001	0.00448	1.26E-05	4.08	0.00132	0.00084	343	0.00291	0.002	0.48	7.46	173	1.28	0.00064	0.000005	0.00064	0.00343	0.02	0.136	0.000292	0.00002	8.1	0.000715	0.006
	08-Nov-23	133	0.298	0.564	0.0017	0.00114	1.21E-05	24.1	0.00109	0.00118		0.00153	0.002			294	0.515	0.000215	0.000005	0.00495	0.00232	0.795	0.05	0.000353	0.00001	11.3	0.00116	0.0031
	12-Nov-23	130	0.213	0.758	0.0102	0.00101	7.2E-06	30.7	0.0005	0.00145	1029	0.00155	0.002	9	8.15	346	0.311	0.000132	0.000005	0.00649	0.00238	1.16	0.05	0.000406	0.00001	5.7	0.00132	0.003
	08-Dec-23	152	0.57	0.0732	0.001	0.00109	1.98E-05	15.1	0.00134	0.00062	392	0.00238	0.002	8.79	7.25	185	1.11	0.000413	0.000005	0.000679	0.00256	0.052	0.05	0.000184	0.00001	21.5	0.000928	0.0075

Table A-4: Water chemistry parameter summary statistics for effluent characterization per Schedule 5 (4), 2020–2023.

Year	Discharge Location	Statistic	**Alkalinity, Total** as CaCO3	Chloride	Conductivity	Field Temp	**Hardness** as CaCO3	**Nitrate** as N	Phosphorus	**Sulfate** as SO4	Aluminum	Cadmium	Chromium	Cobalt	Iron	Manganese	Mercury	Molybdenum	Selenium	Thallium	Uranium
			mg/L	mg/L	us/cm	°C	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
2020	EDL1	N	12	12	12	19	12	12	18	12	17	17	17	17	17	17	12	17	17	17	17
		Mean	134.92	20.40	1349.2	6.3	458.17	4.832	0.026	535.8	0.03878	0.00002	0.00022	0.00169	0.08209	0.01781	0.00003	0.01136	0.00049	0.00002	0.00262
		SD	14.44	6.69	254.1	2.6	45.58	0.951	0.024	157.7	0.02562	0.00000	0.00012	0.00015	0.10475	0.01998	0.00001	0.00253	0.00012	0.00001	0.00044
		Minimum	120.00	10.70	942.0	1.8	376.00	3.600	0.006	279.0	0.01440	0.00001	0.00010	0.00142	0.02200	0.00540	0.00001	0.00653	0.00032	0.00001	0.00212
		Median	126.50	22.35	1430.0	6.2	474.00	4.775	0.013	587.0	0.02440	0.00002	0.00016	0.00168	0.05050	0.01080	0.00003	0.01220	0.00054	0.00002	0.00256
		Maximum	159.00	28.40	1670.0	11.8	504.00	7.370	0.089	685.0	0.09180	0.00003	0.00046	0.00215	0.46700	0.07870	0.00003	0.01420	0.00067	0.00003	0.00411
	EDL2	N	7	7	7	18	7	7	10	7	10	10	10	10	10	10	7	10	10	10	10
		Mean	142.71	26.66	1517.1	3.7	487.43	2.667	0.023	657.0	0.02850	0.00001	0.00015	0.00159	0.17440	0.02472	0.00003	0.01198	0.00032	0.00011	0.00260
		SD	16.52	2.36	76.1	2.6	10.18	1.941	0.019	49.9	0.03028	0.00001	0.00009	0.00017	0.23209	0.02082	0.00000	0.00262	0.00006	0.00013	0.00024
		Minimum	126.00	22.80	1380.0	0.0	475.00	0.560	0.007	550.0	0.01100	0.00000	0.00006	0.00134	0.03350	0.00700	0.00003	0.00762	0.00024	0.00001	0.00223
		Median	139.00	27.80	1540.0	3.6	484.00	3.290	0.011	665.0	0.02250	0.00001	0.00013	0.00168	0.04925	0.01300	0.00003	0.01305	0.00030	0.00003	0.00262
		Maximum	163.00	28.60	1580.0	8.1	500.00	4.690	0.050	697.0	0.11300	0.00002	0.00038	0.00176	0.76700	0.05640	0.00003	0.01490	0.00042	0.00030	0.00305
	Sediment Pond #2	N	15	15	15	10	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
		Mean	162.40	5.76	475.2	7.0	229.53	0.088	0.010	95.4	0.05115	0.00000	0.00033	0.00010	0.04960	0.00479	0.00003	0.00347	0.00044	0.00024	0.00172
		SD	15.09	0.69	28.0	4.0	12.12	0.242	0.002	14.1	0.01603	0.00000	0.00031	0.00002	0.02739	0.00242	0.00001	0.00036	0.00006	0.00012	0.00013
		Minimum	148.00	4.47	402.0	1.0	212.00	0.002	0.007	76.0	0.03220	0.00000	0.00012	0.00007	0.01700	0.00260	0.00001	0.00282	0.00032	0.00000	0.00150
		Median	157.00	5.97	474.0	6.4	230.00	0.020	0.009	97.9	0.04820	0.00000	0.00020	0.00009	0.04800	0.00452	0.00003	0.00353	0.00043	0.00030	0.00170
		Maximum	188.00	6.73	529.0	13.3	247.00	0.960	0.014	121.0	0.08280	0.00001	0.00106	0.00013	0.10300	0.01140	0.00003	0.00434	0.00053	0.00030	0.00206
2021	EDL1	N	8	8	8	30	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
		Mean	124.75	29.69	1536.3	10.9	485.88	1.679	0.014	661.8	0.03073	0.00001	0.00021	0.00117	0.22588	0.05445	0.00003	0.01349	0.00043	0.00002	0.00284
		SD	16.65	7.18	179.7	4.9	31.40	0.991	0.004	85.3	0.03798	0.00000	0.00008	0.00015	0.32566	0.03836	0.00000	0.00094	0.00009	0.00001	0.00068
		Minimum	108.00	24.00	1360.0	1.2	445.00	0.630	0.008	586.0	0.00980	0.00001	0.00014	0.00096	0.02850	0.01560	0.00003	0.01230	0.00024	0.00001	0.00196
		Median	120.50	24.80	1470.0	12.0	489.50	1.410	0.014	620.5	0.01430	0.00001	0.00017	0.00122	0.11175	0.04490	0.00003	0.01345	0.00046	0.00002	0.00296
		Maximum	150.00	39.20	1750.0	17.6	520.00	3.400	0.021	773.0	0.12200	0.00001	0.00034	0.00143	1.01000	0.12600	0.00003	0.01520	0.00052	0.00003	0.00361
	EDL2	N	1	1	1	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		Mean	118.00	39.20	1730.0	13.9	525.00	0.834	0.009	760.0	0.00760	0.00001	0.00006	0.00098	0.12400	0.02460	0.00003	0.01490	0.00025	0.00001	0.00226
		SD	118.00	39.20	1730.0	9.6	525.00	0.834	0.009	760.0	0.00760	0.00001	0.00006	0.00098	0.12400	0.02460	0.00003	0.01490	0.00025	0.00001	0.00226
		Minimum	118.00	39.20	1730.0	13.7	525.00	0.834	0.009	760.0	0.00760	0.00001	0.00006	0.00098	0.12400	0.02460	0.00003	0.01490	0.00025	0.00001	0.00226
		Median	118.00	39.20	1730.0	20.0	525.00	0.834	0.009	760.0	0.00760	0.00001	0.00006	0.00098	0.12400	0.02460	0.00003	0.01490	0.00025	0.00001	0.00226
		Maximum	118.00	39.20	1730.0	20.0	525.00	0.834	0.009	760.0	0.00760	0.00001	0.00006	0.00098	0.12400	0.02460	0.00003	0.01490	0.00025	0.00001	0.00226
	Sediment Pond #2	N	11	11	11	19	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
		Mean	160.36	6.61	583.4	12.3	276.00	2.272	0.017	142.1	0.08587	0.00001	0.00027	0.00023	0.09518	0.01420	0.00003	0.00392	0.00048	0.00014	0.00231
		SD	13.22	1.53	64.6	5.7	25.12	2.884	0.005	27.9	0.08498	0.00000	0.00017	0.00011	0.09325	0.00739	0.00000	0.00036	0.00008	0.00015	0.00033
		Minimum	138.00	4.10	488.0	1.9	239.00	0.100	0.012	93.8	0.00360	0.00000	0.00006	0.00012	0.00500	0.00200	0.00003	0.00357	0.00034	0.00000	0.00197
		Median	161.00	6.84	574.0	12.6	280.00	0.412	0.015	148.0	0.05260	0.00001	0.00018	0.00019	0.05500	0.01590	0.00003	0.00386	0.00050	0.00001	0.00215
		Maximum	183.00	8.59	682.0	22.2	317.00	7.220	0.029	174.0	0.24500	0.00002	0.00056	0.00042	0.26200	0.02460	0.00003	0.00470	0.00059	0.00030	0.00286
2022	EDL1	N	18	18	18	57	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18

Year	Discharge Location	Statistic	**Alkalinity, Total** as CaCO3	Chloride	Conductivity	Field Temp	**Hardness** as CaCO3	**Nitrate** as N	Phosphorus	**Sulfate** as SO4	Aluminum	Cadmium	Chromium	Cobalt	Iron	Manganese	Mercury	Molybdenum	Selenium	Thallium	Uranium
			mg/L	mg/L	us/cm	°C	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
		Mean	181.72	39.03	1498.3	13.0	479.94	1.937	0.022	611.3	0.06394	0.00002	0.00084	0.00133	0.15231	0.04274	0.00001	0.01154	0.00046	0.00006	0.00231
		SD	234.53	6.02	229.8	5.5	73.75	0.579	0.022	116.6	0.08871	0.00001	0.00153	0.00050	0.13019	0.04538	0.00001	0.00233	0.00016	0.00011	0.00067
		Minimum	103.00	28.20	1270.0	4.1	381.00	0.888	0.009	479.0	0.00940	0.00000	0.00008	0.00079	0.02600	0.00758	0.00001	0.00705	0.00023	0.00001	0.00142
		Median	124.00	41.65	1480.0	12.9	468.50	1.995	0.014	599.5	0.02860	0.00002	0.00026	0.00110	0.10175	0.03090	0.00001	0.01275	0.00042	0.00001	0.00228
		Maximum	1120.00	48.40	1920.0	24.6	605.00	3.050	0.079	811.0	0.33200	0.00005	0.00500	0.00207	0.44000	0.20700	0.00003	0.01440	0.00078	0.00030	0.00445
	EDL2	N	13	13	13	32	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
		Mean	143.77	33.43	1481.7	10.2	518.00	1.355	0.038	549.0	0.04317	0.00008	0.00068	0.00153	0.41908	0.15073	0.00001	0.01065	0.00044	0.00005	0.00287
		SD	29.28	12.92	337.3	4.2	89.57	0.978	0.041	209.5	0.04006	0.00014	0.00131	0.00085	0.42993	0.29722	0.00000	0.00297	0.00017	0.00011	0.00165
		Minimum	111.00	5.02	602.0	1.2	411.00	0.100	0.009	137.0	0.00620	0.00000	0.00008	0.00073	0.08300	0.01720	0.00001	0.00427	0.00021	0.00000	0.00140
		Median	131.00	36.60	1490.0	10.5	510.00	1.580	0.015	606.0	0.02980	0.00002	0.00032	0.00132	0.30700	0.05700	0.00001	0.01220	0.00043	0.00001	0.00236
		Maximum	196.00	46.50	1880.0	18.3	734.00	2.700	0.119	786.0	0.14600	0.00045	0.00500	0.00395	1.71000	1.12000	0.00001	0.01380	0.00076	0.00030	0.00738
	Sediment Pond #2	N	19	19	19	23	20	19	20	19	20	20	20	20	20	20	20	20	20	20	20
		Mean	198.98	7.10	598.5	12.5	255.65	2.435	0.015	169.0	0.10465	0.00003	0.00033	0.00022	0.11025	0.01416	0.00001	0.00426	0.00054	0.00014	0.00243
		SD	222.15	8.85	262.6	6.5	33.32	1.660	0.009	108.4	0.13034	0.00005	0.00019	0.00011	0.12433	0.01622	0.00001	0.00071	0.00014	0.00015	0.00038
		Minimum	10.60	3.53	1.0	2.1	202.00	0.352	0.006	98.8	0.01680	0.00000	0.00012	0.00009	0.01600	0.00256	0.00001	0.00265	0.00035	0.00000	0.00195
		Median	156.00	4.96	573.0	13.1	255.50	2.420	0.014	147.0	0.04200	0.00001	0.00028	0.00019	0.04600	0.00660	0.00001	0.00441	0.00055	0.00001	0.00237
		Maximum	1100.00	43.30	1510.0	22.0	359.00	6.470	0.053	608.0	0.56000	0.00021	0.00100	0.00041	0.48600	0.06200	0.00003	0.00522	0.00081	0.00030	0.00335
2023	EDL1	N	15	13	15	34	15	15	15	13	15	15	15	15	15	15	15	15	15	15	15
		Mean	132.40	47.18	1526.7	8.9	520.13	2.548	0.079	654.8	0.02477	0.00002	0.00079	0.00214	0.06460	0.03306	0.00001	0.01404	0.00077	0.00002	0.00219
		SD	28.02	7.22	176.5	6.9	46.15	0.321	0.118	92.9	0.00978	0.00001	0.00119	0.00061	0.07607	0.01993	0.00000	0.00205	0.00012	0.00002	0.00050
		Minimum	117.00	36.60	1270.0	2.1	440.00	2.150	0.014	517.0	0.00920	0.00001	0.00016	0.00144	0.02200	0.00857	0.00001	0.01120	0.00059	0.00001	0.00152
		Median	122.00	47.20	1600.0	5.3	535.00	2.490	0.050	667.0	0.02730	0.00001	0.00050	0.00194	0.04300	0.03260	0.00001	0.01420	0.00080	0.00001	0.00240
		Maximum	224.00	57.30	1750.0	22.5	585.00	3.200	0.500	781.0	0.04120	0.00005	0.00500	0.00304	0.32700	0.07820	0.00001	0.01650	0.00096	0.00010	0.00292
	EDL2	N	16	15	16	31	16	16	16	15	16	16	16	16	16	16	16	16	16	16	16
		Mean	123.69	44.75	1452.1	7.8	496.22	2.370	0.079	613.0	0.02497	0.00002	0.00080	0.00209	0.05472	0.03018	0.00001	0.01317	0.00071	0.00002	0.00203
		SD	41.21	14.06	421.5	6.1	138.29	0.700	0.115	192.4	0.01124	0.00001	0.00114	0.00081	0.03630	0.02233	0.00000	0.00400	0.00021	0.00002	0.00073
		Minimum	2.00	0.10	3.6	2.1	0.59	0.020	0.010	0.3	0.01270	0.00001	0.00022	0.00010	0.01000	0.00010	0.00001	0.00005	0.00005	0.00001	0.00001
		Median	121.50	47.60	1625.0	4.7	545.00	2.475	0.050	661.0	0.02155	0.00002	0.00050	0.00200	0.04525	0.02720	0.00001	0.01445	0.00076	0.00001	0.00222
		Maximum	218.00	56.20	1740.0	21.4	577.00	3.090	0.500	772.0	0.04980	0.00005	0.00500	0.00304	0.11700	0.08200	0.00001	0.01660	0.00090	0.00010	0.00293
	Sediment Pond #2	N	15	14	15	14	15	15	15	14	15	15	15	15	15	15	15	15	15	15	15
		Mean	177.33	7.11	673.8	8.1	305.40	0.811	0.040	213.8	0.09461	0.00001	0.00058	0.00018	0.10373	0.04642	0.00001	0.00409	0.00038	0.00005	0.00231
		SD	62.94	1.75	117.0	6.7	47.61	0.497	0.015	69.2	0.15912	0.00001	0.00029	0.00009	0.15131	0.03326	0.00000	0.00070	0.00005	0.00010	0.00025
		Minimum	110.00	2.99	391.0	1.6	176.00	0.048	0.010	89.1	0.01100	0.00001	0.00022	0.00011	0.01800	0.01080	0.00001	0.00296	0.00030	0.00001	0.00171
		Median	150.00	8.03	741.0	4.5	317.00	0.800	0.050	251.5	0.03180	0.00001	0.00050	0.00014	0.03900	0.03170	0.00001	0.00431	0.00038	0.00001	0.00231
		Maximum	359.00	8.53	799.0	25.3	357.00	1.420	0.050	283.0	0.48300	0.00004	0.00126	0.00039	0.52000	0.10600	0.00001	0.00491	0.00045	0.00030	0.00266

Table A-5: Acute toxicity results for RRM effluent (2020 to 2023).

Location	Date	Percent Mortality (%)	
		Daphnia magna	Rainbow Trout
Sediment Pond 2	16-Jul-20	0	0
	24-Jun-20	0	0
	28-Sep-20	0	0
	13-Oct-20	0	0
	11-Nov-20	0	0
	15-Apr-21	0	0
	12-May-21	0	0
	16-Jun-21	0	0
	22-Sep-21	0	0
	04-Oct-21	0	0
	01-Nov-21	0	0
	20-Apr-22	0	0
	27-Apr-22	0	-
	04-May-22	0	0
	01-Jun-22	0	0
	28-Sep-22	0	0
	02-Nov-22	0	0
	17-Apr-23	0	0
	01-May-23	0	0
	05-Jun-23	0	0
	22-Oct-23	0	0
	05-Nov-23	0	0
EDL2	15-Oct-20	0	0
	29-Sep-21	0	0
	13-Oct-21	0	0
	27-Apr-22	0	-
	20-Apr-22	0	0
	04-May-22	0	0
	01-Jun-22	0	0
	21-Oct-22	0	0
	02-Nov-22	0	0
	17-Apr-23	0	0
	01-May-23	0	0
	05-Jun-23	0	0
	03-Nov-23	0	0
EDL1	29-Apr-20	0	0
	06-May-20	0	0
	07-Sep-20	0	0
	14-Oct-20	0	0
	04-Nov-20	0	0
	19-May-21	0	0
	27-Sep-21	0	0
	13-Oct-21	0	0
	01-Nov-21	0	0
	04-May-22	0	0
	01-Jun-22	0	0
	28-Sep-22	0	0
	02-Nov-22	0	0
	17-Apr-23	0	0
	01-May-23	0	0
	05-Jun-23	0	0
	31-Jul-23	0	0
	03-Nov-23	0	10

New Gold Inc. Rainy River Project
ATTN: Garnet Cornell
24 Marr Rd
Barwick ON P0W 1A0

Date Received: 02-JUN-21
Report Date: 30-JUN-21 14:52 (MT)
Version: FINAL

Client Phone: 807-234-8200

Certificate of Analysis

Lab Work Order #: L2595461

Project P.O. #: 4500038941
Job Reference: DISCHARGE MONITORING
C of C Numbers:
Legal Site Desc:

Christine Paradis
Project Manager

ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
L2595461-1 EDL1_EFF_20210601 Sampled By: Client on 01-JUN-21 @ 08:30 Matrix: EFF							
Physical Tests							
Color, True	8.3		2.0	CU		03-JUN-21	R5477551
Conductivity (EC)	1380		1.0	uS/cm		03-JUN-21	R5478342
Hardness (as CaCO3)	477		0.51	mg/L		07-JUN-21	
pH	7.97		0.10	pH		03-JUN-21	R5478342
Total Suspended Solids	1.5	<DL	3.0	mg/L		04-JUN-21	R5479116
Total Dissolved Solids	990		20	mg/L		04-JUN-21	R5479135
Turbidity	1.15		0.10	NTU		03-JUN-21	R5477690
Anions and Nutrients							
Alkalinity, Total (as CaCO3)	146		2.0	mg/L		03-JUN-21	R5478342
Ammonia, Total (as N)	0.252		0.0050	mg/L		07-JUN-21	R5480103
Chloride (Cl)	25.9		0.20	mg/L	02-JUN-21	03-JUN-21	R5478467
Fluoride (F)	0.046		0.040	mg/L	02-JUN-21	03-JUN-21	R5478467
Nitrate (as N)	<0.002	<W	0.040	mg/L		03-JUN-21	R5478467
Nitrite (as N)	0.063	<T	0.020	mg/L		03-JUN-21	R5478467
Total Kjeldahl Nitrogen	1.29		0.050	mg/L	04-JUN-21	09-JUN-21	R5481265
Orthophosphate-Dissolved (as P)	<0.0030		0.0030	mg/L	02-JUN-21	03-JUN-21	R5478324
Phosphorus (P)-Total	0.0225	<T	0.0030	mg/L		10-JUN-21	R5485357
Sulfate (SO4)	593		0.60	mg/L		03-JUN-21	R5478467
Cyanides							
Cyanide, Weak Acid Diss	0.0012	<DL	0.0020	mg/L		04-JUN-21	R5479638
Cyanide, Total	0.0012	<DL	0.0020	mg/L		04-JUN-21	R5479638
Cyanate	0.50		0.20	mg/L		09-JUN-21	R5482956
Thiocyanate (SCN)	<0.50		0.50	mg/L		10-JUN-21	R5487078
Cyanide, Free	0.0010	<DL	0.0020	mg/L		04-JUN-21	R5479638
Organic / Inorganic Carbon							
Dissolved Organic Carbon	9.98		0.50	mg/L	08-JUN-21	14-JUN-21	R5490459
Total Organic Carbon	11.0		0.50	mg/L		11-JUN-21	R5489823
Bacteriological Tests							
Escherichia Coli	<10		10	MPN/100mL		02-JUN-21	R5477590
Total Metals							
Aluminum (Al)-Total	0.0128	<T	0.0050	mg/L		03-JUN-21	R5478723
Antimony (Sb)-Total	0.0154		0.00060	mg/L		03-JUN-21	R5478723
Arsenic (As)-Total	0.00444	<T	0.0010	mg/L		03-JUN-21	R5478723
Barium (Ba)-Total	0.0603		0.010	mg/L		03-JUN-21	R5478723
Beryllium (Be)-Total	<0.0000001	<W	0.0010	mg/L		03-JUN-21	R5478723
Bismuth (Bi)-Total	<0.00001	<W	0.0010	mg/L		03-JUN-21	R5478723
Boron (B)-Total	0.107		0.050	mg/L		03-JUN-21	R5478723
Cadmium (Cd)-Total	0.000014	<DL	0.000017	mg/L		03-JUN-21	R5478723
Calcium (Ca)-Total	145		0.20	mg/L		03-JUN-21	R5478723
Cesium (Cs)-Total	0.000904		0.000010	mg/L		03-JUN-21	R5478723
Chromium (Cr)-Total	0.00020	<DL	0.0010	mg/L		03-JUN-21	R5478723
Cobalt (Co)-Total	0.00123	<T	0.00050	mg/L		03-JUN-21	R5478723

* Refer to Referenced Information for Qualifiers (if any) and Methodology.

ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
L2595461-1 EDL1_EFF_20210601 Sampled By: Client on 01-JUN-21 @ 08:30 Matrix: EFF							
Total Metals							
Copper (Cu)-Total	0.00446	<T	0.0010	mg/L		03-JUN-21	R5478723
Iron (Fe)-Total	0.226		0.020	mg/L		03-JUN-21	R5478723
Lead (Pb)-Total	0.00028	<T	0.000050	mg/L		03-JUN-21	R5478723
Lithium (Li)-Total	0.0108	<DL	0.050	mg/L		03-JUN-21	R5478723
Magnesium (Mg)-Total	24.5		0.020	mg/L		03-JUN-21	R5478723
Manganese (Mn)-Total	0.0876		0.0010	mg/L		03-JUN-21	R5478723
Mercury (Hg)-Total	<0.000005	<W	0.000030	mg/L		07-JUN-21	R5479674
Molybdenum (Mo)-Total	0.0127		0.0010	mg/L		03-JUN-21	R5478723
Nickel (Ni)-Total	0.00212	<T	0.0020	mg/L		03-JUN-21	R5478723
Phosphorus (P)-Total	0.020	<DL	0.050	mg/L		03-JUN-21	R5478723
Potassium (K)-Total	49.7		0.50	mg/L		03-JUN-21	R5478723
Rubidium (Rb)-Total	0.0259		0.00020	mg/L		03-JUN-21	R5478723
Selenium (Se)-Total	0.000440	<T	0.000050	mg/L		03-JUN-21	R5478723
Silicon (Si)-Total	0.794		0.10	mg/L		03-JUN-21	R5478723
Silver (Ag)-Total	0.000003	<DL	0.00010	mg/L		03-JUN-21	R5478723
Sodium (Na)-Total	109		0.10	mg/L		03-JUN-21	R5478723
Strontium (Sr)-Total	0.604		0.0010	mg/L		03-JUN-21	R5478723
Sulfur (S)-Total	210		0.50	mg/L		03-JUN-21	R5478723
Tellurium (Te)-Total	0.00002	<DL	0.0010	mg/L		03-JUN-21	R5478723
Thallium (Tl)-Total	0.000020	<DL	0.00030	mg/L		03-JUN-21	R5478723
Thorium (Th)-Total	<0.00001	<W	0.00010	mg/L		03-JUN-21	R5478723
Tin (Sn)-Total	0.00005	<DL	0.0010	mg/L		03-JUN-21	R5478723
Titanium (Ti)-Total	0.00043	<DL	0.0020	mg/L		03-JUN-21	R5478723
Tungsten (W)-Total	0.00017	<DL	0.010	mg/L		03-JUN-21	R5478723
Uranium (U)-Total	0.00348	<DL	0.0050	mg/L		03-JUN-21	R5478723
Vanadium (V)-Total	0.00045	<DL	0.0010	mg/L		03-JUN-21	R5478723
Zinc (Zn)-Total	0.0020	<DL	0.0030	mg/L		03-JUN-21	R5478723
Zirconium (Zr)-Total	0.000188	<DL	0.0010	mg/L		03-JUN-21	R5478723
Dissolved Metals							
Dissolved Metals Filtration Location	FIELD					03-JUN-21	R5478327
Aluminum (Al)-Dissolved	0.0068	<T	0.0050	mg/L		03-JUN-21	R5479522
Antimony (Sb)-Dissolved	0.0151		0.00060	mg/L		03-JUN-21	R5479522
Arsenic (As)-Dissolved	0.00390	<T	0.0010	mg/L		03-JUN-21	R5479522
Barium (Ba)-Dissolved	0.0587		0.010	mg/L		03-JUN-21	R5479522
Beryllium (Be)-Dissolved	<0.000002	<W	0.0010	mg/L		03-JUN-21	R5479522
Bismuth (Bi)-Dissolved	<0.000002	<W	0.0010	mg/L		03-JUN-21	R5479522
Boron (B)-Dissolved	0.103		0.050	mg/L		03-JUN-21	R5479522
Cadmium (Cd)-Dissolved	0.0000100	<DL	0.000017	mg/L		03-JUN-21	R5479522
Calcium (Ca)-Dissolved	148		0.20	mg/L		03-JUN-21	R5479522
Cesium (Cs)-Dissolved	0.000873		0.000010	mg/L		03-JUN-21	R5479522
Chromium (Cr)-Dissolved	0.00008	<DL	0.0010	mg/L		03-JUN-21	R5479522

* Refer to Referenced Information for Qualifiers (if any) and Methodology.

ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
L2595461-1 EDL1_EFF_20210601 Sampled By: Client on 01-JUN-21 @ 08:30 Matrix: EFF							
Dissolved Metals							
Cobalt (Co)-Dissolved	0.00114	<T	0.00050	mg/L		03-JUN-21	R5479522
Copper (Cu)-Dissolved	0.00350	<T	0.0010	mg/L		03-JUN-21	R5479522
Iron (Fe)-Dissolved	0.116		0.020	mg/L		03-JUN-21	R5479522
Lead (Pb)-Dissolved	0.00013	<T	0.000050	mg/L		03-JUN-21	R5479522
Lithium (Li)-Dissolved	0.0112	<DL	0.050	mg/L		03-JUN-21	R5479522
Magnesium (Mg)-Dissolved	25.8		0.020	mg/L		03-JUN-21	R5479522
Manganese (Mn)-Dissolved	0.0774		0.0010	mg/L		03-JUN-21	R5479522
Mercury (Hg)-Dissolved	<0.000005	<W	0.000030	mg/L		07-JUN-21	R5479675
Molybdenum (Mo)-Dissolved	0.0120		0.0010	mg/L		03-JUN-21	R5479522
Nickel (Ni)-Dissolved	0.00202	<T	0.0020	mg/L		03-JUN-21	R5479522
Phosphorus (P)-Dissolved	0.010	<DL	0.050	mg/L		03-JUN-21	R5479522
Potassium (K)-Dissolved	49.1		0.50	mg/L		03-JUN-21	R5479522
Rubidium (Rb)-Dissolved	0.0258		0.00020	mg/L		03-JUN-21	R5479522
Selenium (Se)-Dissolved	0.000605	DTSE	0.000050	mg/L		03-JUN-21	R5479522
Silicon (Si)-Dissolved	0.770		0.050	mg/L		03-JUN-21	R5479522
Silver (Ag)-Dissolved	0.0000020	<DL	0.00010	mg/L		03-JUN-21	R5479522
Sodium (Na)-Dissolved	109		0.10	mg/L		03-JUN-21	R5479522
Strontium (Sr)-Dissolved	0.597		0.0010	mg/L		03-JUN-21	R5479522
Sulfur (S)-Dissolved	206		0.50	mg/L		03-JUN-21	R5479522
Tellurium (Te)-Dissolved	0.00003	<DL	0.0010	mg/L		03-JUN-21	R5479522
Thallium (Tl)-Dissolved	0.000016	<DL	0.00030	mg/L		03-JUN-21	R5479522
Thorium (Th)-Dissolved	<0.00001	<W	0.00010	mg/L		03-JUN-21	R5479522
Tin (Sn)-Dissolved	0.000015	<DL	0.0010	mg/L		03-JUN-21	R5479522
Titanium (Ti)-Dissolved	0.00018	<DL	0.0020	mg/L		03-JUN-21	R5479522
Tungsten (W)-Dissolved	0.000162	<DL	0.010	mg/L		03-JUN-21	R5479522
Uranium (U)-Dissolved	0.00334	<DL	0.0050	mg/L		03-JUN-21	R5479522
Vanadium (V)-Dissolved	0.00030	<DL	0.0010	mg/L		03-JUN-21	R5479522
Zinc (Zn)-Dissolved	0.0018	<DL	0.0030	mg/L		03-JUN-21	R5479522
Zirconium (Zr)-Dissolved	0.000178	<DL	0.0010	mg/L		03-JUN-21	R5479522
Aggregate Organics							
Biochemical Oxygen Demand	2.4		2.0	mg/L		03-JUN-21	R5480519
BOD Carbonaceous	3.2		2.0	mg/L		04-JUN-21	R5483098
Chemical Oxygen Demand	32		10	mg/L	04-JUN-21	05-JUN-21	R5479161
Radiological Parameters							
Ra-226	<0.010	<DL	0.010	Bq/L		16-JUN-21	R5492948
Miscellaneous							
Special Request	See Attached					03-JUN-21	R5506293
Special Request	See Attached					03-JUN-21	R5506293
Special Request	See Attached					03-JUN-21	R5506293
Special Request	See Attached					03-JUN-21	R5506293

* Refer to Referenced Information for Qualifiers (if any) and Methodology.

Reference Information

QC Samples with Qualifiers & Comments:

QC Type Description	Parameter	Qualifier	Applies to Sample Number(s)
Matrix Spike	Cyanate	MS-B	L2595461-1
Matrix Spike	Total Kjeldahl Nitrogen	MS-B	L2595461-1

Sample Parameter Qualifier key listed:

Qualifier	Description
<DL	Recorded value = measured amount <LMDL (non-zero)
<T	A Measurable Trace Amount: Interpret With Caution
<W	No Measurable Response (Zero): < Reported Value
DTSE	Dissolved Se concentration exceeds total. Positive bias on D-Se suspected due to signal enhancement from volatile selenium species. Contact ALS if an alternative test to address this interference is needed.
MS-B	Matrix Spike recovery could not be accurately calculated due to high analyte background in sample.
PEHT	Parameter Exceeded Recommended Holding Time Prior to Analysis

Test Method References:

ALS Test Code	Matrix	Test Description	Method Reference**
ALK-MISA-TB	Effluent	Alkalinity, Total (as CaCO3)	APHA 2320 B-Auto-Pot. Titration

This analysis is carried out using procedures adapted from APHA Method 2320 "Alkalinity". Total alkalinity is determined by potentiometric titration to a pH 4.5 endpoint. Bicarbonate, carbonate and hydroxide alkalinity are calculated from phenolphthalein alkalinity and total alkalinity values.

BOD-TB	Water	Biochemical Oxygen Demand (BOD)	APHA 5210 B- BIOCHEMICAL OXYGEN DEMAND

All forms of biochemical oxygen demand (BOD) are determined by diluting and incubating a sample for a specified time period, and measuring the oxygen depletion using a dissolved oxygen meter. Dissolved BOD (SOLUBLE) is determined by filtering the sample through a glass fibre filter prior to dilution. Carbonaceous BOD (CBOD) is determined by adding a nitrification inhibitor to the diluted sample prior to incubation.

CBOD-TB	Water	Carbonaceous BOD	APHA 5210 B- BIOCHEMICAL OXYGEN DEMAND

All forms of biochemical oxygen demand (BOD) are determined by diluting and incubating a sample for a specified time period, and measuring the oxygen depletion using a dissolved oxygen meter. Dissolved BOD (SOLUBLE) is determined by filtering the sample through a glass fibre filter prior to dilution. Carbonaceous BOD (CBOD) is determined by adding a nitrification inhibitor to the diluted sample prior to incubation.

CL-L-IC-N-TB	Water	Chloride in Water by IC (Low Level)	EPA 300.1 (mod)

Inorganic anions are analyzed by Ion Chromatography with conductivity and/or UV detection.

CN-CNO-WT	Water	Cyanate	APHA 4500-CN-L

This analysis is carried out using procedures adapted from APHA method 4500-CN "Cyanide". Cyanate is determined by the Cyanate hydrolysis method using an ammonia selective electrode

CN-FREE-MISA-CFA-WT	Effluent	Free Cyanide by Continuous Flow Analyzer	ASTM D7237-10 (modified)

This analysis is carried out using procedures adapted from ASTM Method 7237 "Free Cyanide with Flow Injection Analysis (FIA) Utilizing Gas Diffusion Separation and Amperometric Detection". Free cyanide is determined by in-line gas diffusion at pH 6 with final determination by colourimetric analysis.

CN-SCN-VA	Water	Thiocyanate by Colour	APHA 4500-CN CYANIDE

This analysis is carried out using procedures adapted from APHA Method 4500-CN- M "Thiocyanate" Thiocyanate is determined by the ferric nitrate colourimetric method.
Water samples containing high levels of hexavalent chromium, cyanide (together with sulfide), reducing agents, or hydrocarbons may cause negative or positive interferences with this method. Contact ALS for additional information if required.

CN-T-MISA-CFA-WT	Effluent	Total Cyanide by CFA	ISO 14403-2:2012 (modified)

This analysis is carried out using procedures adapted from ISO Method 14403:2002 "Determination of Total Cyanide using Flow Analysis (FIA and CFA)". Total or strong acid dissociable (SAD) cyanide is determined by in-line UV digestion along with sample distillation and final determination by colourimetric analysis.
Method Limitation: This method is susceptible to interference from thiocyanate (SCN). If SCN is present in the sample, there could be a positive interference with this method, but it would be less than 1% and could be as low as zero.

CN-WAD-MISA-CFA-WT	Effluent	Weak Acid Dissociable Cyanide by CFA	APHA 4500-CN CYANIDE (modified)

This analysis is carried out using procedures adapted from APHA Method 4500-CN I. "Weak Acid Dissociable Cyanide". Weak Acid Dissociable (WAD) cyanide is determined by in-line sample distillation with final determination by colourimetric analysis.

COD-TB	Water	Chemical Oxygen Demand	APHA 5220D

This analysis is carried out using procedures adapted from APHA Method 5220 "Chemical Oxygen Demand (COD)". Chemical oxygen demand is determined using the closed reflux colourimetric method.

COLOUR-TB	Water	Colour, True	APHA 2120 C

Reference Information

True Colour in aqueous matrices is analyzed using colourimetric detection. This is determined by filtering a sample through a 0.45 micron membrane filter followed by analysis of the filtrate using a platinum-cobalt standard.

DOC-WT	Effluent	Dissolved Organic Carbon for MISA	APHA 5310 B-Instrumental
EC-MISA-TB	Effluent	Conductivity (EC)	APHA 2510 B-ELECTRODE

This analysis is carried out using procedures adapted from APHA Method 2510 "Conductivity". Conductivity is determined using a conductivity electrode.

F-IC-N-TB	Water	Fluoride in Water by IC	EPA 300.1 (mod)

Inorganic anions are analyzed by Ion Chromatography with conductivity and/or UV detection.

HARDNESS-CALC-TB	Effluent	Hardness (as CaCO3)	CALCULATION
HG-DIS-WT	Effluent	Mercury (Hg)-Dissolved for MISA	SW846 7470A
HG-TOT-WT	Effluent	Mercury (Hg)-Total for MISA	SW846 7470A
MET-D-MISA-TB	Effluent	Dissolved Metals in Water (MISA)	APHA 3030B/6020B (mod)

Water samples are filtered (0.45 um), preserved with nitric acid, and analyzed by CRC ICPMS.

Method Limitation (re: Sulfur): Sulfide and volatile sulfur species may not be recovered by this method.

MET-T-MISA-TB	Effluent	Total Metals in Water (MISA)	EPA 200.2/6020B (mod)

Water samples are digested with nitric and hydrochloric acids, and analyzed by CRC ICPMS.

Method Limitation (re: Sulfur): Sulfide and volatile sulfur species may not be recovered by this method.

NH3-MISA-F-TB	Effluent	Ammonia by Discrete Analyzer	catnr 157/158 062217/99321057 (modified)

Ammonia is determined by Flow-injection analysis with fluorescence detection

NO2-MISA-IC-TB	Effluent	Nitrite in Water by IC	EPA 300.1 (mod)

Anions in aqueous matrices are analyzed using ion chromatography with conductivity and/or UV absorbance detectors.

NO3-MISA-IC-TB	Effluent	Nitrate in Water by IC	EPA 300.1 (mod)

Anions in aqueous matrices are analyzed using ion chromatography with conductivity and/or UV absorbance detectors.

P-T-MISA-COL-TB	Effluent	Total Phosphorus by Discrete Analyzer	APHA 4500-P B, F, G (modified)

Phosphorus in aqueous matrices is analyzed using discrete Analyzer with colourimetric detection.

PH-MISA-TB	Effluent	pH	APHA 4500-H-ELECTRODE

This analysis is carried out using procedures adapted from APHA Method 4500-H "pH Value". The pH is determined in the laboratory using a pH electrode

PO4-DO-COL-TB	Water	Dissolved Orthophosphate	APHA 4500-P B, F, G (modified)

Phosphorus in aqueous matrices is analyzed using discrete Analyzer with colourimetric detection.

RA226-MMER-BE	Water	Radium 226	Radium Isotopes by Alpha Spectrometry

Determination of Gamma Emitting Radionuclides In Water and Solids by Gamma Spectrometry.

SO4-MISA-IC-TB	Effluent	Sulfate in Water by IC	EPA 300.1 (mod)

Anions in aqueous matrices are analyzed using ion chromatography with conductivity and/or UV absorbance detectors.

TC,EC-QT97-TB	Water	Total Coliform and E.coli	APHA 9223 B

This analysis is carried out using procedures adapted from APHA Method 9223 "Enzyme Substrate Coliform Test". E. coli and Total Coliform are determined simultaneously. The sample is mixed with a mixture of hydrolyzable substrates and then sealed in a multi-well packet. The packet is incubated for 18 or 24 hours and then the number of wells exhibiting a positive response are counted. The final result is obtained by comparing the positive responses to a probability table.

TDS-MISA-TB	Effluent	Total Dissolved Solids	APHA 2540 C (modified)

Reference Information

	Aqueous matrices are analyzed using gravimetry and evaporation		
TKN-F-TB	Water	TKN in Water by Fluorescence	catnr 157/158, 062818/99334821
	Total Kjeldahl Nitrogen is determined using block digestion followed by Flow-injection analysis with fluorescence detection		
TOC-WT	Water	Total Organic Carbon	APHA 5310B
	Sample is injected into a heated reaction chamber which is packed with an oxidative catalyst. The water is vaporized and the organic cabon is oxidized to carbon dioxide. The carbon dioxide is transported in a carrier gas and is measured by a non-dispersive infrared detector.		
TOX-7DINHIB-LM-TB	Misc.	Growth Inhibition using Lemma minor	SEE SUBLET LAB RESULTS
	Lemna minor 7-day growth toxicity test, based on the protocol "Biological Test Method: Test Method for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, Lemna minor", Report EPS 1/RM/37, Second Edition (January 2007)		
TOX-GROWTH-FH-TB	Misc.	7 Day Survival & Growth Fat Head Minnows	SEE SUBLET LAB RESULTS
	Fathead minnow 7-day test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Environmental Protection Series, Ottawa, ON, Report EPS1/RM/22, Second Edition (February 2011).		
TOX-INHIB-PS-TB	Misc.	Growth Inhibition Pseudokirchneriella	SEE SUBLET LAB RESULTS
	Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) 72-hour growth toxicity test, based upon protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga" Report EPS1/RM/25, Second Edition (March 2007).		
TOX-REPRO-CD-TB	Misc.	Survival & reproduction (Ceriodaphnia)	SEE SUBLET LAB RESULTS
	Ceriodaphnia dubia 3-brood toxicity test, accroding to protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dusbia", Environmental Protection Series, Ottawa, ON, Report EPS 1/RM/21, Second Edition (February 2007).		
TSS-MISA-TB	Effluent	Total Suspended Solids	APHA 2540 D (modified)
	Aqueous matrices are analyzed using gravimetry		
TURBIDITY-TB	Water	Turbidity	APHA 2130 B-Nephelometer
	Aqueous matrices are analyzed using nephelometry with the light scatter measured at a 90” angle.		

** ALS test methods may incorporate modifications from specified reference methods to improve performance.

The last two letters of the above test code(s) indicate the laboratory that performed analytical analysis for that test. Refer to the list below:

Laboratory Definition Code	Laboratory Location
TB	ALS ENVIRONMENTAL - THUNDER BAY, ONTARIO, CANADA
WT	ALS ENVIRONMENTAL - WATERLOO, ONTARIO, CANADA
BE	BECQUEREL LABORATORIES INC. - MISSISSAUGA, ONTARIO, CANADA
VA	ALS ENVIRONMENTAL - VANCOUVER, BRITISH COLUMBIA, CANADA

Chain of Custody Numbers:

GLOSSARY OF REPORT TERMS

Surrogates are compounds that are similar in behaviour to target analyte(s), but that do not normally occur in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery. In reports that display the D.L. column, laboratory objectives for surrogates are listed there.
mg/kg - milligrams per kilogram based on dry weight of sample
mg/kg wwt - milligrams per kilogram based on wet weight of sample
mg/kg lwt - milligrams per kilogram based on lipid weight of sample
mg/L - unit of concentration based on volume, parts per million.
< - Less than.
D.L. - The reporting limit.
N/A - Result not available. Refer to qualifier code and definition for explanation.

Test results reported relate only to the samples as received by the laboratory.
UNLESS OTHERWISE STATED, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.
Analytical results in unsigned test reports with the DRAFT watermark are subject to change, pending final QC review.

Quality Control Report

Workorder: L2595461 Report Date: 30-JUN-21

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0

Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
BOD-TB	**Water**							
Batch R5480519								
WG3546885-8 DUP		**L2595274-1**						
Biochemical Oxygen Demand		53	57		mg/L	7.1	30	03-JUN-21
WG3546885-6 LCS								
Biochemical Oxygen Demand			96.5		%		85-115	03-JUN-21
WG3546885-5 MB								
Biochemical Oxygen Demand			<2.0		mg/L		2	03-JUN-21
CBOD-TB	**Water**							
Batch R5483098								
WG3547819-3 DUP		**L2596003-1**						
BOD Carbonaceous		2.5	3.5	J	mg/L	1.1	4	04-JUN-21
WG3547819-2 LCS								
BOD Carbonaceous			96.8		%		85-115	04-JUN-21
WG3547819-1 MB								
BOD Carbonaceous			<2.0		mg/L		2	04-JUN-21
CL-L-IC-N-TB	**Water**							
Batch R5478467								
WG3546466-3 DUP		**L2594999-31**						
Chloride (Cl)		<0.10	<0.10	RPD-NA	mg/L	N/A	20	03-JUN-21
WG3546466-2 LCS								
Chloride (Cl)			100.5		%		90-110	03-JUN-21
WG3546466-1 MB								
Chloride (Cl)			<0.10		mg/L		0.1	03-JUN-21
CN-CNO-WT	**Water**							
Batch R5482956								
WG3550512-3 DUP		**WG3550512-5**						
Cyanate		14.5	14.1		mg/L	2.5	20	09-JUN-21
WG3550512-2 LCS								
Cyanate			86.9		%		85-115	09-JUN-21
WG3550512-1 MB								
Cyanate			<0.20		mg/L		0.2	09-JUN-21
WG3550512-4 MS		**WG3550512-5**						
Cyanate			N/A	MS-B	%		-	09-JUN-21
CN-SCN-VA	**Water**							
Batch R5487078								
WG3552658-3 DUP		**L2595237-1**						
Thiocyanate (SCN)		<2.5	<2.5	RPD-NA	mg/L	N/A	20	10-JUN-21
WG3552658-2 LCS								

Quality Control Report

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
CN-SCN-VA	**Water**							
Batch R5487078								
WG3552658-2 LCS								
Thiocyanate (SCN)			100.4		%		85-115	10-JUN-21
WG3552658-1 MB								
Thiocyanate (SCN)			<0.50		mg/L		0.5	10-JUN-21
WG3552658-4 MS		**L2595461-1**						
Thiocyanate (SCN)			91.5		%		75-125	10-JUN-21
COD-TB	**Water**							
Batch R5479161								
WG3548225-2 LCS								
Chemical Oxygen Demand			108.5		%		85-115	05-JUN-21
WG3548225-1 MB								
Chemical Oxygen Demand			<10		mg/L		10	05-JUN-21
COLOUR-TB	**Water**							
Batch R5477551								
WG3546454-3 DUP		**L2595461-1**						
Color, True		8.3	8.0		CU	3.9	20	03-JUN-21
WG3546454-2 LCS								
Color, True			91.8		%		85-115	03-JUN-21
WG3546454-1 MB								
Color, True			<2.0		CU		2	03-JUN-21
F-IC-N-TB	**Water**							
Batch R5478467								
WG3546466-3 DUP		**L2594999-31**						
Fluoride (F)		<0.020	<0.020	RPD-NA	mg/L	N/A	20	03-JUN-21
WG3546466-2 LCS								
Fluoride (F)			94.2		%		90-110	03-JUN-21
WG3546466-1 MB								
Fluoride (F)			<0.020		mg/L		0.02	03-JUN-21
PO4-DO-COL-TB	**Water**							
Batch R5478324								
WG3546464-3 DUP		**L2594999-21**						
Orthophosphate-Dissolved (as P)		<0.15	<0.15	RPD-NA	mg/L	N/A	20	03-JUN-21
WG3546464-2 LCS								
Orthophosphate-Dissolved (as P)			97.3		%		80-120	03-JUN-21
WG3546464-1 MB								
Orthophosphate-Dissolved (as P)			<0.0030		mg/L		0.003	03-JUN-21
WG3546464-4 MS		**L2594999-22**						

Quality Control Report

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
PO4-DO-COL-TB	**Water**							
Batch R5478324								
WG3546464-4 MS		**L2594999-22**						
Orthophosphate-Dissolved (as P)			122.6		%		70-130	03-JUN-21
TC,EC-QT97-TB	**Water**							
Batch R5477590								
WG3546463-2 DUP		**L2595412-3**						
Escherichia Coli		5	5		MPN/100mL	0.0	65	02-JUN-21
WG3546463-1 MB								
Escherichia Coli			0		MPN/100mL		1	02-JUN-21
TKN-F-TB	**Water**							
Batch R5481265								
WG3548238-3 DUP		**L2595274-1**						
Total Kjeldahl Nitrogen		2.21	2.18		mg/L	1.4	20	09-JUN-21
WG3548238-2 LCS								
Total Kjeldahl Nitrogen			102.4		%		75-125	09-JUN-21
WG3548238-1 MB								
Total Kjeldahl Nitrogen			<0.050		mg/L		0.05	09-JUN-21
WG3548238-4 MS		**L2595274-2**						
Total Kjeldahl Nitrogen			N/A	MS-B	%		-	09-JUN-21
TOC-WT	**Water**							
Batch R5489823								
WG3551673-3 DUP		**L2596626-3**						
Total Organic Carbon		7.54	7.82		mg/L	3.7	20	11-JUN-21
WG3551673-2 LCS								
Total Organic Carbon			112.9		%		80-120	11-JUN-21
WG3551673-1 MB								
Total Organic Carbon			<0.50		mg/L		0.5	11-JUN-21
WG3551673-4 MS		**L2596626-3**						
Total Organic Carbon			121.4		%		70-130	11-JUN-21
TURBIDITY-TB	**Water**							
Batch R5477690								
WG3547055-2 LCS								
Turbidity			93.5		%		85-115	03-JUN-21
WG3547055-1 MB								
Turbidity			<0.10		NTU		0.1	03-JUN-21
ALK-MISA-TB	**Effluent**							

Quality Control Report

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
ALK-MISA-TB	**Effluent**							
Batch **R5478342**								
WG3546069-2 **LCS**								
Alkalinity, Total (as CaCO3)			103.1		%		85-115	03-JUN-21
WG3546069-1 **MB**								
Alkalinity, Total (as CaCO3)			<0.2		mg/L		2	03-JUN-21
Alkalinity, Phenolphthalein			<0.2		mg/L		2	03-JUN-21
CN-FREE-MISA-CFA-WT	**Effluent**							
Batch **R5479638**								
WG3548509-2 **LCS**								
Cyanide, Free			92.3		%		80-120	04-JUN-21
WG3548509-1 **MB**								
Cyanide, Free			0.0002		mg/L		0.002	04-JUN-21
CN-T-MISA-CFA-WT	**Effluent**							
Batch **R5479638**								
WG3548509-2 **LCS**								
Cyanide, Total			94.3		%		80-120	04-JUN-21
WG3548509-1 **MB**								
Cyanide, Total			<0.0002		mg/L		0.002	04-JUN-21
CN-WAD-MISA-CFA-WT	**Effluent**							
Batch **R5479638**								
WG3548509-2 **LCS**								
Cyanide, Weak Acid Diss			92.6		%		80-120	04-JUN-21
WG3548509-1 **MB**								
Cyanide, Weak Acid Diss			<0.0001		mg/L		0.002	04-JUN-21
DOC-WT	**Effluent**							
Batch **R5490459**								
WG3550586-3 **DUP**		**WG3550586-5**						
Dissolved Organic Carbon		16.1	16.1		mg/L	0.1	25	14-JUN-21
WG3550586-2 **LCS**								
Dissolved Organic Carbon			90.8		%		70-130	14-JUN-21
WG3550586-1 **MB**								
Dissolved Organic Carbon			<0.50		mg/L		0.5	14-JUN-21
EC-MISA-TB	**Effluent**							
Batch **R5478342**								
WG3546069-2 **LCS**								
Conductivity (EC)			101.8		%		90-110	03-JUN-21
WG3546069-1 **MB**								
Conductivity (EC)			1.2		uS/cm		2	03-JUN-21

Quality Control Report

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
HG-DIS-WT	**Effluent**							
Batch R5479675								
WG3549216-3 DUP		**L2596003-1**						
Mercury (Hg)-Dissolved		<0.000005	<0.000005	RPD-NA	mg/L	N/A	20	07-JUN-21
WG3549216-2 LCS								
Mercury (Hg)-Dissolved			103.0		%		80-120	07-JUN-21
WG3549216-1 MB								
Mercury (Hg)-Dissolved			<0.000005		mg/L		0.00003	07-JUN-21
WG3549216-4 MS		**L2596006-1**						
Mercury (Hg)-Dissolved			91.4		%		70-130	07-JUN-21
HG-TOT-WT	**Effluent**							
Batch R5479674								
WG3549207-3 DUP		**L2595443-1**						
Mercury (Hg)-Total		<0.000005	<0.000005	RPD-NA	mg/L	N/A	20	07-JUN-21
WG3549207-2 LCS								
Mercury (Hg)-Total			103.0		%		80-120	07-JUN-21
WG3549207-1 MB								
Mercury (Hg)-Total			<0.000005		mg/L		0.00003	07-JUN-21
WG3549207-4 MS		**L2595443-2**						
Mercury (Hg)-Total			102.4		%		70-130	07-JUN-21
MET-D-MISA-TB	**Effluent**							
Batch R5479522								
WG3547201-10 LCS								
Aluminum (Al)-Dissolved			104.2		%		80-120	03-JUN-21
Antimony (Sb)-Dissolved			104.1		%		80-120	03-JUN-21
Arsenic (As)-Dissolved			101.6		%		80-120	03-JUN-21
Barium (Ba)-Dissolved			102.3		%		80-120	03-JUN-21
Beryllium (Be)-Dissolved			105.2		%		80-120	03-JUN-21
Bismuth (Bi)-Dissolved			104.7		%		80-120	03-JUN-21
Boron (B)-Dissolved			100.3		%		80-120	03-JUN-21
Cadmium (Cd)-Dissolved			102.4		%		80-120	03-JUN-21
Calcium (Ca)-Dissolved			104.2		%		80-120	03-JUN-21
Cesium (Cs)-Dissolved			107.1		%		80-120	03-JUN-21
Chromium (Cr)-Dissolved			103.2		%		80-120	03-JUN-21
Cobalt (Co)-Dissolved			100.2		%		80-120	03-JUN-21
Copper (Cu)-Dissolved			99.8		%		80-120	03-JUN-21
Iron (Fe)-Dissolved			109.2		%		80-120	03-JUN-21
Lead (Pb)-Dissolved			106.6		%		80-120	03-JUN-21

Quality Control Report

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
MET-D-MISA-TB	**Effluent**							
Batch R5479522								
WG3547201-10 LCS								
Lithium (Li)-Dissolved			107.8		%		80-120	03-JUN-21
Magnesium (Mg)-Dissolved			104.1		%		80-120	03-JUN-21
Manganese (Mn)-Dissolved			102.7		%		80-120	03-JUN-21
Molybdenum (Mo)-Dissolved			104.2		%		80-120	03-JUN-21
Nickel (Ni)-Dissolved			100.7		%		80-120	03-JUN-21
Phosphorus (P)-Dissolved			109.8		%		70-130	03-JUN-21
Potassium (K)-Dissolved			105.3		%		80-120	03-JUN-21
Rubidium (Rb)-Dissolved			103.2		%		80-120	03-JUN-21
Selenium (Se)-Dissolved			96.3		%		80-120	03-JUN-21
Silicon (Si)-Dissolved			105.4		%		60-140	03-JUN-21
Silver (Ag)-Dissolved			103.4		%		80-120	03-JUN-21
Sodium (Na)-Dissolved			105.7		%		80-120	03-JUN-21
Strontium (Sr)-Dissolved			112.4		%		80-120	03-JUN-21
Sulfur (S)-Dissolved			101.5		%		80-120	03-JUN-21
Tellurium (Te)-Dissolved			96.9		%		80-120	03-JUN-21
Thallium (Tl)-Dissolved			102.7		%		80-120	03-JUN-21
Thorium (Th)-Dissolved			108.9		%		80-120	03-JUN-21
Tin (Sn)-Dissolved			104.2		%		80-120	03-JUN-21
Titanium (Ti)-Dissolved			101.8		%		80-120	03-JUN-21
Tungsten (W)-Dissolved			102.0		%		80-120	03-JUN-21
Uranium (U)-Dissolved			105.0		%		80-120	03-JUN-21
Vanadium (V)-Dissolved			105.3		%		80-120	03-JUN-21
Zinc (Zn)-Dissolved			99.4		%		80-120	03-JUN-21
Zirconium (Zr)-Dissolved			105.0		%		80-120	03-JUN-21
WG3547201-9 MB								
Aluminum (Al)-Dissolved			<0.0002		mg/L		0.005	03-JUN-21
Antimony (Sb)-Dissolved			<0.000005		mg/L		0.0006	03-JUN-21
Arsenic (As)-Dissolved			0.0000050		mg/L		0.001	03-JUN-21
Barium (Ba)-Dissolved			<0.000005		mg/L		0.01	03-JUN-21
Beryllium (Be)-Dissolved			<0.000002		mg/L		0.001	03-JUN-21
Bismuth (Bi)-Dissolved			<0.000002		mg/L		0.001	03-JUN-21
Boron (B)-Dissolved			0.0010		mg/L		0.05	03-JUN-21
Cadmium (Cd)-Dissolved			<0.0000005		mg/L		0.000017	03-JUN-21
Calcium (Ca)-Dissolved			<0.002		mg/L		0.2	03-JUN-21

Quality Control Report

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
MET-D-MISA-TB	**Effluent**							
Batch R5479522								
WG3547201-9 MB								
Cesium (Cs)-Dissolved			<0.0000005		mg/L		0.00001	03-JUN-21
Chromium (Cr)-Dissolved			<0.00001		mg/L		0.001	03-JUN-21
Cobalt (Co)-Dissolved			<0.000002		mg/L		0.0005	03-JUN-21
Copper (Cu)-Dissolved			<0.00002		mg/L		0.001	03-JUN-21
Iron (Fe)-Dissolved			<0.0005		mg/L		0.02	03-JUN-21
Lead (Pb)-Dissolved			<0.00001		mg/L		0.00005	03-JUN-21
Lithium (Li)-Dissolved			<0.0002		mg/L		0.05	03-JUN-21
Magnesium (Mg)-Dissolved			<0.0005		mg/L		0.02	03-JUN-21
Manganese (Mn)-Dissolved			<0.00002		mg/L		0.001	03-JUN-21
Molybdenum (Mo)-Dissolved			<0.000002		mg/L		0.001	03-JUN-21
Nickel (Ni)-Dissolved			<0.00002		mg/L		0.002	03-JUN-21
Phosphorus (P)-Dissolved			<0.005		mg/L		0.05	03-JUN-21
Potassium (K)-Dissolved			<0.01		mg/L		0.5	03-JUN-21
Rubidium (Rb)-Dissolved			0.000002		mg/L		0.0002	03-JUN-21
Selenium (Se)-Dissolved			<0.000005		mg/L		0.00005	03-JUN-21
Silicon (Si)-Dissolved			<0.005		mg/L		0.05	03-JUN-21
Silver (Ag)-Dissolved			<0.0000005		mg/L		0.0001	03-JUN-21
Sodium (Na)-Dissolved			<0.005		mg/L		0.1	03-JUN-21
Strontium (Sr)-Dissolved			<0.00002		mg/L		0.001	03-JUN-21
Sulfur (S)-Dissolved			<0.2		mg/L		0.5	03-JUN-21
Tellurium (Te)-Dissolved			0.00001		mg/L		0.001	03-JUN-21
Thallium (Tl)-Dissolved			<0.000002		mg/L		0.0003	03-JUN-21
Thorium (Th)-Dissolved			<0.00001		mg/L		0.0001	03-JUN-21
Tin (Sn)-Dissolved			0.000040		mg/L		0.001	03-JUN-21
Titanium (Ti)-Dissolved			<0.00002		mg/L		0.002	03-JUN-21
Tungsten (W)-Dissolved			<0.000002		mg/L		0.01	03-JUN-21
Uranium (U)-Dissolved			<0.0000005		mg/L		0.005	03-JUN-21
Vanadium (V)-Dissolved			0.00002		mg/L		0.001	03-JUN-21
Zinc (Zn)-Dissolved			<0.0002		mg/L		0.003	03-JUN-21
Zirconium (Zr)-Dissolved			<0.000002		mg/L		0.001	03-JUN-21
MET-T-MISA-TB	**Effluent**							

Quality Control Report

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
MET-T-MISA-TB	**Effluent**							
Batch R5478723								
WG3546527-10 LCS								
Aluminum (Al)-Total			109.2		%		80-120	03-JUN-21
Antimony (Sb)-Total			98.4		%		80-120	03-JUN-21
Arsenic (As)-Total			109.9		%		80-120	03-JUN-21
Barium (Ba)-Total			107.6		%		80-120	03-JUN-21
Beryllium (Be)-Total			95.7		%		80-120	03-JUN-21
Bismuth (Bi)-Total			98.8		%		80-120	03-JUN-21
Boron (B)-Total			88.2		%		80-120	03-JUN-21
Cadmium (Cd)-Total			99.1		%		80-120	03-JUN-21
Calcium (Ca)-Total			95.5		%		80-120	03-JUN-21
Cesium (Cs)-Total			100.0		%		80-120	03-JUN-21
Chromium (Cr)-Total			104.2		%		80-120	03-JUN-21
Cobalt (Co)-Total			104.9		%		80-120	03-JUN-21
Copper (Cu)-Total			105.4		%		80-120	03-JUN-21
Iron (Fe)-Total			107.2		%		80-120	03-JUN-21
Lead (Pb)-Total			98.0		%		80-120	03-JUN-21
Lithium (Li)-Total			99.7		%		80-120	03-JUN-21
Magnesium (Mg)-Total			106.9		%		80-120	03-JUN-21
Manganese (Mn)-Total			106.6		%		80-120	03-JUN-21
Molybdenum (Mo)-Total			98.2		%		80-120	03-JUN-21
Nickel (Ni)-Total			105.4		%		80-120	03-JUN-21
Phosphorus (P)-Total			115.6		%		80-120	03-JUN-21
Potassium (K)-Total			108.9		%		80-120	03-JUN-21
Rubidium (Rb)-Total			109.0		%		80-120	03-JUN-21
Selenium (Se)-Total			99.6		%		80-120	03-JUN-21
Silicon (Si)-Total			107.7		%		80-120	03-JUN-21
Silver (Ag)-Total			95.5		%		80-120	03-JUN-21
Sodium (Na)-Total			112.5		%		80-120	03-JUN-21
Strontium (Sr)-Total			103.0		%		80-120	03-JUN-21
Sulfur (S)-Total			97.9		%		80-120	03-JUN-21
Tellurium (Te)-Total			95.2		%		80-120	03-JUN-21
Thallium (Tl)-Total			94.5		%		80-120	03-JUN-21
Thorium (Th)-Total			99.4		%		80-120	03-JUN-21
Tin (Sn)-Total			96.0		%		80-120	03-JUN-21

Quality Control Report

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
MET-T-MISA-TB	**Effluent**							
Batch R5478723								
WG3546527-10 LCS								
Titanium (Ti)-Total			105.5		%		80-120	03-JUN-21
Tungsten (W)-Total			95.8		%		80-120	03-JUN-21
Uranium (U)-Total			97.5		%		80-120	03-JUN-21
Vanadium (V)-Total			107.5		%		80-120	03-JUN-21
Zinc (Zn)-Total			107.0		%		80-120	03-JUN-21
Zirconium (Zr)-Total			96.8		%		80-120	03-JUN-21
WG3546527-9 MB								
Aluminum (Al)-Total			0.0014		mg/L		0.005	03-JUN-21
Antimony (Sb)-Total			<0.000005		mg/L		0.0006	03-JUN-21
Arsenic (As)-Total			0.00002		mg/L		0.001	03-JUN-21
Barium (Ba)-Total			<0.00001		mg/L		0.01	03-JUN-21
Beryllium (Be)-Total			<0.0000001		mg/L		0.001	03-JUN-21
Bismuth (Bi)-Total			<0.00001		mg/L		0.001	03-JUN-21
Boron (B)-Total			0.0010		mg/L		0.05	03-JUN-21
Cadmium (Cd)-Total			<0.000001		mg/L		0.000017	03-JUN-21
Calcium (Ca)-Total			0.002		mg/L		0.2	03-JUN-21
Cesium (Cs)-Total			<0.0000005		mg/L		0.00001	03-JUN-21
Chromium (Cr)-Total			<0.00002		mg/L		0.001	03-JUN-21
Cobalt (Co)-Total			<0.000005		mg/L		0.0005	03-JUN-21
Copper (Cu)-Total			<0.00002		mg/L		0.001	03-JUN-21
Iron (Fe)-Total			0.0010		mg/L		0.02	03-JUN-21
Lead (Pb)-Total			<0.00001		mg/L		0.00005	03-JUN-21
Lithium (Li)-Total			<0.0002		mg/L		0.05	03-JUN-21
Magnesium (Mg)-Total			0.0008		mg/L		0.02	03-JUN-21
Manganese (Mn)-Total			<0.0002		mg/L		0.001	03-JUN-21
Molybdenum (Mo)-Total			<0.000005		mg/L		0.001	03-JUN-21
Nickel (Ni)-Total			<0.00002		mg/L		0.002	03-JUN-21
Phosphorus (P)-Total			0.010		mg/L		0.05	03-JUN-21
Potassium (K)-Total			0.01		mg/L		0.5	03-JUN-21
Rubidium (Rb)-Total			<0.000002		mg/L		0.0002	03-JUN-21
Selenium (Se)-Total			<0.000005		mg/L		0.00005	03-JUN-21
Silicon (Si)-Total			0.018		mg/L		0.1	03-JUN-21
Silver (Ag)-Total			<0.000001		mg/L		0.0001	03-JUN-21
Sodium (Na)-Total			<0.005		mg/L		0.1	03-JUN-21

Quality Control Report

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
MET-T-MISA-TB	**Effluent**							
Batch R5478723								
WG3546527-9 MB								
Strontium (Sr)-Total			0.000010		mg/L		0.001	03-JUN-21
Sulfur (S)-Total			<0.2		mg/L		0.5	03-JUN-21
Tellurium (Te)-Total			0.00002		mg/L		0.001	03-JUN-21
Thallium (Tl)-Total			<0.000005		mg/L		0.0003	03-JUN-21
Thorium (Th)-Total			<0.00001		mg/L		0.0001	03-JUN-21
Tin (Sn)-Total			<0.00001		mg/L		0.001	03-JUN-21
Titanium (Ti)-Total			<0.00001		mg/L		0.002	03-JUN-21
Tungsten (W)-Total			<0.00001		mg/L		0.01	03-JUN-21
Uranium (U)-Total			<0.0000005		mg/L		0.005	03-JUN-21
Vanadium (V)-Total			0.00010		mg/L		0.001	03-JUN-21
Zinc (Zn)-Total			<0.0005		mg/L		0.003	03-JUN-21
Zirconium (Zr)-Total			<0.000002		mg/L		0.001	03-JUN-21
NH3-MISA-F-TB	**Effluent**							
Batch R5480103								
WG3548218-2 LCS								
Ammonia, Total (as N)			92.1		%		85-115	07-JUN-21
WG3548218-1 MB								
Ammonia, Total (as N)			<0.002		mg/L		0.005	07-JUN-21
NO2-MISA-IC-TB	**Effluent**							
Batch R5478467								
WG3546466-2 LCS								
Nitrite (as N)			96.2		%		90-110	03-JUN-21
WG3546466-1 MB								
Nitrite (as N)			<0.001		mg/L		0.01	03-JUN-21
NO3-MISA-IC-TB	**Effluent**							
Batch R5478467								
WG3546466-2 LCS								
Nitrate (as N)			100.2		%		90-110	03-JUN-21
WG3546466-1 MB								
Nitrate (as N)			<0.002		mg/L		0.02	03-JUN-21
P-T-MISA-COL-TB	**Effluent**							
Batch R5485357								
WG3548234-2 LCS								
Phosphorus (P)-Total			98.1		%		80-120	10-JUN-21
WG3548234-1 MB								

Quality Control Report

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed
P-T-MISA-COL-TB	**Effluent**							
Batch R5485357								
WG3548234-1 MB								
Phosphorus (P)-Total			<0.0005		mg/L		0.003	10-JUN-21
PH-MISA-TB	**Effluent**							
Batch R5478342								
WG3546069-2 LCS								
pH			7.01		pH		6.9-7.1	03-JUN-21
SO4-MISA-IC-TB	**Effluent**							
Batch R5478467								
WG3546466-2 LCS								
Sulfate (SO4)			102.4		%		90-110	03-JUN-21
WG3546466-1 MB								
Sulfate (SO4)			<0.05		mg/L		0.3	03-JUN-21
TDS-MISA-TB	**Effluent**							
Batch R5479135								
WG3548002-2 LCS								
Total Dissolved Solids			89.3		%		85-115	04-JUN-21
WG3548002-1 MB								
Total Dissolved Solids			8		mg/L		10	04-JUN-21
TSS-MISA-TB	**Effluent**							
Batch R5479116								
WG3547997-2 LCS								
Total Suspended Solids			99.0		%		85-115	04-JUN-21
WG3547997-1 MB								
Total Suspended Solids			<0.5		mg/L		3	04-JUN-21

Quality Control Report

Workorder: L2595461 Report Date: 30-JUN-21

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Legend:

Limit	ALS Control Limit (Data Quality Objectives)
DUP	Duplicate
RPD	Relative Percent Difference
N/A	Not Available
LCS	Laboratory Control Sample
SRM	Standard Reference Material
MS	Matrix Spike
MSD	Matrix Spike Duplicate
ADE	Average Desorption Efficiency
MB	Method Blank
IRM	Internal Reference Material
CRM	Certified Reference Material
CCV	Continuing Calibration Verification
CVS	Calibration Verification Standard
LCSD	Laboratory Control Sample Duplicate

Sample Parameter Qualifier Definitions:

Qualifier	Description
<W	No Measurable Response (Zero): < Reported Value
DLM	Detection Limit Adjusted due to sample matrix effects (e.g. chemical interference, colour, turbidity).
J	Duplicate results and limits are expressed in terms of absolute difference.
MS-B	Matrix Spike recovery could not be accurately calculated due to high analyte background in sample.
RPD-NA	Relative Percent Difference Not Available due to result(s) being less than detection limit.

Quality Control Report

Workorder: L2595461 Report Date: 30-JUN-21

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Hold Time Exceedances:

ALS Product Description	Sample ID	Sampling Date	Date Processed	Rec. HT	Actual HT	Units	Qualifier
Organic / Inorganic Carbon							
Dissolved Organic Carbon for MISA							
	1	01-JUN-21 08:30	08-JUN-21 00:00	3	7	days	EHT

Legend & Qualifier Definitions:

EHTR-FM:	Exceeded ALS recommended hold time prior to sample receipt. Field Measurement recommended.
EHTR:	Exceeded ALS recommended hold time prior to sample receipt.
EHTL:	Exceeded ALS recommended hold time prior to analysis. Sample was received less than 24 hours prior to expiry.
EHT:	Exceeded ALS recommended hold time prior to analysis.
Rec. HT:	ALS recommended hold time (see units).

Notes*:
Where actual sampling date is not provided to ALS, the date (& time) of receipt is used for calculation purposes.
Where actual sampling time is not provided to ALS, the earlier of 12 noon on the sampling date or the time (& date) of receipt is used for calculation purposes. Samples for L2595461 were received on 02-JUN-21 09:11.

ALS recommended hold times may vary by province. They are assigned to meet known provincial and/or federal government requirements. In the absence of regulatory hold times, ALS establishes recommendations based on guidelines published by the US EPA, APHA Standard Methods, or Environment Canada (where available). For more information, please contact ALS.

The ALS Quality Control Report is provided to ALS clients upon request. ALS includes comprehensive QC checks with every analysis to ensure our high standards of quality are met. Each QC result has a known or expected target value, which is compared against pre-determined data quality objectives to provide confidence in the accuracy of associated test results.

Please note that this report may contain QC results from anonymous Sample Duplicates and Matrix Spikes that do not originate from this Work Order.

pollutech
enviroquatics limited

June 25, 2021

Christine Paradis
ALS Thunder Bay
1081 Barton St.
Thunder Bay, ON P7B 5N3

Dear Christine,

On June 3, 2021, Pollutech EnviroQuatics Limited personnel received one water sample from ALS Environmental, Thunder Bay Site EDL1-Effluent (L2595461-1). The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
EDL1-Effluent (L2595461-1)	Fathead Minnow	7-day LC50 (95% Confidence)	Survival	>100% Volume (Not Applicable)
		7-day IC25 (95% Confidence)	Biomass	>100% Volume[3] (Not Applicable)
#8730-0032104	*Ceriodaphnia dubia*	3-brood LC50 (95% Confidence)	Survival	>100% Volume (Not Applicable)
		3-brood IC25 (95% Confidence)	Reproduction	<0.137% Volume (Not Applicable)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[2] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence)	Growth	0.83% Volume (0.20; 3.91% Volume)
		7-day IC25 Dry Weight (95% Confidence)	Growth	>97% Volume[2] (Not applicable)

1 - Results relate only to the sample tested
2 - Highest concentration tested, based on test method
3 - Inhibition 30% at concentrations 6.25% v/v

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Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 — The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 — The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations, of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 100%, 33%, 11%, 3.7% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations, of 4 replicates each, diluted half by half (*i.e.,* 100%, 50%, 25% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information with regard to quality assurance/quality control procedures and results can be made available if so desired.

If there are any further details which you require, please do not hesitate to contact us.

Yours very truly,
Pollutech EnviroQuatics Limited

R. Clay Ferguson, B.Sc. (Hon.)
Laboratory Manager

File ID: \bioassay\2021\8000\8730-003\8730-003jn2 FCRL

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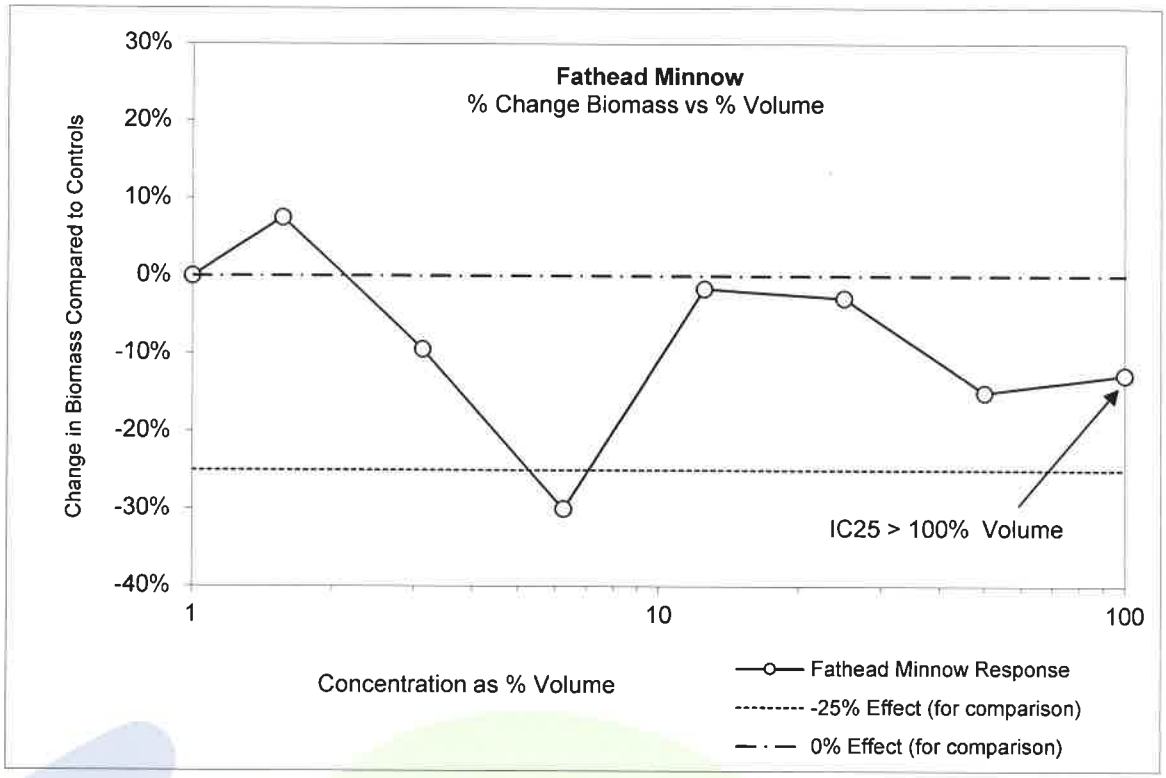
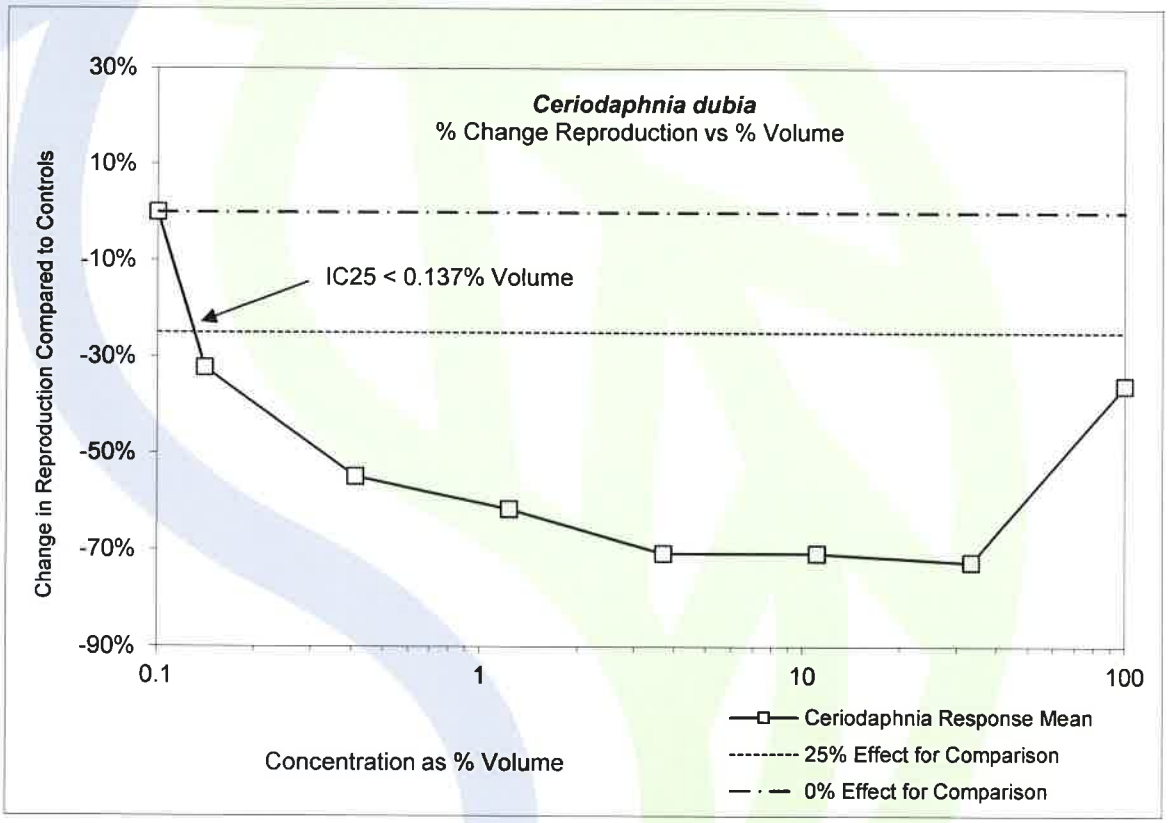
ALS Thunder Bay - EDL1-Effluent (L2595461-1) - June 1, 2021





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Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Pollutech Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay Site		
Sample Type:	Effluent	**Source:**	EDL1-Effluent (L2595461-1)
Date/Time Sampled:	June 1, 2021; 08:30	**Date/Time Received:**	June 3, 2021; 14:30
Date/Time Test Started:	June 3, 2021; 16:45	**Date Test Finished:**	June 10, 2021
Description:	Clear, yellow	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032104	**Sample Collection:**	Grab
Transport:	Air/Road	**Arrival Temp.:**	21.0°C
Collected By:	N/A		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species: Fathead Minnow (*Pimephales promelas*)

Source: Pollutech Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)

Culture Temp.: 22 to 26 °C **Life Stage:** <24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 1.5%

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Fathead Minnow Larval Survival and Growth Study - Continued

Test Conditions

Sample #: 8730-0032104 **Source:** EDL1-Effluent (L2595461-1)

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 1 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Facilities and Apparatus

Testing Laboratory: Pollutech EnviroQuatics Limited, 704 Mara St., Suite 122, Point Edward, Ontario, N7V 1X4

CALA Testing Accreditation No. A1225

This laboratory is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA). The test included in this report is within the scope of this laboratory.

Test Performed By: E. Pasiak / C. D'Andrea / K. Kramer / C. Hamill

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (3.3%)

Average Weight of Controls is ≥ 250 µg: Acceptable (541 µg)

Fathead Minnow Larval Survival and Growth Study - Continued

Test Results

Sample #: 8730-0032104 Sources: EDL1-Effluent (L2595461-1)

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	10	10	10	20	0	20	40	20
	2	0	0	20	20	20	10	10	0
	3	0	0	10	30	30	10	0	30
Mean % Mortality		3.3	3.3	13.3	23.3	16.7	13.3	16.7	16.7
S.D.		5.8	5.8	5.8	5.8	15.3	5.8	20.8	15.3
Survival/Growth Data									
Mean Dry Biomass (mg)	1	0.473	0.540	0.504	0.365	0.619	0.564	0.323	0.474
	2	0.528	0.547	0.432	0.395	0.391	0.405	0.470	0.494
	3	0.569	0.601	0.486	0.339	0.534	0.556	0.541	0.402
% Effect (+ or -)		0.0	7.5	-9.4	-30.0	-1.7	-2.9	-15.0	-12.7
Mean Dry Biomass/ Concentration (mg)		0.523	0.563	0.474	0.366	0.515	0.508	0.445	0.457
S.D.		0.05	0.03	0.04	0.03	0.12	0.09	0.11	0.05

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032104 Sources: EDL1-Effluent (L2595461-1)

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 7-day LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Biomass **(Survival and Growth)** 7-day IC25 (Confidence Interval)[2]	>100% Volume[3] (Not Applicable)	Non-linear Regression 2P Exponential Model

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval
3 - Inhibition 30% at concentrations 6.25% v/v

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Phenol

Date Test Initiated: 05/30/21 **Reference Batch #:** P2107

Method of Analysis: Trimmed Spearman-Karber α = 1.67%

7-Day LC50 (95% Confidence Limits): 28.69 mg/L (24.99 mg/L; 32.93 mg/L)

Historic Geometric Mean LC50: 22.57 mg/L (14.76 mg/L; 34.50 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS - Thunder Bay Sample Name: EDL-1 - Effluent Sample #: 8730-0032104

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 3.3 %

Average Weight of Controls is ≥ 250 µg: (Acceptable) / Not Acceptable 541 µg

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 2 min Days: 1 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
7-day LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
Survival / Growth Biomass	*	Nonlinear regression 2P Exponential model
7-day IC25	>100 % Volume	
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

* Inhibition 30% at conc 6.25%

Analysis Completed: Initials EO Date 06/22/21

Results Verified: Initials CF Date 06/23/21

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** La595461-EDL1 Effluent **Sample #:** 8730.0032104

Day	Date 2021	Initial Variables: Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	Meters/Probes Used: D.O./°C	pH	Cond.	Pre-aeration: yes/no	Rate (bubbles/min)	Duration (min)	Pail Sub-Sampled	Initials
0	0603	24.0	7.4	8.3	1507	6/4	B/84	5/6	~~NO~~ YES	≤100	≤ 20	1	CD
1	4	26.0	7.3	9.4	1573	6/4	B/84	5/6	YES	≤100	≤ 20	1	CD
2	5	24.5	7.5	10.0	1522	6/4	B/84	5/6	YES	≤100	≤ 20	1	CD
3	6	26.3	7.6	9.9	1577	6/4	B/84	5/6	YES	≤100	≤ 20	2	CD
4	7	24.5	7.6	11.1	1517	6/4	B/84	5/6	YES	≤100	≤ 20	2	CD
5	8	25.3	7.7	10.0	1530	6/4	B/84	5/6	YES	≤100	≤ 20	3	CD
6	9	24.7	7.6	10.0	1510	6/4	B/84	5/6	YES	≤100	≤ 20	3	CD
7	10												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? (Yes/No) If yes, state mesh size: Visible daphnia in sample filtered to a 63µm plankton net

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of brine shrimp days 0 to 6? (Yes/No) If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: Control   Sample Name: L2595461 · EDL-1-Effluent   Sample #: 873000382104

Day	Date 2021	Initial °C	Initial pH (units)	Initial D.O. (mg/L)	Initial Cond (µmhos)	Meter / Probe D.O. / °C	Meter / Probe pH	Meter / Probe cond	Initials	Final °C	Final pH (units)	Final D.O. (mg/L)	Meter / Probe D.O. / °C	Meter / Probe pH	Initials	% Mortality / Rep A	% Mortality / Rep B	% Mortality / Rep C	% Atypical / Rep A	% Atypical / Rep B	% Atypical / Rep C	Initials
0	0603	20.9	7.2	7.7	270	6/4	13/84	5/6	CO	25.2	7.7	~~6.9~~ KC 7.0	6/4	13/84	KC							
1	4	26.0	8.0	7.6	280	6/4	13/84	5/6	KC	25.2	7.8	7.0	6/4	13/84	KC	0	0	0	0	0	0	KC
2	5	26.0	8.2	7.7	278	6/4	13/84	5/6	KC	25.2	7.9	6.7	6/4	13/84	C	0	0	0	0	0	0	KC
3	6	25.1	8.1	7.6	275	6/4	13/84	5/6	C	25.5	7.9	7.0	6/4	13/84	CH	0	0	0	0	0	0	CH
4	7	25.0	8.1	7.6	278	6/4	13/84	5/6	C	25.4	7.7	6.5	6/4	13/84	C	0	0	0	0	0	0	CH
5	8	26.3	8.1	7.6	266	6/4	13/84	5/6	C	25.5	7.8	6.1	6/4	13/84	CH	10	0	0	0	0	0	C
6	9	25.4	8.0	7.5	274	6/4	13/84	5/6	C	25.3	CO 7.1	6.2	6/4	13/84	CO	10	0	0	0	0	0	CH
7	10															10	0	0	0	0	0	CO

Observations: ______

Concentration: 1.56% v/v

Day	Date 2021	Initial °C	Initial pH (units)	Initial D.O. (mg/L)	Initial Cond (µmhos)	Meter / Probe D.O. / °C	Meter / Probe pH	Meter / Probe cond	Initials	Final °C	Final pH (units)	Final D.O. (mg/L)	Meter / Probe D.O. / °C	Meter / Probe pH	Initials	% Mortality / Rep A	% Mortality / Rep B	% Mortality / Rep C	% Atypical / Rep A	% Atypical / Rep B	% Atypical / Rep C	Initials
0	0603	24.9	8.1	7.9	295	6/4	13/84	5/6	CO	25.2	7.7	7.0	6/4	13/84	KC							
1	4	25.2	8.0	7.6	299	6/4	13/84	5/6	KC	25.2	7.8	6.7	6/4	13/84	KC	0	0	0	0	0	0	KC
2	5	25.5	8.1	7.8	300	6/4	13/84	5/6	KC	25.2	7.9	6.5	6/4	13/84	C	10	0	0	0	0	0	KC
3	6	24.9	7.8	7.6	300	6/4	13/84	5/6	C	25.4	7.9	7.0	6/4	13/84	CH	10	0	0	0	0	0	CH
4	7	25.1	8.1	8.0	303	6/4	13/84	5/6	CH	25.4	7.7	6.8	6/4	13/84	C	10	0	0	0	0	0	CH
5	8	26.0	8.0	7.8	301	6/4	13/84	5/6	C	25.5	7.7	5.9	6/4	13/84	CH	10	0	0	0	0	0	C
6	9	25.2	8.1	7.6	297	6/4	13/84	5/6	CH	25.3	7.2	6.7	6/4	13/84	CO	10	0	0	0	0	0	CH
7	10															10	0	0	0	0	0	CO

Observations: ______

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13 % v/v Sample Name: L2595461 · EDL-1-Effluent Sample #: 873003321104

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	0603								
1	4								
2	5								
3	6								
4	7								
5	8								
6	9								
7	10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	10 KK	0	0	0	KK
						10	10	0	0	0	0	KK
						10	10	0	0	0	0	CH
						10	10	0	0	0	0	CH
						10	10	10	0	0	0	E
						10	20	10	0	0	0	CH
						10	20	10	0	0	0	CH

Observations: _____

Concentration: 6.25 % v/v

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	0603								
1	4								
2	5								
3	6								
4	7								
5	8								
6	9								
7	10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	KK
						0	0	10	0	0	0	CH
						0	0	10	0	0	0	CH
						20	0	30	0	0	0	E
						20	20	30	0	0	0	CH
						20	20	30	0	0	0	CH

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5% v/v Sample Name: L2595461 · EDL-1-Effluent Sample #: 8730032104

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	0603	24.9	7.9	7.0	444	6/4	13/84	5/6	aO	25.2	7.7	7.1	6/4	13/84	KC							
1	4	25.2	7.9	7.6	455	6/4	13/84	5/6	KC	25.2	7.9	7.1	6/4	13/84	KC	0	0	0	0	0	0	KC
2	5	25.4	~~7.8~~ 8.1 KC	7.8	455	6/4	13/84	5/6	KC	25.2	7.9	6.6	6/4	13/84	T	0	0	0	0	0	0	KC
3	6	25.0	7.8	7.6	449	6/4	13/84	5/6	T	25.4	7.8	6.8	6/4	13/84	CH	0	10	0	0	0	0	CH
4	7	25.1	8.1	8.0	465	6/4	13/84	5/6	CH	25.4	7.8	6.8	6/4	13/84	T	0	10	30	0	0	0	CH
5	8	25.9	8.0	7.6	480	6/4	13/84	5/6	T	25.5	7.6	5.9	6/4	13/84	CH	0	20	30	0	0	0	T
6	9	25.2	8.1	7.7	456	6/4	13/84	5/6	CH	25.3	7.5	6.7	6/4	13/84	aO	0	20	30	0	0	0	CH
7	10															0	20	30	0	0	0	aO

Observations: ______________________

Concentration: 25% v/v

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	0603																					
1	4															0	0	0	0	0	0	KC
2	5															0	10	0	0	0	0	KC
3	6															0	10	0	0	0	0	CH
4	7															0	10	0	0	0	0	CH
5	8															10	~~20~~ 10 CH	10	0	0	0	T
6	9															20	10	10	0	0	0	CH
7	10															20	10	10	0	0	0	aO

Observations: ______________________

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50% v/v

Sample Name: L2595461- EDL-1- Effluent

Sample #: 87300032104

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	0603								
1	4								
2	5								
3	6								
4	7								
5	8								
6	9								
7	10								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0													
1							0	0	0	0	0	0	KK
2							0	0	0	0	0	0	KK
3							10	0	0	0	0	0	CH
4							20	10	0	0	0	0	CH
5							20	10	0	0	0	0	T
6							30	10	0	0	0	0	CH
7							40	10	0	0	0	0	GO

Observations: ____________________

Concentration: 100% v/v

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	0603	24.0	7.4	8.3	1507	6/4	13/84	5/6	GO
1	4	25.3	7.6	8.0	1545	6/4	13/84	5/6	KK
2	5	25.0	7.7	8.9	1536	6/4	13/84	5/6	KK
3	6	25.5	7.7	9.0	1536	6/4	13/84	5/6	T
4	7	25.4	7.8	9.1	1544	6/4	13/84	5/6	CH
5	8	25.3	7.7	9.1	1488	6/4	13/84	5/6	T
6	9	25.2	7.8	8.9	1528	6/4	13/84	5/6	CH
7	10								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	25.2	7.7	6.6	6/4	13/84	KK							
1	25.2	8.0	6.8	6/4	13/84	KK	0	0	0	0	0	0	KK
2	25.1	8.0	6.3	6/4	13/84	T	0	0	0	0	0	0	KK
3	25.4	8.0	6.7	6/4	13/84	CH	10	0	10	0	0	0	CH
4	25.4	7.9	6.7	6/4	13/84	T	20	0	20	0	0	0	CH
5	25.5	7.8	5.8	6/4	13/84	CH	20	0	20	0	0	0	T
6	25.3	7.7	6.8	6/4	13/84	GO	20	0	20	0	0	0	CH
7							20	0	30	0	0	0	GO

Observations: ____________________

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information

Client: ALS
Sample #: 8730-0032104
Date/Time Received: 060321 / 1430
Sample Type: Effluent
100% Hardness: 472

Sample Name: EDL 1 Effluent L2595461
Sample Date/Time: 060121 / 0830
Person Sampling: ________
Arrival Temp: 210 °C
Sample Description: clear, yellow

Test Information

Date/Time Started: 060321 1645
Test started by: aO
Fathead Batch #: FH081200620
Date eggs laid: 05 30/31. 21
Culture mortality within 7 days of egg collection: 1.5%
Swin bladder inflated: (yes) / no CO
Age of Larvae at start of test in hours: <24
Control Hardness: 100
Water Bath Quadrant: C
Average Temperature during Test: 25.3 ± 0.1 °C

Is there any unusual appearance, behavior, or treatment of test organisms before their use in the test? Yes (No) (circle one)

Conc. %	Rep	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	9	0.77726	0.77253	0.473	0.523
	B	10	0.76693	0.76165	0.528	
	C	10	0.77471	0.76902	0.569	
1.56	A	9	0.77452	0.76912	0.540	0.563
	B	10	0.77563	0.77016	0.547	
	C	10	0.77469	0.76868	0.601	
3.13	A	9	0.77797	0.77293	0.504	0.474
	B	8	0.77834	0.77402	0.432	
	C	9	0.78001	0.77515	0.486	
6.25	A	8	0.77503	0.77138	0.365	0.366
	B	8	0.76884	0.76489	0.395	
	C	7	0.76633	0.76294	0.339	
12.5	A	10	0.77089	0.76470	0.619	0.515
	B	8	0.77808	0.77417	0.391	
	C	7	0.78669	0.78135	0.534	
25	A	8	0.77192	0.76628	0.564	0.508
	B	9	0.77283	0.77810	0.405	
	C	9	0.76884	0.76328	0.556	
50	A	6	0.76845	0.76522	0.323	0.445
	B	9	0.76863	0.76393	0.470	
	C	10	0.76932	0.76391	0.541	
100	A	8	0.76582	0.76108	0.474	0.457
	B	10	0.76608	0.76114	0.494	
	C	7	0.76582	0.76180	0.402	
Initials		G	OD	CA	5	C

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8730-0032104 **Sample Name** EDL-1-Effluent

Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug) 541

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	9	0.77726	0.77253	0.526	0.473	0.541	0.523	0.024408	0.04817	4.512813
	10	10	0.76693	0.76165	0.528	0.528					
	10	10	0.77471	0.76902	0.569	0.569					
1.56	10	9	0.77452	0.76912	0.600	0.540	0.583	0.563	0.030892	0.033382	5.30188
	10	10	0.77563	0.77016	0.547	0.547					
	10	10	0.77469	0.76868	0.601	0.601					
3.13	10	9	0.77797	0.77293	0.560	0.504	0.547	0.474	0.011547	0.03747	2.112257
	10	8	0.77834	0.77402	0.540	0.432					
	10	9	0.78001	0.77515	0.540	0.486					
6.25	10	8	0.77503	0.77138	0.456	0.365	0.478	0.366	0.019501	0.028024	4.078975
	10	8	0.76884	0.76489	0.494	0.395					
	10	7	0.76633	0.76294	0.484	0.339					
12.5	10	10	0.77089	0.76470	0.619	0.619	0.624	0.515	0.13711	0.115223	21.98909
	10	8	0.77808	0.77417	0.489	0.391					
	10	7	0.78669	0.78135	0.763	0.534					
25	10	8	0.77192	0.76628	0.705	0.564	0.591	0.508	0.129603	0.089579	21.93224
	10	9	0.77283	0.76878	0.450	0.405					
	10	9	0.76884	0.76328	0.618	0.556					
50	10	6	0.76845	0.76522	0.538	0.323	0.534	0.445	0.010159	0.111186	1.903043
	10	9	0.76863	0.76393	0.522	0.470					
	10	10	0.76932	0.76391	0.541	0.541					
100	10	8	0.76582	0.76108	0.592	0.474	0.554	0.457	0.052408	0.048387	9.466906
	10	10	0.76608	0.76114	0.494	0.494					
	10	7	0.76582	0.76180	0.574	0.402					

SD: Standard Deviation
CV: Coefficient of Variation

CF June 23

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enviroquatics limited

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Pollutech Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay Site		
Sample Type:	Effluent	**Source:**	EDL1-Effluent (L2595461-1)
Date/Time Sampled:	June 1, 2021; 08:30	**Date/Time Received:**	June 3, 2021; 14:30
Date Test Started:	June 3, 2021	**Date Test Finished:**	June 10, 2021
Description:	Clear, yellow	**Days Sample Used:**	Days 0 to 5
Sample #:	8730-0032104	**Sample Collection:**	Grab
Transport:	Air/Road	**Arrival Temp.:**	21.0°C
Collected By:	N/A		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Pollutech Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 0%

Mean Number of Surviving Young Produced Within First 3 Broods: 23.1

Mean Number of Surviving Young Produced Within 7 Days of Testing: 23.8

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Ceriodaphnia dubia Survival and Reproduction Study - Continued

Control and Dilution Water

Sample #: 8730-0032104 **Source:** EDL1-Effluent (L2595461-1)

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 0 to 6

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD21-61

Conditions for Test Validity

Control Mortality is ≤ 20%:	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (16.4 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (80% of controls)

Ceriodaphnia dubia Survival and Reproduction Study - Continued

Test Results

Sample #: 8730-0032104 Sources: EDL1-Effluent (L2595461-1)

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	10	20	20	30	40	0	10
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	18	8	10	13	6	4	4	19
	2	21	9	6	0	12	2	3	3
	3	2	17	5	4	4	3	9	10
	4	19	16	8	10	3	4	10	6
	5	16	12	4	7	5	11	2	10
	6	17	11	0	6	6	11	4	17
	7	15	13	10	4	5	5	3	8
	8	16	7	11	4	2	4	3	10
	9	22	4	11	3	2	2	4	11
	10	18	14	9	12	3	2	3	11
Total Number of Live Neonates in First 3 Broods or Less		164	111	74	63	48	48	45	105
% Effect (+ or -)		0.0	-32.3	-54.9	-61.6	-70.7	-70.7	-72.6	-36.0
Mean Number of Live Neonates in First 3 Broods or Less		16.4	11.1	7.4	6.3	4.8	4.8	4.5	10.5
SD		5.5	4.1	3.6	4.2	2.9	3.4	2.7	4.7

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

Ceriodaphnia dubia Survival and Reproduction Study - Continued

Sample #: 8730-0032104 Sources: EDL1-Effluent (L2595461-1)

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	Insufficient dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	<0.137% Volume (Not Applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (Control; rep. 3). No reason to remove it. Statistics include all data.

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Phenol

Date Test Initiated: 05/30/21 **Reference Batch #:** P2107

Method of Analysis: Trimmed Spearman-Karber α = 10%

3-Brood LC50 (95% Confidence Limits): 7.16 mg/L (5.51 mg/L; 9.31 mg/L)

Historic Geometric Mean LC50: 6.23 mg/L (3.85 mg/L; 10.09 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Test Facilities

Testing Laboratory:

CALA Testing Accreditation No. A1225

Pollutech EnviroQuatics Limited, 704 Mara St., Suite 122, Point Edward, Ontario, N7V 1X4

This laboratory is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA). The test included in this report is within the scope of this laboratory.

Test Performed By: E. Pasiak / C. D'Andrea / K. Kramer / C. Hamill

pollutech enviroquatics limited

CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS - Thunder Bay Sample Name: EDL-1 Effluent L2595461 Sample #: 8730-0032104

Conditions for Test Validity

Control Mortality is< 20%: (Acceptable) / Not Acceptable: 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable) / Not Acceptable: 8 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable) / Not Acceptable: 16.4 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: supersaturation Duration: ≤ 20 min Days: 0 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	> 100 % Volume	insufficient dose response
95% Confidence Interval[2]	N/A % Volume	
REPRODUCTION		NO nonlinear regression models would fit
3-brood IC25	< 0.137 % Volume	IC25 is - linear interpolation
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: (Yes) / No

Concentration(s) & Rep(s): Control ; up 3

Analysis Completed: Initials BV Date 06/22/21

Results Verified: Initials CF Date 06/23/21

Ceriodaphnia dubia Initial Sample Measurements Before Preparation and Use in Toxicity T

Concentration: 100% **Sample Name:** EDL-1 - Effluent L2515461 **Sample #:** 3730-0032104

Day	Date 2021	Initial Measurements: Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	Meters / Probes Used: °C +DO	pH	Cond.	Pre-aeration: yes/no	Rate (bubbles/min)	Duration (min)	Pail Sub-Sampled	Initials
0	0003	24.0	7.4	8.3	1507	6/4	13/84	5/6	no	≤100	≤ 20	1	[illegible]
1	4	26.0	7.3	9.4	1573	6/4	13/84	5/6	yes	≤100	≤ 20	1	[illegible]
2	5	24.5	7.5	10.6	1522	6/4	13/84	5/6	yes	≤100	≤ 20	1	[illegible]
3	6	26.3	7.6	9.9	1577	6/4	13/84	5/6	Yes	≤100	≤ 20	2	[illegible]
4	7	24.5	7.6	11.1	1517	6/4	13/84	5/6	Yes	≤100	≤ 20	2	[illegible]
5	8	25.3	7.7	10.0	1530	6/4	13/84	5/6	Yes	≤100	≤ 20	3	[illegible]
6	9	24.7	7.6	10.0	1510	6/4	13/84	5/6	Yes	≤100	≤ 20	3	[illegible]
7	10									≤100	≤ 20	3	
8	11									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted?	(Yes)/No	If yes, state mesh size: Viable daphnia in sample filtered on a 63µm plankton net
Was sample pH or hardness adjusted?	Yes/(No)	If yes, describe further:
Were alternate concentrations or dilution series used?	Yes/(No)	If yes, describe further:
Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily?	(Yes)/No	If no, describe further:
Were there any other method variations, deviations, or exclusions from method?	Yes/(No)	If yes, describe further:
Was there anything unusual about the test, any problems encountered, or any remedial measures taken?	Yes/(No)	If yes, describe further:

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: Control Sample Name: EDL-1 - Effluent L2545461

Sample #: 8730 0032104

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	0603	25.8	8.3	7.7	459	6/4	13/84	5/6	CH
1	4	25.7	8.0	7.7	462	6/4	13/84	5/6	KK
2	5	25.5	8.2	7.8	442	6/4	13/84	5/6	CH
3	6	25.8	8.1	7.4	449	6/4	13/84	5/6	CH
4	7	25.5	8.0	7.6	447	6/4	13/84	5/6	CH
5	8	26.0	8.2	7.6	464	6/4	13/84	5/6	CH
6	9	25.9	8.2	7.5	467	6/4	13/84	5/6	CH
7	10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
~~24.9~~ 23.3 KK	~~7.89~~ 7.49 KK	~~6.78~~ 7.4 KK	~~6/4~~ 5/5 KK	~~13/84~~ 12/85 KK	KK
24.7	7.4	6.8	6/4	13/84	CH
25.3	7.5	6.1	6/4	13/84	CH
25.6	7.4	6.5	6/4	13/84	CH
25.4	7.5	6.9	6/4	13/84	CH
25.3	7.8	6.6	6/4	13/84	CH
25.1	7.7	6.8	6/4	13/84	CH

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	0603																
1	4	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CH	—
2	5	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CH	—
3	6	3	4	0	4	4	3	0	3	5	3	29	—	0	—	CH	CH
4	7	0	0	2	0	0	0	3	0	0	0	5	—	0	—	CH	CH
5	8	6	7	0	7	5	5	0	5	7	6	48	—	0	—	CH	CH
6	9	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CH	CH
7	10	9	10	0	8	7	9	12	8	10	9	82	—	0	—	CH	CH
8	11																
Total Neonates		18	21	2	19	16	17	15	16	22	18	164					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.137 / 1.x IV Sample Name: EDL-1 - Effluent L2515461 Sample #: 8730 0032104

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	0603	25.7	7.9	7.7	473	6/4	13/84	5/6	CC
1	4	25.6	7.9	7.5	461	6/4	13/84	5/6	KK
2	5	25.4	7.8	7.7	433	6/4	13/84	5/6	CC
3	6	25.8	8.0	7.6	446	6/4	13/84	5/6	C
4	7	25.6	8.0	8.0	456	6/4	13/84	5/6	C
5	8	25.8	8.1	8.3	468	6/4	13/84	5/6	C
6	9	25.8	8.1	8.0	470	6/4	13/84	5/6	C
7	10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.7 KK	7.9	7.3	6/4 5/5 KK	13/84 13/84 KK	KK
24.6	7.6	6.8	6/4	13/84	CC
25.1	7.5	5.5	6/4	13/84	CC
25.6	7.4	5.7	6/4	13/84	CC
25.3	7.7	6.9	6/4	13/84	CC
25.5	7.8	6.1	6/4	13/84	CC
25.1	7.7	6.5	6/4	13/84	CC

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Reched for neo # initia
0	0603																
1	4	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
2	5	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
3	6	3	0	3	4	2	5	3	0	4	3	27	—	0	—	CM	CM
4	7	0	2	0	0	0	0	0	3	0	0	5	—	0	—	CM	CM
5	8	4	0	8	7	5	6	5	0	0	5	40	—	0	—	CM	CM
6	9	dead	0	0	0	0	0	0	0	0	0	1	1	10	—	CM	CM
7	10	↓	7	6	5	5	0	5	4	0	6	38	—	10	—	CC	CC
8	11																
Total Neonates		8	9	17	16	12	11	13	7	4	14	111					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.4115 % v/v   Sample Name: EDL-1- Effluent L2594561   Sample #: 8730 0032104

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	0603														
1	4														
2	5														
3	6														
4	7														
5	8														
6	9														
7	10														

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Rechecked for neo = initials
0	0603																
1	4	0	0	0	0	0	0	0	0	0	0	0	—	0	—	AO	—
2	5	0	0	0	0	0	0	0	0	0	0	0	—	0	—	AO	—
3	6	4	3	4	0	3	0	5	5	2	2	28	—	0	—	CH	CH
4	7	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CH	—
5	8	4	3	1	2	Dead	0	0	6	5	4	26	5	10	—	CH	CH
6	9	0	0	0	0	↓	0	0	0	0	0	0	—	10	—	CH	CH
7	10	2	0	Dead	6		0	5	0	4	3	20	3	20	—	AO	AO
8	11			↓													
Total Neonates		10	6	5	8	4	0	10	11	11	9	74	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 1.2346 1.xN Sample Name: EDL-1 - Effluent L2545461 Sample #: 8730 0032104

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	0603								
1	4								
2	5								
3	6								
4	7								
5	8								
6	9								
7	10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Rechec for neo = initial
0	0603																
1	4	0	0	0	0	0	0	0	0	0	0	0	—	0	—	AA	—
2	5	0	0	0	0	0	0	0	0	0	0	0	—	0	—	AA	—
3	6	4	0	3	4	0	0	3	0	0	4	18	—	0	—	CH	CH
4	7	0	0	0	0	3	3	0	4	3	8	21	—	0	—	CH	CH
5	8	6	0	1	6	4	3	1	0	0	0	21	—	0	—	CH	CH
6	9	0	0	0	0	0	0	0	0	0	0	0	4	10	—	CH	CH
7	10	3	0	0		0	0	0	0	0	0	3	3	20	—	AA	AA
8	11																
Total Neonates		13	0	4	10	7	6	4	4	3	12	63					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 37.1 v/v Sample Name: EDL-1 - Effluent L2545461 Sample #: 8730 0032104

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	0603	25.3	8.1	7.9	502	6/4	13/84	5/6	CH
1	4	25.6	8.0	7.5	499	6/4	13/84	5/6	KK
2	5	25.3	8.0	7.7	483	6/4	13/84	5/6	CH
3	6	25.7	8.1	7.5	486	6/4	13/84	5/6	CH
4	7	25.6	8.2	7.9	490	6/4	13/84	5/6	CH
5	8	25.7	8.2	7.8	505	6/4	13/84	5/6	CH
6	9	25.8	8.3	7.8	507	6/4	13/84	5/6	CH
7	10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.7	7.8	5.9	5/5	12/85	KK
25.0	7.6	6.0	6/4	13/84	CH
25.0	7.6	5.8	6/4	13/84	CH
25.7	7.4	5.7	6/4	13/84	CH
25.1	7.8	7.1	6/4	13/84	CH
25.4	7.9	6.8	6/4	13/84	CH
25.1	7.3	6.3	6/4	13/84	CH

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Rechec for neo = initial
0	0603																
1	4	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CH	—
2	5	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CH	—
3	6	2	1	4	3	0	4	0	2	0	0	16	—	0	—	CH	CH
4	7	0	0	0	0	5	0	0	dead	0	3	8	8	10	—	CH	CH
5	8	4	3	0	0	0	0	5	|	2	0	14	—	10	—	CH	CH
6	9	0	8	0	0	0	0	0	|	0	0	8	—	10	—	CH	CH
7	10	dead	0	0	dead	0	2	0	|	0	0	2	14	30	—	CH	CH
8	11	↓			↓				↓								
Total Neonates		6	12	4	3	5	6	5	2	2	3	48					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 11.11% v/v Sample Name: EDL-1- Effluent L2345461 Sample #: 8730 0032104

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	0603														
1	4														
2	5														
3	6														
4	7														
5	8														
6	9														
7	10														

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neo = initial
0	0603																
1	4	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
2	5	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
3	6	4	2	0	3	0	4	3	4	0	2	22	—	0	—	CA	CA
4	7	0	0	3	0	3	0	0	0	2	0	8	—	0	—	CA	CA
5	8	Dead	0	0	0	6	7	0	0	0	0	13	1	10	—	CA	CA
6	9	↓	0	0	0	0	0	0	0	0	0	0	—	10	—	CA	CA
7	10	↓	Dead	0	1	2	Dead	2	0	Dead	0	5	2,6,9	40	—	ao	ao
8	11	↓	↓				↓			↓							
Total Neonates		4	2	3	4	11	11	5	4	2	2	48					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 33331.v1v Sample Name: EDL-1- Effluent L2595461 Sample #: 8730 0032104

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	0603								
1	4								
2	5								
3	6								
4	7								
5	8								
6	9								
7	10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neo = initial
0	0603																
1	4	0	0	0	0	0	0	0	0	0	0	0	—	0	—	AO	—
2	5	0	0	0	0	0	0	0	0	0	0	0	—	0	—	AO	—
3	6	0	3	1	3	2	0	3	3	4	0	19	—	0	—	CH	CH
4	7	2	0	0	0	0	4	0	0	0	3	9	—	0	—	CH	CH
5	8	0	0	8	7	0	0	0	0	0	0	15	—	0	—	CH	CH
6	9	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CH	CH
7	10	2	0	0	0	0	0	0	0	0	0	2	—	0	—	AO	AO
8	11																
Total Neonates		4	3	9	10	2	4	3	3	4	3	45					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 100 %v/v Sample Name: EDL-1 - Effluent L2515461 Sample #: 8730 00332104

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	0603	24.0	7.4	8.3	1502	6/4	13/84	5/6	[illegible]
1	4	25.5	7.5	8.3	1547	6/4	13/84	5/6	AK
2	5	24.8	7.6	9.3	1512	6/4	13/84	5/6	[illegible]
3	6	25.9	7.7	9.2	1547	6/4	13/84	5/6	C
4	7	25.0	7.8	10.1	1486	6/4	13/84	5/6	C
5	8	25.3	7.7	9.3	1492	6/4	13/84	5/6	C
6	9	25.1	7.8	9.1	1510	6/4	13/84	5/6	C
7	10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.5	7.7	5.4	5/5	12/85	MC
24.8	7.7	5.4	6/4	13/84	[illegible]
25.1	7.9	6.6	6/4	13/84	CH
25.5	7.7	6.2	6/4	13/84	CH
25.3	8.1	7.2	6/4	13/84	CH
25.3	8.0	6.9	6/4	13/84	CH
25.1	7.8	6.8	6/4	13/84	[illegible]

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Reched for neo = initia
0	0603																
1	4	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
2	5	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
3	6	3	0	3	0	4	3	3	2	4	3	25	—	0	—	CH	CH
4	7	0	3	7	0	0	0	0	0	0	0	10	—	0	—	CH	CH
5	8	7	0	0	6	6	7	5	8	7	8	54	—	0	—	CH	CH
6	9	9	0	[illegible]	0	0	7	0	0	0	0	16	3	10	—	CH	CH
7	10	0	0	↓	0	0	0	0	0	0	0	0	—	10	—	[illegible]	[illegible]
8	11			↓													
Total Neonates		19	3	10	6	10	17	8	10	11	11	100					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia Neonate Origin

Sample Information

Client: ALS
Sample #: 8730-0032104
Date/Time Received: 060321C 1430
Sample Type: Effluent
100% Hardness: 472

Sample Name: EDL-1-Effluent L2591546
Date/Time Collected: 060221 / 0830 Person Sampling: n/a
Arrival Temp (°C): 21.0
Sample Description: clear yellow

Test Information

Date Test Started: 060321
Dilution Water Batch Number: CD21 61

Test Started By: AO
Control Hardness: 130

Template Used for Randomization: 1

Individual Culture Health Data

Date Culture Started: 052321
% mortality in previous 7 days (must be ≤20%): 0
Average # neos in 1st 3 broods (must be ≥ 15): 24.7

Culture I.D. (e.g. Wed Row 4): Fri Row 5
Average # neos in previous 7 days (must be ≥15): 24.7
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started: 052321
% mortality in previous 7 days (must be ≤20%): 0
Average # neos in 1st 3 broods (must be ≥ 15): 21.0

Culture I.D. (e.g. Wed Row 4): Fri Row 7
Average # neos in previous 7 days (must be ≥15): 22.9
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____

Culture I.D. (e.g. Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____

Culture I.D. (e.g. Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____

Culture I.D. (e.g. Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days: 0 (add up % mortality for all cultures used / # cultures used)
Mean Number of Young Produced within first 3 broods: 23.1 (avg # 1st 3 broods for all cultures used / # cultures used)
Mean Number of Young Produced in previous 7 days: 23.8 (avg # neos in prev 7 days for all cultures used / # cultures used)
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / **No** (circle one)

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
F.5.1	Y	Y	16	1	AO
3	Y	Y	12	2	AO
5	Y	Y	12	3	AO
6	Y	Y	12	4	AO
8	Y	Y	16	5	AO
F.7.1	Y	Y	14	6	AO
6	Y	Y	13	7	AO
8	Y	Y	10	8	AO
9	Y	Y	13	9	AO
10	Y	Y	16	10	AO
	Y / N	Y / N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Pollutech Test Method PS-GI-R1.14.

Test Material

Client Name/Location:	ALS Environmental, Thunder Bay Site		
Sample #:	8730-0032104	**Sample Name:**	EDL1-Effluent (L2595461-1)
Sample Method:	Grab	**Collected by:**	N/A
Date/Time Collected:	June 1, 2021; 08:30	**Arrival Temp.:**	21.0°C
Date/Time Received:	June 3, 2021; 14:30	**Sample Description:**	Clear, yellow
Sample Point Description:	Other	**Sample Type:**	Effluent
Transportation:	Air/Road		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species (Strain #):	*Raphidocelis subcapitata* (CPCC # 37)
Source:	Pollutech Plant Culture Unit (from CPCC)
Culture Temp.:	24 ± 2 °C
Test Culture Number:	G7(l)a
Culture Age at Test Start:	6 days old
Cell Density in the Microplate Wells at the Start of the Test:	9,750 cells/ml

Test Conditions

Date/Time Test Start:	June 3, 2021; 14:55	**T=0 Control pH:**	6.6
Date/Time Test End:	June 6, 2021; 14:15 - 17:00	**T=72 Control pH:**	6.9
Sample pH Before Dilution:	6.7	**pH Adjustment:**	None
Test Duration:	72 hours		

Test Conditions– continued

Mean Test Temperature (±Standard Deviation): 25.1 (±0.2)°C

Pre-Aeration of Sample: None **Analyst:** C. D'Andrea / E. Pasiak

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Distilled Water

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:

Macronutrient	mg/l	Micronutrient	µg/l
$NaNO_3$	15.94	H_3BO_3	115.95
$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
		$FeCl_3$ $6H_2O$	100
		Na_2EDTA $2H_2O$	46.9

Enumeration Technique: Neubauer Haemacytometer

Test Vessel: 96-Well U-bottomed Polystyrene Microplate

Concentration of Test Solutions: 90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control

 # Replicates/Concentration: 4 reps started; 3 reps counted of test solutions
10 control reps started, 2 used for pH measurement

Design if Specialized Procedure: Not applicable

Method Deviations or Unusual Occurrences: None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times: Acceptable (26.8 times)

pH in controls did not vary by more than 1.5 units: Acceptable (0.3 units)

C V for cell yields is < 20% within the control wells: Acceptable (8.3%)

No inhibitory trend detected across the control wells: Acceptable (no significant trend)

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032104 **Sample Name:** EDL1-Effluent (L2595461-1)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
262,750	310,250	250,250	265,250	237,750	255,250	252,750	252,750	260,875	8.3

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 CV = Coefficient of Variation = (100 x standard deviation / mean)

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	565,250	452,750	280,250	295,250	302,750
2	572,750	450,250	305,250	282,750	270,250
3	560,250	485,250	422,750	250,250	280,250
Mean Cell Yield	566,083	462,750	336,083	276,083	284,417
Coefficient Variation[2]	1	4	23	8	6
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	315,250	300,250			
2	257,750	260,250			
3	272,750	267,750			
Mean Cell Yield	281,917	276,083			
Coefficient Variation[2]	11	8			

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 Coefficient of Variation = (100 x standard deviation / mean)
Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	Result[1]	Method of Calculation
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume[3] (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	no trend	Mann-Kendall

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval for the Bootstrap Estimate
3 - Highest concentration tested, based on test method

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032104 **Sample Name:** EDL1-Effluent (L2595461-1)

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software, 2001-2007 Comprehensive Environmental Toxicity, Information System - CETIS v1.8.1.2.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (10.101% v/v concentration; rep. 3). No reason to remove it. Statistics include all data.

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2106 **Date Test Initiated:** 05/20/21

Method of Analysis: Nonlinear Regression 3P Log-Gompertz Model **Algae Lot #:** G5(l)c

72-hour IC25 (95% Confidence Limits): 66.41 mg/L (41.56 mg/L; 87.29 mg/L)

Historic Geometric Mean IC25: 52.13 mg/L (27.82 mg/L; 97.69 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Test Facilities

Testing Laboratory: Pollutech EnviroQuatics Limited, 704 Mara St., Suite 122, Point Edward, Ontario, N7V 1X4

CALA Testing Accreditation No. A1225

This laboratory is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA). The test included in this report is within the scope of this laboratory.

pollutech enviroquatics limited

Sample Name	EDL-1-Effluent	Sample #	8730-0032104	Date test start	06.03.21

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	107250	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	20	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2145	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	9750	=	26.75641

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	25	43	25	28	25	28	21	32
	29.0	25.0	29.0	22.0	27.0	35.0	25.0	23.0
	21	38.0	29.0	31.0	21.0	23.0	31.0	23.0
	34.0	22.0	21.0	29.0	26.0	20.0	28.0	27.0
total cells	109	128	104	110	99	106	105	105
cells/ul	272.5	320	260	275	247.5	265	262.5	262.5
cells/ml	272500	320000	260000	275000	247500	265000	262500	262500

Cell yield = measured concentration - initial algal cell concentration

	262750	310250	250250	265250	237750	255250	252750	252750

Mean cell yield for the control	= Rc	Standard deviation		coefficient of variation		
Rc	260875	SD	21619.6835	CV	8.2873727	**Must be ≤20**

% inhibition summary

Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	-5.830
0.374	-8.066
1.122	-9.024
3.367	-5.830
10.101	-28.829
30.303	-77.384
90.910	-116.994

enter test data

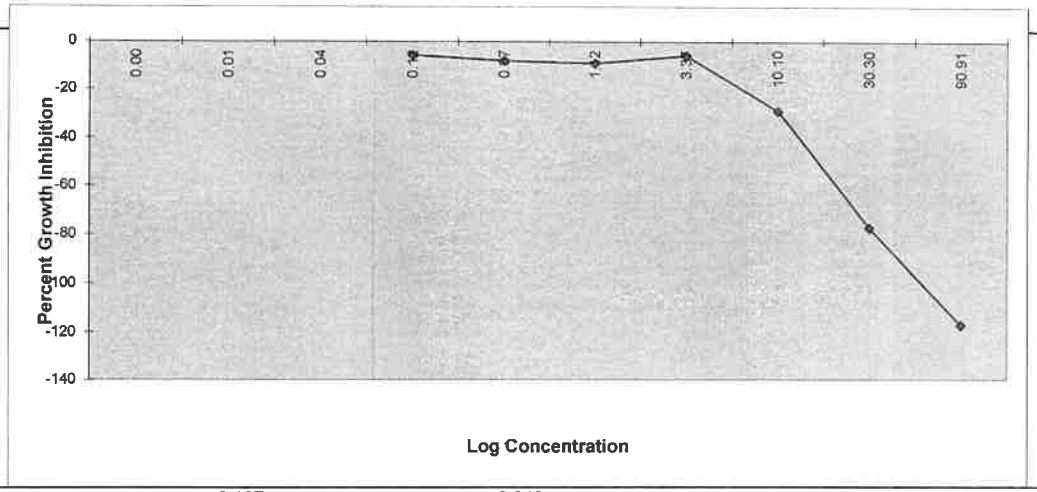
nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	44	58	53	58	42	48	21	36	46	28	27	27	22	26	26
	48.0	57.0	46.0	29.0	56.0	43.0	25.0	30.0	38.0	28.0	22.0	29.0	24.0	25.0	29.0
	71.0	64.0	70.0	42.0	40.0	40.0	31.0	26.0	41.0	28.0	36.0	20.0	49.0	26.0	27.0
	67.0	54.0	59.0	56.0	46.0	67.0	39.0	34.0	48.0	38.0	32.0	28.0	30.0	35.0	34.0
total cells	230	233	228	185	184	198	116	126	173	122	117	104	125	112	116
cells/ul	575	582.5	570	462.5	460	495	290	315	432.5	305	292.5	260	312.5	280	290
cells/ml	575000	582500	570000	462500	460000	495000	290000	315000	432500	305000	292500	260000	312500	280000	290000

Cell yield = measured concentration - initial algal cell concentration

	565250	572750	560250	462750	450250	485250	280250	305250	422750	295250	282750	250250	302750	270250	280250
Mean Yield	566083			462750			336083			276083			284417		
STD Yield	6292			19526			76089			23229			16646		
CV Yield	1			4			23			8			6		

Average % inhibition											
	for 90.910%:	-116.994	for 30.303%	-77.384	for 10.101%	-28.829	for 3.367%	-5.830	for 1.122%	-9.024	

Average % stimulation											
	for 90.910%:	116.994	for 30.303%	77.384	for 10.101%	28.829	for 3.367%	5.830	for 1.122%	9.024	



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
30	25	29	33	27	23									
39.0	22.0	30.0	27.0	27.0	23.0									
27.0	28.0	22.0	31.0	21.0	32.0									
34.0	32.0	32.0	33.0	33.0	33.0									
130	107	113	124	108	111	0	0	0	0	0	0	0	0	0
325	267.5	282.5	310	270	277.5	0	0	0	0	0	0	0	0	0
325000	267500	282500	310000	270000	277500	0	0	0	0	0	0	0	0	0
315250	257750	272750	300250	260250	267750	-9750	-9750	-9750	-9750	-9750	-9750	-9750	-9750	-9750
281917			276083			-9750			-9750			-9750		
29826			21262			0			0			0		
11			8			0			0			0		
for 0.374%	-8.066		for 0.125%	-5.830		for 0.042%	103.737		for 0.014%	103.737		for 0.005%	103.737	
for 0.374%	8.066		for 0.125%	5.830		for 0.042%	-103.737		for 0.014%	-103.737		for 0.005%	-103.737	

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Pollutech EnviroQuatics Limited
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS Sample Name EDL-1-Effluent Sample # 8730-0032104
L2595461

Conditions for Test Validity

Cell increase for control is >16	(Acceptable)/Not acceptable	26.8 (times)
CV among controls ≤ 20	(Acceptable)/Not acceptable	8.3
Result of Mann-Kendall test for trend	(Acceptable)/Not acceptable	no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 107 250 (cells/mL)

Used: (Yes)/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wells at the start of the test 9750 (cells/mL)

Analysis Completed:	Initials: G	Date: 06/09/21
Results Verified:	Initials: CF	Date: 06/23/21

Pollutech EnviroQuatics Limited
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location:	ALS		
Sample #:	8730.0032104	Sample Name:	EDL-1 Effluent L2595461
Collection Method:	grab	Collected By:	n/a
Date/Time Collected:	060121 @ 0730	Arrival Temp.: (meter/probe)	21.0°C (44)
Date/Time Received:	060321 @ 1430	Sample Description:	cloudy yellow
Collection Point Description:	effluent	Sample Type: ☐ Effluent ☐ Chemical ☐ Other:	
Transportation:	Road	Storage:	n/a

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	CF for CD
Source	Pollutech Plant Culture Unit (from CPCC), Test Culture # G7(1)a	CF for CD
Culture Age at Start of Test	6 days old (must be 3 to 7 days old)	CF for CD
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	CF for CD
	Axenic culture? (Yes)/No (Circle one)	CF for CD

Notes:

Test Conditions:

Date / Time Test Start: 060321@1435 Started By: aO		Date / Time Test End: 06.06.21 14:15 – 17:00 Finished By: BY	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 6.7	pH adjustment: Y/N	pH of well D6 at T=0 h 6.6	pH of well D7 at T=72 h 6.9
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # —		Metal mining NUT Lot # NUT 2102
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation)	25.1 ± 0.2 °C		

	Day 1				Day 2			
¼ plate rotation (Initial)	AM	aO	PM	aO	AM	aO	PM	aO
Lights ON (Initial)	AM	aO	PM	aO	AM	aO	PM	aO

* For reference test, record pH of top concentration before use for series dilutions.

Notes:

72-Hour Qualitative Observations:

Condensation: Yes		
Growth: Yes		
Were there any other method variations or deviations from methods? Yes/No		If yes, describe further:
Anything unusual about the test? Yes/No		
Any problems encountered? Yes/No		
Any remedial measures taken? Yes/No		

Pollutech EnviroQuatics Limited
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 8730-0032104	Sample Name: EDL-1 Effluent L2595461

Reference Data:

Reference Chemical Batch #	Phenol P2106	Date test started	05/20/21
Method of Analysis	Nonlinear regression 3P log Gompertz	Algae Lot #	G5(1)C
72-hour IC25 (95% C.I.)[3] mg/L	66.41 (41.56; 87.29)	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	52.13 27.82; 97.69

Test Data:

Statistic	Result[1]		Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	>90.91% (N/A)		No nonlinear regression model with fit
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers	—		ICp IN - linear interpolation
Test for Outliers	No Outliers Present		Grubbs' Test for Residual Outlier Initial T
	If outliers present, indicate Concentration/Rep: 10.101; rep 3		
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed.		
	No statistically significant growth stimulation.		Williams' or Dunnett's Multiple Comparison Test Initial T
	Yes, statistically significant growth stimulation at these concentrations: 10.101; 30.303; 90.91		

1)Results relate only to the sample tested.
2) Tidepool Scientific Software © 2001-2007. Comprehensive Environmental Toxicity Information System – CETIS v. 1.9.6.7
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/No (No circled)

Any outliers and justification for their removal? Yes/No (Yes circled)

Pollutech EnviroQuatics Limited
Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)
72-Hour Growth Inhibition Test – Continued
72-Hour Quantitative Observations of Controls

Sample Name: EDL-1-Effluent Sample Number: 873020032104 Date Test Start: 06.03.21

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D8	Well # D9	Well # D10	Well # D11
1	25	43	25	28	25	28	21	32
2	29	25	29	22	27	35	25	23
3	21	38	29	31	21	23	31	23
4	34	22	21	29	26	20	28	27
5	—	—	—	—	—	—	—	—
Initials	EV	EV	EV	EV	EV	EV	EV	EV

I 107 280

Cell increase for controls = 26.8	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = 8.3	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = no trend	Controls are invalid if there is a trend detected by the Mann-Kendall test

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EOL-1-EF Sample Number: 8730-0032104 Date Test Start: 06.03.21

Theoretical Test Concentration: 100% v/v					Actual Test Concentration: 90.91% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	44	58	53		566 083 (± 6292)
2	48	57	46		Coefficient of Variation of Cell Yield
3	71	64	70		1
4	67	54	59		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-116.994
Initials	EF	EF	EF		EF

Theoretical Test Concentration: 33.33% v/v					Actual Test Concentration: 30.303% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	58	42	48		462 750 (± 19 526)
2	29	56	43		Coefficient of Variation of Cell Yield
3	42	40	40		4
4	56	46	67		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-77.384
Initials	EF	EF	EF		EF

Theoretical Test Concentration: 11.11% v/v					Actual Test Concentration: 10.101% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	21	36	46		336 083 (± 76 089)
2	25	30	38		Coefficient of Variation of Cell Yield
3	31	26	41		23
4	39	34	48		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-28.829
Initials	EF	EF	EF		EF

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL-1-E1f Sample Number: 8730 0032101 Date Test Start: 06.03.21

Theoretical Test Concentration: 3.704% v/v					Actual Test Concentration: 3.367% v/v
Cell count per 0.1 µl or 0.004 µl	Well # E5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation) 276 083 (± 23 229)
1	28	27	27		
2	28	22	29		Coefficient of Variation of Cell Yield 8
3	28	36	20		
4	38	32	28		Average % Inhibition (-ve number = enhancement) -5.830
5	—	—	—		
Initials	EI	E	E		E

Theoretical Test Concentration: 1.235% v/v					Actual Test Concentration: 1.122% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation) 284 417 (± 16646)
1	22	26	26		
2	24	25	29		Coefficient of Variation of Cell Yield 6
3	49	26	27		
4	30	35	34		Average % Inhibition (-ve number = enhancement) -9.024
5	—	—	—		
Initials	EI	E	E		E

Theoretical Test Concentration: 0.412% v/v					Actual Test Concentration: 0.374% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation) 281917 (± 29626)
1	30	25	29		
2	39	22	30		Coefficient of Variation of Cell Yield 11
3	27	28	22		
4	34	32	32		Average % Inhibition (-ve number = enhancement) -8.066
5	—	—	—		
Initials	E	E	E		E

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL-1-E1 Sample Number: 8730-0032104 Date Test Start: 06.05.21

Theoretical Test Concentration: 0.137% v/v					Actual Test Concentration: 0.125% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	33	27	23		276 083 (± 21 362)
2	27	27	23		Coefficient of Variation of Cell Yield
3	31	21	32		8
4	33	33	33		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-5.830
Initials	E	E	E		E

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:
ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected (M/D/Y)	Date Received (M/D/Y)	Date Tested (M/D/Y)	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
EDL1-Effluent (L2595461-1)	8730-0032104	June 1, 2021	June 3, 2021	June 4, 2021	>97% Volume[2] (Not applicable)	>97% Volume[2] (Not applicable)	No

[1] Results relate only to the sample tested.
[2] Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Pollutech Test Method LM-GI-R5.14)

TESTING PERFORMED BY:

Lemna minor Bioassay: C. D'Andrea / E. Pasiak / K. Kramer

TESTING FACILITY:

Pollutech EnviroQuatics Limited, 704 Mara St., Suite 122, Point Edward, Ontario, N7V 1X4
This laboratory is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA).
All of the tests included in this report are within the scope of this laboratory.

CALA Testing Accreditation No. A1225

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	05/22/2021	**Reference Chemical:**	KCl
Frond Increase IC25:	2.65 g/L	**95% Confidence Limits:**	1.56; 3.49 g/L
Historic Geometric Mean IC25:	2.00 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.23; 3.25 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:

Substance	mg/l	Substance	mg/l	Substance	mg/l
$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	$Na_2MoO_4\ 2H_2O$	0.0726
K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$ZnCl_2$	0.00003
KCl	10.1	$MgSO_4\ 7H_2O$	147	$CoCl_2\ 6H_2O$	0.00001
				$CuCl_2\ 2H_2O$	0.00001

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

Date: 06/23/21
(M/D/Y)

R. Clay Ferguson

R. Clay Ferguson, B.Sc. (Hon.)
Laboratory Manager

pollutech enviroquatics limited

Pollutech EnviroQuatics Limited
***Lemna minor* 7-Day Growth Inhibition Test Bench Sheet**

Test Material

Client Name/Location: ALS	L2591546|-1
Sample #: 8730.0032104	Sample Name: EDL-1 effluent
Collection Method: grab	Collected By: n/a
Date/Time Collected: 06 01 21 @ 0830	Arrival Temperature (meter/probe): 2.0 °C (4)
Date/Time Received: 06 03 21 @ 1430	Sample Description: Clear yellow
Collection Point Description: effluent	Sample Type: ☑ Effluent ☐ Chemical ☐ Other:
Transportation: Road	Storage: 4±2 overnight

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	AO
Source:	Pollutech Plant Culture Unit (from CPCC, # 490)	AO
Culture Age at Start of Test:	10 days old, acclimated 23 hours in fresh test solution (mAPHA)	AO
Culture Medium:	Modified Hoagland's E+ medium, Lot # m1121 01	AO
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/No	AO
	Axenic culture? Yes/No	AO
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 31 in HT 1, 31 in HT 2, 39 in HT 3 at finish	AO

Test Conditions and Procedures

Date / Time Test Start: 06 04 21 @ 1430 Started By: AO	Date / Time Test End: 06 11 21 @ 1300 Finished By: AO
Test Type: (Static) (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: ≤20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / (No) (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: (Modified APHA (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): #20 7.6 (13/84)	pH Adjustment: no
Test Volume and Depth: 150ml / 4cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / (No)	If yes, describe further:
Anything unusual about the test? Yes / (No)	
Any problems encountered? Yes / (No)	
Any remedial measures taken? Yes / (No)	Randomization Template: A

Sample #:	8730.032104	Sample Name:	L2309461-1 EDL-1 EFFLUENT

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	25.9	25.8	26.1	26.0	26.0	26.0	25.9	25.4
Low	25.9	25.8	26.1	26.0	26.0	26.0	25.9	25.4
Medium	26.0	25.8	26.1	26.0	26.0	26.0	25.9	25.4
High	26.0	25.8	26.1	26.0	26.0	26.0	25.9	25.4
Initials	KK	KK	[illegible]	[illegible]	[illegible]	[illegible]	KK	KK
meter/probe	44	44	44	44	44	44	44	44
Mean Test Temperature (average of 24h high / low temperatures):	25.6 ± 0.1 °C							

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.3	8.2	8.0	7.7	QO	13584
Day 7	**Control**	**Low**	**Medium**	**High**	**Initials**	**pH meter/probe**
	8.2	8.3	8.10	7.7	QO	13134

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)	—	—	—	—	—	—	—	—

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 06/07/21 (3)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 9					Initials: D
4900	5570	5570	4860	5100	Mean Light Measurement: 5200
±15% Variation of Mean: 4620 – 5980					Acceptable / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) / No
If yes, describe further: None

Reference Data

Reference Date	Frond Increase or Dry Weights (circle one)			
	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
05/22/21	2.65	1.56, 3.49	2.00	1.23, 3.25

Pollutech EnviroQuatics Limited
***Lemna minor* 7-Day Growth Inhibition Test Bench Sheet**

Sample #:	Sample Name:
8730.0032104	L2513461-1 EDL-1 effluent

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 64	B 61	C 48	D 52	56.3

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy							Initials: G
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV: 50.3 (7.5) 14.9							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-8.96	-13.43	-14.93	-2.49	-8.46	14.43	-16.42
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-14.75	-10.91	-12.51	-2.39	-4.09	5.53	-7.82

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: (Yes) Not applicable (no stimulation) (Circle one)

Test Endpoints and Calculations:

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	> 97 % (N/A)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN - linear interpolation
Dry Weights		
IC25 (95% C.I.)[3]	> 97% (N/A)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN - linear interpolation

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2001-2007. Comprehensive Environmental Toxicity Information System CETISv 1.8.1.2
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / (No)

Any outliers and justification for their removal? (Yes) / No
wrong (3.1; rep 4)

Lemna minor Weights

Client	ALS - TB	Site	EDL - 1 - Effluent	Sample number	8730-00321/04
In Oven Date/Time/ °C:	06/11/21 15:00 62°	Out Oven Date/Time/°C:	06/12/21 15:00 62°C		

Conc. -1-v/v	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	58	50.5 (7.5)	0.77133	0.76678	5.05	5.02 (0.6)
	B	55		0.76077	0.76000	5.77	
	C	42		0.77145	0.76705	4.40	
	D	46		0.77610	0.77125	4.85	
0.097	A	45	45.8 (11.2)	0.77589	0.77202	3.87	4.28 (0.5)
	B	43		0.77642	0.77190	4.52	
	C	34		0.77790	0.77411	3.79	
	D	61		0.77105	0.76612	4.93	
0.29	A	52	43.5 (5.7)	0.77195	0.76663	5.32	4.47 (0.6)
	B	40		0.77367	0.76933	4.34	
	C	40		0.76894	0.76450	4.44	
	D	42		0.77002	0.76624	3.78	
0.97	A	47	42.8 (3.0)	0.77100	0.76619	4.81	4.39 (0.5)
	B	42		0.77302	0.76911	3.91	
	C	40		0.77497	0.77020	4.77	
	D	42		0.76962	0.76555	4.07	
3.1	A	48	49.0 (14.2)	0.76637	0.76196	4.41	4.90 (1.5)
	B	43		0.77172	0.76765	4.07	
	C	36		0.77033	0.76687	3.96	
	D	69		0.77727	0.77012	7.15	
9.7	A	43	46.0 (2.9)	0.76854	0.76410	4.44	4.81 (0.3)
	B	50		0.76951	0.76432	5.19	
	C	45		0.76950	0.76465	4.85	
	D	46		0.77638	0.77161	4.77	
31	A	54	57.5 (9.8)	0.76833	0.76362	4.71	5.30 (1.1)
	B	50		0.77997	0.77541	4.56	
	C	54		0.77893	0.77398	4.95	
	D	72		0.78088	0.77392	6.96	
97	A	44	42.0 (3.7)	0.76733	0.76309	4.79	4.63 (0.5)
	B	38		0.77260	0.76810	4.50	
	C	46		0.77255	0.76729	5.26	
	D	40		0.77711	0.77316	3.95	
Initials		EG	EC	AO	EG	EG	EC

Notes:

Sample name EDL-1-Effluent Date started 06.04.21 sample # 8730-0032104

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

56.3 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	64	51	58	53	54	49	60	50
	61	49	46	48	49	56	56	44
	48	40	46	46	42	51	60	52
	52	67	48	48	75	52	78	46
Total Fronds	225	207	198	195	220	208	254	192

Increase in Frond Number = Total # Fronds - 6 Starting Fronds

	58	45	52	47	48	43	54	44
	55	43	40	42	43	50	50	38
	42	34	40	40	36	45	54	46
	46	61	42	42	69	46	72	40
Total Increase	201	183	174	171	196	184	230	168
Mean Increase	50.3	45.8	43.5	42.8	49.0	46.0	57.5	42.0
SD Increase	7.5	11.2	5.7	3.0	14.2	2.9	9.8	3.7
CV Increase	14.9	24.6	13.2	7.0	29.0	6.4	17.1	8.7
% Stimulation		**-8.96**	**-13.43**	**-14.93**	**-2.49**	**-8.46**	**14.43**	**-16.42**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.77183	0.77589	0.77195	0.77100	0.76637	0.76854	0.76833	0.76788
Pan + Plant	0.76577	0.77642	0.77367	0.77302	0.77172	0.76951	0.77997	0.77260
	0.77145	0.77790	0.76894	0.77497	0.77083	0.76950	0.77893	0.77255
	0.77610	0.77105	0.77002	0.76962	0.77727	0.77638	0.78088	0.77711
Initial Weight (g)	0.76678	0.77202	0.76663	0.76619	0.76196	0.76410	0.76362	0.76309
Pan Only	0.76000	0.77190	0.76933	0.76911	0.76765	0.76432	0.77541	0.76810
	0.76705	0.77411	0.76450	0.77020	0.76687	0.76465	0.77398	0.76729
	0.77125	0.76612	0.76624	0.76555	0.77012	0.77161	0.77392	0.77316
Plant Only (mg)	5.05	3.87	5.32	4.81	4.41	4.44	4.71	4.79
	5.77	4.52	4.34	3.91	4.07	5.19	4.56	4.50
	4.40	3.79	4.44	4.77	3.96	4.85	4.95	5.26
	4.85	4.93	3.78	4.07	7.15	4.77	6.96	3.95
Mean Dry Weight	5.017	4.277	4.470	4.390	4.897	4.812	5.295	4.625
SD Dry Weight	0.6	0.5	0.6	0.5	1.5	0.3	1.1	0.5
CV Dry Weight	11.4	12.7	14.2	10.6	30.9	6.4	21.2	11.9
% Stimulation		**-14.75**	**-10.91**	**-12.51**	**-2.39**	**-4.09**	**5.53**	**-7.82**

For Data Transfer to CETIS

	# fronds	total mass	tare
0	1	58	0.77183	0.76678
	2	55	0.76577	0.76000
	3	42	0.77145	0.76705
	4	46	0.77610	0.77125
0.1	1	45	0.77589	0.77202
	2	43	0.77642	0.77190
	3	34	0.77790	0.77411
	4	61	0.77105	0.76612
0.3	1	52	0.77195	0.76663
	2	40	0.77367	0.76933
	3	40	0.76894	0.76450
	4	42	0.77002	0.76624
1	1	47	0.77100	0.76619
	2	42	0.77302	0.76911
	3	40	0.77497	0.77020
	4	42	0.76962	0.76555
3.1	1	48	0.76637	0.76196
	2	43	0.77172	0.76765
	3	36	0.77083	0.76687
	4	69	0.77727	0.77012
9.7	1	43	0.76854	0.76410
	2	50	0.76951	0.76432
	3	45	0.76950	0.76465
	4	46	0.77638	0.77161
31	1	54	0.76833	0.76362
	2	50	0.77997	0.77541
	3	54	0.77893	0.77398
	4	72	0.78088	0.77392
97	1	44	0.76788	0.76309
	2	38	0.77260	0.76810
	3	46	0.77255	0.76729
	4	40	0.77711	0.77316

June 23/21

Lemna minor L. / 7 Observations

Client: ALS	**Sample number:** 9730.0032104	**Date Started:** 06/04/21	
Site: L2595461-1		**Date Ended:** 06/11/21	
Concentration: Control	**Observations By:** AD	**Concentration:** 0.097 / √√	**Observations By:** AD

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	64	61	43	52	**Number of**	51	49	40	67
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	√	√	√
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	X	X	X	X	**Abnormally sized fronds**	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations	Algae on Bottom	—	—	—	**Other Observations**	Algae on Bottom	—	—	—
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO N/A					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor / 7 Observations

Client: ALS	Sample number: 9730 0032104	Date Started: 06 04 21	
Site: L2595446-1		Date Ended: 06 11 21	
Concentration: 0.29	Observations By: aD	Concentration: 0.97	Observations By: aD

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	58	46	46	47	Number of	53	43	46	48
Chlorosis (loss of pigment)	✓	✓	✓	✓	Chlorosis (loss of pigment)	✓	✓	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	✓	✓	✓	✓	Yellow fronds	✓	✓	X	✓
Abnormally sized fronds	X	X	X	X	Abnormally sized fronds	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	—	—	—	—	Other Observations	—	—	—	—
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor – 7 Observations

Client: ALS	Sample number: 9730 0032104	Date Started: 06/04/21	
Site: L2595461-1		Date Ended: 06/11/21	
Concentration: 3.1	Observations By: aO	Concentration: 9.7	Observations By: aO

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	64	49	42	75	Number of	49	56	51	52
Chlorosis (loss of pigment)	✓	✓	X	X	Chlorosis (loss of pigment)	✓	✓	X	✓
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X✓	✓	✓	✓	Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X	Abnormally sized fronds	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	✓	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	light algae on Bottom→				Other Observations	algae on the bottom→			
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor – 7 Observations

Client: ALS	Sample number: 9730 0032104	Date Started: 06/4/21	
Site: L2595446d - 1		Date Ended: 06/11/21	
Concentration: 31	Observations By: aD	Concentration: 97	Observations By: aD

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	60	56	60	78	Number of	50	44	52	46
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	√	√	√	√
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	√	Yellow fronds	√	√	√	√
Abnormally sized fronds	X	X	X	X	Abnormally sized fronds	X	X	X	X
Gibbosity (humped or swollen appearance)	√	√	√	√	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	lots of algae on bottom				Other Observations	lots of	algae	on	Bottom
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

L2595461-COFC

Chain of Custody (COC) / Analytical Request Form

COC Number: 20 -

Page of

Canada Toll Free: 1 800 668 9878

Report To	Contact and company name below will appear on the final report
Company:	New Gold
Contact:	Garnet Cornell
Phone:	
	Company address below will appear on the final report
Street:	
City/Province:	
Postal Code:	
Invoice To	Same as Report To ☐ YES ☐ NO
	Copy of Invoice with Report ☐ YES ☐ NO
Company:	
Contact:	

Reports / Recipients

Select Report Format: ☐ PDF ☐ EXCEL ☐ EDD (DIGITAL)

Merge QC/QCI Reports with COA ☐ YES ☐ NO ☐ N/A

☐ Compare Results to Criteria on Report - provide details below if box checked

Select Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Email 3

Invoice Recipients

Select Invoice Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Turnaround Time (TAT) Requested

☐ Routine [R] if received by 3pm M-F - no surcharges apply
☐ 4 day [P4] if received by 3pm M-F - 20% rush surcharge minimum
☐ 3 day [P3] if received by 3pm M-F - 25% rush surcharge minimum
☐ 2 day [P2] if received by 3pm M-F - 50% rush surcharge minimum
☐ 1 day [E] if received by 3pm M-F - 100% rush surcharge minimum
☐ Same day [E2] if received by 10am M-S - 200% rush surcharge.

Additional fees may apply to rush requests on weekends, statutory holidays and for non-routine tests.

Date and Time Required for all E&P TATs: dd-mmm-yy hh:mm am/pm

For all tests with rush TATs requested, please contact your AM to confirm availability.

AFFIX ALS BARCODE LABEL HERE (ALS use only)

Project Information

ALS Account # / Quote #:	
Job #:	
PO / AFE:	
LSD:	
ALS Lab Work Order # (ALS use only):	L2595461

Oil and Gas Required Fields (client use)

AFE/Cost Center:	PO#
Major/Minor Code:	Routing Code:
Requisitioner:	
Location:	
ALS Contact:	Sampler:

Analysis Request

Indicate Filtered (F), Preserved (P) or Filtered and Preserved (F/P) below

ALS Sample # (ALS use only)	Sample Identification and/or Coordinates (This description will appear on the report)	Date (dd-mmm-yy)	Time (hh:mm)	Sample Type	NUMBER OF CONTAINERS	NG-ODM-P-TB	NG-ST-P-TB	SAMPLES ON HOLD	EXTENDED STORAGE REQUIRED	SUSPECTED HAZARD (see notes)
1	EDL1_Effluent	1-Jun-21	8:30	Water	22	x	x			

Drinking Water (DW) Samples[1] (client use)

Are samples taken from a Regulated DW System? ☐ YES ☐ NO

Are samples for human consumption/ use? ☐ YES ☐ NO

Notes / Specify Limits for result evaluation by selecting from drop-down below (Excel COC only)

SAMPLE RECEIPT DETAILS (ALS use only)

Cooling Method: ☐ NONE ☐ ICE ☑ ICE PACKS ☐ FROZEN ☐ COOLING INITIATED

Submission Comments identified on Sample Receipt Notification: ☐ YES ☐ NO

Cooler Custody Seals Intact: ☐ YES ☐ N/A Sample Custody Seals Intact: ☐ YES ☐ N/A

INITIAL COOLER TEMPERATURES °C			FINAL COOLER TEMPERATURES °C		
13.4					

SHIPMENT RELEASE (client use)			INITIAL SHIPMENT RECEPTION (ALS use only)			FINAL SHIPMENT RECEPTION (ALS use only)		
Released by:	Date:	Time:	Received by:	Date:	Time:	Received by:	Date:	Time:
				2021 06 02	911 hrs			

REFER TO BACK PAGE FOR ALS LOCATIONS AND SAMPLING INFORMATION

WHITE - LABORATORY COPY YELLOW - CLIENT COPY

AUG 2020 FRONT

Failure to complete all portions of this form may delay analysis. Please fill in this form LEGIBLY. By the use of this form the user acknowledges and agrees with the Terms and Conditions as specified on the back page of the white - report copy.

1. If any water samples are taken from a Regulated Drinking Water (DW) System, please submit using an Authorized DW COC form.

CB

New Gold Inc. Rainy River Project
ATTN: Garnet Cornell
24 Marr Rd
Barwick ON P0W 1A0

Date Received: 04-OCT-21
Report Date: 04-NOV-21 12:07 (MT)
Version: FINAL

Client Phone: 807-234-8200

Certificate of Analysis

Lab Work Order #: L2647105

Project P.O. #: 4500038941
Job Reference: SUBLETHAL TOXICITY
C of C Numbers:
Legal Site Desc:

Christine Paradis
Project Manager

ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
L2647105-1 SUBLETHAL TOXICITY- SED 2 DISCHARGE Sampled By: Client on 04-OCT-21 @ 08:45 Matrix: Discharge							
Miscellaneous							
Special Request	See Attached					13-OCT-21	R5634929
Special Request	See Attached					13-OCT-21	R5634929
Special Request	See Attached					13-OCT-21	R5634929
Special Request	See Attached					13-OCT-21	R5634929

* Refer to Referenced Information for Qualifiers (if any) and Methodology.

Reference Information

Test Method References:

ALS Test Code	Matrix	Test Description	Method Reference**
TOX-7DINHIB-LM-TB	Misc.	Growth Inhibition using Lemma minor	SEE SUBLET LAB RESULTS
Lemna minor 7-day growth toxicity test, based on the protocol "Biological Test Method: Test Method for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, Lemna minor", Report EPS 1/RM/37, Second Edition (January 2007)			
TOX-GROWTH-FH-TB	Misc.	7 Day Survival & Growth Fat Head Minnows	SEE SUBLET LAB RESULTS
Fathead minnow 7-day test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Environmental Protection Series, Ottawa, ON, Report EPS1/RM/22, Second Edition (February 2011).			
TOX-INHIB-PS-TB	Misc.	Growth Inhibition Pseudokirchneriella	SEE SUBLET LAB RESULTS
Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) 72-hour growth toxicity test, based upon protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga" Report EPS1/RM/25, Second Edition (March 2007).			
TOX-REPRO-CD-TB	Misc.	Survival & reproduction (Ceriodaphnia)	SEE SUBLET LAB RESULTS
Ceriodaphnia dubia 3-brood toxicity test, accroding to protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dusbia", Environmental Protection Series, Ottawa, ON, Report EPS 1/RM/21, Second Edition (February 2007).			

** ALS test methods may incorporate modifications from specified reference methods to improve performance.

The last two letters of the above test code(s) indicate the laboratory that performed analytical analysis for that test. Refer to the list below:

Laboratory Definition Code	Laboratory Location
TB	ALS ENVIRONMENTAL - THUNDER BAY, ONTARIO, CANADA

Chain of Custody Numbers:

GLOSSARY OF REPORT TERMS

Surrogates are compounds that are similar in behaviour to target analyte(s), but that do not normally occur in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery. In reports that display the D.L. column, laboratory objectives for surrogates are listed there.
mg/kg - milligrams per kilogram based on dry weight of sample
mg/kg wwt - milligrams per kilogram based on wet weight of sample
mg/kg lwt - milligrams per kilogram based on lipid weight of sample
mg/L - unit of concentration based on volume, parts per million.
< - Less than.
D.L. - The reporting limit.
N/A - Result not available. Refer to qualifier code and definition for explanation.

Test results reported relate only to the samples as received by the laboratory.
UNLESS OTHERWISE STATED, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.
Analytical results in unsigned test reports with the DRAFT watermark are subject to change, pending final QC review.

Quality Control Report

Workorder: L2647105 Report Date: 04-NOV-21

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0

Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed

Quality Control Report

Workorder: L2647105 Report Date: 04-NOV-21

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Legend:

Limit	ALS Control Limit (Data Quality Objectives)
DUP	Duplicate
RPD	Relative Percent Difference
N/A	Not Available
LCS	Laboratory Control Sample
SRM	Standard Reference Material
MS	Matrix Spike
MSD	Matrix Spike Duplicate
ADE	Average Desorption Efficiency
MB	Method Blank
IRM	Internal Reference Material
CRM	Certified Reference Material
CCV	Continuing Calibration Verification
CVS	Calibration Verification Standard
LCSD	Laboratory Control Sample Duplicate

Hold Time Exceedances:

All test results reported with this submission were conducted within ALS recommended hold times.

ALS recommended hold times may vary by province. They are assigned to meet known provincial and/or federal government requirements. In the absence of regulatory hold times, ALS establishes recommendations based on guidelines published by the US EPA, APHA Standard Methods, or Environment Canada (where available). For more information, please contact ALS.

The ALS Quality Control Report is provided to ALS clients upon request. ALS includes comprehensive QC checks with every analysis to ensure our high standards of quality are met. Each QC result has a known or expected target value, which is compared against pre-determined data quality objectives to provide confidence in the accuracy of associated test results.

Please note that this report may contain QC results from anonymous Sample Duplicates and Matrix Spikes that do not originate from this Work Order.

pollutech
enviroquatics limited

October 29, 2021

Christine Paradis
ALS Thunder Bay
1081 Barton St.
Thunder Bay, ON P7B 5N3

Dear Christine,

On October 6, 2021, Pollutech EnviroQuatics Limited personnel received one water sample from ALS Environmental, Thunder Bay Site Sublethal Toxicity - Sed 2 Discharge (L2647105-1). The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
Sublethal Toxicity - Sed 2 Discharge (L2647105-1) #8730-0032112	Fathead Minnow	7-day LC50 (95% Confidence)	Survival	>100% Volume (Not Applicable)
		7-day IC25 (95% Confidence)	Biomass	>100% Volume (Not Applicable)
	Ceriodaphnia dubia	3-brood LC50 (95% Confidence)	Survival	>100% Volume (Not Applicable)
		3-brood IC25 (95% Confidence)	Reproduction	<0.137% Volume (Not Applicable)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[2] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence)	Growth	>97% Volume (Not applicable)
		7-day IC25 Dry Weight (95% Confidence)	Growth	>97% Volume[2] (Not applicable)

1 - Results relate only to the sample tested
2 - Highest concentration tested, based on test method

704 Mara Street, Suite 122, Point Edward, Ontario, Canada N7V 1X4 • T: 519.339.8787 • F: 519.336.6965
Email: info@pollutechgroup.com • www.pollutechgroup.com

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 — The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 — The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.*, the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.*, 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations, of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 100%, 33%, 11%, 3.7% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations, of 4 replicates each, diluted half by half (*i.e.,* 100%, 50%, 25% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor.*

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor,* and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information with regard to quality assurance/quality control procedures and results can be made available if so desired.

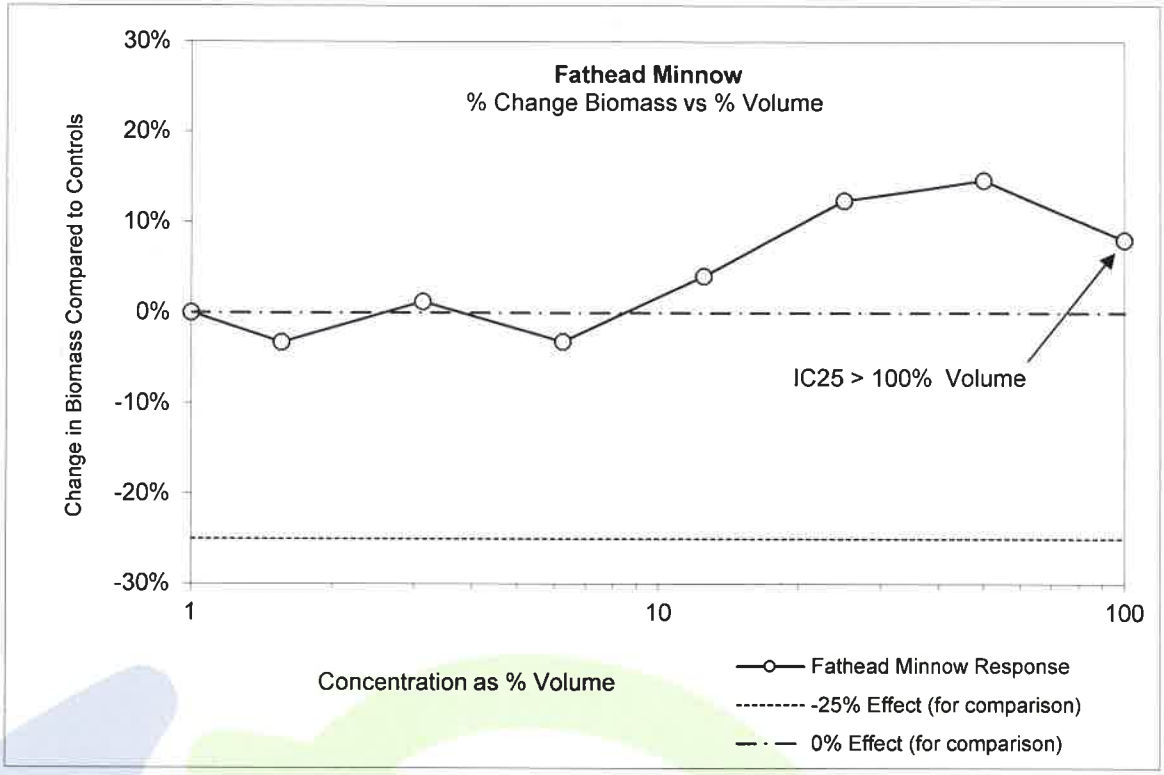
If there are any further details which you require, please do not hesitate to contact us.

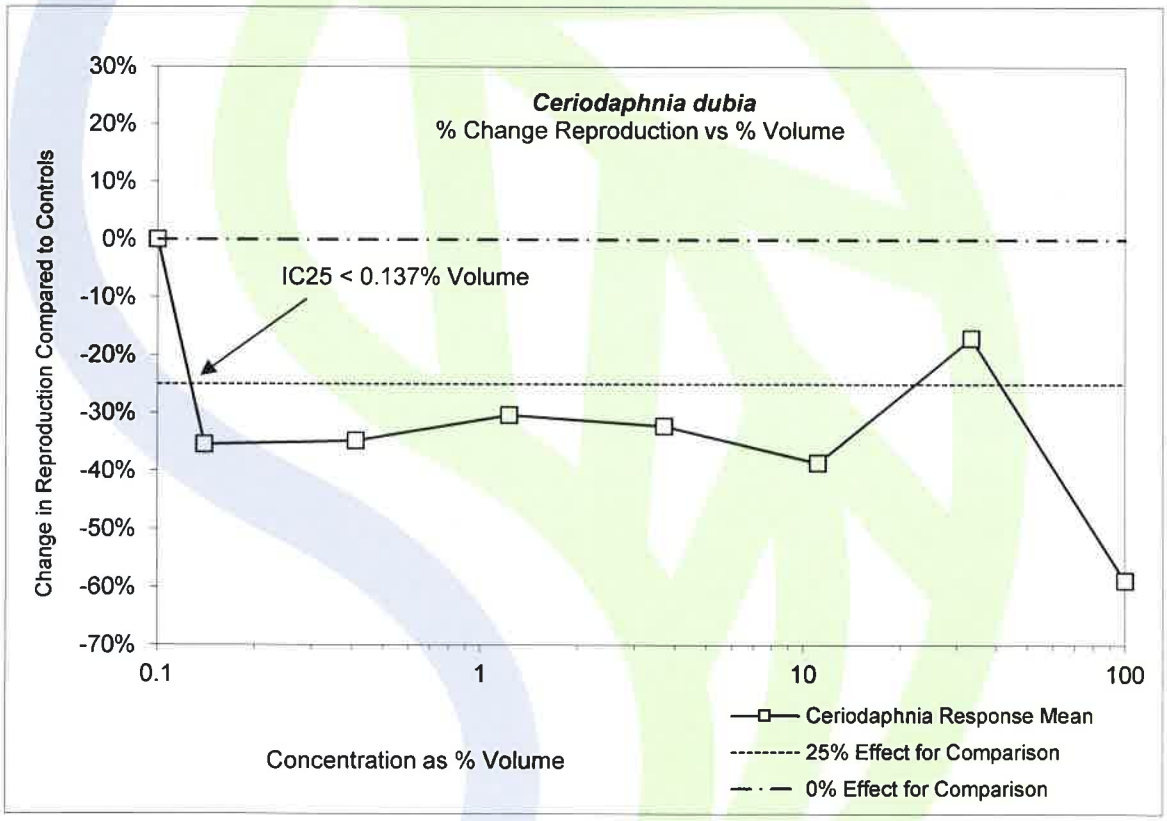
Yours very truly,
Pollutech EnviroQuatics Limited

KF Ken Ferguson, B.Sc. (Hon.)
Senior Laboratory Leader

File ID: \bioassay\2021\8000\8730-003\8730-003oc1 FCRL

ALS Thunder Bay - Sublethal Toxicity - Sed 2 Discharge (L2647105-1) - October 4, 2021





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Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Pollutech Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay Site		
Sample Type:	N/A	**Source:**	Sublethal Toxicity - Sed 2 Discharge (L2647105-1)
Date/Time Sampled:	October 4, 2021; 08:45	**Date/Time Received:**	October 6, 2021; 14:00
Date/Time Test Started:	October 6, 2021; 15:50	**Date Test Finished:**	October 13, 2021
Description:	Clear, light yellow	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032112	**Sample Collection:**	Grab
Transport:	Air/Road	**Arrival Temp.:**	19.5°C
Collected By:	N/A		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species: Fathead Minnow (*Pimephales promelas*)

Source: Pollutech Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)

Culture Temp.: 22 to 26 °C **Life Stage:** <24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 0%

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Fathead Minnow Larval Survival and Growth Study - Continued

Test Conditions

Sample #: 8730-0032112 **Source:** Sublethal Toxicity - Sed 2 Discharge (L2647105-1)

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 1 to 5

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Facilities and Apparatus

Testing Laboratory: Pollutech EnviroQuatics Limited, 704 Mara St., Suite 122, Point Edward, Ontario, N7V 1X4

CALA Testing Accreditation No. A1225

This laboratory is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA). The test included in this report is within the scope of this laboratory.

Test Performed By: E. Pasiak / C. D'Andrea / K. Kramer

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (3.3%)

Average Weight of Controls is ≥ 250 µg: Acceptable (612 µg)

Fathead Minnow Larval Survival and Growth Study - Continued

Test Results

Sample #: 8730-0032112 **Source:** Sublethal Toxicity - Sed 2 Discharge (L2647105-1)

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	20	20	0	0	10	20	0
	2	0	10	0	0	10	0	10	20
	3	10	10	0	10	20	10	0	10
Mean % Mortality		3.3	13.3	6.7	3.3	10.0	6.7	10.0	10.0
S.D.		5.8	5.8	11.5	5.8	10.0	5.8	10.0	10.0
Survival/Growth Data									
Mean Dry Biomass	1	0.686	0.513	0.493	0.577	0.652	0.706	0.591	0.719
(mg)	2	0.551	0.614	0.658	0.546	0.613	0.624	0.649	0.546
	3	0.539	0.591	0.647	0.596	0.583	0.666	0.796	0.654
% Effect (+ or -)		0.0	-3.3	1.2	-3.2	4.1	12.4	14.6	8.1
Mean Dry Biomass/ Concentration (mg)		0.592	0.573	0.599	0.573	0.616	0.665	0.679	0.640
S.D.		0.08	0.05	0.09	0.03	0.03	0.04	0.11	0.09

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032112 **Source:** Sublethal Toxicity - Sed 2 Discharge (L2647105-1)

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 7-day LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Biomass **(Survival and Growth)** 7-day IC25 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No non-linear regression models fit ICPIN-linear interpolation

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Phenol

Date Test Initiated: 10/08/21 **Reference Batch #:** P2116

Method of Analysis: Trimmed Spearman-Karber α = 6.67%

7-Day LC50 (95% Confidence Limits): 19.95 mg/L (16.39 mg/L; 24.30 mg/L)

Historic Geometric Mean LC50: 22.85 mg/L (14.58 mg/L; 35.83 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS - Thunder Bay Sample Name: Sublethal Tox - Sed 2 Discharge Sample #: 8730-0032112

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 3.3 %

Average Weight of Controls is ≥ 250 µg: (Acceptable) / Not Acceptable 612 µg

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 1 - 5

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
7-day LC50	> 100 % Volume	no dose response
95% Confidence Interval[2]	n/a % Volume	
Survival / Growth Biomass		No nonlinear regression models found fit
7-day IC25	> 100 % Volume	IC25 = linear interpolation
95% Confidence Interval[2]	n/a % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 10/14/21

Results Verified: Initials AD Date 10.15.21

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** Sublethal TOX – Sed 2 Discharge **Sample #:** 8730-00321 12

Day	Date 2021	Initial Variables: Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	Meters/Probes Used: D.O./°C	pH	Cond.	Pre-aeration: yes/no	Rate (bubbles/min)	Duration (min)	Pail Sub-Sampled	Initials
0	10.06	25.3	7.7	7.7	667	6/4	13/84	5/6	NO	≤100	< 20	1	EF
1	07	24.7	7.7	8.7	650	6/4	13/84	5/6	yes	≤100	< 20	1	ao
2	08	20.7	7.7	9.7	674	6/4	13/84	5/6	yes	≤100	< 20	1	ao
3	09	20.0	7.6	8.4	686	6/4	13/84	5/6	yes	≤100	< 20	2	ao
4	10	24.3	7.6	9.6	641	6/4	13/84	5/6	yes	≤100	< 20	2	ao
5	11	24.7	7.8	9.2	655	6/4	13/84	5/6	yes	≤100	< 20	3	ao
6	12	24.1	7.8	9.7	652	6/4	13/84	5/6	YES	≤100	< 20	3	EF
7	13												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further _____

Was test fed 100µl of brine shrimp days 0 to 6? (Yes)/No If no, describe further _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further _____

Fathead Minnow 7-day, Growth Toxicity Test

Concentration: Control Sample Name: Sublethal Toxicity - Seal 2 Discharge Sample #: 8130-00321-12

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	10.06	24.0	8.0	7.7	249	6/4	13/84	5/6	C
1	07	24.1	7.8	8.0	253	6/4	13/84	5/6	KK
2	08	25.2	8.2	7.8	256	6/4	13/84	5/6	KK
3	09	24.8	8.2	7.5	255	6/4	13/84	5/6	CO
4	10	24.3	8.2	7.6	252	6/4	13/84	5/6	CO
5	11	25.0	8.3	7.5	253	6/4	13/84	5/6	CO
6	12	24.4	8.2	7.6	252	6/4	13/84	5/6	KK
7	13								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.2	7.8	7.3	6/4	13/84	KK							
24.4	7.8	6.9	6/4	13/84	KK	0	0	0	0	0	0	KK
24.4	7.4	7.0	6/4	13/84	CO	0	0	10	0	0	0	KK
24.3	7.8	6.5	6/4	13/84	CO	0	0	10	0	0	0	CO
24.4	8.0	6.5	6/4	13/84	CO	0	0	10	0	0	0	CO
24.6	7.9	6.5	6/4	13/84	KK	0	0	10	0	0	0	CO
24.5	7.3	6.5	6/4	13/84	C	0	0	10	0	0	0	CO
						0	10	10	0	0	0	C

Observations: _____

Concentration: 1.56 % v/v

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	10.06	24.0	8.0	7.4	254	6/4	13/84	5/6	C
1	07	23.8	7.9	8.2	254	6/4	13/84	5/6	KK
2	08	24.6	8.1	7.9	257	6/4	13/84	5/6	KK
3	09	24.4	8.0	7.7	255	6/4	13/84	5/6	CO
4	10	24.3	8.0	7.6	256	6/4	13/84	5/6	CO
5	11	25.1	8.2	7.5	253	6/4	13/84	5/6	CO
6	12	24.1	8.3	7.7	254	6/4	13/84	5/6	KK
7	13								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.2	7.9	7.5	6/4	13/84	KK							
24.4	7.8	7.0	6/4	13/84	KK	20	10	10	0	0	0	KK
24.3	7.4	6.8	6/4	13/84	CO	20	10	10	0	0	0	KK
24.5	7.3	6.5	6/4	13/84	CO	20	10	10	0	0	0	CO
24.4	8.0	6.8	6/4	13/84	CO	20	10	10	0	0	0	CO
24.6	7.9	6.4	6/4	13/84	KK	20	10	10	0	0	0	CO
24.5	7.9	6.3	6/4	13/84	C	20	10	10	0	0	0	CO
						20	10	10	0	0	0	C

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13% v/v Sample Name: Sublethal Toxicity - Seal 2 Discharge Sample #: 8130-00321 12

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	10_06								
1	07								
2	08								
3	09								
4	10								
5	11								
6	12								
7	13								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	KK
						10	0	0	0	0	0	
						10	0	0	0	0	0	
						10	0	0	0	0	0	
						10	0	0	0	0	0	
						20	0	0	0	0	0	

Observations: _____

Concentration: 6.25% v/v

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	10_06								
1	07								
2	08								
3	09								
4	10								
5	11								
6	12								
7	13								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	10	0	0	0	KK
						0	0	10	0	0	0	KK
						0	0	10	0	0	0	
						0	0	10	0	0	0	
						0	0	10	0	0	0	
						0	0	10	0	0	0	
						0	0	10	0	0	0	

Observations: _____

Fathead Minnow 7-day, Growth Toxicity Test

Concentration: 12.5 % v/v Sample Name: Sublethal Toxicity - Seal 2 Discharge Sample # 8730-00321-12

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	10_06	24.1	8.0	7.4	301	6/4	13/84	5/6	[illegible]
1	07	23.9	7.9	8.1	302	6/4	13/84	5/6	KK
2	08	24.6	8.1	7.9	302	6/4	13/84	5/6	KK
3	09	24.7	8.0	7.8	300	6/4	13/84	5/6	[illegible]
4	10	24.5	8.0	7.6	303	6/4	13/84	5/6	[illegible]
5	11	25.0	8.2	7.5	298	6/4	13/84	5/6	[illegible]
6	12	24.2	8.2	7.7	301	6/4	13/84	5/6	KK
7	13								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.2	8.0	7.6	6/4	13/84	KK							
24.4	7.8	6.9	6/4	13/84	KK	0	0	10	0	0	0	KK
24.3	7.6	6.9	6/4	13/84	[illegible]	0	0	10	0	0	0	KK
24.3	7.4	6.7	6/4	13/84	[illegible]	0	0	20	0	0	0	[illegible]
24.4	8.0	6.9	6/4	13/84	[illegible]	0	10	20	0	0	0	[illegible]
24.6	7.9	6.3	6/4	13/84	KK	0	10	20	0	0	0	[illegible]
24.5	7.8	6.2	6/4	13/84	[illegible]	0	10	20	0	0	0	[illegible]
						0	10	20	0	0	0	[illegible]

Observations: _____

Concentration: 25 % v/v

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	10_06								
1	07								
2	08								
3	09								
4	10								
5	11								
6	12								
7	13								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						10	0	10	0	0	0	KK
						10	0	10	0	0	0	KK
						10	0	10	0	0	0	[illegible]
						10	0	10	0	0	0	[illegible]
						10	0	10	0	0	0	[illegible]
						10	0	10	0	0	0	[illegible]
						10	0	10	0	0	0	[illegible]

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50% v/v Sample Name: Sublethal Toxicity - Seal 2 Discharge Sample #: 8730-00321-12

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	10.06								
1	07								
2	08								
3	09								
4	10								
5	11								
6	12								
7	13								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						20	0	0	0	0	0	KK
						20	0	0	0	0	0	KK
						20	0	0	0	0	0	
						20	0	0	0	0	0	
						20	0	0	0	0	0	
						20	1	0	0	0	0	
						20	0	0	0	0	0	

Observations: _____

Concentration: 100% v/v

Day	Date 2021	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	10.06	24.2	7.8	7.6	659	6/4	13/84	5/6	C
1	07	24.3	7.8	8.3	652	6/4	13/84	5/6	KK
2	08	24.4	7.9	8.5	655	6/4	13/84	5/6	KK
3	09	24.9	7.7	8.8	641	6/4	13/84	5/6	C
4	10	24.4	7.7	8.9	640	6/4	13/84	5/6	C
5	11	24.6	7.9	8.8	646	6/4	13/84	5/6	C
6	12	24.3	8.0	8.4	658	6/4	13/84	5/6	KK
7	13								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.2	8.0	7.4	6/4	13/84	KK							
24.4	8.0	7.0	6/4	13/84	KK	0	20	0	0	0	0	KK
24.3	7.7	6.6	6/4	13/84	C	0	20	0	0	0	0	KK
24.5	7.7	6.6	6/4	13/84	C	0	20	1	0	0	0	C
24.4	8.0	6.6	6/4	13/84	C	0	20	1	0	0	0	C
24.5	8.1	6.4	6/4	13/84	KK	0	20	1	0	0	0	C
24.5	7.8	6.0	6/4	13/84	C	0	20	1	0	0	0	C
						0	20	1	0	0	0	C

Observations: _____

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information
Client: ALS – Thunder Bay
Sample #: 8730 0032 12
Date/Time Received: 10/06/21 , 14:00
Sample Type: Other
100% Hardness: 270

Sample Name: Sublethal Tox – Sed 2 Discharge
Sample Date/Time: 10/04/21 , 8:45
Arrival Temp: 19.5 °C
Sample Description: cloudy, light yellow
Person Sampling: N/A

Test Information
Date/Time Started: 10/06/21 , 15:30
Test started by: G
Fathead Batch #: FH 0106 20
Date eggs laid: 10/01/21
Culture mortality within 7 days of egg collection: 0
Swim bladder inflated; yes / no: G
Age of Larvae at start of test in hours: <24.0
Control Hardness: 100
Water Bath Quadrant: A
Average Temperature during Test: 25.4 ± 0.1 °C
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / No (circle one)

Conc.	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	10	0.68138	0.67452	0.686	0.592
	B	10	0.67702	0.67151	0.551	
	C	9	0.67856	0.67317	0.539	
1.56	A	8	0.67278	0.66765	0.513	0.573
	B	9	0.68358	0.67744	0.614	
	C	9	0.67952	0.67361	0.591	
3.13	A	8	0.67908	0.67415	0.493	0.599
	B	10	0.67801O	0.67352	0.658	
	C	10	0.68625	0.67978	0.647	
6.25	A	10	0.66953	0.66376	0.577	0.573
	B	10	0.68712	0.68166	0.546	
	C	9	0.67765	0.67169	0.596	
12.5	A	10	0.675457	0.66905	0.652	0.616
	B	9	0.67629	0.67016	0.613	
	C	8	0.68329	0.67746	0.583	
25	A	9	0.68329	0.67623	0.706	0.665
	B	10	0.67049	0.66425	0.624	
	C	9	0.68814	0.68140	0.666	
50	A	8	0.68325	0.67734	0.591	0.679
	B	9	0.67725	0.67076	0.649	
	C	10	0.68210	0.67414	0.796	
100	A	10	0.67076	0.66357	0.719	0.640
	B	8	0.68181	0.67635	0.546	
	C	9	0.67919	0.67265	0.654	
Initials		D	G	✓	G	C

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8/730-0032112 **Sample Name** Sublethal Tox-sed 2 Disc **Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug)** 612

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	10	0.68138	0.67452	0.686	0.686	0.612	0.592	0.068443	0.081627	11.18418
	10	10	0.67702	0.67151	0.551	0.551					
	10	9	0.67856	0.67317	0.599	0.539					
1.56	10	8	0.67278	0.66765	0.641	0.513	0.660	0.573	0.020694	0.052937	3.135255
	10	9	0.68358	0.67744	0.682	0.614					
	10	9	0.67952	0.67361	0.657	0.591					
3.13	10	8	0.67908	0.67415	0.616	0.493	0.640	0.599	0.02164	0.092251	3.378982
	10	10	0.68010	0.67352	0.658	0.658					
	10	10	0.68625	0.67978	0.647	0.647					
6.25	10	10	0.66953	0.66376	0.577	0.577	0.595	0.573	0.060182	0.025239	10.11341
	10	10	0.68712	0.68166	0.546	0.546					
	10	9	0.67765	0.67169	0.662	0.596					
12.5	10	10	0.67557	0.66905	0.652	0.652	0.687	0.616	0.038746	0.034598	5.637518
	10	9	0.67629	0.67016	0.681	0.613					
	10	8	0.68329	0.67746	0.729	0.583					
25	10	9	0.68329	0.67623	0.784	0.706	0.716	0.665	0.082839	0.041004	11.56729
	10	10	0.67049	0.66425	0.624	0.624					
	10	9	0.68814	0.68148	0.740	0.666					
50	10	8	0.68325	0.67734	0.739	0.591	0.752	0.679	0.039151	0.105671	5.206637
	10	9	0.67725	0.67076	0.721	0.649					
	10	10	0.68210	0.67414	0.796	0.796					
100	10	10	0.67076	0.66357	0.719	0.719	0.709	0.640	0.0236	0.087386	3.326788
	10	8	0.68181	0.67635	0.683	0.546					
	10	9	0.67919	0.67265	0.727	0.654					

SD: Standard Deviation
CV: Coefficient of Variation

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10.15.21

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Pollutech Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay Site		
Sample Type:	Effluent	**Source:**	Sublethal Toxicity - Sed 2 Discharge (L2647105-1)
Date/Time Sampled:	October 4, 2021; 08:45	**Date/Time Received:**	October 6, 2021; 14:00
Date Test Started:	October 6, 2021	**Date Test Finished:**	October 12, 2021
Description:	Clear, light yellow	**Days Sample Used:**	Days 0 to 5
Sample #:	8730-0032112	**Sample Collection:**	Grab
Transport:	Air/Road	**Arrival Temp.:**	19.5°C
Collected By:	N/A		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Pollutech Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 0%

Mean Number of Surviving Young Produced Within First 3 Broods: 24.3

Mean Number of Surviving Young Produced Within 7 Days of Testing: 52.4

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Ceriodaphnia dubia Survival and Reproduction Study - Continued

Control and Dilution Water

Sample #: 8730-0032112 **Source:** Sublethal Toxicity - Sed 2 Discharge (L2647105-1)

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 1 to 5

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD21-115

Conditions for Test Validity

Control Mortality is ≤ 20%:	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (15.8 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (70% of controls)

Ceriodaphnia dubia Survival and Reproduction Study - Continued

Test Results

Sample #: 8730-0032112 **Source:** Sublethal Toxicity - Sed 2 Discharge (L2647105-1)

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	10	0	10	0	0	0	10
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	4	14	5	10	10	10	11	4
	2	18	15	9	8	11	9	18	6
	3	20	13	5	5	5	9	11	1
	4	25	7	20	5	7	2	18	5
	5	25	10	6	8	17	2	11	11
	6	9	10	11	16	10	9	10	7
	7	17	13	14	15	13	13	13	0
	8	16	7	13	14	4	9	14	8
	9	17	11	8	16	13	13	18	9
	10	7	2	12	13	17	21	7	14
Total Number of Live Neonates in First 3 Broods or Less		158	102	103	110	107	97	131	65
% Effect (+ or -)		0.0	-35.4	-34.8	-30.4	-32.3	-38.6	-17.1	-58.9
Mean Number of Live Neonates in First 3 Broods or Less		15.8	10.2	10.3	11.0	10.7	9.7	13.1	6.5
SD		7.1	4.0	4.7	4.3	4.5	5.5	3.8	4.3

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

Ceriodaphnia dubia Survival and Reproduction Study - Continued

Sample #: 8730-0032112 **Source:** Sublethal Toxicity - Sed 2 Discharge (L2647105-1)

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	<0.137% Volume (Not Applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Phenol

Date Test Initiated: 10/08/21 **Reference Batch #:** P2116

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 9.90 mg/L (8.68 mg/L; 11.29 mg/L)

Historic Geometric Mean LC50: 6.52 mg/L (3.76 mg/L; 11.31 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Test Facilities

Testing Laboratory: Pollutech EnviroQuatics Limited, 704 Mara St., Suite 122, Point Edward, Ontario, N7V 1X4

CALA Testing Accreditation No. A1225

This laboratory is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA). The test included in this report is within the scope of this laboratory.

Test Performed By: E. Pasiak / C. D'Andrea / K. Kramer

pollutech enviroquatics limited

CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: AES-TB Sample Name: Sublethal Tox - Sed 2 Discharge Sample #: 8730 20321 12

Conditions for Test Validity

Control Mortality is< 20%: (Acceptable) / Not Acceptable 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable) / Not Acceptable 7 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable) / Not Acceptable 15.8 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 1 to 5

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	> 100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
REPRODUCTION		
3-brood IC25	< 0.137 % Volume	NO nonlinear regression models would fit IC25 IN – linear interpolation
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 10/15/21

Results Verified: Initials AO Date 10/15/21

Ceriodaphnia dubia Initial Sample Measurements Before Preparation and Use in Toxicity T

Concentration: 100% **Sample Name:** Sublethal tox - Sed 2 Discharge **Sample #:** 8730-100321 12

Day	Date 2021	Initial Measurements: Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	Meters / Probes Used: °C	pH	Cond.	Pre-aeration: yes/no	Rate (bubbles/min)	Duration (min)	Pail Sub-Sampled	Initials
0	10.06	25.3	7.7	7.7	667	6/4	13/84	5/6	NO	≤100	≤ 20	1	C
1	07	24.7	7.7	8.7	6550	6/4	13/84	5/6	yes	≤100	≤ 20	1	CO
2	08	25.7	7.7	9.7	674	6/4	13/84	5/6	yes	≤100	≤ 20	1	CO
3	09	26.0	7.6	8.9	686	6/4	13/84	5/6	yes	≤100	≤ 20	2	CO
4	10	24.3	7.6	9.6	641	6/4	13/84	5/6	yes	≤100	≤ 20	2	CO
5	11	24.7	7.8	9.2	655	6/4	13/84	5/6	yes	≤100	≤ 20	3	CO
6	12									≤100	≤ 20	3	
7	13									≤100	≤ 20	3	
8	14									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted?	Yes/No	If yes, state mesh size:
Was sample pH or hardness adjusted?	Yes/No	If yes, describe further:
Were alternate concentrations or dilution series used?	Yes/No	If yes, describe further:
Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily?	Yes/No	If no, describe further:
Were there any other method variations, deviations, or exclusions from method?	Yes/No	If yes, describe further:
Was there anything unusual about the test, any problems encountered, or any remedial measures taken?	Yes/No	If yes, describe further:

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: Control Sample Name: Sublethal Toxicity Test 2 Dilution Sample #: 8720 0052117

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	10.06	24.0	8.2	7.6	394	6/4	13/84	5/6	EC	24.0	7.4	7.2	6/4	13/84	CC
1	07	24.4	8.0	7.9	399	6/4	13/84	5/6	CC	24.4	8.0	7.2	6/4	13/84	KC
2	08	24.5	8.2	7.8	398	6/4	13/84	5/6	CC	23.7	8.0	7.3	6/4	13/84	CC
3	09	24.5	8.3	7.5	417	6/4	13/84	5/6	CC	24.0	7.9	7.2	6/4	13/84	CC
4	10	24.0	8.3	7.5	415	6/4	13/84	5/6	CC	24.3	8.0	6.9	6/4	13/84	CC
5	11	24.7	8.4	7.6	415	6/4	13/84	5/6	CC	24.5	7.9	7.6	6/4	13/84	CC
6	12														
7	13														

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	07	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
2	08	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
3	09	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
4	10	2	2	3	3	2	5	2	3	3	2	27	—	0	—	CC	CC
5	11	0	8	5	7	8	4	10	6	9	5	62	—	0	—	CC	CC
6	12	2	8	12	15	15	0	5	7	5	0	69	—	0	—	EC	CC
7	13																
8	14																
Total Neonates		4	18	20	25	25	9	17	16	17	7	158	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.137 v/v Sample Name: Sublethal Toxicity Test 2 Pisces Sample #: 8730 0052117

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06	24.1	8.1	7.6	392	6/4	13/84	5/6	C
1	07	24.4	7.9	7.8	405	6/4	13/84	5/6	∅
2	08	24.4	8.0	8.0	401	6/4	13/84	5/6	∅
3	09	24.6	8.0	7.7	430	6/4	13/84	5/6	∅
4	10	24.9	7.9	7.7	440	6/4	13/84	5/6	∅
5	11	25.1	8.0	7.5	447	6/4	13/84	5/6	∅
6	12								
7	13								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.0	7.4	7.2	6/4	13/84	∅
24.5	8.0	7.2	6/4	13/84	∅
24.0	7.9	7.2	6/4	13/84	∅
24.0	7.8	7.1	6/4	13/84	∅
24.0	8.1	6.9	6/4	13/84	∅
24.5	8.0	7.1	6/4	13/84	C

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	07	0	0	0	0	0	0	0	0	0	0	0	—	0	—	∅	—
2	08	0	0	0	0	0	0	0	0	0	0	0	—	0	—	∅	—
3	09	0	0	0	0	0	0	0	0	0	0	0	—	0	—	∅	—
4	10	4	3	2	2	4	2	0	3	3	2	20	—	0	—	∅	∅
5	11	5	4	3	0	6	8	5	4	6	0	41	—	0	—	∅	∅
6	12	5	8	8	5	0	0	8	Dead	2	0	36	8	10	—	C	C
7	13								↓								
8	14																
Total Neonates		14	15	13	7	10	10	13	7	11	∅/2	102	Notes: ^ = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.41 T.U. Sample Name: Sublethal Toxicity Sed 2 Pre Sed Sample #: 8720 005217

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	07								
2	08								
3	09								
4	10								
5	11								
6	12								
7	13								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	07	0	0	0	0	0	0	0	0	0	0	0	—	0	—		—
2	08	0	0	0	0	0	0	0	0	0	0	0	—	0	—		—
3	09	0	0	0	0	0	0	0	0	0	0	0	—	0	—		—
4	10	2	0	0	3	0	2	2	0	2	0	11	—	0	—		—
5	11	0	6	5	8	6	5	3	6	6	2	47	—	0	—		—
6	12	3	3	0	9	0	4	9	7	0	10	45	—	0	—		—
7	13																
8	14																
Total Neonates		5	9	5	20	6	11	14	13	8	12	103	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 1.23% v/v Sample Name: Sublethal Toxicity Sed 2 Discharge Sample #: 8730-005217

Day	Date 2021	Initial Variables °C	Initial Variables pH	Initial Variables D.O. (mg/L)	Initial Variables Cond (umhos)	Meter/Probe D.O. / °C	Meter/Probe pH	Meter/Probe Cond	Initials	Final Variables °C	Final Variables pH	Final Variables D.O. (mg/L)	Meter/Probe D.O. / °C	Meter/Probe pH	Initials
0	10.06														
1	07														
2	08														
3	09														
4	10														
5	11														
6	12														
7	13														

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	% Mortality / day Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	07	0	0	0	0	0	0	0	0	0	0	0	—	0	—	al	—
2	08	0	0	0	0	0	0	0	0	0	0	0	—	0	—	al	—
3	09	0	0	0	0	0	0	0	0	0	0	0	—	0	—	al	—
4	10	3	2	2	2	2	0	2	2	5	2	22	—	0	—	al	al
5	11	7	2	3	0	6	7	5	6	9	4	49	—	0	—	al	al
6	12	0	4	DEAD	3	0	9	8	6	2	7	39	3	10	—	E	e
7	13			↓													
8	14																
Total Neonates		10	8	5	5	8	16	15	14	16	13	110	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 3.7% v/v Sample Name: Sublethal Toxicity Test 2 Pisidia Sample #: 8720 005211

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06	24.3	8.1	7.5	404	6/4	13/84	5/6	C
1	07	24.3	7.7	7.8	415	6/4	13/84	5/6	CC
2	08	24.4	8.1	8.0	411	6/4	13/84	5/6	CC
3	09	24.7	8.1	7.6	429	6/4	13/84	5/6	CC
4	10	24.7	~~7.6~~ 8.0	7.7	430	6/4	13/84	5/6	CC
5	11	24.8	8.2	7.7	434	6/4	13/84	5/6	CC
6	12								
7	13								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.0	7.6	7.1	6/4	13/84	CC
24.3	8.0	7.2	6/4	13/84	KK
24.0	7.9	7.0	6/4	13/84	CC
24.0	7.8	7.1	6/4	13/84	CC
24.3	8.1	6.8	6/4	13/84	CC
24.5	8.0	7.0	6/4	13/84	C

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	07	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
2	08	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
3	09	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
4	10	0	2	0	0	2	0	0	0	0	0	4	—	0	—	CC	CC
5	11	0	9	0	2	7	3	6	4	5	7	43	—	0	—	CC	CC
6	12	10	0	5	5	8	7	7	0	8	10	60	—	0	—	C	C
7	13																
8	14																
Total Neonates		10	11	5	7	17	10	13	4	13	17	107	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 11.7 v/v Sample Name: Sublethal Toxicity Sed 2 Pisciary Sample #: 8720 005211

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	07								
2	08								
3	09								
4	10								
5	11								
6	12								
7	13								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	07	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ag	—
2	08	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ag	—
3	09	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ag	—
4	10	2	0	1	0	0	2	2	0	2	3	12	—	0	—	ag	ag
5	11	3	3	3	2	0	3	5	6	5	8	38	—	0	—	ag	ag
6	12	5	6	5	0	2	4	6	3	6	10	47	—	0	—	ag	ag
7	13																
8	14																
Total Neonates		10	9	9	2	2	9	13	9	13	21	97	Notes: ^ = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 33.3% v/v Sample Name: Sublethal Toxicity Sed 2 Pisha Sample #: 8720 0052117

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	07								
2	08								
3	09								
4	10								
5	11								
6	12								
7	13								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	07	0	0	0	0	0	0	0	0	0	0	0	—	0	—	∞	—
2	08	0	0	0	0	0	0	0	0	0	0	0	—	0	—	∞	—
3	09	0	0	0	0	0	0	0	0	0	0	0	—	0	—	∞	—
4	10	0	0	0	4	0	3	2	2	1	2	14	—	0	—	∞	∞
5	11	4	7	4	7	6	7	6	7	10	0	60	—	0	—	∞	∞
6	12	7	11	7	7	5	0	5	5	7	3	57	—	0	—	C	C
7	13																
8	14																
Total Neonates		11	18	11	18	11	10	13	14	18	7	131	Notes: ^ = > 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 100% v/v Sample Name: Sublethal Toxicity Test 2 Dishes Sample #: 8730.0052117

Day	Date 2021	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06	24.3	7.8	7.6	646	6/4	13/84	5/6	C
1	07	24.5	7.6	8.3	657	6.4	13174	5/6	CC
2	08	24.9	7.8	7.7	656	6.4	13184	5/6	CC
3	09	25.3	7.7	8.5	660	6.4	13184	5/6	CC
4	10	24.6	7.6	8.0	656	6.4	13184	5/6	CC
5	11	24.8	7.9	8.6	655	6.4	13184	5/6	CC
6	12								
7	13								

Day	Date 2021	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	10.06	24.0	7.8	7.2	6.4	13184	CC
1	07	24.3	8.2	7.4	6/4	13/84	Kc
2	08	24.0	7.9	6.9	6.4	13184	CC
3	09	25.3	7.9	7.4	6.4	13184	CC
4	10	24.3	8.1	7.0	6.4	13184	CC
5	11	24.5	8.0	6.9	6/4	13/84	C
6	12						
7	13						

Day	Date 2021	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	07	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
2	08	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
3	09	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
4	10	0	2	0	2	2	2	0	0	2	2	10	—	0	—	CC	CC
5	11	0	0	1	2	2	3	0	0	4	6	20	7	10	—	CC	CC
6	12	4	4	0	1	7	2	↓	6	3	6	33	—	10	—	C	C
7	13																
8	14																
Total Neonates		4	6	1	5	11	7	0	8	9	14	65	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia Neonate Origin

Sample Information

Client: ALS- Thunder Bay
Sample #: 8730 0032112
Date/Time Received: 10/06/21 14:00
Sample Type: Other
100% Hardness: 270

Sample Name: Sublethal Tox - Sed 2 Discharge
Date/Time Collected: 10/04/21 8:45 Person Sampling: N/A
Arrival Temp (°C): 19.5
Sample Description: clear, light yellow

Test Information

Date Test Started: ~~CD21 115 ao~~ 10.06.21
Dilution Water Batch Number: CD21 115
Test Started By: ao
Control Hardness: 120
Template Used for Randomization: T2

Individual Culture Health Data

Date Culture Started: 09.22.21
% mortality in previous 7 days (must be ≤20%): 0
Average # neos in 1st 3 broods (must be ≥ 15): 25.5
Culture I.D. (e.g., Wed Row 4): Wed Row 6
Average # neos in previous 7 days (must be ≥15): 55.0
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started: 09.22.21
% mortality in previous 7 days (must be ≤20%): 0
Average # neos in 1st 3 broods (must be ≥ 15): 23.0
Culture I.D. (e.g., Wed Row 4): Wed Row 7
Average # neos in previous 7 days (must be ≥15): 49.8
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (e.g., Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (e.g., Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (e.g., Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 0 (add up % mortality for all cultures used / # cultures used)
Mean Number of Young Produced within first 3 broods 24.3 (avg # 1st 3 broods for all cultures used / # cultures used)
Mean Number of Young Produced in previous 7 days 52.4 (avg # neos in prev 7 days for all cultures used / # cultures usec
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / No (circle one) — No circled

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
W.6.1	(Y) / N	(Y) / N	13	1	ao
2	(Y) / N	(Y) / N	13	2	ao
3	(Y) / N	(Y) / N	14	3	ao
4	(Y) / N	(Y) / N	10	4	ao
6	(Y) / N	(Y) / N	14	5	ao
W.7.1	(Y) / N	(Y) / N	13	6	ao
2	(Y) / N	(Y) / N	15	7	ao
4	(Y) / N	(Y) / N	12	8	ao
7	(Y) / N	(Y) / N	10	9	ao
9	(Y) / N	(Y) / N	11	10	
	Y / N	Y / N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Pollutech Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay Site

Sample #:	8730-0032112	**Sample Name:**	Sublethal Toxicity - Sed 2 Discharge (L2647105-1)
Sample Method:	Grab	**Collected by:**	N/A
Date/Time Collected:	October 4, 2021; 08:45	**Arrival Temp.:**	19.5°C
Date/Time Received:	October 6, 2021; 14:00	**Sample Description:**	Clear, light yellow
Sample Point Description:	Other	**Sample Type:**	N/A

Transportation: Air/Road

Storage: 4 ±2 °C

Container: Polyethylene pails lined with polyethylene bags

N/A - Not Available

Test Organisms

Species (Strain #): *Raphidocelis subcapitata* (CPCC # 37)

Source: Pollutech Plant Culture Unit (from CPCC)

Culture Temp.: 24 ± 2 °C

Test Culture Number: G6(l)a

Culture Age at Test Start: 3 days old

Cell Density in the Microplate Wells at the Start of the Test: 10,909.04 cells/ml

Test Conditions

Date/Time Test Start:	October 7, 2021; 12:05	**T=0 Control pH:**	7.0
Date/Time Test End:	October 10, 2021; 12:00 - 14:30	**T=72 Control pH:**	7.0
Sample pH Before Dilution:	7.7	**pH Adjustment:**	None

Test Duration: 72 hours

Test Conditions– continued

Mean Test Temperature (±Standard Deviation): 25.4 (±0.3)°C

Pre-Aeration of Sample: None **Analyst:** E. Pasiak / C. D'Andrea

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Distilled Water

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2\ 6H_2O$	6.25	$MnCl_2\ 4H_2O$	259.76
	$CaCl_2\ 2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4\ 7H_2O$	9.19	$CoCl_2\ 6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2\ 2H_2O$	0.008
	$NaHCO_3$	9.38	$Na_2MoO_4\ 2H_2O$	4.54
			$FeCl_3\ 6H_2O$	100
			$Na_2EDTA\ 2H_2O$	46.9

Enumeration Technique: Neubauer Haemacytometer

Test Vessel: 96-Well U-bottomed Polystyrene Microplate

Concentration of Test Solutions: 90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control

 # Replicates/Concentration: 4 reps started; 3 reps counted of test solutions
10 control reps started, 2 used for pH measurement

Design if Specialized Procedure: Not applicable

Method Deviations or Unusual Occurrences: None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times: Acceptable (18.1 times)

pH in controls did not vary by more than 1.5 units: Acceptable (0 units)

C V for cell yields is < 20% within the control wells: Acceptable (6.8%)

No inhibitory trend detected across the control wells: Acceptable (no significant trend)

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032112 **Sample Name:** Sublethal Toxicity - Sed 2 Discharge (L2647105-1)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
219,091	206,591	189,091	211,591	181,591	184,091	194,091	199,091	198,153	6.8

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 CV = Coefficient of Variation = (100 x standard deviation / mean)

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	714,091	604,091	549,091	359,091	226,591
2	594,091	776,591	544,091	421,591	286,591
3	631,591	641,591	429,091	341,591	326,591
Mean Cell Yield	646,591	674,091	507,424	374,091	279,924
Coefficient Variation[2]	9	13	13	11	18
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	194,091	201,591			
2	191,591	206,591			
3	174,091	194,091			
Mean Cell Yield	186,591	200,758			
Coefficient Variation[2]	6	3			

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 Coefficient of Variation = (100 x standard deviation / mean)
Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	Result[1]	Method of Calculation
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume[3] (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	no trend	Mann-Kendall

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval for the Bootstrap Estimate
3 - Highest concentration tested, based on test method

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032112 **Sample Name:** Sublethal Toxicity - Sed 2 Discharge (L2647105-1)

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software, 2001-2007 Comprehensive Environmental Toxicity, Information System - CETIS v1.8.1.2.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (10.101% v/v concentration; rep. 3). No reason to remove it. Statistics include all data.

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2114 **Date Test Initiated:** 10/01/21

Method of Analysis: Linear Interpolation (ICPIN) **Algae Lot #:** G3(l)b

72-hour IC25 (95% Confidence Limits): 64.62 mg/L (55.66 mg/L; 71.11 mg/L)

Historic Geometric Mean IC25: 49.94 mg/L (25.67 mg/L; 97.16 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Test Facilities

Testing Laboratory:

CALA Testing Accreditation No. A1225

Pollutech EnviroQuatics Limited, 704 Mara St., Suite 122, Point Edward, Ontario, N7V 1X4

This laboratory is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA). The test included in this report is within the scope of this laboratory.

pollutech enviroquatics limited

Sample Name	S2 Discharge	Sample #	8730-0032112	Date test start	10.07.21

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	120000	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	20	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2400	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	10909.0909	=	18,164063

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	19	21	20	27	23	21	26	20
	21.0	22.0	17.0	24.0	15.0	19.0	17.0	23.0
	25	26.0	21.0	15.0	20.0	22.0	16.0	19.0
	27.0	18.0	22.0	23.0	19.0	16.0	23.0	22.0
total cells	92	87	80	89	77	78	82	84
cells/ul	230	217.5	200	222.5	192.5	195	205	210
cells/ml	230000	217500	200000	222500	192500	195000	205000	210000
Cell yield = measured concentration - initial algal cell concentration								
	219091	206591	189091	211591	181591	184091	194091	199091

% inhibition summary

Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	-1.314
0.374	5.835
1.122	-41.266
3.367	-88.789
10.101	-156.076
30.303	-240.186
90.910	-226.308

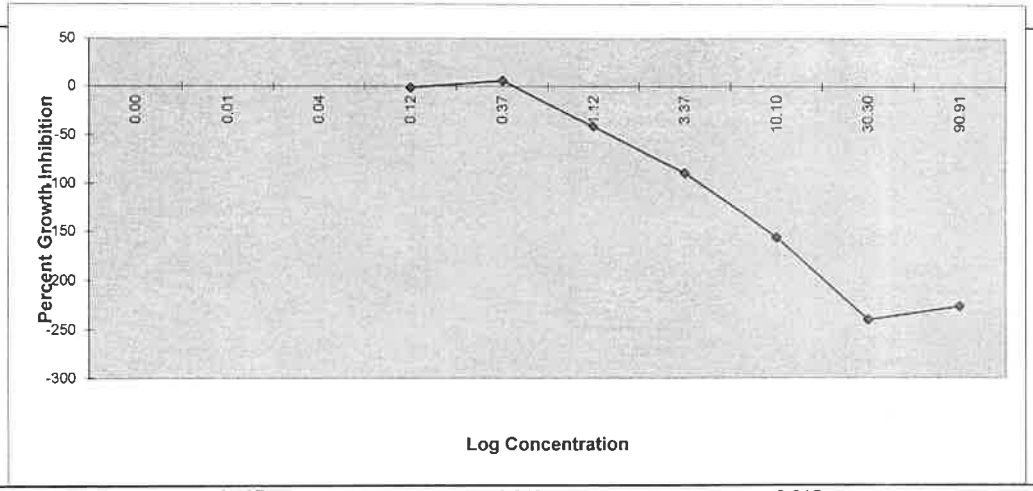
Mean cell yield for the control	= Rc	Standard deviation		coefficient of variation	
Rc	198153	SD	13425.5553	CV	6.775334 **Must be ≤20**

enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	73	56	61	61	71	60	56	49	48	37	42	36	27	31	30
	73.0	76.0	72.0	52.0	78.0	61.0	65.0	63.0	43.0	41.0	46.0	34.0	25.0	27.0	30.0
	70.0	50.0	60.0	69.0	84.0	65.0	55.0	50.0	45.0	40.0	47.0	33.0	20.0	34.0	39.0
	74.0	60.0	64.0	64.0	82.0	75.0	48.0	60.0	40.0	30.0	38.0	38.0	23.0	27.0	36.0
total cells	290	242	257	246	315	261	224	222	176	148	173	141	95	119	135
cells/ul	725	605	642.5	615	787.5	652.5	560	555	440	370	432.5	352.5	237.5	297.5	337.5
cells/ml	725000	605000	642500	615000	787500	652500	560000	555000	440000	370000	432500	352500	237500	297500	337500
Cell yield = measured concentration - initial algal cell concentration															
	714091	594091	631591	604091	776591	641591	549091	544091	429091	359091	421591	341591	226591	286591	326591
Mean Yield	646591			674091			507424			374091			279924		
STD Yield	61390			90726			67885			42057			50332		
CV Yield	9			13			13			11			18		

Average % inhibition											
	for 90.910%:	-226.308	for 30.303%	-240.186	for 10.101%	-156.076	for 3.367%	-88.789	for 1.122%	-41.266	

Average % stimulation											
	for 90.910%:	226.308	for 30.303%	240.186	for 10.101%	156.076	for 3.367%	88.789	for 1.122%	41.266	



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
17	21	16	26	24	19									
24.0	26.0	18.0	16.0	21.0	21.0									
19.0	15.0	20.0	25.0	19.0	20.0									
22.0	19.0	20.0	18.0	23.0	22.0									
82	81	74	85	87	82	0	0	0	0	0	0	0	0	0
205	202.5	185	212.5	217.5	205	0	0	0	0	0	0	0	0	0
205000	202500	185000	212500	217500	205000	0	0	0	0	0	0	0	0	0
194091	191591	174091	201591	206591	194091	-10909	-10909	-10909	-10909	-10909	-10909	-10909	-10909	-10909
186591			200758			-10909			-10909			-10909		
10897			6292			0			0			0		
6			3			0			0			0		
for 0.374%	5.835		for 0.125%	-1.314		for 0.042%	105.505		for 0.014%	105.505		for 0.005%	105.505	
for 0.374%	-5.835		for 0.125%	1.314		for 0.042%	-105.505		for 0.014%	-105.505		for 0.005%	-105.505	

Pollutech EnviroQuatics Limited
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS- Thunder Bay Sample Name Sublethal tox - Sed 2 Discharge L 2647105.1 Sample # 8730-0032112

Conditions for Test Validity

Cell increase for control is >16 — (Acceptable)/Not acceptable 18.1 (times)

CV among controls ≤ 20 — (Acceptable)/Not acceptable 6.8

Result of Mann-Kendall test for trend — (Acceptable)/Not acceptable no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 120 000 (cells/mL)

Used: (Yes)/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wells at the start of the test 10909.09 (cells/mL)

Analysis Completed: Initials: EV Date: 10/20/21

Results Verified: Initials: aD Date: 10/29/21

Pollutech EnviroQuatics Limited
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location: ALS Thunder Bay	L2647105-1
Sample #: 8730-00321 12	Sample Name: Sublethal toxicity - Eel 2 Discharge
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 10/04/21 8:45	Arrival Temp.: (meter/probe) 19.5 °C (34)
Date/Time Received: 10/06/21 14:00	Sample Description: clear, light yellow
Collection Point Description: other	Sample Type: ☐ Effluent ☐ Chemical ☒ Other: N/A
Transportation: Road / ~~Air~~	Storage: 4±2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	EC
Source	Pollutech Plant Culture Unit (from CPCC), Test Culture # G6(1)a	EC
Culture Age at Start of Test	3 days old (must be 3 to 7 days old)	EC
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	EC
	Axenic culture? Yes/No (Circle one)	C

Notes:

Test Conditions:

Date / Time Test Start: 10.07.21 12:05 Started By: CP		Date / Time Test End: 10/10/21 1200 - 1430 Finished By: CP	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 7.7	pH adjustment: Y/N	pH of well D6 at T=0 h 7.0	pH of well D7 at T=72 h 7.0
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # —		Metal mining NUT Lot # NUT 2104
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation)		25.4 ± 0.3 °C	

	Day 1		Day 2	
¼ plate rotation (Initial)	AM EC	PM EC	AM CP	PM CP
Lights ON (Initial)	AM EC	PM EC	AM CP	PM CP

* For reference test, record pH of top concentration before use for series dilutions.

Notes: Millipore H2O

72-Hour Qualitative Observations:

Condensation: no		
Growth: yes		
Were there any other method variations or deviations from methods? Yes/No		If yes, describe further:
Anything unusual about the test? Yes/No		
Any problems encountered? Yes/No		
Any remedial measures taken? Yes/No		

Pollutech EnviroQuatics Limited
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 8730-0032112	Sample Name: Sublethal Toxicity – Seal 2 Discharge

Reference Data:

Reference Chemical Batch #	Phenol P2114	Date test started	10.01.21
Method of Analysis	ICPIN – Linear Interpolation	Algae Lot #	6-5(2)c
72-hour IC25 (95% C.I.)[3] mg/L	64.62 55.66; 71.11	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	49.99 25.67; 97.16

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	> 90.91% (N/A)	No nonlinear regression model could fit
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers	–	ICPIN – Linear Interpolation –
Test for Outliers	No Outliers Present ✓	Grubbs' Test for Residual Outlier Initial C
	If outliers present, indicate Concentration/Rep:	
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed.	
	No statistically significant growth stimulation.	Williams' or Dunnett's Multiple Comparison Test Initial C
	Yes, statistically significant growth stimulation at these concentrations: 90.91; 30.303; 10.101; 3.367; 1.122	

1)Results relate only to the sample tested.
2) Tidepool Scientific Software © 2001-2007. Comprehensive Environmental Toxicity Information System – CETIS v. 1.9.6.7
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/No

Any outliers and justification for their removal? Yes/No

Pollutech EnviroQuatics Limited

Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)

72-Hour Growth Inhibition Test – Continued

72-Hour Quantitative Observations of Controls

Sample Name: Sublethal toxicity - Scd 2 Discharge Sample Number: 8730-032112 Date Test Start: 10.07.21

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D6	Well # D9	Well # D10	Well # D11
1	19	21	20	27	23	21	26	20
2	21	22	17	24	15	19	17	23
3	25	26	21	15	20	22	16	19
4	27	18	22	23	19	16	23	22
5	—	—	—	—	—	—	—	—
Initials	ao	ao	ao	ao	ao	ao	ao	ao

I = 120,000

Cell increase for controls = 18.2	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = 6.8	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = no significant trend	Controls are invalid if there is a trend detected by the Mann-Kendall test

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: Sublethal Tox - Set 2 Discharge Sample Number: 8730003212 Date Test Start: 10.07.21

Theoretical Test Concentration: 100.00% v/v					Actual Test Concentration: 90.91% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	73	96	61		646 591 (± 61 390)
2	73	76	72		Coefficient of Variation of Cell Yield
3	70	50	60		9
4	74	60	64		Average % Inhibition (-ve number = enhancement)
5	—	—	—		−226.308
Initials	aO	aO	aO		C

Theoretical Test Concentration: 33.33% v/v					Actual Test Concentration: 30.303% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	61	71	60		674 091 (± 90726)
2	52	78	61		Coefficient of Variation of Cell Yield
3	69	84	63		13
4	64	82	75		Average % Inhibition (-ve number = enhancement)
5	—	—	—		−240.186
Initials	aO	aO	aO		C

Theoretical Test Concentration: 11.111% v/v					Actual Test Concentration: 10.101% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	56	49	48		507 424 (± 67885)
2	65	63	43		Coefficient of Variation of Cell Yield
3	55	50	45		13
4	48	60	40		Average % Inhibition (-ve number = enhancement)
5	—	—	—		−156.076
Initials	aO	aO	aO		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)

Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: Sublethal for [illegible] 2 Discharge Sample Number: STS0003212 Date Test Start: [illegible] 21

Theoretical Test Concentration: 3.704 % v/v				Actual Test Concentration: 3.367 % v/v	
Cell count per 0.1 µl or 0.004 µl	Well = B5	Well = C5	Well = F5	Well =	Average Cell Yield (±Standard Deviation)
1	37	42	36		374091 (± 42057)
2	41	46	34		Coefficient of Variation of Cell Yield
3	40	47	33		11
4	30	38	38		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-88.789
Initials	aO	aO	aO		C

Theoretical Test Concentration: 1.235 % v/v				Actual Test Concentration: 1.122 % v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation)
1	27	31	30		279924 (± 50332)
2	25	27	30		Coefficient of Variation of Cell Yield
3	20	34	39		18
4	23	27	36		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-41.266
Initials	aO	aO	aO		C

Theoretical Test Concentration: 0.412 % v/v				Actual Test Concentration: 0.374 % v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation)
1	17	21	16		186591 (± 10897)
2	24	26	18		Coefficient of Variation of Cell Yield
3	19	15	20		6
4	22	19	20		Average % Inhibition (-ve number = enhancement)
5	—	—	—		5.835
Initials	aO	aO	aO		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: Sublethal tox - Set 2 Discharge Sample Number: 8830U321L Date Test Start: 10.07.21

Theoretical Test Concentration: 0.137 %v/v					Actual Test Concentration: 0.125 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # F3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	26	24	19		200758 (± 6292)
2	16	21	21		Coefficient of Variation of Cell Yield
3	25	19	20		3
4	18	23	22		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-1.314
Initials	aD	aD	aD		G

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:
ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected (M/D/Y)	Date Received (M/D/Y)	Date Tested (M/D/Y)	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
Sublethal Toxicity - Sed 2 Discharge (L2647105-1)	8730-0032112	October 4, 2021	October 6, 2021	October 7, 2021	>97% Volume[2] (Not applicable)	>97% Volume[2] (Not applicable)	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Pollutech Test Method LM-GI-R5.14)

TESTING PERFORMED BY:

Lemna minor Bioassay: E. Pasiak / K. Kramer / C. D'Andrea / K. Ferguson

TESTING FACILITY:

Pollutech EnviroQuatics Limited, 704 Mara St., Suite 122, Point Edward, Ontario, N7V 1X4
This laboratory is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA).
All of the tests included in this report are within the scope of this laboratory.

CALA Testing Accreditation No. A1225

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	10/08/2021	**Reference Chemical:**	KCl
Frond Increase IC25:	2.60 g/L	**95% Confidence Limits:**	2.22; 2.97 g/L
Historic Geometric Mean IC25:	2.06 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.22; 3.48 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:

Substance	mg/l	Substance	mg/l	Substance	mg/l
$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	$Na_2MoO_4\ 2H_2O$	0.0726
K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$ZnCl_2$	0.00003
KCl	10.1	$MgSO_4\ 7H_2O$	147	$CoCl_2\ 6H_2O$	0.00001
				$CuCl_2\ 2H_2O$	0.00001

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

Date: 10.29.21
(M/D/Y)

C D'Andrea
per: **Ken Ferguson, B.Sc. (Hon.)**
Senior Laboratory Leader

pollutech enviroquatics limited

Test Material

Client Name/Location: MLS - Thunder Bay L2647105-1	
Sample #: 8730-00321 12	Sample Name: Sublethal toxicity - Sed 2 Disc
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 10/04/21 8:45	Arrival Temperature (meter/probe): 19.5 °C (34)
Date/Time Received: 10/06/21 14:00	Sample Description: clear, light yellow
Collection Point Description: other	Sample Type: ☐ Effluent ☐ Chemical ☒ Other:
Transportation: Air / Road	Storage: 4±2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	C
Source:	Pollutech Plant Culture Unit (from CPCC, # 490)	C
Culture Age at Start of Test:	8 days old, acclimated 19 hours in fresh test solution (mAPHA)	C
Culture Medium:	Modified Hoagland's E+ medium, Lot # M42103	C
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/No	C
	Axenic culture? Yes/No	C
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 30 in HT 1, 30 in HT 2, 25 in HT 3 at finish	C

Test Conditions and Procedures

Date / Time Test Start: 10.07.21 11:00 Started By: C	Date / Time Test End: 10.14.21 11:35 Finished By: C
Test Type: (Static (no renewal)) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / (No) (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: (Modified APHA) (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 8.5 (12187)	pH Adjustment: none
Test Volume and Depth: 150 mL / 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / (No)	If yes, describe further:
Anything unusual about the test? Yes / (No)	
Any problems encountered? Yes / (No)	
Any remedial measures taken? Yes / (No)	Randomization Template: D

Sample #: 8730-0032112	Sample Name: Sublethal toxicity - Sed 2 Discharge

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	24.6	25.4	25.7	25.6	25.6	25.4	25.0	25.4
Low	24.4	25.3	25.7	25.6	25.6	25.4	25.1	25.2
Medium	24.5	25.3	25.7	25.5	25.7	25.3	25.1	25.1
High	24.4	25.3	25.7	25.5	25.6	25.4	25.1	25.4
Initials	TV	KK	[illegible]	[illegible]	[illegible]	KK	TV	TV
meter/probe	52	52	52	52	52	52	52	52
Mean Test Temperature (average of 24h high / low temperatures):	25.3 ± 0.1 °C							

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

	Control	Low	Medium	High	Initials	pH meter/probe
Day 0	8.4	8.6	8.6	8.5	TV	12/87
Day 7	8.4	8.6	9.1	10.3	TV	12/87

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)	—	—	—	—	—	—	—	—

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 10/13/21 (6)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 9					Initials: TV
5200	4890	5140	4690	4380	Mean Light Measurement: 4852
±15% Variation of Mean: 4125 - 5580					(Acceptable) / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) / No
If yes, describe further: Algae

Reference Data

Reference Date	(Frond Increase) or Dry Weights (circle one)			
	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
10/08/21	2.60	2.22; 2.97	2.06	1.22; 3.48

Sample # 8730-0032112	Sample Name: Sublethal toxicity - Sed 2 Discharge

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 50	B 53	C 49	D 48	50.0

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants 3 fronds each in each vessel, dark green and healthy						Initials	
						CO	
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV: 44.0 (2.2) 4.9							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control (% v/v) g/L	0.097	0.29	0.97	3.1	9.7 *	31 *	97
Mean % Stimulation	-12.80	-10.80	-19.89	11.93	25.57	30.68	5.11
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control (% v/v) g/L	0.097	0.29	0.97	3.1	9.7	31 *	97 *
Mean % Stimulation	-7.16	-7.41	-15.62	9.77	17.67	33.98	49.53

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using (Dunnett's) or Williams' Multiple Comparison Test.

Stimulation calculation completed: (Yes) / Not applicable (no stimulation) (Circle one)

Test Endpoints and Calculations: Fronds / Weights

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	> 97% (N/A)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN - linear interpolation
Dry Weights		
IC25 (95% C.I.)[3]	> 97% (N/A)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN - linear interpolation

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2001-2007. Comprehensive Environmental Toxicity Information System CETISv 1.8.1.2
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / (No)

Any outliers and justification for their removal? Yes / (No)

Lemna minor Weights

Client: ALS-Thunder Bay	Site: Sublethal toxicity Seal 2 - Discharge	Sample number: 8730 0032 12
In Oven Date/Time/°C: 10.14.21 11:55 62°C	Out Oven Date/Time/°C: 11/15/21 11:55 62°C	

Conc.	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	44	44.0 (2.2)	0.68361	0.67954	4.07	4.02 (0.5)
	B	47		0.68510	0.678050	4.60	
	C	43		0.67671	0.67324	3.47	
	D	42		0.67393	0.67000	3.93	
0.097	A	40	38.5 (5.8)	0.67278	0.676921	3.57	3.73 (0.5)
	B	45		0.67283	0.66856	4.27	
	C	31		0.66251	0.65933	3.18	
	D	38		0.67880	0.67490	3.90	
0.29	A	41	39.3 (3.5)	0.66716	0.66373	3.43	3.72 (0.5)
	B	38		0.68546	0.68169	3.77	
	C	35		0.71905	0.71575	3.30	
	D	43		0.71708	0.71270	4.38	
0.97	A	33	35.3 (2.9)	0.70658	0.70310	3.48	3.39 (0.4)
	B	36		0.73366	0.73032	3.34	
	C	33		0.72089	0.71796	2.93	
	D	39		0.69919	0.69538	3.81	
3.1	A	52	49.3 (2.1)	0.708540	0.70370	4.70	4.41 (0.4)
	B	47		0.70795	0.70403	3.92	
	C	49		0.67205	0.66772	4.33	
	D	49		0.68153	0.67684	4.69	
9.7	A	52	55.3 (5.9)	0.68754	0.68314	4.40	4.73 (0.6)
	B	53		0.68182	0.67742	4.40	
	C	64		0.68690	0.68128	5.62	
	D	52		0.67692	0.67243	4.49	
31	A	60	57.5 (2.4)	0.67747	0.67186	5.61	5.38 (0.2)
	B	56		0.66314	0.65797	5.22	
	C	59		0.67731	0.67185	5.46	
	D	55		0.67620	0.67096	5.24	
97	A	42	46.3 (6.1)	0.68517	0.68065	4.52	6.01 (1.2)
	B	55		0.68801	0.68165	6.36	
	C	42		0.68148	0.67412	7.36	
	D	46		0.68157	0.67578	5.79	
Initials		EG	EG	EG	EG	EG	EG

Notes:

Sample name: Sublethal toxicity-Sed 2 Discharge Date started: 10/7/2021 sample # 8730-0032112

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

50.0 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	50	46	47	39	58	58	66	48
	53	51	44	42	53	59	62	61
	49	37	41	39	55	70	65	48
	48	44	49	45	55	58	61	52
Total Fronds	200	178	181	165	221	245	254	209
Increase in Frond Number = Total # Fronds - 6 Starting Fronds								
	44	40	41	33	52	52	60	42
	47	45	38	36	47	53	56	55
	43	31	35	33	49	64	59	42
	42	38	43	39	49	52	55	46
Total Increase	176	154	157	141	197	221	230	185
Mean Increase	44.0	38.5	39.3	35.3	49.3	55.3	57.5	46.3
SD Increase	2.2	5.8	3.5	2.9	2.1	5.9	2.4	6.1
CV Increase	4.9	15.1	8.9	8.1	4.2	10.6	4.1	13.3
% Stimulation		**-12.50**	**-10.80**	**-19.89**	**11.93**	**25.57**	**30.68**	**5.11**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.68361	0.67278	0.66716	0.70658	0.70840	0.68754	0.67747	0.68517
Pan + Plant	0.68510	0.67283	0.68546	0.73366	0.70795	0.68182	0.66319	0.68801
	0.67671	0.66251	0.71905	0.72089	0.67205	0.68690	0.67731	0.68148
	0.67393	0.67880	0.71708	0.69919	0.68153	0.67692	0.67620	0.68157
Initial Weight (g)	0.67954	0.66921	0.66373	0.70310	0.70370	0.68314	0.67186	0.68065
Pan Only	0.68050	0.66856	0.68169	0.73032	0.70403	0.67742	0.65797	0.68165
	0.67324	0.65933	0.71575	0.71796	0.66772	0.68128	0.67185	0.67412
	0.67000	0.67490	0.71270	0.69538	0.67684	0.67243	0.67096	0.67578
Plant Only (mg)	4.07	3.57	3.43	3.48	4.70	4.40	5.61	4.52
	4.60	4.27	3.77	3.34	3.92	4.40	5.22	6.36
	3.47	3.18	3.30	2.93	4.33	5.62	5.46	7.36
	3.93	3.90	4.38	3.81	4.69	4.49	5.24	5.79
Mean Dry Weight	4.018	3.730	3.720	3.390	4.410	4.728	5.382	6.007
SD Dry Weight	0.5	0.5	0.5	0.4	0.4	0.6	0.2	1.2
CV Dry Weight	11.6	12.5	13.0	10.8	8.4	12.6	3.5	19.7
% Stimulation		**-7.16**	**-7.41**	**-15.62**	**9.77**	**17.67**	**33.98**	**49.53**

For Data Transfer to CETIS

		# fronds	total mass	tare
0	1	44	0.68361	0.67954
	2	47	0.68510	0.68050
	3	43	0.67671	0.67324
	4	42	0.67393	0.67000
0.1	1	40	0.67278	0.66921
	2	45	0.67283	0.66856
	3	31	0.66251	0.65933
	4	38	0.67880	0.67490
0.3	1	41	0.66716	0.66373
	2	38	0.68546	0.68169
	3	35	0.71905	0.71575
	4	43	0.71708	0.71270
1	1	33	0.70658	0.70310
	2	36	0.73366	0.73032
	3	33	0.72089	0.71796
	4	39	0.69919	0.69538
3.1	1	52	0.70840	0.70370
	2	47	0.70795	0.70403
	3	49	0.67205	0.66772
	4	49	0.68153	0.67684
9.7	1	52	0.68754	0.68314
	2	53	0.68182	0.67742
	3	64	0.68690	0.68128
	4	52	0.67692	0.67243
31	1	60	0.67747	0.67186
	2	56	0.66319	0.65797
	3	59	0.67731	0.67185
	4	55	0.67620	0.67096
97	1	42	0.68517	0.68065
	2	55	0.68801	0.68165
	3	42	0.68148	0.67412
	4	46	0.68157	0.67578

Lemna mi[illegible] y 7 Observations

Client: ALS - Thornhill Bay				**Sample number:** 8730 0032112			**Date Started:** 10.07.21		
Site: Sublethal tox - Set 2 Dish							**Date Ended:** 10.14.21		
Concentration: Control			**Observations By:** C	**Concentration:** 0.097 T. vit.			**Observations By:** C		
Observations	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Observations**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
Number of	50	53	49	48	**Number of**	46	51	37	44
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	X	X	X	X	**Abnormally sized fronds**	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations					**Other Observations**				
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO N/A Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna min[illegible] y 7 Observations

Client: ALS - Thornhill Bay	**Sample number:** 8730 0032112	**Date Started:** 10.07.21	
Site: Sublethal tox - Set 2 Dish		**Date Ended:** 10.14.21	
Concentration: 0.29 l. /ml	**Observations By:** C	**Concentration:** 0.97 l. /ml	**Observations By:** C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	47	44	41	49	**Number of**	39	42	39	45
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	**Abnormally sized fronds**	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	✓	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	✓	X	**Loss of Buoyancy**	X	X	✓	X
Other Observations					**Other Observations**				
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna mir… y 7 Observations

Client: ALS - Thunder Bay	Sample number: 8730 0032112	Date Started: 10.07.21	
Site: Sublethal tox - Set 2 Dish		Date Ended: 10.14.21	

Concentration: 3.1% v/v — Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	58	53	55	55
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: (YES) / NO
Weights: (YES) / NO

Concentration: 9.7% v/v — Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	58	59	70	58
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: (YES) / NO
Weights: (YES) / NO

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor y 7 Observations

Client: ALS - Thunder Bay				**Sample number:** 8730-0032112				**Date Started:** 10.07.21	
Site: Sublethal tox - Set 2 Dish								**Date Ended:** 10.14.21	
Concentration: 31% v/v				**Observations By:** C	**Concentration:** 97% v/v				**Observations By:** C
Observations	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Observations**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
Number of	66	62	65	61	**Number of**	48	61	48	52
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	X	X	X	X	**Abnormally sized fronds**	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations	algae	→	→	→	**Other Observations**	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

L2647105-COFC

Chain of Custody (COC) / Analytical Request Form

COC Number: 20 -

Page of

Canada Toll Free: 1 800 668 9878

ALS www.alsglobal.com

Report To	Contact and company name below will appear on the final report
Company:	New Gold
Contact:	Garnet Cornell
Phone:	
	Company address below will appear on the final report
Street:	
City/Province:	
Postal Code:	
Invoice To	Same as Report To ☐ YES ☐ NO
	Copy of Invoice with Report ☐ YES ☐ NO
Company:	
Contact:	

Reports / Recipients

Select Report Format: ☐ PDF ☐ EXCEL ☐ EDD (DIGITAL)

Merge QC/QCI Reports with COA ☐ YES ☐ NO ☐ N/A

☐ Compare Results to Criteria on Report - provide details below if box checked

Select Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Email 3

Invoice Recipients

Select Invoice Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Turnaround Time (TAT) Requested

☐ Routine [R] if received by 3pm M-F - no surcharges apply
☐ 4 day [P4] if received by 3pm M-F - 20% rush surcharge minimum
☐ 3 day [P3] if received by 3pm M-F - 25% rush surcharge minimum
☐ 2 day [P2] if received by 3pm M-F - 50% rush surcharge minimum
☐ 1 day [E] if received by 3pm M-F - 100% rush surcharge minimum
☐ Same day [E2] if received by 10am M-S - 200% rush surcharge.

Additional fees may apply to rush requests on weekends, statutory holidays and for non-routine tests.

Date and Time Required for all E&P TATs: dd-mmm-yy hh:mm am/pm

For all tests with rush TATs requested, please contact your AM to confirm availability.

AFFIX ALS BARCODE LABEL HERE (ALS use only)

Project Information

ALS Account # / Quote #:	
Job #:	
PO / AFE:	
LSD:	
ALS Lab Work Order # (ALS use only):	L2647105

Oil and Gas Required Fields (client use)

AFE/Cost Center:	PO#
Major/Minor Code:	Routing Code:
Requisitioner:	
Location:	
ALS Contact:	Sampler:

Analysis Request

Indicate Filtered (F), Preserved (P) or Filtered and Preserved (F/P) below

ALS Sample # (ALS use only)	Sample Identification and/or Coordinates (This description will appear on the report)	Date (dd-mmm-yy)	Time (hh:mm)	Sample Type	NUMBER OF CONTAINERS	NG-ST-P-TB	SAMPLES ON HOLD	EXTENDED STORAGE REQUIRED	SUSPECTED HAZARD (see notes)
-1	Sublethal Toxicity- Sed 2 Discharge	4-Oct-21	8:45	Discharge	4	X			

Drinking Water (DW) Samples[1] (client use)

Are samples taken from a Regulated DW System? ☐ YES ☐ NO

Are samples for human consumption/ use? ☐ YES ☐ NO

Notes / Specify Limits for result evaluation by selecting from drop-down below (Excel COC only)

SAMPLE RECEIPT DETAILS (ALS use only)

Cooling Method: ☐ NONE ☐ ICE ☐ ICE PACKS ☐ FROZEN ☐ COOLING INITIATED

Submission Comments identified on Sample Receipt Notification: ☐ YES ☐ NO

Cooler Custody Seals Intact: ☐ YES ☐ N/A Sample Custody Seals Intact: ☐ YES ☐ N/A

INITIAL COOLER TEMPERATURES °C			FINAL COOLER TEMPERATURES °C		
16.0					

SHIPMENT RELEASE (client use)			INITIAL SHIPMENT RECEPTION (ALS use only)			FINAL SHIPMENT RECEPTION (ALS use only)		
Released by:	Date:	Time:	Received by:	Date:	Time:	Received by:	Date:	Time:
				16/04/21	17:00			

REFER TO BACK PAGE FOR ALS LOCATIONS AND SAMPLING INFORMATION

WHITE - LABORATORY COPY YELLOW - CLIENT COPY

ALS 2020 FRONT

Failure to complete all portions of this form may delay analysis. Please fill in this form LEGIBLY. By the use of this form the user acknowledges and agrees with the Terms and Conditions as specified on the back page of the white - report copy.

1. If any water samples are taken from a Regulated Drinking Water (DW) System, please submit using an Authorized DW COC form.

New Gold Inc. Rainy River Project
ATTN: Garnet Cornell
24 Marr Rd
Barwick ON P0W 1A0

Date Received: 28-APR-22
Report Date: 27-MAY-22 13:07 (MT)
Version: FINAL

Client Phone: 807-234-8200

Certificate of Analysis

Lab Work Order #: L2701854

Project P.O. #: 4500058071
Job Reference:
C of C Numbers:
Legal Site Desc:

Christine Paradis
Project Manager

ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
L2701854-1 EDL1 Sampled By: Client on 27-APR-22 @ 09:00 Matrix: EFF							
Miscellaneous							
Special Request	See attached					29-APR-22	R5786394
Special Request	See Attached					24-MAY-22	R5786394
Special Request	See Attached					24-MAY-22	R5786394
Special Request	See Attached					24-MAY-22	R5786394
L2701854-2 SP2 Sampled By: Client on 27-APR-22 @ 09:10 Matrix: EFF							
Miscellaneous							
Special Request	See Attached					24-MAY-22	R5786394
Special Request	See Attached					24-MAY-22	R5786394
Special Request	See Attached					24-MAY-22	R5786394
Special Request	See Attached					24-MAY-22	R5786394

* Refer to Referenced Information for Qualifiers (if any) and Methodology.

Reference Information

Test Method References:

ALS Test Code	Matrix	Test Description	Method Reference**
TOX-7DINHIB-LM-TB	Misc.	Growth Inhibition using Lemma minor	SEE SUBLET LAB RESULTS
Lemna minor 7-day growth toxicity test, based on the protocol "Biological Test Method: Test Method for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, Lemna minor", Report EPS 1/RM/37, Second Edition (January 2007)			
TOX-GROWTH-FH-TB	Misc.	7 Day Survival & Growth Fat Head Minnows	SEE SUBLET LAB RESULTS
Fathead minnow 7-day test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Environmental Protection Series, Ottawa, ON, Report EPS1/RM/22, Second Edition (February 2011).			
TOX-INHIB-PS-TB	Misc.	Growth Inhibition Pseudokirchneriella	SEE SUBLET LAB RESULTS
Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) 72-hour growth toxicity test, based upon protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga" Report EPS1/RM/25, Second Edition (March 2007).			
TOX-REPRO-CD-TB	Misc.	Survival & reproduction (Ceriodaphnia)	SEE SUBLET LAB RESULTS
Ceriodaphnia dubia 3-brood toxicity test, accroding to protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dusbia", Environmental Protection Series, Ottawa, ON, Report EPS 1/RM/21, Second Edition (February 2007).			

** ALS test methods may incorporate modifications from specified reference methods to improve performance.

The last two letters of the above test code(s) indicate the laboratory that performed analytical analysis for that test. Refer to the list below:

Laboratory Definition Code	Laboratory Location
TB	ALS ENVIRONMENTAL - THUNDER BAY, ONTARIO, CANADA

Chain of Custody Numbers:

GLOSSARY OF REPORT TERMS

Surrogates are compounds that are similar in behaviour to target analyte(s), but that do not normally occur in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery. In reports that display the D.L. column, laboratory objectives for surrogates are listed there.
mg/kg - milligrams per kilogram based on dry weight of sample
mg/kg wwt - milligrams per kilogram based on wet weight of sample
mg/kg lwt - milligrams per kilogram based on lipid weight of sample
mg/L - unit of concentration based on volume, parts per million.
< - Less than.
D.L. - The reporting limit.
N/A - Result not available. Refer to qualifier code and definition for explanation.

Test results reported relate only to the samples as received by the laboratory.
UNLESS OTHERWISE STATED, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.
Analytical results in unsigned test reports with the DRAFT watermark are subject to change, pending final QC review.

Quality Control Report

Workorder: L2701854 Report Date: 27-MAY-22

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0

Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed

Quality Control Report

Workorder: L2701854 Report Date: 27-MAY-22

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Legend:

Limit	ALS Control Limit (Data Quality Objectives)
DUP	Duplicate
RPD	Relative Percent Difference
N/A	Not Available
LCS	Laboratory Control Sample
SRM	Standard Reference Material
MS	Matrix Spike
MSD	Matrix Spike Duplicate
ADE	Average Desorption Efficiency
MB	Method Blank
IRM	Internal Reference Material
CRM	Certified Reference Material
CCV	Continuing Calibration Verification
CVS	Calibration Verification Standard
LCSD	Laboratory Control Sample Duplicate

Hold Time Exceedances:

All test results reported with this submission were conducted within ALS recommended hold times.

ALS recommended hold times may vary by province. They are assigned to meet known provincial and/or federal government requirements. In the absence of regulatory hold times, ALS establishes recommendations based on guidelines published by the US EPA, APHA Standard Methods, or Environment Canada (where available). For more information, please contact ALS.

The ALS Quality Control Report is provided to ALS clients upon request. ALS includes comprehensive QC checks with every analysis to ensure our high standards of quality are met. Each QC result has a known or expected target value, which is compared against pre-determined data quality objectives to provide confidence in the accuracy of associated test results.

Please note that this report may contain QC results from anonymous Sample Duplicates and Matrix Spikes that do not originate from this Work Order.

May 19, 2022

Christine Paradis,
ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Dear Christine:

On April 29, 2022, Nautilus Environmental Company Inc. personnel received one water sample (EDL1 L2701854-1) from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected April 27, 2022

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
EDL1 L2701854-1	Fathead Minnow	7-day LC50 (95% Confidence)	Survival	>100% Volume (Not Applicable)
		7-day IC25 (95% Confidence)	Biomass	>100% Volume (Not Applicable)
#8730-0032203	*Ceriodaphnia dubia*	3-brood LC50 (95% Confidence)	Survival	>100% Volume (Not Applicable)
		3-brood IC25 (95% Confidence)	Reproduction	50.69% Volume (0.26% Volume; N/A)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	79.31% Volume[2] (74.42; 87.13% Volume)
	Lemna minor	7-day IC25 Frond Number (95% Confidence)	Growth	79.25% Volume (60.5% Volume; N/A)
		7-day IC25 Dry Weight (95% Confidence)	Growth	>97% Volume[3] (Not applicable)

1 - Results relate only to the sample tested
2 - Concentration 0.125% v/v - 3.367% v/v replaced with control data for hormetic response.
3 - Highest concentration tested, based on test method

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 100%, 33%, 11%, 3.7% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted half by half (*i.e.,* 100%, 50%, 25% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

If there are any further details which you require, please do not hesitate to contact us.

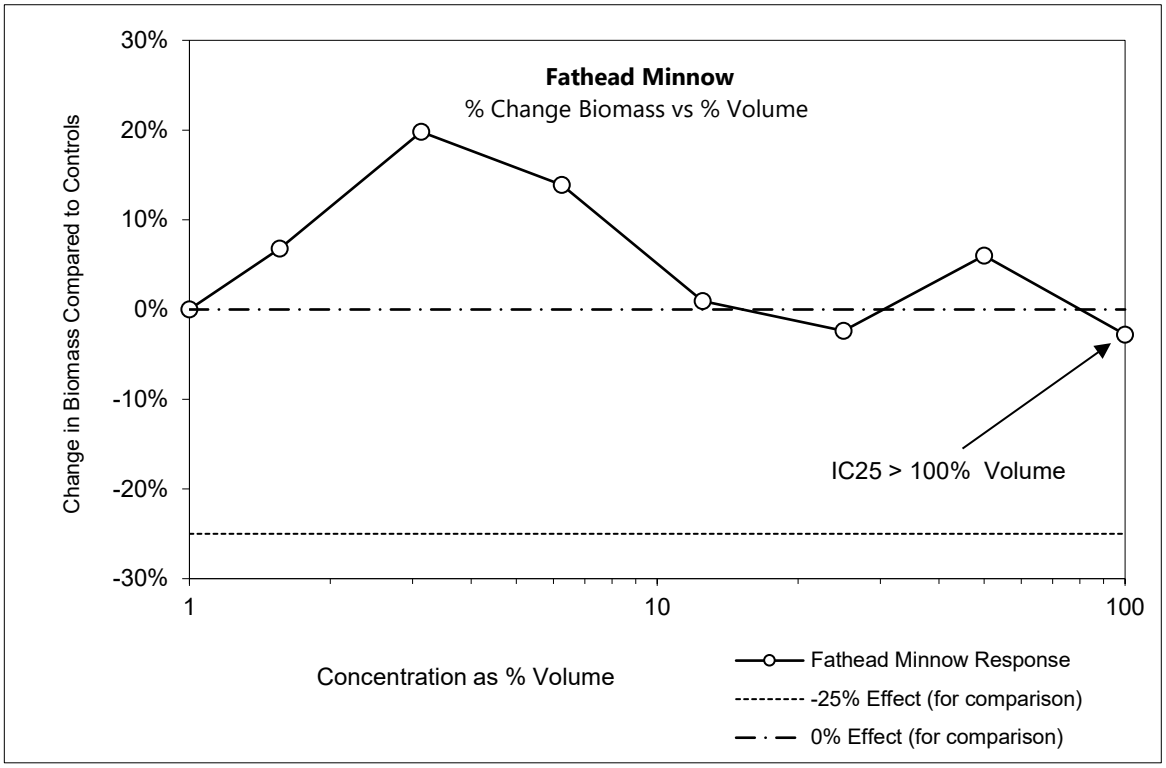
Yours very truly,
Nautilus Environmental Company Inc.

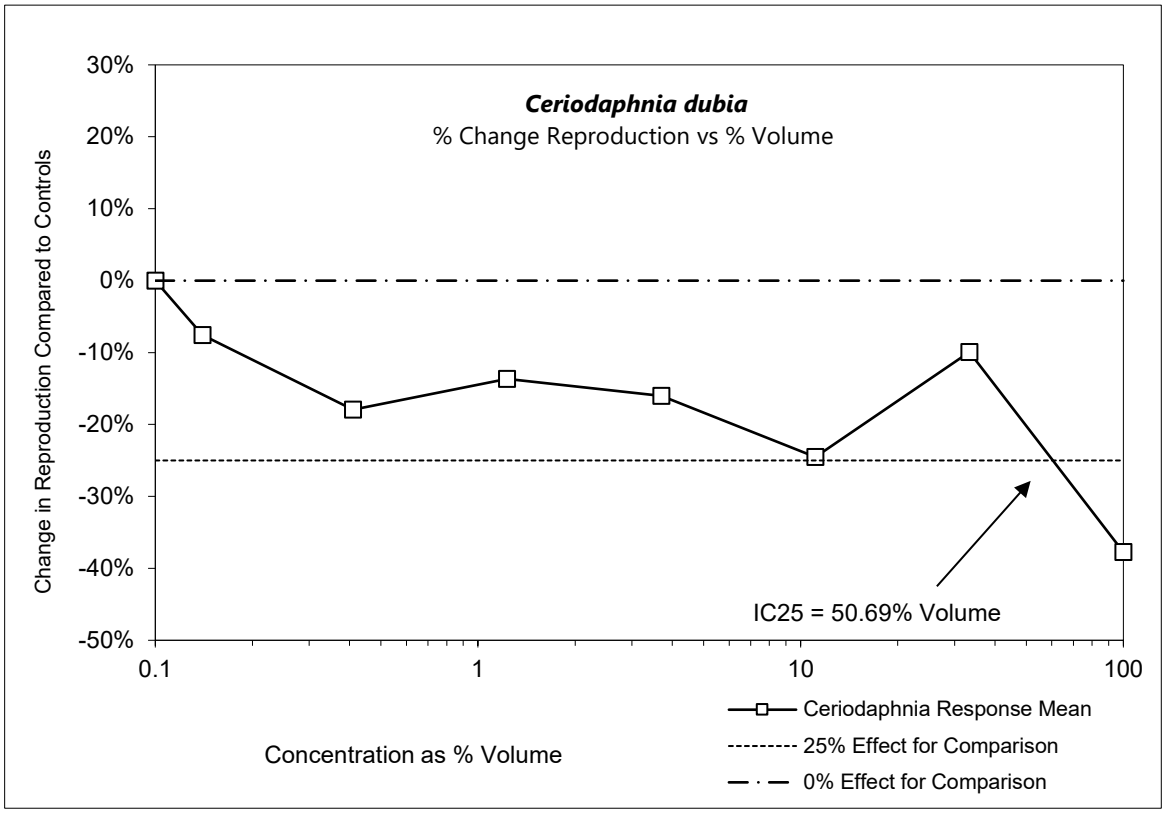
C. D'Andrea

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2021\1000\8730-003\8730-0032203 FCRL

ALS Thunder Bay - EDL1 (L2701854-1) - April 27, 2022





P:\Data\IC25\2022\8730-0032203

Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	EDL1
Date/Time Sampled:	April 27, 2022; 09:00	**Date/Time Received:**	April 29, 2022; 15:00
Date/Time Test Started:	April 29, 2022; 16:00	**Date Test Finished:**	May 6, 2022
Description:	Clear, light green	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032203	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	11.7°C
Collected By:	N/A		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species:	Fathead Minnow (*Pimephales promelas*)		
Source:	Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)		
Culture Temp.:	22 to 26 °C	**Life Stage:**	<24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 0%

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032203 **Sources:** EDL1

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Test Conditions

Test Volume: 533 ml/rep **Temp.:**25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 0 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (0%)

Average Weight of Controls is ≥ 250 µg: Acceptable (717 µg)

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032203 **Sources:** EDL1

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	10	10
	3	0	0	0	0	0	0	0	10
Mean % Mortality		0.0	0.0	0.0	0.0	0.0	0.0	3.3	10.0
S.D.		0.0	0.0	0.0	0.0	0.0	0.0	5.8	0.0
Survival/Growth Data									
Mean Dry Biomass	1	0.673	0.863	0.838	0.887	0.741	0.683	0.816	0.628
(mg)	2	0.703	0.724	0.879	0.804	0.674	0.675	0.707	0.653
	3	0.776	0.711	0.861	0.760	0.757	0.743	0.758	0.811
% Effect (+ or -)		0.0	6.8	19.8	13.9	0.9	-2.4	6.0	-2.8
Mean Dry Biomass/ Concentration (mg)		0.717	0.766	0.859	0.817	0.724	0.700	0.760	0.697
S.D.		0.05	0.08	0.02	0.06	0.04	0.04	0.05	0.10

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032203 **Sources:** EDL1

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 7-day LC50 (Confidence Interval)[2]	 >100% Volume (Not Applicable)	 No dose response
Biomass **(Survival and Growth)** 7-day IC25 (Confidence Interval)[2]	 >100% Volume (Not Applicable)	 Non-linear Regression 2P Exponential Model

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 21-Apr-2022 **Reference Batch #:** Zn2102

Method of Analysis: Trimmed Spearman-Karber α = 1.67%

7-Day LC50 (95% Confidence Limits): 0.51 mg/L (0.44 mg/L; 0.59 mg/L)

Historic Geometric Mean LC50: 0.73 mg/L (0.43 mg/L; 1.21 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS - Thunder Bay Sample Name: EDL 1 Sample #: 8730-003 2203

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 0 %

Average Weight of Controls is ≥ 250 µg: (Acceptable) / Not Acceptable 717 µg

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 0 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
7-day LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
Survival / Growth Biomass		Nonlinear regression 2P Exponential model
7-day IC25	>100 % Volume	
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 11/05/22

Results Verified: Initials GO Date 11/05/22

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** EDL 1 "03" **Sample #:** 8730-0032203

Day	Date 2022	Initial Variables				Meters/Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	D.O. / °C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	29-04	24.9	7.5	9.7	1769	0064 5/5	13/88	5/6	yes	≤100	≤ 20	1	KC
1	30	26.3	7.91	10.3	1825	0064 5/5	13/88	5/6	yes	≤100	≤ 20	1	KC
2	01-05	25.7	7.80	11.0	1779	0064 5/5	13/88	5/6	yes	≤100	≤ 20	1	KC
3	02	25.1	7.6	11.0	1791	6/4	13/88	5/6	yes	≤100	≤ 20	2	WL
4	03	25.0	7.7	11.2	1781	6/4	13/88	5/6	yes	≤100	≤ 20	2	KC
5	04	26.4	7.6	11.1	1880	6/4	13/8	5/6	Yes	≤100	≤ 20	3	KC
6	05	25.0	7.6	11.7	1853	6/4	13/88	5/6	yes	≤100	≤ 20	3	KC
7	06												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of brine shrimp days 0 to 6? (Yes)/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: control Sample Name: EDL 1 ~~"04" 58~~ "03" Sample #: 8730-0032203

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.04	25.9	7.9	7.8	239	6/4	13/88	5/6	CS
1	30.	26.3	8.2	7.7	245	6/4	13/88	5/6	JM
2	01.05	26.0	8.1	7.7	238	6/4	13/88	5/6	JM
3	02	26.1	8.3	7.7	238	6/4	13/88	5/6	WL
4	03	25.5	8.1	7.8	236	6/4	13/88	5/6	WL
5	04	25.6	8.2	7.8	237	6/4	13/88	5/6	WL
6	05	24.9	8.0	8.0	244	6/4	13/88	5/6	WL
7	06								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25.1	8.1	6.7	6/4	13/88	JM							
25.1	7.7	6.3	6/4	13/88	JM	0	0	0	0	0	0	JM
24.9	7.8	6.9	6/4	13/88	KK	0	0	0	0	0	0	JM
24.9	7.6	5.9	6/4	13/88	WL	0	0	0	0	0	0	KK
24.9	7.5	5.5	6/4	13/88	WL	0	0	0	0	0	0	WL
24.8	7.7	6.5	6/4	13/88	WL	0	0	0	0	0	0	KK
24.9	7.6	6.3	6/4	13/88	KK	0	0	0	0	0	0	WL
						0	0	0	0	0	0	WL

Observations:

Concentration: 1.56 %v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.04	23.8	8.0	8.0	256	6/4	13/88	5/6	CS
1	30	25.6	8.3	7.5	263	6/4	13/88	5/6	JM
2	01.05	25.8	8.0	7.5	~~237~~ JM 261	6/4	13/88	5/6	JM
3	02	25.1	8.2	7.8	260	6/4	13/88	5/6	WL
4	03	24.9	8.2	7.8	260	6/4	13/88	5/6	WL
5	04	25.0	8.2	7.9	262	6/4	13/88	5/6	WL
6	05	24.4	8.1	7.9	272	6/4	13/88	5/6	WL
7	06								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25.1	8.0	6.8	6/4	13/88	JM							
~~24.9~~ 25.2	7.7	6.6	6/4	13/88	JM	0	0	0	0	0	0	JM
24.9	7.9	7.0	6/4	13/88	KK	0	0	0	0	0	0	JM
24.9	7.6	6.1	6/4	13/88	WL	0	0	0	0	0	0	KK
24.9	7.6	5.6	6/4	13/88	WL	0	0	0	0	0	0	WL
24.8	7.6	6.0	6/4	13/88	WL	0	0	0	0	0	0	KK
24.9	7.6	6.1	6/4	13/88	KK	0	0	0	0	0	0	WL
						0	0	0	0	0	0	WL

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13 % Sample Name: EDL1 ~~"04"~~ "03" Sample #: 8730-0032203

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.04								
1	30.								
2	01.05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	SM
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	0	0	0	0	0	WL

Observations: _____

Concentration: 6.25 %

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.04								
1	30								
2	01.05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	SM
						0	0	0	0	0	0	SM
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	0	0	0	0	0	WL

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5 % Sample Name: EDL 1 ~~"04"~~ "03" Sample #: 8730-0032203

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.04	24.0	8.0	8.0	456	6/4	13/88	5/6	CS
1	30.	25.7	8.3	7.4	475	6/4	13/88	5/6	JM
2	01.05	25.6	8.0	7.6	450	6/4	13/88	5/6	JM
3	02	25.1	8.1	7.8	459	6/4	13/88	5/6	WL
4	03	24.9	8.1	7.8	461	6/4	13/88	5/6	WL
5	04	25.1	8.1	7.9	463	6/4	13/88	5/6	WL
6	05	24.5	8.1	8.0	481	6/4	13/88	5/6	WL
7	06								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25.0	8.1	6.7	6/4	13/88	JM							
25.2	7.8	6.7	6/4	13/88	JM	0	0	0	0	0	0	JM
24.9	7.9	6.8	6/4	13/88	KK	0	0	0	0	0	0	JM
24.9	7.7	5.7	6/4	13/88	WL	0	0	0	0	0	0	KK
24.8	7.6	5.4	6/4	13/88	WL	0	0	0	0	0	0	WL
24.8	7.6	5.7	6/4	13/88	WL	0	0	0	0	0	0	KK
24.9	7.6	KK 25.8	6/4	13/88	KK	0	0	0	0	0	0	WL
						0	0	0	0	0	0	WL

Observations:

Concentration: 25 %

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.04								
1	30								
2	01.05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	0	0	0	0	0	WL

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50 %v/v Sample Name: EDL 1 ~~"04"~~ SB "03" Sample #: 8730-0032203

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.04	~~25.0~~	~~7.6~~	~~9.1~~	~~1777~~	6/4	13/88 CS	5/6	CS
1	30.								
2	01.05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	0	0	0	0	0	KK
						0	10	0	0	10A	0	WL
						0	10	0	0	10A	0	WL

A - crooked tail

Observations:

Concentration: 100 %v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.04	25.0	7.6	9.1	1777	6/4	13/88	5/6	CS
1	30	25.9	8.0	8.8	1816	6/4	13/88	5/6	JM
2	01.05	25.2	7.8	10.1	1784	6/4	13/88	5/6	JM
3	02	25.0	~~7.8~~ 8.9 WL	7.8	1786	6/4	13/88	5/6	WL
4	03	25.0	7.8	8.9	1778	6/4	13/88	5/6	WL
5	04	25.8	7.7	9.1	1804	6/4	13/88	5/6	WL
6	05	24.7	7.8	~~9.5~~ 9.4 WL	1850	6/4	13/88	5/6	WL
7	06								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25.1	8.2	6.7	6/4	13/88	JM							
25.2	7.8	6.5	6/4	13/88	JM	0	0	0	0	0	0	JM
24.9	8.0	6.5	6/4	13/88	KK	0	0	0	0	0	0	JM
24.9	7.9	6.1	6/4	13/88	WL	0	0	0	0	0	0	KK
24.8	8.0	6.1	6/4	13/88	WL	0	0	0	0	0	0	WL
24.7	8.0	6.3	6/4	13/88	WL	0	10	0	0	0	0	KK
24.8	7.~~8~~ 9 KK	6.1	6/4	13/88	KK	10	10	0	0	0	0	WL
						10	10	10	0	0	0	WL

Observations:

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information

Client ALS | Sample Name EDL1 "03"

Sample # 8730-0032203 | Sample Date/Time 27/04/22 / 0900 | Person Sampling N/A

Date/Time Received 29.04.22 / 15:00 | Arrival Temp 11.7 °C

Sample Type Water | Sample Description clear, light green

100% Hardness 426

Test Information

Date/Time Started 29.04.22 / 16:00 | Test started by KC | Fathead Batch # FH0121/FH0621

Date eggs laid 23/24/25/26 04.22 | Culture mortality within 7 days of egg collection 0% | Swim bladder inflated; yes / no Yes

Age of Larvae at start of test in hours <24 | Control Hardness 94 | Water Bath Quadrant B

Average Temperature during Test: 25.4 ± 0.1 °C

Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / No (circle one)

Conc. % v/v	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	10	0.67246	0.66573	0.673	0.717
	B	10	0.67106	0.66403	0.703	
	C	10	0.68054	0.67279	0.776	
1.56	A	10	0.67692	0.66829	0.863	0.766
	B	10	0.67999	0.67275	0.724	
	C	10	0.67143	0.66432	0.711	
3.13	A	10	0.68038	0.67200	0.838	0.859
	B	10	0.68099	0.67220	0.879	
	C	10	0.67369	0.66508	0.861	
6.25	A	10	0.67789	0.66902	0.887	0.817
	B	10	0.68089	0.67285	0.804	
	C	10	0.68350	0.67590	0.760	
12.5	A	10	0.67746	0.67005	0.741	0.724
	B	10	0.687911	0.67237	0.674	
	C	10	0.67889	0.67132	0.757	
25	A	10	0.68119	0.67436	0.683	0.700
	B	10	0.68146	0.67471	0.675	
	C	10	0.67708	0.66965	0.743	
50	A	10	0.67677	0.66861	0.816	0.760
	B	9	0.68311	0.67604	0.707	
	C	10	0.66709	0.65951	0.758	
100	A	9	0.67727	0.67099	0.628	0.697
	B	9	0.67429	0.66776	0.653	
	C	9	0.66679	0.65868	0.811	
Initials		C	D	020522 D	D	D

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8730-0032203 **Sample Name** EDL1

Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug) 717

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	10	0.67246	0.66573	0.673	0.673	0.717	0.717	0.052975	0.052975	7.384968
	10	10	0.67106	0.66403	0.703	0.703					
	10	10	0.68054	0.67278	0.776	0.776					
1.56	10	10	0.67692	0.66829	0.863	0.863	0.766	0.766	0.084256	0.084256	10.99942
	10	10	0.67999	0.67275	0.724	0.724					
	10	10	0.67143	0.66432	0.711	0.711					
3.13	10	10	0.68038	0.67200	0.838	0.838	0.859	0.859	0.020551	0.020551	2.391476
	10	10	0.68099	0.67220	0.879	0.879					
	10	10	0.67369	0.66508	0.861	0.861					
6.25	10	10	0.67789	0.66902	0.887	0.887	0.817	0.817	0.06449	0.06449	7.893551
	10	10	0.68089	0.67285	0.804	0.804					
	10	10	0.68350	0.67590	0.760	0.760					
12.5	10	10	0.67746	0.67005	0.741	0.741	0.724	0.724	0.044034	0.044034	6.082055
	10	10	0.67911	0.67237	0.674	0.674					
	10	10	0.67889	0.67132	0.757	0.757					
25	10	10	0.68119	0.67436	0.683	0.683	0.700	0.700	0.037166	0.037166	5.306943
	10	10	0.68146	0.67471	0.675	0.675					
	10	10	0.67708	0.66965	0.743	0.743					
50	10	10	0.67677	0.66861	0.816	0.816	0.787	0.760	0.029012	0.054537	3.688659
	10	9	0.68311	0.67604	0.786	0.707					
	10	10	0.66709	0.65951	0.758	0.758					
100	10	9	0.67727	0.67099	0.698	0.628	0.775	0.697	0.110254	0.099229	14.22974
	10	9	0.67429	0.66776	0.726	0.653					
	10	9	0.66679	0.65868	0.901	0.811					

SD: Standard Deviation
CV: Coefficient of Variation

NAUTILUS ENVIRONMENTAL

11/05/22

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	EDL1
Date/Time Sampled	April 27, 2022; 09:00	**Date/Time Received:**	April 29, 2022; 15:00
Date Test Started:	April 29, 2022	**Date Test Finished:**	May 5, 2022
Description:	Clear, light green	**Days Sample Used:**	Days 0 to 5
Sample #:	8730-0032203	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	11.7°C
Collected By:	N/A		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032203 **Sources:** EDL1

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 0%

Mean Number of Surviving Young Produced Within First 3 Broods: 22.5

Mean Number of Surviving Young Produced Within 7 Days of Testing: 41.4

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 0 to 5

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032203 **Sources:** EDL1

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD22-45

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (21.2 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (70% of controls)

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032203 **Sources:** EDL1

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	20	0	0	0	0	0	0
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	27	19	21	24	21	23	20	20
	2	23	24	20	24	23	21	24	26
	3	24	22	30	8	12	2	16	4
	4	26	12	23	12	22	9	25	14
	5	24	10	22	16	13	9	23	22
	6	12	15	5	20	23	21	15	18
	7	22	24	17	11	9	18	20	13
	8	24	17	12	20	22	25	19	0
	9	16	25	17	20	19	15	17	6
	10	14	28	7	28	14	17	12	9
Total Number of Live Neonates in First 3 Broods or Less		212	196	174	183	178	160	191	132
% Effect (+ or -)		0.0	-7.5	-17.9	-13.7	-16.0	-24.5	-9.9	-37.7
Mean Number of Live Neonates in First 3 Broods or Less		21.2	19.6	17.4	18.3	17.8	16.0	19.1	13.2
SD		5.2	6.0	7.6	6.4	5.3	7.3	4.2	8.4

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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Ceriodaphnia dubia **Survival and Reproduction Study -** Continued

Sample #: 8730-0032203 **Sources:** EDL1

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	50.69% Volume (0.26% Volume; N/A)	No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 21-Apr-2022 **Reference Batch #:** Zn2102

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 0.13 mg/L (0.10 mg/L; 0.18 mg/L)

Historic Geometric Mean LC50: 0.08 mg/L (0.02 mg/L; 0.26 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS – Thunder Bay Sample Name: EDL 1 Sample #: 8730-00322 03

Conditions for Test Validity

Control Mortality is< 20%: (Acceptable / Not Acceptable): 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable / Not Acceptable): 7 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable / Not Acceptable): 21.2 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 40 min Days: 0 to 5

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	> 100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
REPRODUCTION		
3-brood IC25	50.69 % Volume	No nonlinear regression models would fit
95% Confidence Interval[2]	0.26 ; N/A % Volume	ICPIN – linear interpolation

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials [illegible] Date 11/05/22

Results Verified: Initials [illegible] Date 11/05/22

Ceriodaphnia dubia Initial Sample Measureme Before Preparation and Use in Toxicity Te

Concentration: 100% **Sample Name:** EDL 1 "03" **Sample #:** 8730-0073 2203

Day	Date 2022	Initial Measurements				Meters / Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	°C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	29-04	24.9	7.5	9.7	1769	6/4	13/88	5/6	yes	≤100	≤ 20	1	ao
1	30	26.3	7.9	10.5	1885	6/4	13/88	5/6	yes	≤100	≤ 20	1	ao
2	01-05	25.8	7.6	11.0	1779	6/4	13/88	5/6	yes	≤100	≤ 20	1	ao
3	02	25.1	7.6	11.0	1791	6/4	13/88	5/6	yes	≤100	≤ 20	2	HC
4	03	25.0	7.7	11.2	1781	6/4	13/88	5/6	yes	≤100	≤ 20	2	
5	04	26.4	7.6	11.1	1880	6/4	13/88	5/6	yes	≤100	≤ 20	3	G
6	05									≤100	≤ 20	3	
7	06									≤100	≤ 20	3	
8	07									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted?	Yes/No	If yes, state mesh size:
Was sample pH or hardness adjusted?	Yes/No	If yes, describe further:
Were alternate concentrations or dilution series used?	Yes/No	If yes, describe further:
Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily?	Yes/No	If no, describe further:
Were there any other method variations, deviations, or exclusions from method?	Yes/No	If yes, describe further:
Was there anything unusual about the test, any problems encountered, or any remedial measures taken?	Yes/No	If yes, describe further:

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentrati Control Sample Name: EDL 1 "03" Sample #: 8730-003220 3

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	29-04	26.0	8.0	7.8	398	6/4	13/88	5/6	CS	25.0	7.6	6.6	6/4	13/88	QD
1	30	25.8	7.5	7.8	391	6/4	13/88	5/6	QD	25.0	7.9	6.7	6/4	13/88	QD
2	01-05	26.3	8.2	7.7	389	6/4	13/88	5/6	JM	23.9	7.9	7.1	6/4	13/88	QD
3	02	26.3	8.4	7.7	395	6/4	13/88	5/6	QD	24.2	8.1	7.3	6/4	13/88	WL
4	03	25.5	8.2	7.8	393	6/4	13/88	5/6	KK	24.2	8.0	7.5	6/4	13/88	C
5	04	25.3	8.2	7.8	391	6/4	13/88	5/6	C	24.2	8.2	7.7	6/4	13/88	QD
6	05														
7	06														

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	QD	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	QD	—
3	02	0	0	0	0	0	0	0	0	0	0	0	—	0	—	QD	—
4	03	6	4	1	5	4	5	4	4	2	6	41	—	0	—	KK	KK
5	04	8	7	9	8	8	7	7	7	5	8	74	—	0	—	C	C
6	05	13	12	14	13	12	0	11	13	9	0	97	—	0	—	QD	QD
7	06																
8	07																
Total Neonates		27	23	24	26	24	12	22	24	16	14	212	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.137 %v/v Sample Name: EDC 1 "03" Sample #: 8730-003220 3

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29-04	25.4	8.1	7.3	391	6/4	13/88	5/6	CS
1	30	26.3	8.5	7.7	438	6/4	13/88	5/6	CO
2	01-05	26.2	8.2	7.4	388	6/4	13/88	5/6	JM
3	02	25.6	8.3	7.7	354	6/4	13/88	5/6	CO
4	03	25.4	8.3	7.6	392	6/4	13/88	5/6	KK
5	04	25.0	8.2	7.5	397	6/4	13/88	5/6	EC
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.9	7.9	6.7	6/4	13/88	CO
25.1	7.9	6.4	6/4	13/88	CO
24.0	8.0	6.9	6/4	13/88	CO
24.0	8.1	7.2	6/4	13/88	WL
24.1	8.1	7.4	6/4	13/88	EC
24.4	8.2	7.6	6/4	13/88	CO

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CO	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CO	—
3	02	0	0	0	0	0	0	0	0	3+1	3	7 ~~8~~ KK	—	0	—	CO	CO
4	03	3	5	4	5	5	5	4	4	KK 0	0	35	—	0	—	KK	KK
5	04	6	6	7	7	5	7	9	5	7	9	~~68~~ 72	—	0	—	EC	EC
6	05	10	13	11	0	0 Dead	3 Dead	11	8	14	16	86	56	20	—	CO	CO
7	06					↓	↓										
8	07																
Total Neonates		19	24	22	12	10	~~13~~ 10	24	17	25	28	196	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.41 rN Sample Name: EDC 1 "03" Sample #: 8730-003220 3

Day	Date 2022	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	29-04								
1	30								
2	01-05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	

Day	Date 2022	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	0	—	0	LO	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	0	—	0	LO	—
3	02	0	0	0	2	0	0	0	0	0	0	2	0	—	0	20	—
4	03	4	2	4	0	2	3	4	4	0	1	24	0	—	0	14C	KK
5	04	5	8	13	9	10	2	9	8	10	6	80	—	0	—	12	0
6	05	12	10	13	12	10	0	4	0	7	0	68	—	0	—	20	20
7	06																
8	07																
Total Neonates		21	20	30	23	22	5	17	12	17	7	174	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 1.23% v/v Sample Name: EDC 1 "03" Sample #: 8730-003220 3

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29-04								
1	30								
2	01-05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ad	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ad	—
3	02	0	0	0	0	3	0	0	3	0	3	9	—	0	—	ad	ad
4	03	4	4	2	5	6	3	4	0	3	0	31	—	0	—	KK	KK
5	04	8	10	6	7	7	9	7	8	9	12	83	—	0	—	E	C
6	05	12	10	0	0	*	8	0	9	8	13	60	—	0	—	ad	ad
7	06																
8	07																
Total Neonates		24	24	8	12	16	20	11	20	20	27	183					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 37% v/v Sample Name: EDC 1 "03" Sample #: 8730-003220 3

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29-04	25.4	8.2	7.5	446	6/4	13/88	5/6	CS
1	30	26.3	8.5	7.7	454	6/4	13/88	5/6	CO
2	01-05	26.0	8.2	7.5	438	6/4	13/88	5/6	JM
3	02	25.6	8.3	7.10	447	6/4	13/88	5/6	CO
4	03	25.3	8.3	7.7	443	6/4	13/88	5/6	KK
5	04	24.8	8.3	7.7	456	6/4	13/88	5/6	C
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
25.0	8.0	6.6	6/4	13/88	CO
25.0	7.8	6.4	6/4	13/88	CO
24.0	7.9	7.0	6/4	13/88	CO
24.0	8.1	7.2	6/4	13/88	WL
24.3	8.1	7.2	6/4	13/88	C
24.3	8.2	7.6	6/4	13/88	CO

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CO	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CO	—
3	02	0	0	0	0	0	0	0	2	0	0	2	—	0	—	CO	CO
4	03	4	7	5	3	3	5	1	0	6	3	37	—	0	—	KK	KK
5	04	11	6	7	6	8	8	4	10	7	5	72	—	0	—	C	C
6	05	6	10	0	13	2	10	4	10	6	6	67	—	0	—	CO	CO
7	06																
8	07																
Total Neonates		21	23	12	22	13	23	9	22	19	14	177	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 11.11.1.RN Sample Name: EDC 1 "03" Sample #: 8730-003220 3

Day	Date 2022	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	29-04								
1	30								
2	01-05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	

Day	Date 2022	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	∞	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	∞	—
3	02	2	0	0	0	0	0	0	0	0	0	2	—	0	—	∞	∞
4	03	0	3	0	3	0	3	4	6	3	4	26	—	0	—	KK	KK
5	04	7	8	0	6	3	8	6	9	5	6	58	—	0	—	C	6
6	05	14	10	2	0	6	10	8	10	7	7	74	—	0	—	∞	∞
7	06																
8	07																
Total Neonates		23	21	2	9	9	21	18	25	15	17	160	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 33.3% v/v Sample Name: EDL 1 "03" Sample #: 8730-003220 3

Day	Date 2022	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	29-04								
1	30								
2	01-05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	

Day	Date 2022	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
3	02	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
4	03	4	5	3	4	3	0	5	3	3	2	32	—	0	—	KK	KK
5	04	7	7	7	9	6	5	5	7	6	5	64	—	0	—	C	C
6	05	9	12	6	12	14	10	10	9	8	5	95	—	0	—	ao	ao
7	06																
8	07																
Total Neonates		20	24	16	25	23	15	20	19	17	12	191	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 100% V/V Sample Name: EDC 1 "03" Sample #: 8730-0032203

Day	Date 2022	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	29-04	25.5	7.8	8.2	1794	6/4	13/88	5/6	CS
1	30	25.3	7.9	8.9	1781	6/4	13/88	5/6	"
2	01-05	25.3	7.8	8.9	1776	6/4	13/88	5/6	JM
3	02	25.4	7.8	9.1	1770	6/4	13/88	5/6	"
4	03	25.2	7.9	8.9	1773	6/4	13/88	5/6	KC
5	04	25.2	7.8	9.0	1781	6/4	13/88	5/6	C
6	05								
7	06								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	
25.0	7.80	6.3	6/4	13/88	"
25.0	7.8	6.7	6/4	13/88	"
25.3	7.9	7.4	6/4	13/88	"
24.1	8.2	7.4	6/4	13/88	WL
24.2	8.2	7.5	6/4	13/88	C
24.1	7.9	7.6	6/4	13/88	"

Day	Date 2022	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	"	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	"	—
3	02	0	0	0	0	3	0	0	0	0	0	3	—	0	—	"	"
4	03	3	4	0	5	0	4	3	0	2	1	22	—	0	—	KC	KC
5	04	7	10	1	5	7	6	4	0	4	4	48	—	0	—	C	C
6	05	10	12	3	4	12	8	6	0	0	4	59	—	0	—	"	"
7	06																
8	07																
Total Neonates		20	26	4	14	22	18	13	0	6	9	132	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia Neonate Origin

Sample Information

Client ALS

Sample # 8730-0032203

Date/Time Received 29.04.22/15:00

Sample Type Water

100% Hardness ~~232 426 212~~ 426

Sample Name EDL/"03"

Date/Time Collected 27/04/22 / 0900

Person Sampling N/A

Arrival Temp (°C) 11.7

Sample Description cloudy, light green

Test Information

Date Test Started 29.04.22

Dilution Water Batch Number CD22.45

Test Started By GO

Control Hardness 118

Template Used for Randomization 3

Individual Culture Health Data

Date Culture Started 20.04.22
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 22.3
Culture I.D. (*e.g.*, Wed Row 4) Wed Row 7
Average # neos in previous 7 days (must be ≥15) 36.1
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 20.04.22
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 22.7
Culture I.D. (*e.g.*, Wed Row 4) Wed Row 8
Average # neos in previous 7 days (must be ≥15) 46.6
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 0 (add up % mortality for all cultures used / # cultures used)

Mean Number of Young Produced within first 3 broods 22.5 (avg # 1st 3 broods for all cultures used / # cultures used)

Mean Number of Young Produced in previous 7 days 41.4 (avg # neos in prev 7 days for all cultures used / # cultures used)

Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / (No) (circle one)

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
W.7.2	(Y) / N	(Y) / N	12	1	GO
3	(Y) / N	(Y) / N	9	2	GO
4	(Y) / N	(Y) / N	8	3	GO
5	(Y) / N	(Y) / N	12	4	GO
6	(Y) / N	(Y) / N	8	5	GO
W.8.1	(Y) / N	(Y) / N	9	6	GO
2	(Y) / N	(Y) / N	10	7	GO
3	(Y) / N	(Y) / N	11	8	GO
4	(Y) / N	(Y) / N	10	9	GO
5	(Y) / N	(Y) / N	12	10	GO
	Y / N	Y / N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032203	**Sample Name:**	EDL1
Sample Method:	Grab	**Collected by:**	Not Available
Date/Time Collected:	April 27, 2022; 09:00	**Arrival Temp.:**	11.7°C
Date/Time Received:	April 29, 2022; 15:00	**Sample Description:**	Clear, light green
Sample Point Description:	Other	**Sample Type:**	Effluent
Transportation:	Road		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species (Strain #): *Raphidocelis subcapitata* (CPCC # 37)

Source: Nautilus Plant Culture Unit (from CPCC)

Culture Temp.: 24 ± 2 °C

Test Culture Number: G4(l)a

Culture Age at Test Start: 4 days old

Cell Density in the Microplate Wells at the Start of the Test: 10,454.55 cells/ml

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*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032203 **Sample Name:** EDL1

Test Conditions

Date/Time Test Start:	April 30, 2022; 12:30	**T=0 Control pH:**	6.3
Date/Time Test End:	May 3, 2022; 10:20 - 13:30	**T=72 Control pH:**	6.5
Sample pH Before Dilution:	7.0	**pH Adjustment:**	None
Test Duration:	72 hours		

Mean Test Temperature (±Standard Deviation): 25.3 (±0.1)°C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Distilled Water

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032203 **Sample Name:** EDL1

Test Conditions - continued

Enumeration Technique: Neubauer Haemacytometer

Test Vessel: 96-Well U-bottomed Polystyrene Microplate

Concentration of Test Solutions: 90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control

 # Replicates/Concentration: 4 reps started; 3 reps counted of test solutions
10 control reps started, 2 used for pH measurement

Design if Specialized Procedure: Not applicable

Method Deviations or Unusual Occurrences: None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times: Acceptable (21.2 times)

pH in controls did not vary by more than 1.5 units: Acceptable (0.2 units)

C V for cell yields is < 20% within the control wells: Acceptable (9.6%)

No inhibitory trend detected across the control wells: Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
239,545	249,545	197,045	194,545	224,545	214,545	209,545	244,545	221,733	9.6

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 CV = Coefficient of Variation = (100 x standard deviation / mean)

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032203 **Sample Name:** EDL1

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	184,545	427,045	292,045	272,045	267,045
2	182,045	462,045	252,045	267,045	209,545
3	189,545	404,545	287,045	344,545	249,545
Mean Cell Yield	185,379	431,212	277,045	294,545	242,045
Coefficient Variation[2]	2	7	8	15	12
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	227,045	257,045			
2	227,045	232,045			
3	229,545	214,545			
Mean Cell Yield	227,879	234,545			
Coefficient Variation[2]	1	9			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	**Result[1]**	**Method of Calculation**
IC25 (95% CI)[2] for Cell Yield	79.31% Volume[2,3] (74.42; 87.13% Volume)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	no trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

3 - Concentration 0.125% v/v - 3.367% v/v replaced with control data for hormetic response.

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032203 **Sample Name:** EDL1

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software, 2001-2007 Comprehensive Environmental Toxicity, Information System - CETIS v1.8.1.2.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (30.303% v/v concentration; rep. 2). No reason to remove it. Statistics include all data.

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2206 **Date Test Initiated:** 02-May-2022

Method of Analysis: Linear Interpolation (ICPIN) **Algae Lot #:** G4(l)b

72-hour IC25 (95% Confidence Limits): 40.18 mg/L (34.42 mg/L; 44.43 mg/L)

Historic Geometric Mean IC25: 49.59 mg/L (22.46 mg/L; 109.48 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS - Thunder Bay Sample Name EDL1 L270 1854-1 Sample # 8730-0032203

Conditions for Test Validity

Cell increase for control is >16	Acceptable/Not acceptable	21.2 (times)
CV among controls ≤ 20	Acceptable/Not acceptable	9.6
Result of Mann-Kendall test for trend	Acceptable/Not acceptable	no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 115 000 (cells/mL)

Used: Yes/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10454.55 (cells/mL)

Analysis Completed: Initials: EF Date: 11/05/22

Results Verified: Initials: AO Date: 17/05/22

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location:	ALS – Thunder Bay	L 2701854-1	
Sample #:	8730-0032203	Sample Name:	EDL 1
Collection Method:	Grab	Collected By:	N/A
Date/Time Collected:	27.04.22 9:00	Arrival Temp.: (meter/probe)	11.7 °C (44)
Date/Time Received:	29.04.22 15:00	Sample Description:	clear, light green
Collection Point Description:	other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:	
Transportation:	Air / Road	Storage:	4±2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	∞
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # G4(2) ~	∞
Culture Age at Start of Test	4 days old (must be 3 to 7 days old)	∞
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	∞
	Axenic culture? Yes/No (Circle one)	∞

Notes:

Test Conditions:

Date / Time Test Start: 30.04.22 1235 Started By: ∞		Date / Time Test End: 03.05.22 10:20 – 13:30 Finished By: ∞	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 7.0	pH adjustment: Y(N)	pH of well D6 at T=0 h 8.3	pH of well D7 at T=72 h 6.5
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # —		Metal mining NUT Lot # NUT 2202
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation)	25.3 ± 0.1 °C		

	Day 1		Day 2	
¼ plate rotation (Initial)	AM ∞	PM ∞	AM ∞	PM ∞
Lights ON (Initial)	AM ∞	PM ∞	AM ∞	PM ∞

* For reference test, record pH of top concentration before use for series dilutions.

Notes:

72-Hour Qualitative Observations:

Condensation: Yes		
Growth: Yes		
Were there any other method variations or deviations from methods? Yes/No	No	If yes, describe further:
Anything unusual about the test? Yes/No	No	
Any problems encountered? Yes/No	No	
Any remedial measures taken? Yes/No	No	

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 3730-003 2203	Sample Name: EPL 1 L2701854-1

Reference Data:

Reference Chemical Batch #	Phenol P2206	Date test started	02/05/22
Method of Analysis	ICPIN – linear interpolation	Algae Lot #	G4(2)6
72-hour IC25 (95% C.I.)[3] mg/L	40.18 (34.42; 44.43)	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	49.59 22.46; 109.48

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	79.31* (74.42; 87.13) %.v/v	NO hormesis models would fit
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers		ICPIN – linear interpolation
Test for Outliers	No Outliers Present If outliers present, indicate Concentration/Rep: 30.303; rep 2	Grubbs' Test for Residual Outlier Initial C
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed. No statistically significant growth stimulation. Yes, statistically significant growth stimulation at these concentrations: 30.303; 10.101; 3.367	Williams' or Dunnett's Multiple Comparison Test Initial C

1)Results relate only to the sample tested.
2) Tidepool Scientific Software © 2001-2007. Comprehensive Environmental Toxicity Information System – CETIS v. 1.9.6.7
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/No

Any outliers and justification for their removal? Yes/No

* concentrations 0.125 – 3.367 / replaced with control data for hormetic response

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EQ1 Sample Number: 8730-0032203 Date Test Start: 30.04.22

Theoretical Test Concentration: 100.00 %v/v Actual Test Concentration: 90.91 %v/v

Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	18	15	15		185 379 (± 3819)
2	23	23	27		Coefficient of Variation of Cell Yield
3	18	21	17		2
4	19	18	21		Average % Inhibition (-ve number = enhancement)
5	—	—	—		16.395
Initials	[illegible]	[illegible]	[illegible]		[illegible]

Theoretical Test Concentration: 33.33 %v/v Actual Test Concentration: 30.303 %v/v

Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	481	41	38		431212 (± 28976)
2	42	52	43		Coefficient of Variation of Cell Yield
3	41	50	45		7
4	51	43	40		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-94.474
Initials	[illegible]	[illegible]	[illegible]		

Theoretical Test Concentration: 11.11 %v/v Actual Test Concentration: 10.101 %v/v

Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	35	33	26		277 045 (± 21794)
2	29	21	36		Coefficient of Variation of Cell Yield
3	24	28	28		8
4	33	23	29		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-24.946
Initials	[illegible]	[illegible]	[illegible]		[illegible]

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL1 Sample Number: 8730-0032203 Date Test Start: 30.04.22

Theoretical Test Concentration: 3.704 %v/v					Actual Test Concentration: 3.367 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation)
1	28	25	33		294545 (± 43373)
2	27	26	34		Coefficient of Variation of Cell Yield
3	31	26	40		15
4	27	34	35		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-32.838
Initials					

Theoretical Test Concentration: 1.235 %v/v					Actual Test Concentration: 1.122 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation)
1	29	23	24		242045 (± 29475)
2	23	20	25		Coefficient of Variation of Cell Yield
3	28	22	27		12
4	31	23	28		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-9.161
Initials					

Theoretical Test Concentration: 0.412 %v/v					Actual Test Concentration: 0.374 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation)
1	24	25	24		227879 (± 1443)
2	23	27	20		Coefficient of Variation of Cell Yield
3	27	21	24		1
4	21	22	28		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-2.772
Initials					

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EQ1 Sample Number: 8730-0032203 Date Test Start: 30.04.22

Theoretical Test Concentration: 0.137 %v/v					Actual Test Concentration: 0.125 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	27	25	21		234 545 (± 21360)
2	28	25	23		Coefficient of Variation of Cell Yield
3	29	18	22		9
4	23	29	24		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-5.778
Initials	E	E	E		E

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	EDL1	Sample #	8730-0032203	Date test start	30.04.22

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	115000	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	20	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2300	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	10454.5455	=	21.209239

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	21	38	20	20	28	24	24	24
	25.0	23.0	20.0	21.0	23.0	20.0	22.0	24.0
	30	20.0	23.0	21.0	23.0	26.0	21.0	28.0
	24.0	23.0	20.0	20.0	20.0	20.0	21.0	26.0
total cells	100	104	83	82	94	90	88	102
cells/ul	250	260	207.5	205	235	225	220	255
cells/ml	250000	260000	207500	205000	235000	225000	220000	255000
Cell yield = measured concentration - initial algal cell concentration								
	239545	249545	197045	194545	224545	214545	209545	244545

Mean cell yield for the control = Rc | Standard deviation | coefficient of variation

Rc 221733 | SD 21273.6213 | CV 9.5942533 **Must be ≤20**

% inhibition summary

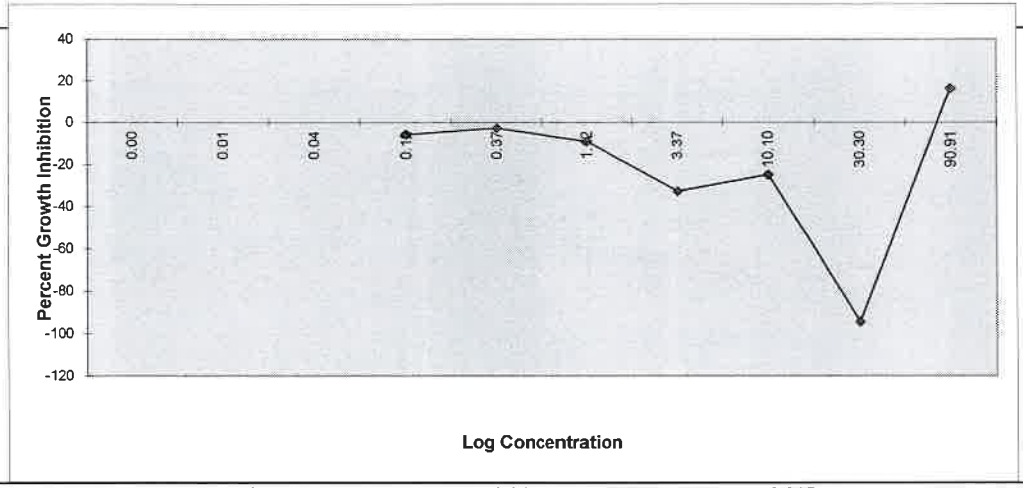
Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	-5.778
0.374	-2.772
1.122	-9.161
3.367	-32.838
10.101	-24.946
30.303	-94.474
90.910	16.395

enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	18	15	15	41	41	38	35	33	26	28	25	33	29	23	24
	23.0	23.0	27.0	42.0	52.0	43.0	29.0	21.0	36.0	27.0	26.0	34.0	23.0	20.0	25.0
	18.0	21.0	17.0	41.0	53.0	45.0	24.0	28.0	28.0	31.0	26.0	40.0	28.0	22.0	27.0
	19.0	18.0	21.0	51.0	43.0	40.0	33.0	23.0	29.0	27.0	34.0	35.0	31.0	23.0	28.0
total cells	78	77	80	175	189	166	121	105	119	113	111	142	111	88	104
cells/ul	195	192.5	200	437.5	472.5	415	302.5	262.5	297.5	282.5	277.5	355	277.5	220	260
cells/ml	195000	192500	200000	437500	472500	415000	302500	262500	297500	282500	277500	355000	277500	220000	260000
Cell yield = measured concentration - initial algal cell concentration															
	184545	182045	189545	427045	462045	404545	292045	252045	287045	272045	267045	344545	267045	209545	249545
Mean Yield	185379			431212			277045			294545			242045		
STD Yield	3819			28976			21794			43373			29475		
CV Yield	2			7			8			15			12		

Average % inhibition											
	for 90.910%:	16.395	for 30.303%	-94.474	for 10.101%	-24.946	for 3.367%	-32.838	for 1.122%	-9.161	

Average % stimulation											
	for 90.910%:	-16.395	for 30.303%	94.474	for 10.101%	24.946	for 3.367%	32.838	for 1.122%	9.161	



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
24	25	24	27	25	21									
23.0	27.0	20.0	28.0	25.0	23.0									
27.0	21.0	24.0	29.0	18.0	22.0									
21.0	22.0	28.0	23.0	29.0	24.0									
95	95	96	107	97	90	0	0	0	0	0	0	0	0	0
237.5	237.5	240	267.5	242.5	225	0	0	0	0	0	0	0	0	0
237500	237500	240000	267500	242500	225000	0	0	0	0	0	0	0	0	0
227045	227045	229545	257045	232045	214545	-10455	-10455	-10455	-10455	-10455	-10455	-10455	-10455	-10455
227879			234545			-10455			-10455			-10455		
1443			21360			0			0			0		
1			9			0			0			0		
for 0.374%	-2.772		for 0.125%	-5.778		for 0.042%	104.715		for 0.014%	104.715		for 0.005%	104.715	
for 0.374%	2.772		for 0.125%	5.778		for 0.042%	-104.715		for 0.014%	-104.715		for 0.005%	-104.715	

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
EDL1	8730-0032203	April 27, 2022	April 29, 2022	April 29, 2022	79.25% Volume (60.5% Volume; N/A)	>97% Volume[2] (Not applicable)	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	22-Apr-2022	**Reference Chemical:**	KCl
Frond Increase IC25:	2.27 g/L	**95% Confidence Limits:**	1.92; 2.62 g/L
Historic Geometric Mean IC25:	2.24 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.5; 3.33 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	Na_2MoO_4	0.0726
	K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$2H_2O$	0.00003
	KCl	10.1	$MgSO_4\ 7H_2O$	147	$ZnCl_2$	0.00001
					$CoCl_2\ 6H_2O$	0.00001
					$CuCl_2\ 2H_2O$	

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

Date: May 19, 2022

C. D'Andrea

Carol D'Andrea
Laboratory Supervisor

Nautilus Environmental Company Inc.
***Lemna minor* 7-Day Growth Inhibition Test Bench Sheet**

Test Material

Client Name/Location: ALS – Thunder Bay L2701854-1	
Sample #: 8730-00322 03	Sample Name: EDL 1
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 27.04.22 9:00	Arrival Temperature (meter/probe): 11.7 °C (44)
Date/Time Received: 29.04.22 15:00	Sample Description: clear, light green
Collection Point Description: open	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: Air / Road	Storage: none

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	
Culture Age at Start of Test:	9 days old, acclimated 24.0 hours in fresh test solution (mAPHA)	
Culture Medium:	Modified Hoagland's E+ medium, Lot # mh2201	
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/No	
	Axenic culture? Yes/No	
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 27 in HT 1, 31 in HT 2, 201 in HT 3 at finish	

Test Conditions and Procedures

Date / Time Test Start: 29.04.22 17:00 Started By:	Date / Time Test End: 06.05.22 9:15 Finished By:
Test Type: Static (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / No (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: Modified APHA (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 7.8 (12/89)	pH Adjustment: none
Test Volume and Depth: 180 mL / 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / No	If yes, describe further:
Anything unusual about the test? Yes / No	
Any problems encountered? Yes / No	
Any remedial measures taken? Yes / No	Randomization Template: A

Sample #: 8730-0032203	Sample Name: EDL 1

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	24.4	24.9	25.1	25.2	25.1	25.3	25.1	25.2
Low	24.2	24.8	25.2	25.2	25.1	25.3	25.1	25.2
Medium	24.2	24.9	25.2	25.2	25.2	25.3	25.1	25.2
High	24.1	24.9	25.2	25.2	25.2	25.3	25.1	25.3
Initials	[illegible]	GO	GO	CS	CS	[illegible]	[illegible]	[illegible]
meter/probe	44	44	44	44	44	44	44	44
Mean Test Temperature (average of 24h high / low temperatures):	25.3±0.1 °C							

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day	Control	Low	Medium	High	Initials	pH meter/probe
Day 0	8.3	8.3	8.2	~~8.2~~ 8.0	[illegible]	~~12/84~~ 13/88
Day 7	8.3	8.6	8.6	9.0	[illegible]	13/88

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)	—							

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 02/05/22 (3)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 12					Initials: [illegible]
5280	5490	5320	4430	5070	Mean Light Measurement: 5112
±15% Variation of Mean: 4345 – 5879					(Acceptable) / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) / No
If yes, describe further: Algae in upper concentrations

Reference Data

Reference Date	(Frond Increase) or Dry Weights (circle one)			
	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
21/04/22	2.27	1.92; 2.62	2.24	1.50; 3.33

Sample #: 8130-0032203	Sample Name: EDL 1

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 50	B 50	C 51	D 50	50.3

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy						Initials	
						C	
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV : 44.3 (0.5) 1.1							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-5.08	-6.78	-3.39	-6.78	2.26	6.21	-29.95
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-12.30	-4.97	-1.80	-10.93	-0.68	9.07	0.12

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: (Yes) / Not applicable (no stimulation) (Circle one)

Test Endpoints and Calculations: Fronds / Weights

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	79.25 (60.5 ; N/A)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN – linear interpolation
Dry Weights		
IC25 (95% C.I.)[3]	> 97% (N/A)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN – linear interpolation

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019. Comprehensive Environmental Toxicity Information System CETISv 1.9.6.7
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / (No)

Any outliers and justification for their removal? Yes / (No)

Lemna minor D Observations

Client:	ALS – Thunder Bay	Sample number:	8730-00322205	Date Started:	29.04.22
Site:	GDL1			Date Ended:	06.05.22

Concentration: Control			Observations By: C		Concentration: 0.097% v/v			Observations By: C	
Observations	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Observations**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
Number of	50	50	51	50	**Number of**	47	52	49	44
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	X	X	X	X	**Abnormally sized fronds**	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations					**Other Observations**				

Control: Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO; Weights: YES / NO — N/A

0.097% v/v: Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO (NO circled); Weights: YES / NO (NO circled)

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor D Observations

Client:	AES – Thunder Bay			**Sample number:**	8730-003220		**Date Started:**	29.04.22	
Site:	EDL1						**Date Ended:**	06.05.22	
Concentration:	0.29% v/v			**Observations By:**	C		**Concentration:**	0.97% v/v	**Observations By:** C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	48	46	46	49	**Number of**	48	47	48	52
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	✓	✓	✓	✓
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	**Abnormally sized fronds**	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations					**Other Observations**				
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor D: Observations

Client:	AFS – Thunder Bay	**Sample number:**	8730-0032203
Site:	EDL1	**Date Started:**	29.04.22
		Date Ended:	06.05.22
Concentration:	3.1 % v/v	**Observations By:**	C
Concentration:	9.7 % v/v	**Observations By:**	C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	46	46	48	49	Number of	43	58	55	49
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations					Other Observations				
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: (YES) / NO Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor D. Observations

Client: AFS – Thunder Bay	**Sample number:** 8730-003220	**Date Started:** 29.04.22	
Site: ODL1		**Date Ended:** 06.05.22	
Concentration: 31.1. √√	**Observations By:** C	**Concentration:** 97.1. √√	**Observations By:** C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	54	55	51	52	**Number of**	36	35	34	43
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	✓	✓	✓	✓
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	**Abnormally sized fronds**	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations	algae	→	→	→	**Other Observations**	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Weights

Client	Site	Sample number
ALS - Thunder Bay	EDL1	8730 003 22 03

In Oven Date/Time/ °C:	Out Oven Date/Time/°C:
06/05/22 10:20 64°	07/05/22 10:20 68°

Conc. 1	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	44	44.3 (0.5)	0.68041	0.67672	3.69	4.03 (0.2)
	B	44		0.67718	0.67311	4.07	
	C	45		0.67558	0.67139	4.19	
	D	44		0.67689	0.67274	4.15	
0.097	A	41	42.0 (3.4)	0.67334	0.66985	3.49	3.53 (0.4)
	B	46		0.68371	0.67989	3.82	
	C	43		0.67748	0.67371	3.77	
	D	38		0.66599	0.66295	3.04	
0.29	A	42	41.3 (1.5)	0.67895	0.67497	3.98	3.83 (0.2)
	B	40		0.68167	0.67799	3.68	
	C	40		0.67462	0.67093	3.69	
	D	43		0.66812	0.66417	3.95	
0.97	A	42	42.8 (2.2)	0.68244	0.67873	3.71	3.95 (0.3)
	B	41		0.67695	0.67310	3.83	
	C	42		0.68119	0.66869	3.90	
	D	46		0.67306	0.67229	4.37	
3.1	A	40	41.3 (1.5)	0.68007	0.67676	3.31	3.59 (0.3)
	B	40		0.67597	0.67252	3.45	
	C	42		0.67527	0.67157	3.70	
	D	43		0.67224	0.66836	3.88	
9.7	A	37	45.3 (6.7)	0.68357	0.68005	3.52	4.00 (0.5)
	B	52		0.67486	0.67030	4.56	
	C	49		0.68188	0.67770	4.18	
	D	43		0.67197	0.66824	3.73	
31	A	48	47.0 (1.8)	0.68242	0.67785	~~4.57~~ ~~6.67~~	4.39 (0.2)
	B	49		0.67825	0.67367	4.58	
	C	45		0.67705	0.67293	4.12	
	D	46		0.67875	0.67446	4.29	
97	A	30	31.0 (4.1)	0.67864	0.67478	3.86	4.03 (0.2)
	B	29		0.68025	0.67603	4.22	
	C	28		0.67805	0.67421	3.84	
	D	37		0.67593	0.67173	4.20	
Initials		NF	NF	TP	AO	NF	NF

Notes:

Sample name: EDL Date started 29/[illegible]/22 sample # 8730-0032203

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

50.3 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	50	47	48	48	46	43	54	36
	50	52	46	47	46	58	55	35
	51	49	46	48	48	55	51	34
	50	44	49	52	49	49	52	43
Total Fronds	201	192	189	195	189	205	212	148

Increase in Frond Number = Total # Fronds - 6 Starting Fronds

	44	41	42	42	40	37	48	30
	44	46	40	41	40	52	49	29
	45	43	40	42	42	49	45	28
	44	38	43	46	43	43	46	37
Total Increase	177	168	165	171	165	181	188	124
Mean Increase	44.3	42.0	41.3	42.8	41.3	45.3	47.0	31.0
SD Increase	0.5	3.4	1.5	2.2	1.5	6.7	1.8	4.1
CV Increase	1.1	8.0	3.6	5.2	3.6	14.7	3.9	13.2
% Stimulation		**-5.08**	**-6.78**	**-3.39**	**-6.78**	**2.26**	**6.21**	**-29.94**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.68041	0.67334	0.67895	0.68244	0.68007	0.68357	0.68242	0.67864
Pan + Plant	0.67718	0.68371	0.68167	0.67695	0.67597	0.67486	0.67825	0.68025
	0.67558	0.67748	0.67462	0.68119	0.67527	0.68188	0.67705	0.67805
	0.67689	0.66599	0.66812	0.67306	0.67224	0.67197	0.67875	0.67593
Initial Weight (g)	0.67672	0.66985	0.67497	0.67873	0.67676	0.68005	0.67785	0.67478
Pan Only	0.67311	0.67989	0.67799	0.67312	0.67252	0.67030	0.67367	0.67603
	0.67139	0.67371	0.67093	0.67729	0.67157	0.67770	0.67293	0.67421
	0.67274	0.66295	0.66417	0.66869	0.66836	0.66824	0.67446	0.67173
Plant Only (mg)	3.69	3.49	3.98	3.71	3.31	3.52	4.57	3.86
	4.07	3.82	3.68	3.83	3.45	4.56	4.58	4.22
	4.19	3.77	3.69	3.90	3.70	4.18	4.12	3.84
	4.15	3.04	3.95	4.37	3.88	3.73	4.29	4.20
Mean Dry Weight	4.025	3.530	3.825	3.953	3.585	3.998	4.390	4.030
SD Dry Weight	0.2	0.4	0.2	0.3	0.3	0.5	0.2	0.2
CV Dry Weight	5.7	10.1	4.2	7.3	7.1	11.6	5.1	5.2
% Stimulation		**-12.30**	**-4.97**	**-1.80**	**-10.93**	**-0.68**	**9.07**	**0.12**

For Data Transfer to CETIS

	# fronds	total mass	tare
0	1	44	0.68041	0.67672
	2	44	0.67718	0.67311
	3	45	0.67558	0.67139
	4	44	0.67689	0.67274
0.1	1	41	0.67334	0.66985
	2	46	0.68371	0.67989
	3	43	0.67748	0.67371
	4	38	0.66599	0.66295
0.3	1	42	0.67895	0.67497
	2	40	0.68167	0.67799
	3	40	0.67462	0.67093
	4	43	0.66812	0.66417
1	1	42	0.68244	0.67873
	2	41	0.67695	0.67312
	3	42	0.68119	0.67729
	4	46	0.67306	0.66869
3.1	1	40	0.68007	0.67676
	2	40	0.67597	0.67252
	3	42	0.67527	0.67157
	4	43	0.67224	0.66836
9.7	1	37	0.68357	0.68005
	2	52	0.67486	0.67030
	3	49	0.68188	0.67770
	4	43	0.67197	0.66824
31	1	48	0.68242	0.67785
	2	49	0.67825	0.67367
	3	45	0.67705	0.67293
	4	46	0.67875	0.67446
97	1	30	0.67864	0.67478
	2	29	0.68025	0.67603
	3	28	0.67805	0.67421
	4	37	0.67593	0.67173

AO 17/05/22

L2701854-COFC

Chain of Custody (COC) / Analytical Request Form

AJ

Canada Toll Free: 1 800 668 9878

COC Number: 20 -

Page of

Report To	Contact and company name below will appear on the final report
Company:	New Gold
Contact:	Garnet Cornell
Phone:	
	Company address below will appear on the final report
Street:	
City/Province:	
Postal Code:	
Invoice To	Same as Report To ☐ YES ☐ NO
	Copy of Invoice with Report ☐ YES ☐ NO
Company:	
Contact:	

Reports / Recipients

Select Report Format: ☐ PDF ☐ EXCEL ☐ EDD (DIGITAL)

Merge QC/QCI Reports with COA ☐ YES ☐ NO ☐ N/A

☐ Compare Results to Criteria on Report - provide details below if box checked

Select Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Email 3

Invoice Recipients

Select Invoice Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Turnaround Time (TAT) Requested

☐ Routine [R] if received by 3pm M-F - no surcharges apply
☐ 1 day [P4] if received by 3pm M-F - 20% rush surcharge minimum
☐ 3 day [P3] if received by 3pm M-F - 25% rush surcharge minimum
☐ 2 day [P2] if received by 3pm M-F - 50% rush surcharge minimum
☐ 1 day [E] if received by 3pm M-F - 100% rush surcharge minimum
☐ Same day [E2] if received by 10am M-S - 200% rush surcharge.

Additional fees may apply to rush requests on weekends, statutory holidays and for non-routine tests.

Date and Time Required for all E&P TATs: dd-mmm-yy hh:mm am/pm

For all tests with rush TATs requested, please contact your AM to confirm availability.

AFFIX ALS BARCODE LABEL HERE (ALS use only)

Project Information		Oil and Gas Required Fields (client use)	
ALS Account # / Quote #:		AFE/Cost Center:	PO#:
Job #:		Major/Minor Code:	Routing Code:
PO / AFE:		Requisitioner:	
LSD:		Location:	
ALS Lab Work Order # (ALS use only): L2701854		ALS Contact:	Sampler:

Analysis Request

Indicate Filtered (F), Preserved (P) or Filtered and Preserved (F/P) below

ALS Sample # (ALS use only)	Sample Identification and/or Coordinates (This description will appear on the report)	Date (dd-mmm-yy)	Time (hh:mm)	Sample Type	NUMBER OF CONTAINERS	NG-ST-P-TB	SAMPLES ON HOLD	EXTENDED STORAGE REQUIRED	SUSPECTED HAZARD (see notes)
1	EDL1	27-Apr-22	9:00	EFF	19	x			
2	SP2	27-Apr-22	9:10	EFF	19	x			

Drinking Water (DW) Samples[1] (client use)

Are samples taken from a Regulated DW System? ☐ YES ☐ NO

Are samples for human consumption/ use? ☐ YES ☐ NO

Notes / Specify Limits for result evaluation by selecting from drop-down below (Excel COC only)

SAMPLE RECEIPT DETAILS (ALS use only)

Cooling Method: ☐ NONE ☐ ICE ☒ ICE PACKS ☐ FROZEN ☐ COOLING INITIATED

Submission Comments identified on Sample Receipt Notification: ☐ YES ☐ NO

Cooler Custody Seals Intact: ☐ YES ☐ N/A Sample Custody Seals Intact: ☐ YES ☐ N/A

INITIAL COOLER TEMPERATURES °C				FINAL COOLER TEMPERATURES °C			
4.1							

SHIPMENT RELEASE (client use)			INITIAL SHIPMENT RECEPTION (ALS use only)			FINAL SHIPMENT RECEPTION (ALS use only)		
Released by:	Date:	Time:	Received by: AJ	Date: 04/28/22	Time: 9:20	Received by:	Date:	Time:

REFER TO BACK PAGE FOR ALS LOCATIONS AND SAMPLING INFORMATION

WHITE - LABORATORY COPY YELLOW - CLIENT COPY

AUG 2020 FRONT

Failure to complete all portions of this form may delay analysis. Please fill in this form LEGIBLY. By the use of this form the user acknowledges and agrees with the Terms and Conditions as specified on the back page of the white - report copy.

1. If any water samples are taken from a **Regulated Drinking Water (DW) System**, please submit using an **Authorized DW COC form**.

AJ

May 19, 2022

Christine Paradis,
ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Dear Christine:

On April 29, 2022, Nautilus Environmental Company Inc. personnel received one water sample (SP2 L2701854-2) from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected April 27, 2022

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
SP2 L2701854-2	Fathead Minnow	7-day LC50 (95% Confidence)	Survival	>100% Volume (Not Applicable)
		7-day IC25 (95% Confidence)	Biomass	>100% Volume (Not Applicable)
#8730-0032204	*Ceriodaphnia dubia*	3-brood LC50 (95% Confidence)	Survival	Non-lethal (Not Applicable)
		3-brood IC25 (95% Confidence)	Reproduction	>100% Volume[2] (Not Applicable)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[3] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence)	Growth	>97% Volume[3] (Not applicable)
		7-day IC25 Dry Weight (95% Confidence)	Growth	>97% Volume[3] (Not applicable)

1 - Results relate only to the sample tested
2 - Inhibition >25% at concentration 0.137% v/v
3 - Highest concentration tested, based on test method

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 100%, 33%, 11%, 3.7% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted half by half (*i.e.,* 100%, 50%, 25% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

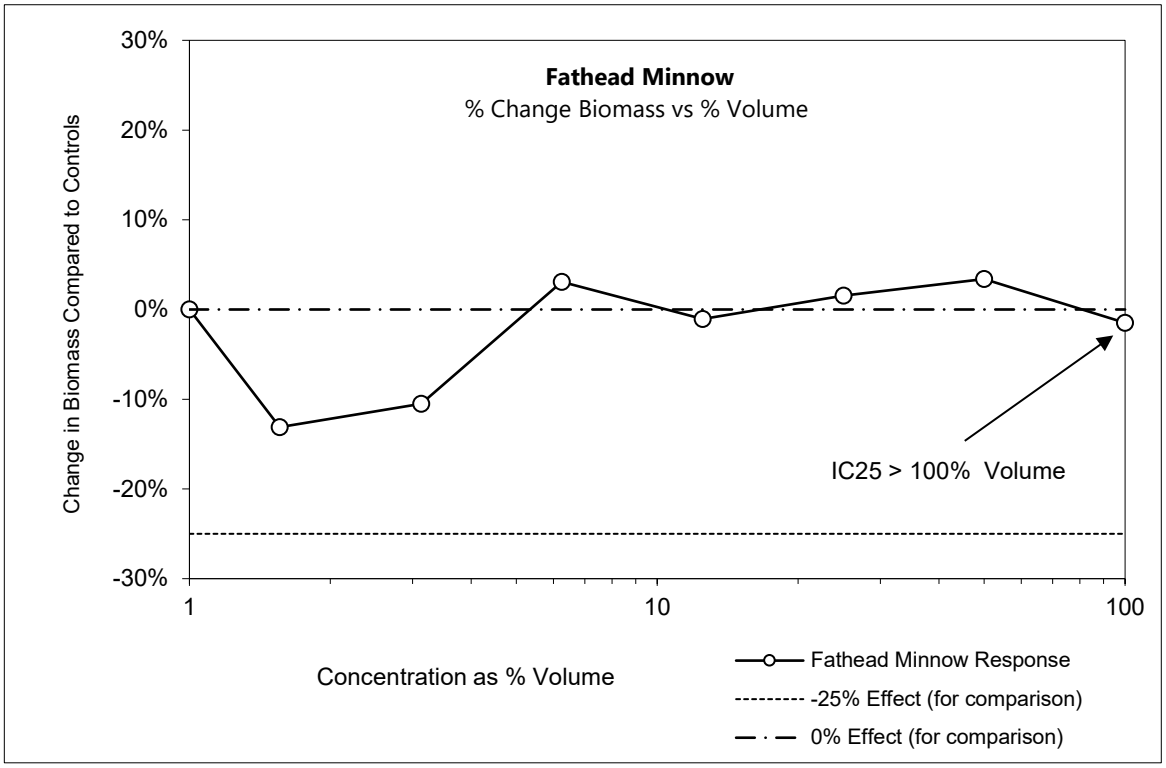
If there are any further details which you require, please do not hesitate to contact us.

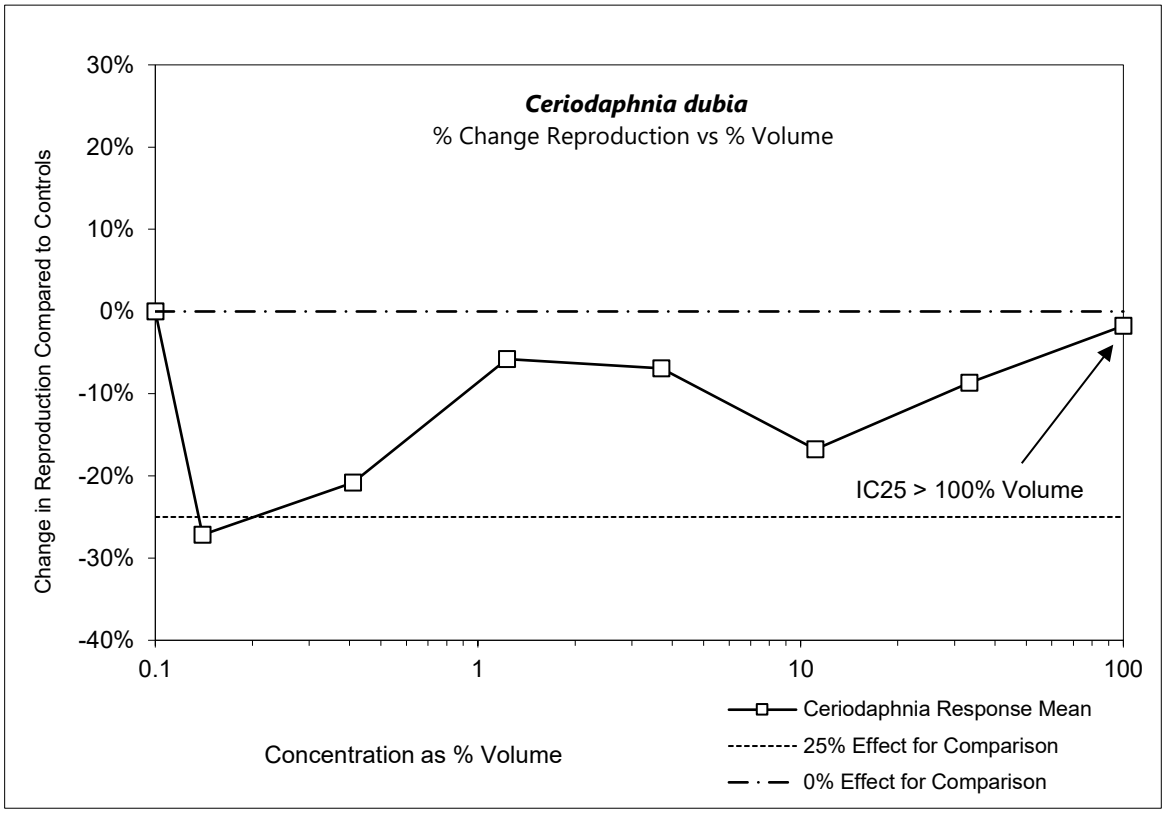
Yours very truly,
Nautilus Environmental Company Inc.

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2021\1000\8730-003\8730-0032204 FCRL

ALS Thunder Bay - SP2 (L2701854-2) - April 27, 2022





P:\Data\IC25\2022\8730-0032204

Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	SP2
Date/Time Sampled:	April 27, 2022; 09:10	**Date/Time Received:**	April 29, 2022; 15:00
Date/Time Test Started:	April 29, 2022; 17:00	**Date Test Finished:**	May 6, 2022
Description:	Clear, light green	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032204	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	12.7°C
Collected By:	N/A		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species:	Fathead Minnow (*Pimephales promelas*)		
Source:	Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)		
Culture Temp.:	22 to 26 °C	**Life Stage:**	<24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 0%

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032204 **Sources:** SP2

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Test Conditions

Test Volume: 533 ml/rep **Temp.:**25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 0 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (0%)

Average Weight of Controls is ≥ 250 µg: Acceptable (717 µg)

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032204 **Sources:** SP2

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	0	0	0	0	0	0	0
	2	0	0	0	10	0	10	10	10
	3	0	0	0	0	0	10	0	0
Mean % Mortality		0.0	0.0	0.0	3.3	0.0	6.7	3.3	3.3
S.D.		0.0	0.0	0.0	5.8	0.0	5.8	5.8	5.8
Survival/Growth Data									
Mean Dry Biomass	1	0.676	0.590	0.596	0.646	0.617	0.676	0.777	0.700
(mg)	2	0.690	0.630	0.699	0.746	0.772	0.732	0.674	0.668
	3	0.785	0.649	0.630	0.825	0.739	0.776	0.773	0.751
% Effect (+ or -)		0.0	-13.1	-10.5	3.1	-1.1	1.5	3.4	-1.5
Mean Dry Biomass/ Concentration (mg)		0.717	0.623	0.642	0.739	0.709	0.728	0.741	0.706
S.D.		0.06	0.03	0.05	0.09	0.08	0.05	0.06	0.04

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032204 **Sources:** SP2

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 7-day LC50 (Confidence Interval)[2]	 >100% Volume (Not Applicable)	 No dose response
Biomass **(Survival and Growth)** 7-day IC25 (Confidence Interval)[2]	 >100% Volume (Not Applicable)	 Non-linear Regression 3P Log-Logistic Model

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 21-Apr-2022 **Reference Batch #:** Zn2102

Method of Analysis: Trimmed Spearman-Karber α = 1.67%

7-Day LC50 (95% Confidence Limits): 0.51 mg/L (0.44 mg/L; 0.59 mg/L)

Historic Geometric Mean LC50: 0.73 mg/L (0.43 mg/L; 1.21 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS - Thunder Bay Sample Name: SP 2 Sample #: 873002003 2204

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 0 %

Average Weight of Controls is ≥ 250 µg: (Acceptable) / Not Acceptable 717 µg

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 0 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
7-day LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
Survival / Growth Biomass		Nonlinear regression
7-day IC25	>100 % Volume	3P Log logistic model
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 11/05/22

Results Verified: Initials GO Date 11/05/22

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** SP 2 "03" 04 **Sample #:** 8730-0032204

Day	Date 2022	Initial Variables: Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	Meters/Probes Used: D.O. / °C	pH	Cond.	Pre-aeration: yes/no	Rate (bubbles/min)	Duration (min)	Pail Sub-Sampled	Initials
0	29.04	24.3	7.6	10.0	489	6/4	13/88	5/6	yes	≤100	≤ 20	1	[illegible]
1	30	26.3	7.1	10.3	507	6/4	13/88	5/6	yes	≤100	≤ 20	1	[illegible]
2	01.05	24.9	7.7	11.0	434	6/4	13/88	5/6	yes	≤100	≤ 20	1	[illegible]
3	02	24.9	7.8	10.9	473	6/4	13/88	5/6	yes	≤100	≤ 20	2	WL
4	03	24.9	7.8	11.3	473	6/4	13/88	5/6	yes	≤100	≤ 20	2	KK
5	04	26.4	7.7	10.9	502	6/4	13/88	5/6	yes	≤100	≤ 20	3	[illegible]
6	05	24.4	7.7	11.5	488	6/4	13/88	5/6	yes	≤100	≤ 20	3	KK
7	06												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of brine shrimp days 0 to 6? (Yes)/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: control Sample Name: SP2 "04" Sample #: 8730-0032204

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29-04	25.9	7.9	7.8	239	6/4	13/88	~~6~~ 5/6	CS
1	30-04	26.3	8.2	7.7	245	6/4	13/88	5/6	JM
2	01-05	26.0	8.1	7.7	238	6/4	13/88	5/6	JM
3	02-05	26.1	8.3	7.7	238	6/4	13/88	5/6	WL
4	03-05	25.5	8.1	7.8	236	6/4	13/88	5/6	WL
5	04-05	25.6	8.2	7.8	237	6/4	13/88	5/6	WL
6	05-05	24.9	8.0	8.0	244	6/4	13/88	5/6	WL
7	06-05								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25.0	8.1	6.7	6/4	13/88	JM							
25.2	7.5	6.7	6/4	13/88	JM	0	0	0	0	0	0	JM
24.9	7.8	6.5	6/4	13/88	KK	0	0	0	0	0	0	JM
24.8	7.6	~~5.6~~ WL 5.7	6/4	13/88	WL	0	0	0	0	0	0	KK
~~24.9~~ WL 24.8	7.5	5.4	6/4	13/88	WL	0	0	0	0	0	0	WL
24.8	~~7.4~~ WL 7.5	6.4	6/4	13/88	WL	0	0	0	0	0	0	KK
24.8	7.6	6.1	6/4	13/88	KK	0	0	0	0	0	0	WL
						0	0	0	0	0	0	WL

Observations: _____

Concentration: 1.56 %

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29-04	23.7	7.9	8.1	232	6/4	13/88	5/6	CS
1	30-04	25.3	8.4	7.5	236	6/4	13/88	5/6	JM
2	01-05	25.7	8.1	7.4	238	6/4	13/88	5/6	JM
3	02-05	25.0	8.2	7.8	234	6/4	13/88	5/6	WL
4	03-05	24.8	8.1	7.8	233	6/4	13/88	5/6	WL
5	04-05	25.1	8.1	7.9	236	6/4	13/88	5/6	WL
6	05-05	24.5	8.1	7.9	245	6/4	13/88	5/6	WL
7	06-05								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25.0	8.1	6.6	6/4	13/88	JM							
25.3	7.6	6.1	6/4	13/88	JM	0	0	0	0	0	0	0
24.8	7.8	6.5	6/4	13/88	KK	0	0	0	0	0	0	JM
24.9	7.6	5.6	6/4	13/88	WL	0	0	0	0	0	0	KK
24.8	7.6	5.6	6/4	13/88	WL	0	0	0	0	0	0	WL
24.7	7.6	6.3	6/4	13/88	WL	0	0	0	0	0	0	KK
24.8	7.6	5.9	6/4	13/88	KK	0	0	0	0	0	0	WL
						0	0	0	0	0	0	WL

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13 % v/v Sample Name: SP2 "04" Sample #: 8730-0032204

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29-04								
1	30-04								
2	01-05								
3	02-05								
4	03-05								
5	04-05								
6	05-05								
7	06-05								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	0	0	0	0	0	WL

Observations:

Concentration: 6.25 % v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29-04								
1	30-04								
2	01-05								
3	02-05								
4	03-05								
5	04-05								
6	05-05								
7	06-05								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	10	0	0	0	0	JM
						0	10	0	0	0	0	JM
						0	10	0	0	0	0	JM
						0	10	0	0	0	0	KK
						0	10	0	0	0	0	WL
						0	10	0	0	0	0	KK
						0	10	0	0	0	0	WL
						0	10	0	0	0	0	WL

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5% Sample Name: SP2 "04" Sample #: 8730-0032204

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29-04	23.9	8.0	8.1	259	6/4	13/88	~~6/6~~ 5/6	cs
1	30-04	25.4	8.4	7.5	266	6/4	13/88	5/6	JM
2	01-05	28.7	8.1	7.4	268	6/4	13/88	5/6	JM
3	02-05	25.1	8.2	7.8	262	6/4	13/88	5/6	WL
4	03-05	24.9	8.1	7.8	260	6/4	13/88	5/6	WL
5	04-05	25.2	8.1	7.9	263	6/4	13/88	5/6	WL
6	05-05	24.5	~~8.0~~ WL 8.1	~~8.1~~ WL 7.9	274	6/4	13/88	5/6	WL
7	06-05								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25.0	8.1	6.7	6/4	13/88	JM	~~0~~	0	0	0	0	0	JM
25.3	7.6	6.4	6/4	13/88	JM	0	0	0	0	0	0	JM
24.9	7.7	6.3	6/4	13/88	KK	0	0	0	0	0	0	JM
24.7	7.6	5.7	6/4	13/88	WL	0	0	0	0	0	0	KK
24.7	7.6	5.6	6/4	13/88	WL	0	0	0	0	0	0	WL
24.7	7.6	6.1	6/4	13/88	WL	0	0	0	0	0	0	KK
24.8	7.6	6.0	6/4	13/88	KK	0	0	0	0	0	0	WL
						0	0	0	0	0	0	WL

Observations:

Concentration: 25%

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29-04								
1	30-04								
2	01-05								
3	02-05								
4	03-05								
5	04-05								
6	05-05								
7	06-05								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						~~0~~	0	~~0~~	0	0	0	JM
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	JM
						0	10	10	0	0	0	KK
						0	10	10	0	0	0	WL
						0	10	10	0	0	0	KK
						0	10	10	0	0	0	WL
						0	10	10	0	0	0	WL

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50 %v/v Sample Name: SP2 "04" Sample #: 8730-00322204

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29-04								
1	30-04								
2	01-05								
3	02-05								
4	03-05								
5	04-05								
6	05-05								
7	06-05								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	JM
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	WL
						0	10	0	0	0	0	WL

Observations:

Concentration: 100 %v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29-04	24.7	7.7	9.3	472	6/4	13/88	~~6/6~~ 5/6	CS
1	30-04	25.7	8.2	8.6	482	6/4	13/88	5/6	JM
2	01-05	25.2	7.8	9.4	478	6/4	13/88	5/6	JM
3	02-05	25.0	7.9	8.9	474	6/4	13/88	5/6	WL
4	03-05	24.9	7.9	9.0	472	6/4	13/88	5/6	WL
5	04-05	25.9	7.9	9.1	482	6/4	13/88	5/6	WL
6	05-05	24.5	7.8	9.4	489	6/4	13/88	5/6	WL
7	06-05								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25.0	8.2	6.8	6/4	13/88	JM							
25.2	7.7	6.7	6/4	13/88	JM	0	0	0	0	0	0	JM
24.8	8.0	~~6.8~~ 6.7 KK	6/4	13/88	KK	0	0	0	0	0	0	JM
24.7	~~7.8~~ 7.9 WL	5.7	6/4	13/88	WL	0	0	0	0	0	0	KK
24.7	7.7	5.1	6/4	13/88	WL	0	0	0	0	0	0	WL
24.7	7.8	6.2	6/4	13/88	WL	0	0	0	0	0	0	KK
24.7	7.8	5.8	6/4	13/88	KK	0	10	0	0	0	0	WL
						0	10	0	0	0	0	WL

Observations:

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information

Client ALS
Sample # 8730-0032204
Date/Time Received 29-04-22 / 15:00
Sample Type Water
100% Hardness 212

Sample Name SP2 "04"
Sample Date/Time 27/04/22 / 0910
Person Sampling N/A
Arrival Temp 12.7 °C
Sample Description cloudy, light green

Test Information

Date/Time Started 29-04-22 / 17:00
Test started by IK
Fathead Batch # FH0121/0621
Date eggs laid 23/24/25/26-04-22
Culture mortality within 7 days of egg collection 0%
Swim bladder inflated; yes / no Yes
Age of Larvae at start of test in hours <24
Control Hardness 94
Water Bath Quadrant C
Average Temperature during Test: 25.4 ± 0.1 °C

Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / No (circle one)

Conc.	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	10	0.73065	0.72379	0.676	0.717
	B	10	0.74228	0.73538	0.690	
	C	10	0.74845	0.74060	0.785	
1.56	A	10	0.73415	0.72825	0.590	0.623
	B	10	0.73564	0.72934	0.630	
	C	10	0.72870	0.72221	0.649	
3.13	A	10	0.74393	0.73797	0.596	0.642
	B	10	0.74757	0.74058	0.699	
	C	10	0.73634	0.73004	0.630	
6.25	A	10	0.73898	0.73252	0.646	0.739
	B	9	0.73013	0.72267	0.746	
	C	10	0.74060	0.73235	0.825	
12.5	A	10	0.74410	0.73793	0.617	0.709
	B	10	0.73528	0.72756	0.772	
	C	10	0.74757	0.74018	0.739	
25	A	10	0.73295	0.72619	0.676	0.728
	B	9	0.73804	0.73072	0.732	
	C	9	0.74970	0.74194	0.776	
50	A	10	0.72730	0.71953	0.777	0.741
	B	9	0.75547	0.74873	0.674	
	C	10	0.74168	0.73395	0.773	
100	A	10	0.75346	0.74646	0.700	0.706
	B	9	0.73846	0.73178	0.668	
	C	10	0.73120	0.72369	0.751	
Initials		IK	IK	AO	IK	IK

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8730-0032204 **Sample Name** SP2

Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug) 717

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	10	0.73065	0.72389	0.676	0.676	0.717	0.717	0.059304	0.059304	8.271172
	10	10	0.74228	0.73538	0.690	0.690					
	10	10 ✓	0.74845	0.74060	0.785	0.785					
1.56	10	10	0.73415	0.72825	0.590	0.590	0.623	0.623	0.030116	0.030116	4.8341
	10	10	0.73564	0.72934	0.630	0.630					
	10	10 ✓	0.72870	0.72221	0.649	0.649					
3.13	10	10	0.74393	0.73797	0.596	0.596	0.642	0.642	0.052482	0.052482	8.178973
	10	10	0.74757	0.74058	0.699	0.699					
	10	10 ✓	0.73634	0.73004	0.630	0.630					
6.25	10	10	0.73898	0.73252	0.646	0.646	0.767	0.739	0.104486	0.089705	13.62932
	10	9	0.73013	0.72267	0.829	0.746					
	10	10 ✓	0.74060	0.73235	0.825	0.825					
12.5	10	10	0.74410	0.73793	0.617	0.617	0.709	0.709	0.081648	0.081648	11.51047
	10	10	0.73528	0.72756	0.772	0.772					
	10	10 ✓	0.74757	0.74018	0.739	0.739					
25	10	10	0.73295	0.72619	0.676	0.676	0.784	0.728	0.096548	0.05012	12.31714
	10	9	0.73804	0.73072	0.813	0.732					
	10	9 ✓	0.74970	0.74194	0.862	0.776					
50	10	10	0.72730	0.71953	0.777	0.777	0.766	0.741	0.015207	0.058347	1.984526
	10	9	0.75547	0.74873	0.749	0.674					
	10	10 ✓	0.74168	0.73395	0.773	0.773					
100	10	10	0.75346	0.74646	0.700	0.700	0.731	0.706	0.027266	0.041861	3.729647
	10	9	0.73846	0.73178	0.742	0.668					
	10	10 ✓	0.73120	0.72369	0.751	0.751					

SD: Standard Deviation
CV: Coefficient of Variation

NAUTILUS ENVIRONMENTAL

@0 11/05/22

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	SP2
Date/Time Sampled	April 27, 2022; 09:10	**Date/Time Received:**	April 29, 2022; 15:00
Date Test Started:	April 29, 2022	**Date Test Finished:**	May 5, 2022
Description:	Clear, light green	**Days Sample Used:**	Days 0 to 5
Sample #:	8730-0032204	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	12.7°C
Collected By:	N/A		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032204 **Sources:** SP2

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 0%

Mean Number of Surviving Young Produced Within First 3 Broods: 23.5

Mean Number of Surviving Young Produced Within 7 Days of Testing: 32.5

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 0 to 5

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032204 **Sources:** SP2

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD22-45

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (17.3 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (60% of controls)

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032204 **Sources:** SP2

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	0	0	0	0	0	0	0
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	17	10	15	0	11	13	9	15
	2	20	9	11	24	18	10	14	7
	3	14	8	9	13	22	19	8	9
	4	23	23	20	26	10	16	20	26
	5	8	15	19	21	10	22	11	18
	6	14	20	3	11	11	14	21	28
	7	13	5	2	25	19	13	21	13
	8	23	12	22	14	13	10	18	24
	9	14	11	12	7	23	10	14	7
	10	27	13	24	22	24	17	22	23
Total Number of Live Neonates in First 3 Broods or Less		173	126	137	163	161	144	158	170
% Effect (+ or -)		0.0	-27.2	-20.8	-5.8	-6.9	-16.8	-8.7	-1.7
Mean Number of Live Neonates in First 3 Broods or Less		17.3	12.6	13.7	16.3	16.1	14.4	15.8	17.0
SD		5.8	5.5	7.7	8.7	5.7	4.1	5.3	8.0

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032204 **Sources:** SP2

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	Non-lethal (Not Applicable)	No mortality
Reproduction 3-Brood IC25 (Confidence Interval)[2]	>100% Volume[3] (Not Applicable)	No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval
3 - Inhibition >25% at concentration 0.137% v/v

Test Method Deviation: None

Outliers and Justification for Their Removal: None

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 21-Apr-2022 **Reference Batch #:** Zn2102

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 0.13 mg/L (0.10 mg/L; 0.18 mg/L)

Historic Geometric Mean LC50: 0.08 mg/L (0.02 mg/L; 0.26 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS Thunder Bay Sample Name: SP2 Sample #: 8730-0032204

Conditions for Test Validity

Control Mortality is< 20%: (Acceptable) / Not Acceptable: 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable) / Not Acceptable: 6 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable) / Not Acceptable: 17.3 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤40 min Days: 0 to 5

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	non-lethal % Volume	no mortality
95% Confidence Interval[2]	/ % Volume	
REPRODUCTION	*	No non-linear regression models would fit
3-brood IC25	>100 % Volume	IC IR - linear interpolation
95% Confidence Interval[2]	n/a % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

* inhibition > 25% at conc 0.137%

Analysis Completed: Initials TV Date 11/05/22

Results Verified: Initials [illegible] Date 11/05/22

daphnia dubia Initial Sample Measureme Before Preparation and Use in Toxicity Te

Concentration: 100% **Sample Name:** SP2 "04" **Sample #:** 8730-0032204

Day	Date 2022	Initial Measurements				Meters / Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	°C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	29-01	24.5	7.6	10.0	469	6/6 5/6	13/80	5/6	yes	≤100	≤ 20	1	QO
1	30	20.3	7.1	10.5	507	6/6 5/6	13/80	5/6	yes	≤100	≤ 20	1	QO
2	01-05	24.9	7.7	11.0	484	6/6 5/6	13/88	5/6	yes	≤100	≤ 20	1	QO
3	02	24.9	7.8	10.9	473	6/4	13/88	5/6	yes	≤100	≤ 20	2	KK
4	03	24.9	7.8	11.3	473	6/4	13/88	5/6	yes	≤100	≤ 20	2	KK
5	04	26.4	7.7	10.9	502	6/4	13/88	5/6	yes	≤100	≤ 20	3	C
6	05									≤100	≤ 20	3	
7	06									≤100	≤ 20	3	
8	07									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily? (Yes)/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: Control | Sample Name: SP2 "04" | Sample #: 8730-003 220

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29-04	26.0	8.0	7.8	398	6/14	13/88	5/6	CS
1	30	~~25.8~~	8.3	7.8	391	6/4	13/88	5/6	ao
2	01-05	26.3	8.2	7.7	389	6/4	13/88	5/6	JM
3	02	~~26.3~~	8.4	7.7	~~395~~	6/4	13/88	5/6	ao
4	03	25.5	8.2	7.8	KK 3893	6/4	13/88	5/6	KK
5	04	25.3	8.2	7.8	391	6/4	13/88	5/4	C
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.0	8.0	7.9	6/4	13/88	ao
24.4	8.0	7.1	6/4	13/88	ao
24.0	8.0	7.1	6/4	13/88	ao
24.1	8.0	7.3	6/4	13/88	KK
24.6	~~7.7~~ 8.2	7.4	6/4	13/88	C
24.0	8.3	7.8	6/4	13/88	ao

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
3	02	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
4	03	5	6	6	4	3	4	3	4	3	4	42	—	0	—	KK	KK
5	04	2	2	8	6	5	10	10	7	3	9	62	—	0	—	C	C
6	05	10	12	0	13	0	0	0	12	7	14	64	—	0	—	ao	ao
7	06																
8	07																
Total Neonates		17	20	14	23	8	14	13	23	14	27	173	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.137 % v/v Sample Name: SP2 "04" Sample #: 8730-003 22a4

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29-04	25.4	8.2	7.6	388	6/4	13/88	5/6	cs
1	30	26.0	8.5	7.8	407	6/4	13/88	5/6	QD
2	01-05	26.1	8.2	7.4	386	6/4	13/88	5/6	JM
3	02	25.9	8.3	7.7	420	6/4	13/88	5/6	QD
4	03	25.3	8.3	7.7	KK 2390	6/4	13/88	5/6	KK
5	04	25.2	8.3	7.6	397	6/4	13/88	5/6	C
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.4	8.3	7.3	6/4	13/88	QD
24.6	8.0	7.1	6/4	13/88	QD
23.9	8.1	7.1	6/4	13/88	QD
23.9	8.1	7.3	6/4	13/88	KK
24.4	8.1	7.2	6/4	13/88	C
24.0	8.2	7.7	6/4	13/88	QD

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	QD	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	QD	—
3	02	0	0	0	0	0	2	0	0	0	0	2	—	0	—	QD	QD
4	03	3	3	5	4	3	7	0	5	4	5	39	—	0	—	KK	KK
5	04	7	6	3	7	4	0	5	7	7	8	54	—	0	—	C	6
6	05	0	0	0	12	3	11	0	0	0	0	31	—	0	—	QD	QD
7	06																
8	07																
Total Neonates		10	9	8	23	15	20	5	12	11	13	126	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.41 l.v.N Sample Name: SP2 "04" Sample #: 8730-003 22q

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29 · 04								
1	30								
2	01 · 05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29 · 04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
2	01 · 05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
3	02	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
4	03	3	4	4	4	3	0	0	5	4	4	31	—	0	—	KK	KK
5	04	7	7	5	7	3	3	2	7	8	7	56	—	0	—	7	6
6	05	5	0	0	9	13	0	0	10	0	13	50	—	0	—	ao	ao
7	06																
8	07																
Total Neonates		15	11	9	20	19	3	2	22	12	24	137	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 1.23% v/v Sample Name: SP2 "04" Sample #: 8730-003 22d

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29-04								
1	30								
2	01-05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
3	02	0	0	0	0	0	2	2	0	0	0	4	—	0	—	[illegible]	[illegible]
4	03	0	3	5	5	3	3	9	5	1	5	34	—	0	—	KK	KK
5	04	0	8	8	9	5	6	0	9	6	8	59	—	0	—	[illegible]	G
6	05	0	13	0	12	13	0	14	0	0	9	61	—	0	—	[illegible]	[illegible]
7	06																
8	07																
Total Neonates		0	24	13	26	21	9	25	14	7	22	161					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 3.7 % v/v Sample Name: SP2 "04" Sample #: 8730-003 22-04

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29-04	25.4	8.2	7.4	391	6/4	13/88	5/6	CS
1	30	25.3	8.5	7.8	400	6/4	13/88	5/6	CS
2	01-05	25.8	8.3	7.4	387	6/4	13/88	5/6	JM
3	02	25.8	8.3	7.7	402	6/4	13/88	5/6	CS
4	03	25.4	8.3	7.7	394	6/4	13/88	5/6	KK
5	04	25.2	8.3	7.6	395	6/4	13/88	5/6	C
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.4	8.3	7.2	6/4	13/88	CS
24.8	7.9	6.9	6/4	13/88	CS
24.0	8.0	6.7	6/4	13/88	CS
24.9 (3 KK)	8.1	7.2	6/4	13/88	KK
24.4	8.1	7.2	6/4	13/88	C
23.8	8.3	7.7	6/4	13/88	CS

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CS	—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CS	—
3	02	0	0	0	0	0	0	2	0	0	0	2	—	0	—	CS	CS
4	03	2	4	2	0	2	3	7	4	6	6	36	—	0	—	KK	KK
5	04	9	6	10	5	8	8	0	7	7	7	67	—	0	—	C	C
6	05	0	8	12	5	0	0	10	2	9	11	57	—	0	—	CS	CS
7	06																
8	07																
Total Neonates		11	18	22	10	10	11	19	13	23	24	161	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentratio 11.11.1.vlv Sample Name: SP2 "04" Sample #: 8730-003 224

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29 · 04								
1	30								
2	01 · 05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29 · 04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
2	01 · 05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
3	02	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
4	03	4	5	4	6	2	2	4	3	2	6	38	—	0	—	KK	KK
5	04	9	0	4	0	6	12	9	7	8	11	66	—	0	—	C	C
6	05	0	5	11	10	14	0	0	0	0	0	40	—	0	—	ao	ao
7	06																
8	07																
Total Neonates		13	10	19	16	22	14	13	10	10	17	144	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentratic 33.3% v/v Sample Name: SP2 "04" Sample #: 8730-003 22q

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29 · 04								
1	30								
2	01 · 05								
3	02								
4	03								
5	04								
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29 · 04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
2	01 · 05	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
3	02	0	0	0	0	0	2	0	0	0	0	2	—	0	—	ao	ao
4	03	4	6	2	4	3	8	0	5	4	5	41	—	0	—	KK	KK
5	04	5	8	6	6	8	0	9	5	10	7	64	—	0	—	C	6
6	05	0	0	0	10	0	11	12	3	0	10	51	—	0	—	ao	ao
7	06																
8	07																
Total Neonates		9	14	8	20	11	21	21	13	14	22	157	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentratio: 100 % v/v Sample Name: SP2 "04" Sample #: 8730-003 22nd

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	29-04	25.3	7.9	8.4	475	6/14	13/88	5/6	CS
1	30	26.0	8.2	7.9	490	6/4	13/88	5/6	
2	01-05	25.6	~~7.3~~ 7.9 JM	JM ~~9.3~~ 7.9	483	6/4	13/88	5/6	JM
3	02	~~25.8~~	7.9	9.1	477	6/4	13/88	5/6	
4	03	25.2	8.0	9.0	476	6/4	13/88	5/6	KK
5	04	25.4	7.9	9.1	476	6/4	13/88	5/6	C
6	05								
7	06								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.5	7.3	7.4	6/4	13/88	
24.6	8.0	6.8	6/4	13/88	
24.0	8.1	7.3	6/4	13/88	
24.0	8.3	7.7	6/4	13/88	KK
24.5	8.2	7.3	6/4	13/88	C
23.7	~~8.3~~	7.7	6/4	13/88	

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	29-04																
1	30	0	0	0	0	0	0	0	0	0	0	0	—	0	—		—
2	01-05	0	0	0	0	0	0	0	0	0	0	0	—	0	—		—
3	02	0	0	0	0	0	0	0	0	0	3	3	—	0	—		
4	03	1	2	3	4	2	6	0	5	0	8	31	—	0	—	KK	KK
5	04	8	5	6	8	7	10	6	9	7	0	66	—	0	—	C	C
6	05	6	0	0	14	9	12	7	10	0	12	70	—	0	—		
7	06																
8	07																
Total Neonates		15	7	9	20	17	28	13	24	7	23	170	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia Neonate Origin

Sample Information

Client ALS

Sample # 8730-0032204

Date/Time Received 29.04.22 /15:00

Sample Type Water

100% Hardness 212

Sample Name 8"SE SP2 "04"

Date/Time Collected 27/04/22 10910

Person Sampling N/A

Arrival Temp (°C) 11.7

Sample Description Clear, light green

Test Information

Date Test Started 29.04.22 @1633

Dilution Water Batch Number CD22-45

Test Started By GO

Control Hardness 118

Template Used for Randomization 1

Individual Culture Health Data

Date Culture Started 22.04.22
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 24.0
Culture I.D. (e.g., Wed Row 4) FRI ROW 2
Average # neos in previous 7 days (must be ≥15) 34.5
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 22.04.22
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 22.9
Culture I.D. (e.g., Wed Row 4) FRI ROW 3
Average # neos in previous 7 days (must be ≥15) 30.5
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (e.g., Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (e.g., Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (e.g., Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 0 (add up % mortality for all cultures used / # cultures used)

Mean Number of Young Produced within first 3 broods 23.5 (avg # 1st 3 broods for all cultures used / # cultures used)

Mean Number of Young Produced in previous 7 days 32.5 (avg # neos in prev 7 days for all cultures used / # cultures used)

Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / No (circle one) [No circled]

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
F.2.1	(Y) / N	(Y) / N	16	1	GO
4	(Y) / N	(Y) / N	13	2	GO
5	(Y) / N	(Y) / N	11	3	GO
6	(Y) / N	(Y) / N	11	4	GO
8	(Y) / N	(Y) / N	10	5	GO
3.1	(Y) / N	(Y) / N	15	6	GO
2	(Y) / N	(Y) / N	11	7	GO
4	(Y) / N	(Y) / N	12	8	GO
5	(Y) / N	(Y) / N	14	9	GO
7	(Y) / N	(Y) / N	11	10	GO
	Y / N	Y / N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032204	**Sample Name:**	SP2
Sample Method:	Grab	**Collected by:**	Not Available
Date/Time Collected:	April 27, 2022; 09:10	**Arrival Temp.:**	12.7°C
Date/Time Received:	April 29, 2022; 15:00	**Sample Description:**	Clear, light green
Sample Point Description:	Other	**Sample Type:**	Effluent
Transportation:	Road		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species (Strain #): *Raphidocelis subcapitata* (CPCC # 37)

Source: Nautilus Plant Culture Unit (from CPCC)

Culture Temp.: 24 ± 2 °C

Test Culture Number: G4(l)a

Culture Age at Test Start: 4 days old

Cell Density in the Microplate Wells at the Start of the Test: 10,454.55 cells/ml

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032204 **Sample Name:** SP2

Test Conditions

Date/Time Test Start:	April 30, 2022; 13:00	**T=0 Control pH:**	6.0
Date/Time Test End:	May 3, 2022; 11:30 - 15:15	**T=72 Control pH:**	6.0
Sample pH Before Dilution:	7.0	**pH Adjustment:**	None
Test Duration:	72 hours		

Mean Test Temperature (±Standard Deviation): 25.4 (±0.1)°C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Distilled Water

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032204 **Sample Name:** SP2

Test Conditions - continued

Enumeration Technique: Neubauer Haemacytometer

Test Vessel: 96-Well U-bottomed Polystyrene Microplate

Concentration of Test Solutions: 90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control

 # Replicates/Concentration: 4 reps started; 3 reps counted of test solutions
10 control reps started, 2 used for pH measurement

Design if Specialized Procedure: Not applicable

Method Deviations or Unusual Occurrences: None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times: Acceptable (24.0 times)

pH in controls did not vary by more than 1.5 units: Acceptable (0 units)

C V for cell yields is < 20% within the control wells: Acceptable (9.9%)

No inhibitory trend detected across the control wells: Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
252,045	247,045	254,545	297,045	242,045	217,045	269,545	227,045	250,795	9.9

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 CV = Coefficient of Variation = (100 x standard deviation / mean)

Nautilus Environmental Company Inc.
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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032204 **Sample Name:** SP2

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	999,545	947,045	417,045	277,045	294,545
2	1,184,545	714,545	294,545	387,045	312,045
3	1,197,045	919,545	379,545	384,545	309,545
Mean Cell Yield	1,127,045	860,379	363,712	349,545	305,379
Coefficient Variation[2]	10	15	17	18	3
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	302,045	269,545			
2	244,545	329,545			
3	297,045	282,045			
Mean Cell Yield	281,212	293,712			
Coefficient Variation[2]	11	11			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	**Result[1]**	**Method of Calculation**
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume[3] (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	no trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

3 - Highest concentration tested, based on test method

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032204 **Sample Name:** SP2

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software, 2001-2007 Comprehensive Environmental Toxicity, Information System - CETIS v1.8.1.2.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (90.91% v/v concentration; rep. 3). No reason to remove it. Statistics include all data.

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2206 **Date Test Initiated:** 02-May-2022

Method of Analysis: Linear Interpolation (ICPIN) **Algae Lot #:** G4(l)b

72-hour IC25 (95% Confidence Limits): 40.18 mg/L (34.42 mg/L; 44.43 mg/L)

Historic Geometric Mean IC25: 49.59 mg/L (22.46 mg/L; 109.48 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS- Thunder Bay Sample Name SP2 L270-1854.2 Sample # 8730-0032204

Conditions for Test Validity

Cell increase for control is >16	(Acceptable)/Not acceptable	24.0 (times)
CV among controls ≤ 20	(Acceptable)/Not acceptable	9.9
Result of Mann-Kendall test for trend	(Acceptable)/Not acceptable	no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 115 000 (cells/mL)

Used: Yes/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10454.55 (cells/mL)

Analysis Completed: Initials: [illegible] Date: 10/05/22

Results Verified: Initials: GO Date: 11/05/22

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location: ALS - Thunder Bay	L270 1854-2
Sample #: 8730-0032204	Sample Name: SP2
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 27.04.22 9:10	Arrival Temp.: (meter/probe) 12.7 °C (44)
Date/Time Received: 29.04.22 15:00	Sample Description: cloudy, light green
Collection Point Description: other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: Air / Road	Storage: 4±2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	GO
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # G411	GO
Culture Age at Start of Test	4 days old (must be 3 to 7 days old)	GO
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	GO
	Axenic culture? Yes/No (Circle one)	GO

Notes:

Test Conditions:

Date / Time Test Start: 30042022 1300 Started By: GO		Date / Time Test End: 03.05.22 11:30 - 15:15 Finished By: GO	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 6.9	pH adjustment: Y/N	pH of well D6 at T=0 h 6.9	pH of well D7 at T=72 h 6.0
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # ———		Metal mining NUT Lot # MCT 2202
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation)		25.4±0.1 °C	

		Day 1		Day 2	
¼ plate rotation	(Initial)	AM GO	PM GO	AM GO	PM GO
Lights ON	(Initial)	AM GO	PM GO	AM GO	PM GO

* For reference test, record pH of top concentration before use for series dilutions.

Notes:

72-Hour Qualitative Observations:

Condensation: YN (1/3)		
Growth: YN		
Were there any other method variations or deviations from methods?	Yes/No	If yes, describe further:
Anything unusual about the test?	Yes/No	
Any problems encountered?	Yes/No	
Any remedial measures taken?	Yes/No	

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 8730-0032204	Sample Name: SP2

Reference Data:

Reference Chemical Batch #	Phenol P2206	Date test started	02/05/22
Method of Analysis	ICPIN – linear interpolation	Algae Lot #	G411) b
72-hour IC25 (95% C.I.)[3] mg/L	40.18 34.42; 44.43	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	49.99 22.46; 109.48

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	> 90.91 % (N/A)	No nonlinear regression models would fit
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers		ICPIN – linear interpolation
Test for Outliers	No Outliers Present If outliers present, indicate Concentration/Rep: 90.91; rep 3	Grubbs' Test for Residual Outlier Initial C
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed. No statistically significant growth stimulation. (Yes) statistically significant growth stimulation at these concentrations: 90.91; 30.303; 10.101	Williams' or (Dunnett's) Multiple Comparison Test Initial C

1)Results relate only to the sample tested.
2) Tidepool Scientific Software © 2001-2007. Comprehensive Environmental Toxicity Information System – CETIS v. 1.9.6.7
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/(No)

Any outliers and justification for their removal? (Yes)/No

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SP2 Sample Number: 8730-0032204 Date Test Start: 30.04.22

Theoretical Test Concentration: 100.00% v/v					Actual Test Concentration: 90.91% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	101	129	123		1127045 (± 110595)
2	93	95	111		Coefficient of Variation of Cell Yield
3	99	124	113		10
4	111	130	136		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-349.388
Initials	EV	EV	EV		EV

Theoretical Test Concentration: 33.33% v/v					Actual Test Concentration: 30.303% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	93	66	94		860379 (± 127042)
2	96	76	99		Coefficient of Variation of Cell Yield
3	87	72	86		15
4	107	76	93		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-243.060
Initials	EV	EV	EV		EV

Theoretical Test Concentration: 11.11% v/v					Actual Test Concentration: 10.101% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	36	32	41		363712 (± 62766)
2	39	31	37		Coefficient of Variation of Cell Yield
3	43	29	42		17
4	53	30	36		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-45.623
Initials	EV	EV	EV		EV

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SP2 Sample Number: 8730-0032204 Date Test Start: 30.04.22

Theoretical Test Concentration: 3.704 %v/v					Actual Test Concentration: 3.367 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation)
1	22	39	40		349545 (± 62749)
2	27	35	36		Coefficient of Variation of Cell Yield
3	28	35	37		18
4	38	50	45		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-39.375
Initials	C	C	C		C

Theoretical Test Concentration: 1.235 %v/v					Actual Test Concentration: 1.122 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation)
1	33	30	34		305379 (± 9465)
2	31	31	30		Coefficient of Variation of Cell Yield
3	36	29	35		3
4	22	39	29		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-21.764
Initials	C	C	C		C

Theoretical Test Concentration: 0.412 %v/v					Actual Test Concentration: 0.374 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation)
1	38	27	30		281 212 (± 31853)
2	26	21	30		Coefficient of Variation of Cell Yield
3	35	28	27		11
4	26	26	36		Average % Inhibition (-ve number = enhancement)
5	—	—	—		- 12.128
Initials	C	C	C		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SP2 Sample Number: 8730-0032204 Date Test Start: 30.04.22

Theoretical Test Concentration: 0.137 %.v/v				Actual Test Concentration: 0.125 %.v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	28	29	29		8293712 (± 31656)
2	32	41	31		Coefficient of Variation of Cell Yield
3	31	31	33		11
4	21	35	24		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-17.112
Initials	[illegible]	[illegible]	[illegible]		[illegible]

Theoretical Test Concentration:				Actual Test Concentration:	
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:				Actual Test Concentration:	
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	SP2	Sample #	8730-0032204	Date test start	30.04.22

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	115000	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	20	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2300	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	10454.5455	=	23.98913

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	31	28	27	29	24	24	36	22
	24.0	24.0	26.0	30.0	24.0	22.0	28.0	29.0
	26	26.0	31.0	35.0	23.0	23.0	19.0	24.0
	24.0	25.0	22.0	29.0	30.0	22.0	29.0	20.0
total cells	105	103	106	123	101	91	112	95
cells/ul	262.5	257.5	265	307.5	252.5	227.5	280	237.5
cells/ml	262500	257500	265000	307500	252500	227500	280000	237500
Cell yield = measured concentration - initial algal cell concentration								
	252045	247045	254545	297045	242045	217045	269545	227045

Mean cell yield for the control	= Rc	Standard deviation	coefficient of variation	
Rc	250795	SD	24784.788	CV 9.882471 **Must be ≤20**

% inhibition summary

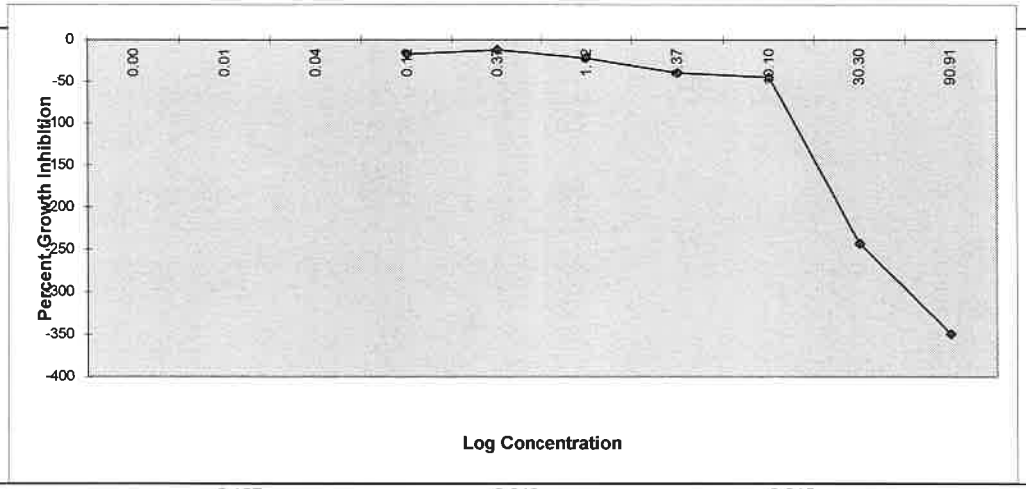
Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	-17.112
0.374	-12.128
1.122	-21.764
3.367	-39.375
10.101	-45.023
30.303	-243.060
90.910	-349.388

enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	101	129	123	93	66	94	36	32	41	22	39	40	33	30	34
	93.0	95.0	111.0	96.0	76.0	99.0	39.0	31.0	37.0	27.0	35.0	36.0	31.0	31.0	30.0
	99.0	124.0	113.0	87.0	72.0	86.0	43.0	29.0	42.0	28.0	35.0	37.0	36.0	29.0	35.0
	111.0	130.0	136.0	107.0	76.0	93.0	53.0	30.0	36.0	38.0	50.0	45.0	22.0	39.0	29.0
total cells	404	478	483	383	290	372	171	122	156	115	159	158	122	129	128
cells/ul	1010	1195	1207.5	957.5	725	930	427.5	305	390	287.5	397.5	395	305	322.5	320
cells/ml	1010000	1195000	1207500	957500	725000	930000	427500	305000	390000	287500	397500	395000	305000	322500	320000
Cell yield = measured concentration - initial algal cell concentration															
	999545	1184545	1197045	947045	714545	919545	417045	294545	379545	277045	387045	384545	294545	312045	309545
Mean Yield	1127045			860379			363712			349545			305379		
STD Yield	110595			127042			62766			62799			9465		
CV Yield	10			15			17			18			3		

Average % inhibition											
	for 90.910%:	-349.388	for 30.303%	-243.060	for 10.101%	-45.023	for 3.367%	-39.375	for 1.122%	-21.764	

Average % stimulation											
	for 90.910%:	349.388	for 30.303%	243.060	for 10.101%	45.023	for 3.367%	39.375	for 1.122%	21.764	



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
38	27	30	28	29	29									
26.0	21.0	30.0	32.0	41.0	31.0									
35.0	28.0	27.0	31.0	31.0	33.0									
26.0	26.0	36.0	21.0	35.0	24.0									
125	102	123	112	136	117	0	0	0	0	0	0	0	0	0
312.5	255	307.5	280	340	292.5	0	0	0	0	0	0	0	0	0
312500	255000	307500	280000	340000	292500	0	0	0	0	0	0	0	0	0
302045	244545	297045	269545	329545	282045	-10455	-10455	-10455	-10455	-10455	-10455	-10455	-10455	-10455
281212			293712			-10455			-10455			-10455		
31853			31656			0			0			0		
11			11			0			0			0		
for 0.374%	-12.128		for 0.125%	-17.112		for 0.042%	104.169		for 0.014%	104.169		for 0.005%	104.169	
for 0.374%	12.128		for 0.125%	17.112		for 0.042%	-104.169		for 0.014%	-104.169		for 0.005%	-104.169	

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
SP2	8730-0032204	April 27, 2022	April 29, 2022	April 29, 2022	>97% Volume[2] (Not applicable)	>97% Volume[2] (Not applicable)	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	22-Apr-2022	**Reference Chemical:**	KCl
Frond Increase IC25:	2.27 g/L	**95% Confidence Limits:**	1.92; 2.62 g/L
Historic Geometric Mean IC25:	2.24 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.5; 3.33 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	Na_2MoO_4	0.0726
	K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$2H_2O$	0.00003
	KCl	10.1	$MgSO_4\ 7H_2O$	147	$ZnCl_2$	0.00001
					$CoCl_2\ 6H_2O$	0.00001
					$CuCl_2\ 2H_2O$	

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

Date: May 19, 2022

C. D'Andrea

Carol D'Andrea
Laboratory Supervisor

Nautilus Environmental Company Inc.
***Lemna minor* 7-Day Growth Inhibition Test Bench Sheet**

Test Material

Client Name/Location: ALS – Thunder Bay	L2701854-2
Sample #: 8730-0032204	Sample Name: SP2
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 27.04.22 9:10	Arrival Temperature (meter/probe): 12.7 °C ( )
Date/Time Received: 29.04.22 15:00	Sample Description: cloudy, light green
Collection Point Description: other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: Air / Road	Storage: none

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	EB
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	EB
Culture Age at Start of Test:	9 days old, acclimated 24.0 hours in fresh test solution (mAPHA)	EB
Culture Medium:	Modified Hoagland's E+ medium, Lot # mh2201	EB
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/(No)	EB
	Axenic culture? (Yes)/No	EB
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 27 in HT 1, 31 in HT 2, 29 in HT 3 at finish	EB

Test Conditions and Procedures

Date / Time Test Start: 29.04.22 16:45 Started By: EB	Date / Time Test End: 06.05.22 10:10 Finished By: EB
Test Type: (Static (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / (No) (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: (Modified APHA (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 8.0 (1489)	pH Adjustment: none
Test Volume and Depth: 150 mL / 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / (No)	If yes, describe further:
Anything unusual about the test? Yes / (No)	
Any problems encountered? Yes / (No)	
Any remedial measures taken? Yes / (No)	Randomization Template: 5

Sample #: 8730-003 22 04	Sample Name: SP 2

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	26.4	24.3	25.1	25.3	25.1	25.3	25.2	25.2
Low	26.4	24.4	25.1	25.2	25.2	25.3	25.2	25.1
Medium	26.5	24.4	25.2	25.2	25.2	25.3	25.2	25.2
High	26.4	24.3	25.2	25.2	25.3	25.3	25.2	25.2
Initials	EB	DO	DO	CS	CS	EB	EB	EB
meter/probe	44	44	44	44	44	44	44	44
Mean Test Temperature (average of 24h high / low temperatures):					25.3 ± 0.1 °C			

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.2	8.0	8.1	7.9	EB	12/89
Day 7	**Control**	**Low**	**Medium**	**High**	**Initials**	**pH meter/probe**
	8.3	8.6	9.0	9.3	EB	13/88

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)								

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 02/05/22 (3)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 12					Initials: EB
5220	4980	4540	4830	4240	Mean Light Measurement: 4762
±15% Variation of Mean: 4048 – 5476					(Acceptable) / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) / No
If yes, describe further: Algae

Reference Data

Reference Date	(Frond Increase) or Dry Weights (circle one)			
	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
22/04/22	2.27	1.92; 2.62	2.24	1.80; 3.33

Sample #: 8730-003 2204	Sample Name: SP2

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 52	B 49	C 48	D 51	50.0

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy							Initials: C
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV : 44.0 (1.8) 4.1							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control (% v/v g/L)	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-5.11	-1.70	-14.20	-10.80	2.27	1.70	-2.84
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control (% v/v g/L)	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-3.75	-8.01	-14.99	-21.33	-12.16	-8.41	-13.14

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: (Yes) / Not applicable (no stimulation) (Circle one)

Test Endpoints and Calculations: Fronds

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	>97% (N/A)	Nonlinear regression 2P Exponential model
IC25 (95% C.I.)[3] If calculated without outlier[4]	—	—
Dry Weights		
IC25 (95% C.I.)[3]	>97% (N/A)	No nonlinear regression model would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN - linear interpolation

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019. Comprehensive Environmental Toxicity Information System CETISv 1.9.6.7
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / (No)

Any outliers and justification for their removal? (Yes) / No

Fronds (rep 1; conc 9.7% v/v)

Lemna minor D Observations

Client: ALS Thunder Bay	Sample number: 8730-0032204	Date Started: 29.04.22	
Site: SP 2		Date Ended: 06.05.22	
Concentration: Control	Observations By: D	Concentration: 0.097	Observations By: D

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	52	49	48	51	Number of	48	47	48	48
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X	Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations					Other Observations				

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO
Weights: YES / NO
N/A

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO)
Weights: YES / (NO)

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Observations

Client: ALS Thunder Bay	Sample number: 8730-0032204	Date Started: 29.04.22	
Site: SP2		Date Ended: 06.05.22	
Concentration: 0.29% v/v	Observations By: D	Concentration: 0.97% v/v	Observations By: D

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	47	46	49	55	Number of	45	42	42	46
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations					Other Observations				
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor D Observations

Client: ALS Thunder Bay	Sample number: 8730-0032204	Date Started: 24.04.22	
Site: SP2		Date Ended: 06.05.22	
Concentration: 3.1% v/v	Observations By: D	Concentration: 9.7% v/v	Observations By: D

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	46	42	46	47	Number of	61	55	43	45
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations					Other Observations				
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: (YES) / NO Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor D. Observations

Client: ALS Thunder Bay	**Sample number:** 8730-0032204	**Date Started:** 29.04.22	
Site: SP 2		**Date Ended:** 06.05.22	
Concentration: 31% v/v	**Observations By:** D	**Concentration:** 47% v/v	**Observations By:** D

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	49	54	54	46	**Number of**	52	50	51	42
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	X	√√ Smaller	**Abnormally sized fronds**	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations	algae →			→	**Other Observations**	algae →			→
Growth Stimulation (Hormesis) at this concentration? Fronds: (YES) / NO Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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(02)

Lemna minor Weights

Client	Site	Sample number
ALS - Thunder Bay	SP2	8730 20032204

In Oven Date/Time/ °C:	Out Oven Date/Time/°C:
06.05.22 10:20 64°	07/05/22 10:20 68°

Conc. 1.	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	46	44.0 (1.8)	0.67194	0.66766	4.28	4.34 (0.1)
	B	43		0.67544	0.67114	4.30	
	C	42		0.67688	0.67256	4.32	
	D	45		0.67791	0.67346	4.45	
0.097	A	42	41.8 (0.5)	0.67205	0.66799	4.06	4.18 (0.2)
	B	41		0.67250	0.66810	4.34	
	C	42		0.68192	0.67797	3.95	
	D	42		0.67926	0.67491	4.35	
0.29	A	41	43.3 (4.0)	0.66873	0.66483	3.90	3.99 (0.3)
	B	40		0.67638	0.67276	3.62	
	C	43		0.66476	0.66069	4.07	
	D	49		0.66754	0.66317	4.37	
0.97	A	39	37.8 (2.1)	0.66527	0.66159	3.68	3.69 (0.2)
	B	36		0.65935	0.65588	3.47	
	C	36		0.67550	0.67192	3.58	
	D	40		0.67345	0.66943	4.02	
3.1	A	40	39.3 (2.2)	0.67913	0.67608	3.05	3.41 (0.3)
	B	36		0.67398	0.67066	3.32	
	C	40		0.66747	0.66402	3.45	
	D	41		0.67129	0.66746	3.83	
9.7	A	55	45.0 (8.5)	0.67200	0.66757	4.43	3.81 (0.5)
	B	49		0.68507	0.68116	3.91	
	C	37		0.67668	0.67324	3.44	
	D	39		0.67522	0.67176	3.46	
31	A	43	44.8 (3.9)	0.67609	0.67251	3.58	3.97 (0.4)
	B	48		0.67329	0.66909	4.20	
	C	48		0.68368	0.67923	4.45	
	D	40		0.68000	0.67634	3.66	
97	A	46	42.8 (4.6)	0.67086	0.66690	3.96	3.77 (0.3)
	B	44		0.68468	0.68070	3.98	
	C	45		0.66736	0.66350	3.86	
	D	36		0.68183	0.67856	3.27	
Initials		NF	NP	TB	AD	NP	NF

Notes:

RG

Sample name: SP2 | Date started 29/04/22 | sample # 8730-0032204

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

50.0 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	52	48	47	45	46	61	49	52
	49	47	46	42	42	55	54	50
	48	48	49	42	46	43	54	51
	51	48	55	46	47	45	46	42
Total Fronds	200	191	197	175	181	204	203	195
Increase in Frond Number = Total # Fronds - 6 Starting Fronds								
	46	42	41	39	40	55	43	46
	43	41	40	36	36	49	48	44
	42	42	43	36	40	37	48	45
	45	42	49	40	41	39	40	36
Total Increase	176	167	173	151	157	180	179	171
Mean Increase	44.0	41.8	43.3	37.8	39.3	45.0	44.8	42.8
SD Increase	1.8	0.5	4.0	2.1	2.2	8.5	3.9	4.6
CV Increase	4.1	1.2	9.3	5.5	5.6	18.9	8.8	10.7
% Stimulation		**-5.11**	**-1.70**	**-14.20**	**-10.80**	**2.27**	**1.70**	**-2.84**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.67194	0.67205	0.66873	0.66527	0.67913	0.67200	0.67609	0.67086
Pan + Plant	0.67544	0.67250	0.67638	0.65935	0.67398	0.68507	0.67329	0.68468
	0.67688	0.68192	0.66476	0.67550	0.66747	0.67668	0.68368	0.66736
	0.67791	0.67926	0.66754	0.67345	0.67129	0.67522	0.68000	0.68183
Initial Weight (g)	0.66766	0.66799	0.66483	0.66159	0.67608	0.66757	0.67251	0.66690
Pan Only	0.67114	0.66816	0.67276	0.65588	0.67066	0.68116	0.66909	0.68070
	0.67256	0.67797	0.66069	0.67192	0.66402	0.67324	0.67923	0.66350
	0.67346	0.67491	0.66317	0.66943	0.66746	0.67176	0.67634	0.67856
Plant Only (mg)	4.28	4.06	3.90	3.68	3.05	4.43	3.58	3.96
	4.30	4.34	3.62	3.47	3.32	3.91	4.20	3.98
	4.32	3.95	4.07	3.58	3.45	3.44	4.45	3.86
	4.45	4.35	4.37	4.02	3.83	3.46	3.66	3.27
Mean Dry Weight	4.338	4.175	3.990	3.688	3.412	3.810	3.972	3.767
SD Dry Weight	0.1	0.2	0.3	0.2	0.3	0.5	0.4	0.3
CV Dry Weight	1.8	4.8	7.9	6.4	9.5	12.3	10.6	8.9
% Stimulation		**-3.75**	**-8.01**	**-14.99**	**-21.33**	**-12.16**	**-8.41**	**-13.14**

For Data Transfer to CETIS

	# fronds	total mass	tare
0	1	46	0.67194	0.66766
	2	43	0.67544	0.67114
	3	42	0.67688	0.67256
	4	45	0.67791	0.67346
0.1	1	42	0.67205	0.66799
	2	41	0.67250	0.66816
	3	42	0.68192	0.67797
	4	42	0.67926	0.67491
0.3	1	41	0.66873	0.66483
	2	40	0.67638	0.67276
	3	43	0.66476	0.66069
	4	49	0.66754	0.66317
1	1	39	0.66527	0.66159
	2	36	0.65935	0.65588
	3	36	0.67550	0.67192
	4	40	0.67345	0.66943
3.1	1	40	0.67913	0.67608
	2	36	0.67398	0.67066
	3	40	0.66747	0.66402
	4	41	0.67129	0.66746
9.7	1	55	0.67200	0.66757
	2	49	0.68507	0.68116
	3	37	0.67668	0.67324
	4	39	0.67522	0.67176
31	1	43	0.67609	0.67251
	2	48	0.67329	0.66909
	3	48	0.68368	0.67923
	4	40	0.68000	0.67634
97	1	46	0.67086	0.66690
	2	44	0.68468	0.68070
	3	45	0.66736	0.66350
	4	36	0.68183	0.67856

AO 17/05/22

New Gold Inc. Rainy River Project
ATTN: Garnet Cornell
24 Marr Rd
Barwick ON P0W 1A0

Date Received: 09-JUN-22
Report Date: 06-JUL-22 21:00 (MT)
Version: FINAL

Client Phone: 807-234-8200

Certificate of Analysis

Lab Work Order #: L2713601

Project P.O. #: 4500062842
Job Reference: SUBLETHAL TOXICITY
C of C Numbers:
Legal Site Desc:

Christine Paradis
Project Manager

ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
L2713601-1 EDL1 Sampled By: Client on 08-JUN-22 @ 08:10 Matrix: Effluent							
Miscellaneous							
Special Request	See Attached					18-JUN-22	R5812758
Special Request	See Attached					18-JUN-22	R5812758
Special Request	See Attached					14-JUN-22	R5812758
Special Request	See Attached					17-JUN-22	R5812758
L2713601-2 EDL2 Sampled By: Client on 08-JUN-22 @ 08:10 Matrix: Effluent							
Miscellaneous							
Special Request	See Attached					18-JUN-22	R5812758
Special Request	See Attached					18-JUN-22	R5812758
Special Request	See Attached					14-JUN-22	R5812758
Special Request	See Attached					16-JUN-22	R5812758
L2713601-3 SED 2 DIS Sampled By: Client on 08-JUN-22 @ 08:40 Matrix: Effluent							
Miscellaneous							
Special Request	See Attached					18-JUN-22	R5812758
Special Request	See Attached					18-JUN-22	R5812758
Special Request	See Attached					14-JUN-22	R5812758
Special Request	See Attached					16-JUN-22	R5812758

* Refer to Referenced Information for Qualifiers (if any) and Methodology.

Reference Information

Test Method References:

ALS Test Code	Matrix	Test Description	Method Reference**
TOX-7DINHIB-LM-TB	Misc.	Growth Inhibition using Lemma minor	SEE SUBLET LAB RESULTS
Lemna minor 7-day growth toxicity test, based on the protocol "Biological Test Method: Test Method for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, Lemna minor", Report EPS 1/RM/37, Second Edition (January 2007)			
TOX-GROWTH-FH-TB	Misc.	7 Day Survival & Growth Fat Head Minnows	SEE SUBLET LAB RESULTS
Fathead minnow 7-day test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Environmental Protection Series, Ottawa, ON, Report EPS1/RM/22, Second Edition (February 2011).			
TOX-INHIB-PS-TB	Misc.	Growth Inhibition Pseudokirchneriella	SEE SUBLET LAB RESULTS
Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) 72-hour growth toxicity test, based upon protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga" Report EPS1/RM/25, Second Edition (March 2007).			
TOX-REPRO-CD-TB	Misc.	Survival & reproduction (Ceriodaphnia)	SEE SUBLET LAB RESULTS
Ceriodaphnia dubia 3-brood toxicity test, accroding to protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dusbia", Environmental Protection Series, Ottawa, ON, Report EPS 1/RM/21, Second Edition (February 2007).			

** ALS test methods may incorporate modifications from specified reference methods to improve performance.

The last two letters of the above test code(s) indicate the laboratory that performed analytical analysis for that test. Refer to the list below:

Laboratory Definition Code	Laboratory Location
TB	ALS ENVIRONMENTAL - THUNDER BAY, ONTARIO, CANADA

Chain of Custody Numbers:

GLOSSARY OF REPORT TERMS

Surrogates are compounds that are similar in behaviour to target analyte(s), but that do not normally occur in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery. In reports that display the D.L. column, laboratory objectives for surrogates are listed there.
mg/kg - milligrams per kilogram based on dry weight of sample
mg/kg wwt - milligrams per kilogram based on wet weight of sample
mg/kg lwt - milligrams per kilogram based on lipid weight of sample
mg/L - unit of concentration based on volume, parts per million.
< - Less than.
D.L. - The reporting limit.
N/A - Result not available. Refer to qualifier code and definition for explanation.

Test results reported relate only to the samples as received by the laboratory.
UNLESS OTHERWISE STATED, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.
Analytical results in unsigned test reports with the DRAFT watermark are subject to change, pending final QC review.

Quality Control Report

Workorder: L2713601 Report Date: 06-JUL-22

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed

Quality Control Report

Workorder: L2713601 Report Date: 06-JUL-22

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Legend:

Limit	ALS Control Limit (Data Quality Objectives)
DUP	Duplicate
RPD	Relative Percent Difference
N/A	Not Available
LCS	Laboratory Control Sample
SRM	Standard Reference Material
MS	Matrix Spike
MSD	Matrix Spike Duplicate
ADE	Average Desorption Efficiency
MB	Method Blank
IRM	Internal Reference Material
CRM	Certified Reference Material
CCV	Continuing Calibration Verification
CVS	Calibration Verification Standard
LCSD	Laboratory Control Sample Duplicate

Hold Time Exceedances:

All test results reported with this submission were conducted within ALS recommended hold times.

ALS recommended hold times may vary by province. They are assigned to meet known provincial and/or federal government requirements. In the absence of regulatory hold times, ALS establishes recommendations based on guidelines published by the US EPA, APHA Standard Methods, or Environment Canada (where available). For more information, please contact ALS.

The ALS Quality Control Report is provided to ALS clients upon request. ALS includes comprehensive QC checks with every analysis to ensure our high standards of quality are met. Each QC result has a known or expected target value, which is compared against pre-determined data quality objectives to provide confidence in the accuracy of associated test results.

Please note that this report may contain QC results from anonymous Sample Duplicates and Matrix Spikes that do not originate from this Work Order.

June 30, 2022

Christine Paradis,
ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Dear Christine:

On June 10, 2022, Nautilus Environmental Company Inc. personnel received one water sample (EDL1 L2713601-1) from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected June 8, 2022

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
EDL1 L2713601-1 #8730-0032234	Fathead Minnow	7-day LC50 (95% Confidence) 7-day IC25 (95% Confidence)	Survival Biomass	>100% Volume (Not Applicable) >100% Volume (Not Applicable)
	Ceriodaphnia dubia	3-brood LC50 (95% Confidence) 3-brood IC25 (95% Confidence)	Survival Reproduction	>100% Volume (Not Applicable) 0.32% Volume (0.13; 44.36% Volume)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[2] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence) 7-day IC25 Dry Weight (95% Confidence)	Growth Growth	39.99% Volume (28.36; 71.02% Volume) 0.27% Volume (Not applicable)

1 - Results relate only to the sample tested
2 - Highest concentration tested, based on test method

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 100%, 33%, 11%, 3.7% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted half by half (*i.e.,* 100%, 50%, 25% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

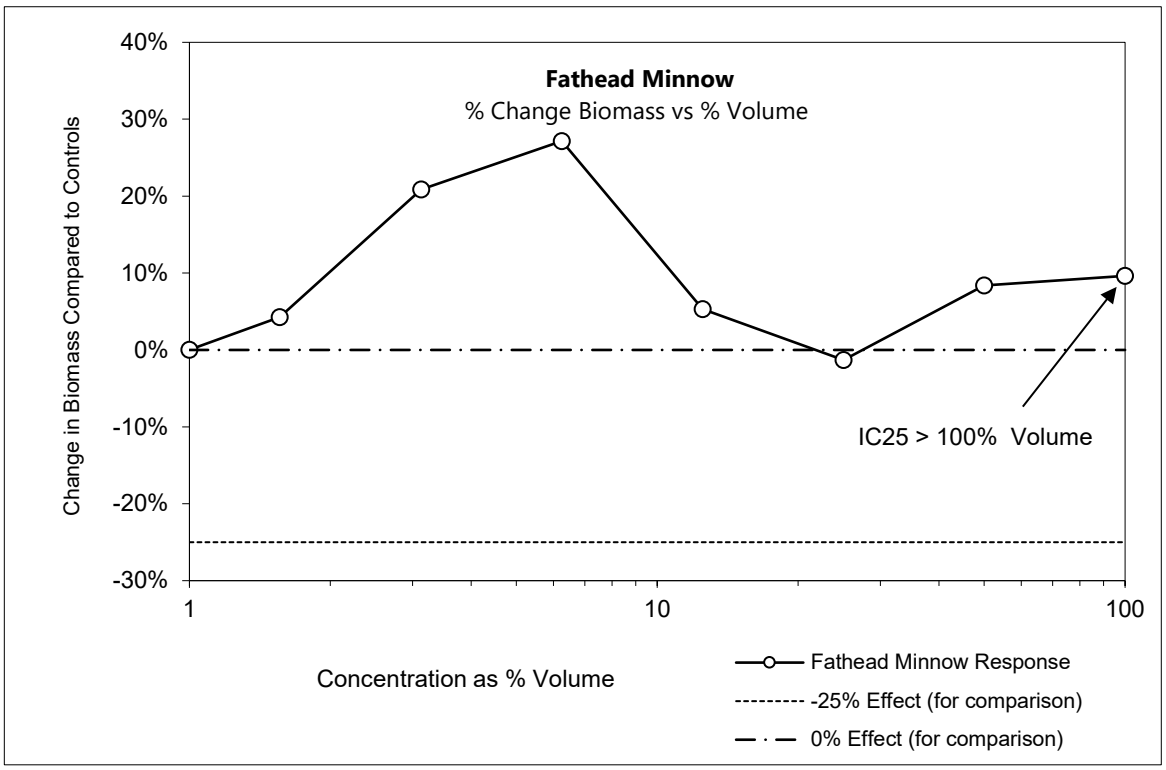
If there are any further details which you require, please do not hesitate to contact us.

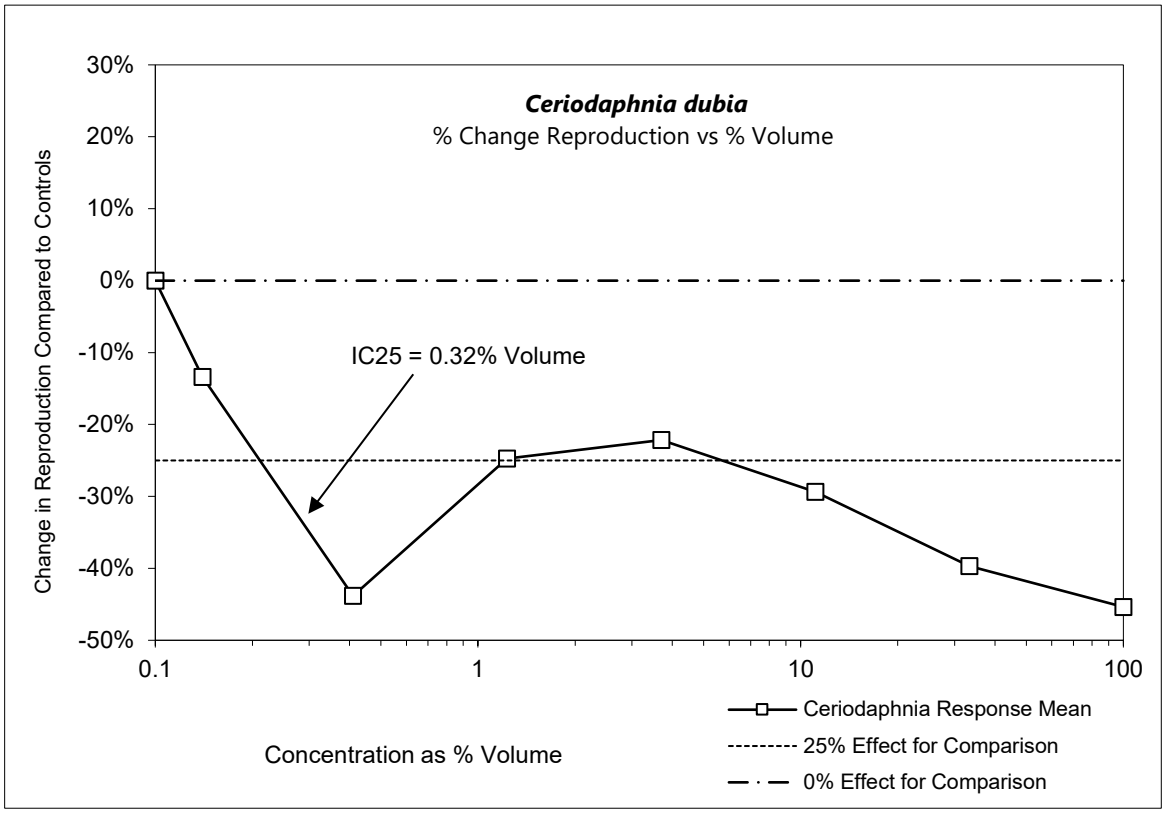
Yours very truly,
Nautilus Environmental Company Inc.

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2022\8000\8730-003\8730-0032234 FCRL

ALS Thunder Bay - EDL1 (L2713601-1) - June 8, 2022





P:\Data\IC25\2022\8730-0032234

Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	EDL1 L2713601-1
Date/Time Sampled:	June 8, 2022; 08:10	**Date/Time Received:**	June 10, 2022; 11:10
Date/Time Test Started:	June 11, 2022; 15:10	**Date Test Finished:**	June 18, 2022
Description:	Clear, light green	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032234	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.5°C
Collected By:	Not available		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species:	Fathead Minnow (*Pimephales promelas*)		
Source:	Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)		
Culture Temp.:	22 to 26 °C	**Life Stage:**	<24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 1.0%

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032234 **Sources:** EDL1 L2713601-1

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Test Conditions

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 0 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (3.3%)

Average Weight of Controls is ≥ 250 µg: Acceptable (527 µg)

Nautilus Environmental Company Inc.
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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032234 **Sources:** EDL1 L2713601-1

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	10	0	0	10	0	0	0	0
	2	0	0	0	0	0	10	0	10
	3	0	0	0	10	10	10	30	10
Mean % Mortality		3.3	0.0	0.0	6.7	3.3	6.7	10.0	6.7
S.D.		5.8	0.0	0.0	5.8	5.8	5.8	17.3	5.8
Survival/Growth Data									
Mean Dry Biomass	1	0.484	0.513	0.655	0.602	0.495	0.523	0.582	0.572
(mg)	2	0.511	0.545	0.671	0.590	0.530	0.533	0.607	0.610
	3	0.533	0.535	0.521	0.751	0.584	0.452	0.467	0.493
% Effect (+ or -)		0.0	4.3	20.9	27.2	5.3	-0.7	8.4	9.6
Mean Dry Biomass/ Concentration (mg)		0.509	0.531	0.616	0.648	0.536	0.503	0.552	0.558
S.D.		0.02	0.02	0.08	0.09	0.04	0.04	0.07	0.06

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032234 **Sources:** EDL1 L2713601-1

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival		
7-day LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Biomass **(Survival and Growth)**		
7-day IC25 (Confidence Interval)[2]	>100% Volume (Not Applicable)	Non-linear Regression 2P Linear Model

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 02-Jun-2022 **Reference Batch #:** Zn2102

Method of Analysis: Trimmed Spearman-Karber α = 10%

7-Day LC50 (95% Confidence Limits): 0.61 mg/L (0.49 mg/L; 0.76 mg/L)

Historic Geometric Mean LC50: 0.70 mg/L (0.41 mg/L; 1.21 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS - TB Sample Name: EDL-1 Sample #: 8730-0032234
L2713601-1

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable / Not Acceptable) 3.3 %

Average Weight of Controls is ≥ 250 µg: (Acceptable / Not Acceptable) 527 µg

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 60 min Days: 0 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
7-day LC50	> 100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
Survival / Growth Biomass		Nonlinear regression 2P Linear ~~regression~~ model
7-day IC25	> 100 % Volume	
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 23/06/22

Results Verified: Initials al Date 26/06/22

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** EDL-1 L2713601-1 **Sample #:** 3730.00322934

Day	Date 2020	Initial Variables				Meters/Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	D.O. / °C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	11.06	23.9	7.9	8.6	1313	6/4	13/88	5/6	Yes	≤100	≤ 20	1	C
1	12	26.1	7.9	9.4	1375	6/4	13/88	5/6	Yes	≤100	≤ 20	1	C
2	13	26.2	7.9	9.8	1411	6/4	13/88	5/6	Yes	≤100	≤ 20	1	KK
3	14	25.0	7.8	9.9	1391	6/4	13/88	5/6	yes	≤100	≤ 20	2	KK
4	15	25.0	7.8	10.4	1388	6/4	13/88	5/6	yes	≤100	≤ 20	2	KK
5	16	24.5	7.6	10.8	1360	6/4	13/88	5/6	Yes	≤100	≤ 20	3	C
6	17	25.8 ~~26.4~~ KK	7.9	11.2	1322	6/4	13/88	5/6	yes	≤100	≤ 20	3	KK
7	18												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of brine shrimp days 0 to 6? (Yes)/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: Control   Sample Name: EDL-1 L2713601-1   Sample #: 8730.00322234

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06	24.7	8.0	7.4	269	6/4	13/88	5/6	C
1	12	24.6	8.2	7.9	259	6/4	13/88	5/6	C
2	13	25.0	7.8	7.7	270	6/4	13/88	5/6	SO
3	14	24.6	8.1	7.4	264	6/4	13/88	5/6	SO
4	15	24.6	7.7	7.6	267	6/4	13/88	5/6	SO
5	16	25.6	7.9	7.9	256	6/4	13/88	5/6	KK
6	17	25.7	8.2	7.7	~~245~~ 250 KK	6/4	13/88	5/6	~~KK~~ KK
7	18								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	23.5	8.0	7.9	6/4	13/88	C							
1	23.6	7.9	7.9	5/5	12/89	KK	0	0	0	0	0	0	CS
2	23.6	7.9	7.3	5/5	12/89	KK	0	0	0	0	0	0	CS
3	23.7	7.6	6.3	6/4	13/88	SO	0	0	0	0	0	0	KK
4	~~23.7~~ 24.1 SO	~~7.6~~ 7.5 SO	6.3	6/4	13/88	KK	~~10~~ 0 SO	0	0	10[A]	0	0	SO
5	23.8	7.7	6.6	6/4	13/88	KK	10	0	0	0	0	0	CS
6	25.4	7.6	5.6	6/4	13/88	SO	10	0	0	0	0	0	KK
7							10	0	0	0	0	0	KK

Observations: ____________________   A kinked tail

Concentration: 1.56% WW

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06	24.5	7.8	8.3	276	6/4	13/88	5/6	C
1	12	24.5	8.0	7.9	278	6/4	13/88	5/6	C
2	13	24.0	8.0	~~8.0~~ 7.7 SO	282	6/4	13/88	5/6	SO
3	14	24.7	8.1	~~8~~ 7.5 SO	286	6/4	13/88	5/6	SO
4	15	24.5	7.8	7.5	289	6/4	13/88	5/6	SO
5	16	25.0	7.9	7.9	264	6/4	13/88	5/6	KK
6	17	25.3	8.2	7.8	267	6/4	13/88	5/6	KK
7	18								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	23.8	7.9	6.8	6/4	13/88	C							
1	23.6	7.9	7.8	5/5	12/89	KK	0	0	0	0	0	0	CS
2	23.6	7.8	7.0	5/5	12/89	KK	0	0	0	0	0	0	CS
3	23.7	7.6	6.2	6/4	13/88	SO	0	0	0	0	0	0	KK
4	24.0	7.5	6.1	6/4	13/88	KK	0	0	0	0	10[B]	0	SO
5	23.8	7.7	6.5	6/4	13/88	KK	0	0	0	0	0	0	CS
6	25.5	7.5	5.0	6/4	13/88	SO	0	0	0	0	0	0	KK
7							0	0	0	0	0	0	KK

Observations: ____________________

B LOE

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13% v/v   Sample Name: EDC-1 L2713601-1   Sample #: 8730.0032234

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	11.06																					
1	12															0	0	0	0	0	0	CJ
2	13															0	0	0	0	0	0	CJ
3	14															0	0	0	0	0	0	KK
4	15															0	0	0	0	0	0	SO
5	16															0	0	0	0	0	0	CJ
6	17															0	0	0	0	0	0	KK
7	18															0	0	0	0	0	0	KK

Observations: ______________________

Concentration: 6.25% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	11.06																					
1	12															10	0	10	0	0	0	CJ
2	13															10	0	10	0	0	0	CJ
3	14															10	0	10	0	0	0	KK
4	15															10	0	10	0	0	0	SO
5	16															10	0	10	0	0	0	CJ
6	17															10	0	10	0	0	0	KK
7	18															10	0	10	0	0	0	KK

Observations: ______________________

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5% v/v   Sample Name: EDL-1 L0713601-1   Sample #: 3730.00320234

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06	24.5	7.91	8.1	414	6/4	13/88	5/6	[illegible]
1	12	24.6	8.0	7.9	407	6/4	13/88	5/6	[illegible]
2	13	24.3	8.1	7.7	423	6/4	13/88	5/6	SO
3	14	24.7	8.0	7.3	426	6/4	13/88	5/6	SO
4	15	24.7	7.8	7.6	429	6/4	13/88	5/6	SO
5	16	25.1	8.0	7.9	389	6/4	13/88	5/6	KK
6	17	25.4	8.1	7.9	393	6/4	13/88	5/6	KK
7	18								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	23.8	7.9	7.4	6/9	13/88	[illegible]							
1	23.6	7.8	7.9	5/5	12/89	KK	0	0	0	0	0	0	CS
2	23.6	7.8	7.2	5/5	12/89	KK	0	0	0	0	0	0	CS
3	23.7	7.6	6.0	6/4	13/88	SO	0	0	0	0	0	0	KK
4	24.1	7.5	5.5	6/4	13/88	KK	0	0	10	0	0	0	[illegible]
5	23.7	7.7	6.4	6/4	13/88	KK	0	0	10	0	0	0	CS
6	25.4	7.6	5.5	6/4	13/88	SO	0	0	10	0	0	0	KK
7							0	0	10	0	0	0	KK

Observations: ____________________

Concentration: 25% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06								
1	12								
2	13								
3	14								
4	15								
5	16								
6	17								
7	18								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0													
1							0	0	0	0	0	0	CS
2							0	0	0	0	0	0	CS
3							0	0	10	0	0	0	KK
4							0	0	10	0	0	0	[illegible]
5							0	10	10	0	0	0	CS
6							0	10	10	0	0	0	KK
7							0	10	10	0	0	0	KK

Observations: ____________________

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50% v/v Sample Name: EDL-1 L2713601-1 Sample #: 3730.00322234

Day	Date 2022	Initial °C	Initial pH (units)	Initial D.O. (mg/L)	Initial Cond (µmhos)	Meter/Probe D.O. / °C	Meter/Probe pH	Meter/Probe cond	Initials	Final °C	Final pH (units)	Final D.O. (mg/L)	Meter/Probe D.O. / °C	Meter/Probe pH	Initials	% Mortality / Rep A	% Mortality / Rep B	% Mortality / Rep C	% Atypical / Rep A	% Atypical / Rep B	% Atypical / Rep C	Initials
0	11.06																					
1	12															0	0	0	0	0	0	CS
2	13															0	0	10	0	0	0	CS
3	14															0	0	30	0	0	0	KK
4	15															0	0	30	0	0	0	[illegible]
5	16															0	0	30	0	0	0	CS
6	17															0	0	30	0	0	0	KK
7	18															0	0	30	0	0	0	KK

Observations: ______________________

Concentration: 100% v/v

Day	Date 2022	Initial °C	Initial pH (units)	Initial D.O. (mg/L)	Initial Cond (µmhos)	Meter/Probe D.O. / °C	Meter/Probe pH	Meter/Probe cond	Initials	Final °C	Final pH (units)	Final D.O. (mg/L)	Meter/Probe D.O. / °C	Meter/Probe pH	Initials	% Mortality / Rep A	% Mortality / Rep B	% Mortality / Rep C	% Atypical / Rep A	% Atypical / Rep B	% Atypical / Rep C	Initials
0	1106	24.6	7.9	8.8	1304	6/4	13/88	5/6	U	23.8	7.9	7.3	6/4	13/88	U							
1	12	25.4	7.9	8.5	1325	6/4	13/88	5/6	U	23.6	8.0	7.9	5/5	12/89	KK	0	0	0	0	0	0	CS
2	13	25.4	7.9	8.5	1390	6/4	13/88	5/6	SO	23.6	7.9	7.2	5/5	12/89	KK	0	0	0	0	0	0	CS
3	14	25.0	7.9	8.3	1390	6/4	13/88	5/6	SO	23.6	7.9	6.3	6/4	13/88	SO	0	0	0	0	0	0	KK
4	15	25.4	7.9	8.3	1402	6/4	13/88	5/6	SO	23.9	7.7	5.7	6/4	13/88	KK	0	10	0	0	0	0	[illegible]
5	16	25.0	7.9	8.6	1270	6/4	13/88	5/6	KK	23.7	7.9	6.6	6/4	13/88	KK	0	10	0	0	0	A 10	CS
6	17	25.6	8.0	8.9	1316	6/4	13/88	5/6	KK	25.4	7.8	5.4	6/4	13/88	SO	0	10	0	0	0	0	KK
7	18															0	10	10	0	0	0	KK

Observations: ______________________

ALOE

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information
Client ALS EB
Sample # 330-483234
Date/Time Received 101022 / 1110
Sample Type effluent
100% Hardness 374

Sample Name EDL-1 L2713601-1
Sample Date/Time 070622 / 0810
Person Sampling n/a
Arrival Temp 17.5 °C
Sample Description clear light green

Test Information
Date/Time Started 11.06.22 / 1510
Test started by CD
Fathead Batch # FH0121 6621
Date eggs laid 06/07/08.06.22
Culture mortality within 7 days of egg collection 1.0%
Swim bladder inflated; yes / no yes
Age of Larvae at start of test in hours 24
Control Hardness 90
Water Bath Quadrant H
Average Temperature during Test: 25.4 ± 0.1 °C
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / No (circle one)

Conc.	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	9	0.76488	0.76004	0.484	0.509
	B	10	0.75327	0.74816	0.511	
	C	10	0.76055	0.75522	0.533	
1.56	A	10	0.75603	0.75090	0.513	0.531
	B	10	0.76909	0.76364	0.545	
	C	10	0.75881	0.75346	0.535	
3.13	A	10	0.76691	0.76036	0.655	0.616
	B	10	0.75826	0.75155	0.671	
	C	10	0.76494	0.75973	0.521	
6.25	A	9	0.77208	0.76606	0.602	0.648
	B	10	0.76045	0.75455	0.590	
	C	9	0.75942	0.75486	0.751	
12.5	A	10	0.75553	0.75058	0.495	0.536
	B	10	0.75817	0.75287	0.530	
	C	9	0.75847	0.75263	0.584	
25	A	10	0.76166	0.7563	0.523	0.503
	B	9	0.77186	0.76608	0.603	
	C	9	0.76497	0.76045	0.452	
50	A	10	0.77353	0.76771	0.582	0.552
	B	10	0.76585	0.75979	0.607	
	C	7	0.76103	0.75636	0.467	
100	A	10	0.75719	0.75147	0.572	0.558
	B	9	0.76433	0.75823	0.610	
	C	9	0.76993	0.76500	0.493	
Initials		CG	SO	SO	CG	CG

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8730-0032234 **Sample Name** EDL-1 / L2713601-1

Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug) 527

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	9	0.76488	0.76004	0.538	0.484	0.527	0.509	0.014282	0.024542	2.70875
	10	10	0.75327	0.74816	0.511	0.511					
	10	10	0.76055	0.75522	0.533	0.533					
1.56	10	10	0.75603	0.75090	0.513	0.513	0.531	0.531	0.016371	0.016371	3.082995
	10	10	0.76909	0.76364	0.545	0.545					
	10	10	0.75881	0.75346	0.535	0.535					
3.13	10	10	0.76691	0.76036	0.655	0.655	0.616	0.616	0.082373	0.082373	13.3795
	10	10	0.75826	0.75155	0.671	0.671					
	10	10	0.76494	0.75973	0.521	0.521					
6.25	10	9	0.77208	0.76606	0.669	0.602	0.698	0.648	0.124757	0.08969	17.87912
	10	10	0.76045	0.75455	0.590	0.590					
	10	9	0.75942	0.75191	0.834	0.751					
12.5	10	10	0.75553	0.75058	0.495	0.495	0.558	0.536	0.080665	0.044837	14.45711
	10	10	0.75817	0.75287	0.530	0.530					
	10	9	0.75847	0.75263	0.649	0.584					
25	10	10	0.76166	0.75643	0.523	0.523	0.539	0.503	0.047123	0.044163	8.740259
	10	9	0.77141	0.76608	0.592	0.533					
	10	9	0.76497	0.76045	0.502	0.452					
50	10	10	0.77353	0.76771	0.582	0.582	0.619	0.552	0.043764	0.074666	7.073299
	10	10	0.76585	0.75978	0.607	0.607					
	10	7	0.76103	0.75636	0.667	0.467					
100	10	10	0.75719	0.75147	0.572	0.572	0.599	0.558	0.069132	0.059685	11.53772
	10	9	0.76433	0.75823	0.678	0.610					
	10	9	0.76993	0.76500	0.548	0.493					

SD: Standard Deviation
CV: Coefficient of Variation

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Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	EDL1 L2713601-1
Date/Time Sampled	June 8, 2022; 08:10	**Date/Time Received:**	June 10, 2022; 11:10
Date Test Started:	June 10, 2022; 17:10	**Date Test Finished:**	June 17, 2022
Description:	Clear, light green	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032234	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.5°C
Collected By:	Not available		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032234 **Sources:** EDL1 L2713601-1

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 0%

Mean Number of Surviving Young Produced Within First 3 Broods: 21.6

Mean Number of Surviving Young Produced Within 7 Days of Testing: 31.3

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 0 to 6

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032234 **Sources:** EDL1 L2713601-1

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD22-73

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (19.4 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (100% of controls)

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032234 **Sources:** EDL1 L2713601-1

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	0	20	10	0	0	0	0
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	18	18	8	3	7	15	12	17
	2	24	16	8	7	7	16	6	3
	3	20	21	0	16	14	4	16	16
	4	20	13	14	16	18	20	8	8
	5	22	20	19	16	20	10	34	18
	6	19	20	13	24	10	11	13	0
	7	21	10	2	22	25	8	2	6
	8	11	18	12	11	22	19	0	10
	9	18	13	17	20	13	18	15	19
	10	21	19	16	11	15	16	11	9
Total Number of Live Neonates in First 3 Broods or Less		194	168	109	146	151	137	117	106
% Effect (+ or -)		0.0	-13.4	-43.8	-24.7	-22.2	-29.4	-39.7	-45.4
Mean Number of Live Neonates in First 3 Broods or Less		19.4	16.8	10.9	14.6	15.1	13.7	11.7	10.6
SD		3.5	3.7	6.3	6.6	6.2	5.2	9.5	6.6

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032234 **Sources:** EDL1 L2713601-1

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	0.32% Volume (0.13; 44.36% Volume)	Non-linear regression models didn't produce reasonable IC25 value Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (33.33% v/v concentration; rep. 5). No reason to remove it. Statistics include all data.

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 02-Jun-2022 **Reference Batch #:** Zn2102

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 0.15 mg/L (0.12 mg/L; 0.19 mg/L)

Historic Geometric Mean LC50: 0.08 mg/L (0.03 mg/L; 0.27 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS - TB Sample Name: EDL-1 Sample #: 8730 0032234
L 2713601-1

Conditions for Test Validity

Control Mortality is< 20%: (Acceptable) / Not Acceptable: 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable) / Not Acceptable: 10 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable) / Not Acceptable: 19.64 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 0 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	> 100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
REPRODUCTION		Non linear regression models didn't produce reasonable IC_{25} value
3-brood IC25	0.32 % Volume	
95% Confidence Interval[2]	0.13 ; 44.36 % Volume	ICPIN – linear interpolation

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: (Yes) / No

Concentration(s) & Rep(s): 33.33 ; rep 5

Analysis Completed: Initials EV Date 23/06/22

Results Verified: Initials al Date 26/06/22

Ceriodaphnia dubia Initial Sample Measurements Before Preparation and Use in Toxicity Te

Concentration: 100% **Sample Name:** EDL-1 L2713601-1 **Sample #:** 9730 0082234

Day	Date 2022	Initial Measurements				Meters / Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	°C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	10:06	25.4	8.0	8.7	1361	6/4	13/88	5/6	yes	≤100	≤ 20	1	WL
1	11	23.7	7.9	9.2	1260	6/4	13/88	5/6	Yes	≤100	≤ 20	1	C
2	12	24.6	7.9	11.1	1324	6/4	13/88	5/6	Yes	≤100	≤ 20	1	C
3	13	23.8	7.7	10.6	1334	6/4	13/88	5/6	Yes	≤100	≤ 20	2	KK
4	14	24.7	7.8	10.4	1363	6/4	13/88	5/6	yes	≤100	≤ 20	2	SO
5	15	25.5	7.7	10.4	1370	6/4	13/88	5/6	Yes	≤100	≤ 20	3	C
6	16	25.4	7.8	11.3	1285	6/4	13/88	5/6	yes	≤100	≤ 20	3	KK
7	17									≤100	≤ 20	3	
8	18									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted?	Yes/(No)	If yes, state mesh size:
Was sample pH or hardness adjusted?	Yes/(No)	If yes, describe further:
Were alternate concentrations or dilution series used?	Yes/(No)	If yes, describe further:
Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily?	(Yes)/No	If no, describe further:
Were there any other method variations, deviations, or exclusions from method?	Yes/(No)	If yes, describe further:
Was there anything unusual about the test, any problems encountered, or any remedial measures taken?	Yes/(No)	If yes, describe further:

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra. Control Sample Name: EDL-1 L2713601-1 Sample #: 3730.008 2234

Day	Date 2020	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	10.06	26.1	8.2	7.5	442	6/4	13/88	5/6	WL
1	11	25.2	8.2	7.5	424	6/4	13/88	5/6	[illegible]
2	12	24.9	8.2	8.1	424	6/4	13/88	5/6	[illegible]
3	13	25.6	8.2	7.6	435	6/4	13/88	5/6	-S
4	14	25.5	8.2	7.2	448	6/4	13/88	5/6	SO
5	15	25.1	8.2	7.7	477	6/4	13/88	5/6	KK
6	16	25.2	7.9	8.1	417	6/4	13/88	5/6	VO
7	17								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	
24.5	7.9	7.0	6/4	13/88	[illegible]
24.6	8.0	6.7	6/4	13/88	[illegible]
25.3	8.0	6.8	6/4	13/88	SO
25.2	7.0	6.7	6/4	13/88	SO
25.0	8.0	6.7	6/4	13/88	SO
24.5	8.1	7.3	6/4	13/88	KK
24.5	8.0	6.4	6/4	13/88	KK

Day	Date 2020	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
4	14	4	8	3	6	6	3	4	3	3	3	37	—	0	—	[illegible]	[illegible]
5	15	3+1	0	7	5	6	5+1	7	3	3	5	46	—	0	0	[illegible]	[illegible]
6	16	*9/0	10	*7 10	*4 9	*4 10	*7 10	0	*0 5	0	0	54*	—	0	0	[illegible]	[illegible]
7	17	10	12	0	0	0	0	10	0	12	13	57	—	0	—	[illegible]	[illegible]
8	18																
Total Neonates		17	24	20	20	22	19	21	11	18	21	194	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra.. 0.137 l.v.v Sample Name: EDL-1 L2743601-1 Sample #: 3730008 2234

Day	Date 2020	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06	25.3	8.3	7.8	441	6/4	13/88	5/6	WL
1	11	25.2	8.2	7.5	424	6/4	13/88	5/6	C
2	12	25.6	8.3	8.2	436	6/4	13/88	5/6	C
3	13	25.6	8.3	7.4	436	6/4	13/88	5/6	CS
4	14	25.5	8.2	7.6	452	6/4	13/88	5/6	SO
5	15	25.6	8.2	7.1	447	6/4	13/88	5/6	KK
6	16	25.7	8.0	7.2	425	6/4	13/88	5/6	KK
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.6	8.0	7.0	6/4	13/88	C
24.3	7.9	6.3	6/4	13/88	C
25.0	7.8	6.4	6/4	13/88	SO
24.9	8.0	6.6	6/4	13/88	SO
24.7	8.0	6.6	6/4	13/88	SO
23.9	8.1	7.2	6/4	13/88	KK
25.1	8.0	6.0	6/4	13/88	KK

Day	Date 2020	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10 06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CO	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	CO	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CO	—
4	14	3	3	2	2	3	3	2	4	0	3	22	—	0	—	CO	CO
5	15	7	4+2	5+4	3	9	8	4	9	0	6	61	—	0	0	KK	KK
6	16	8	0	0	8	8	9	4	5	3	0	45	—	0	0	KK	KK
7	17	0	10	10	0	0	0	0	0	10	10	40	—	0	—	CO	CO
8	18																
Total Neonates		18	16	21	13	20	20	10	18	13*	19	168	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra: 0.411.vN Sample Name: EDL-1 L2713601-1 Sample #: 3730.008 2234

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	✓	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	✓	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	✓	—
4	14	2	3	dead	2	3	3	2	3	2	0	22	3	10	—	✓	✓
5	15	0	2	|	6	9	8+2	dead	9	8	6	50	7	20	—	0	2
6	16	6	3	|	6	7	0	|	0	7	8	37	6	20	0	✓	✓
7	17	0	0	↓	0	0	0	|	0	0	0	0	—	20	—	✓	✓
8	17																
Total Neonates		8	8	0	14	19	13	2	12	17	14	109	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra: 1.23 1.vN Sample Name: EDL-1 L2713601-1 Sample #: 3730.003 2234

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
4	14	3	2	0	2	0	3	0	3	3	0	16	—	0	—	[illegible]	[illegible]
5	15	0	0	3	5	7	9	9	6	8	4	51	—	0	0	[illegible]	2
6	16	dead	5	7	9	9	12	13	2	9	7	73	1	10	0	[illegible]	[illegible]
7	17	1	0	0	0	0	0	0	0	0	0	0	—	10	—	[illegible]	[illegible]
8	17																
Total Neonates		3	7	10	16	16	24	22	11	20	11	146	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra: 3.71. v/v Sample Name: EDL-1 L2733601-1 Sample #: 8730.008 2234

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06	25.1	8.3	7.9	473	6/4	13/88	5/6	WL
1	11	25.1	8-2	7.8	424	6/4	13/88	5/6	C
2	12	25.6	8-3	7.6	467	6/4	13/88	5/6	C
3	13	25.6	8.2	7.5	480	6/4	13/88	5/6	C
4	14	25.6	8.2	7.3	488	6/4	13/88	5/6	SO
5	15	25.6	8.2	7.1	490	6/4	13/88	5/6	KK
6	16	25.8	8.0	7.2	459	6/4	13/88	5/6	W
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.9	8.0	6.9	6/4	13/88	C
24.2	7.9	6.9	6/4	13/88	C
25.2	7.8	6.2	6/4	13/88	SO
25.1	7.9	6.7	6/4	13/88	SO
24.7	7.9	6.4	6/4	13/88	SO
23.8	8.1	7.3	6/4	13/88	KK
25.2	8.0	6.4	6/4	13/88	KK

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10 06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	aO	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	✓	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	aO	—
4	14	0	4	3	3	3	0	3	5	0	3	24	—	0	—	aO	aO
5	15	0	3	3	7	7	4	8	7	4	8	51	—	0	0	✓	✓
6	16	7	0	8	8	10	2	14	10	9	4	74*	—	0	0	✓	✓
7	17	0	0	0	0	0	4	0	0	0	0	4	—	0	0	aO	aO
8	19																
Total Neonates		7	7	14	18	20	10	25	22	13	15	151	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra: 11.1 +23.1 v1v Sample Name: EDL-1 L2733601-1 Sample #: 3730.003 2234

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	[illegible]
4	14	4	6	0	4	0	3	2	4	3	4	30	—	0	—	[illegible]	[illegible]
5	15	6	4	0	0	0	0	4	5	6	8	33	—	0	0	[illegible]	[illegible]
6	16	5	6	4	5	10	8	2	10	9	4	63	—	0	0	[illegible]	[illegible]
7	17	0	0	0	11	0	0	0	0	0	0	11	—	0	—	[illegible]	[illegible]
8	18																
Total Neonates		15	16	4	20	10	11	8	19	18	16	137	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: ~~0.41~~ 33.3 % v/v   Sample Name: EDL-1   L2743601-1   Sample #: 3730.003   2234

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Day	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0						
1						
2						
3						
4						
5						
6						
7						

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CO	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	✓	—
3	13	0	0	0	0	0	0	0	0	0	0	0	~~6~~	0	—	CO	—
4	14	3	4	2	2	4	2	2	0	3	2	24	—	0	—	CO	CO
5	15	2	0	4	0	12	2	0	0	6	5	31	—	0	0	[illegible]	[illegible]
6	16	7	2	10	6	13	9 ~~dead~~	0	0	6	4	62	—	0	0	[illegible]	[illegible]
7	17	0	0	0	0	0	|	0	0	0	0	0	—	0	—	CO	CO
8	17																
Total Neonates		12	6	16	8	34	13	2	0	15	11	117	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra...: 100% v/v Sample Name: EDL-1 L2733601-1 Sample #: 3730008 2234

Day	Date 2020	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10:00	25.2	8.1	8.0	1360	6/4	13/88	5/6	WL
1	11	25.2	8.2	7.9	1330	6/4	13/88	5/6	C
2	12	25.3	8.0	8.5	1351	6/4	13/88	5/6	C
3	13	25.3	8.0	8.1	1385	6/4	13/88	5/6	CC
4	14	25.4	7.9	8.2	1390	6/4	13/88	5/6	SO
5	15	25.7	8.0	8.1	1364	6/4	13/88	5/6	KK
6	16	25.8	7.8	8.3	1263	6/4	13/88	5/6	KK
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.7	8.0	6.9	6/4	13/88	C
24.2	7.4	7.6	6/4	13/88	C
25.0	7.9	6.3	6/4	13/88	SO
25.1	7.9	6.4	6/4	13/88	SO
24.9	8.1	6.7	6/4	13/88	SO
24.5	8.2	7.2	6/4	13/88	KK
25.4	8.1	6.9	6/4	13/88	KK

Day	Date 2020	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10:00																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	CC	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
4	14	4	0	0	2	2	0	6	2	3	0	19	—	0	—	CC	—
5	15	0	0	3	2	4	0	0	0	6	0	15	—	0	0	SO	SO
6	16	13	3	13	4	12	0	0	8	10	9	72	—	0	0	SO	SO
7	17	0	0	0	0	0	6	0	0	0	0	0	—	0	—	CC	CC
8	19																
Total Neonates		17	3	16	8	18	0	6	18	19	9	106	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia Neonate Origin

Sample Information

Client ALS – TB

Sample # 8730.0032234

Date/Time Received 10/06/22 11:10

Sample Type Effluent

100% Hardness 374

Sample Name '34' EDL 1 L2713601-1

Date/Time Collected 08/06/22 / 8:10 Person Sampling N/A

Arrival Temp (°C) 17.5

Sample Description Clear, light green

Test Information

Date Test Started 10.06.22 @ 1710

Dilution Water Batch Number DW22-73

Test Started By VS

Control Hardness 126

Template Used for Randomization 1

Individual Culture Health Data

Date Culture Started 031022
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 22.4
Culture I.D. (*e.g.*, Wed Row 4) Fri Row 1
Average # neos in previous 7 days (must be ≥15) 34.0
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 030622
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 20.7
Culture I.D. (*e.g.*, Wed Row 4) Fri Row 2
Average # neos in previous 7 days (must be ≥15) 28.6
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ____
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ____
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ____
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 0 (add up % mortality for all cultures used / # cultures used)

Mean Number of Young Produced within first 3 broods 21.6 (avg # 1st 3 broods for all cultures used / # cultures used)

Mean Number of Young Produced in previous 7 days 31.3 (avg # neos in prev 7 days for all cultures used / # cultures used)

Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / (No) (circle one)

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
F.1.1	(Y) / N	(Y) / N	13	1	CO
2	(Y) / N	(Y) / N	11	2	CO
5	(Y) / N	(Y) / N	14	3	CO
6	(Y) / N	(Y) / N	11	4	CO
7	(Y) / N	(Y) / N	15	5	CO
8	(Y) / N	(Y) / N	12	6	CO
9	(Y) / N	(Y) / N	14	7	CO
10	(Y) / N	(Y) / N	14	8	CO
F.2.2	(Y) / N	(Y) / N	12	9	CO
10	(Y) / N	(Y) / N	14	10	CO
	Y / N	Y / N			

VS

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032234	**Sample Name:**	EDL1 L2713601-1
Sample Method:	Grab	**Collected by:**	Not available
Date/Time Collected:	June 8, 2022; 08:10	**Arrival Temp.:**	17.5°C
Date/Time Received:	June 10, 2022; 11:10	**Sample Description:**	Clear, light green
Sample Point Description:	Other	**Sample Type:**	Effluent
Transportation:	Road		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species (Strain #): *Raphidocelis subcapitata* (CPCC # 37)

Source: Nautilus Plant Culture Unit (from CPCC)

Culture Temp.: 24 ± 2 °C

Test Culture Number: G4(l)a

Culture Age at Test Start: 4 days old

Cell Density in the Microplate Wells at the Start of the Test: 10,568.18 cells/ml

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*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032234 **Sample Name:** EDL1 L2713601-1

Test Conditions

Date/Time Test Start:	June 11, 2022; 10:35	**T=0 Control pH:**	6.9
Date/Time Test End:	June 14, 2022; 09:30 - 12:15	**T=72 Control pH:**	6.9
Sample pH Before Dilution:	7.6	**pH Adjustment:**	None
Test Duration:	72 hours		

Mean Test Temperature (±Standard Deviation): 25.4 (±0.1)°C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Millipore

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032234 **Sample Name:** EDL1 L2713601-1

Test Conditions - continued

Enumeration Technique:	Neubauer Haemacytometer
Test Vessel:	96-Well U-bottomed Polystyrene Microplate
Concentration of Test Solutions:	90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control
# Replicates/Concentration:	4 reps started; 3 reps counted of test solutions 10 control reps started, 2 used for pH measurement
Design if Specialized Procedure:	Not applicable
Method Deviations or Unusual Occurrences:	None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times:	Acceptable (31.5 times)
pH in controls did not vary by more than 1.5 units:	Acceptable (0 units)
C V for cell yields is < 20% within the control wells:	Acceptable (9.4%)
No inhibitory trend detected across the control wells:	Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
309,432	351,932	296,932	309,432	331,932	336,932	396,932	329,432	332,869	9.4

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 CV = Coefficient of Variation = (100 x standard deviation / mean)

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032234 **Sample Name:** EDL1 L2713601-1

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	521,932	1,106,932	1,066,932	686,932	259,432
2	594,432	1,196,932	1,036,932	701,932	259,432
3	584,432	1,199,432	969,432	554,432	294,432
Mean Cell Yield	566,932	1,167,765	1,024,432	647,765	271,098
Coefficient Variation[2]	7	5	5	13	7
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	354,432	334,432			
2	301,932	344,432			
3	299,432	271,932			
Mean Cell Yield	318,598	316,932			
Coefficient Variation[2]	10	12			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	**Result[1]**	**Method of Calculation**
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume[3] (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	no trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

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*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032234 **Sample Name:** EDL1 L2713601-1

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software, 2001-2007 Comprehensive Environmental Toxicity, Information System - CETIS v1.8.1.2.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: None

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2209 **Date Test Initiated:** 17-Jun-2022

Method of Analysis: Linear Interpolation (ICPIN) **Algae Lot #:** G4(l)a

72-hour IC25 (95% Confidence Limits): 67.48 mg/L (48.16 mg/L; 83.28 mg/L)

Historic Geometric Mean IC25: 51.76 mg/L (23.79 mg/L; 112.61 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS - TB Sample Name EDL-1 L271360-1 Sample # 8730-00322234

Conditions for Test Validity

Cell increase for control is >16	(Acceptable)/Not acceptable	31.5 (times)
CV among controls ≤ 20	(Acceptable)/Not acceptable	9.4
Result of Mann-Kendall test for trend	(Acceptable)/Not acceptable	no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 116 250 (cells/mL)

Used: (Yes)/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10568.18 (cells/mL)

Analysis Completed:	Initials: TB	Date: 27/06/22
Results Verified:	Initials: GD	Date: 29/06/22

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location:	ALS Thunder Bay		
Sample #:	8730 003 22 34	Sample Name:	EDL 1 L 271 3601-1
Collection Method:	Grab	Collected By:	N/A
Date/Time Collected:	08/06/22 8:10	Arrival Temp.: (meter/probe)	17.5 °C (47)
Date/Time Received:	10/06/22 11:10	Sample Description:	clear, light green
Collection Point Description:	other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:	
Transportation:		Storage:	4±2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # 6411a	
Culture Age at Start of Test	4 days old (must be 3 to 7 days old)	
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	
	Axenic culture? Yes/No (Circle one)	

Notes:

Test Conditions:

Date / Time Test Start: 11.06.22 10:35 Started By:		Date / Time Test End: 14.06.22 9:30-10:15 Finished By:	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 7.6	pH adjustment: Y/N	pH of well D6 at T=0 h 6.9	pH of well D7 at T=72 h 6.9
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # —		Metal mining NUT Lot # NUT 2203
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation) 25.4 ± 0.1 °C			

	Day 1		Day 2	
¼ plate rotation (Initial)	AM	PM	AM	PM
Lights ON (Initial)	AM	PM	AM	PM

* For reference test, record pH of top concentration before use for series dilutions.

Notes: Millipore

72-Hour Qualitative Observations:

Condensation: Yes		
Growth: Yes		
Were there any other method variations or deviations from methods? Yes/No		If yes, describe further:
Anything unusual about the test? Yes/No		
Any problems encountered? Yes/No		
Any remedial measures taken? Yes/No		

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 8730-0032234	Sample Name: EDL-1 L271360-1

Reference Data:

Reference Chemical Batch #	Phenol G P22089	Date test started	17.06.22
Method of Analysis	ICPIN – linear interpolation	Algae Lot #	G 4(1)a
72-hour IC25 (95% C.I.)[3] mg/L	67.48 48.16; 83.28	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	51.76 23.79; 112.61

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	> 90.91% (N/A)	No nonlinear regression models could fit
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers	—	ICPIN – linear interpolation
Test for Outliers	No Outliers Present	Grubbs' Test for Residual Outlier Initial C
	If outliers present, indicate Concentration/Rep: —	
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed.	
	No statistically significant growth stimulation.	Williams' or (Dunnett's) Multiple Comparison Test Initial C
	(Yes) statistically significant growth stimulation at these concentrations: 90.91; 30.303; 10.101; 3.367	

1)Results relate only to the sample tested.
2) Tidepool Scientific Software © 2001-2007. Comprehensive Environmental Toxicity Information System – CETIS v. 1.9.6.7
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/(No)

Any outliers and justification for their removal? Yes/(No)

Nautilus Environmental Company Inc.

Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)

72-Hour Growth Inhibition Test – Continued

72-Hour Quantitative Observations of Controls

Sample Name: EDL 1 Sample Number: 8730-0032234 Date Test Start: 11.06.22

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D8	Well # D9	Well # D10	Well # D11
1	34	42	32	27	45	37	41	32
2	28	40	28	33	34	37	47	32
3	33	32	31	27	25	34	37	34
4	33	31	32	41	33	31	38	38
5	—	—	—	—	—	—	—	—
Initials	E	E	E	C	E	E	E	C

I 116 280

Cell increase for controls = 31.5	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = 9.4	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = no trend	Controls are invalid if there is a trend detected by the Mann-Kendall test

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL 1 Sample Number: 873000032234 Date Test Start: 11.06.22

Theoretical Test Concentration: 100.00 % v/v					Actual Test Concentration: 90.91 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	49	47	63		566932 (± 39291)
2	54	67	49		Coefficient of Variation of Cell Yield
3	49	62	63		7
4	61	66	63		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-70.317
Initials	G	G	G		G

Theoretical Test Concentration: 33.33 % v/v					Actual Test Concentration: 30.303 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	114	120	136		1167765 (± 52698)
2	101	119	122		Coefficient of Variation of Cell Yield
3	119	131	107		5
4	113	113	119		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-250.818
Initials	G	G	G		G

Theoretical Test Concentration: 11.11 % v/v					Actual Test Concentration: 10.101 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	101	109	114		1024432 (± 49937)
2	134	109	84		Coefficient of Variation of Cell Yield
3	96	84	104		5
4	100	117	90		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-207.758
Initials	G	G	G		G

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL 1 Sample Number: 873000032234 Date Test Start: 11.06.22

Theoretical Test Concentration: 3.704 %v/v					Actual Test Concentration: 3.367 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation)
1	56	79	61		647765 (± 81176)
2	77	55	65		Coefficient of Variation of Cell Yield
3	71	76	41		13
4	75	75	59		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-94.600
Initials	C	C	C		C

Theoretical Test Concentration: 1.235 %v/v					Actual Test Concentration: 1.122 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation)
1	26	29	28		271098 (± 20207)
2	29	29	27		Coefficient of Variation of Cell Yield
3	23	23	43		7
4	30	27	24		Average % Inhibition (-ve number = enhancement)
5	—	—	—		18.557
Initials	C	C	C		C

Theoretical Test Concentration: 0.412 %v/v					Actual Test Concentration: 0.374 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation)
1	45	40	26		318598 (± 31058)
2	29	30	31		Coefficient of Variation of Cell Yield
3	36	30	36		10
4	36	25	31		Average % Inhibition (-ve number = enhancement)
5	—	—	—		4.287
Initials	C	C	C		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL 1 Sample Number: 873000032234 Date Test Start: 11.06.22

Theoretical Test Concentration: 0.137 % v/v					Actual Test Concentration: 0.125 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	25	23	24		316932 (± 39291)
2	36	46	34		Coefficient of Variation of Cell Yield
3	36	34	22		12
4	41	39	33		Average % Inhibition (-ve number = enhancement)
5	–	–	–		4.788
Initials	G	G	G		G

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	EDL-1	Sample #	8730-0032234	Date test start	11.06.22

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	116250	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	20	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2325	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	10568.1818	=	31.497312

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	34	42	32	27	45	37	41	32
	28.0	40.0	28.0	33.0	34.0	37.0	47.0	32.0
	33	32.0	31.0	27.0	25.0	34.0	37.0	34.0
	33.0	31.0	32.0	41.0	33.0	31.0	38.0	38.0
total cells	128	145	123	128	137	139	163	136
cells/ul	320	362.5	307.5	320	342.5	347.5	407.5	340
cells/ml	320000	362500	307500	320000	342500	347500	407500	340000
Cell yield = measured concentration - initial algal cell concentration								
	309432	351932	296932	309432	331932	336932	396932	329432

% inhibition summary

Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	4.788
0.374	4.287
1.122	18.557
3.367	-94.600
10.101	-207.758
30.303	-250.818
90.910	-70.317

Mean cell yield for the control	= Rc	Standard deviation		coefficient of variation		
Rc	332869	SD	31365.8567	CV	9.4228741	**Must be ≤20**

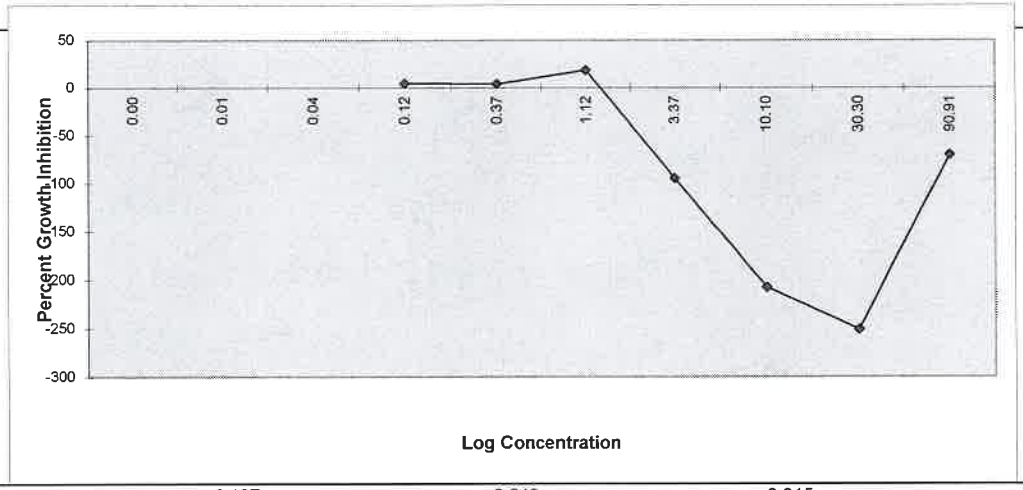
enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	49	47	63	114	120	136	101	109	114	56	79	61	26	29	28
	54.0	67.0	49.0	101.0	119.0	122.0	134.0	109.0	84.0	77.0	55.0	65.0	29.0	29.0	27.0
	49.0	62.0	63.0	119.0	131.0	107.0	96.0	84.0	104.0	71.0	76.0	41.0	23.0	23.0	43.0
	61.0	66.0	63.0	113.0	113.0	119.0	100.0	117.0	90.0	75.0	75.0	59.0	30.0	27.0	24.0
total cells	213	242	238	447	483	484	431	419	392	279	285	226	108	108	122
cells/ul	532.5	605	595	1117.5	1207.5	1210	1077.5	1047.5	980	697.5	712.5	565	270	270	305
cells/ml	532500	605000	595000	1117500	1207500	1210000	1077500	1047500	980000	697500	712500	565000	270000	270000	305000
Cell yield = measured concentration - initial algal cell concentration															
	521932	594432	584432	1106932	1196932	1199432	1066932	1036932	969432	686932	701932	554432	259432	259432	294432
Mean Yield	566932			1167765			1024432			647765			271098		
STD Yield	39291			52698			49937			81176			20207		
CV Yield	7			5			5			13			7		

Average % inhibition											
	for 90.910%:	-70.317	for 30.303%	-250.818	for 10.101%	-207.758	for 3.367%	-94.600	for 1.122%	18.557	

Average % stimulation											
	for 90.910%:	70.317	for 30.303%	250.818	for 10.101%	207.758	for 3.367%	94.600	for 1.122%	-18.557	

29/06/22



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
45	40	26	25	23	24									
29.0	30.0	31.0	36.0	46.0	34.0									
36.0	30.0	36.0	36.0	34.0	22.0									
36.0	25.0	31.0	41.0	39.0	33.0									
146	125	124	138	142	113	0	0	0	0	0	0	0	0	0
365	312.5	310	345	355	282.5	0	0	0	0	0	0	0	0	0
365000	312500	310000	345000	355000	282500	0	0	0	0	0	0	0	0	0
354432	301932	299432	334432	344432	271932	-10568	-10568	-10568	-10568	-10568	-10568	-10568	-10568	-10568
318598			316932			-10568			-10568			-10568		
31058			39291			0			0			0		
10			12			0			0			0		
for 0.374%	4.287		for 0.125%	4.788		for 0.042%	103.175		for 0.014%	103.175		for 0.005%	103.175	
for 0.374%	-4.287		for 0.125%	-4.788		for 0.042%	-103.175		for 0.014%	-103.175		for 0.005%	-103.175	

✓ 29/06/22

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
EDL1 L2713601-1	8730-0032234	June 8, 2022	June 10, 2022	June 10, 2022	39.99% Volume (28.36; 71.02% Volume)	0.27% Volume (Not applicable)	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	03-Jun-2022	**Reference Chemical:**	KCl
Frond Increase IC25:	2.71 g/L	**95% Confidence Limits:**	2.21; 3.19 g/L
Historic Geometric Mean IC25:	2.24 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.50; 3.34 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2$ $2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2$ $6H_2O$	121.7	Na_2MoO_4 $2H_2O$	0.0726
	K_2HPO_4	10.4	$FeCl_3$ $6H_2O$	1.6	$ZnCl_2$	0.00003
	KCl	10.1	$MgSO_4$ $7H_2O$	147	$CoCl_2$ $6H_2O$	0.00001
					$CuCl_2$ $2H_2O$	0.00001

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

Date: June 30, 2022

C. D'Andrea

Carol D'Andrea
Laboratory Supervisor

Test Material

Client Name/Location:	ALS - Thunder Bay		
Sample #:	8730-00322 34	Sample Name:	EDL 1 L271360-1
Collection Method:	Grab	Collected By:	N/A
Date/Time Collected:	08 / 06 / 22 8:10	Arrival Temperature (meter/probe):	17.5°C (4)
Date/Time Received:	10 / 06 / 22 11:10	Sample Description:	Clear, light green
Collection Point Description:	other	Sample Type: ☒Effluent ☐ Chemical ☐ Other:	
Transportation:	Road / Air	Storage:	4±2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	EV
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	EV
Culture Age at Start of Test:	9 days old, acclimated 20 hours in fresh test solution (mAPHA)	EV
Culture Medium:	Modified Hoagland's E+ medium, Lot # M M2201	EV
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/No	EV
	Axenic culture? Yes/No	EV
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 29 in HT 1, 31 in HT 2, 29 in HT 3 at finish	EV

Test Conditions and Procedures

Date / Time Test Start: 10.06.22 16:00 Started By: EV	Date / Time Test End: 17.06.22 8:25 Finished By: EV
Test Type: Static (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / No (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: Modified APHA (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 8.2 (13/18)	pH Adjustment: none
Test Volume and Depth: 150 mL / 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / No	If yes, describe further:
Anything unusual about the test? Yes / No	
Any problems encountered? Yes / No	
Any remedial measures taken? Yes / No	Randomization Template: 6

Sample #: 8730-00522 34	Sample Name: EDL1

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	24.0	25.1	24.8	25.2	25.5	25.7	25.3	25.1
Low	24.0	25.1	24.8	25.2	25.5	25.7	25.2	25.1
Medium	24.0	25.1	24.8	25.2	25.3	25.7	25.2	25.1
High	23.9	25.1	24.8	25.3	25.3	25.7	25.2	25.1
Initials	EV	EV	CS	EV	EV	EV	CS	EV
meter/probe	51	44	51	51	44	44	44	44
Mean Test Temperature (average of 24h high / low temperatures):					25.4 ± 0.2 °C			

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.3	7.9	8.0	8.1	EV	13/88
Day 7	Control	Low	Medium	High	Initials	pH meter/probe
	8.4	9.5	9.7	9.4	EV	12/89

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)								

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 14/06/22 (4)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 12					Initials: EV
5440	5180	5060	5560	4950	Mean Light Measurement: 5238
±15% Variation of Mean: 4482 - 5994					Acceptable / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: Yes / No
If yes, describe further: Algae

Reference Data

Reference Date	Frond Increase or Dry Weights (circle one)			
03/06/22	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
	2.71	2.21 ; 3.19	2.24	1.50 ; 3.34

Sample #: 8730-0032234	Sample Name: EDL 1

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 49	B 49	C 50	D 51	49.8

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy							Initials: C
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV: 43.8 (1.0) 2.2							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-8.00	-25.71	-22.29	-29.43	-19.43	-78.86	-36.57
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-16.44	-31.60	-29.67	-29.93	-21.19	-21.32	-22.16

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: Yes / Not applicable (no stimulation) (Circle one)

Test Endpoints and Calculations:

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	39.99 (28.36; 71.02)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]	—	ICPIN - linear interpolation
Dry Weights		
IC25 (95% C.I.)[3]	0.297 (N/A)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN - linear interpolation

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019. Comprehensive Environmental Toxicity Information System CETISv 1.9.6.7
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / No

Any outliers and justification for their removal? Yes / No weights (Control, up 3

Lemna minor Observations

Client: ALS - TB			Sample number: 8730-0032234		Date Started: 10.06.22				
Site: EDL1					Date Ended: 17.06.22				
Concentration: Control			Observations By: C		Concentration: 0.047 1.25			Observations By: C	
Observations	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Observations**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
Number of	49	49	50	51	**Number of**	48	47	44	46
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	X	X	X	X	**Abnormally sized fronds**	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	√	√	√	√	**Loss of Buoyancy**	√	√	√	√
Other Observations					**Other Observations**	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO N/A Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Observations

Client: ALS - TB				Sample number: 8730-0032234			Date Started: 10.06.22		
Site: EDL1							Date Ended: 17.06.22		
Concentration: 0.29 % v/v				Observations By: C	Concentration: 0.97 % v/v				Observations By: C
Observations	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Observations**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
Number of	40	38	38	38	Number of	42	41	38	39
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→	Other Observations	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor L Observations

Client:	ALS - TB			Sample number:	8730-0032234	Date Started:	10.06.22		
Site:	EDL1					Date Ended:	17.06.22		
Concentration:	3.1% v/v			Observations By:	C	Concentration:	9.7% v/v		Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	36	43	43	38	Number of	42	43	42	38
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→	Other Observations	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Observations

Client: ALS - TB				Sample number: 8730-0032234			Date Started: 10.06.22		
Site: EDL1							Date Ended: 17.06.22		
Concentration: 31% v/v			Observations By: C	Concentration: 97% v/v			Observations By: C		
Observations	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Observations**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
Number of	42	41	39	44	Number of	33	38	27	37
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	✓	✓	✓	✓
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→	Other Observations	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Weights

Client	ALS-TB	Site	EDL 1	Sample number	8730-00322 34
In Oven Date/Time/ °C:	17/06/22 8:35 63°	Out Oven Date/Time/°C:	18/06/22 8:35 59°		

Conc. % v/v	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	43	43.8 (1.0)	0.82361	0.82001	3.60	3.89 (0.3)
	B	43		0.79212	0.78821	3.91	
	C	44		0.81315	0.80879	4.36	
	D	45		0.81377	0.801007	3.70	
0.097	A	42	40.3 (1.7)	0.80151	0.79820	3.31	3.25 (0.2)
	B	41		0.81211	0.80866	3.45	
	C	38		0.~~8~~79793	0.79484	3.09	
	D	40		0.80141	0.79825	3.16	
0.29	A	34	32.5 (1.0)	0.808~~52~~ 42 50	0.80570	2.72	2.66 (0.3)
	B	32		0.80908	0.80623	2.85	
	C	32		0.80~~09~~ 999 50	0.80715	2.84	
	D	~~33~~ 32		0.80215	0.79991	2.24	
0.97	A	36	34.0 (1.8)	~~0.79982~~ 0.80727	0.80430	2.97	2.74 (0.4)
	B	35		0.80430	0.80143	2.87	
	C	32		0.80278	0.80068	2.10	
	D	33		0.79982	0.79681	3.01	
3.1	A	30	34.0 (3.6)	0.81510	0.81254	2.56	2.73 (0.2)
	B	37		0.81092	0.80832	2.60	
	C	37		0.80989	0.80685	3.04	
	D	32		0.80557	0.80286	2.71	
9.7	A	36	35.3 (2.2)	0.79973	0.79666	3.07	3.07 (0.2)
	B	37		0.80663	0.80336	3.27	
	C	36		0.80229	0.79923	3.06	
	D	32		0.81083	0.80796	2.87	
31	A	36	35.5 (2.1)	0.81259	0.80938	3.21	3.06 (0.1)
	B	35		0.80856	0.80550	3.06	
	C	33		0.81406	0.81110	2.96	
	D	38		0.80256	0.79954	3.02	
97	A	27	27.8 (5.0)	0.80301	0.80022	2.79	3.03 (0.2)
	B	32		0.80901	0.80591	3.10	
	C	21		0.80946	0.80658	2.88	
	D	31		0.81665	0.81330	3.35	
Initials		TG	C	SO	TG	TG	TG

Notes:

RG

Sample name EDL1 L2713601-1 Date started 6/1[illegible]/22 sample # 8730-0032234

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

49.8 (must be ≥48)

Frond Data Control

Conc (real % v/v)	0	0.097	0.29	0.97	3.1	9.7	31	97
	49	48	40	42	36	42	42	33
	49	47	38	41	43	43	41	38
	50	44	38	38	43	42	39	27
	51	46	38	39	38	38	44	37
Total Fronds	199	185	154	160	160	165	166	135
Increase in Frond Number = Total # Fronds - 6 Starting Fronds								
	43	42	34	36	30	36	36	27
	43	41	32	35	37	37	35	32
	44	38	32	32	37	36	33	21
	45	40	32	33	32	32	38	31
Total Increase	175	161	130	136	136	141	142	111
Mean Increase	43.8	40.3	32.5	34.0	34.0	35.3	35.5	27.8
SD Increase	1.0	1.7	1.0	1.8	3.6	2.2	2.1	5.0
CV Increase	2.2	4.2	3.1	5.4	10.5	6.3	5.9	18.0
% Stimulation		**-8.00**	**-25.71**	**-22.29**	**-22.29**	**-19.43**	**-18.86**	**-36.57**

Weight data Control

Conc (real %v/v)	0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.82361	0.80151	0.80842	0.80727	0.81510	0.79973	0.81259	0.80301
Pan + Plant	0.79212	0.81211	0.80908	0.80430	0.81092	0.80663	0.80856	0.80901
	0.81315	0.79793	0.80999	0.80278	0.80989	0.80229	0.81406	0.80946
	0.81377	0.80141	0.80215	0.79982	0.80557	0.81083	0.80256	0.81665
Initial Weight (g)	0.82001	0.79820	0.80570	0.80430	0.81254	0.79666	0.80938	0.80022
Pan Only	0.78821	0.80866	0.80623	0.80143	0.80832	0.80336	0.80550	0.80591
	0.80879	0.79484	0.80715	0.80068	0.80685	0.79923	0.81110	0.80658
	0.81007	0.79825	0.79991	0.79681	0.80286	0.80796	0.79954	0.81330
Plant Only (mg)	3.60	3.31	2.72	2.97	2.56	3.07	3.21	2.79
	3.91	3.45	2.85	2.87	2.60	3.27	3.06	3.10
	4.36	3.09	2.84	2.10	3.04	3.06	2.96	2.88
	3.70	3.16	2.24	3.01	2.71	2.87	3.02	3.35
Mean Dry Weight	3.893	3.252	2.663	2.738	2.727	3.068	3.063	3.030
SD Dry Weight	0.3	0.2	0.3	0.4	0.2	0.2	0.1	0.2
CV Dry Weight	8.7	4.9	10.8	15.7	8.0	5.3	3.5	8.2
% Stimulation		**-16.44**	**-31.60**	**-29.67**	**-29.93**	**-21.19**	**-21.32**	**-22.16**

For Data Transfer to CETIS

	# fronds	total mass	tare
0	1	43	0.82361	0.82001
	2	43	0.79212	0.78821
	3	44	0.81315	0.80879
	4	45	0.81377	0.81007
0.1	1	42	0.80151	0.79820
	2	41	0.81211	0.80866
	3	38	0.79793	0.79484
	4	40	0.80141	0.79825
0.3	1	34	0.80842	0.80570
	2	32	0.80908	0.80623
	3	32	0.80999	0.80715
	4	32	0.80215	0.79991
1	1	36	0.80727	0.80430
	2	35	0.80430	0.80143
	3	32	0.80278	0.80068
	4	33	0.79982	0.79681
3.1	1	30	0.81510	0.81254
	2	37	0.81092	0.80832
	3	37	0.80989	0.80685
	4	32	0.80557	0.80286
9.7	1	36	0.79973	0.79666
	2	37	0.80663	0.80336
	3	36	0.80229	0.79923
	4	32	0.81083	0.80796
31	1	36	0.81259	0.80938
	2	35	0.80856	0.80550
	3	33	0.81406	0.81110
	4	38	0.80256	0.79954
97	1	27	0.80301	0.80022
	2	32	0.80901	0.80591
	3	21	0.80946	0.80658
	4	31	0.81665	0.81330

AO 29 06 22

June 30, 2022

Christine Paradis,
ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Dear Christine:

On June 10, 2022, Nautilus Environmental Company Inc. personnel received one water sample (EDL2 L2713601-2) from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected June 8, 2022

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
EDL2 L2713601-2 #8730-0032235	Fathead Minnow	7-day LC50 (95% Confidence) 7-day IC25 (95% Confidence)	Survival Biomass	>100% Volume (Not Applicable) >100% Volume (Not Applicable)
	Ceriodaphnia dubia	3-brood LC50 (95% Confidence) 3-brood IC25 (95% Confidence)	Survival Reproduction	>100% Volume (Not Applicable) 48.1% Volume (6.28% Volume; N/A)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[2] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence) 7-day IC25 Dry Weight (95% Confidence)	Growth Growth	79.11% Volume (14.14% Volume; N/A) 0.25% Volume (Not applicable)

1 - Results relate only to the sample tested
2 - Highest concentration tested, based on test method

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 100%, 33%, 11%, 3.7% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted half by half (*i.e.,* 100%, 50%, 25% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

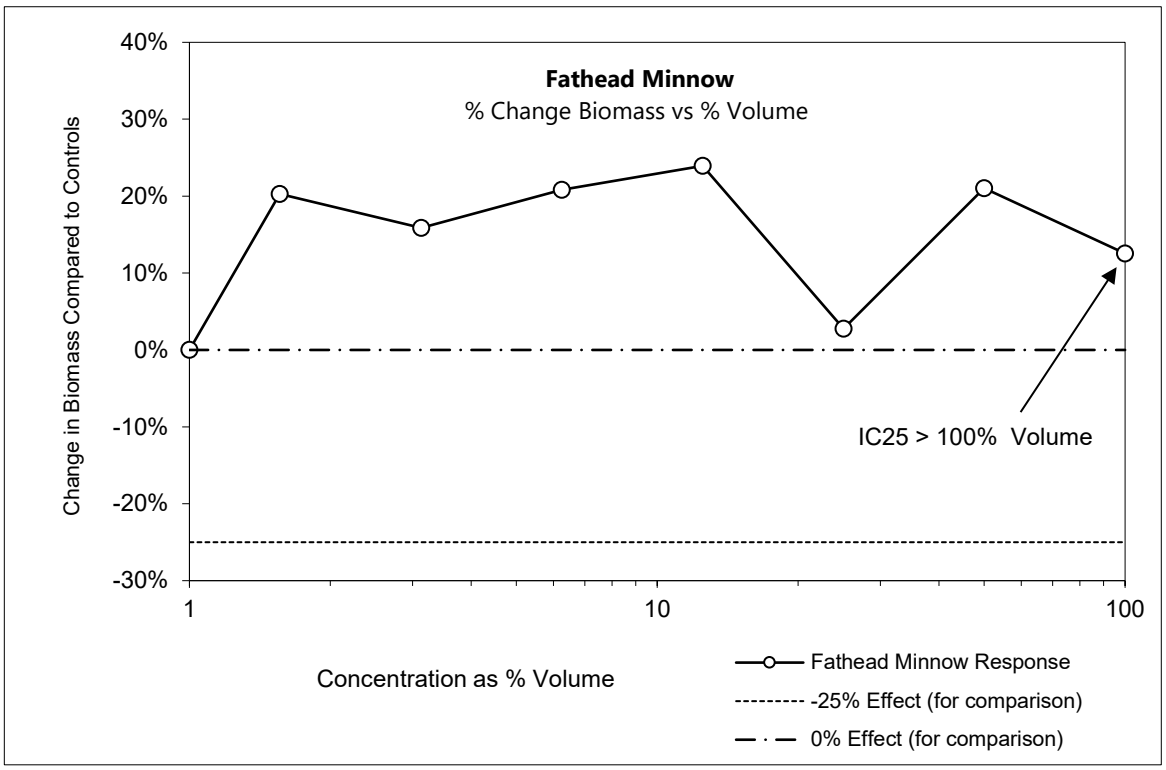
If there are any further details which you require, please do not hesitate to contact us.

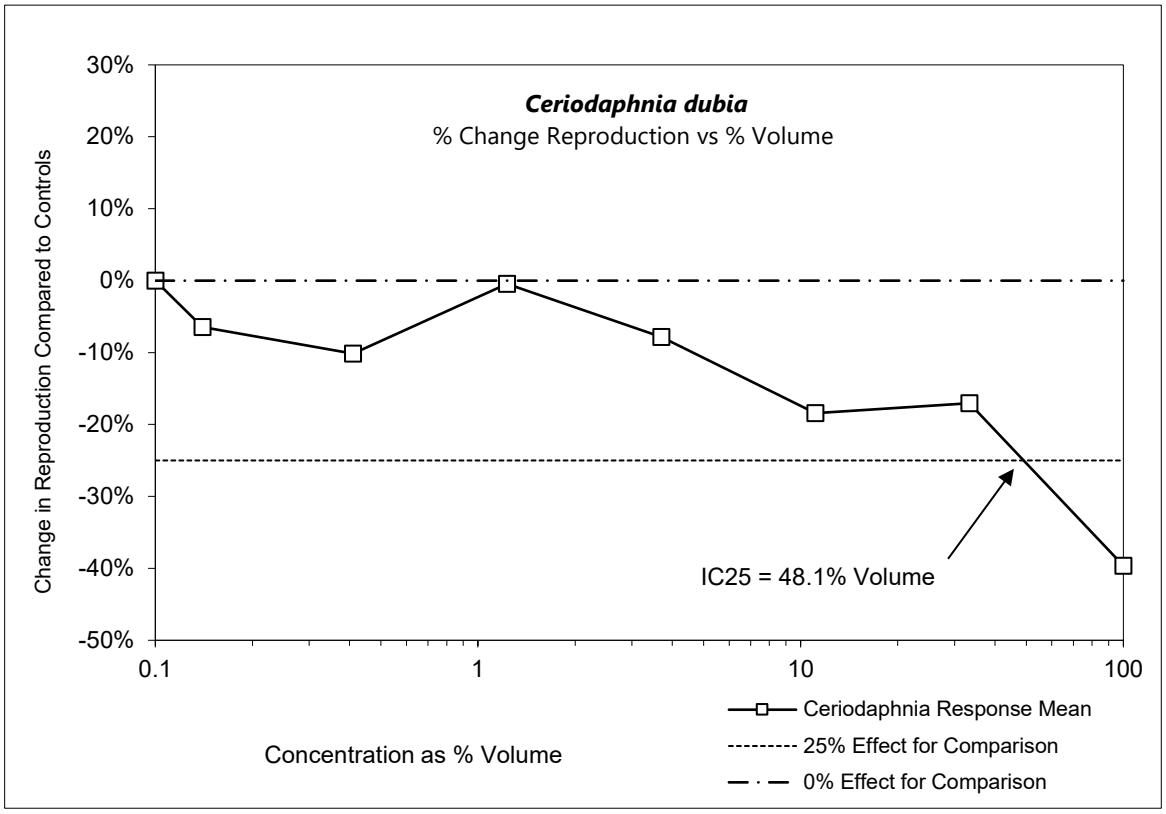
Yours very truly,
Nautilus Environmental Company Inc.

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2022\8000\8730-003\8730-0032235 FCRL

ALS Thunder Bay - EDL2 (L2713601-2) - June 8, 2022





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Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	EDL2 L2713601-2
Date/Time Sampled:	June 8, 2022; 08:10	**Date/Time Received:**	June 10, 2022; 11:10
Date/Time Test Started:	June 11, 2022; 15:35	**Date Test Finished:**	June 18, 2022
Description:	Clear, light green	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032235	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.7°C
Collected By:	Not available		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species:	Fathead Minnow (*Pimephales promelas*)		
Source:	Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)		
Culture Temp.:	22 to 26 °C	**Life Stage:**	<24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 1.0%

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032235 **Sources:** EDL2 L2713601-2

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Test Conditions

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 4 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (0%)

Average Weight of Controls is ≥ 250 µg: Acceptable (447 µg)

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032235 **Sources:** EDL2 L2713601-2

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	0	10	10	0	20	0	20
	2	0	0	0	10	10	0	20	10
	3	0	0	0	0	0	30	0	30
Mean % Mortality		0.0	0.0	3.3	6.7	3.3	16.7	6.7	20.0
S.D.		0.0	0.0	5.8	5.8	5.8	15.3	11.5	10.0
Survival/Growth Data									
Mean Dry Biomass	1	0.540	0.478	0.473	0.530	0.580	0.398	0.612	0.514
(mg)	2	0.373	0.513	0.593	0.534	0.498	0.597	0.403	0.583
	3	0.428	0.622	0.488	0.556	0.584	0.383	0.608	0.412
% Effect (+ or -)		0.0	20.3	15.9	20.8	23.9	2.8	21.0	12.5
Mean Dry Biomass/ Concentration (mg)		0.447	0.538	0.518	0.540	0.554	0.459	0.541	0.503
S.D.		0.09	0.08	0.07	0.01	0.05	0.12	0.12	0.09

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032235 **Sources:** EDL2 L2713601-2

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival		
7-day LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Biomass **(Survival and Growth)**		
7-day IC25 (Confidence Interval)[2]	>100% Volume (Not Applicable)	Non-linear Regression 2P Linear Model

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 02-Jun-2022 **Reference Batch #:** Zn2102

Method of Analysis: Trimmed Spearman-Karber α = 10%

7-Day LC50 (95% Confidence Limits): 0.61 mg/L (0.49 mg/L; 0.76 mg/L)

Historic Geometric Mean LC50: 0.70 mg/L (0.41 mg/L; 1.21 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS - TB Sample Name: EDL-2 L2713601.2 Sample #: 8730-0032235

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 4% 0

Average Weight of Controls is ≥ 250 µg: (Acceptable) / Not Acceptable 447 µg

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 4-6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
7-day LC50	> 100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
Survival / Growth Biomass		
7-day IC25	> 100 % Volume	Nonlinear regression 2P Linear model
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials [illegible] Date 23/06/22

Results Verified: Initials [illegible] Date 20/06/22

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** EDL-2 L2713801-2 **Sample #:** 8730.00322935

Day	Date 2022	Initial Variables				Meters/Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	D.O./°C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	11.06	24.2	7.3	4.0	1372	6/4	13/88	5/6	NO	≤100	≤ 20	1	CJ
1	12	25.9	7.3	5.6	1415	6/4	13/88	5/6	NO	≤100	≤ 20	1	CJ
2	13	24.6	7.3	7.2	1402	6/4	13/88	5/6	No	≤100	≤ 20	1	CS
3	14	25.6	7.2	6.7	1457	6/4	13/88	5/6	No	≤100	≤ 20	2	KK
4	15	25.8	7.32 KK	8.5	1459	6/4	13/88	5/6	Yes	≤100	≤ 20	2	KK
5	16	24.1	7.1	7.9	1380	6/4	13/88	5/6	Yes	≤100	≤ 20	3	C
6	17	26.4	7.1	8.7	1388	6/4	13/88	5/6	Yes	≤100	≤ 20	3	KK
7	18												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of brine shrimp days 0 to 6? (Yes)/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: Contr

Sample Name: EDL-2 L2713601-2

Sample #: 8730.003229 35 28 90

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06	24.7	8.0	7.4	269	6/4	13/88	5/6	C
1	12	24.6	8.2	7.9	259	6/4	13/88	5/6	C
2	13	25.0	7.8	7.7	270	6/4	13/88	5/6	SO
3	14	24.6	8.1	7.4	264	6/4	13/88	5/6	SO
4	15	24.87 50	8.02	7.86	240 276	6/4	13/88	5/6	SO
5	16	25.6	7.9	7.5	256	6/4	13/88	5/6	KK
6	17	25.7	8.2	7.7	250	6/4	13/88	5/6	KK
7	18								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
23.6	7.9	7.3	6/4	13/88	C							
23.6	7.8	7.8	5/5	12/89	KK	0	0	0	0	0	0	[illegible]
23.6	7.7	7.0	5/5	12/89	KK	0	0	0	0	0	0	KK
23.7	7.5	5.8	6/4	13/88	SO	0	0	0	0	0	0	KK
24.2	7.5	5.9	6/4	13/88	KK	0	0	0	0	0	0	[illegible]
24.0	7.7	6.4	6/4	13/88 KK	KK	0	10 KK	0	0	0	0	VO
25.2	7.8	6.5	6/4	13/88	SO	0	0	0	0	0	0	KK
						0	0	0	0	0	0	SO

Observations:

Concentration: 1.56% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06	24.4	8.1	8.1	279	6/4	13/86	5/6	C
1	12	24.4	8.1	8.1	279	6/4	13/88	5/6	C
2	13	24.0	8.0	8.0 7.7	281	6/4	13/88	5/6	SO
3	14	24.6	8.0	8.7	287	6/4	13/88	5/6	SO
4	15	24.6	8.0	7.5	290	6/4	13/88	5/6	SO
5	16	25.1	8.1	7.7	267	6/4	13/88	5/6	KK
6	17	25.2	8.2	7.8	267	6/4	13/88	5/6	KK
7	18								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
23.6	7.9	7.2	6/4	13/88	C							
23.6	7.8	7.8 KK	5/5	12/89	KK	0	0	0	0	0	0	[illegible]
23.6	7.7	7.2	5/5	12/89	KK	0	0	0	0	0	0	KK
23.7	7.5	5.5	6/4	13/88	SO	0	0	0	0	0	0	KK
24.1	7.5	5.9	6/4	13/88	KK	0	0	0	0	0	0	[illegible]
23.9	7.7	6.5	6/4	13/88	KK	0	0	0	0	0	0	[illegible]
25.1	7.7	6.0	6/4	13/88	SO	0	0	0	0	0	0	KK
						0	0	0	0	0	0	SO

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13% v/v Sample Name: EDL-2 L2713601-2 Sample #: 3730.0032228 35.0

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	11.06																					
1	12															0	0	~~8~~0 KC	0	0	0	[illegible]
2	13															0	0	0	0	0	0	KK
3	14															0	0	0	0	0	0	KK
4	15															10	0	0	0	0	0	[illegible]
5	16															10	0	0	0	0	0	[illegible]
6	17															10	0	0	0	0	0	KC
7	18															10	0	0	0	0	0	SO

Observations:

Concentration: 6.25% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	11.06																					
1	12															0	0	0	0	0	0	CS
2	13															0	0	0	0	0	0	KK
3	14															0	0	0	0	0	0	KK
4	15															0	0	0	0	0	0	[illegible]
5	16															10	10	0	0	0	0	[illegible]
6	17															10	10	0	0	0	0	KK
7	18															10	10	0	0	0	0	SO

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5 % v/v

Sample Name: EDL-2 L2713601-2

Sample #: 3730.003222 35 2800

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06	24.5	8.0	7.8	415	6/4	13/88	5/6	C
1	12	24.4	8.0	8.1	412	6/4	13/88	5/6	O
2	13	24.1	~~8.~~ 7.9	7.7	427	6/4	13/88	5/6	SO
3	14	24.6	7.9	8.2	428	6/4	13/88	5/6	SO
4	15	24.6	7.9	~~8.~~ 7.4	435	6/4	13/88	5/6	SO
5	16	25.1	7.9	7.7	408	6/4	13/88	5/6	KK
6	17	25.2	8.0	7.9	406	6/4	13/88	5/6	KK
7	18								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	23.6	7.9	7.4	6/4	13/88	C							
1	23.6	7.8	7.8	5/5	12/89	KK	0	0	0	0	0	0	[illegible]
2	23.6	7.7	7.~~1~~2 KK	5/5	12/89	KK	0	0	0	0	0	0	KK
3	23.7	7.6	5.9	6/4	13/88	SO	0	0	0	0	0	0	KK
4	24.1	7.6	5.9	6/4	13/88	KK	0	0	0	0	0	0	[illegible]
5	24.0	7.8	6.7	6/4	13/88	KK	0	10	0	0	0	0	[illegible]
6	25.1	7.8	6.1	6/4	13/88	SO	0	10	0	0	0	0	KK
7							0	10	0	0	0	0	SO

Observations: ______________________

Concentration: 25 % v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06								
1	12								
2	13								
3	14								
4	15								
5	16								
6	17								
7	18								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0													
1							0	0	0	0	0	0	[illegible]
2							0	0	0	0	0	0	KK
3							0	0	0	0	0	0	KK
4							0	0	10	0	0	0	[illegible]
5							20	0	20	0	0	0	[illegible]
6							20	0	30	0	0	0	KK
7							20	0	30	0	0	0	SO

Observations: ______________________

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50% v/v   Sample Name: EDL-2 L2713601-2   Sample #: 3730.003222 35

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06								
1	12								
2	13								
3	14								
4	15								
5	16								
6	17								
7	18								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	CS
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	✓
						0	0	~~KK~~ 10	0	0	0	✓
						0	10	KK ~~10~~	0	0	0	KK
						0	20	0	0	0	0	SO

Observations: ____________

Concentration: 100% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	1106	24.8	7.5	4.5	1381	6/4	13/88	5/6	C
1	12	25.0	7.9	6.3	1376	6/4	13/88	5/6	C
2	13	24.5	7.4	7.5	1403 SO ~~13~~	6/4	13/88	5/6	SO
3	14	25.2	7.3 SO	7.3	1445	6/4	13/88	5/6	SO
4	15	25.6	7.4	7.1	1457	6/4	13/88	5/6	SO
5	16	25.2	7.2	7.4	1329	6/4	13/88	5/6	KK
6	17	25.5	7.3	7.8	1365	6/4	13/88	5/6	KK
7	18								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
23.5	7.8	6.5	6/4	13/88	CT							
23.6	7.9	7.4	5/5	12/89	KK	20	0	0	0	0	0	CS
23.6	7.8	6.6	5/5	12/89	KK	20	0	0	0	0	0	KK
23.7	7.9	5.5	6/4	13/88	SO	20	0	0	0	0	0	KK
24.1	7.9	5.9	6/4	13/88	KK	20	10	20	0	0	0	✓
24.0	7.9	6.3	6/4	13/88	KK	20	10	20	0	0	0	✓
25.1	7.8	5.6	6/4	13/88	SO	20	10	20	0	0	0	KK
						20	10	30	0	0	0	SO

Observations: ____________

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information

Client AW IB

Sample # 8730-W32235

Date/Time Received 100622 / 1110

Sample Type Effluent

100% Hardness 402

Sample Name ED-2 L2713601-2

Sample Date/Time 080622 / 0810

Arrival Temp 17.7 °C

Sample Description Clear light green

Person Sampling DKW

Test Information

Date/Time Started 11.06.22 / 15:35

Test started by CD

Fathead Batch # FH0121/0621

Date eggs laid 06/07/08.06.22

Culture mortality within 7 days of egg collection 1.0%

Swim bladder inflated; yes / no Yes

Age of Larvae at start of test in hours <24

Control Hardness 90

Water Bath Quadrant I

Average Temperature during Test: 25.4±0.1 °C

Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / No (circle one)

Conc.	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	10	0.77021	0.76481	0.540	0.447
	B	10	0.75975	~~0.75588~~ 0.75602	0.373	
	C	10	0.76229	0.75801	0.428	
1.56	A	10	0.76038	0.75560	0.478	0.538
	B	10	0.76187	0.75674	0.513	
	C	10	0.77155	0.76533	0.622	
3.13	A	9	0.76189	0.75716	0.473	0.518
	B	10	0.76368	0.75775	0.593	
	C	10	0.76609	0.76121	0.488	
6.25	A	9	0.73432	0.72902	0.530	0.540
	B	9	0.75583	0.75049	0.534	
	C	10	0.74498	0.73942	0.556	
12.5	A	10	0.73713	0.73133	0.580	0.554
	B	9	0.73823	0.73325	0.498	
	C	10	0.73204	0.72620	0.584	
25	A	8	0.74007	0.73609	0.398	0.459
	B	10	0.74352	0.73755	0.597	
	C	7	0.73139	0.72756	0.383	
50	A	10	0.72073	0.71461	0.612	0.541
	B	8	0.73817	0.73414	0.403	
	C	10	0.75263	0.74655	0.608	
100	A	8	0.72421	0.71907	0.514	0.503
	B	9	0.73448	0.72865	0.583	
	C	7	0.73711	0.73299	0.412	
Initials			SO	SO	CG	CG

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

 RG

Sample # 8730-0032235 **Sample Name** EDL-2 / L2713601-2

Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug) 447

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	10	0.77021	0.76481	0.540	0.540	0.447	0.447	0.085106	0.085106	19.03933
	10	10	0.75975	0.75602	0.373	0.373					
	10	10	0.76229	0.75801	0.428	0.428					
1.56	10	10	0.76038	0.75560	0.478	0.478	0.538	0.538	0.075102	0.075102	13.96816
	10	10	0.76187	0.75674	0.513	0.513					
	10	10	0.77155	0.76533	0.622	0.622					
3.13	10	9	0.76189	0.75716	0.526	0.473	0.536	0.518	0.053204	0.065383	9.935096
	10	10	0.76368	0.75775	0.593	0.593					
	10	10	0.76609	0.76121	0.488	0.488					
6.25	10	9	0.73432	0.72902	0.589	0.530	0.579	0.540	0.020393	0.014	3.519605
	10	9	0.75583	0.75049	0.593	0.534					
	10	10	0.74498	0.73942	0.556	0.556					
12.5	10	10	0.73713	0.73133	0.580	0.580	0.572	0.554	0.016671	0.048539	2.912267
	10	9	0.73823	0.73325	0.553	0.498					
	10	10	0.73204	0.72620	0.584	0.584					
25	10	8	0.74007	0.73609	0.497	0.398	0.547	0.459	0.04975	0.119459	9.091509
	10	10	0.74352	0.73755	0.597	0.597					
	10	7	0.73139	0.72756	0.547	0.383					
50	10	10	0.72073	0.71461	0.612	0.612	0.575	0.541	0.061376	0.119528	10.68184
	10	8	0.73817	0.73414	0.504	0.403					
	10	10	0.75263	0.74655	0.608	0.608					
100	10	8	0.72421	0.71907	0.643	0.514	0.626	0.503	0.032766	0.086029	5.231768
	10	9	0.73448	0.72865	0.648	0.583					
	10	7	0.73711	0.73299	0.589	0.412					

SD: Standard Deviation
CV: Coefficient of Variation

NAUTILUS ENVIRONMENTAL

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	EDL2 L2713601-2
Date/Time Sampled	June 8, 2022; 08:10	**Date/Time Received:**	June 10, 2022; 11:10
Date Test Started:	June 10, 2022	**Date Test Finished:**	June 16, 2022
Description:	Clear, light green	**Days Sample Used:**	Days 0 to 5
Sample #:	8730-0032235	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.7°C
Collected By:	Not available		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032235 **Sources:** EDL2 L2713601-2

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 3.3%

Mean Number of Surviving Young Produced Within First 3 Broods: 25.8

Mean Number of Surviving Young Produced Within 7 Days of Testing: 50.3

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 0 to 5

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032235 **Sources:** EDL2 L2713601-2

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD22-73

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (21.7 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (90% of controls)

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032235 **Sources:** EDL2 L2713601-2

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	0	0	0	0	0	0	10
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	28	9	14	17	8	14	12	11
	2	23	17	19	22	28	14	20	23
	3	24	22	24	20	19	12	23	0
	4	24	22	26	16	20	15	0	19
	5	21	16	14	25	26	23	28	10
	6	21	20	22	24	23	27	25	13
	7	22	22	19	24	24	24	8	21
	8	22	18	16	21	15	7	25	19
	9	19	26	16	22	21	21	18	0
	10	13	31	25	25	16	20	21	15
Total Number of Live Neonates in First 3 Broods or Less		217	203	195	216	200	177	180	131
% Effect (+ or -)		0.0	-6.5	-10.1	-0.5	-7.8	-18.4	-17.1	-39.6
Mean Number of Live Neonates in First 3 Broods or Less		21.7	20.3	19.5	21.6	20.0	17.7	18.0	13.1
SD		3.9	5.9	4.5	3.2	5.9	6.3	8.8	8.1

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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Ceriodaphnia dubia **Survival and Reproduction Study -** Continued

Sample #: 8730-0032235 **Sources:** EDL2 L2713601-2

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	48.1% Volume (6.28% Volume; N/A)	No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 02-Jun-2022 **Reference Batch #:** Zn2102

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 0.15 mg/L (0.12 mg/L; 0.19 mg/L)

Historic Geometric Mean LC50: 0.08 mg/L (0.03 mg/L; 0.27 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: AES - TB Sample Name: EDL - 2 Sample #: 8730-0032235
L2713601-2

Conditions for Test Validity

Control Mortality is< 20%: (Acceptable) / Not Acceptable: 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable) / Not Acceptable: 01 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable) / Not Acceptable: 21.7 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 0 to 5

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
REPRODUCTION		No nonlinear regression models would fit
3-brood IC25	48.1 % Volume	ICPIN - linear interpolation
95% Confidence Interval[2]	6.28 ; N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 23/06/22

Results Verified: Initials CO Date 26/06/22

Ceriodaphnia dubia Initial Sample Measurements Before Preparation and Use in Toxicity Te

Concentration: 100% **Sample Name:** EDL2 L2713601-2 **Sample #:** 9730 00822 35

Day	Date 2022	Initial Measurements: Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	Meters / Probes Used: °C	pH	Cond.	Pre-aeration: yes/no	Rate (bubbles/min)	Duration (min)	Pail Sub-Sampled	Initials
0	10th	25.3	7.2	2.7	1410	6/4	13/88	5/6	yes	≤100	≤ 20	1	WL
1	11	24.7	7.4	6.1	1371	6/4	13/88	5/6	Ves	≤100	≤ 20	1	E
2	12	25.1	7.3	9.2	1382	6/4	13/88	5/6	Ves	≤100	≤ 20	1	C
3	13	26.4	7.3	9.1	1470	6/4	13/88	5/6	yes	≤100	≤ 20	2	KK
4	14	24.6	7.4	9.6	1411	6/4	13/88	5/6	yes	≤100	≤ 20	2	SO
5	15	24.5	7.5	9.9	1406	6/4	13/88	5/6	Ves	≤100	≤ 20	3	C
6	16									≤100	≤ 20	3	
7	17									≤100	≤ 20	3	
8	18									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted?	Yes/No	If yes, state mesh size:
Was sample pH or hardness adjusted?	Yes/No	If yes, describe further:
Were alternate concentrations or dilution series used?	Yes/No	If yes, describe further:
Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily?	Yes/No	If no, describe further:
Were there any other method variations, deviations, or exclusions from method?	Yes/No	If yes, describe further:
Was there anything unusual about the test, any problems encountered, or any remedial measures taken?	Yes/No	If yes, describe further:

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: Control Sample Name: EDL2 L271301-2 Sample #: 3730003 2235

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06	26.1	8.2	7.5	442	6/4	13/88	5/6	WL
1	11	25.7	8.4	7.3	445	6/4	13/88	5/6	CB
2	12	24.9	8.2	8.1	424	6/4	13/88	5/6	CB
3	13	26.1	8.1	7.5	449	6/4	13/88	5/6	CS
4	14	25.6	8.3	7.1	475	6/4	13/88	5/6	SO
5	15	25.1	8.2	7.7	477	6/4	13/88	5/6	KK
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.5	7.9	6.7	6/4	13/88	CB
23.7	7.9	6.9	6/4	13/88	CB
24.3	7.7	7.6	6/4	13/88	CB
24.9	7.7	7.6	6/4	13/88	CB
24.4	8.0	6.8	6/4	13/88	SO
25.0	8.1	7.3	6/4	13/88	KK

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10Oct																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CB	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	CB	—
3	13	0	0	6	6	3	0	4	4	2	0	25	—	0	—	CB	CB
4	14	6	5	0	0	0	0	0	0	0	3	14	—	0	—	CB	CB
5	15	12	10	7	8	8	11	10	7	8	3	84	—	0	0	CB	CB
6	16	10	8	11	10	10	10	8	11	9	7	94	—	0	—	CB	CB
7	17																
8	17																
Total Neonates		28	23	24	24	21	21	22	22	19	13	217	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 1.337% v/v Sample Name: EDL2 L271301-2 Sample #: 3730008 2235

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10:06	25.0	8.2	7.7	442	6/4	13/88	5/6	WL
1	11	25.3	8.2	7.6	454	6/4	13/88	5/6	C
2	12	25.8	8.2	7.6	437	6/4	13/88	5/6	C
3	13	25.8	8.2	7.3	449	6/4	13/88	5/6	CS
4	14	25.7	8.2	7.1	459	6/4	13/88	5/6	SO
5	15	25.4	8.1	7.2	457	6/4	13/88	5/6	KK
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.7	7.9	6.6	6/4	13/88	C
23.4	7.9	6.5	6/4	13/88	C
24.7	7.7	7.1	6/4	13/88	CO
24.9	7.9	7.1	6/4	13/88	CO
24.2	8.0	6.7	6/4	13/88	SO
24.6	8.1	7.2	6/4	13/88	KK

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10:06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CO	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	CO	—
3	13	0	0	6	4	4	6	4	0	4	5	33	—	0	—	CO	CO
4	14	2	3	0	0	0	0	0	0	0	13	18	—	0	—	CO	CO
5	15	7	6	8	7	9	5	10	9	9	0	70	—	0	0	CO	CO
6	16	0	8	8	11	3	9	8	9	13	13	82	—	0	—	CO	CO
7	17																
8	19																
Total Neonates		9	17	22	22	16	20	22	18	26	31	203	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.41 1.vW Sample Name: EDL2 L2713001-2 Sample #: 3730.003 2235

Day	Date 2020	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2020	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10 06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ao	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ao	—
3	13	0	4	4	0	0	3	3	3	0	3	20	—	0	—	ao	ao
4	14	4	0	0	0	6	0	0	6	0	0	16	—	0	—	ao	ao
5	15	10	8	10	10	8	8	8	7	7	10	86	—	0	0	ao	ao
6	16	0	7	10	16	0	11	8	✱	9	12	73	—	0	—	B	0
7	17																
8	17																
Total Neonates		14	19	24	26	14	22	19	16	16	25	195	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra: 1.23 1.vV Sample Name: EDL2 L2713001-2 Sample #: 3730.003 2235

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
3	13	0	0	0	0	0	0	4	0	3	5	12	—	0	—	[illegible]	[illegible]
4	14	5	4	4	0	6	0	0	2	0	8	29	—	0	—	[illegible]	[illegible]
5	15	12	8	9	5	8	10	10	8	9	0	78	—	0	0	[illegible]	[illegible]
6	16	0	10	8	11	11	14	10	11	10	12	97	—	0	—	[illegible]	[illegible]
7	17																
8	19																
Total Neonates		17	22	20	16	25	24	24	21	22	25	216	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra: 3.71.VN Sample Name: EDL2 L2713601-2 Sample #: 3730.003 2235

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10:06	25.0	8.2	7.8	477	6/4	13/88	5/6	WL
1	11	25.1	8.2	7.7	480	6/4	13/88	5/6	C
2	12	25.8	8.2	7.5	461	6/4	13/88	5/6	C
3	13	25.9	8.2	7.4	480	6/4	13/88	5/6	C
4	14	25.5	8.2	7.1	500	6/4	13/88	5/6	SO
5	15	25.5	8.1	7.2	496	6/4	13/88	5/6	KC
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.6	7.9	6.2	6/4	13/88	C
23.4	7.9	6.8	6/4	13/88	C
24.5	7.8	7.3	6/4	13/88	C
24.0	7.9	7.9	6/4	13/88	C
24.1	8.0	6.7	6/4	13/88	SO
24.3	8.0	7.1	6/4	13/88	KC

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10:06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	SO	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	KC	—
3	13	0	0	0	0	0	6	3	0	2	4	15	—	0	—	SO	SO
4	14	0	4	0	0	5	0	0	0	4	0	13	—	0	—	SO	SO
5	15	8	10	9	9	9	11	9	6	8+7	2	88	—	0	6	5	2
6	16	0	14	10	11	12	6	12	9	*	10	84	—	0	—	2	0
7	17																
8	19																
Total Neonates		8	28	19	20	26	23	24	15	21	16	200	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra . 11.1'1.vV   Sample Name: EDL2   L2713601-2   Sample #: 3730 003   2235

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	10.06														
1	11														
2	12														
3	13														
4	14														
5	15														
6	16														
7	17														

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10 06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	AO	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
3	13	0	0	0	0	0	5	5	0	3	4	17	—	0	—	AO	AO
4	14	0	2	2	4	3	0	0	0	0	0	11	—	0	—	AO	AO
5	15	11	6	7	7	9	9	9	5	11	6	80	—	0	0	[illegible]	[illegible]
6	16	3	6	3	4	11	13	10	2	7	10	69	—	0	—	[illegible]	[illegible]
7	17																
8	17																
Total Neonates		14	14	12	15	23	27	24	7	21	20	177					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra: 33.3 %v/v Sample Name: EDL2 L271301-2 Sample #: 3730.003 2235

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	AO	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	AO	—
3	13	0	4	3	0	0	5	4	3	4	4	27	—	0	—	AO	96
4	14	4	0	0	0	7	0	0	0	0	7	18	—	0	—	AO	96
5	15	7+1	10	9	0	10	9	3+1	9	7	0	66	—	0	0	AO	—
6	16	0	6	11	0	11	11	0	13	7	10	69	—	0	—	AO	—
7	17																
8	19																
Total Neonates		12	20	23	0	28	25	8	25	18	21	180	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra: 100% v/v Sample Name: EDL2 L2713001-2 Sample #: 3730.008 2235

Day	Date 2020	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06	24.9	7.6	5.7 WC / 5.5	1392	6/4	13/88	5/6	WC
1	11	25.5	7.6	6.1	1402	6/4	13/87	5/6	C
2	12	25.7	7.7	7.5	+91404	6/4	13/88	5/6	C
3	13	25.8	7.7	7.4	14428	6/4	13/88	5/4	CS
4	14	25.6	7.7	7.3	1454	6/4	13/88	5/4	SO
5	15	25.3	7.5	8.5	1434	6/4	13/88	5/6	KK
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.7	7.91	6.6	6/4	13/88	C
23.5	8.1	7.0	6/4	13/88	CO
24.4	7.9	6.7	6/4	13/88	CO
24.0	7.9	6.8	6/4	13/88	CO
24.3	8.1	6.4	6/4	13/88	SO
24.4	8.2	7.2	6/4	13/88	KK

Day	Date 2020	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10th																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CO	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	CO	—
3	13	0	4	0	0	0	4	6	5	0	5	20	—	0	—	CO	CO
4	14	4	0	0	3	2	0	0	0	0	0	9	—	0	—	CO	CO
5	15	7	9	0	8	6	3	6	7	dead	3	49	9 —	10	0	SO	SO
6	16	0	10	0	8	0	6	9	7		7	47	—	10	—	KK	KK
7	17																
8	19																
Total Neonates		11	23	0	19	10	13	21	19	0	15	131	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia Neonate Origin

Sample Information

Client ALS Thunder bay
Sample # 8330-W32235
Date/Time Received 10.06.22 / 1110
Sample Type Effluent
100% Hardness 402

Sample Name EDL2 L2713001-2
Date/Time Collected 08.06.22 / 0810 Person Sampling N/A
Arrival Temp (°C) 17.7
Sample Description Clear light green

Test Information

Date Test Started 101022
Dilution Water Batch Number 0822-F3

Test Started By GD
Control Hardness 120

Template Used for Randomization T1

Individual Culture Health Data

Date Culture Started 01 0022
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 25.4

Culture I.D. (*e.g.*, Wed Row 4) WED ROW5
Average # neos in previous 7 days (must be ≥15) 49.8
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 01 0622
% mortality in previous 7 days (must be ≤20%) 10
Average # neos in 1st 3 broods (must be ≥ 15) 26.2

Culture I.D. (*e.g.*, Wed Row 4) WED ROW6
Average # neos in previous 7 days (must be ≥15) 49.8
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 01 06.22
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 25.4

Culture I.D. (*e.g.*, Wed Row 4) WED ROW7
Average # neos in previous 7 days (must be ≥15) 51.2
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started _____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____

Culture I.D. (*e.g.*, Wed Row 4) _____
Average # neos in previous 7 days (must be ≥15) _____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started _____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____

Culture I.D. (*e.g.*, Wed Row 4) _____
Average # neos in previous 7 days (must be ≥15) _____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 3.3 (add up % mortality for all cultures used / # cultures used)
Mean Number of Young Produced within first 3 broods 25.7 (avg # 1st 3 broods for all cultures used / # cultures used)
Mean Number of Young Produced in previous 7 days 50.3 (avg # neos in prev 7 days for all cultures used / # cultures used)
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / No (circle one)

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
W.5.7	Y / N	Y / N	18	1	GD
8	Y / N	Y / N	15	2	GD
9	Y / N	Y / N	7	3	GD
W.6.6	Y / N	Y / N	14	4	GD
7	Y / N	Y / N	15	5	GD
9	Y / N	Y / N	13	6	GD
10	Y / N	Y / N	9	7	GD
W.7.1	Y / N	Y / N	16	8	GD
W.7. 1	Y / N	Y / N	13	9	GD
2	Y / N	Y / N	16	10	GD
	Y / N	Y / N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032235	**Sample Name:**	EDL2 L2713601-2
Sample Method:	Grab	**Collected by:**	Not available
Date/Time Collected:	June 8, 2022; 08:10	**Arrival Temp.:**	17.7°C
Date/Time Received:	June 10, 2022; 11:10	**Sample Description:**	Clear, light green
Sample Point Description:	Other	**Sample Type:**	Effluent
Transportation:	Road		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species (Strain #): *Raphidocelis subcapitata* (CPCC # 37)

Source: Nautilus Plant Culture Unit (from CPCC)

Culture Temp.: 24 ± 2 °C

Test Culture Number: G4(l)a

Culture Age at Test Start: 4 days old

Cell Density in the Microplate Wells at the Start of the Test: 10,568.18 cells/ml

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*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032235 **Sample Name:** EDL2 L2713601-2

Test Conditions

Date/Time Test Start:	June 11, 2022; 11:50	**T=0 Control pH:**	6.9
Date/Time Test End:	June 14, 2022; 11:30 - 14:30	**T=72 Control pH:**	6.9
Sample pH Before Dilution:	7.9	**pH Adjustment:**	None
Test Duration:	72 hours		

Mean Test Temperature (±Standard Deviation): 25.3 (±0.1)°C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Millipore

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032235 **Sample Name:** EDL2 L2713601-2

Test Conditions - continued

Enumeration Technique:	Neubauer Haemacytometer
Test Vessel:	96-Well U-bottomed Polystyrene Microplate
Concentration of Test Solutions:	90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control
# Replicates/Concentration:	4 reps started; 3 reps counted of test solutions 10 control reps started, 2 used for pH measurement
Design if Specialized Procedure:	Not applicable
Method Deviations or Unusual Occurrences:	None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times:	Acceptable (24.2 times)
pH in controls did not vary by more than 1.5 units:	Acceptable (0 units)
C V for cell yields is < 20% within the control wells:	Acceptable (12.1%)
No inhibitory trend detected across the control wells:	Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
231,932	244,432	286,932	304,432	226,932	279,432	221,932	246,932	255,369	12.1

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 CV = Coefficient of Variation = (100 x standard deviation / mean)

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032235 **Sample Name:** EDL2 L2713601-2

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	686,932	964,432	854,432	439,432	314,432
2	729,432	1,021,932	674,432	466,932	261,932
3	821,932	1,096,932	749,432	476,932	361,932
Mean Cell Yield	746,098	1,027,765	759,432	461,098	312,765
Coefficient Variation[2]	9	6	12	4	16
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	344,432	329,432			
2	346,932	314,432			
3	321,932	329,432			
Mean Cell Yield	337,765	324,432			
Coefficient Variation[2]	4	3			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	**Result[1]**	**Method of Calculation**
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	no trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032235 **Sample Name:** EDL2 L2713601-2

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software, 2001-2007 Comprehensive Environmental Toxicity, Information System - CETIS v1.8.1.2.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: None

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2209 **Date Test Initiated:** 17-Jun-2022

Method of Analysis: Linear Interpolation (ICPIN) **Algae Lot #:** G4(l)a

72-hour IC25 (95% Confidence Limits): 67.48 mg/L (48.16 mg/L; 83.28 mg/L)

Historic Geometric Mean IC25: 51.76 mg/L (23.79 mg/L; 112.61 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS - TB Sample Name EDL 2 L2713601-2 Sample # 8730-0032235

Conditions for Test Validity

Cell increase for control is >16	(Acceptable)/Not acceptable	24.2 (times)
CV among controls ≤ 20	(Acceptable)/Not acceptable	12.1
Result of Mann-Kendall test for trend	(Acceptable)/Not acceptable	no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 116 250 (cells/mL)

Used: (Yes)/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10568.18 (cells/mL)

Analysis Completed: Initials: EA Date: 27/06/22

Results Verified: Initials: AD Date: 29/06/22

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location: ALS Thunder Bay	
Sample #: 8730 2003 2235	Sample Name: EDL 2 L 2736601-2
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 08/06/22 8:10	Arrival Temp.: (meter/probe) 17.7 °C (47)
Date/Time Received: 10/06/22 11:10	Sample Description: clear light green
Collection Point Description: other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: Air / Road	Storage: 4±2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # G 411)a	
Culture Age at Start of Test	4 days old (must be 3 to 7 days old)	
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	
	Axenic culture? Yes/No (Circle one)	

Notes:

Test Conditions:

Date / Time Test Start: 11.06.22 11:50 Started By:		Date / Time Test End: 14.06.22 11:30 – 14:30 Finished By:	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 7.9	pH adjustment: Y/N	pH of well D6 at T=0 h 6.9	pH of well D7 at T=72 h 6.9
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # —		Metal mining NUT Lot # NV CT 2203
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation) 25.3 ± 0.1 °C			

	Day 1		Day 2	
¼ plate rotation (Initial)	AM	PM	AM	PM
Lights ON (Initial)	AM	PM	AM	PM

* For reference test, record pH of top concentration before use for series dilutions.

Notes: Millipore

72-Hour Qualitative Observations:

Condensation: Yes		
Growth: Yes		
Were there any other method variations or deviations from methods? Yes/No		If yes, describe further:
Anything unusual about the test? Yes/No		
Any problems encountered? Yes/No		
Any remedial measures taken? Yes/No		

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #:	8730-0032235	Sample Name:	EDL 2 L2713601-2

Reference Data:

Reference Chemical Batch #	Phenol B P22089	Date test started	17.06.22
Method of Analysis	ICPIN – linear interpolation	Algae Lot #	G 4(1) a
72-hour IC25 (95% C.I.)[3] mg/L	67.48 48.16; 83.28	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	51.76 23.79; 112.61

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	>90.91 v/v (N/A)	No no linear regression model could fit
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers		ICPIN – linear interpolation
Test for Outliers	No Outliers Present ✓	Grubbs' Test for Residual Outlier Initial C
	If outliers present, indicate Concentration/Rep: —	
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed.	
	No statistically significant growth stimulation.	Williams' or (Dunnett's) Multiple Comparison Test Initial C
	(Yes), statistically significant growth stimulation at these concentrations: 90.91; 30.303; 10.101; 3.367	

1)Results relate only to the sample tested.
2) Tidepool Scientific Software © 2001-2007. Comprehensive Environmental Toxicity Information System – CETIS v. 1.9.6.7
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/(No)

Any outliers and justification for their removal? Yes/(No)

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Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)

72-Hour Growth Inhibition Test – Continued

72-Hour Quantitative Observations of Controls

Sample Name: EDL 2 Sample Number: 8730-003235 Date Test Start: 11.06.22

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D8	Well # D9	Well # D10	Well # D11
1	20	27	32	28	22	24	26	29
2	27	27	34	35	24	30	22	23
3	23	22	31	36	26	24	20	25
4	27	26	22	27	23	38	25	26
5	—	—	—	—	—	—	—	—
Initials	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Cell increase for controls = 24.2	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = 12.1	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = no trend	Controls are invalid if there is a trend detected by the Mann-Kendall test

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL2 Sample Number: 8730-00322 Date Test Start: 11.06.22

Theoretical Test Concentration: 100.00 %. v/v					Actual Test Concentration: 90.91 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	75	79	89		746098 (± 69026)
2	69	67	78		Coefficient of Variation of Cell Yield
3	59	72	78		9
4	76	78	88		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-192.164
Initials	C	C	C		C

Theoretical Test Concentration: 33.33 %. v/v					Actual Test Concentration: 30.303 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	89	104	98		1027765 (± 66462)
2	101	113	111		Coefficient of Variation of Cell Yield
3	108	89	143		6
4	92	107	91		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-302.462
Initials	C	C	C		C

Theoretical Test Concentration: 11.11 %. v/v					Actual Test Concentration: 10.101 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	82	81	82		759432 (± 90416)
2	91	57	84		Coefficient of Variation of Cell Yield
3	93	56	53		12
4	80	80	85		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-197.386
Initials	C	C	C		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL2 Sample Number: 8730 00322 3 Date Test Start: 11.06.22

Theoretical Test Concentration: 3.704 %v/v					Actual Test Concentration: 3.367 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation)
1	46	46	48		461098 (± 19419)
2	46	49	46		Coefficient of Variation of Cell Yield
3	49	47	39		4
4	39	49	62		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-80.561
Initials	C	C	C		C

Theoretical Test Concentration: 1.235 %v/v					Actual Test Concentration: 1.122 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation)
1	36	23	33		312765 (± 50021)
2	30	24	46		Coefficient of Variation of Cell Yield
3	29	34	43		16
4	35	28	27		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-22.476
Initials	C	C	C		C

Theoretical Test Concentration: 0.412 %v/v					Actual Test Concentration: 0.374 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation)
1	41	42	31		337765 (± 13769)
2	32	35	35		Coefficient of Variation of Cell Yield
3	34	37	29		4
4	35	29	38		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-32.265
Initials	C	C	C		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL2 Sample Number: 8730 00322 35 Date Test Start: 11.06.22

Theoretical Test Concentration: 0.137 %v/v					Actual Test Concentration: 0.125 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	32	37	31		324432 (± 8660)
2	38	29	42		Coefficient of Variation of Cell Yield
3	39	34	35		3
4	27	30	28		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-27.044
Initials	[illegible]	[illegible]	[illegible]		[illegible]

Theoretical Test Concentration:			Actual Test Concentration:		
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:			Actual Test Concentration:		
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	EDL2			Sample #	8730-0032235			Date test start	11.06.22							
Calculate initial algal cell concentration																
	Concentration of innocculum (cells/ml)				116250			Use last count algae/nutrient mixture or algae only								
	Volume of algae addition (uL)				20			Algae/nutrient mixture = 20uL, algae only 10uL								
	Cells added to each test well				2325			**Cell yield (must be >16 times in controls)**								
	Cells/ml in well at T=0				10568.1818			=	24.163978							
enter control data																
	D2	D3	D4	D5	D8	D9	D10	D11								
	20	27	32	28	22	24	26	29								
	27.0	27.0	34.0	35.0	24.0	30.0	22.0	23.0								
	23	22.0	31.0	36.0	26.0	24.0	20.0	25.0								
	27.0	26.0	22.0	27.0	23.0	38.0	25.0	26.0								
total cells	97	102	119	126	95	116	93	103								
cells/ul	242.5	255	297.5	315	237.5	290	232.5	257.5								
cells/ml	242500	255000	297500	315000	237500	290000	232500	257500								
Cell yield = measured concentration - initial algal cell concentration																
	231932	244432	286932	304432	226932	279432	221932	246932								
Mean cell yield for the control	= Rc			Standard deviation			coefficient of variation									
Rc	255369			SD	30820.2595		CV	12.068897	**Must be ≤20**							

% inhibition summary

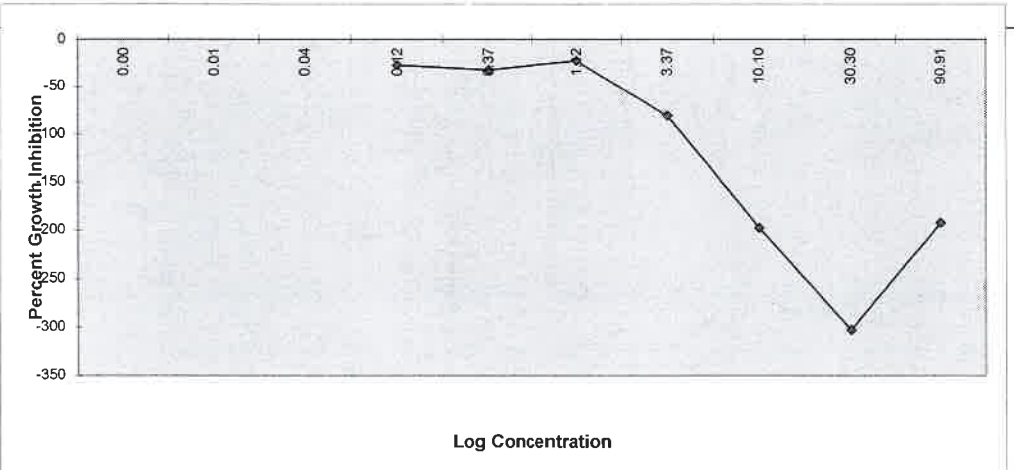
Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	-27.044
0.374	-32.265
1.122	-22.476
3.367	-80.561
10.101	-197.386
30.303	-302.462
90.910	-192.164

enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	75	79	89	89	104	98	82	81	82	46	46	48	36	23	33
	69.0	67.0	78.0	101.0	113.0	111.0	91.0	57.0	84.0	46.0	49.0	46.0	30.0	24.0	46.0
	59.0	72.0	78.0	108.0	89.0	143.0	93.0	56.0	53.0	49.0	47.0	39.0	29.0	34.0	43.0
	76.0	78.0	88.0	92.0	107.0	91.0	80.0	80.0	85.0	39.0	49.0	62.0	35.0	28.0	27.0
total cells	279	296	333	390	413	443	346	274	304	180	191	195	130	109	149
cells/ul	697.5	740	832.5	975	1032.5	1107.5	865	685	760	450	477.5	487.5	325	272.5	372.5
cells/ml	697500	740000	832500	975000	1032500	1107500	865000	685000	760000	450000	477500	487500	325000	272500	372500
Cell yield = measured concentration - initial algal cell concentration															
	686932	729432	821932	964432	1021932	1096932	854432	674432	749432	439432	466932	476932	314432	261932	361932
Mean Yield	746098			1027765			759432			461098			312765		
STD Yield	69026			66442			90416			19419			50021		
CV Yield	9			6			12			4			16		

Average % inhibition												
	for 90.910%:	-192.164	for 30.303%	-302.462	for 10.101%	-197.386	for 3.367%	-80.561	for 1.122%	-22.476		

Average % stimulation												
	for 90.910%:	192.164	for 30.303%	302.462	for 10.101%	197.386	for 3.367%	80.561	for 1.122%	22.476		



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
41	42	31	32	37	31									
32.0	35.0	35.0	38.0	29.0	42.0									
34.0	37.0	29.0	39.0	34.0	35.0									
35.0	29.0	38.0	27.0	30.0	28.0									
142	143	133	136	130	136	0	0	0	0	0	0	0	0	0
355	357.5	332.5	340	325	340	0	0	0	0	0	0	0	0	0
355000	357500	332500	340000	325000	340000	0	0	0	0	0	0	0	0	0
344432	346932	321932	329432	314432	329432	-10568	-10568	-10568	-10568	-10568	-10568	-10568	-10568	-10568
337765			324432			-10568			-10568			-10568		
13769			8660			0			0			0		
4			3			0			0			0		
for 0.374%	-32.265		for 0.125%	-27.044		for 0.042%	104.138		for 0.014%	104.138		for 0.005%	104.138	
for 0.374%	32.265		for 0.125%	27.044		for 0.042%	-104.138		for 0.014%	-104.138		for 0.005%	-104.138	

ao 2907022

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
EDL2 L2713601-2	8730-0032235	June 8, 2022	June 10, 2022	June 10, 2022	79.11% Volume (14.14% Volume; N/A)	0.25% Volume (Not applicable)	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	03-Jun-2022	**Reference Chemical:**	KCl
Frond Increase IC25:	2.71 g/L	**95% Confidence Limits:**	2.21; 3.19 g/L
Historic Geometric Mean IC25:	2.24 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.50; 3.34 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	Na_2MoO_4	0.0726
	K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$2H_2O$	0.00003
	KCl	10.1	$MgSO_4\ 7H_2O$	147	$ZnCl_2$	0.00001
					$CoCl_2\ 6H_2O$	0.00001
					$CuCl_2\ 2H_2O$	

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

Date: June 30, 2022

C. D'Andrea

Carol D'Andrea
Laboratory Supervisor

Test Material

Client Name/Location:	AMS - Thunder Bay		
Sample #:	8730-0032235	Sample Name:	EDL 2 L2713601-2
Collection Method:	Grab	Collected By:	N/A
Date/Time Collected:	08/06/22 8:40	Arrival Temperature (meter/probe):	°C (4.7)
Date/Time Received:	10/06/22 11:10	Sample Description:	clear, light green
Collection Point Description:	Other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:	
Transportation:	Air / Road	Storage:	none

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	EV
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	EV
Culture Age at Start of Test:	9 days old, acclimated 24 hours in fresh test solution (mAPHA)	EV
Culture Medium:	Modified Hoagland's E+ medium, Lot # MH220	EV
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/No	EV
	Axenic culture? Yes/No	EV
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 29 in HT 1, 31 in HT 2, 29 in HT 3 at finish	EV

Test Conditions and Procedures

Date / Time Test Start: 10.06.22 15:15 17:00 Started By: EV	Date / Time Test End: 17.06.22 9:05 Finished By: EV
Test Type: Static (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / No (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: Modified APHA (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 7.6 (13/88	pH Adjustment: none
Test Volume and Depth: 150 mL / 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / No	If yes, describe further:
Anything unusual about the test? Yes / No	
Any problems encountered? Yes / No	
Any remedial measures taken? Yes / No	Randomization Template: A

Sample #: 8730-0032235	Sample Name: EDL 2

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	24.5	24.1	24.2	24.3	24.3	24.7	24.8	24.8
Low	24.5	24.1	24.1	24.2	24.3	24.7	24.7	24.8
Medium	24.7	24.1	24.1	24.3	24.3	24.7	24.7	24.8
High	24.8	24.1	24.1	24.3	24.3	24.7	24.7	24.8
Initials	TV	TV	CS	TV	TV	TV	CS	TV
meter/probe	51	51	51	51	44	44	44	44
Mean Test Temperature (average of 24h high / low temperatures):					25.4 ± 0.1		°C	

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.3	8.1	8.0	7.6	TV	13/80
Day 7	**Control**	**Low**	**Medium**	**High**	**Initials**	**pH meter/probe**
	8.3	8.9	9.6	9.7	TV	12/89

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)								

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 11/06/22 (1)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 12					Initials: TV
4430	4150	4930	4430	4360	Mean Light Measurement: 4460
±15% Variation of Mean: 3791 – 5129					(Acceptable) / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) No
If yes, describe further: Algae in upper concentrations

Reference Data

Reference Date	(Frond Increase) or Dry Weights (circle one)			
03/06/22	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
	2.71	2.21 ; 3.19	2.24	1.50 ; 3.34

Sample #: 8730-003-2235	Sample Name: EDL 2

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 52	B 48	C 53	D 50	50.8

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy						Initials	
						G	
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV : 44.8 (2.2) 5.0							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control (% v/v) g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-2.79	-17.32	-11.73	-13.97	-13.97	-20.67	-27.93
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control (% v/v) g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-13.80	-36.28	-22.54	-28.70	-22.12	-32.91	-21.96

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: Yes / (Not applicable (no stimulation) (Circle one)

Test Endpoints and Calculations:

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	79.11 (14.14; N/A)	No nonlinear regression 3P Log Gompertz model
IC25 (95% C.I.)[3] If calculated without outlier[4]		
Dry Weights		
IC25 (95% C.I.)[3]	0.25 1. (N/A)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN – Linear interpolation

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019. Comprehensive Environmental Toxicity Information System CETISv 1.9.6.7
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / (No)

Any outliers and justification for their removal? Yes / (No)

Lemna minor Observations

Client:	ALS - TB	Sample number:	8730 00 32235
Site:	EDL 2	Date Started:	10.06.22
Concentration:	Control	Observations By:	(T)
		Date Ended:	17.06.22

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	52	48	53	50
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	√	√	√	√
Other Observations				

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO
N/A Weights: YES / NO

Concentration:	0.0977 w/v	Observations By:	(T)

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	47	48	48	55
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	X	√	√	√
Other Observations	algae	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO)
Weights: YES / (NO)

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Observations

Client:	ALS- TB	Sample number: 8730-0032235	Date Started: 10.06.22
Site:	EDL 2		Date Ended: 17.06.22
Concentration:	0.29 %v/v	Observations By: (T)	Concentration: 0.97 %v/v Observations By: (T)

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	38	45	44	45	Number of	52	44	47	39
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→	Other Observations	algae	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO)
Weights: YES / (NO)

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO)
Weights: YES / (NO)

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Observations

Client:	ALS- TB	Sample number:	8730-00322351
Site:	EDL 2	Date Started:	10.06.22
Concentration:	3.1 % v/v	Observations By:	CJ
		Date Ended:	17.06.22

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	43	46	48	41
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	X	√	√	√
Other Observations	algae	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO)
Weights: YES / (NO)

Concentration: 9.7 % v/v — Observations By: CJ

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	43	46	51	38
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	√	√	√	√
Other Observations	algae	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO)
Weights: YES / (NO)

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Observations

Client:	ALS- TB	Sample number: 8730-00322351	Date Started: 10.06.22
Site:	EDL 2		Date Ended: 17.06.22
Concentration:	31% v/v	Observations By: (T)	Concentration: 97% v/v Observations By: (T)

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	38	41	46	41	Number of	39	39	37	38
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→	Other Observations	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Weights

Client	ALS – TB	Site	EDL 2	Sample number	8730-00322
In Oven Date/Time/ °C:	17/06/22 9:20 64°	Out Oven Date/Time/°C:	18/06/22 9:20 62°		

Conc.	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	46	44.8 (2.6)	0.80327	0.79806	5.21	4.75 (0.4)
	B	42		0.81638	0.81211	4.27	
	C	47		0.81780	0.80294	4.86	
	D	44		0.79813	0.79348	4.65	
0.097	A	41	43.5 (3.7)	0.81069	0.80713	3.56	4.09 (0.5)
	B	42		0.80283	0.79871	3.98	
	C	42		0.80531	0.80125	4.06	
	D	49		0.80539	0.80062	4.77	
0.29	A	32	37.0 (3.4)	0.81379	0.80932	1.04	3.03 (0.7)
	B	39		0.81286	0.80943	3.43	
	C	38		0.81391	0.81053	3.38	
	D	39		0.80947	0.80612	3.35	
0.97	A	46	39.5 (5.4)	0.80389	0.79927	4.62	3.68 (0.8)
	B	38		0.80998	0.80633	3.65	
	C	41		0.80747	0.80370	3.77	
	D	33		0.81208	0.80941	2.67	
3.1	A	37	38.5 (3.1)	0.81118	0.79460	2.73	3.39 (0.6)
	B	40		0.80622	0.80251	3.71	
	C	42		0.80356	0.79952	4.04	
	D	35		0.81383	0.81077	3.06	
9.7	A	37	38.5 (5.4)	0.79874	0.79534	3.40	3.70 (0.5)
	B	40		0.81181	0.80793	3.88	
	C	45		0.80690	0.80265	4.25	
	D	32		0.79869	0.79543	3.26	
31	A	32	35.5 (3.3)	0.81115	0.80898	2.17	3.19 (0.7)
	B	35		0.80925	0.80563	3.62	
	C	40		0.81462	0.81086	3.76	
	D	35		0.80587	0.80268	3.19	
97	A	33	32.3 (1.0)	0.80531	0.80146	3.85	3.71 (0.3)
	B	33		0.81448	0.81084	3.64	
	C	31		0.80950	0.80544	4.06	
	D	32		0.80548	0.80221	3.27	
Initials		EC	EC	SO	EC	EC	EC

Notes:

Sample name: EDL2 L2713601-2 | Date started: 6/10/22 | sample # 8730-0032235

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

50.8 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	52	47	38	52	43	43	38	39
	48	48	45	44	46	46	41	39
	53	48	44	47	48	51	46	37
	50	55	45	39	41	38	41	38
Total Fronds	203	198	172	182	178	178	166	153
Increase in Frond Number = Total # Fronds - 6 Starting Fronds								
	46	41	32	46	37	37	32	33
	42	42	39	38	40	40	35	33
	47	42	38	41	42	45	40	31
	44	49	39	33	35	32	35	32
Total Increase	179	174	148	158	154	154	142	129
Mean Increase	44.8	43.5	37.0	39.5	38.5	38.5	35.5	32.3
SD Increase	2.2	3.7	3.4	5.4	3.1	5.4	3.3	1.0
CV Increase	5.0	8.5	9.1	13.8	8.1	14.1	9.3	3.0
% Stimulation		-2.79	-17.32	-11.73	-13.97	-13.97	-20.67	-27.93

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.80327	0.81069	0.81126	0.80389	0.79733	0.79874	0.81115	0.80531
Pan + Plant	0.81638	0.80269	0.81286	0.80998	0.80622	0.81181	0.80925	0.81448
	0.81780	0.80531	0.81391	0.80747	0.80356	0.80690	0.81462	0.80950
	0.79813	0.80539	0.80947	0.81208	0.81383	0.79869	0.80587	0.80548
Initial Weight (g)	0.79806	0.80713	0.80932	0.79927	0.79460	0.79534	0.80898	0.80146
Pan Only	0.81211	0.79871	0.80943	0.80633	0.80251	0.80793	0.80563	0.81084
	0.81294	0.80125	0.81053	0.80370	0.79952	0.80265	0.81086	0.80544
	0.79348	0.80062	0.80612	0.80941	0.81077	0.79543	0.80268	0.80221
Plant Only (mg)	5.21	3.56	1.94	4.62	2.73	3.40	2.17	3.85
	4.27	3.98	3.43	3.65	3.71	3.88	3.62	3.64
	4.86	4.06	3.38	3.77	4.04	4.25	3.76	4.06
	4.65	4.77	3.35	2.67	3.06	3.26	3.19	3.27
Mean Dry Weight	4.748	4.093	3.025	3.678	3.385	3.697	3.185	3.705
SD Dry Weight	0.4	0.5	0.7	0.8	0.6	0.5	0.7	0.3
CV Dry Weight	8.3	12.3	23.9	21.7	17.6	12.3	22.6	9.1
% Stimulation		-13.80	-36.28	-22.54	-28.70	-22.12	-32.91	-21.96

For Data Transfer to CETIS

		# fronds	total mass	tare
0	1	46	0.80327	0.79806
	2	42	0.81638	0.81211
	3	47	0.81780	0.81294
	4	44	0.79813	0.79348
0.1	1	41	0.81069	0.80713
	2	42	0.80269	0.79871
	3	42	0.80531	0.80125
	4	49	0.80539	0.80062
0.3	1	32	0.81126	0.80932
	2	39	0.81286	0.80943
	3	38	0.81391	0.81053
	4	39	0.80947	0.80612
1	1	46	0.80389	0.79927
	2	38	0.80998	0.80633
	3	41	0.80747	0.80370
	4	33	0.81208	0.80941
3.1	1	37	0.79733	0.79460
	2	40	0.80622	0.80251
	3	42	0.80356	0.79952
	4	35	0.81383	0.81077
9.7	1	37	0.79874	0.79534
	2	40	0.81181	0.80793
	3	45	0.80690	0.80265
	4	32	0.79869	0.79543
31	1	32	0.81115	0.80898
	2	35	0.80925	0.80563
	3	40	0.81462	0.81086
	4	35	0.80587	0.80268
97	1	33	0.80531	0.80146
	2	33	0.81448	0.81084
	3	31	0.80950	0.80544
	4	32	0.80548	0.80221

ao 29.06.22

June 30, 2022

Christine Paradis,
ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Dear Christine:

On June 10, 2022, Nautilus Environmental Company Inc. personnel received one water sample (SED 2 DIS L2713601-3) from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected June 8, 2022

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
SED 2 DIS L2713601-3	Fathead Minnow	7-day LC50 (95% Confidence) 7-day IC25 (95% Confidence)	Survival Biomass	>100% Volume (Not Applicable) >100% Volume[2] (Not Applicable)
#8730-0032236	*Ceriodaphnia dubia*	3-brood LC50 (95% Confidence) 3-brood IC25 (95% Confidence)	Survival Reproduction	>100% Volume (Not Applicable) 5.66% Volume (1.03; 13.39% Volume)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[3] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence) 7-day IC25 Dry Weight (95% Confidence)	Growth Growth	>97% Volume[3] (Not applicable) >97% Volume[3] (Not applicable)

1 - Results relate only to the sample tested
2 - Inhibition 29.93% at concentrations 12.5% v/v
3 - Highest concentration tested, based on test method

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 100%, 33%, 11%, 3.7% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted half by half (*i.e.,* 100%, 50%, 25% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

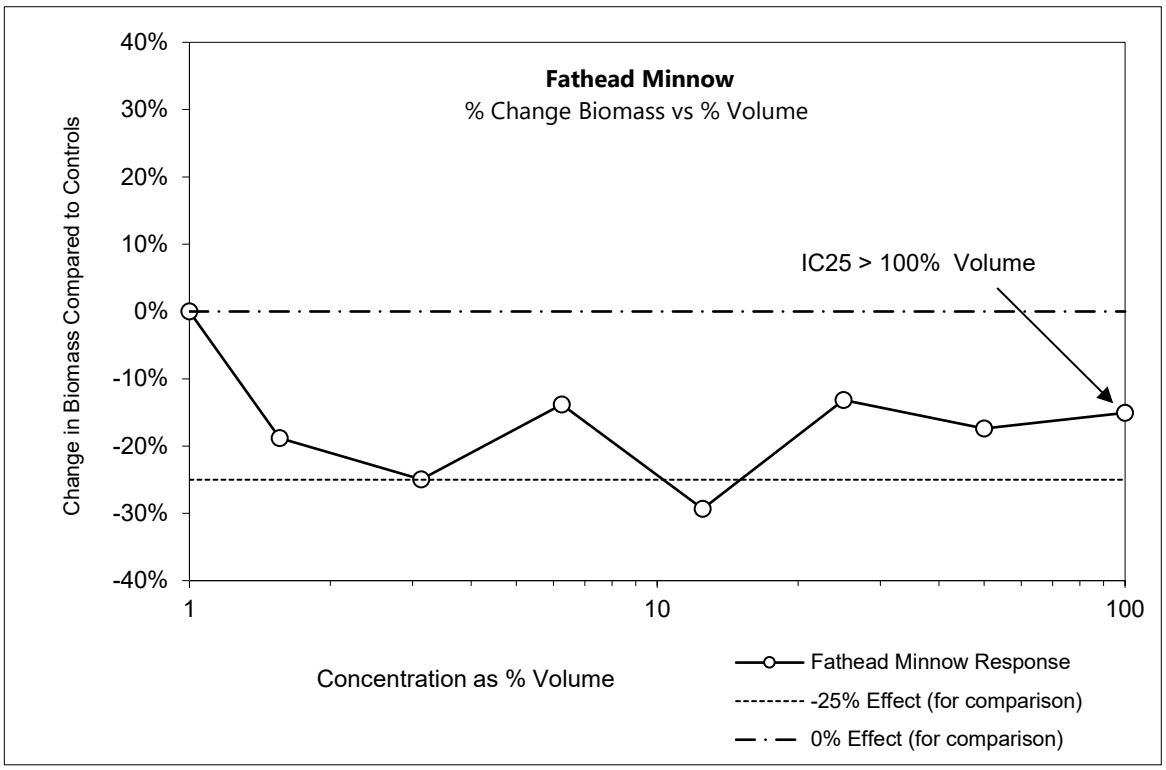
If there are any further details which you require, please do not hesitate to contact us.

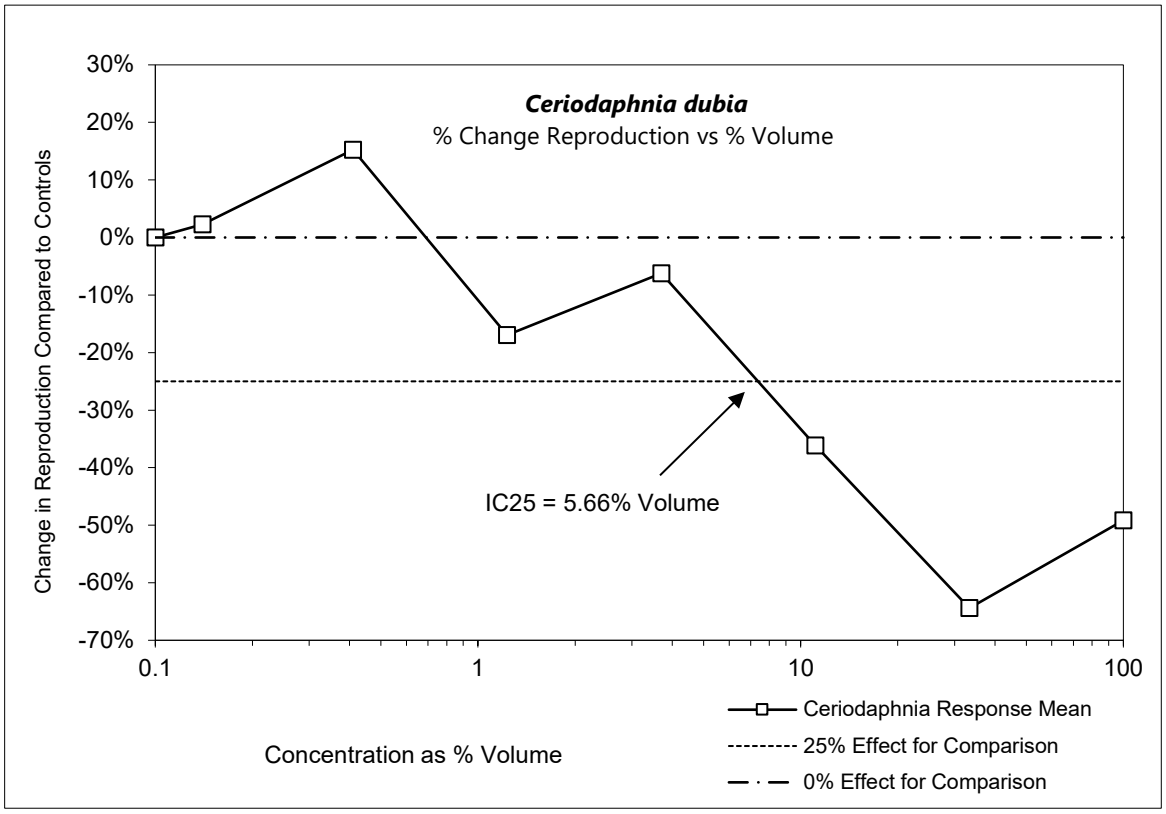
Yours very truly,
Nautilus Environmental Company Inc.

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2022\8000\8730-003\8730-0032236 FCRL

ALS Thunder Bay - SED 2 DIS (L2713601-3) - June 8, 2022





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Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	SED 2 DIS L2713601-3
Date/Time Sampled:	June 8, 2022; 08:40	**Date/Time Received:**	June 10, 2022; 11:10
Date/Time Test Started:	June 11, 2022; 16:10	**Date Test Finished:**	June 18, 2022
Description:	Clear, light brown	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032236	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.3°C
Collected By:	Not available		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species:	Fathead Minnow (*Pimephales promelas*)		
Source:	Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)		
Culture Temp.:	22 to 26 °C	**Life Stage:**	<24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 1.0%

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032236 **Sources:** SED 2 DIS L2713601-3

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Test Conditions

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 0 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (0%)

Average Weight of Controls is ≥ 250 µg: Acceptable (441 µg)

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032236 **Sources:** SED 2 DIS L2713601-3

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	0	0	0	40	10	20	30
	2	0	40	0	40	10	10	30	20
	3	0	0	0	0	0	10	10	10
Mean % Mortality		0.0	13.3	0.0	13.3	16.7	10.0	20.0	20.0
S.D.		0.0	23.1	0.0	23.1	20.8	0.0	10.0	10.0
Survival/Growth Data									
Mean Dry Biomass	1	0.417	0.429	0.313	0.440	0.215	0.433	0.352	0.312
(mg)	2	0.470	0.197	0.358	0.223	0.358	0.280	0.298	0.473
	3	0.436	0.448	0.322	0.477	0.362	0.436	0.443	0.339
% Effect (+ or -)		0.0	-18.8	-24.9	-13.8	-29.3	-13.2	-17.4	-15.0
Mean Dry Biomass/ Concentration (mg)		0.441	0.358	0.331	0.380	0.312	0.383	0.364	0.375
S.D.		0.03	0.14	0.02	0.14	0.08	0.09	0.07	0.09

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032236 **Sources:** SED 2 DIS L2713601-3

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival		
7-day LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Biomass (Survival and Growth)		
7-day IC25 (Confidence Interval)[2]	>100% Volume[3] (Not Applicable)	No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval
3 - Inhibition 29.93% at concentrations 12.5% v/v

Test Method Deviation: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (Control; rep. 2). No reason to remove it. Statistics include all data.

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 02-Jun-2022 **Reference Batch #:** Zn2102

Method of Analysis: Trimmed Spearman-Karber α = 10%

7-Day LC50 (95% Confidence Limits): 0.61 mg/L (0.49 mg/L; 0.76 mg/L)

Historic Geometric Mean LC50: 0.70 mg/L (0.41 mg/L; 1.21 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS - TB Sample Name: SED - 2 - DIS Sample #: 8730 0032236
L 2713601-3

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 0 %

Average Weight of Controls is ≥ 250 µg: (Acceptable) / Not Acceptable 441 µg

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 0 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
7-day LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
Survival / Growth Biomass	*	No nonlinear regression models would fit
7-day IC25	>100 % Volume	ICPIN - linear interpolation
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

* inhibition 29.93% at conc 12.5%

Are there any outliers present: (Yes) / No

Concentration(s) & Rep(s): Control; rep 2

Analysis Completed: Initials EV Date 21/06/22

Results Verified: Initials CP Date 22/06/22

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** SED-2.DS L2713601-3 **Sample #:** 3730.00322936

Day	Date 2020	Initial Variables				Meters/Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	D.O. / °C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	11.06	24.1	8.0	8.4	621	6/4	13/88	5/6	Yes	≤100	≤ 20	1	[illegible]
1	12	26.4	8.1	9.4	628	6/4	13/88	5/4	Yes	≤100	≤ 20	1	[illegible]
2	13	25.0	8.0	9.8	625	6/4	13/88	5/6	yes	≤100	≤ 20	1	KK
3	14	25.4	8.0	10.0	639	6/4	13/88	5/6	yes	≤100	≤ 20	2	KK
4	15	26.4	7.9	10.4	656	6/4	13/88	5/6	yes	≤100	≤ 20	2	KK
5	16	23.6	7.7	10.9	563	6/4	13/88	5/6	Yes	≤100	≤ 20	3	[illegible]
6	17	26.4	8.0	10.9	610	6/4	13/88	5/6	yes	≤100	≤ 20	3	KK
7	18												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of brine shrimp days 0 to 6? (Yes)/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: Control   Sample Name: 2022 - R5 L2713601-3   Sample #: 3780.00322936

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06	24.7	8.0	7.4	269	6/4	13/88	5/6	C
1	12	24.6	8.2	7.01	259	6/4	13/88	5/6	C
2	13	25.0	7.8	7.7	270	6/4	13/88	5/6	SO
3	14	24.8	8.1	7.4	264	6/4	13/88	5/6	SO
4	15	24.7	8.2	7.6	276	6/4	13/88	5/6	SO
5	16	24.6	7.6	8.1	247	6/4	13/88	5/6	KK
6	17	25.7	8.2	KK 7.7	250	6/4	13/88	5/6	KK
7	18								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	23.6	7.9	7.3	6/4	13/88	C							
1	23.6	7.7	7.6	5/5	12/89	KK	0	0	0	0	0	0	SO
2	23.7	7.5	7.1	5/5	12/89	KK	0	0	0	0	0	0	KK
3	23.6	7.5	5.8	6/4	13/88	SO	0	0	0	0	0	0	KK
4	24.5	7.5	5.7	6/4	13/88	KK	0	0	0	0	0	0	[illegible]
5	23.9	7.8	6.8	6/4	13/88	KK	0	0	0	0	0	0	SO
6	25.3	7.8	6.2	6/4	13/88	SO	0	0	0	0	0	0	SO
7							0	0	0	0	0	0	SO

Observations:

Concentration: 1.56 % v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06	24.5	8.1	8.0	263	6/4	13/88	5/6	C
1	12	24.4	8.2	7.9	267	6/4	13/88	5/6	C
2	13	24.0	8.1	7.7	267	6/4	13/88	5/6	SO
3	14	24.5	8.0	7.9	270	6/4	13/88	5/6	SO
4	15	24.6	8.0	7.5	274	6/4	13/88	5/6	SO
5	16	24.6	7.6	8.1	251	6/4	13/88	5/6	KK
6	17	25.3	8.2	7.8	255	6/4	13/88	5/6	KK
7	18								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	23.6	7.8	6.8	6/4	13/88	C							
1	23.6	7.6	7.5	5/5	12/89	KK	0	0	0	0	0	0	SO
2	23.8	7.6	7.1	5/5	12/89	KK	0	0	0	0	0	0	KK
3	23.6	7.5	5.6	6/4	13/88	SO	0	0	0	0	0	0	KK
4	24.4	7.5	5.6	6/4	13/88	KK	0	30	0	0	0	0	[illegible]
5	23.9	7.7	6.4	6/4	13/88	KK	0	40	0	0	0	0	SO
6	25.4	7.7	5.7	6/4	13/88	SO	0	40	0	0	0	0	SO
7							0	40	0	0	0	0	SO

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13 % v/v Sample Name: 2022 - RS L2713601-3 Sample #: 3730.00350936

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06								
1	12								
2	13								
3	14								
4	15								
5	16								
6	17								
7	18								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	CS
						0	0	0	0	0	0	KK
						0	0	0	0	6	6	KK
						0	0	0	0	0	0	JS
						0	0	0	0	0	0	SO
						0	0	0	0	0	0	SO
						0	0	0	0	0	0	SO

Observations: _____

Concentration: 6.25 % v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06								
1	12								
2	13								
3	14								
4	15								
5	16								
6	17								
7	18								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	CS
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	KK
						0	20	0	0	0	0	JS
						0	40	0	0	0	6	SO
						0	40	0	0	0	0	SO
						0	40	0	0	0	0	SO

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5 % v/v   Sample Name: 2022 - RS L2713001-3   Sample #: 3780.0032236

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06	24.5	8.1	8.1	302	6/4	13/88	5/6	C
1	12	24.4	8.1	7.9	296	6/4	13/88	5/6	C
2	13	24.0	8.1	7.7	307	6/4	13/88	5/6	SO
3	14	24.5	8.0	7.9	314	6/4	13/88	5/6	SO
4	15	24.8	8.0	7.6	318	6/4	13/88	5/6	SO
5	16	24.6	7.6	8.1	290	6/4	13/88	5/6	KC
6	17	25.4	8.2	7.9	294	6/4	13/88	5/6	KC
7	18								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0	23.6	7.8	7.0	6/4	13/88	C							
1	23.6	7.7	7.4	5/5	12/89	KK	0	0	0	0	0	0	CS
2	23.7	7.6	7.2	5/5	12/89	KK	0	0	0	0	0	0	KC
3	23.6	7.6	5.7	6/4	13/88	SO	0	0	0	0	0	0	KK
4	24.5	7.6	6.1	6/4	13/88	KK	20	10	0	0	0	0	[illegible]
5	23.8	7.7	6.6	6/4	13/88	KK	30	10	0	0	0	0	SO
6	25.3	7.8	6.3	6/4	13/88	SO	30	10	10	0	0	0	SO
7							40	10	10	0	0	0	SO

Observations: ____________________

Concentration: 25 % v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06								
1	12								
2	13								
3	14								
4	15								
5	16								
6	17								
7	18								

Day	Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
0													
1							0	0	0	0	0	0	CS
2							0	0	0	0	0	0	KK
3							0	0	0	0	0	0	KK
4							10	0	10	0	0	0	[illegible]
5							10	0	10	0	0	0	SO
6							10	10	10	0	0	0	SO
7							10	10	10	0	0	0	SO

Observations: ____________________

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50% v/v Sample Name: 2022 - DS L2713601-3 Sample #: 3730.00329236

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06								
1	12								
2	13								
3	14								
4	15								
5	16								
6	17								
7	18								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	
						6	0	0	0	0	0	KK
						0	0	10	0	0	0	KK
						10	20	10	0	0	0	
						10	20	10	0	0	0	50
						10	20	10	0	0	0	50
						20	30	10	0	0	0	50

Observations:

Concentration: 100% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	11.06	24.8	8.1	8.1	610	6/4	13/88	5/6	
1	12	25.0	8.1	8.5	604	6/4	13/88	5/6	
2	13	24.5	8.1	8.5	621	6/4	13/88	5/6	50
3	14	25.0	8.1	8.5	635	6/4	13/88	5/6	50
4	15	25.7	8.10	8.2	645	6/4	13/88	5/6	50
5	16	24.9	8.0	9.0	582	6/4	13/88	5/6	KK
6	17	25.5	8.1	9.0	597	6/4	13/88	5/6	KK
7	18								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
23.6	7.9	7.1	6/4	13/88								
23.6	~~7.9~~ 8.0 KK	7.3	5/5	12/89	KK	0	0	0	0	0	0	
23.7	7.9	6.8	5/5	12/89	KK	0	0	0	0	0	0	KK
23.6	7.9	5.4	6/4	13/88	50	0	0	10	0	0	0	KK
24.3	8.0	6.2	6/4	13/88	KK	0	10	10	0	0	0	
23.8	8.0	6.0	6/4	13/88	KK	10	20	10	0	0	0	50
25.3	8.0	5.5	6/4	13/88	50	10	20	10	0	0	0	50
						30	20	10	0	0	0	50

Observations:

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information
Client ALS TB
Sample # B730.0032036
Date/Time Received 10/06/22 / 1110
Sample Type effluent
100% Hardness 232

Sample Name 2002 DLS - L2713601-3
Sample Date/Time 05/06/22 / 0940 Person Sampling n/a
Arrival Temp 17.3 °C
Sample Description clear light brown

Test Information
Date/Time Started 11.06.22 / 16:10 Test started by CD Fathead Batch # FH0121/0621
Date eggs laid 06/07/08.06.22 Culture mortality within 7 days of egg collection 1.0% Swim bladder inflated; yes / no YES
Age of Larvae at start of test in hours <24 Control Hardness 90 Water Bath Quadrant J
Average Temperature during Test: 25.4 ± 0.1 °C
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / No (circle one)

Conc.	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	10	0.74509	0.74092	0.417	0.441
	B	10	0.74527	0.74057	0.470	
	C	10	0.73985	0.73549	0.436	
1.56	A	10	0.73725	0.73296	0.429	0.358
	B	6 ~~10~~	0.73988	0.73791	0.197	
	C	10	0.73789	0.73345	0.448	
3.13	A	10	0.73648	0.73385	0.313	0.331
	B	10	0.73461	0.73103	0.358	
	C	10	0.73089	0.72767	0.322	
6.25	A	10	0.73807	0.73367	0.440	0.380
	B	~~10~~ 6	0.72711	0.72488	0.223	
	C	10	0.73136	0.72659	0.477	
12.5	A	~~10~~ 6	0.73760	0.73545	0.215	0.312
	B	~~6~~ 9	0.74265	0.73907	0.358	
	C	10	0.73408	0.73046	0.362	
25	A	~~6~~ 9	0.73404	0.72971	0.433	0.383
	B	~~9~~ 9	0.73380	0.73100	0.280	
	C	~~9~~ 9	0.73888	0.73452	0.436	
50	A	8	0.74651	0.74299	0.352	0.364
	B	7	0.72709	0.72411	0.298	
	C	9	0.73474	0.73031	0.443	
100	A	7	0.72872	0.72560	0.312	0.375
	B	8	0.73889	0.73416	0.473	
	C	9	0.74207	0.73861	0.339	
Initials		50	50	50	CG	CG

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8730-0032236 **Sample Name** "36" / L2713601-3 **Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug)** 441

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	10	0.74509	0.74092	0.417	0.417	0.441	0.441	0.026851	0.026851	6.088763
	10	10	0.74527	0.74057	0.470	0.470					
	10	10	0.73985	0.73549	0.436	0.436					
1.56	10	10	0.73725	0.73296	0.429	0.429	0.402	0.358	0.06431	0.139753	16.00644
	10	6	0.73988	0.73791	0.328	0.197					
	10	10	0.73789	0.73341	0.448	0.448					
3.13	10	10	0.73698	0.73385	0.313	0.313	0.331	0.331	0.023812	0.023812	7.193886
	10	10	0.73461	0.73103	0.358	0.358					
	10	10	0.73089	0.72767	0.322	0.322					
6.25	10	10	0.73807	0.73367	0.440	0.440	0.430	0.380	0.053438	0.137219	12.44024
	10	6	0.72711	0.72488	0.372	0.223					
	10	10	0.73136	0.72659	0.477	0.477					
12.5	10	6	0.73760	0.73545	0.358	0.215	0.373	0.312	0.021792	0.08374	5.847015
	10	9	0.74265	0.73907	0.398	0.358					
	10	10	0.73408	0.73046	0.362	0.362					
25	10	9	0.73404	0.72971	0.481	0.433	0.426	0.383	0.099126	0.089213	23.29327
	10	9	0.73380	0.73100	0.311	0.280					
	10	9	0.73888	0.73452	0.484	0.436					
50	10	8	0.74651	0.74299	0.440	0.352	0.453	0.364	0.035011	0.073283	7.734712
	10	7	0.72709	0.72411	0.426	0.298					
	10	9	0.73474	0.73031	0.492	0.443					
100	10	7	0.72872	0.72560	0.446	0.312	0.471	0.375	0.10954	0.086223	23.24654
	10	8	0.73889	0.73416	0.591	0.473					
	10	9	0.74200	0.73861	0.377	0.339					

SD: Standard Deviation
CV: Coefficient of Variation

NAUTILUS ENVIRONMENTAL

22100122

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	SED 2 DIS L2713601-3
Date/Time Sampled	June 8, 2022; 08:40	**Date/Time Received:**	June 10, 2022; 11:10
Date Test Started:	June 10, 2022; 17:20	**Date Test Finished:**	June 16, 2022
Description:	Clear, light brown	**Days Sample Used:**	Days 0 to 5
Sample #:	8730-0032236	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.3°C
Collected By:	Not available		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032236 **Sources:** SED 2 DIS L2713601-3

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 0%

Mean Number of Surviving Young Produced Within First 3 Broods: 23.2

Mean Number of Surviving Young Produced Within 7 Days of Testing: 35.6

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 0 to 5

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032236 **Sources:** SED 2 DIS L2713601-3

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD22-73

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (17.7 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (80% of controls)

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032236 **Sources:** SED 2 DIS L2713601-3

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	0	0	0	0	10	0	0
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	23	20	17	22	19	22	4	4
	2	22	24	23	13	6	7	5	4
	3	19	21	22	15	18	2	13	5
	4	19	7	20	18	19	20	7	8
	5	18	18	21	18	22	7	3	11
	6	17	22	19	10	18	12	2	11
	7	13	24	14	18	8	6	7	13
	8	13	17	22	9	19	17	10	11
	9	21	17	21	19	11	10	7	15
	10	12	11	25	5	26	10	5	8
Total Number of Live Neonates in First 3 Broods or Less		177	181	204	147	166	113	63	90
% Effect (+ or -)		0.0	2.3	15.3	-16.9	-6.2	-36.2	-64.4	-49.2
Mean Number of Live Neonates in First 3 Broods or Less		17.7	18.1	20.4	14.7	16.6	11.3	6.3	9.0
SD		3.9	5.5	3.1	5.3	6.3	6.5	3.3	3.8

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032236 **Sources:** SED 2 DIS L2713601-3

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	5.66% Volume (1.03; 13.39% Volume)	No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 02-Jun-2022 **Reference Batch #:** Zn2102

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 0.15 mg/L (0.12 mg/L; 0.19 mg/L)

Historic Geometric Mean LC50: 0.08 mg/L (0.03 mg/L; 0.27 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS - Thunder Bay Sample Name: Sed-2-Dish L2713601-3 Sample #: 873000032236

Conditions for Test Validity

Control Mortality is< 20%: (Acceptable) / Not Acceptable: 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable) / Not Acceptable: 8 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable) / Not Acceptable: 17.7 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤20 min Days: 0 to 5

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	n/a % Volume	
REPRODUCTION		No nonlinear regression models would fit
3-brood IC25	5.66 % Volume	
95% Confidence Interval[2]	1.03; 13.39 % Volume	IC25 - linear interpolation

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 27/06/22

Results Verified: Initials AO Date 27/06/22

eriodaphnia dubia Initial Sample Measureme s Before Preparation and Use in Toxicity Te

Concentration: 100% **Sample Name:** SED-2-DUS L27136001-3 **Sample #:** 9730 0382236

Day	Date 2020	Initial Measurements				Meters / Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	°C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	10:06	25.3	8.2	8.7	619	6/4	13/88	5/6	yes	≤100	≤ 20	1	WL
1	11	24.0	8.0	9.0	597	6/4	13/88	5/4	Y	≤100	≤ 20	1	EP
2	12	25.2	8.0	10.6	627	6/4	13/88	5/6	Yes	≤100	≤ 20	1	EP
3	13	26.4	8.0	10.2	655	6/4	13/88	5/6	Yes	≤100	≤ 20	2	ao
4	14	25.9	8.0	10.1	639	6/4	13/88	5/6	yes	≤100	≤ 20	2	SD
5	15	25.5	7.9	10.8	640	6/4	13/88	5/4	Yes	≤100	≤ 20	3	EP
6	16									≤100	≤ 20	3	
7	17									≤100	≤ 20	3	
8	18									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/No If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/No If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/No If yes, describe further: _____

Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily? Yes/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/No If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/No If yes, describe further: _____

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: Control Sample Name: SED2 DS L2713601-3 Sample #: 3730 003 2236

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06	26.1	8.2	7.5	442	6/4	13/88	5/6	WL
1	11	25.7	8.4	7.3	445	6/4	13/88	5/6	C
2	12	24.9	8.2	8.1	424	6/4	13/88	5/6	C
3	13	25.6	8.2	7.6	433	6/4	13/88	5/6	KS
4	14	25.5	8.2	7.2	448	6/4	13/88	5/6	SO
5	15	25.1	8.2	7.7	477	6/4	13/88	5/6	SO
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.4	8.0	7.3	6/4	13/88	C
24.4	8.1	7.1	6/4	13/88	CC
25.1	8.0	7.0	6/4	13/88	SO
24.1	7.9	7.0	6/4	13/88	C
25.1	7.9	6.3	6/4	13/88	SO
25.4	7.9	6.9	6/4	13/88	KK

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10 06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	CC	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CC	—
4	14	9	4	5	5	4	4	2	2	4	5	44	—	0	—	CC	CC
5	15	9	7	8	9	6	8	0	4	8	7	66	—	0	—	0	6
6	16	5	11	6	5	8	5	11	7	9	0	67	—	0	—	0	6
7	17																
8	17																
Total Neonates		23	22	19	19	18	17	13	13	21	12	177	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra . 0.137 % v/v Sample Name: SED2 DIS L2713601-3 Sample #: 8730.003 2236

Day	Date 2022	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	10.06	25.3	8.3	7.8	441	6/4	13/88	5/6	WL
1	11	25.1	8.1	7.8	423	6/4	13/88	5/6	CB
2	12	25.7	8.2	7.6	441	6/4	13/88	5/6	L
3	13	25.8	8.2	7.4	432	6/4	13/88	5/6	CS
4	14	25.6	8.2	7.1	452	6/4	13/88	5/6	SO
5	15	25.7	8.2	7.1	448	6/4	13/88	5/6	SO
6	16								
7	17								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	
24.7	8.1	6.9	6/4	13/88	C
23.9	8.0	6.8	6/4	13/88	CA
25.2	8.0	6.9	6/4	13/88	SO
24.9	8.0	6.7	6/4	13/88	CB
24.7	7.9	6.3	6/4	13/88	SO
25.1	7.9	6.9	6/4	13/88	KK

Day	Date 2022	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CB	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	L	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CB	—
4	14	2	3	2	2	3	3	4	3	2	4	28	—	0	—	CB	CB
5	15	10	7	8	5	7	9	10	8	5	7	76	—	0	—	L	L
6	16	8	14	11	0	8	10	10	6	10	0	77	—	0	—	L	L
7	17																
8	19																
Total Neonates		20	24	21	7	18	22	24	17	17	11	181	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.41 v/v Sample Name: SED2 DI L2713601-3 Sample #: 3730-003 2236

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
4	14	2	4	2	4	2	5	4	3	5	6	37	—	0	—	[illegible]	[illegible]
5	15	6	9	8	6	9	5	2	10	7	9	71	—	0	—	[illegible]	6
6	16	9	10	12	10	10	9	8	9	9	10	96	—	0	—	[illegible]	6
7	17																
8	17																
Total Neonates		17	23	22	20	21	19	14	22	21	25	204	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentrat... 1.231 v/v Sample Name: SED2 DIS L2713601-3 Sample #: 3730.003 2236

Day	Date 2022	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	

Day	Date 2022	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—		—
2	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0		—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—		—
4	14	2	2	3	5	2	4	3	3	6	3	33	—	0	—		—
5	15	9	9	3	7	8	6	5	6	8	2	63	—	0	—		
6	16	11	2	9	6	8	0	10	0	5	0	51	—	0	—		
7	17																
8	19																
Total Neonates		22	13	15	18	18	10	18	9	19	5	147	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra: 3.7%v/v Sample Name: SED2DIS L2713601-3 Sample #: 3730.008 2236

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	10.06	25.2	8.3	7.8	447	6/4	13/88	5/6	WL	24.7	8.0	7.0	6/4	13/88	O
1	11	25.0	8.2	7.5	443	6/4	13/88	5/6	O	23.7	7.7	6.8	6/4	13/88	CQ
2	12	25.7	8.2	7.5	436	6/4	13/88	5/6	C	25.1	7.9	6.7	6/4	13/88	SO
3	13	26.0	8.2	7.4	444	6/4	13/88	5/6	CS	24.3	8.0	6.9	6/4	13/88	CO
4	14	25.5	8.2	7.1	458	6/4	13/88	5/6	SO	24.7	7.8	6.1	6/4	13/88	SO
5	15	25.6	8.1	7.2	454	6/4	13/88	5/6	SO	25.1	7.9	6.9	6/4	13/88	KC
6	16														
7	17														

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CQ	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	C	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CO	—
4	14	6	4	3	3	2	4	4	2	2	6	36	—	0	—	CO	SO
5	15	4	0	9	7	8	2	2	8	0	10	50	—	0	—	U	L
6	16	9	2	6	9	12	12	2	9	9	10	80	—	0	—	U	L
7	17																
8	17																
Total Neonates		19	6	18	19	22	18	8	19	11	26	166	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 11.1 v/v Sample Name: SED2 DS L2713601-3 Sample #: 3730 003 2236

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	AD	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	AD	—
4	14	4	5	2	2	5	2	0	4	2	0	26	—	0	—	AD	—
5	15	8	2	0	6	2	2	6	3	8	10	47	—	0	—	B	6
6	16	10	0	6	12	0	8	DEAD	10	0	0	40	7	10	—	E	12
7	17							↓									
8	17																
Total Neonates		22	7	2	20	7	12	6	17	10	10	113	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentra. 33.3 1.v/v Sample Name: SED2 D15 L2713601-3 Sample #: 3730.003 2236

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06								
1	11								
2	12								
3	13								
4	14								
5	15								
6	16								
7	17								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	[illegible]	—
4	14	4	2	3	0	3	0	2	2	0	3	19	—	0	—	[illegible]	—
5	15	0	2+1	10	4	0	2	0	0	2	2	23	—	0	—	[illegible]	[illegible]
6	16	0	0	0	3	0	0	5	8	5	0	21	—	0	—	[illegible]	[illegible]
7	17																
8	19																
Total Neonates		4	5	13	7	3	2	7	10	7	5	63	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentrat.. 100% v/v Sample Name: SED2 DIS L2713601-3 Sample #: 8730.003 22360

Day	Date 2020	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	10.06	25.3	8.2	7.9	618	6/4	13/88	5/6	WL
1	11	25.2	8.1	7.9	611	6/4	13/88	5/6	C
2	12	25.7	8.1	8.4	618	6/4	13/88	5/6	C
3	13	25.9	8.1	7.7	642	6/4	13/88	5/6	KS
4	14	25.7	8.1	7.8	646	6/4	13/88	5/6	SO
5	15	25.8	8.1	7.9	643	6/4	13/88	5/6	SO
6	16								
7	17								

Day	Date 2020	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	10.06	24.7	8.1	7.1	6/4	13/88	C
1	11	23.5	8.0	7.0	6/4	13/88	CA
2	12	25.0	8.1	6.7	6/4	13/88	SO
3	13	24.3	8.1	7.0	6/4	13/88	SO
4	14	25.0	8.1	6.3	6/4	13/88	SO
5	15	25.3	8.1	7.0	6/4	13/88	KK
6	16						
7	17						

Day	Date 2020	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	10.06																
1	11	0	0	0	0	0	0	0	0	0	0	0	—	0	—	CA	—
2	12	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SO	—
3	13	0	0	0	0	0	0	0	0	0	0	0	—	0	—	SO	—
4	14	3	3	2	3	2	0	2	2	3	0	20	—	0	—	SO	SO
5	15	0	1	3	2	5	5	5	2	5	0	26	—	0	—	KK	G
6	16	1	0	0	3	4	8	6	7	7	2	38	—	0	—	KK	C
7	17																
8	19																
Total Neonates		4	4	5	8	11	11	13	11	15	8	90	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia Neonate Origin

Sample Information

Client ALS
Sample # 8730.00322236
Date/Time Received 10.06.22 / 1110
Sample Type Effluent
100% Hardness 232

Sample Name '36' SED 2' DO L27B601-3
Date/Time Collected 08.06.22 / 0840
Person Sampling N/A
Arrival Temp (°C) 17.7 3 CR
Sample Description Clear light ~~green~~ CG Brown

Test Information

Date Test Started 10.06.22 1720
Dilution Water Batch Number CD22-73
Test Started By
Control Hardness 126
Template Used for Randomization 2

Individual Culture Health Data

Date Culture Started 030622
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 23.1
Culture I.D. (*e.g.*, Wed Row 4) FRi ROW3
Average # neos in previous 7 days (must be ≥15) 35.7
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 030622
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 23.3
Culture I.D. (*e.g.*, Wed Row 4) FRi ROW4
Average # neos in previous 7 days (must be ≥15) 35.4
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 0 (add up % mortality for all cultures used / # cultures used)
Mean Number of Young Produced within first 3 broods 23.2 (avg # 1st 3 broods for all cultures used / # cultures used)
Mean Number of Young Produced in previous 7 days 35.6 (avg # neos in prev 7 days for all cultures used / # cultures used)
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / **No** (circle one)

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
F.3.1	**Y** / N	**Y** / N	13	1	CD
2	**Y** / N	**Y** / N	15	2	CD
3	**Y** / N	**Y** / N	10	3	CD
4	**Y** / N	**Y** / N	12	4	CD
5	**Y** / N	**Y** / N	14	5	CD
4.1	**Y** / N	**Y** / N	15	6	CD
3	**Y** / N	**Y** / N	14	7	CD
4	**Y** / N	**Y** / N	12	8	CD
5	**Y** / N	**Y** / N	9	9	CD
6	**Y** / N	**Y** / N	10	10	CD
	Y / N	Y / N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032236	**Sample Name:**	SED 2 DIS L2713601-3
Sample Method:	Grab	**Collected by:**	Not available
Date/Time Collected:	June 8, 2022; 08:40	**Arrival Temp.:**	17.3°C
Date/Time Received:	June 10, 2022; 11:10	**Sample Description:**	Clear, light brown
Sample Point Description:	Other	**Sample Type:**	Effluent
Transportation:	Road		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species (Strain #):	*Raphidocelis subcapitata* (CPCC # 37)
Source:	Nautilus Plant Culture Unit (from CPCC)
Culture Temp.:	24 ± 2 °C
Test Culture Number:	G4(l)a
Culture Age at Test Start:	4 days old
Cell Density in the Microplate Wells at the Start of the Test:	10,568.18 cells/ml

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*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032236 **Sample Name:** SED 2 DIS L2713601-3

Test Conditions

Date/Time Test Start:	June 11, 2022; 13:05	**T=0 Control pH:**	7.0
Date/Time Test End:	June 14, 2022; 12:45 - 15:45	**T=72 Control pH:**	7.0
Sample pH Before Dilution:	7.3	**pH Adjustment:**	None
Test Duration:	72 hours		

Mean Test Temperature (±Standard Deviation): 25.4 (±0.1)°C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Millipore

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032236 **Sample Name:** SED 2 DIS L2713601-3

Test Conditions - continued

Enumeration Technique: Neubauer Haemacytometer

Test Vessel: 96-Well U-bottomed Polystyrene Microplate

Concentration of Test Solutions: 90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control

 # Replicates/Concentration: 4 reps started; 3 reps counted of test solutions
10 control reps started, 2 used for pH measurement

Design if Specialized Procedure: Not applicable

Method Deviations or Unusual Occurrences: None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times: Acceptable (27.7 times)

pH in controls did not vary by more than 1.5 units: Acceptable (0 units)

C V for cell yields is < 20% within the control wells: Acceptable (11.7%)

No inhibitory trend detected across the control wells: Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
281,932	266,932	311,932	294,432	364,432	294,432	256,932	266,932	292,244	11.7

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 CV = Coefficient of Variation = (100 x standard deviation / mean)

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032236 **Sample Name:** SED 2 DIS L2713601-3

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	576,932	1,061,932	764,432	279,432	219,432
2	489,432	1,381,932	749,432	316,932	224,432
3	544,432	1,356,932	671,932	266,932	236,932
Mean Cell Yield	536,932	1,266,932	728,598	287,765	226,932
Coefficient Variation[2]	8	14	7	9	4
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	234,432	241,932			
2	211,932	266,932			
3	226,932	276,932			
Mean Cell Yield	224,432	261,932			
Coefficient Variation[2]	5	7			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	**Result[1]**	**Method of Calculation**
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume[3] (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	no trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032236 **Sample Name:** SED 2 DIS L2713601-3

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software, 2001-2007 Comprehensive Environmental Toxicity, Information System - CETIS v1.8.1.2.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (30.303% v/v concentration; rep. 1). No reason to remove it. Statistics include all data.

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2209 **Date Test Initiated:** 17-Jun-2022

Method of Analysis: Linear Interpolation (ICPIN) **Algae Lot #:** G4(l)a

72-hour IC25 (95% Confidence Limits): 67.48 mg/L (48.16 mg/L; 83.28 mg/L)

Historic Geometric Mean IC25: 51.76 mg/L (23.79 mg/L; 112.61 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS - TB Sample Name Sed 2 Discharge L 2713601.3 Sample # 8730-003 2236

Conditions for Test Validity

Cell increase for control is >16	Acceptable/Not acceptable	27.7 (times)
CV among controls ≤ 20	Acceptable/Not acceptable	11.7
Result of Mann-Kendall test for trend	Acceptable/Not acceptable	no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 116 250 (cells/mL)

Used: Yes/No (Circle one)

Algae only ~ (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10 568. 18 (cells/mL)

Analysis Completed:	Initials:	Date: 27/06/22
Results Verified:	Initials:	Date: 29 06 22

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location:	AMS Thunder Bay	L 2713601 · 3	
Sample #:	8730-003 22 36	Sample Name:	SED 2 Discharge
Collection Method:	Grab	Collected By:	N/A
Date/Time Collected:	08/06/22 8:40	Arrival Temp.: (meter/probe)	17.3 °C (47)
Date/Time Received:	10/06/22 11:10	Sample Description:	clear, light brown
Collection Point Description:	Other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:	
Transportation:	Air / Road	Storage:	4 ± 2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	EV
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # G 4(11)a	EV
Culture Age at Start of Test	4 days old (must be 3 to 7 days old)	EV
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	EV
	Axenic culture? Yes/No (Circle one)	EV

Notes:

Test Conditions:

Date / Time Test Start: 11.06.22 13:05 Started By: EV		Date / Time Test End: 14.06.22 12:45 – 15:45 Finished By: EV	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 7.6	pH adjustment: Y/N	pH of well D6 at T=0 h 7.0	pH of well D7 at T=72 h 7.0
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # ———		Metal mining NUT Lot # NUT 2203
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation) 25.4 ± 0.1 °C			

	Day 1		Day 2	
¼ plate rotation (Initial)	AM EV	PM EV	AM ✓	PM EV
Lights ON (Initial)	AM EV	PM EV	AM EV	PM EV

* For reference test, record pH of top concentration before use for series dilutions.

Notes:

72-Hour Qualitative Observations:

Condensation:	Yes	
Growth:	Yes	
Were there any other method variations or deviations from methods? Yes/No	No	If yes, describe further:
Anything unusual about the test? Yes/No	No	
Any problems encountered? Yes/No	No	
Any remedial measures taken? Yes/No	No	

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #:	8730-0032236	Sample Name:	Sed 2 Discharge L 2713601.3

Reference Data:

Reference Chemical Batch #	Phenol P22089	Date test started	17.06.22
Method of Analysis	ICPIN – linear interpolation	Algae Lot #	G 4(11)a
72-hour IC25 (95% C.I.)[3] mg/L	67.48 48.16; 83.28	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	51.76 23.79; 112.61

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	>90.91% (N/A)	No nonlinear regression model could fit
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers	—	ICPIN – linear interpolation
Test for Outliers	No Outliers Present	Grubbs' Test for Residual Outlier Initial G
	If outliers present, indicate Concentration/Rep: 30.303; rep 1	
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed.	Williams' or (Dunnett's) Multiple Comparison Test Initial G
	No statistically significant growth stimulation.	
	(Yes), statistically significant growth stimulation at these concentrations: 90.91; 30.303; 10.101	

1)Results relate only to the sample tested.
2) Tidepool Scientific Software © 2001-2007. Comprehensive Environmental Toxicity Information System – CETIS v. 1.9.6.7
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/(No)

Any outliers and justification for their removal? Yes/(No)

Nautilus Environmental Company Inc.

Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)

72-Hour Growth Inhibition Test – Continued

72-Hour Quantitative Observations of Controls

Sample Name: SED 2 Discharge Sample Number: 873020322 36 Date Test Start: 11. 06. 22

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D8	Well # D9	Well # D10	Well # D11
1	35	25	30	27	41	33	21	31
2	25	36	38	27	28	29	33	28
3	26	24	32	31	36	30	23	24
4	31	26	24	37	45	30	30	28
5	—	—	—	—	—	—	—	—
Initials	E	C	C	C	E	C	C	C

Cell increase for controls = 27.7	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = 11.7	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = no trend	Controls are invalid if there is a trend detected by the Mann-Kendall test

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SED 2 Discharge Sample Number: 8730003236 Date Test Start: 11. 06. 22

Theoretical Test Concentration: 100.00 % v/v					Actual Test Concentration: 90.91 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	58	46	57		536932 (± 44230)
2	67	51	50		Coefficient of Variation of Cell Yield
3	58	48	65		8
4	52	55	50		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-83.727
Initials					

Theoretical Test Concentration: 33.33 % v/v					Actual Test Concentration: 30.303 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	106	135	121		1266932 (± 177975)
2	114	138	146		Coefficient of Variation of Cell Yield
3	103	140	149		14
4	106	144	131		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-333.518
Initials					

Theoretical Test Concentration: 11.11 % v/v					Actual Test Concentration: 10.101 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	78	84	84		728598 (± 49695)
2	81	57	69		Coefficient of Variation of Cell Yield
3	76	82	57		7
4	75	81	63		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-149.311
Initials					

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SED 2 Discharge Sample Number: 8730 0032236 Date Test Start: 11. 06. 22

Theoretical Test Concentration: 3.704 % v/v				Actual Test Concentration: 3.367 % v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	32	40	27		287765 (± 26021)
2	29	38	25		Coefficient of Variation of Cell Yield
3	22	21	36		9
4	33	32	23		Average % Inhibition (-ve number = enhancement)
5	—	—	—		1.533
Initials	GV	GV	G		C

Theoretical Test Concentration: 1.235 % v/v				Actual Test Concentration: 1.122 % v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation)
1	20	26	23		226932 (± 9014)
2	25	27	28		Coefficient of Variation of Cell Yield
3	23	20	27		4
4	24	21	21		Average % Inhibition (-ve number = enhancement)
5	—	—	—		22.349
Initials	GV	GV	G		C

Theoretical Test Concentration: 0.412 % v/v				Actual Test Concentration: 0.374 % v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	21	22	19		224432 (± 11456)
2	27	23	27		Coefficient of Variation of Cell Yield
3	26	24	26		5
4	24	20	23		Average % Inhibition (-ve number = enhancement)
5	—	—	—		23.204
Initials	C	GV	C		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SED 2 Discharge Sample Number: 8730-003236 Date Test Start: 11. 06. 22

Theoretical Test Concentration: 0.137 % v/v				Actual Test Concentration: 0.125 % v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	21	24	32		261932 (± 18028)
2	22	28	30		Coefficient of Variation of Cell Yield
3	27	27	29		7
4	31	32	24		Average % Inhibition (-ve number = enhancement)
5	—	—	—		10.372
Initials	CV	CV	CV		CV

Theoretical Test Concentration:				Actual Test Concentration:	
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:				Actual Test Concentration:	
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	SED2 Discharge	Sample #	8730-0032236	Date test start	11.06.22

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	116250	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	20	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2325	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	10568.1818	=	27.653226

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	35	25	30	27	41	33	21	31
	25.0	36.0	38.0	27.0	28.0	29.0	33.0	28.0
	26	24.0	32.0	31.0	36.0	30.0	23.0	24.0
	31.0	26.0	29.0	37.0	45.0	30.0	30.0	28.0
total cells	117	111	129	122	150	122	107	111
cells/ul	292.5	277.5	322.5	305	375	305	267.5	277.5
cells/ml	292500	277500	322500	305000	375000	305000	267500	277500
Cell yield = measured concentration - initial algal cell concentration								
	281932	266932	311932	294432	364432	294432	256932	266932

Mean cell yield for the control	= Rc	Standard deviation		coefficient of variation	
Rc	292244	SD	34315.6956	CV	11.742126 **Must be ≤20**

% inhibition summary

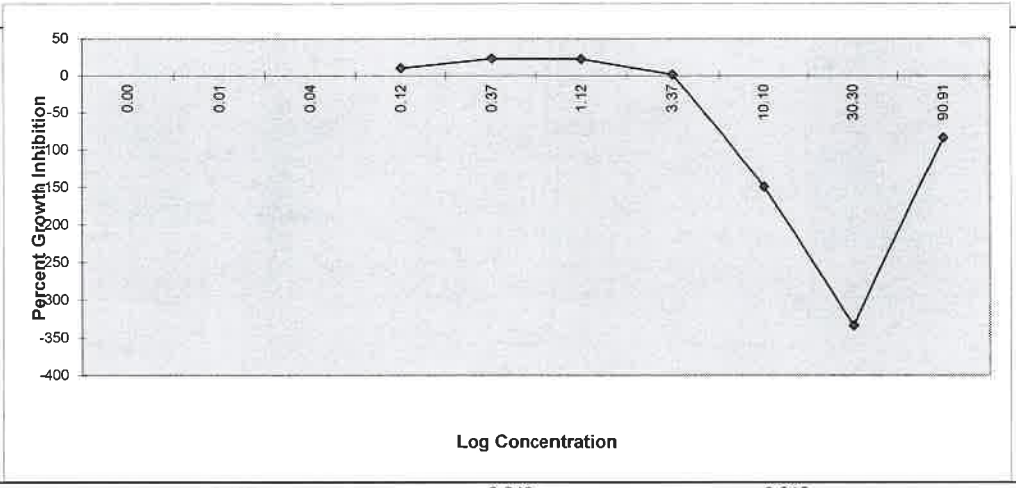
Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	10.372
0.374	23.204
1.122	22.349
3.367	1.533
10.101	-149.311
30.303	-333.518
90.910	-83.727

enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	58	46	57	106	135	121	78	84	84	32	40	27	20	26	23
	67.0	51.0	50.0	114.0	138.0	146.0	81.0	57.0	69.0	29.0	38.0	25.0	25.0	27.0	28.0
	58.0	48.0	65.0	103.0	140.0	149.0	76.0	82.0	57.0	22.0	21.0	36.0	23.0	20.0	27.0
	52.0	55.0	50.0	106.0	144.0	131.0	75.0	81.0	63.0	33.0	32.0	23.0	24.0	21.0	21.0
total cells	235	200	222	429	557	547	310	304	273	116	131	111	92	94	99
cells/ul	587.5	500	555	1072.5	1392.5	1367.5	775	760	682.5	290	327.5	277.5	230	235	247.5
cells/ml	587500	500000	555000	1072500	1392500	1367500	775000	760000	682500	290000	327500	277500	230000	235000	247500
Cell yield = measured concentration - initial algal cell concentration															
	576932	489432	544432	1061932	1381932	1356932	764432	749432	671932	279432	316932	266932	219432	224432	236932
Mean Yield	536932			1266932			728598			287765			226932		
STD Yield	44230			177975			49645			26021			9014		
CV Yield	8			14			7			9			4		

Average % inhibition												
	for 90.910%:	-83.727		for 30.303%	-333.518		for 10.101%	-149.311		for 3.367%	1.533		for 1.122%	22.349	

Average % stimulation												
	for 90.910%:	83.727		for 30.303%	333.518		for 10.101%	149.311		for 3.367%	-1.533		for 1.122%	-22.349	



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
21	22	19	21	24	32									
27.0	23.0	27.0	22.0	28.0	30.0									
26.0	24.0	26.0	27.0	27.0	29.0									
24.0	20.0	23.0	31.0	32.0	24.0									
98	89	95	101	111	115	0	0	0	0	0	0	0	0	0
245	222.5	237.5	252.5	277.5	287.5	0	0	0	0	0	0	0	0	0
245000	222500	237500	252500	277500	287500	0	0	0	0	0	0	0	0	0
234432	211932	226932	241932	266932	276932	-10568	-10568	-10568	-10568	-10568	-10568	-10568	-10568	-10568
224432			261932			-10568			-10568			-10568		
11456			18028			0			0			0		
5			7			0			0			0		
for 0.374%	23.204		for 0.125%	10.372		for 0.042%	103.616		for 0.014%	103.616		for 0.005%	103.616	
for 0.374%	-23.204		for 0.125%	-10.372		for 0.042%	-103.616		for 0.014%	-103.616		for 0.005%	-103.616	

JO 29.X022

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
SED 2 DIS L2713601-3	8730-0032236	June 8, 2022	June 10, 2022	June 10, 2022	>97% Volume[2] (Not applicable)	>97% Volume[2] (Not applicable)	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	03-Jun-2022	**Reference Chemical:**	KCl
Frond Increase IC25:	2.71 g/L	**95% Confidence Limits:**	2.21; 3.19 g/L
Historic Geometric Mean IC25:	2.24 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.50; 3.34 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	Na_2MoO_4	0.0726
	K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$2H_2O$	0.00003
	KCl	10.1	$MgSO_4\ 7H_2O$	147	$ZnCl_2$	0.00001
					$CoCl_2\ 6H_2O$	0.00001
					$CuCl_2\ 2H_2O$	

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

Date: June 30, 2022

C. D'Andrea

Carol D'Andrea
Laboratory Supervisor

Test Material

Client Name/Location: AMS – Thunder Bay	
Sample #: 8730-003 22 36	Sample Name: SED 2 Discharge
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 08/06/22 8:40	Arrival Temperature (meter/probe): 4.3 °C (47)
Date/Time Received: 10/06/22 11:10	Sample Description: clear, light brown
Collection Point Description: Other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: Air / Road	Storage: none

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	G
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	G
Culture Age at Start of Test:	9 days old, acclimated 23 hours in fresh test solution (mAPHA)	G
Culture Medium:	Modified Hoagland's E+ medium, Lot # MM2201	G
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/No	G
	Axenic culture? Yes/No	G
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 29 in HT 1, 31 in HT 2, 29 in HT 3 at finish	C

Test Conditions and Procedures

Date / Time Test Start: 10.06.22 15:15 Started By: G	Date / Time Test End: 17.06.22 10:00 Finished By: G
Test Type: Static (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / No (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: Modified APHA (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 8.2 (13/8)	pH Adjustment: none
Test Volume and Depth: 150 mL / 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / No	If yes, describe further:
Anything unusual about the test? Yes / No	
Any problems encountered? Yes / No	
Any remedial measures taken? Yes / No	Randomization Template: A

Sample #: 8730-0032236	Sample Name: SED 2 Discharge

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	24.6	24.0	24.1	24.3	24.3	24.7	24.8	24.4
Low	24.6	24.0	24.1	24.2	24.4	24.7	24.7	24.4
Medium	24.6	24.0	24.1	24.2	24.3	24.7	24.7	24.4
High	24.7	24.0	24.1	24.2	24.3	24.7	24.7	24.4
Initials	EP	EP	CS	VB	EP	EP	CS	EP
meter/probe	51	44	51	51	44	44	44	44
Mean Test Temperature (average of 24h high / low temperatures):					25.4 ±0.1 °C			

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.3	8.2	8.2	8.2	EP	13188
Day 7	Control	Low	Medium	High	Initials	pH meter/probe
	8.3	9.1	9.4	10.3	EP	12189

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)								

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 11/06/22 (1)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 12					Initials: EP
4680	4270	4890	4530	4260	Mean Light Measurement: 4526
±15% Variation of Mean: 3837 - 5215					(Acceptable) / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) / No
If yes, describe further: Algae in upper concentrations

Reference Data

Reference Date	(Frond Increase) or Dry Weights (circle one)			
	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
03/06/22	2.71	2.21 ; 3.19	2.24	1.50 ; 3.34

Sample #: 8730-003 2236	Sample Name: Seal 2 Disch

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 52	B 51	C 51	D 51	51.3

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy						Initials	
						C	
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV: 45.3 (0.5) 1.1							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control (% v/v g/L)	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-4.97	-8.29	-6.63	-9.94	-1.66	-18.78	-6.08
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control (% v/v g/L)	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-10.59	-11.10	-7.71	-17.73	-7.82	-23.57	-10.14

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: Yes / Not applicable (no stimulation) (Circle one)

Test Endpoints and Calculations:

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	>97 % (N/A)	No nonlinear regression models invalid fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN – linear interpolation
Dry Weights		
IC25 (95% C.I.)[3]	>97 % (N/A)	Nonlinear regression log Expobenmole model
IC25 (95% C.I.)[3] If calculated without outlier[4]		

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019. Comprehensive Environmental Toxicity Information System CETISv 1.9.6.7
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / No

Any outliers and justification for their removal? Yes / No Fronds (9.7 % up 3)

Lemna minor Observations

Client: MLS – TB	Sample number: 8730-0032236	Date Started: 10.06.22	
Site:		Date Ended: 17.06.22	
Concentration: Control	Observations By: C	Concentration: 0.0971 v/v	Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	52	51	51	51	Number of	48	48	52	48
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X	Abnormally sized fronds	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations					Other Observations	algae			
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO			N/A		Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Observations

Client:	MLS – TB				Sample number:	8730-0032236		Date Started:	10.06.22
Site:								Date Ended:	17.06.22
Concentration:	0.29 l. v/v			Observations By: C	Concentration:	0.97 l. v/v			Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	51	42	48	49	Number of	40	56	53	44
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X	Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	algae	→			Other Observations				
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Observations

Client:	MLS – TB	Sample number:	8730-0032236	Date Started: 10.06.22
Site:				Date Ended: 17.06.22
Concentration:	3.1 % v/v	Observations By:	C	

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	48	47	51	41
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO)
Weights: YES / (NO)

Concentration:	9.7 % v/v	Observations By:	C	

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	52	45	48	57
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO)
Weights: YES / (NO)

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Observations

Client:	MLS – TB	Sample number:	8730-0032236	Date Started:	10.06.22				
Site:				Date Ended:	17.06.22				
Concentration:	31% v/v	Observations By:	C	Concentration:	97% v/v	Observations By:	C		

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	39	42	48	42	Number of	49	49	51	45
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	Abnormally sized fronds	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	√	X	X	Loss of Buoyancy	√	X	X	X
Other Observations	algae	→	→	→	Other Observations	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor Weights

Client	ALS – ~~Thorn~~ TB	Site	Scd – 2 – Disch	Sample number	8730-0032236
In Oven Date/Time/ °C:	17/06/22 10:10 63°	Out Oven Date/Time/°C:	18/06/22 10:10 60°		

Conc.	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	46	45.3 (0.5)	0.80324	0.79846	4.78	4.41 (0.31)
	B	45		0.80762	0.80328	4.34	
	C	45		0.80143	0.79696	4.47	
	D	45		0.80476	0.80070	4.06	
0.097	A	42	43.0 (2.0)	0.80418	0.79997	4.21	3.95 (0.3)
	B	42		0.81033	0.80642	3.91	
	C	46		0.81036	0.80629	4.07	
	D	42		0.~~8~~79721	0.79362	3.59	
0.29	A	45	41.5 (3.9)	0.80641	0.80210	4.31	3.92 (0.4)
	B	36		0.80829	0.80501	3.28	
	C	42		0.80919	0.80509	4.10	
	D	43		0.80~~8~~39	0.80438	4.00	
0.97	A	34	42.3 (7.5)	0.79941	0.79579	3.62	4.07 (0.6)
	B	50		0.81~~0~~766	0.80573	4.93	
	C	47		0.80969	0.80553	4.16	
	D	38		0.80980	0.80622	3.58	
3.1	A	42	40.8 (4.2)	0.80907	0.80531	3.76	3.63 (0.5)
	B	41		0.80129	0.79719	4.10	
	C	45		0.80256	0.79889	3.67	
	D	35		0.80545	0.80246	2.99	
9.7	A	46	44.5 (5.2)	0.80276	0.79893	3.83	4.07 (0.4)
	B	39		0.80125	0.79764	3.61	
	C	42		0.80730	0.80295	4.35	
	D	51		0.79976	0.79528	4.48	
31	A	33	36.8 (3.8)	0.80010	0.79717	2.93	3.37 (0.5)
	B	36		0.804~~50~~42	0.80151	2.91	
	C	42		0.810~~8~~5	0.80613	3.92	
	D	36		0.813~~59~~	0.80972	3.73	
97	A	45	42.5 (2.5)	0.80564	0.80217	3.47	3.97 (0.4)
	B	43		0.8~~0~~79931	0.79520	4.11	
	C	45		0.81031	0.80645	3.86	
	D	39		0.81806	0.81364	4.42	
Initials		EG	EG	SO	EG	EG	EG

Notes:

RG

Sample name SED 2 DIS L2713601-3 Date started 6/1[illegible]/22 sample # 8730-0032236

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

51.3 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	52	48	51	40	48	52	39	49
	51	48	42	56	47	45	42	49
	51	52	48	53	51	48	48	51
	51	48	49	44	41	57	42	45
Total Fronds	205	196	190	193	187	202	171	194
Increase in Frond Number = Total # Fronds - 6 Starting Fronds								
	46	42	45	34	42	46	33	43
	45	42	36	50	41	39	36	43
	45	46	42	47	45	42	42	45
	45	42	43	38	35	51	36	39
Total Increase	181	172	166	169	163	178	147	170
Mean Increase	45.3	43.0	41.5	42.3	40.8	44.5	36.8	42.5
SD Increase	0.5	2.0	3.9	7.5	4.2	5.2	3.8	2.5
CV Increase	1.1	4.7	9.3	17.8	10.3	11.7	10.3	5.9
% Stimulation		**-4.97**	**-8.29**	**-6.63**	**-9.94**	**-1.66**	**-18.78**	**-6.08**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.80324	0.80418	0.80641	0.79941	0.80907	0.80276	0.80010	0.80564
Pan + Plant	0.80762	0.81033	0.80829	0.81066	0.80129	0.80125	0.80442	0.79931
	0.80143	0.81036	0.80919	0.80969	0.80256	0.80730	0.81005	0.81031
	0.80476	0.79721	0.80838	0.80980	0.80545	0.79976	0.81345	0.81806
Initial Weight (g)	0.79846	0.79997	0.80210	0.79579	0.80531	0.79893	0.79717	0.80217
Pan Only	0.80328	0.80642	0.80501	0.80573	0.79719	0.79764	0.80151	0.79520
	0.79696	0.80629	0.80509	0.80553	0.79889	0.80295	0.80613	0.80645
	0.80070	0.79362	0.80438	0.80622	0.80246	0.79528	0.80972	0.81364
Plant Only (mg)	4.78	4.21	4.31	3.62	3.76	3.83	2.93	3.47
	4.34	3.91	3.28	4.93	4.10	3.61	2.91	4.11
	4.47	4.07	4.10	4.16	3.67	4.35	3.92	3.86
	4.06	3.59	4.00	3.58	2.99	4.48	3.73	4.42
Mean Dry Weight	4.413	3.945	3.922	4.073	3.630	4.068	3.373	3.965
SD Dry Weight	0.3	0.3	0.4	0.6	0.5	0.4	0.5	0.4
CV Dry Weight	6.8	6.8	11.4	15.5	12.8	10.2	15.7	10.1
% Stimulation		**-10.59**	**-11.10**	**-7.71**	**-17.73**	**-7.82**	**-23.57**	**-10.14**

For Data Transfer to CETIS

		# fronds	total mass	tare
0	1	46	0.80324	0.79846
	2	45	0.80762	0.80328
	3	45	0.80143	0.79696
	4	45	0.80476	0.80070
0.1	1	42	0.80418	0.79997
	2	42	0.81033	0.80642
	3	46	0.81036	0.80629
	4	42	0.79721	0.79362
0.3	1	45	0.80641	0.80210
	2	36	0.80829	0.80501
	3	42	0.80919	0.80509
	4	43	0.80838	0.80438
1	1	34	0.79941	0.79579
	2	50	0.81066	0.80573
	3	47	0.80969	0.80553
	4	38	0.80980	0.80622
3.1	1	42	0.80907	0.80531
	2	41	0.80129	0.79719
	3	45	0.80256	0.79889
	4	35	0.80545	0.80246
9.7	1	46	0.80276	0.79893
	2	39	0.80125	0.79764
	3	42	0.80730	0.80295
	4	51	0.79976	0.79528
31	1	33	0.80010	0.79717
	2	36	0.80442	0.80151
	3	42	0.81005	0.80613
	4	36	0.81345	0.80972
97	1	43	0.80564	0.80217
	2	43	0.79931	0.79520
	3	45	0.81031	0.80645
	4	39	0.81806	0.81364

✓ ✓ ✓

✓ 29/6/22

Chain of Custody (COC)

L2713601-COFC

Canada Toll

www.alsglobal.com

Report To	Contact and company name below will appear on the final report
Company:	New Gold
Contact:	Garnet Cornell
Phone:	
	Company address below will appear on the final report
Street:	
City/Province:	
Postal Code:	
Invoice To	Same as Report To ☐ YES ☐ NO
	Copy of Invoice with Report ☐ YES ☐ NO
Company:	
Contact:	

Reports / Recipients

Select Report Format: ☐ PDF ☐ EXCEL ☐ EDD (DIGITAL)

Merge QC/QCI Reports with COA ☐ YES ☐ NO ☐ N/A

☐ Compare Results to Criteria on Report - provide details below if box checked

Select Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Email 3

Invoice Recipients

Select Invoice Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Turnaround Time (TAT) Requested

☐ Routine [R] if received by 3pm M-F - no surcharges apply
☐ 4 day [P4] if received by 3pm M-F - 20% rush surcharge minimum
☐ 3 day [P3] if received by 3pm M-F - 25% rush surcharge minimum
☐ 2 day [P2] if received by 3pm M-F - 50% rush surcharge minimum
☐ 1 day [E] if received by 3pm M-F - 100% rush surcharge minimum
☐ Same day [E2] if received by 10am M-S - 200% rush surcharge.

Additional fees may apply to rush requests on weekends, statutory holidays and for non-routine tests.

Date and Time Required for all E&P TATs: dd-mmm-yy hh:mm am/pm

For all tests with rush TATs requested, please contact your AM to confirm availability.

AFFIX ALS BARCODE LABEL HERE (ALS use only)

Project Information

ALS Account # / Quote #:	
Job #:	
PO / AFE:	
LSD:	
ALS Lab Work Order # (ALS use only):	L2713601

Oil and Gas Required Fields (client use)

AFE/Cost Center:	PO#:
Major/Minor Code:	Routing Code:
Requisitioner:	
Location:	
ALS Contact:	Sampler:

Analysis Request

Indicate Filtered (F), Preserved (P) or Filtered and Preserved (F/P) below

ALS Sample # (ALS use only)	Sample Identification and/or Coordinates (This description will appear on the report)	Date (dd-mmm-yy)	Time (hh:mm)	Sample Type	NUMBER OF CONTAINERS	F/P: NG-ST-P-TB	SAMPLES ON HOLD	EXTENDED STORAGE REQUIRED	SUSPECTED HAZARD (see notes)
1	EDL1	08-Jun-22	8:10	EFF	3	X			
2	EDL2	8-Jun-22	8:10	EFF	3	X			
3	SED 2 DIS	8-Jun-22	8:40	EFF	3	X			

Drinking Water (DW) Samples[1] (client use)

Are samples taken from a Regulated DW System? ☐ YES ☐ NO

Are samples for human consumption/ use? ☐ YES ☐ NO

Notes / Specify Limits for result evaluation by selecting from drop-down below (Excel COC only)

SAMPLE RECEIPT DETAILS (ALS use only)

Cooling Method: ☐ NONE ☐ ICE ☐ ICE PACKS ☐ FROZEN ☐ COOLING INITIATED

Submission Comments Identified on Sample Receipt Notification: ☐ YES ☐ NO

Cooler Custody Seals Intact: ☐ YES ☐ N/A Sample Custody Seals Intact: ☐ YES ☐ N/A

INITIAL COOLER TEMPERATURES °C	FINAL COOLER TEMPERATURES °C
13.7	

SHIPMENT RELEASE (client use)			INITIAL SHIPMENT RECEPTION (ALS use only)			FINAL SHIPMENT RECEPTION (ALS use only)		
Released by:	Date:	Time:	Received by: NP3	Date: June 9, 22	Time: 9:30 AM	Received by:	Date:	Time:

REFER TO BACK PAGE FOR ALS LOCATIONS AND SAMPLING INFORMATION

WHITE - LABORATORY COPY YELLOW - CLIENT COPY

AUG 2020 FRONT

Failure to complete all portions of this form may delay analysis. Please fill in this form LEGIBLY. By the use of this form the user acknowledges and agrees with the Terms and Conditions as specified on the back page of the white - report copy.

1. If any water samples are taken from a **Regulated Drinking Water (DW) System**, please submit using an **Authorized DW COC form**.

KC17

New Gold Inc. Rainy River Project
ATTN: Garnet Cornell
24 Marr Rd
Barwick ON P0W 1A0

Date Received: 28-SEP-22
Report Date: 04-NOV-22 16:49 (MT)
Version: FINAL REV. 2

Client Phone: 807-234-8200

Certificate of Analysis

Lab Work Order #: L2734791

Project P.O. #: 4500062842
Job Reference: SUBLETHAL TOXICITY
C of C Numbers:
Legal Site Desc:

Christine Paradis
Project Manager

ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
L2734791-1 EDL1 Sampled By: Client on 28-SEP-22 @ 08:15 Matrix: EFF							
Miscellaneous							
Special Request	See Attached					29-SEP-22	R5885925
Special Request	See Attached					29-SEP-22	R5885925
Special Request	See Attached					29-SEP-22	R5885925
Special Request	See Attached					29-SEP-22	R5885925
L2734791-2 SED2DIS Sampled By: Client on 28-SEP-22 @ 08:10 Matrix: EFF							
Miscellaneous							
Special Request	See Attached					30-SEP-22	R5885925
Special Request	See Attached					30-SEP-22	R5885925
Special Request	See Attached					30-SEP-22	R5885925
Special Request	See Attached					30-SEP-22	R5885925

* Refer to Referenced Information for Qualifiers (if any) and Methodology.

Reference Information

Test Method References:

ALS Test Code	Matrix	Test Description	Method Reference**
TOX-7DINHIB-LM-TB	Misc.	Growth Inhibition using Lemma minor	SEE SUBLET LAB RESULTS
Lemna minor 7-day growth toxicity test, based on the protocol "Biological Test Method: Test Method for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, Lemna minor", Report EPS 1/RM/37, Second Edition (January 2007)			
TOX-GROWTH-FH-TB	Misc.	7 Day Survival & Growth Fat Head Minnows	SEE SUBLET LAB RESULTS
Fathead minnow 7-day test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Environmental Protection Series, Ottawa, ON, Report EPS1/RM/22, Second Edition (February 2011).			
TOX-INHIB-PS-TB	Misc.	Growth Inhibition Pseudokirchneriella	SEE SUBLET LAB RESULTS
Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) 72-hour growth toxicity test, based upon protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga" Report EPS1/RM/25, Second Edition (March 2007).			
TOX-REPRO-CD-TB	Misc.	Survival & reproduction (Ceriodaphnia)	SEE SUBLET LAB RESULTS
Ceriodaphnia dubia 3-brood toxicity test, accroding to protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dusbia", Environmental Protection Series, Ottawa, ON, Report EPS 1/RM/21, Second Edition (February 2007).			

** ALS test methods may incorporate modifications from specified reference methods to improve performance.

The last two letters of the above test code(s) indicate the laboratory that performed analytical analysis for that test. Refer to the list below:

Laboratory Definition Code	Laboratory Location
TB	ALS ENVIRONMENTAL - THUNDER BAY, ONTARIO, CANADA

Chain of Custody Numbers:

GLOSSARY OF REPORT TERMS

Surrogates are compounds that are similar in behaviour to target analyte(s), but that do not normally occur in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery. In reports that display the D.L. column, laboratory objectives for surrogates are listed there.
mg/kg - milligrams per kilogram based on dry weight of sample
mg/kg wwt - milligrams per kilogram based on wet weight of sample
mg/kg lwt - milligrams per kilogram based on lipid weight of sample
mg/L - unit of concentration based on volume, parts per million.
< - Less than.
D.L. - The reporting limit.
N/A - Result not available. Refer to qualifier code and definition for explanation.

Test results reported relate only to the samples as received by the laboratory.
UNLESS OTHERWISE STATED, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.
Analytical results in unsigned test reports with the DRAFT watermark are subject to change, pending final QC review.

Quality Control Report

Workorder: L2734791 Report Date: 04-NOV-22

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0

Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed

Quality Control Report

Workorder: L2734791 Report Date: 04-NOV-22

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Legend:

Limit	ALS Control Limit (Data Quality Objectives)
DUP	Duplicate
RPD	Relative Percent Difference
N/A	Not Available
LCS	Laboratory Control Sample
SRM	Standard Reference Material
MS	Matrix Spike
MSD	Matrix Spike Duplicate
ADE	Average Desorption Efficiency
MB	Method Blank
IRM	Internal Reference Material
CRM	Certified Reference Material
CCV	Continuing Calibration Verification
CVS	Calibration Verification Standard
LCSD	Laboratory Control Sample Duplicate

Hold Time Exceedances:

All test results reported with this submission were conducted within ALS recommended hold times.

ALS recommended hold times may vary by province. They are assigned to meet known provincial and/or federal government requirements. In the absence of regulatory hold times, ALS establishes recommendations based on guidelines published by the US EPA, APHA Standard Methods, or Environment Canada (where available). For more information, please contact ALS.

The ALS Quality Control Report is provided to ALS clients upon request. ALS includes comprehensive QC checks with every analysis to ensure our high standards of quality are met. Each QC result has a known or expected target value, which is compared against pre-determined data quality objectives to provide confidence in the accuracy of associated test results.

Please note that this report may contain QC results from anonymous Sample Duplicates and Matrix Spikes that do not originate from this Work Order.

November 2, 2022

ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Attention Lab:

On September 29, 2022, Nautilus Environmental Company Inc. personnel received one water sample (EDL1 L2734791-1) from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected September 28, 2022

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
EDL1 L2734791-1	Fathead Minnow	7-day LC50 (95% Confidence) 7-day IC25 (95% Confidence)	Survival Biomass	>100% Volume[2] (Not Applicable) >100% Volume[2] (Not Applicable)
8730-0032242	*Ceriodaphnia dubia*	3-brood LC50 (95% Confidence) 3-brood IC25 (95% Confidence)	Survival Reproduction	>100% Volume[2] (Not Applicable) >100% Volume[2,3] (Not Applicable)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	37.95% Volume (15.05; 55.42% Volume)
	Lemna minor	7-day IC25 Frond Number (95% Confidence) 7-day IC25 Dry Weight (95% Confidence)	Growth Growth	10.9% Volume (N/A; 22.96% Volume) <0.097% Volume (Not available)

1 - Results relate only to the sample tested
2 - Highest concentration tested, based on test method
3 - Inhibition >25% at concentrations 3.7%; 33.3% v/v.

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 100%, 33%, 11%, 3.7% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted by a factor of 0.33 (i.e., 97%, 31%, 9.7% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

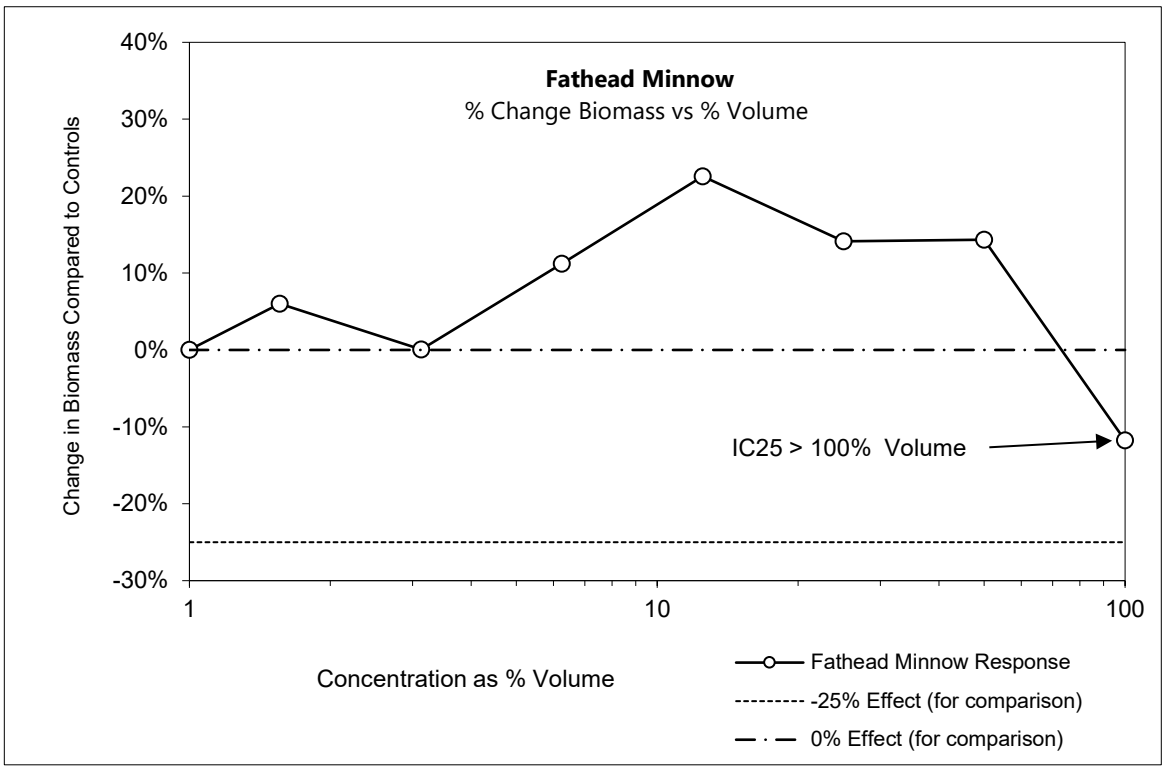
If there are any further details which you require, please do not hesitate to contact us.

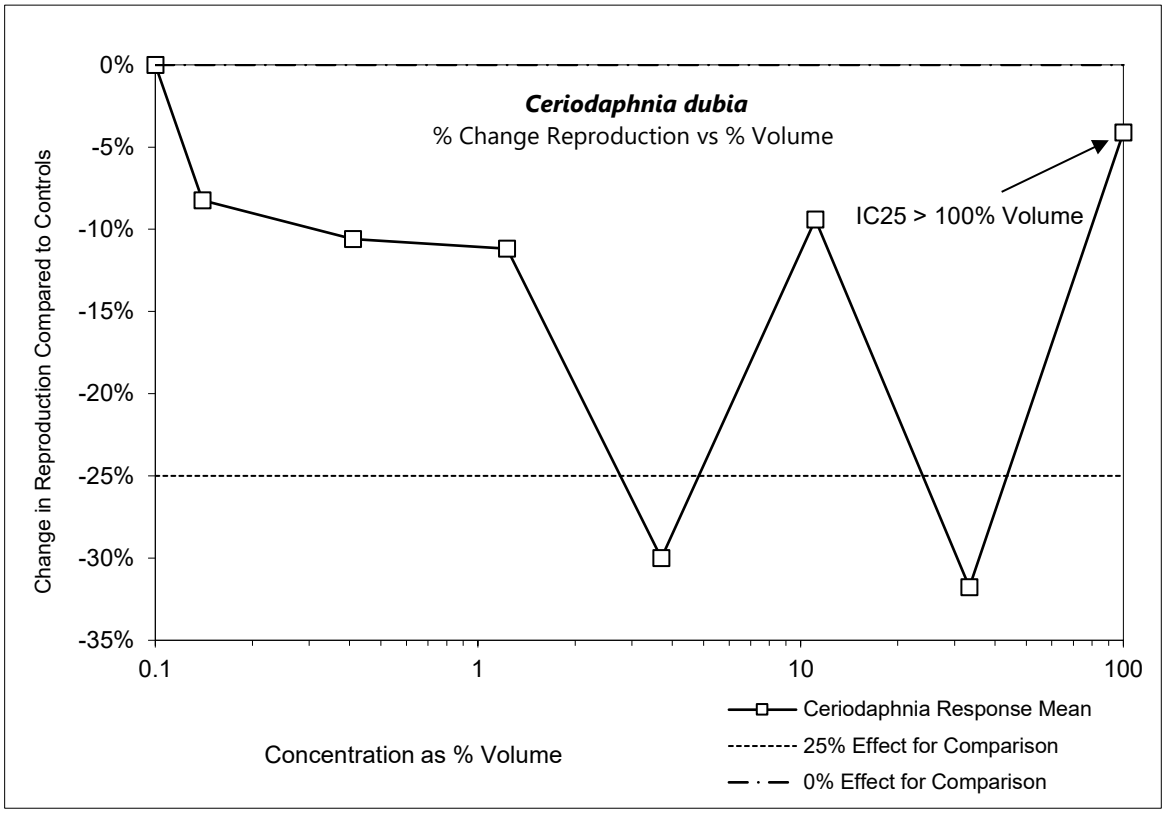
Yours very truly,
Nautilus Environmental Company Inc.

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2022\8000\8730-003\8730-0032242 FCRL

ALS Thunder Bay - EDL1 L2734791-1 - September 28, 2022





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Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	EDL1 L2734791-1
Date/Time Sampled:	September 28, 2022; 08:15	**Date/Time Received:**	September 29, 2022; 11:00
Date/Time Test Started:	September 29, 2022; 16:45	**Date Test Finished:**	October 6, 2022
Description:	Clear, light green	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032242	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	14.0°C
Collected By:	Not available		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species:	Fathead Minnow (*Pimephales promelas*)		
Source:	Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)		
Culture Temp.:	22 to 26 °C	**Life Stage:**	<24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 0%

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032242 **Sources:** EDL1 L2734791-1

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Test Conditions

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 0 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (3.3%)

Average Weight of Controls is ≥ 250 µg: Acceptable (497 µg)

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032242 **Sources:** EDL1 L2734791-1

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	0	0	0	0	0	0	20
	2	0	0	0	10	0	0	0	10
	3	10	0	0	0	0	0	0	30
Mean % Mortality		3.3	0.0	0.0	3.3	0.0	0.0	0.0	20.0
S.D.		5.8	0.0	0.0	5.8	0.0	0.0	0.0	10.0
Survival/Growth Data									
Mean Dry Biomass	1	0.478	0.521	0.516	0.485	0.673	0.593	0.588	0.363
(mg)	2	0.536	0.520	0.430	0.555	0.536	0.598	0.507	0.516
	3	0.430	0.490	0.499	0.566	0.561	0.457	0.556	0.395
% Effect (+ or -)		0.0	6.0	0.1	11.2	22.6	14.1	14.3	-11.8
Mean Dry Biomass/ Concentration (mg)		0.481	0.510	0.482	0.535	0.590	0.549	0.550	0.425
S.D.		0.05	0.02	0.05	0.04	0.07	0.08	0.04	0.08

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032242 **Sources:** EDL1 L2734791-1

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival		
7-day LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Biomass **(Survival and Growth)**		
7-day IC25 (Confidence Interval)[2]	>100% Volume (Not Applicable)	Non-linear Regression 2P Linear Model

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Phenol

Date Test Initiated: 22-Sep-2022 **Reference Batch #:** P2213

Method of Analysis: Trimmed Spearman-Karber α = 3.33%

7-Day LC50 (95% Confidence Limits): 20.14 mg/L (16.77 mg/L; 24.20 mg/L)

Historic Geometric Mean LC50: 23.38 mg/L (15.00 mg/L; 36.44 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS-TB Sample Name: EDL-1 L2734791 Sample #: 8730-0032242

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 3.3 %

Average Weight of Controls is ≥ 250 µg: Acceptable / Not Acceptable 497 ~~572~~ µg

Summary of Test Results

Pre-aeration: Yes Reason: supersaturation Duration: ≤ 20 min Days: 0 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
7-day LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
Survival / Growth Biomass		
7-day IC25	>100 % Volume	Non linear regression 2P Linear
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials TB Date 20/10/22

Results Verified: Initials CeO Date 01/11/22

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** L2734791 / EDL 1 **Sample #:** 8730.0032242

Day	Date 2022	Initial Variables				Meters/Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	D.O. / °C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	29.09	24.3	7.9	9.1	1577	6/4	13/88	5/6	yes	≤100	≤ 20	1	KK
1	30.09	25.3	7.9	10.1	1510	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
2	01.10	24.8	7.9	10.7	1511	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
3	02.10	25.3	7.9	10.9	1523	6/4	13/88	5/6	yes	≤100	≤ 20	2	SO
4	03.10	25.1	7.9	12.1	1546	6/4	13/88	5/6	yes	≤100	≤ 20	2	SO
5	04.10	26.4	7.9	10.5	1612	6/4	13/88	5/6	yes	≤100	≤ 20	3	SO
6	05.10	26.3	7.8	11.0	1632	6/4	13/88	5/6	yes	≤100	≤ 20	3	KK
7	06.10												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/No If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/No If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/No If yes, describe further: _____

Was test fed 100µl of brine shrimp days 0 to 6? Yes/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/No If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/No If yes, describe further: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: Control Sample Name: L2734791-1 / EDL 1 Sample #: 8730.0032242

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.09	23.9	8.0	7.7	259	6/4	13/88	5/6	KP
1	30.09	24.4	8.3	8.2	240	6/4	13/88	5/6	ET
2	01.10	~~24.6 24.4 24.1~~	8.4	7.4	256	6/4	13/88	5/6	SO
3	02.10	24.8	8.4	7.9	245	6/4	13/88	5/6	VS
4	03.10	24.8	8.3	~~8.2~~ 7.9 ET	~~269~~ 245	6/4	13/88	5/6	ET
5	04.10	26.2	8.3	7.6	259	6/4	13/88	5/6	KP
6	05.10	24.3	8.2	8.0	255	6/4	13/88	5/6	KP
7	06.10	24.5	7.8						

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.9	8.0	7.4	6/4	13/88	KP	0	0	0	0	0	0	ET
24.9	7.8	6.8	6/4	13/88	SO	↓	↓	↓	↓	↓	↓	↓
24.7	7.7	5.9	6/4	13/88	VS	0	0	0	0	0	0	VS
24.2	~~8.0~~ 7.9	6.8	6/4	13/88	ET	0	0	0	0	0	0	ET
24.2	7.7	5.8	6/4	13/88	KP	0	0	0	0	0	0	ET
24.3	7.7	6.1	6/4	13/88	KP	0	0	10	0	0	0	ET
24.5	7.8	6.5	6/4	13/88	ET	0	0	10	0	0	0	ET
						0	0	10	0	0	0	SO

Observations:

Concentration: 0.156% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	28.09	24.0	8.0	7.8	283	6/4	13/88	5/6	KP
1	30.09	24.3	8.3	8.6	270	6/4	13/88	5/6	ET
2	01.10	25.8	8.4	7.6	272	6/4	13/88	5/6	SO
3	02.10	24.4	8.2	7.3	267	6/4	13/88	5/6	VS
4	03.10	24.0	8.3	8.2	269	6/4	13/88	5/6	ET
5	04.10	25.2	8.3	7.7	281	6/4	13/88	5/6	KP
6	05.10	24.1	8.2	7.9	280	6/4	13/88	5/6	KP
7	06.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.9	8.0	7.4	6/4	13/88	KP	0	0	0	10 (A)	0	0	ET
24.9	7.8	6.5	6/4	13/88	SO	↓	↓	↓	↓	↓	↓	↓
24.7	7.9	5.6	6/4	13/88	VS	0	0	0	0	0	0	VS
24.1	7.9	6.8	6/4	13/88	ET	0	0	0	0	0	0	ET
24.1	7.8	6.5	6/4	13/88	KP	0	0	0	0	0	0	ET
24.4	7.8	6.7	6/4	13/88	KP	0	0	0	0	0	0	ET
24.5	7.8	6.5	6/4	13/88	ET	0	0	0	0	0	0	ET
						0	0	0	0	0	0	SO

Observations:

(A) Kinked tail

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13 %. v/v Sample Name: L2734791-1 / EDL 1 Sample #: 8730.0032242

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.09								
1	30.09								
2	01.10								
3	02.10								
4	03.10								
5	04.10								
6	05.10								
7	06.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	ET
						↓	↓	↓	↓	↓	↓	↓
						0	0	0	0	0	0	
						0	0	0	0	0	0	
						0	0	0	0	0	0	ET
						0	0	0	0	0	0	ET
						0	0	0	0	0	0	ET
						0	0	0	0	0	0	SO

Observations: _____

Concentration: 6.25 %. v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	28.09								
1	30.09								
2	01.10								
3	02.10								
4	03.10								
5	04.10								
6	05.10								
7	06.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	ET
						↓	↓	↓	↓	↓	↓	↓
						0	0	0	0	0	0	
						0	0	0	0	0	0	
						0	10	0	0	0	0	ET
						0	10	0	0	0	0	ET
						0	10	0	0	0	0	ET
						0	10	0	0	0	0	SO

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5% v/v Sample Name: L2734791-1 / EDL 1 Sample #: 8730.0032242

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.09	24.0	8.0	7.8	448	6/4	13/88	5/6	KP
1	30.09	24.4	8.2	8.4	424	6/4	13/88	5/6	ET
2	01.10	25.7	8.3	7.7	431	6/4	13/88	5/6	SO
3	02.10	24.5	8.2	7.4	~~336~~ 430	6/4	13/86	5/6	VK
4	03.10	24.0	8.3	8.2	424	6/4	13/88	5/6	ET
5	04.10	25.2	8.3	7.7	446	6/4	13/88	5/6	KP
6	05.10	24.4	8.2	7.8	~~438~~ 436	6/4	13/88	5/6	KP
7	06.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.9	7.9	7.2	6/4	13/88	KP	0	0	0	0	0	0	ET
24.9	7.8	6.2	6/4	13/88	SO	↓	↓	↓	↓	↓	↓	↓
24.7	7.9	5.4	6/4	13/86	VK	0	0	0	0	0	0	VK
24.2	7.8	6.1	6/4	13/88	ET	0	0	0	0	0	0	✓
24.1	7.8	6.2	6/4	13/88	KP	0	0	0	0	0	0	ET
24.5	7.8	6.6	6/4	13/88	KP	0	0	0	0	0	0	ET
24.5	7.8	6.5	6/4	13/88	ET	0	0	0	0	0	0	ET
						0	0	0	0	0	0	SO

Observations: _____

Concentration: 25% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	28.09								
1	30.09								
2	01.10								
3	02.10								
4	03.10								
5	04.10								
6	05.10								
7	06.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	ET
						↓	↓	↓	↓	↓	↓	↓
						0	0	0	0	0	0	✓
						0	0	0	0	0	0	✓
						0	0	0	0	0	0	ET
						0	0	0	0	0	0	ET
						0	0	0	0	0	0	ET
						~~20~~	~~18~~	~~20~~	0	0	0	SO

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50% v/v Sample Name: L2734791-1 / EDL 1 Sample #: 8730.0032242

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	29.09								
1	30.09								
2	01.10								
3	02.10								
4	03.10								
5	04.10								
6	05.10								
7	06.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	ET
						↓	↓	↓	↓	↓	↓	↓
						0	0	0	0	0	0	
						0	0	0	0	0	0	
						0	0	0	0	0	0	ET
						0	0	0	0	0	0	ET
						0	0	0	0	0	0	ET
						0	0	0	0	0	0	SO

Observations: _____

Concentration: 100% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	28.09	24.2	7.9	8.5	1570	6/4	13/88	5/6	KP
1	30.09	24.9	8.0	8.8	1495	6/4	13/88	5/6	ET
2	01.10	25.1	8.0	8.9	1519	6/4	13/88	5/6	SO
3	02.10	25.0	7.9	9.0	1521	6/4	13/88	5/6	
4	03.10	24.5	8.0	9.6	1525	6/4	13/88	5/6	ET
5	04.10	25.0	8.0	9.0	1562	6/4	13/88	5/6	KP
6	05.10	25.2	8.0	8.7	1595	6/4	13/88	5/6	KP
7	06.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.8	8.1	7.2	6/4	13/88	KP	0	0	0	0	0	0	ET
24.9	7.9	5.8	6/4	13/88	SO	↓	↓	↓	↓	↓	↓	↓
24.7	7.9	5.2	6/4	13/88		0	0	0	0	0	0	
24.1	7.8	5.8	6/4	13/88	ET	0	0	0	0	0	0	
24.1	7.9	6.2	6/4	13/88	KP	10	10	20	0	0	0	ET
24.5	7.9	6.5	6/4	13/88	KP	10	10	30	0	0	0	ET
24.6	7.6	5.4	6/4	13/88	ET	10	10	30	0	0	0	ET
						20	10	30	0	0	0	SO

Observations: _____

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information

Client ALS Thunder Bay
Sample # 8730.0032242
Date/Time Received 29/09/22 / 1100
Sample Type Water
100% Hardness 378

Sample Name L2734791-EDL
Sample Date/Time 28/09/22 / 0815
Arrival Temp 14.5 °C
Sample Description clear, light green

Person Sampling ALS - N/A

Test Information

Date/Time Started 29/09/22 / 1645
Test started by WL
Fathead Batch # FH 0621/0122
Date eggs laid 23-25/09/22
Culture mortality within 7 days of egg collection 0%
Swim bladder inflated; (yes) / no WL
Age of Larvae at start of test in hours <24h
Control Hardness 98
Water Bath Quadrant A
Average Temperature during Test: 25.0 ± 0.5 °C

Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / (No) (circle one)

Conc. % v/v	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	10	0.80643	0.80165	0.478	0.491
	B	10	0.81328	0.80792	0.536	
	C	9	0.80819 ET 196	0.79766	0.430	
1.56	A	10	0.80555	0.80034	0.521	0.510
	B	10	0.80212	0.79692	0.520	
	C	10	0.82815 ET 266	0.81776	0.490	
3.13	A	10	0.81815	0.81299	0.516	0.482
	B	10	0.81400	0.80970	0.430	
	C	10	0.79962	0.79463	0.499	
6.25	A	10	0.80567	0.80082	0.485	0.535
	B	9	0.80727	0.80172	0.555	
	C	10	0.80364	0.79798	0.566	
12.5	A	10	0.81422	0.80749	0.673	0.590
	B	10	0.82151	0.81615	0.536	
	C	10	0.81559	0.80998	0.561	
25	A	10	0.81647	0.81054	0.593	0.549
	B	10	0.81550	0.80952	0.598	
	C	10	0.82567	0.82110	0.457	
50	A	10	0.82167	0.81579	0.588	0.550
	B	10	0.82300	0.81793	0.507	
	C	10	0.81967	0.81411	0.556	
100	A	8	0.81948	0.81585	0.363	0.425
	B	9	0.81903	0.81387	0.516	
	C	7	0.82132	0.81737	0.395	
Initials		30	ET	KK/ET	U	U

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8730-0032242 **Sample Name** EDL

Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug) 497

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	10	0.80643	0.80165	0.478	0.478	0.497	0.481	0.033551	0.053079	6.747114
	10	10	0.81328	0.80792	0.536	0.536					
	10	9 ✓	0.80196	0.79766	0.478	0.430					
1.56	10	10	0.80555	0.80034	0.521	0.521	0.510	0.510	0.017616	0.017616	3.451916
	10	10	0.80212	0.79692	0.520	0.520					
	10	10 ✓	0.82266	0.81776	0.490	0.490					
3.13	10	10	0.81815	0.81299	0.516	0.516	0.482	0.482	0.045545	0.045545	9.455678
	10	10	0.81400	0.80970	0.430	0.430					
	10	10 ✓	0.79962	0.79463	0.499	0.499					
6.25	10	10	0.80567	0.80082	0.485	0.485	0.556	0.535	0.066413	0.043936	11.94719
	10	9	0.80727	0.80172	0.617	0.555					
	10	10 ✓	0.80364	0.79798	0.566	0.566					
12.5	10	10	0.81422	0.80749	0.673	0.673	0.590	0.590	0.072959	0.072959	12.36591
	10	10 ✓	0.82151	0.81615	0.536	0.536					
	10	10	0.81559	0.80998	0.561	0.561					
25	10	10	0.81647	0.81054	0.593	0.593	0.549	0.549	0.080002	0.080002	14.56349
	10	10	0.81550	0.80952	0.598	0.598					
	10	10 ✓	0.82567	0.82110	0.457	0.457					
50	10	10	0.82167	0.81579	0.588	0.588	0.550	0.550	0.040796	0.040796	7.413006
	10	10 ✓	0.82300	0.81793	0.507	0.507					
	10	10	0.81967	0.81411	0.556	0.556					
100	10	8	0.81948	0.81585	0.454	0.363	0.530	0.425	0.066584	0.080699	12.55212
	10	9 ✓	0.81903	0.81387	0.573	0.516					
	10	7 ✓	0.82132	0.81737	0.564	0.395					

SD: Standard Deviation
CV: Coefficient of Variation

NAUTILUS ENVIRONMENTAL

01.11.22

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	EDL1 L2734791-1
Date/Time Sampled	September 28, 2022; 08:15	**Date/Time Received:**	September 29, 2022; 11:00
Date Test Started:	September 30, 2022; 11:15	**Date Test Finished:**	October 6, 2022
Description:	Clear, light green	**Days Sample Used:**	Days 0 to 5
Sample #:	8730-0032242	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	14.0°C
Collected By:	Not available		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032242 **Sources:** EDL1 L2734791-1

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 0%

Mean Number of Surviving Young Produced Within First 3 Broods: 20.1

Mean Number of Surviving Young Produced Within 7 Days of Testing: 44.8

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 0 to 5

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032242 **Sources:** EDL1 L2734791-1

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD22-159

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (10%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (18.9 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (60% of controls)

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032242 **Sources:** EDL1 L2734791-1

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		10	0	0	0	10	10	0	10
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	22	18	19	15	10	18	9	21
	2	19	13	20	7	6	17	7	0
	3	21	10	3	16	0	17	22	10
	4	21	22	24	15	19	16	22	18
	5	19	26	15	26	15	14	0	19
	6	23	19	7	10	0	20	0	22
	7	11	8	24	17	17	18	21	21
	8	26	12	17	30	7	0	16	17
	9	0	18	11	11	20	16	11	13
	10	8	10	12	4	25	18	8	22
Total Number of Live Neonates in First 3 Broods or Less		170	156	152	151	119	154	116	163
% Effect (+ or -)		0.0	-8.2	-10.6	-11.2	-30.0	-9.4	-31.8	-4.1
Mean Number of Live Neonates in First 3 Broods or Less		17.0	15.6	15.2	15.1	11.9	15.4	11.6	16.3
SD		8.1	5.9	7.0	8.0	8.6	5.6	8.4	7.0

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032242 **Sources:** EDL1 L2734791-1

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	>100% Volume[3] (Not Applicable)	No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval
3 - Inhibition >25% at concentrations 3.7%; 33.3% v/v.

Test Method Deviation: None

Outliers and Justification for Their Removal: None

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Phenol

Date Test Initiated: 22-Sep-2022 **Reference Batch #:** P2213

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 8.25 mg/L (7.23 mg/L; 9.41 mg/L)

Historic Geometric Mean LC50: 6.80 mg/L (4.17 mg/L; 11.10 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS - Thunder Bay Sample Name: EDL 1 L2734791-1 Sample #: 873020032242

Conditions for Test Validity

Control Mortality is< 20%: (Acceptable / Not Acceptable): 10 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable / Not Acceptable): 6 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable / Not Acceptable): 18.9 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 0 to 5

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	- % Volume	
REPRODUCTION	*	
3-brood IC25	>100 % Volume	No nonlinear regression models would fit IC25 is - linear interpolation
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

* inhibition > 25% at conc: 3.7 and 33.3%

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EB Date 13/10/22

Results Verified: Initials CO Date 01.11.22

riodaphnia dubia Initial Sample Measureme Before Preparation and Use in Toxicity Tes

Concentration: 100% **Sample Name:** L2734791-1 / EDL 1 **Sample #:** 8730.0032242

Day	Date 2022	Initial Measurements				Meters / Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	°C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	30.09	25.5	7.9	10.1	1516	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
1	01.10	24.8	7.9	11.2	1508	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
2	02.10	24.6	7.9	11.9	1499	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
3	03.10	25.5	7.9	11.6	1551	6/4	13/88	5/6	yes	≤100	≤ 20	2	SO
4	04.10	24.6	8.0	11.9	1549	6/4	13/88	5/6	yes	≤100	≤ 20	2	SO
5	05.10	25.6	7.9	11.4	1608	6/4	13/88	5/6	yes	≤100	≤ 20	3	KK
6	06.10									≤100	≤ 20	3	
7	07.10									≤100	≤ 20	3	
8	08.10									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily? (Yes)/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: Control Sample Name: L2734791-VEDL Sample #: 8730.0032242

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	30.09	25.7	8.4	7.9	431	6/4	13/88	5/6	KP
1	01.10	24.9	8.4	8.0	431	6/4	13/88	5/6	SO
2	02.10	25.2	~~8.3~~ 8.5	~~7.1~~ 7.8	~~442~~ 440	6/4	13/86	5/6	KP
3	03.10	25.4	8.5	7.8	449	6/4	13/88	5/6	ET
4	04.10	25.8	8.5	7.7	450	6/4	13/88	5/6	KP
5	05.10	25.1	8.3	7.8	454	6/4	13/88	5/6	KP
6	06.10								
7	07.10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
23.6	8.1	6.9	6/4	13/88	SO
24.5	7.9	6.7	6/4	13/86	KP
23.8	8.3	7.6	6/4	13/88	KK
24.3	8.4	7.5	6/4	13/88	KP
24.6	8.2	7.1	6/4	13/88	KP
23.6	8.2	7.6	6/4	13/88	ET

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0 KK	—	0	—	KK	—
2	02.10	0	0	0	0	0	0	0	0	dead	0	10	9 ~~50~~	10	0	SO	—
3	03.10	0	3	3	1	0	4	0	4	|	0	15	—	10	0	KK	KK
4	04.10	2	4+2	0	3	5	0	0	0	|	3	19	—	10	—	KP	6
5	05.10	10	0	7	6	0	8	6	10	|	5	52	—	10	—	KP	6
6	06.10	10	10	11	11	14	11	5	12	|	0	84	—	10	—	KP	6
7	07.10									|							
8	08.10									✓							
Total Neonates		22	19	21	21	19	23	11	26	0	8	170	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.137% v/v Sample Name: L2734791-VEDL Sample #: 8730.0032242

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	30.09	25.0	8.4	7.6	436	6/4	13/88	5/6	KP
1	01.10	25.1	8.4	7.8	438	6/4	13/88	5/6	SO
2	02.10	25.1	8.5	7.0	440	6/4	13/88	5/6	↓
3	03.10	25.1	8.4	7.9	442	6/4	13/188	5/6	ET
4	04.10	24.7	8.4	7.7	446	6/4	13/88	5/6	KP
5	05.10	24.8	8.2	7.8	456	6/4	13/88	5/6	KP
6	06.10								
7	07.10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
23.6	~~8.6~~ 8.0 SO	86.8	6/4	13/88	SO
24.5	7.9	6.5	6/4	13/86	↓
23.6	8.2	7.3	6/4	13/88	KP
24.2	8.3	7.4	6/4	13/88	KP
24.6	8.1	7.1	6/4	13/88	KP
23.6	8.2	7.5	6/4	13/188	ET

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	—	KP	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SO	—
3	03.10	4	1	0	4	5	4	0	3	4	2	27	—	0	—	KP	KK
4	04.10	0	0	2	0	0	0	0	0	0	0	2	—	0	—	(1)	4
5	05.10	5	2	8	5	7	5	0	8+1	8	8	57	—	0	—	(7)	0
6	06.10	9	10	0	13	14	10	8	0	6	0	70	—	0	—	(2)	2
7	07.10																
8	08.10																
Total Neonates		18	13	10	22	26	19	8	12	18	10	156	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Surv[illegible] and Reproduction Toxicity Test

Concentrati[illegible]: 0.410% v/v Sample Name: L2734791-VEDL Sample #: 8730.0032242

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	30.09								
1	01.10								
2	02.10								
3	03.10								
4	04.10								
5	05.10								
6	06.10								
7	07.10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	—	KF	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SO	—
3	03.10	0	3	0	3+1	1	2	3	2	2	5	22	—	0	—	KK	KK
4	04.10	11	0	2	0	0	0	0	0	0	0	13	—	0	—	[illegible]	L
5	05.10	0	5	0	5	6	5	7	7	8+1	7	51	—	0	—	[illegible]	L
6	06.10	8	12	1	15	8	0	14	8	0	0	66	—	0	—	[illegible]	L
7	07.10																
8	08.10																
Total Neonates		19	20	3	24	15	7	24	17	11	12	152	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Surv[illegible] and Reproduction Toxicity Test

Concentrati[illegible] 1.23% v/v Sample Name: L2734791-VEDL Sample #: 8730.0032242

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	30.09								
1	01.10								
2	02.10								
3	03.10								
4	04.10								
5	05.10								
6	06.10								
7	07.10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	—	KF	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SO	—
3	03.10	4	3	0	1	5+1	0	0	5	4	4	27	—	0	—	KK	KK
4	04.10	0	0	8	0	0	0	9	0	0	0	17	—	0	—	CJ	4
5	05.10	1	3+1	0	7	8	8	0	10	2	0	40	—	0	—	CJ	0
6	06.10	10	0	8	7	12	2	8	15	5	0	67	—	0	—	CJ	0
7	07.10																
8	08.10																
Total Neonates		15	7	16	15	26	10	17	30	11	4	151	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 3.7 %vw Sample Name: LZ734791-VEDL Sample #: 8730.0032242

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	30.09	24.9	8.4	7.7	474	6/4	13/88	5/6	KP
1	01.10	25.1	8.4	7.9	490	6/4	13/88	5/6	SO
2	02.10	25.2	8.4	7.9	489	6/4	13/88	5/6	ET
3	03.10	25.1	8.4	8.0	490	6/4	13/88	5/6	ET
4	04.10	24.5	8.4	7.7	496	6/4	13/88	5/6	KP
5	05.10	24.7	8.3	7.7	498	6/4	13/88	5/6	KP
6	06.10								
7	07.10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
23.6	8.0	6.7	6/4	13/88	SO
24.6	7.9	6.1	6/4	13/88	ET
23.9	8.1	6.9	6/4	13/88	KK
24.2	8.2	7.2	6/4	13/88	KP
24.5	8.1	7.1	6/4	13/88	KP
23.6	8.2	7.2	6/4	13/88	ET

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	—	KF	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SO	—
3	03.10	2	0	0	4	3	0	3	0	4	4	20	—	0	—	KK	KK
4	04.10	0	6	DEAD	0	0	0	0	7	0	0	13	3	10	—	ET	8
5	05.10	8	0	|	7	4	0	5	0	8	10	42	—	10	—	ET	8
6	06.10	0	0	|	8	8	0	9	0	8	11	44	—	10	—	ET	7
7	07.10																
8	08.10			↓													
Total Neonates		10	6	0	19	15	0	17	7	20	25	119					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Surv[illegible] and Reproduction Toxicity Test

Concentrati[illegible] 11.11% V/V Sample Name: L2734791-VEDL Sample #: 8730.0032242

Day	Date 2022	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	30.09								
1	01.10								
2	02.10								
3	03.10								
4	04.10								
5	05.10								
6	06.10								
7	07.10								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	

Day	Date 2022	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	—	KF	—
2	02.10	0	0	0	0	0	0	0	dead	0	0	0	8	10	0	SO	—
3	03.10	0	2	0	2	1	3	0		1	4	13	—	10	—	KC	KC
4	04.10	0	0	0	0	0	0	10		0	0	10	—	10	—	C	4
5	05.10	5	5	6	5	6	5	0		7	6	45	—	10	—	C	8
6	06.10	13	10	11	9	7	12	8		8	8	86	—	10	—	C	6
7	07.10																
8	08.10																
Total Neonates		18	17	17	16	14	20	18	0	16	18	154	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 33.33% v/v Sample Name: L2734791-VEDL Sample #: 8730.0032242

Day	Date 2022	Initial Variables				Meter/Probe			Initials	Final Variables			Meter/Probe		Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond		°C	pH	D.O. (mg/L)	D.O. / °C	pH	
0	30.09														
1	01.10														
2	02.10														
3	03.10														
4	04.10														
5	05.10														
6	06.10														
7	07.10														

Day	Date 2022	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	—	KF	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SO	—
3	03.10	3	0	2	3	0	0	3	3	3	0	17	—	0	—	KC	KC
4	04.10	0	0	0	0	0	0	0	0	0	0	0	—	0	—	C	
5	05.10	6	7	9	7	0	0	7	0	6+2	3	~~50~~ 47	—	0	—	C	C
6	06.10	0	0	11	12	0	0	11	13	0	5	52	—	0	—	C	C
7	07.10																
8	08.10																
Total Neonates		9	7	22	22	0	0	21	16	11	8	119~~6~~	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 100% VW Sample Name: L2734791-VEDL Sample #: 8730.0032242

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	30.09	24.9	8.0	8.4	1494	6/4	13/88	5/6	KP
1	01.10	25.1	~~8.1~~ 8.0	9.1	1524	6/4	13/88	5/6	SO
2	02.10	25.1	8.0	9.5	1508	6/4	13/88	5/6	KP
3	03.10	25.1	8.1	9.5	1506	6/4	13/88	5/6	ET
4	04.10	24.5	8.1	9.1	1555	6/4	13/88	5/6	KP
5	05.10	24.9	8.0	8.9	1589	6/4	13/88	5/6	KP
6	06.10								
7	07.10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24.5	8.0	6.5	6/4	13/88	SO
24.6	7.9	6.3	6/4	13/88	SO
23.9	8.3	7.1	6/4	13/88	KP
24.3	8.3	7.3	6/4	13/88	KP
24.6	8.2	7.4	6/4	13/88	KP
23.7	8.3	7.5	6/4	13/88	ET

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	—	KF	
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SO	—
3	03.10	2	0	0	4	2	2	3	0	0	3	16	—	0	—	KK	KK
4	04.10	0	0	2	0	6	0	0	7	2	0	17	—	0	—	C	C
5	05.10	4	DEAD	0	4	0	7	6	0	4	9	34	2	10	—	C	C
6	06.10	15	1	8	10	11	13	12	10	7	10	96	—	10	—	C	C
7	07.10		↓														
8	08.10																
Total Neonates		21	0	10	18	19	22	21	17	13	22	163	Notes: * = ≥ 4th brood (not counted)				

W.O#L2734791

Ceriodaphnia dubia Neonate Origin

Sample Information

Client ALS Thunder Bay
Sample # 8730.0032242
Date/Time Received 29/09/22 - 1100
Sample Type Water
100% Hardness 378

Sample Name L2734791-1/EDL1 ~~DLS~~ ET
Date/Time Collected 28/09/22/0815 Person Sampling N/A
Arrival Temp (°C) 14.0°C
Sample Description clear, light green

Test Information

Date Test Started 30/09/22 / 11:15
Dilution Water Batch Number CD22159

Test Started By KK
Control Hardness 126

Template Used for Randomization T1

Individual Culture Health Data

Date Culture Started 16 KK 09.09.22
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 22.7

Culture I.D. (*e.g.*, Wed Row 4) FRi Row 5
Average # neos in previous 7 days (must be ≥15) 55.0
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 16 KK 09.09.22
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 21.5

Culture I.D. (*e.g.*, Wed Row 4) FRi Row 6
Average # neos in previous 7 days (must be ≥15) 51.1
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 21 KK 14.09.22
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 19.3

Culture I.D. (*e.g.*, Wed Row 4) WED Row 5
Average # neos in previous 7 days (must be ≥15) 34.6
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 21 KK 14.09.22
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 17

Culture I.D. (*e.g.*, Wed Row 4) WED Row 6
Average # neos in previous 7 days (must be ≥15) 38.6
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started _____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____

Culture I.D. (*e.g.*, Wed Row 4) _____
Average # neos in previous 7 days (must be ≥15) _____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 0 (add up % mortality for all cultures used / # cultures used)
Mean Number of Young Produced within first 3 broods 20.1 (avg # 1st 3 broods for all cultures used / # cultures used)
Mean Number of Young Produced in previous 7 days 44.8 (avg # neos in prev 7 days for all cultures used / # cultures used)
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / (No) (circle one)

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
F5.2	(Y)/N	(Y)/N	9	1	KK
5.5	(Y)/N	(Y)/N	15	2	KK
5.6	(Y)/N	(Y)/N	9	3	KK
5.10	(Y)/N	(Y)/N	12	4	KK
F6.2	(Y)/N	(Y)/N	9	5, 6	KK
6.3	(Y)/N	(Y)/N	11	7	KK
6.7	(Y)/N	(Y)/N	9	8	KK
W5.4	(Y)/N	(Y)/N	9	9	KK
6.2	(Y)/N	(Y)/N	~~9~~ 8	10	KK
	Y/N	Y/N	8 KK		
	Y/N	Y/N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032242	**Sample Name:**	EDL1 L2734791-1
Sample Method:	Grab	**Collected by:**	Not available
Date/Time Collected:	September 28, 2022; 08:15	**Arrival Temp.:**	14.0°C
Date/Time Received:	September 29, 2022; 11:00	**Sample Description:**	Clear, light green
Sample Point Description:	Other	**Sample Type:**	Effluent

Transportation: Road

Storage: None

Container: Polyethylene pails lined with polyethylene bags

Test Organisms

Species (Strain #): *Raphidocelis subcapitata* (CPCC # 37)

Source: Nautilus Plant Culture Unit (from CPCC)

Culture Temp.: 24 ± 2 °C

Test Culture Number: G7(l)c

Culture Age at Test Start: 7 days old

Cell Density in the Microplate Wells at the Start of the Test: 10,795.45 cells/ml

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032242 **Sample Name:** EDL1 L2734791-1

Test Conditions

Date/Time Test Start:	September 30, 2022; 09:50	**T=0 Control pH:**	6.8
Date/Time Test End:	October 3, 2022; 08:30 - 11:10	**T=72 Control pH:**	6.8
Sample pH Before Dilution:	7.7	**pH Adjustment:**	None

Test Duration: 72 hours

Mean Test Temperature (±Standard Deviation): 25.3 (±0.1)°C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Millipore

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

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***Raphidocelis subcapitata*72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032242 **Sample Name:** EDL1 L2734791-1

Test Conditions - continued

Enumeration Technique:	Neubauer Haemacytometer
Test Vessel:	96-Well U-bottomed Polystyrene Microplate
Concentration of Test Solutions:	90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control
# Replicates/Concentration:	4 reps started; 3 reps counted of test solutions 10 control reps started, 2 used for pH measurement
Design if Specialized Procedure:	Not applicable
Method Deviations or Unusual Occurrences:	None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times:	Acceptable (19.1 times)
pH in controls did not vary by more than 1.5 units:	Acceptable (0 units)
C V for cell yields is < 20% within the control wells:	Acceptable (12.5%)
No inhibitory trend detected across the control wells:	Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
229,205	234,205	189,205	176,705	226,705	226,705	174,205	189,205	205,767	12.5

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 CV = Coefficient of Variation = (100 x standard deviation / mean)

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032242 **Sample Name:** EDL1 L2734791-1

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	81,705	149,205	229,205	191,705	156,705
2	81,705	174,205	309,205	204,205	194,205
3	124,205	184,205	326,705	201,705	171,705
Mean Cell Yield	95,871	169,205	288,371	199,205	174,205
Coefficient Variation[2]	26	11	18	3	11
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	176,705	196,705			
2	171,705	174,205			
3	181,705	176,705			
Mean Cell Yield	176,705	182,538			
Coefficient Variation[2]	3	7			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	**Result[1]**	**Method of Calculation**
IC25 (95% CI)[2] for Cell Yield	37.95% Volume (15.05; 55.42% Volume)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	no trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032242 **Sample Name:** EDL1 L2734791-1

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software, 2001-2007 Comprehensive Environmental Toxicity, Information System - CETIS v1.8.1.2.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (10.101% v/v concentration; rep. 3). No reason to remove it. Statistics include all data.

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2213 **Date Test Initiated:** 26-Sep-2022

Method of Analysis: Linear Interpolation (ICPIN) **Algae Lot #:** G6(l)b

72-hour IC25 (95% Confidence Limits): 52.47 mg/L (45.83 mg/L; 60.40 mg/L)

Historic Geometric Mean IC25: 54.33 mg/L (26.16 mg/L; 112.84 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS - Thunder Bay Sample Name EDL1 L2734791 Sample # 8730-0032242

Conditions for Test Validity

Cell increase for control is >16	Acceptable/Not acceptable	19.1 (times)
CV among controls ≤ 20	Acceptable/Not acceptable	12.5
Result of Mann-Kendall test for trend	Acceptable/Not acceptable	no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 118 750 (cells/mL)

Used: Yes/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10 795.45 (cells/mL)

Analysis Completed:	Initials: EV	Date: 21/10/22
Results Verified:	Initials: AO	Date: 01/Nov/11/22

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location: ALS Thunder Bay	
Sample #: 8730-0032242	Sample Name: EDL1 L2734791
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 28/09/22 8:15	Arrival Temp.: (meter/probe) 14.0 °C (51)
Date/Time Received: 29/09/22 11:00	Sample Description: clear, light green
Collection Point Description: OPMW	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: Driv / Road	Storage: 4±2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	G
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # G 711)C	G
Culture Age at Start of Test	7 days old (must be 3 to 7 days old)	G
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	G
	Axenic culture? Yes/No (Circle one)	G

Notes:

Test Conditions:

Date / Time Test Start: 30.09.22 9:50 Started By: G		Date / Time Test End: 03.10.22 8.50 - 11:10 Finished By: G	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 7.7	pH adjustment: Y/N	pH of well D6 at T=0 h 6.8	pH of well D7 at T=72 h 6.8
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # —		Metal mining NUT Lot # NUT 2203
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation) 25.3 ± 0.1 °C			

¼ plate rotation (Initial)	Day 1		Day 2	
	AM SO	PM VG	AM G	PM G
Lights ON (Initial)	AM ✓	PM G	AM G	PM G

* For reference test, record pH of top concentration before use for series dilutions.

Notes:

72-Hour Qualitative Observations:

Condensation: Yes		
Growth: Yes		
Were there any other method variations or deviations from methods? Yes/No		If yes, describe further:
Anything unusual about the test? Yes/No		
Any problems encountered? Yes/No		
Any remedial measures taken? Yes/No		

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 8730-0032242	Sample Name: EDL 1

Reference Data:

Reference Chemical Batch #	Phenol P2213	Date test started	26/09/22
Method of Analysis	ICPIN – Linear interpolation	Algae Lot #	G 6116
72-hour IC25 (95% C.I.)[3] mg/L	52.47 45.83; 60.40	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	54.33 26.16; 112.84

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	37.95 (15.05; 55.42)	No hormesis models would fit
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers	—	ICPIN – Linear interpolation —
Test for Outliers	No Outliers Present	Grubbs' Test for Residual Outlier Initial C
	If outliers present, indicate Concentration/Rep: 10.101; rep 3	
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed.	
	No statistically significant growth stimulation.	Williams' or Dunnett's Multiple Comparison Test Initial C
	Yes, statistically significant growth stimulation at these concentrations: 10.101	

1)Results relate only to the sample tested.
2) Tidepool Scientific Software © 2001-2007. Comprehensive Environmental Toxicity Information System – CETIS v. 1.9.6.7
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/No

Any outliers and justification for their removal? Yes/No

Nautilus Environmental Company Inc.

Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)

72-Hour Growth Inhibition Test – Continued

72-Hour Quantitative Observations of Controls

Sample Name: EDL1 Sample Number: 8730-0032242 Date Test Start: 30.09.22

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D8	Well # D9	Well # D10	Well # D11
1	20	22	21	18	24	21	19	15
2	30	28	16	21	17	25	22	18
3	16	22	22	16	31	27	16	25
4	30	26	21	20	23	22	17	22
5	—	—	—	—	—	—	—	—
Initials	EV	EV	EV	EV	EV	EV	EV	EV

1 118 750

Cell increase for controls = 19.1	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = 12.5	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = no significant trend	Controls are invalid if there is a trend detected by the Mann-Kendall test

Mann-Kendall Trend Test for *Selenastrum capricornutum* Growth Inhibtion Test

EPS Method 1/RM/25
Version 1.1, Nov. 2000
Pollutech EnviroQuatics

Sample #: "8730-0032242
Client #: "8730-003
Date tested: "30.09.22

Instructions:

Enter control data below cells labelled D2..D5 and D8..D11.
The test of significance is completed immediately following data entry.

D2	D3	D4	D5	D8	D9	D10	D11
229205	234205	189205	176705	226705	226705	174205	189205

Results:

There is no significant trend.

Notes:

The test is a two-sided alternative using a nominal alpha value of 0.05%.
Due to the discrete nature of the test statistic, the true alpha value is 0.062%.

01.11.22

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL1 Sample Number: 8730-203242 Date Test Start: 30.09.22

Theoretical Test Concentration: 100.00% v/v					Actual Test Concentration: 90.91% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	8	11	10		95871 (± 24537)
2	11	12	13		Coefficient of Variation of Cell Yield
3	9	6	15		26
4	9	8	16		Average % Inhibition (-ve number = enhancement)
5	—	—	—		53.408
Initials	EV	EV	E		E

Theoretical Test Concentration: 33.33% v/v					Actual Test Concentration: 30.303% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	19	19	23		169 205 (± 18028)
2	13	20	18		Coefficient of Variation of Cell Yield
3	14	16	22		11
4	18	19	15		Average % Inhibition (-ve number = enhancement)
5	—	—	—		17.769
Initials	E	EV	E		E

Theoretical Test Concentration: 11.11% v/v					Actual Test Concentration: 10.101% v/v
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	21	37	37		288371 (± 51982)
2	23	27	25		Coefficient of Variation of Cell Yield
3	23	31	28		18
4	29	33	45		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-40.145
Initials	EV	E	E		E

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL1 Sample Number: 8730-032242 Date Test Start: 30.09.22

Theoretical Test Concentration: 3.704 %. v/v					Actual Test Concentration: 3.367 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation)
1	18	18	25		199 205 (± 6614)
2	21	23	21		Coefficient of Variation of Cell Yield
3	22	25	22		3
4	20	20	17		Average % Inhibition (-ve number = enhancement)
5	—	—	—		3.189
Initials	C	C	C		C

Theoretical Test Concentration: 1.235 %. v/v					Actual Test Concentration: 1.122 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation)
1	13	21	19		174 205 (± 1887)
2	17	21	19		Coefficient of Variation of Cell Yield
3	21	20	19		11
4	16	20	16		Average % Inhibition (-ve number = enhancement)
5	—	—	—		15.339
Initials	C	C	C		C

Theoretical Test Concentration: 0.412 %. v/v					Actual Test Concentration: 0.374 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation)
1	16	20	16		176 705 (± 5000)
2	20	15	23		Coefficient of Variation of Cell Yield
3	19	19	18		3
4	20	19	20		Average % Inhibition (-ve number = enhancement)
5	—	—	—		14.129
Initials	C	C	C		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: EDL1 Sample Number: 8730-032242 Date Test Start: 30.09.22

Theoretical Test Concentration: 0.137 % v/v					Actual Test Concentration: 0.125 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	17	15	22		182 538 (± 12332)
2	20	21	15		Coefficient of Variation of Cell Yield
3	26	20	17		7
4	20	18	21		Average % Inhibition (-ve number = enhancement)
5	—	—	—		11.289
Initials	[illegible]	[illegible]	[illegible]		[illegible]

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	EDL1	Sample #	8730-0032242	Date test start	30.09.22

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	118750	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	20	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2375	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	10795.4545	=	19.060526

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	20	22	21	18	24	21	19	15
	30.0	28.0	16.0	21.0	17.0	25.0	22.0	18.0
	16	22.0	22.0	16.0	31.0	27.0	16.0	25.0
	30.0	26.0	21.0	20.0	23.0	22.0	17.0	22.0
total cells	96	98	80	75	95	95	74	80
cells/ul	240	245	200	187.5	237.5	237.5	185	200
cells/ml	240000	245000	200000	187500	237500	237500	185000	200000
Cell yield = measured concentration - initial algal cell concentration								
	229205	234205	189205	176705	226705	226705	174205	189205

Mean cell yield for the control = Rc — Standard deviation — coefficient of variation

Rc 205767 — SD 25702.1921 — CV 12.490918 **Must be ≤20**

% inhibition summary

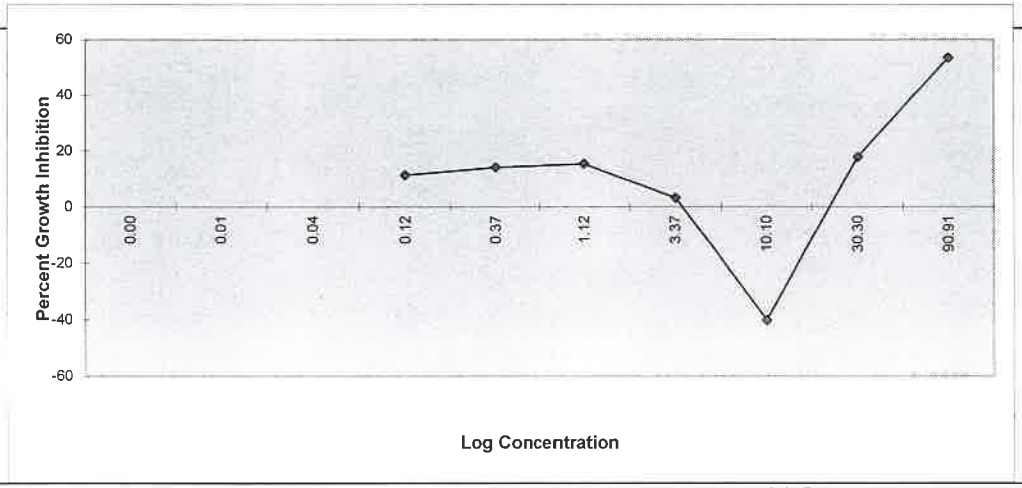
Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	11.289
0.374	14.124
1.122	15.339
3.367	3.189
10.101	-40.145
30.303	17.769
90.910	53.408

enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	8	11	10	19	19	23	21	37	37	18	18	25	13	21	19
	11.0	12.0	13.0	13.0	20.0	18.0	23.0	27.0	25.0	21.0	23.0	21.0	17.0	21.0	19.0
	9.0	6.0	15.0	14.0	16.0	22.0	23.0	31.0	28.0	22.0	25.0	22.0	21.0	20.0	19.0
	9.0	8.0	16.0	18.0	19.0	15.0	29.0	33.0	45.0	20.0	20.0	17.0	16.0	20.0	16.0
total cells	37	37	54	64	74	78	96	128	135	81	86	85	67	82	73
cells/ul	92.5	92.5	135	160	185	195	240	320	337.5	202.5	215	212.5	167.5	205	182.5
cells/ml	92500	92500	135000	160000	185000	195000	240000	320000	337500	202500	215000	212500	167500	205000	182500
Cell yield = measured concentration - initial algal cell concentration															
	81705	81705	124205	149205	174205	184205	229205	309205	326705	191705	204205	201705	156705	194205	171705
Mean Yield	95871			169205			288371			199205			174205		
STD Yield	24537			18028			51982			6614			18875		
CV Yield	26			11			18			3			11		

Average % inhibition														
	for 90.910%:	53.408		for 30.303%	17.769		for 10.101%	-40.145		for 3.367%	3.189		for 1.122%	15.339	

Average % stimulation														
	for 90.910%:	-53.408		for 30.303%	-17.769		for 10.101%	40.145		for 3.367%	-3.189		for 1.122%	-15.339	



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
16	20	16	17	15	22									
20.0	15.0	23.0	20.0	21.0	15.0									
19.0	19.0	18.0	26.0	20.0	17.0									
20.0	19.0	20.0	20.0	18.0	21.0									
75	73	77	83	74	75	0	0	0	0	0	0	0	0	0
187.5	182.5	192.5	207.5	185	187.5	0	0	0	0	0	0	0	0	0
187500	182500	192500	207500	185000	187500	0	0	0	0	0	0	0	0	0
176705	171705	181705	196705	174205	176705	-10795	-10795	-10795	-10795	-10795	-10795	-10795	-10795	-10795
176705			182538			-10795			-10795			-10795		
5000			12332			0			0			0		
3			7			0			0			0		
for 0.374%	14.124		for 0.125%	11.289		for 0.042%	105.246		for 0.014%	105.246		for 0.005%	105.246	
for 0.374%	-14.124		for 0.125%	-11.289		for 0.042%	-105.246		for 0.014%	-105.246		for 0.005%	-105.246	

AO 01.11.22

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
EDL1 L2734791-1	8730-0032242	September 28, 2022	September 29, 2022	September 30, 2022	10.9% Volume (N/A; 22.96% Volume)	<0.097% Volume (Not available)	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	23-Sep-2022	**Reference Chemical:**	KCl
Frond Increase IC25:	2.52 g/L	**95% Confidence Limits:**	1.83; 3.89 g/L
Historic Geometric Mean IC25:	2.22 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.41; 3.49 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	Na_2MoO_4	0.0726
	K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$2H_2O$	0.00003
	KCl	10.1	$MgSO_4\ 7H_2O$	147	$ZnCl_2$	0.00001
					$CoCl_2\ 6H_2O$	0.00001
					$CuCl_2\ 2H_2O$	

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

C. D'Andrea

Date: November 2, 2022

Carol D'Andrea
Laboratory Supervisor

Nautilus Environmental Company Inc.
***Lemna minor* 7-Day Growth Inhibition Test Bench Sheet**

Test Material

Client Name/Location: ALS Thunder Bay	
Sample #: 8730-0032241	Sample Name: EDL1 L2734791
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 28/09/22 8:15	Arrival Temperature (meter/probe): 14.0 °C (5.1)
Date/Time Received: 29/09/22 11:00	Sample Description: clear, light green
Collection Point Description: Other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: Air / Road	Storage: 4±2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	ET
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	ET
Culture Age at Start of Test:	9 days old, acclimated 22.5 hours in fresh test solution (mAPHA)	ET
Culture Medium:	Modified Hoagland's E+ medium, Lot # MH220L	ET
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/No	ET
	Axenic culture? Yes/No	ET
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 30 in HT 1, 25 in HT 2, 27 in HT 3 at finish	ET

Test Conditions and Procedures

Date / Time Test Start: 30.09.22 14:30 Started By: ET	Date / Time Test End: 07.10.22 14:25 Finished By: ET
Test Type: (Static) (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / (No) (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: (Modified APHA) (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 8.0	pH Adjustment: none
Test Volume and Depth: 150 mL / 4cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / (No)	If yes, describe further:
Anything unusual about the test? Yes / (No)	
Any problems encountered? Yes / (No)	
Any remedial measures taken? Yes / (No)	Randomization Template: D

Sample #: 8730-0032242	Sample Name: EDL 1

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	26.4	25.2	25.3	25.3	24.6	24.8	25.1	24.7
Low	26.2	25.3	25.3	25.3	24.8	24.8	25.3	25.0
Medium	26.2	25.4	25.4	25.3	24.9	25.0	25.4	25.0
High	26.3	25.3	25.3	25.3	24.7	24.6	25.4	25.0
Initials	EV	SO	✓	EV	KP	KP	KP	KP
meter/probe	51	52	52	52	52	52	52	52
Mean Test Temperature (average of 24h high / low temperatures):					25.3 ± 0.1 °C			

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.2	8.2	8.2	8.0	EV	12/86
Day 7	Control	Low	Medium	High	Initials	pH meter/probe
	8.4	9.4	9.5	9.1	EV	14/90

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)								

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 05/10/22 (5)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 12					Initials: EV
4730	4410	4580	5190	4580	Mean Light Measurement: 4698
±15% Variation of Mean: 3993 – 5430					Acceptable / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) / No
If yes, describe further: None

Reference Data

Reference Date	**Frond Increase** or **Dry Weights** (circle one)			
	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
23/09/22	2.52	1.83; 3.89	2.22	1.41; 3.49

Sample #: 8730-0032242	Sample Name: EDL 1

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 48	B 51	C 51	D 49	49.8

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy							Initials: C
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV: 43.8 (1.5) 3.4							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-9.71	-21.71	-21.14	-20.57	-24.00	-34.29	-56.57
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-27.08	-34.13	-24.75	-35.77	-39.92	-34.70	-36.27

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: Yes / (Not applicable (no stimulation)) (Circle one)

Test Endpoints and Calculations:

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	10.9 (N/A; 22.96)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]	—	ICPIN – linear interpolation
Dry Weights		
IC25 (95% C.I.)[3]	< 0.097 (N/A)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN – linear interpolation

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019. Comprehensive Environmental Toxicity Information System CETISv 1.9.6.7
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / (No)

Any outliers and justification for their removal? Yes / (No)

Lemna minor Day 7 Observations

Client: ALS – Thunder Bay	**Sample number:** 8730-0032242	**Date Started:** 30. 09. 22	
Site: EDL1		**Date Ended:** 07. 10. 22	
Concentration: Control	**Observations By:** EV	**Concentration:** 0.047 % v/v	**Observations By:** C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	48	51	51	49	**Number of**	45	43	46	48
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	X	X	X	X	**Abnormally sized fronds**	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	√	**Loss of Buoyancy**	X	√	√	√
Other Observations					**Other Observations**	algae			
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO N/A Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor Day 7 Observations

Client: ALS – The Water Bay	Sample number: 8730-0032242	Date Started: 30. 09. 22	
Site: EDL1		Date Ended: 07. 10. 22	
Concentration: 0.29% v/v	Observations By: EV	Concentration: 0.97% v/v	Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	40	40	40	41	Number of	42	41	40	39
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	√	√	√	Loss of Buoyancy	X	√	√	√
Other Observations	algae	→	→	→	Other Observations	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Day 7 Observations

Client: ALS – Thunder Bay			**Sample number:** 8730-0032242		**Date Started:** 30. 09. 22				
Site: EDL1					**Date Ended:** 07. 10. 22				
Concentration: 3.1 % v/v			**Observations By:** CR		**Concentration:** 9.7 % v/v			**Observations By:** C	
Observations	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Observations**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
Number of	38	44	41	40	**Number of**	42	39	35	41
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	**Abnormally sized fronds**	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	√	√	√
Other Observations	algae	→	→	→	**Other Observations**	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Day 7 Observations

Client: ALS – The Water Bay			**Sample number:** 8730-0032242		**Date Started:** 30. 09. 22				
Site: EDL1					**Date Ended:** 07. 10. 22				
Concentration: 31% v/v			**Observations By:** CF		**Concentration:** 97 % v/v			**Observations By:** CF	
Observations	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Observations**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
Number of	39	35	35	30	**Number of**	26	23	27	24
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	√	√	√	√
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	**Abnormally sized fronds**	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	√	√	**Loss of Buoyancy**	X	X	X	X
Other Observations	algae	→	→	→	**Other Observations**	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Weights

Client	ALS - Thunder Bay	Site	EDL 1	Sample number	8730-003224 2
In Oven Date/Time/ °C:	07/10/22 15:00 63°	Out Oven Date/Time/°C:	08/10/22 15:00 62°		

Conc. -/. v/v	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	42	43.8 (1.5)	0.81303	0.80887	4.16	3.97 (0.1)
	B	45		0.80465	0.80080	3.85	
	C	45		0.80065	0.79663	4.02	
	D	43		0.79954	0.79569	3.85	
0.097	A	39	39.5 (2.1)	0.82320	0.82018	3.02	2.90 (0.3)
	B	37		0.79922	0.79668	2.54	
	C	40		0.81140	0.80827	3.13	
	D	42		0.81277	0.80988	2.89	
0.29	A	34	34.3 (0.5)	0.82002	0.81715	2.87	2.62 (0.3)
	B	34		0.81353	0.81080	2.73	
	C	34		0.81561	0.81338	2.23	
	D	35		0.80686	0.80423	2.63	
0.97	A	36	34.5 (1.3)	0.81510	0.81190	3.20	2.99 (0.2)
	B	35		0.80891	0.80592	2.99	
	C	34		0.813269	0.81045	2.84	
	D	33		0.80987	0.80695	2.92	
3.1	A	32	34.8 (2.5)	0.80800	0.80609	1.911	2.55 (0.5)
	B	38		0.809752	0.80668	3.04	
	C	35		0.80540	0.80267	2.73	
	D	34		0.80496	0.80244	2.52	
9.7	A	36	33.3 (3.1)	0.80811	0.80537	2.74	2.39 (0.5)
	B	33		0.80775	0.80598	1.77	
	C	29		0.790828	0.78806	2.22	
	D	35		0.80455	0.80174	2.81	
31	A	33	28.8 (3.7)	0.80640	0.80382	2.58	2.59 (0.2)
	B	29		0.81142	0.80862	2.80	
	C	29		0.81834	0.81609	2.25	
	D	24		0.81246	0.80972	2.74	
97	A	20	19.0 (1.8)	0.81502	0.81234	2.68	2.53 (0.3)
	B	17		0.81205	0.80996	2.09	
	C	21		0.80934	0.80670	2.64	
	D	18		0.80939	0.80668	2.71	
Initials		TO	TC	CS	SO	TV	TC

Notes:

RG

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Sample name EDL1 Date started 30/09/22 sample # "8730-0032242

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

49.8 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	48	45	40	42	38	42	39	26
	51	43	40	41	44	39	35	23
	51	46	40	40	41	35	35	27
	49	48	41	39	40	41	30	24
Total Fronds	199	182	161	162	163	157	139	100

Increase in Frond Number = Total # Fronds - 6 Starting Fronds

	42	39	34	36	32	36	33	20
	45	37	34	35	38	33	29	17
	45	40	34	34	35	29	29	21
	43	42	35	33	34	35	24	18
Total Increase	175	158	137	138	139	133	115	76
Mean Increase	43.8	39.5	34.3	34.5	34.8	33.3	28.8	19.0
SD Increase	1.5	2.1	0.5	1.3	2.5	3.1	3.7	1.8
CV Increase	3.4	5.3	1.5	3.7	7.2	9.3	12.8	9.6
% Stimulation		**-9.71**	**-21.71**	**-21.14**	**-20.57**	**-24.00**	**-34.29**	**-56.57**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.81303	0.82320	0.82002	0.81510	0.80800	0.80811	0.80640	0.81502
Pan + Plant	0.80465	0.79922	0.81353	0.80891	0.80972	0.80775	0.81142	0.81205
	0.80065	0.81140	0.81561	0.81329	0.80540	0.79028	0.81834	0.80934
	0.79954	0.81277	0.80686	0.80987	0.80496	0.80455	0.81246	0.80939
Initial Weight (g)	0.80887	0.82018	0.81715	0.81190	0.80609	0.80537	0.80382	0.81234
Pan Only	0.80080	0.79668	0.81080	0.80592	0.80668	0.80598	0.80862	0.80996
	0.79663	0.80827	0.81338	0.81045	0.80267	0.78806	0.81609	0.80670
	0.79569	0.80988	0.80423	0.80695	0.80244	0.80174	0.80972	0.80668
Plant Only (mg)	4.16	3.02	2.87	3.20	1.91	2.74	2.58	2.68
	3.85	2.54	2.73	2.99	3.04	1.77	2.80	2.09
	4.02	3.13	2.23	2.84	2.73	2.22	2.25	2.64
	3.85	2.89	2.63	2.92	2.52	2.81	2.74	2.71
Mean Dry Weight	3.970	2.895	2.615	2.988	2.550	2.385	2.593	2.530
SD Dry Weight	0.1	0.3	0.3	0.2	0.5	0.5	0.2	0.3
CV Dry Weight	3.8	8.8	10.5	5.2	18.7	20.4	9.5	11.6
% Stimulation		**-27.08**	**-34.13**	**-24.75**	**-35.77**	**-39.92**	**-34.70**	**-36.27**

For Data Transfer to CETIS

	# fronds	total mass	tare
0	1	42	0.81303	0.80887
	2	45	0.80465	0.80080
	3	45	0.80065	0.79663
	4	43	0.79954	0.79569
0.1	1	39	0.82320	0.82018
	2	37	0.79922	0.79668
	3	40	0.81140	0.80827
	4	42	0.81277	0.80988
0.3	1	34	0.82002	0.81715
	2	34	0.81353	0.81080
	3	34	0.81561	0.81338
	4	35	0.80686	0.80423
1	1	36	0.81510	0.81190
	2	35	0.80891	0.80592
	3	34	0.81329	0.81045
	4	33	0.80987	0.80695
3.1	1	32	0.80800	0.80609
	2	38	0.80972	0.80668
	3	35	0.80540	0.80267
	4	34	0.80496	0.80244
9.7	1	36	0.80811	0.80537
	2	33	0.80775	0.80598
	3	29	0.79028	0.78806
	4	35	0.80455	0.80174
31	1	33	0.80640	0.80382
	2	29	0.81142	0.80862
	3	29	0.81834	0.81609
	4	24	0.81246	0.80972
97	1	20	0.81502	0.81234
	2	17	0.81205	0.80996
	3	21	0.80934	0.80670
	4	18	0.80939	0.80668

AD 011122

November 2, 2022

ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Attention Lab:

On September 29, 2022, Nautilus Environmental Company Inc. personnel received one water sample (SED2DIS L2734791-2) from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected September 28, 2022

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
SED2DIS L2734791-2	Fathead Minnow	7-day LC50 (95% Confidence) 7-day IC25 (95% Confidence)	Survival Biomass	>100% Volume[2] (Not Applicable) >100% Volume[2] (Not Applicable)
#8730-0032243	*Ceriodaphnia dubia*	3-brood LC50 (95% Confidence) 3-brood IC25 (95% Confidence)	Survival Reproduction	>100% Volume[2] (Not Applicable) 10.58% Volume (N/A; 45.47% Volume)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[2] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence) 7-day IC25 Dry Weight (95% Confidence)	Growth Growth	>97% Volume[2] (Not applicable) >97% Volume[2] (Not applicable)

1 - Results relate only to the sample tested
2 - Highest concentration tested, based on test method

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 100%, 33%, 11%, 3.7% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted by a factor of 0.33 (i.e., 97%, 31%, 9.7% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

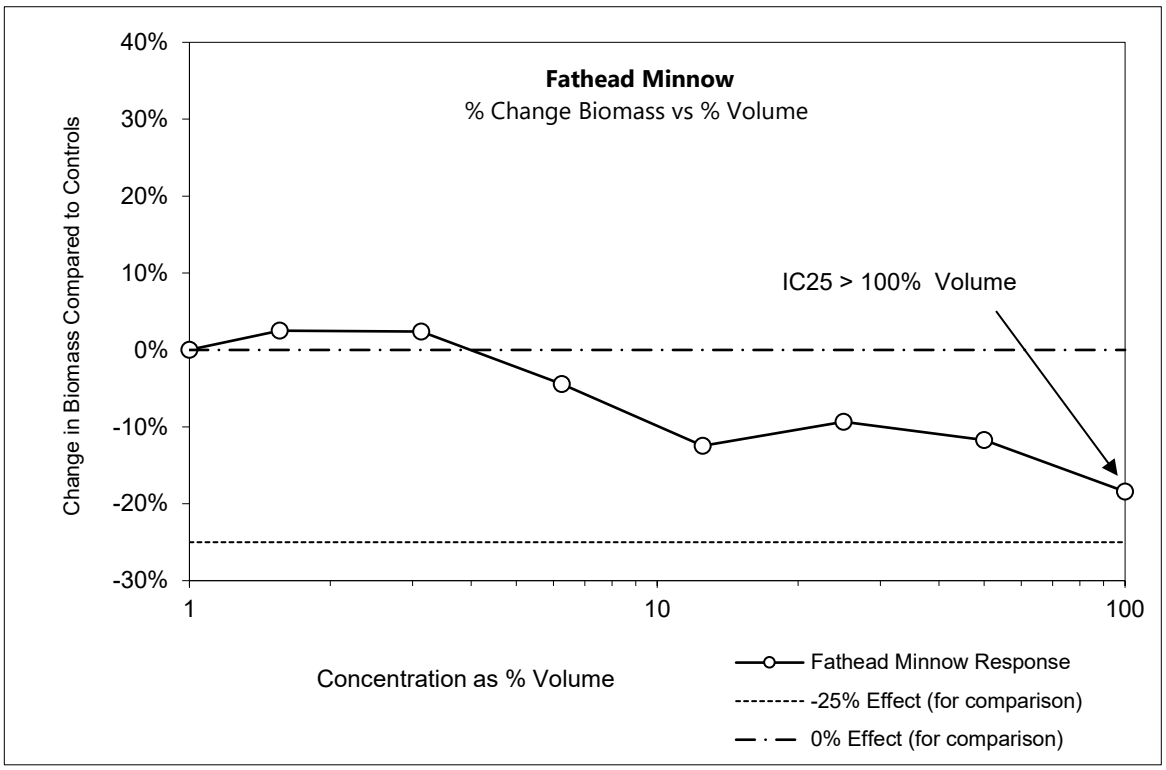
If there are any further details which you require, please do not hesitate to contact us.

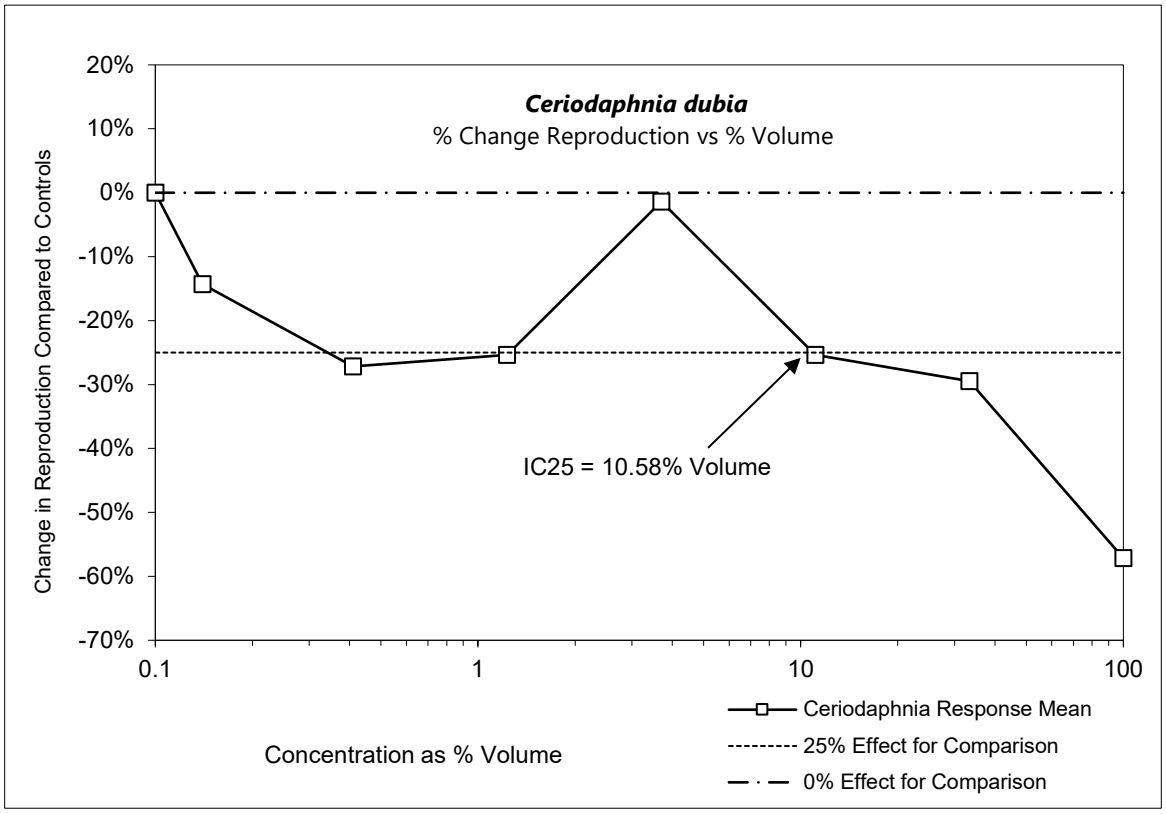
Yours very truly,
Nautilus Environmental Company Inc.

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2022\8000\8730-003\8730-0032243 FCRL

ALS Thunder Bay - SED2DIS L2734791-2 - September 28, 2022





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Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	SED2DIS L2734791-2
Date/Time Sampled:	September 28, 2022; 08:10	**Date/Time Received:**	September 29, 2022; 11:00
Date/Time Test Started:	September 30, 2022; 09:15	**Date Test Finished:**	October 7, 2022
Description:	Clear, light yellow	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032243	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	14.0°C
Collected By:	Not available		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species: Fathead Minnow (*Pimephales promelas*)

Source: Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)

Culture Temp.: 22 to 26 °C **Life Stage:** <24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 0.5%

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032243 **Sources:** SED2DIS L2734791-2

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Test Conditions

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 0 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (0%)

Average Weight of Controls is ≥ 250 µg: Acceptable (572 µg)

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032243 **Sources:** SED2DIS L2734791-2

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	0	10	10	0	0	10	10
	2	0	0	0	0	10	0	20	10
	3	0	0	10	10	20	30	10	10
Mean % Mortality		0.0	0.0	6.7	6.7	10.0	10.0	13.3	10.0
S.D.		0.0	0.0	5.8	5.8	10.0	17.3	5.8	0.0
Survival/Growth Data									
Mean Dry Biomass	1	0.561	0.656	0.602	0.514	0.555	0.607	0.480	0.501
(mg)	2	0.575	0.650	0.599	0.476	0.480	0.526	0.486	0.498
	3	0.580	0.453	0.556	0.650	0.467	0.423	0.549	0.401
% Effect (+ or -)		0.0	2.5	2.4	-4.4	-12.5	-9.3	-11.7	-18.4
Mean Dry Biomass/ Concentration (mg)		0.572	0.586	0.586	0.547	0.501	0.519	0.505	0.467
S.D.		0.01	0.12	0.03	0.09	0.05	0.09	0.04	0.06

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032243 **Sources:** SED2DIS L2734791-2

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival		
7-day LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Biomass **(Survival and Growth)**		
7-day IC25 (Confidence Interval)[2]	>100% Volume (Not Applicable)	Non-linear Regression 3P Log-Logistic Model

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Phenol

Date Test Initiated: 22-Sep-2022 **Reference Batch #:** P2213

Method of Analysis: Trimmed Spearman-Karber α = 3.33%

7-Day LC50 (95% Confidence Limits): 20.14 mg/L (16.77 mg/L; 24.20 mg/L)

Historic Geometric Mean LC50: 23.38 mg/L (15.00 mg/L; 36.44 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS - TB Sample Name: SED2 DIS L2734711-2 Sample #: 8730-0032243

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 0 %

Average Weight of Controls is ≥ 250 µg: (Acceptable) / Not Acceptable 572 µg

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 0 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
7-day LC50	> 100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
Survival / Growth Biomass		
7-day IC25	> 100 % Volume	Non-linear regression 3P Log logistic model
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 19/10/22

Results Verified: Initials AO Date 01/11/22

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** L2734791-2/SED 2 DIS **Sample #:** 8730.0032243

Day	Date 2022	Initial Variables				Meters/Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	D.O. / °C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	30.09	25.3	8.3	10.4	577	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
1	01.10	24.0	8.3	11.3	566	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
2	02.10	25.2	8.3	10.9	581	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
3	03.10	24.3	8.3	10.9	580	6/4	13/88	5/6	yes	≤100	≤ 20	2	SO
4	04.10	25.4	8.3	10.8	603	6/4	13/88	5/6	yes	≤100	≤ 20	2	SO
5	05.10	24.6	8.2	10.7	603	6/4	13/88	5/6	yes	≤100	≤ 20	3	KK
6	06.10	24.5	8.2	10.9	596	6/4	13/88	5/6	yes	≤100	≤ 20	3	SO
7	07.10												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of brine shrimp days 0 to 6? (Yes)/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: Control Sample Name: L2734791-2/SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	30.09	24.4	8.3	8.2	240	6/14	13/188	5/6	ET
1	01.10	26.4	8.4	7.4	256	6/14	13/88	5/6	SO
2	02.10	24.8	8.4	7.8	245	6/4	13/88	5/6	✓
3	03.10	24.3	8.3	7.9	248	6/14	13/188	5/6	ET
4	04.10	26.2	8.3	7.6	259	6/4	13/88	5/6	KP
5	05.10	24.3	8.2	8.0	255	6/4	13/88	5/6	KP
6	06.10	24.7	8.3	7.8	258	6/14	13/188	5/6	ET
7	07.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.8	7.9	6.5	6/4	13/88	SO							
24.6	7.7	5.6	6/4	13/86	✓	0	0	0	0	0	0	✓
24.3	7.9	6.7	6/14	13/188	ET	0	0	0	0	0	0	✓
24.2	7.8	6.3	6/4	13/88	KP	0	0	0	0	0	0	KP
24.5	7.8	6.4	6/4	13/88	KP	0	0	0	0	0	0	ET
24.5	7.6	6.1	6/14	13/188	ET	0	0	0	0	0	0	ET
24.4	7.6	5.8	6/14	13/188	ET	0	0	0	0	0	0	ET
						0	0	0	0	0	0	~~KP~~ SO SO

Observations: _____

Concentration: 1.56 %v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	30.09	24.4	8.2	8.3	245	6/14	13/188	5/6	ET
1	01.10	25.8	8.4	~~8.0~~ 7.5	254	6/4	13/88	5/6	SO
2	02.10	24.5	8.2	7.2	249	6/4	13/88	5/6	✓
3	03.10	24.0	8.3	8.1	251	6/14	13/188	5/6	ET
4	04.10	25.0	8.4	7.8	259	6/4	13/88	5/6	KP
5	05.10	24.2	8.2	7.9	261	6/4	13/88	5/6	KP
6	06.10	24.3	8.3	8.0	266	6/14	13/188	5/6	ET
7	07.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.8	7.9	6.7	6/4	13/88	SO							
24.6	7.9	5.7	6/4	13/86	✓	0	0	0	0	0	0	✓
24.3	7.8	6.3	6/14	13/188	ET	0	0	0	0	0	0	✓
24.1	7.8	6.2	6/4	13/88	KP	0	0	0	0	0	0	KP
24.5	7.9	6.7	6/4	13/88	KP	0	0	0	0	0	0	ET
24.5	7.7	6.2	6/14	13/188	ET	0	0	0	0	0	0	ET
24.4	7.5	5.2	6/14	13/188	ET	0	0	0	0	0	0	~~KP~~
						0	0	0	0	0	0	SO

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13% v/v Sample Name: L2734791-2/SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	30.09								
1	01.10								
2	02.10								
3	03.10								
4	04.10								
5	05.10								
6	06.10								
7	07.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	[illegible]
						0	0	0	0	0	0	[illegible]
						10	0	0	0	0	0	KP
						10	0	10	0	0	0	ET
						10	0	10	0	0	0	ET
						10	0	10	0	0	0	KP
						10	0	10	0	0	0	SO

Observations: _____

Concentration: 6.25% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	30.09								
1	01.10								
2	02.10								
3	03.10								
4	04.10								
5	05.10								
6	06.10								
7	07.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						10	0	0	0	0	0	[illegible]
						10	0	0	0	0	0	[illegible]
						10	0	0	0	0	0	KP
						10	0	10	0	0	0	ET
						10	0	10	0	0	0	ET
						10	0	10	0	0	0	KP
						10	0	10	0	0	0	SO

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5% v/v Sample Name: L2734791-2/SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	30.09	24.5	8.3	8.2	284	6/4	13/188	5/6	ET
1	01.10	25.8	8.4	7.7	293	6/4	13/188	5/6	SO
2	02.10	24.5	8.3	7.1	286	6/4	13/66	5/6	[illegible]
3	03.10	24.0	8.3	8.3	292	6/4	13/188	5/6	ET
4	04.10	25.0	8.4	7.9	299	6/4	13/188	5/6	KP
5	05.10	24.3	8.2	8.0	297	6/4	13/188	5/6	KP
6	06.10	24.3	8.3	8.0	300	6/4	13/188	5/6	ET
7	07.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.8	7.9	6.4	6/4	13/188	SO							
24.6	8.0	5.5	6/4	13/66	[illegible]	0	0	0	0	0	0	[illegible]
24.2	7.8	6.2	6/4	13/188	ET	0	0	0	0	0	0	[illegible]
24.1	7.8	6.0	6/4	13/188	KP	0	0	20	0	0	0	KP
24.5	7.8	6.3	6/4	13/188	KP	0	10	20	0	0	0	ET
24.5	7.7	6.1	6/4	13/188	ET	0	10	20	0	0	0	ET
24.3	7.6	5.5	6/4	13/188	ET	0	10	20	0	0	0	KP
						0	10	20	0	0	0	SO

Observations: _____

Concentration: 25% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	30.09								
1	01.10								
2	02.10								
3	03.10								
4	04.10								
5	05.10								
6	06.10								
7	07.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	[illegible]
						0	0	0	0	0	0	[illegible]
						0	0	0	0	0	0	KP
						0	0	10	0	0	0	ET
						0	0	10	0	0	0	ET
						0	0	30	0	0	0	KP
						0	0	30	0	0	0	SO

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50% v/v Sample Name: L2734791-2/SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	30.09								
1	01.10								
2	02.10								
3	03.10								
4	04.10								
5	05.10								
6	06.10								
7	07.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	
						0	0	0	0	0	0	
						0	0	0	0	0	0	KP
						0	0	0	0	0	0	ET
						0	0	0	0	0	0	ET
						10	20	10	0	0	0	KP
						10	20	10	0	0	0	SO

Observations: _____

Concentration: 100% v/v

Day	Date 2022	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	30.09	25.1	8.3	9.4	574	6/4	13/188	5/6	ET
1	01.10	24.9	8.3	9.2	579	6/4	13/88	5/6	SO
2	02.10	24.9	8.2	9.0	560	6/4	13/88	5/6	
3	03.10	24.3	8.3	9.3	580	6/4	13/188	5/6	ET
4	04.10	24.7	8.3	9.3	594	6/4	13/88	5/6	KP
5	05.10	25.0	8.2	8.9	605	6/4	13/88	5/6	KP
6	06.10	24.7	8.3	9.1	599	6/4	13/188	5/6	ET
7	07.10								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
24.8	8.2	6.4	6/4	13/88	SO							
24.6	8.1	5.4	6/4	13/88		0	0	0	0	0	0	
24.2	8.1	6.0	6/4	13/188	ET	0	0	0	0	0	0	
24.1	8.0	5.5	6/4	13/88	KP	0	0	10	0	0	0	KP
24.2	8.1	6.5	6/4	13/88	KP	10	0	10	0	0	0	ET
24.4	7.9	5.5	6/4	13/188	ET	10	10	10	0	0	0	ET
24.2	8.0	5.6	6/4	13/188	ET	10	10	10	0	0	0	KP
						10	10	10	0	0	0	SO

Observations: _____

WO # L2734791

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information

Client: ALS Thunder Bay
Sample #: 8730.00343
Date/Time Received: 29/09/22 / 1100
Sample Type: Water
100% Hardness: 220

Sample Name: ~~8730.003~~ L2734791-2/SED 2 DIS
Sample Date/Time: 28/09/22 / 0810
Person Sampling: n/a
Arrival Temp: 14.0 °C
Sample Description: clear, light yellow

Test Information

Date/Time Started: 30/09/22 / 0915
Test started by: SO
Fathead Batch #: FH 0621 / 0122
Date eggs laid: 24,25,26/09/22
Culture mortality within 7 days of egg collection: 0.5%
Swim bladder inflated: (yes) / no: yes
Age of Larvae at start of test in hours: <24
Control Hardness: 98
Water Bath Quadrant: B
Average Temperature during Test: 25.0 ± 0.5 °C

Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes (No) (circle one)

Conc.	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	10	0.81661	0.81100	0.561	0.572
	B	10	0.80225	0.79650	0.575	
	C	10	0.81307	0.80727	0.580	
1.56	A	10	0.82433	0.81777	0.656	0.586
	B	10	0.82153	0.81503	0.650	
	C	10	0.81565	0.81112	0.453	
3.13	A	9	0.81240	0.80638	0.602	0.586
	B	10	0.81671	0.81072	0.599	
	C	9	0.82122	0.81566	0.556	
6.25	A	9	0.82205	0.81691	0.514	0.547
	B	10	0.81349	0.80873	0.476	
	C	9	0.82620	0.81970	0.650	
12.5	A	10	0.82015	0.81460	0.555	0.501
	B	9	0.82432 E	0.81951	0.480	
	C	8	0.81153	0.80686	0.467	
25.	A	10	0.82213	0.81606	0.607	0.519
	B	10	0.81366	0.80840	0.526	
	C	7	0.81541	0.81118	0.423	
50	A	9	0.81883	0.81403	0.480	0.505
	B	8	0.82855	0.82369	0.486	
	C	9	0.81615	0.81066	0.549	
100	A	9	0.82086	0.81585	0.501	0.467
	B	9	0.81549	0.81051	0.498	
	C	9	0.82230	0.81829	0.401	
Initials		SO	ET	SO	G	G

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8730-0032243 **Sample Name** SED2-DIS

Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug) 572

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	10	0.81661	0.81100	0.561	0.561	0.572	0.572	0.009849	0.009849	1.721828
	10	10	0.80225	0.79650	0.575	0.575					
	10	10 ✓	0.81307	0.80727	0.580	0.580					
1.56	10	10	0.82433	0.81777	0.656	0.656	0.586	0.586	0.115509	0.115509	19.70023
	10	10	0.82153	0.81503	0.650	0.650					
	10	10 ✓	0.81565	0.81112	0.453	0.453					
3.13	10	9	0.81240	0.80638	0.669	0.602	0.629	0.586	0.03617	0.025736	5.754388
	10	10	0.81671	0.81072	0.599	0.599					
	10	9 ✓	0.82122	0.81566	0.618	0.556					
6.25	10	9	0.82205	0.81691	0.571	0.514	0.590	0.547	0.124168	0.091484	21.05334
	10	10 ✓	0.81349	0.80873	0.476	0.476					
	10	9 ✓	0.82620	0.81970	0.722	0.650					
12.5	10	10	0.82015	0.81460	0.555	0.555	0.557	0.501	0.025291	0.047501	4.537656
	10	9 ✓	0.82431	0.81951	0.533	0.480					
	10	8 ✓	0.81153	0.80686	0.584	0.467					
25	10	10	0.82213	0.81606	0.607	0.607	0.579	0.519	0.046002	0.092219	7.943745
	10	10	0.81366	0.80840	0.526	0.526					
	10	7 ✓	0.81541	0.81118	0.604	0.423					
50	10	9	0.81883	0.81403	0.533	0.480	0.584	0.505	0.04356	0.038223	7.463835
	10	8 ✓	0.82855	0.82369	0.607	0.486					
	10	9 ✓	0.81615	0.81066	0.610	0.549					
100	10	9	0.82086	0.81585	0.557	0.501	0.519	0.467	0.06321	0.056889	12.19045
	10	9	0.81549	0.81051	0.553	0.498					
	10	9 ✓	0.82230 ✓	0.81829 ✓	0.446	0.401					

SD: Standard Deviation
CV: Coefficient of Variation

AO 01.11.23

NAUTILUS ENVIRONMENTAL

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Effluent	**Source:**	SED2DIS L2734791-2
Date/Time Sampled	September 28, 2022; 08:10	**Date/Time Received:**	September 29, 2022; 11:00
Date Test Started:	September 30, 2022; 11:50	**Date Test Finished:**	October 6, 2022
Description:	Clear, light yellow	**Days Sample Used:**	Days 0 to 5
Sample #:	8730-0032243	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	14.0°C
Collected By:	Not available		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

Nautilus Environmental Company Inc.
Suite 122, 704 Mara Street, Point Edward, ON N7V 1X4 519-339-8787

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032243 **Sources:** SED2DIS L2734791-2

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 0%

Mean Number of Surviving Young Produced Within First 3 Broods: 20.6

Mean Number of Surviving Young Produced Within 7 Days of Testing: 39.0

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 0 to 5

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032243 **Sources:** SED2DIS L2734791-2

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD22-159

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (21.7 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (90% of controls)

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***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032243 **Sources:** SED2DIS L2734791-2

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	0	0	0	0	0	10	10
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	25	17	23	1	13	19	23	3
	2	24	23	20	13	16	20	18	7
	3	23	15	14	18	26	6	9	0
	4	14	25	12	23	19	7	6	8
	5	19	19	8	0	23	25	0	9
	6	22	0	2	21	24	15	13	8
	7	24	20	17	20	20	25	21	9
	8	18	16	24	23	25	10	21	14
	9	23	25	15	25	22	14	22	18
	10	25	26	23	18	26	21	20	17
Total Number of Live Neonates in First 3 Broods or Less		217	186	158	162	214	162	153	93
% Effect (+ or -)		0.0	-14.3	-27.2	-25.3	-1.4	-25.3	-29.5	-57.1
Mean Number of Live Neonates in First 3 Broods or Less		21.7	18.6	15.8	16.2	21.4	16.2	15.3	9.3
SD		3.6	7.6	7.1	8.9	4.4	6.9	7.9	5.7

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2019. Comprehensive Environmental Toxicity Information System – CETIS v1.9.6.7.

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Ceriodaphnia dubia **Survival and Reproduction Study -** Continued

Sample #: 8730-0032243 **Sources:** SED2DIS L2734791-2

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	10.58% Volume (N/A; 45.47% Volume)	No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Phenol

Date Test Initiated: 22-Sep-2022 **Reference Batch #:** P2213

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 8.25 mg/L (7.23 mg/L; 9.41 mg/L)

Historic Geometric Mean LC50: 6.80 mg/L (4.17 mg/L; 11.10 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS - Thunder Bay Sample Name: SED2 DIS L2734791-2 Sample #: 8730-00322 43

Conditions for Test Validity

Control Mortality is< 20%: (Acceptable) / Not Acceptable: 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable) / Not Acceptable: 9 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable) / Not Acceptable: 21.7 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 0 to 5

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
REPRODUCTION		
3-brood IC25	10.58 % Volume	No nonlinear regression models would fit IC25 – linear interpolation
95% Confidence Interval[2]	N/A; 45.47 % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 19/10/22

Results Verified: Initials AO Date 01/11/22

riodaphnia dubia Initial Sample Measureme Before Preparation and Use in Toxicity Tes

Concentration: 100% **Sample Name:** L2734791-2 / SED 2 DIS **Sample #:** 8730.0032243

Day	Date 2022	Initial Measurements				Meters / Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	°C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	30.09	25.3	8.3	10.4	577	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
1	01.10	24.0	8.3	11.3	566	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
2	02.10	25.2	8.3	10.9	581	6/4	13/88	5/6	yes	≤100	≤ 20	1	SO
3	03.10	24.3	8.3	10.9	580	6/4	13/88	5/6	yes	≤100	≤ 20	2	SO
4	04.10	25.4	8.3	10.8	603	6/4	13/88	5/6	yes	≤100	≤ 20	2	SO
5	05.10	24.6	8.2	10.7	603	6/4	13/88	5/6	yes	≤100	≤ 20	3	
6	06.10									≤100	≤ 20	3	
7	07.10									≤100	≤ 20	3	
8	08.10									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted?	Yes/No	If yes, state mesh size: _____
Was sample pH or hardness adjusted?	Yes/No	If yes, describe further: _____
Were alternate concentrations or dilution series used?	Yes/No	If yes, describe further: _____
Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily?	Yes/No	If no, describe further: _____
Were there any other method variations, deviations, or exclusions from method?	Yes/No	If yes, describe further: _____
Was there anything unusual about the test, any problems encountered, or any remedial measures taken?	Yes/No	If yes, describe further: _____

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentrati: Control Sample Name: L2734791-2, SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	30.09	25.7	8.4	7.9	431	6/5	13/88	5/6	KP
1	01.10	24.9	8.4	8.0	431	6/5	13/88	5/6	SO
2	02.10	25.6	8.5	7.8	442	6/5	13/88	5/6	ET
3	03.10	25.4	8.5	7.8	449	6/4	13/88	5/6	ET
4	04.10	25.8	8.5	7.7	450	6/4	13/88	5/6	KP
5	05.10	25.2	8.3	7.8	454	6/4	13/88	5/6	KP
6	06.10								
7	07.10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
23.6	8.0	6.9	6/4	13/88	SO
25.1	7.9	6.0	6/4	13/88	SO
24.0	8.3	7.5	6/4	13/88	ET
24.3	8.4	7.3	6/4	13/88	KP
24.7	7.7	7.3	6/4	13/88	ET
23.6	8.3	7.6	6/4	13/88	ET

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SO	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SO	0
3	03.10	1	4	4	2	4	0	3	4	4	4	30	—	0	—	KK	KK
4	04.10	0	0	0	0	0	0	0	0	0	0	0	—	0	—	ET	0
5	05.10	12	10	6	6	9	9	8	6	6	10	82	—	0	—	ET	0
6	06.10	12	10	13	6	6	13	13	8	13	11	105	—	0	—	ET	0
7	07.10																
8	08.10																
Total Neonates		25	24	23	14	19	22	24	18	23	25	217	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.137 % v/v ✓ Sample Name: L2734791-2, SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Variables °C	Initial Variables pH	Initial Variables D.O. (mg/L)	Initial Variables Cond (umhos)	Meter/Probe D.O. / °C	Meter/Probe pH	Meter/Probe Cond	Initials
0	30.09	24.8	8.4	7.6	429	6/4	13/88	5/6	KP
1	01.10	25.3	8.34	7.8	439	6/4	13/88	5/6	SO
2	02.10	25.0	8.4	7.1	435	6/4	13/88	5/6	[illegible]
3	03.10	25.0	8.5	7.9	443	6/4	13/88	5/6	ET
4	04.10	24.8	8.4	7.7	443	6/4	13/88	5/6	KP
5	05.10	24.6	8.3	7.6	452	6/4	13/88	5/6	KP
6	06.10								
7	07.10								

Final Variables °C	Final Variables pH	Final Variables D.O. (mg/L)	Meter/Probe D.O. / °C	Meter/Probe pH	Initials
23.6	8.1	7.2	6/4	13/88	SO
25.1	7.9	5.8	6/4	13/88	SO
23.7	8.2	7.3	6/4	13/88	ET
24.1	8.3	7.4	6/4	13/88	KP
24.7	7.6	7.4	6/4	13/88	C
23.6	8.3	7.7	6/4	13/88	ET

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	% Mortality / day Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
3	03.10	3	5	2	3	1	0	0	2	2	4	22	—	0	0 lqc	KK	lqc
4	04.10	0	0	0	0	0	~~0~~ 0	0	0	0	0	0	—	0	—	C	C
5	05.10	4	9	7	11	5	0	8	6	10	10	70	—	0	—	C	C
6	06.10	10	9	6	11	13	0	12	8	13	12	94	—	0	—	C	C
7	07.10																
8	08.10											186					
Total Neonates		17	23	15	25	19	0	20	16	25	26	~~258~~	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.410% YN Sample Name: L2734791-2, SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	30.09								
1	01.10								
2	02.10								
3	03.10								
4	04.10								
5	05.10								
6	06.10								
7	07.10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	✓	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	So	—
3	03.10	4	3	0	4	3	0	0	4	5	4	27	—	0	—	KC	KC
4	04.10	0	0	0	0	0	1	0	0	0	0	1	—	0	—	CE	CE
5	05.10	8	9	8	7H	5	0	7	7	2	7	61	—	0	—	C	CE
6	06.10	11	8	6	0	0	1	10	13	8	12	69	—	0	—	CE	CE
7	07.10																
8	08.10																
Total Neonates		23	20	14	12	8	2	17	24	15	23	158					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 1.23% v/v Sample Name: L2734791-2, SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	30.09														
1	01.10														
2	02.10														
3	03.10														
4	04.10														
5	05.10														
6	06.10														
7	07.10														

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	—	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SD	—
3	03.10	0	0	0	10	0	0	0	0	0	0	10	—	0	—	CE	0
4	04.10	0	3	6	0	0	9	5	6	7	7	43	—	0	—	CE	u
5	05.10	1	10	12	13	0	0	5	6	8	0	55	—	0	—	CE	U
6	06.10	0	0	0	0	0	12	10	11	10	11	54	—	0	—	CE	U
7	07.10																
8	08.10																
Total Neonates		1	13	18	23	0	21	20	23	25	18	162	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 3.7% v/v Sample Name: L2734791-2, SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	30.09	24.8	8.4	8.0 7.6	432	6/4	13/88	6/4	KP	23.6	8.1	7.2	6/4	13/88	SO
1	01.10	25.2	8.5	7.9	447	6/4	13/88	5/6	SO	25.1	8.0	6.0	6/4	13/88	SO
2	02.10	25.1	8.5	7.1	446	6/4	13/88	5/6	ET	24.0	8.2	7.1	6/4	13/88	ET
3	03.10	24.9	8.5	7.8	446	6/4	13/88	5/6	ET	23.9	8.2	7.1	6/4	13/88	KP
4	04.10	24.8	8.4	7.6	448	6/4	13/88	5/6	KP	24.7	7.8	7.2	6/4	13/88	C
5	05.10	24.6	8.4	7.7	458	6/4	13/88	5/6	KP	23.6	8.2	7.3	6/4	13/88	ET
6	06.10														
7	07.10														

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	✓	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SO	—
3	03.10	0	2	5	0	4	4	4	5	4+1	4	33	—	0	—	KK	KK
4	04.10	1	0	0	4	0	0	0	8	0	0	13	—	0	—	C	6
5	05.10	3	7	8	7	5	7	6	0	8	9	60	—	0	—	C	8
6	06.10	9	7	13	8	14	13	10	12	9	13	108	—	0	—	C	6
7	07.10																
8	08.10																
Total Neonates		13	16	26	19	23	24	20	25	22	26	214	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 11.11% v/v Sample Name: L2734791-2, SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Variables °C	Initial Variables pH	Initial Variables D.O. (mg/L)	Initial Variables Cond (umhos)	Meter/Probe D.O. / °C	Meter/Probe pH	Meter/Probe Cond	Initials
0	30.09								
1	01.10								
2	02.10								
3	03.10								
4	04.10								
5	05.10								
6	06.10								
7	07.10								

Final Variables °C	Final Variables pH	Final Variables D.O. (mg/L)	Meter/Probe D.O. / °C	Meter/Probe pH	Initials

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	% Mortality / day Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SD	—
3	03.10	0	3	4	3	4	3	4	0	2	4	27	—	0	—	KK	KK
4	04.10	4	0	0	0	0	0	0	0	0	0	4	—	0	—	C	C
5	05.10	5	7	0	4	9	7	7	9+1	4	6	59	—	0	—	D	D
6	06.10	10	10	2	0	12	5	14	0	8	11	72	—	0	—	E	E
7	07.10																
8	08.10																
Total Neonates		19	20	6	7	25	15	25	10	14	21	162	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 33.33% v/v Sample Name: L2734791-2, SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	30.09														
1	01.10														
2	02.10														
3	03.10														
4	04.10														
5	05.10														
6	06.10														
7	07.10														

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	✓	—
2	02.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SV	—
3	03.10	4	1	0	3	0 Dead	0	2	4	3	2	19	5	10	0	KC	KC
4	04.10	0	0	4	3	/	0	0	0	0	0	7	—	10	—	C	2
5	05.10	7	7	0	0	/	6	9	6	7	5	47	—	10	—	C	C
6	06.10	12	10	5	0	/	7	10	11	12	13	80	—	10	—	C	6
7	07.10					/											
8	08.10					/											
Total Neonates		23	18	9	6	0	13	21	21	22	20	153	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentrati. 100% v/v Sample Name: L2734791-2, SED 2 DIS Sample #: 8730.0032243

Day	Date 2022	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	30.09	24.8	8.3	8.4	572	6/4	13/88	5/6	KP
1	01.10	25.2	8.3	9.6	584	6/4	13/88	5/6	SO
2	02.10	25.0	8.3	9.5	581	6/4	13/88	5/6	ET
3	03.10	24.8	8.3	9.0	582	6/4	13/88	5/6	ET
4	04.10	24.8	8.3	9.0	598	6/4	13/88	5/6	KP
5	05.10	24.6	8.2	8.7	601	6/4	13/88	5/6	KP
6	06.10								
7	07.10								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
23.9	8.3	7.2	6/4	13/88	SO
25.2	8.4	6.8	6/4	13/88	SO
24.0	8.5	7.4	6/4	13/88	ET
24.2	8.5	7.4	6/4	13/88	KP
24.8	8.2	7.4	6/4	13/88	ET
23.6	8.5	7.8	6/4	13/88	ET

Day	Date 2022	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	30.09																
1	01.10	0	0	0	0	0	0	0	0	0	0	0	—	0	0	—	—
2	02.10	0	0	dead	0	0	0	0	0	0	0	0	3	10	0	SO	—
3	03.10	0	2	|	3	4	1	0	0	2	3	15	—	10	—	KC	KC
4	04.10	0	0	|	0	0	0	3	0	7	8	18	—	10	—	ET	6
5	05.10	0	3	|	0	3	1	2	7	0	0	16	—	10	—	ET	8
6	06.10	3	2	|	5	2	6	4	7	9	6	44	—	10	—	ET	0
7	07.10			|													
8	08.10			|													
Total Neonates		3	7	0	8	9	8	9	14	18	17	93	Notes: * = ≥ 4th brood (not counted)				

WO# L2734791

Ceriodaphnia dubia Neonate Origin

Sample Information

Client ALS Thunder Bay
Sample # 8730.0032243
Date/Time Received 29/09/22 – 1100
Sample Type Water
100% Hardness ~~80~~ 220

Sample Name L2734791-2/SED 2 DIS
Date/Time Collected 28/09/22/0810 Person Sampling N/A
Arrival Temp (°C) 14.0°C
Sample Description clear, light yellow

Test Information

Date Test Started 30/09/22 /11:50
Dilution Water Batch Number CD22159
Test Started By KC
Control Hardness 126
Template Used for Randomization T2

Individual Culture Health Data

Date Culture Started ~~19~~ 21.09.22 KK
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 17
Culture I.D. (*e.g.*, Wed Row 4) WED Row 6
Average # neos in previous 7 days (must be ≥15) 38.6
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ~~19~~ 21.09.22 KK
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 24.2
Culture I.D. (*e.g.*, Wed Row 4) WED Row 7
Average # neos in previous 7 days (must be ≥15) 39.4
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ______
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (*e.g.*, Wed Row 4) ______
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 0 (add up % mortality for all cultures used / # cultures used)
Mean Number of Young Produced within first 3 broods 20.6 (avg # 1st 3 broods for all cultures used / # cultures used)
Mean Number of Young Produced in previous 7 days 39.0 (avg # neos in prev 7 days for all cultures used / # cultures used)
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / (No) (circle one)

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
W 6.10	(Y) / N	(Y) / N	14	1	KC
W7.1	(Y) / N	(Y) / N	15	2,3	KC
7.3	(Y) / N	(Y) / N	12	4,5	KC
7.4	(Y) / N	(Y) / N	10	6	KC
7.7	(Y) / N	(Y) / N	13	7	KC
7.8	(Y) / N	(Y) / N	10	8	KC
7.10	(Y) / N	(Y) / N	15	9,10	KC
	Y / N	Y / N			
	Y / N	Y / N			
	Y / N	Y / N			
	Y / N	Y / N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032243	**Sample Name:**	SED2DIS L2734791-2
Sample Method:	Grab	**Collected by:**	Not available
Date/Time Collected:	September 28, 2022; 08:10	**Arrival Temp.:**	14.0°C
Date/Time Received:	September 29, 2022; 11:00	**Sample Description:**	Clear, light yellow
Sample Point Description:	Other	**Sample Type:**	Effluent
Transportation:	Road		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species (Strain #):	*Raphidocelis subcapitata* (CPCC # 37)
Source:	Nautilus Plant Culture Unit (from CPCC)
Culture Temp.:	24 ± 2 °C
Test Culture Number:	G7(l)c
Culture Age at Test Start:	7 days old
Cell Density in the Microplate Wells at the Start of the Test:	10,795.45 cells/ml

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*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032243 **Sample Name:** SED2DIS L2734791-2

Test Conditions

Date/Time Test Start:	September 30, 2022; 10:05	**T=0 Control pH:**	6.8
Date/Time Test End:	October 3, 2022; 09:30 - 12:45	**T=72 Control pH:**	6.8
Sample pH Before Dilution:	8.3	**pH Adjustment:**	None

Test Duration: 72 hours

Mean Test Temperature (±Standard Deviation): 25.4 (±0.1)°C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Millipore

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032243 **Sample Name:** SED2DIS L2734791-2

Test Conditions - continued

Enumeration Technique: Neubauer Haemacytometer

Test Vessel: 96-Well U-bottomed Polystyrene Microplate

Concentration of Test Solutions: 90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control

 # Replicates/Concentration: 4 reps started; 3 reps counted of test solutions
10 control reps started, 2 used for pH measurement

Design if Specialized Procedure: Not applicable

Method Deviations or Unusual Occurrences: None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times: Acceptable (21.6 times)

pH in controls did not vary by more than 1.5 units: Acceptable (0 units)

C V for cell yields is < 20% within the control wells: Acceptable (16.1%)

No inhibitory trend detected across the control wells: Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
199,205	264,205	211,705	211,705	234,205	296,705	256,705	186,705	232,642	16.1

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 CV = Coefficient of Variation = (100 x standard deviation / mean)

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032243 **Sample Name:** SED2DIS L2734791-2

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	769,205	1,189,205	1,034,205	201,705	196,705
2	981,705	1,174,205	1,129,205	211,705	191,705
3	1,094,205	1,296,705	1,074,205	169,205	194,205
Mean Cell Yield	948,371	1,220,038	1,079,205	194,205	194,205
Coefficient Variation[2]	17	5	4	11	1
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	181,705	201,705			
2	191,705	199,205			
3	181,705	196,705			
Mean Cell Yield	185,038	199,205			
Coefficient Variation[2]	3	1			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	**Result[1]**	**Method of Calculation**
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	no trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

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***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032243 **Sample Name:** SED2DIS L2734791-2

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software, 2001-2007 Comprehensive Environmental Toxicity, Information System - CETIS v1.8.1.2.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (90.91% v/v concentration; rep. 1). No reason to remove it. Statistics include all data.

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2213 **Date Test Initiated:** 26-Sep-2022

Method of Analysis: Linear Interpolation (ICPIN) **Algae Lot #:** G6(l)b

72-hour IC25 (95% Confidence Limits): 52.47 mg/L (45.83 mg/L; 60.40 mg/L)

Historic Geometric Mean IC25: 54.33 mg/L (26.16 mg/L; 112.84 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

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Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS- Thunder Bay Sample Name SED 2 DIS L2734791.2 Sample # 8730-003 2243

Conditions for Test Validity

Cell increase for control is >16	Acceptable/Not acceptable	21.6 (times)
CV among controls ≤ 20	Acceptable/Not acceptable	16.1
Result of Mann-Kendall test for trend	Acceptable/Not acceptable	no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 118 750 (cells/mL)

Used: Yes/No (Circle one)

Algae only _____ (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10 795.45 (cells/mL)

Analysis Completed:	Initials: EO	Date: 21/10/22
Results Verified:	Initials: aO	Date: 01.11.22

Nautilus Environmental Company Inc.

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)

72-Hour Growth Inhibition Test

Test Material

Client Name/Location:	ALS Thunder Bay	L2734791-2	
Sample #:	8730-00322 43	Sample Name:	SED 2 DIS
Collection Method:	Grab	Collected By:	N/A
Date/Time Collected:	28 | 09 | 22 8:10	Arrival Temp.: (meter/probe)	14.0 °C (51)
Date/Time Received:	29 | 09 | 22 11:00	Sample Description:	Clear, light yellow
Collection Point Description:	Other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:	
Transportation:	Air | Road	Storage:	4 ± 2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	E
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # G 7(1)C	E
Culture Age at Start of Test	7 days old (must be 3 to 7 days old)	E
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	E
	Axenic culture? Yes/No (Circle one)	E

Notes:

Test Conditions:

Date / Time Test Start: Started By:	30.09.22 10:05	Date / Time Test End: Finished By:	03.10.22 8:30 - 12:45
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 8.3	pH adjustment: Y/N	pH of well D6 at T=0 h 6.8	pH of well D7 at T=72 h 6.8
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # —		Metal mining NUT Lot # NUT 2203
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation)	25.4 ± 0.1 °C		

	Day 1		Day 2	
¼ plate rotation (Initial)	AM SD	PM ✓	AM ✓	PM ✓
Lights ON (Initial)	AM ✓	PM ✓	AM ✓	PM ✓

* For reference test, record pH of top concentration before use for series dilutions.

Notes:

72-Hour Qualitative Observations:

Condensation:	Y/N	
Growth:	Y/N	
Were there any other method variations or deviations from methods?	Yes/No	If yes, describe further:
Anything unusual about the test?	Yes/No	
Any problems encountered?	Yes/No	
Any remedial measures taken?	Yes/No	

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 8730-003243	Sample Name: SED2 DIS

Reference Data:

Reference Chemical Batch #	Phenol P2213	Date test started	26/09/22
Method of Analysis	ICPIN – linear interpolation	Algae Lot #	G 6(1) b
72-hour IC25 (95% C.I.)[3] mg/L	52.47 45.83; 60.40	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	54.833 26.16; 112.84

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	>90.91 % (N/A)	No nonlinear regression models would fit ICPIN – linear interpolation
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers	—	—
Test for Outliers	No Outliers Present If outliers present, indicate Concentration/Rep: 90.91% rep 1	Grubbs' Test for Residual Outlier Initial E
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed. No statistically significant growth stimulation. Yes, statistically significant growth stimulation at these concentrations: 90.91; 30.303; 10.101	Williams' or Dunnett's Multiple Comparison Test Initial E

1)Results relate only to the sample tested.
2) Tidepool Scientific Software © 2001-2007. Comprehensive Environmental Toxicity Information System – CETIS v. 1.9.6.7
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/No

Any outliers and justification for their removal? Yes/No

Nautilus Environmental Company Inc.

Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)

72-Hour Growth Inhibition Test – Continued

72-Hour Quantitative Observations of Controls

Sample Name: SED 2 D15 Sample Number: 8730 003 2243 Date Test Start: 30. 09. 22

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D8	Well # D9	Well # D10	Well # D11
1	24	31	21	20	22	31	24	15
2	24	17	26	19	22	26	30	20
3	18	36	22	29	20	32	27	23
4	18	26	20	21	34	34	26	21
5	—	—	—	—	—	—	—	—
Initials	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

I 118750

Cell increase for controls = 21.6	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = 16.1	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = no trend	Controls are invalid if there is a trend detected by the Mann-Kendall test

Mann-Kendall Trend Test for *Selenastrum capricornutum* Growth Inhibtion Test

EPS Method 1/RM/25
Version 1.1, Nov. 2000
Pollutech EnviroQuatics

Sample #: "8730-0032243
Client #: "8730-003
Date tested: "30.09.22

Instructions:

Enter control data below cells labelled D2..D5 and D8..D11.
The test of significance is completed immediately following data entry.

D2	D3	D4	D5	D8	D9	D10	D11
199205	264205	211705	211705	234205	296705	256705	186705

✓

Results:

There is no significant trend.

AO 01.11.22

Notes:

The test is a two-sided alternative using a nominal alpha value of 0.05%.
Due to the discrete nature of the test statistic, the true alpha value is 0.062%.

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SED2 DIS Sample Number: 8730-0032243 Date Test Start: 30.09.22

Theoretical Test Concentration: 100.00% v/v Actual Test Concentration: 90.91% v/v

Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	100	83	119		948 371 (± 165044)
2	70	115	87		Coefficient of Variation of Cell Yield
3	54	104	118		17
4	88	95	118		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-307.653
Initials	CV	CV	CV		CV

Theoretical Test Concentration: 33.33% v/v Actual Test Concentration: 30.303% v/v

Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	117	118	119		1220038 (± 66818)
2	115	118	136		Coefficient of Variation of Cell Yield
3	127	125	134		5
4	121	113	134		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-424.427
Initials	CV	CV	CV		CV

Theoretical Test Concentration: 11.11% v/v Actual Test Concentration: 10.101% v/v

Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	118	114	118		1079205 (± 47697)
2	100	110	98		Coefficient of Variation of Cell Yield
3	99	125	105		4
4	101	107	113		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-363.891
Initials	CV	CV	CV		CV

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SED2 DIS Sample Number: 8730 2032249 Date Test Start: 30. 09 . 22

Theoretical Test Concentration: 3.704 %. v/v					Actual Test Concentration: 3.367 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation)
1	18	22	15		194205 (± 22220)
2	26	22	20		Coefficient of Variation of Cell Yield
3	20	25	15		11
4	21	20	22		Average % Inhibition (-ve number = enhancement)
5	—	—	—		16.522
Initials	C	C	C		C

Theoretical Test Concentration: 1.235 %. v/v					Actual Test Concentration: 1.122 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation)
1	20	18	19		194205 (± 2600)
2	24	20	22		Coefficient of Variation of Cell Yield
3	20	18	18		1
4	19	25	23		Average % Inhibition (-ve number = enhancement)
5	—	—	—		16.522
Initials	C	C	C		C

Theoretical Test Concentration: 0.412 %. v/v					Actual Test Concentration: 0.374 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation)
1	16	21	20		185038 (± 5774)
2	21	21	18		Coefficient of Variation of Cell Yield
3	20	19	20		3
4	20	20	19		Average % Inhibition (-ve number = enhancement)
5	—	—	—		20.462
Initials	C	C	C		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SED2 DIS Sample Number: 8730 2003249 Date Test Start: 30.09.22

Theoretical Test Concentration: 0.137 % v/v					Actual Test Concentration: 0.125 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	23	19	20		199205 (± 2500)
2	20	18	21		Coefficient of Variation of Cell Yield
3	20	23	23		1
4	22	24	19		Average % Inhibition (-ve number = enhancement)
5	–	–	–		14.373
Initials	G	G	G		G

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	SED2 DIS	Sample #	8730-0032243	Date test start	30.09.22

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	118750	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	20	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2375	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	10795.4545	=	21.55

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	24	31	21	20	22	31	24	15
	24.0	17.0	26.0	19.0	22.0	26.0	30.0	20.0
	18	36.0	22.0	29.0	20.0	32.0	27.0	23.0
	18.0	26.0	20.0	21.0	34.0	34.0	26.0	21.0
total cells	84	110	89	89	98	123	107	79
cells/ul	210	275	222.5	222.5	245	307.5	267.5	197.5
cells/ml	210000	275000	222500	222500	245000	307500	267500	197500
Cell yield = measured concentration - initial algal cell concentration								
	199205	264205	211705	211705	234205	296705	256705	186705

Mean cell yield for the control = Rc — Standard deviation — coefficient of variation

Rc 232642 — SD 37391.2112 — CV 16.072422 **Must be ≤20**

% inhibition summary

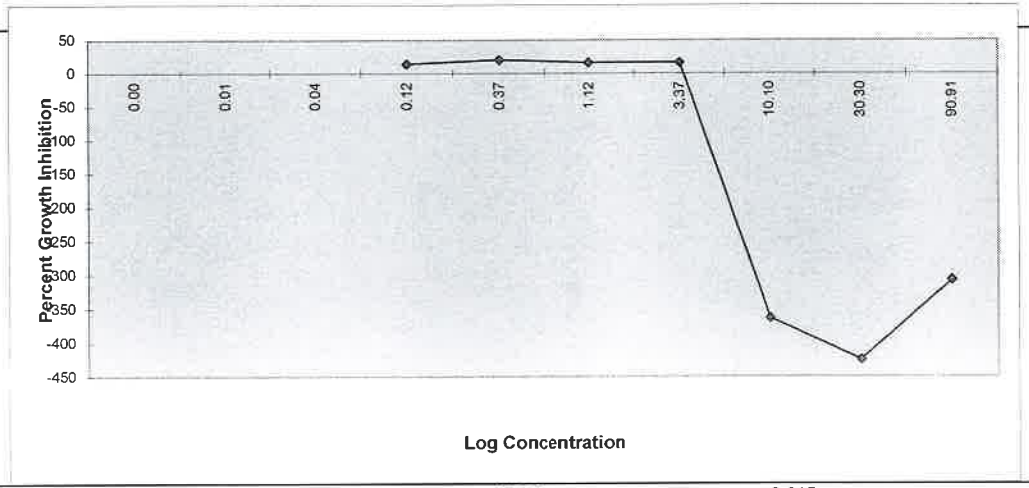
Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	14.373
0.374	20.462
1.122	16.522
3.367	16.522
10.101	-363.891
30.303	-424.427
90.910	-307.653

enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	100	83	119	117	118	119	118	114	118	18	22	15	20	18	19
	70.0	115.0	87.0	115.0	118.0	136.0	100.0	110.0	98.0	26.0	22.0	20.0	24.0	20.0	22.0
	54.0	104.0	118.0	127.0	125.0	134.0	99.0	125.0	105.0	20.0	25.0	15.0	20.0	18.0	18.0
	88.0	95.0	118.0	121.0	113.0	134.0	101.0	107.0	113.0	21.0	20.0	22.0	19.0	25.0	23.0
total cells	312	397	442	480	474	523	418	456	434	85	89	72	83	81	82
cells/ul	780	992.5	1105	1200	1185	1307.5	1045	1140	1085	212.5	222.5	180	207.5	202.5	205
cells/ml	780000	992500	1105000	1200000	1185000	1307500	1045000	1140000	1085000	212500	222500	180000	207500	202500	205000
Cell yield = measured concentration - initial algal cell concentration															
	769205	981705	1094205	1189205	1174205	1296705	1034205	1129205	1074205	201705	211705	169205	196705	191705	194205
Mean Yield	948371			1220038			1079205			194205			194205		
STD Yield	165044			66818			47697			22220			2500		
CV Yield	17			5			4			11			1		

Average % inhibition											
	for 90.910%:	-307.653	for 30.303%	-424.427	for 10.101%	-363.891	for 3.367%	16.522	for 1.122%	16.522	

Average % stimulation											
	for 90.910%:	307.653	for 30.303%	424.427	for 10.101%	363.891	for 3.367%	-16.522	for 1.122%	-16.522	



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
16	21	20	23	19	20									
21.0	21.0	18.0	20.0	18.0	21.0									
20.0	19.0	20.0	20.0	23.0	23.0									
20.0	20.0	19.0	22.0	24.0	19.0									
77	81	77	85	84	83	0	0	0	0	0	0	0	0	0
192.5	202.5	192.5	212.5	210	207.5	0	0	0	0	0	0	0	0	0
192500	202500	192500	212500	210000	207500	0	0	0	0	0	0	0	0	0
181705	191705	181705	201705	199205	196705	-10795	-10795	-10795	-10795	-10795	-10795	-10795	-10795	-10795
185038			199205			-10795			-10795			-10795		
5774			2500			0			0			0		
3			1			0			0			0		
for 0.374%	20.462		for 0.125%	14.373		for 0.042%	104.640		for 0.014%	104.640		for 0.005%	104.640	
for 0.374%	-20.462		for 0.125%	-14.373		for 0.042%	-104.640		for 0.014%	-104.640		for 0.005%	-104.640	

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
SED2DIS L2734791-2	8730-0032243	September 28, 2022	September 29, 2022	September 30, 2022	>97% Volume[2] (Not applicable)	>97% Volume[2] (Not applicable)	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	23-Sep-2022	**Reference Chemical:**	KCl
Frond Increase IC25:	2.52 g/L	**95% Confidence Limits:**	1.83; 3.89 g/L
Historic Geometric Mean IC25:	2.22 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.41; 3.49 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	Na_2MoO_4	0.0726
	K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$2H_2O$	0.00003
	KCl	10.1	$MgSO_4\ 7H_2O$	147	$ZnCl_2$	0.00001
					$CoCl_2\ 6H_2O$	0.00001
					$CuCl_2\ 2H_2O$	

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

Date: November 2, 2022

Carol D'Andrea
Laboratory Supervisor

Test Material

Client Name/Location: AUS – Thunder Bay L2734791·2	
Sample #: 8730003 2243	Sample Name: SED 2 DIS
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 28/09/22 8:10	Arrival Temperature (meter/probe): 14.0 °C (51)
Date/Time Received: 29/09/22 11:00	Sample Description: clear, light yellow
Collection Point Description: other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: Air / Road	Storage: 4 ± 2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	GI
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	GI
Culture Age at Start of Test:	9 days old, acclimated 22 hours in fresh test solution (mAPHA)	GI
Culture Medium:	Modified Hoagland's E+ medium, Lot # MH2202	GI
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/(No)	GI
	Axenic culture? (Yes)/No	GI
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 30 in HT 1, 27 in HT 2, 25 in HT 3 at finish	GI

Test Conditions and Procedures

Date / Time Test Start: 30.09.22 14:45 Started By: GI	Date / Time Test End: 07.10.22 15:00 Finished By: GI
Test Type: (Static) (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / (No) (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: (Modified APHA) (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 8.2 (12/86)	pH Adjustment: none
Test Volume and Depth: 150 mL / 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / (No)	If yes, describe further:
Anything unusual about the test? Yes / (No)	
Any problems encountered? Yes / (No)	
Any remedial measures taken? Yes / (No)	Randomization Template: B

Sample #: 8730 0032243	Sample Name: SED 2 DIS

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	27.4	25.0	25.3	25.2	24.8	24.9	25.5	25.1
Low	27.3	25.3	25.2	25.1	24.5	25.0	25.5	25.0
Medium	27.3	25.3	25.2	25.1	24.9	24.9	25.5	25.0
High	27.3	25.3	25.3	25.1	25.1	25.1	25.5	25.1
Initials	TV	SO	VB	TV	KP	KP	KP	KP
meter/probe	51	52	52	52	52	52	52	52
Mean Test Temperature (average of 24h high / low temperatures):					25.4±0.1°C			

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.3	8.2	8.2	8.1	TV	12/86
Day 7	Control	Low	Medium	High	Initials	pH meter/probe
	8.4	8.8	9.3	9.9	TV	14/90

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)								

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 05/10/22 (5)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 12					Initials: TV
4200	4980	4710	4570	5020	Mean Light Measurement: 5020
±15% Variation of Mean: 3992 – 5238					Acceptable / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) / No
If yes, describe further: Algae

Reference Data

Reference Date	(Frond Increase) or Dry Weights (circle one)			
	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
23/09/22	2.52	1.83; 3.89	2.22	1.41; 3.69

Sample #: 8730-003243	Sample Name: SED 2 DIS

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 48	B 50	C 50	D 49	49.3

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy						Initials	
						CE	
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV : 43.3 (1.0) 2.2							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-10.98	-19.65	-9.25	-22.54	-10.40	-8.09	-16.18
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-19.57	-24.56	-20.43	-24.60	-24.56	-15.30	-18.79

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: Yes / Not applicable (no stimulation) (Circle one)

Test Endpoints and Calculations:

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	>97 % (N/A)	No nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN – linear interpolation
Dry Weights		
IC25 (95% C.I.)[3]	>97 % (N/A)	Nonlinear regression 2P Exponential model
IC25 (95% C.I.)[3] If calculated without outlier[4]		

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019. Comprehensive Environmental Toxicity Information System CETISv 1.9.6.7
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / No

Any outliers and justification for their removal? Yes / No

Fronds (9.7 %; ~~control~~; up 2)
Weights (control; up 2)

Lemna minor Day 7 Observations

Client: AIS Thunder Bay	Sample number: 8730-0322 43	Date Started: 30. 09. 22	
Site: SED 2 DIS		Date Ended: 07. 10. 22	
Concentration: Control	Observations By: E	Concentration: 0.097 1.11	Observations By: E

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	48	50	50	49	Number of	45	45	45	43
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X	Abnormally sized fronds	X	✓ smaller	✓ smaller	✓ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	✓	✓	✓
Other Observations					Other Observations	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO			NM		Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor Day 7 Observations

Client:	AIS Thunder Bay			**Sample number:**	8730-032243		**Date Started:**	30.09.22	
Site:	SED 2 DIS						**Date Ended:**	07.10.22	
Concentration:	0.29% v/v			**Observations By:**	C		**Concentration:**	0.97% v/v	
Observations	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Observations**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
Number of	41	41	38	43	**Number of**	44	51	40	46
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	**Abnormally sized fronds**	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	√	X	X	X
Other Observations	algae	→	→	→	**Other Observations**	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Day 7 Observations

Client: AIS Thunder Bay	Sample number: 8730-0322 43	Date Started: 30. 09. 22	
Site: SED 2 DIS		Date Ended: 07. 10. 22	
Concentration: 3.1 % √√	Observations By: C	Concentration: 9.7 % √√	Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	40	40	38	40	Number of	43	51	40	45
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	√	X	√	Loss of Buoyancy	√	√	X	X
Other Observations	algae	→	→	→	Other Observations	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor Day 7 Observations

Client:	AIS Thunder Bay		**Sample number:**	8730-030322 43		**Date Started:**	30. 09. 22		
Site:	SED 2 DIS					**Date Ended:**	07. 10. 22		
Concentration:	31 % v/v		**Observations By:**	C		**Concentration:**	97 % v/v		**Observations By:**	C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	45	49	46	43	**Number of**	41	43	45	40
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	X	X	X	X	**Abnormally sized fronds**	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations	algae	→	→	→	**Other Observations**	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Weights

Client	ALS-TB	Site	SED2 DIS	Sample number	8730-003224
In Oven Date/Time/ °C:	07/10/22 15:00 63	Out Oven Date/Time/°C:	08/10/22 15:00	62°C	

Conc.	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	42	43.3 (1.0)	0.80328	0.80095	2.33	3.51 (0.8)
	B	44		0.80598	0.80212	3.86	
	C	44		0.82408	0.81709	3.99	
	D	43		0.81219	0.80832	3.87	
0.097	A	39	38.5 (1.0)	0.80389	0.80104	2.85	2.83 (0.2)
	B	39		0.79879	0.79573	3.06	
	C	39		0.81090	0.80828	2.62	
	D	37		0.82261	0.81984	2.77	
0.29	A	35	34.8 (2.1)	0.82180	0.81840	3.40	2.65 (0.6)
	B	35		0.82056	0.81827	2.29	
	C	32		0.81987	0.81769	2.18	
	D	37		0.81491	0.81218	2.73	
0.97	A	38	39.3 (4.6)	0.81756	0.81442	3.14	2.80 (0.3)
	B	45		0.81719	0.81484	2.35	
	C	34		0.81942	0.81671	2.71	
	D	40		0.82088	0.81432	2.98	
3.1	A	34	33.5 (1.0)	0.81404	0.81157	2.47	2.65 (0.4)
	B	34		0.81385	0.81149	2.36	
	C	32		0.81292	0.81034	2.58	
	D	34		0.82067	0.81749	3.18	
9.7	A	37	38.8 (4.6)	0.82040	0.81811	2.29	2.65 (0.3)
	B	45		0.82213	0.81914	2.99	
	C	34		0.81891	0.81648	2.43	
	D	39		0.79241	0.78952	2.89	
31	A	39	39.8 (2.5)	0.82260	0.82002	2.58	2.98 (0.3)
	B	43		0.80328	0.80035	2.93	
	C	40		0.82205	0.81868	3.37	
	D	37		0.82153	0.81851	3.02	
97	A	35	36.3 (2.2)	0.80584	0.80281	3.03	2.85 (0.4)
	B	37		0.80431	0.80192	2.39	
	C	39		0.81740	0.81415	3.25	
	D	34		0.80794	0.80520	2.73	
Initials		TB	TC	LJ	SO	TC	

Notes:

Sample name SED2 DIS Date started 30.09.22 sample # 8730-0032243

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

49.3 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	48	45	41	44	40	43	45	41
	50	45	41	51	40	51	49	43
	50	45	38	40	38	40	46	45
	49	43	43	46	40	45	43	40
Total Fronds	197	178	163	181	158	179	183	169
Increase in Frond Number = Total # Fronds - 6 Starting Fronds								
	42	39	35	38	34	37	39	35
	44	39	35	45	34	45	43	37
	44	39	32	34	32	34	40	39
	43	37	37	40	34	39	37	34
Total Increase	173	154	139	157	134	155	159	145
Mean Increase	43.3	38.5	34.8	39.3	33.5	38.8	39.8	36.3
SD Increase	1.0	1.0	2.1	4.6	1.0	4.6	2.5	2.2
CV Increase	2.2	2.6	5.9	11.7	3.0	12.0	6.3	6.1
% Stimulation		**-10.98**	**-19.65**	**-9.25**	**-22.54**	**-10.40**	**-8.09**	**-16.18**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.80328	0.80389	0.82180	0.81756	0.81404	0.82040	0.82260	0.80584
Pan + Plant	0.80598	0.79879	0.82056	0.81719	0.81385	0.82213	0.80328	0.80431
	0.82108	0.81090	0.81987	0.81942	0.81292	0.81891	0.82205	0.81740
	0.81219	0.82261	0.81491	0.81730	0.82067	0.79241	0.82153	0.80794
Initial Weight (g)	0.80095	0.80104	0.81840	0.81442	0.81157	0.81811	0.82002	0.80281
Pan Only	0.80212	0.79573	0.81827	0.81484	0.81149	0.81914	0.80035	0.80192
	0.81709	0.80828	0.81769	0.81671	0.81034	0.81648	0.81868	0.81415
	0.80832	0.81984	0.81218	0.81432	0.81749	0.78952	0.81851	0.80520
Plant Only (mg)	2.33	2.85	3.40	3.14	2.47	2.29	2.58	3.03
	3.86	3.06	2.29	2.35	2.36	2.99	2.93	2.39
	3.99	2.62	2.18	2.71	2.58	2.43	3.37	3.25
	3.87	2.77	2.73	2.98	3.18	2.89	3.02	2.74
Mean Dry Weight	3.512	2.825	2.650	2.795	2.647	2.650	2.975	2.852
SD Dry Weight	0.8	0.2	0.6	0.3	0.4	0.3	0.3	0.4
CV Dry Weight	22.5	6.5	20.9	12.4	13.8	12.9	10.9	13.1
% Stimulation		**-19.57**	**-24.56**	**-20.43**	**-24.63**	**-24.56**	**-15.30**	**-18.79**

For Data Transfer to CETIS

	# fronds	total mass	tare
0	1	42	0.80328	0.80095
	2	44	0.80598	0.80212
	3	44	0.82108	0.81709
	4	43	0.81219	0.80832
0.1	1	39	0.80389	0.80104
	2	39	0.79879	0.79573
	3	39	0.81090	0.80828
	4	37	0.82261	0.81984
0.3	1	35	0.82180	0.81840
	2	35	0.82056	0.81827
	3	32	0.81987	0.81769
	4	37	0.81491	0.81218
1	1	38	0.81756	0.81442
	2	45	0.81719	0.81484
	3	34	0.81942	0.81671
	4	40	0.81730	0.81432
3.1	1	34	0.81404	0.81157
	2	34	0.81385	0.81149
	3	32	0.81292	0.81034
	4	34	0.82067	0.81749
9.7	1	37	0.82040	0.81811
	2	45	0.82213	0.81914
	3	34	0.81891	0.81648
	4	39	0.79241	0.78952
31	1	39	0.82260	0.82002
	2	43	0.80328	0.80035
	3	40	0.82205	0.81868
	4	37	0.82153	0.81851
97	1	35	0.80584	0.80281
	2	37	0.80431	0.80192
	3	39	0.81740	0.81415
	4	34	0.80794	0.80520

AO 01.11.22

Chain of Custody (COC) / Analytical Request Form

www.alsglobal.com

Canada Toll Free: 1 800 668 9878

2734791 COFC

Report To	Contact and company name below will appear on the final report
Company:	New Gold
Contact:	Garnet Cornell
Phone:	
	Company address below will appear on the final report
Street:	
City/Province:	
Postal Code:	
Invoice To	Same as Report To ☐ YES ☐ NO
	Copy of Invoice with Report ☐ YES ☐ NO
Company:	
Contact:	

Reports / Recipients

Select Report Format: ☐ PDF ☐ EXCEL ☐ EDD (DIGITAL)

Merge QC/QCI Reports with COA ☐ YES ☐ NO ☐ N/A

☐ Compare Results to Criteria on Report - provide details below if box checked

Select Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Email 3

Invoice Recipients

Select Invoice Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Turnaround Time (TAT) Requested

☐ Routine [R] if received by 3pm M-F - no surcharges apply
☐ 4 day [P4] if received by 3pm M-F - 20% rush surcharge minimum
☐ 3 day [P3] if received by 3pm M-F - 25% rush surcharge minimum
☐ 2 day [P2] if received by 3pm M-F - 50% rush surcharge minimum
☐ 1 day [E] if received by 3pm M-F - 100% rush surcharge minimum
☐ Same day [E2] if received by 10am M-S - 200% rush surcharge.

Additional fees may apply to rush requests on weekends, statutory holidays and for non-routine tests.

Date and Time Required for all E&P TATs: dd-mmm-yy hh:mm am/pm

For all tests with rush TATs requested, please contact your AM to confirm availability.

AFFIX ALS BARCODE LABEL HERE (ALS use only)

Project Information

ALS Account # / Quote #:	
Job #:	
PO / AFE:	
LSD:	
ALS Lab Work Order # (ALS use only):	L2734791

Oil and Gas Required Fields (client use)

AFE/Cost Center:	PO#
Major/Minor Code:	Routing Code:
Requisitioner:	
Location:	
ALS Contact:	Sampler:

Analysis Request

Indicate Filtered (F), Preserved (P) or Filtered and Preserved (F/P) below

ALS Sample # (ALS use only)	Sample Identification and/or Coordinates (This description will appear on the report)	Date (dd-mmm-yy)	Time (hh:mm)	Sample Type	NUMBER OF CONTAINERS	NG-ST-P-TB	F/P	SAMPLES ON HOLD	EXTENDED STORAGE REQUIRED	SUSPECTED HAZARD (see notes)
[redacted]	EDL1	28-Sep-22	8:15	EFF	3	X				
[redacted]	SED2DIS	28-Sep-22	8:10	EFF	3	X				
[redacted]										
[redacted]										
[redacted]										
[redacted]										
[redacted]										
[redacted]										
[redacted]										
[redacted]										
[redacted]										
[redacted]										

Drinking Water (DW) Samples[1] (client use)

Are samples taken from a Regulated DW System?
☐ YES ☐ NO

Are samples for human consumption/ use?
☐ YES ☐ NO

Notes / Specify Limits for result evaluation by selecting from drop-down below (Excel COC only)

SAMPLE RECEIPT DETAILS (ALS use only)

Cooling Method: ☐ NONE ☐ ICE ☒ ICE PACKS ☐ FROZEN ☐ COOLING INITIATED

Submission Comments identified on Sample Receipt Notification: ☐ YES ☐ NO

Cooler Custody Seals Intact: ☐ YES ☐ N/A Sample Custody Seals Intact: ☐ YES ☐ N/A

INITIAL COOLER TEMPERATURES °C				FINAL COOLER TEMPERATURES °C			
12.1							

SHIPMENT RELEASE (client use)

Released by:	Date:	Time:

INITIAL SHIPMENT RECEPTION (ALS use only)

Received by:	Date:	Time:
GP	28-Sep-22	15:30

FINAL SHIPMENT RECEPTION (ALS use only)

Received by:	Date:	Time:

Failure to complete all portions of this form may delay analysis. Please fill in this form LEGIBLY. By the use of this form the user acknowledges and agrees with the Terms and Conditions as specified on the back page of the white - report copy.

1. If any water samples are taken from a Regulated Drinking Water (DW) System, please submit using an Authorized DW COC form.

New Gold Inc. Rainy River Project
ATTN: Garnet Cornell
24 Marr Rd
Barwick ON P0W 1A0

Date Received: 10-NOV-22
Report Date: 23-DEC-22 12:03 (MT)
Version: FINAL

Client Phone: 807-234-8200

Certificate of Analysis

Lab Work Order #: L2740058

Project P.O. #: 4500062842
Job Reference: SUBLETHAL TOXICITY
C of C Numbers:
Legal Site Desc:

Christine Paradis
Project Manager

ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
L2740058-1 EDL1 Sampled By: Client on 09-NOV-22 @ 15:15 Matrix: EFF							
Miscellaneous							
Special Request	See Attached					15-NOV-22	R5911057
L2740058-2 SED2DIS Sampled By: Client on 09-NOV-22 @ 16:00 Matrix: EFF							
Miscellaneous							
Special Request	See Attached					15-NOV-22	R5911057
L2740058-3 EDL2 Sampled By: Client on 09-NOV-22 @ 12:20 Matrix: EFF							
Miscellaneous							
Special Request	See Attached					15-NOV-22	R5911057

* Refer to Referenced Information for Qualifiers (if any) and Methodology.

Reference Information

Test Method References:

ALS Test Code	Matrix	Test Description	Method Reference**
SPECIAL REQUEST-AQT	Misc.	Special Request Aquatox	SEE SUBLET LAB RESULTS

** ALS test methods may incorporate modifications from specified reference methods to improve performance.

The last two letters of the above test code(s) indicate the laboratory that performed analytical analysis for that test. Refer to the list below:

Laboratory Definition Code	Laboratory Location

Chain of Custody Numbers:

GLOSSARY OF REPORT TERMS

Surrogates are compounds that are similar in behaviour to target analyte(s), but that do not normally occur in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery. In reports that display the D.L. column, laboratory objectives for surrogates are listed there.
mg/kg - milligrams per kilogram based on dry weight of sample
mg/kg wwt - milligrams per kilogram based on wet weight of sample
mg/kg lwt - milligrams per kilogram based on lipid weight of sample
mg/L - unit of concentration based on volume, parts per million.
< - Less than.
D.L. - The reporting limit.
N/A - Result not available. Refer to qualifier code and definition for explanation.

Test results reported relate only to the samples as received by the laboratory.
UNLESS OTHERWISE STATED, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.
Analytical results in unsigned test reports with the DRAFT watermark are subject to change, pending final QC review.

Quality Control Report

Workorder: L2740058 Report Date: 23-DEC-22

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Test	Matrix	Reference	Result	Qualifier	Units	RPD	Limit	Analyzed

Quality Control Report

Workorder: L2740058 Report Date: 23-DEC-22

Client: New Gold Inc. Rainy River Project
24 Marr Rd
Barwick ON P0W 1A0
Contact: Garnet Cornell

Legend:

Limit	ALS Control Limit (Data Quality Objectives)
DUP	Duplicate
RPD	Relative Percent Difference
N/A	Not Available
LCS	Laboratory Control Sample
SRM	Standard Reference Material
MS	Matrix Spike
MSD	Matrix Spike Duplicate
ADE	Average Desorption Efficiency
MB	Method Blank
IRM	Internal Reference Material
CRM	Certified Reference Material
CCV	Continuing Calibration Verification
CVS	Calibration Verification Standard
LCSD	Laboratory Control Sample Duplicate

Hold Time Exceedances:

All test results reported with this submission were conducted within ALS recommended hold times.

ALS recommended hold times may vary by province. They are assigned to meet known provincial and/or federal government requirements. In the absence of regulatory hold times, ALS establishes recommendations based on guidelines published by the US EPA, APHA Standard Methods, or Environment Canada (where available). For more information, please contact ALS.

The ALS Quality Control Report is provided to ALS clients upon request. ALS includes comprehensive QC checks with every analysis to ensure our high standards of quality are met. Each QC result has a known or expected target value, which is compared against pre-determined data quality objectives to provide confidence in the accuracy of associated test results.

Please note that this report may contain QC results from anonymous Sample Duplicates and Matrix Spikes that do not originate from this Work Order.

AquaTox Testing & Consulting Inc.
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Tel. (519) 763-4412
Fax. (519) 763-4419

TOXICITY TEST REPORT

Ceriodaphnia dubia
EPS 1/RM/21

Work Order : 250143
Sample Number : 75492

SAMPLE IDENTIFICATION

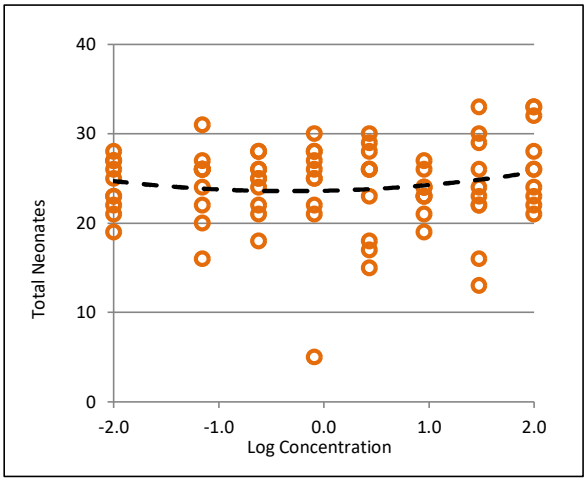
Company :	ALS Laboratory Group - Thunder Bay		
Location :	Thunder Bay ON	Sample Date :	2022-11-09
Job Number :	L2740058-1	Time Collected :	15:15
Substance :	L2740058-1 EDL1	Date Received :	2022-11-14
Sampling Method :	Not provided	Time Received :	11:45
Sampled By :	Not provided	Temperature at Receipt :	15 °C
Sample Description :	Clear, light yellow with settled solids	Date Tested :	2022-11-15

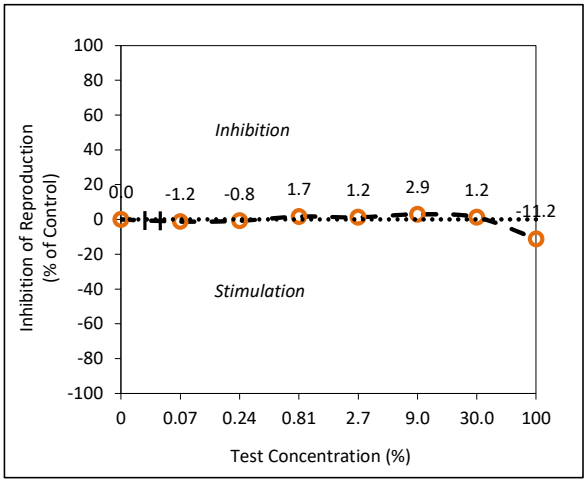
Test Method : Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia* . Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/21, 2nd ed. (February 2007).

7-DAY TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Reproduction)	>100%	–	–
LC50	>100%	–	–

The results reported relate only to the sample tested and as received.





COMMENTS

Noted Deviations: Due to a temperature system malfunction, the daily average temperature fell below the allowable range of 25 ± 1°C as per the test method. Since all validity criteria were satisfied, this deviation is not considered to have significantly altered the outcome of the test, and the test is considered to be valid. The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

•All test validity criteria as specified in the test method cited above were satisfied.

Approved By : _____

Project Manager

Work Order : 250143
Sample Number : 75492

TEST ORGANISM

Test Organism :	*Ceriodaphnia dubia*	Range of Age (at start of test) :	20:20 h - 22:20 h
Organism Batch :	Cd22-11	Mean Brood Organism Mortality :	5% (previous 7 days)
Organism Origin :	Single in-house mass culture	Average Total Neonates :	20.1 (first three broods)
Test Organism Origin :	Individual in-house cultures	Average Neonates :	12.9 (3rd or subsequent brood)
Ephippia in Culture :	None		

No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.

TEST CONDITIONS

Test Type :	Static renewal	Control/Dilution Water :	Well water [2]
Renewal Method :	Transferred to fresh solutions	Test Volume per Replicate :	15 mL
Renewal Frequency :	≤ 24 hours	Test Vessel :	20 mL glass vial
Sample Filtration :	None	Depth of Test Solution :	4 cm
Test Aeration :	None	Organisms per Replicate :	1
pH Adjustment :	None	Number of Replicates :	10
Hardness Adjustment :	None	Test Method Deviation(s) :	Yes (see 'Comments')

[2]no additional chemicals

REFERENCE TOXICANT DATA

Toxicant :	Sodium Chloride	Analyst(s) :	CL2, ELS, CGR, AW, XD
Date Tested :	2022-11-01	Test Duration :	6 days
IC25 (Reproduction) :	1.13 g/L	LC50 :	2.21 g/L
95% Confidence Limits :	0.64 - 1.27 g/L	95% Confidence Limits :	2.00 - 2.43 g/L
Statistical Method :	Linear Interpolation (CETIS)[a]	Statistical Method :	Spearman-Kärber (CETIS)[a]
Historical Mean IC25 :	1.06 g/L	Historical Mean LC50 :	2.02 g/L
Warning Limits (± 2SD) :	0.65 - 1.74 g/L	Warning Limits (± 2SD) :	1.58 - 2.58 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

CUMULATIVE DAILY MORTALITY DATA

		Test Concentration (%)							
Date	Test Day	**Control**	**0.07**	**0.24**	**0.81**	**2.7**	**9**	**30**	**100**
2022-11-16	1	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0
2022-11-19	4	0	0	0	0	0	0	0	0
2022-11-20	5	0	0	0	0	0	0	0	0
2022-11-21	6	0	0	0	0	0	0	0	0
2022-11-22	7	0	0	0	0	0	0	0	0
Total Mortality (%) :		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

REFERENCES

[a] CETIS™, © 2000-2018. V.1.9.4.7. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

[b]Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11* :1-21.

Work Order : 250143
Sample Number : 75492

SURVIVAL AND REPRODUCTION

Test Initiation Date : 2022-11-15
Initiated By : SJC
Initiation Time : 14:20
Test Completion Date : 2022-11-22

Control

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)	Analyst(s)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0	SK
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0	JJ (VBC)
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0	JW
2022-11-19	4	5	4	5	5	5	7	6	5	2	4	4.8	SK
2022-11-20	5	10	7	10	7	10	11	9	10	8	11	9.3	SK
2022-11-21	6	11	0	0	0	0	0	0	0	0	0	1.1	PG (VBC)
2022-11-22	7	0	12	13	7	12	4	6	12	13	10	8.9	SJC (PC)
Total		**26**	**23**	**28**	**19**	**27**	**22**	**21**	**27**	**23**	**25**	**24.1 (±3.0)**	

2.7%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	6	4	5	4	3	4	6	4	4	5	4.5
2022-11-20	5	10	9	11	6	11	9	10	8	9	11	9.4
2022-11-21	6	14	0	12	0	0	0	0	0	0	10	3.6
2022-11-22	7	0	13	0	7	12	16	7	3	5	0	6.3
Total		**30**	**26**	**28**	**17**	**26**	**29**	**23**	**15**	**18**	**26**	**23.8 (±5.3)**

0.07%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	6	3	4	6	5	6	5	5	4	5	4.9
2022-11-20	5	10	8	8	10	9	11	10	11	10	9	9.6
2022-11-21	6	10	0	0	0	0	0	0	0	0	13	2.3
2022-11-22	7	0	11	12	10	2	14	11	10	6	0	7.6
Total		**26**	**22**	**24**	**26**	**16**	**31**	**26**	**26**	**20**	**27**	**24.4 (±4.2)**

9%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	4	4	5	6	4	4	6	6	4	4	4.7
2022-11-20	5	9	8	11	8	10	8	8	8	8	9	8.7
2022-11-21	6	10	0	11	0	0	0	0	0	0	13	3.4
2022-11-22	7	1	11	0	9	9	12	5	7	12	0	6.6
Total		**24**	**23**	**27**	**23**	**23**	**24**	**19**	**21**	**24**	**26**	**23.4 (±2.3)**

0.24%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	6	4	5	2	4	6	5	4	4	5	4.5
2022-11-20	5	10	9	11	6	8	9	9	9	10	11	9.2
2022-11-21	6	12	0	12	0	0	0	0	0	10	0	3.4
2022-11-22	7	0	12	0	10	10	11	7	12	0	10	7.2
Total		**28**	**25**	**28**	**18**	**22**	**26**	**21**	**25**	**24**	**26**	**24.3 (±3.2)**

30%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	7	4	6	6	5	6	5	6	4	6	5.5
2022-11-20	5	12	7	9	11	7	5	9	7	6	12	8.5
2022-11-21	6	13	0	11	12	0	0	10	0	0	12	5.8
2022-11-22	7	1	12	0	0	10	5	0	9	3	0	4
Total		**33**	**23**	**26**	**29**	**22**	**16**	**24**	**22**	**13**	**30**	**23.8 (±6.1)**

0.81%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	4	4	6	6	2	7	6	6	3	5	4.9
2022-11-20	5	10	7	10	10	3	6	9	7	9	11	8.2
2022-11-21	6	13	0	14	12	0	12	0	0	13	11	7.5
2022-11-22	7	0	11	0	0	0	1	6	12	0	1	3.1
Total		**27**	**22**	**30**	**28**	**5[3]**	**26**	**21**	**25**	**25**	**28**	**23.7 (±7.1)**

100%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	4	4	7	5	5	3	5	5	4	6	4.8
2022-11-20	5	8	9	10	12	9	7	11	9	7	11	9.3
2022-11-21	6	10	0	16	0	0	0	12	0	0	15	5.3
2022-11-22	7	0	11	0	15	12	11	0	12	12	1	7.4
Total		**22**	**24**	**33**	**32**	**26**	**21**	**28**	**26**	**23**	**33**	**26.8 (±4.5)**

NOTES : •All young produced by a test organism during its fourth and subsequent broods were discarded and not included in the above counts. The presence of two or more neonates in any test chamber, during any given day of the test, constitutes a brood.

•[3] Outlier according to Grubbs Test[b]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

x = test organism mortality

* = accidental test organism mortality

– =4th brood (see 'NOTES')

Test Data Reviewed By : KP

Date : 2022-11-29

Work Order : 250143
Sample Number : 75492

WATER CHEMISTRY DATA

			Day 0 - 1	Day 1 - 2	Day 2 - 3	Day 3 - 4	Day 4 - 5	Day 5 - 6	Day 6 - 7
	Date :		2022-11-15	2022-11-16	2022-11-17	2022-11-18	2022-11-19	2022-11-20	2022-11-21
Initial Chemistry (100 %)	Sub-sample Used		1	1	1	2	2	3	3
	Temperature (°C)		24	24	24	24	24	26	24
	Dissolved O_2 (mg/L)		8.6	8.1	8.5	9.1	8.5	9.4	8.8
	Dissolved O_2 (% Sat.)[4]		106	99	106	112	104	120	107
	pH		7.8	7.9	7.8	7.9	8.0	7.8	7.9
	Conductivity (µmhos/cm)		1535	1531	1535	1532	1527	1543	1528
	Pre-aeration Time (min)[5]		20	0	20	20	20	20	20
	Analyst(s)	Initial	PG (VBC)	CL2 (VBC)	CL2 (VBC)	SK	ELS(SK)	ELS(SK)	CL2 (VBC)
		Final	SK	JJ (VBC)	JW	JJ (SK)	SK	VC	SJC (PC)
Control	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (% Sat.)[4]	Initial	101	101	100	101	96	97	100
	Dissolved O_2 (mg/L)	Initial	8.1	8.3	7.9	8.1	7.7	7.8	8.1
		Final	7.3	6.7	7.4	7.0	7.5	7.1	6.7
	pH	Initial	8.3	8.4	8.3	8.3	8.3	8.4	8.4
		Final	8.0	8.0	8.2	8.1	8.2	8.1	8.0
	Conductivity (µmhos/cm)	Initial	491	540	531	448	430	417	419
0.07 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (mg/L)	Initial	7.8	8.0	7.6	7.9	7.6	7.7	7.5
		Final	7.3	6.8	7.4	7.0	7.4	7.2	6.7
	pH	Initial	8.2	8.1	8.2	8.3	8.3	8.3	8.3
		Final	8.0	8.0	8.1	8.2	8.2	8.1	8.0
	Conductivity (µmhos/cm)	Initial	493	530	482	450	434	407	418
9 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (mg/L)	Initial	8.0	8.1	7.7	8.0	7.9	7.9	7.7
		Final	7.3	6.8	7.4	7.1	7.4	7.2	6.9
	pH	Initial	8.2	8.2	8.2	8.3	8.3	8.3	8.3
		Final	8.0	8.1	8.2	8.2	8.2	8.1	8.1
	Conductivity (µmhos/cm)	Initial	591	623	585	558	553	527	536
100 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (mg/L)	Initial	8.2	8.2	8.0	8.3	8.0	8.4	8.2
		Final	7.1	6.8	7.3	7.0	7.4	7.2	6.8
	pH	Initial	8.0	8.0	8.0	8.0	7.9	8.0	8.0
		Final	7.9	8.0	8.1	8.1	8.1	8.0	8.0
	Conductivity (µmhos/cm)	Initial	1549	1539	1533	1538	1530	1534	1535
	Hardness (mg/L as $CaCO_3$)		470	–	–	–	–	–	–

"–" = not measured/not required
[4] adjusted for temperature and barometric pressure
[5] ≤100 bubbles/minute

Test Data Reviewed By : KP
Date : 2022-11-29

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TOXICITY TEST REPORT

Fathead Minnow
EPS 1/RM/22

Work Order : 250143
Sample Number : 75492

SAMPLE IDENTIFICATION

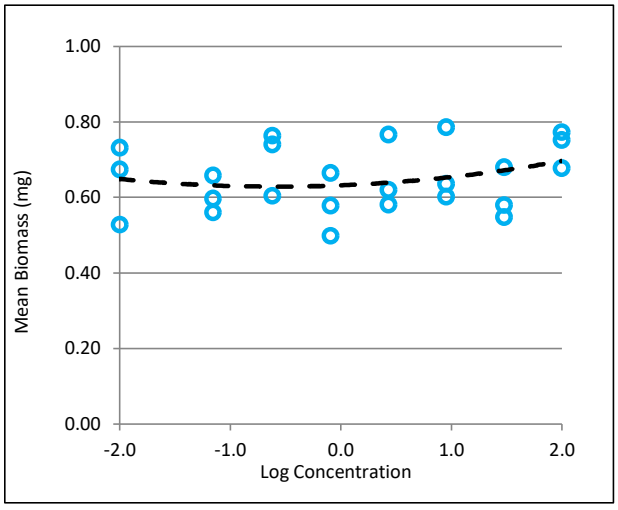
Company :	ALS Laboratory Group - Thunder Bay		
Location :	Thunder Bay ON	Sample Date :	2022-11-09
Job Number :	L2740058-1	Time Collected :	15:15
Substance :	L2740058-1 EDL1	Date Received :	2022-11-14
Sampling Method :	Not provided	Time Received :	11:45
Sampled By :	Not provided	Temperature at Receipt :	15 °C
Sample Description :	Clear, light yellow with settled solids	Date Tested :	2022-11-15

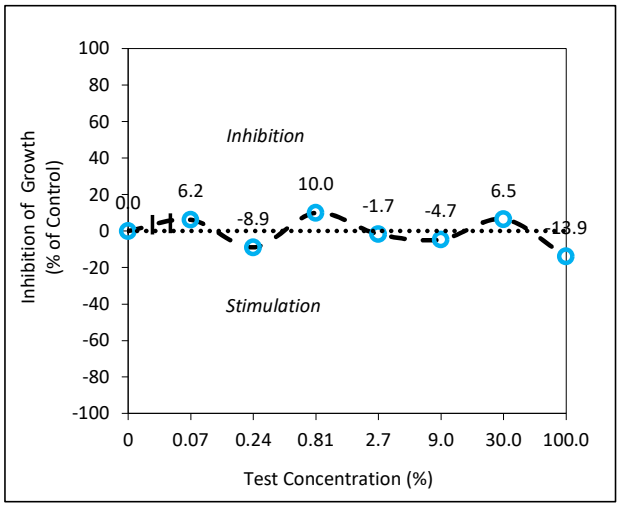
Test Method : Test of Larval Growth and Survival Using Fathead Minnows. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/22 , 2nd ed. (February 2011).

7-DAY TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Biomass)[1]	>100%	–	–
LC50	>100%	–	–

The results reported relate only to the sample tested and as received.





COMMENTS

Noted Deviation: The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

[1]as a measure of Growth

•All test validity criteria as specified in the test method cited above were satisfied.

Approved By : _____

Project Manager

Work Order : 250143
Sample Number : 75492

TEST ORGANISM

Test Organism :	*Pimephales promelas*	Culture Mortality/Diseased :	0 % (previous 7 days)
Organism Batch :	Fm22-11	Organism Age :	~07:00 - 23:00 h at test start
Source :	In-house culture		

•No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.
•Inflated swim bladders were confirmed in all test organisms used in this test.

TEST CONDITIONS

Test Type :	Static Renewal	Control/Dilution Water :	Well water [3]
Renewal Method :	80-85% syphoned and replaced	Test Volume / Replicate :	300 mL
Renewal Frequency :	≤ 24 hours	Test Vessel :	420 mL polystyrene beaker
Sample Filtration :	None	Depth of Test Solution :	8 cm
Test Aeration :	None	Organisms per Replicate :	10
pH Adjustment :	None	Number of Replicates :	3
Hardness Adjustment :	None	Test Method Deviation(s):	Yes (see 'Comments')

[3]no additional chemicals

REFERENCE TOXICANT DATA

Toxicant :	Potassium Chloride	Analyst(s) :	CGR, NP, XD
Date Tested :	2022-11-01	Test Duration :	7 days
IC25 (Biomass)1 :	0.98 g/L	LC50 :	1.07 g/L
95% Confidence Limits :	0.83 - 1.07 g/L	95% Confidence Limits :	1.00 - 1.14 g/L
Statistical Method :	Non-Linear Regression (CETIS)[a]	Statistical Method :	Linear Regression (MLE) (CETIS)[a]
Historical Mean IC25 :	1.01 g/L	Historical Mean LC50 :	1.14 g/L
Warning Limits (± 2SD) :	0.92 - 1.10 g/L	Warning Limits (± 2SD) :	1.01 - 1.29 g/L

[1]as a measure of Growth

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

REFERENCES

[a] CETIS™, © 2000-2018. V.1.9.4.7. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

[b]Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11* :1-21.

Work Order : 250143
Sample Number : 75492

CUMULATIVE DAILY CONTROL MORTALITY AND IMPAIRMENT

Date :	2022-11-15	2022-11-16	2022-11-17	2022-11-18	2022-11-19	2022-11-20	2022-11-21	2022-11-22
Mortality/Impairment :	0.00%	0.00%	0.00%	0.00%	0.00%	3.33%	3.33%	3.33%
Standard Deviation :	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±5.8)	(±5.8)	(±5.8)

CUMULATIVE DAILY MORTALITY

Initiation Time : 15:00
Initiation Date : 2022-11-15
Completion Date : 2022-11-22

		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Treatment
Date :		2022-11-15		2022-11-16		2022-11-17		2022-11-18		2022-11-19		2022-11-20		2022-11-21		2022-11-22		Mean Mortality (± SD)
Analyst(s):		AW		CN		JW (VBC)		AW		JJ (SK)		NP		PC		ELS (PC)		
Concentration		Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	**%**
%	**Replicate**																	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**3.33**
Control	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±5.77)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	1	**10**	1	**10**	1	**10**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	1	**10**	1	**10**	**3.33**
0.07	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±5.77)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
0.24	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	1	**10**	**3.33**
0.81	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±5.77)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	1	**10**	1	**10**	1	**10**	1	**10**	1	**10**	1	**10**	**3.33**
2.7	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±5.77)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
9	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**3.33**
30	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±5.77)
	C	0	**0**	0	**0**	0	**0**	0	**0**	1	**10**	1	**10**	1	**10**	1	**10**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
100	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	

Aberrant behaviour or swimming impairment : 2022-11-20: One small fish is swimming in circles in the 0.07% concentration, Replicate C (NP).
2022-11-21: One fish is swimming in circles in the 0.07% concentration, Replicate C (PC).

Test Data Reviewed By : KP
Date : 2022-11-30

Work Order : 250143
Sample Number : 75492

DRY WEIGHT AND BIOMASS DATA

Concentration %	Replicate	Number Exposed	Replicate Mean Dry Weight (mg)	Treatment Mean Biomass (mg)	Standard Deviation
Control	A	10	0.732	0.645	0.105
	B	10	0.674		
	C	10	0.528		
0.07	A	10	0.658	0.605	0.049
	B	10	0.597		
	C	10	0.560		
0.24	A	10	0.763	0.702	0.086
	B	10	0.740		
	C	10	0.604		
0.81	A	10	0.578	0.580	0.084
	B	10	0.498		
	C	10	0.665		
2.7	A	10	0.581	0.656	0.098
	B	10	0.766		
	C	10	0.620		
9	A	10	0.602	0.675	0.098
	B	10	0.636		
	C	10	0.786		
30	A	10	0.680	0.603	0.069
	B	10	0.580		
	C	10	0.548		
100	A	10	0.678	0.734	0.050
	B	10	0.752		
	C	10	0.773		

NOTES : •No outlying data points were detected according to Grubbs Test[b].

• Control average dry weight per surviving organism = 0.664 mg

Test Data Reviewed By : KP

Date : 2022-11-30

Work Order : 250143
Sample Number : 75492

WATER CHEMISTRY DATA

			Day 0 - 1 2022-11-15	Day 1 - 2 2022-11-16	Day 2 - 3 2022-11-17	Day 3 - 4 2022-11-18	Day 4 - 5 2022-11-19	Day 5 - 6 2022-11-20	Day 6 - 7 2022-11-21
Initial Chemistry (100%)	Sub-sample Used		1	1	1	2	2	3	3
	Temperature (°C)		24	24	24	24	24	26	24
	Dissolved O_2 (mg/L)		8.6	8.1	8.5	9.1	8.5	9.4	8.8
	Dissolved O_2 % Sat.[5]		106	99	106	112	104	120	107
	pH		7.8	7.9	7.8	7.9	8.0	7.8	7.9
	Conductivity (µmhos/cm)		1535	1531	1535	1532	1527	1543	1528
	Pre-aeration Time (min)[6]		20	0	20	20	20	20	20
	Analyst(s) :	Initial	PG (VBC)	CL2 (VBC)	CL2 (VBC)	SK	ELS(SK)	ELS(SK)	CL2 (VBC)
		Final	SK	JW	SJC (KP)	SK	NP	CL2 (VBC)	ELS (PC)
Control	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 % Sat.[5]	Initial	101	101	100	101	96	97	100
	Dissolved O_2 (mg/L)	Initial	8.1	8.3	7.9	8.1	7.7	7.8	8.1
		Final	7.4	6.8	7.3	5.9	6.3	6.7	6.7
	pH	Initial	8.3	8.4	8.3	8.3	8.3	8.4	8.4
		Final	8.1	8.0	8.1	7.9	7.9	8.0	8.0
	Conductivity (µmhos/cm)	Initial	491	540	531	448	430	417	419
0.07 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 (mg/L)	Initial	7.8	8.0	7.6	7.9	7.6	7.7	7.5
		Final	7.6	6.9	7.5	6.3	6.4	6.8	6.9
	pH	Initial	8.2	8.1	8.2	8.3	8.3	8.3	8.3
		Final	8.2	8.0	8.1	8.0	7.9	8.1	8.1
	Conductivity (µmhos/cm)	Initial	493	530	482	450	434	407	418
9 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 (mg/L)	Initial	8.0	8.1	7.7	8.0	7.9	7.9	7.7
		Final	7.6	6.8	7.5	5.8	6.4	6.7	6.9
	pH	Initial	8.2	8.2	8.2	8.3	8.3	8.3	8.3
		Final	8.2	8.0	8.1	7.5	7.9	8.0	8.0
	Conductivity (µmhos/cm)	Initial	591	623	585	558	553	527	536
100 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 (mg/L)	Initial	8.2	8.2	8.0	8.3	8.0	8.4	8.2
		Final	7.6	6.7	7.5	5.6	6.3	6.5	6.9
	pH	Initial	8.0	8.0	8.0	8.0	7.9	8.0	8.0
		Final	8.0	7.9	8.0	7.8	7.8	7.9	7.9
	Conductivity (µmhos/cm)	Initial	1549	1539	1533	1538	1530	1534	1535
	Hardness (mg/L as $CaCO_3$)		470	–	–	–	–	–	–

"–" = not measured/not required

[5] adjusted for temperature and barometric pressure

[6] ≤100 bubbles/minute

Test Data Reviewed By : KP

Date : 2022-11-30

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TOXICITY TEST REPORT

Pseudokirchneriella subcapitata
EPS 1/RM/25

Work Order : 250143
Sample Number : 75492

SAMPLE IDENTIFICATION

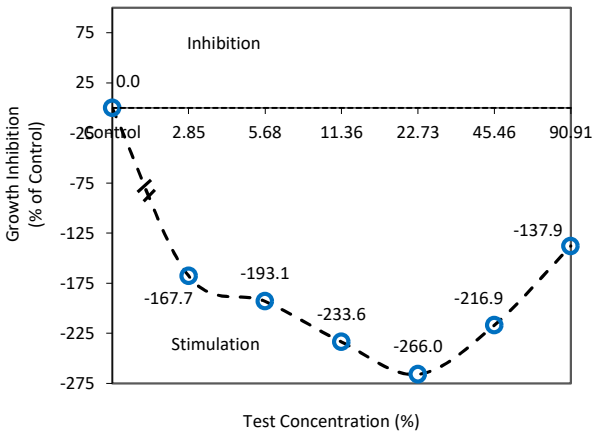
Company :	ALS Laboratory Group - Thunder Bay	Sample Date :	2022-11-09
Location :	Thunder Bay ON	Time Collected :	15:15
Job Number:	L2740058-1	Date Received :	2022-11-14
Substance :	L2740058-1 EDL1	Time Received :	11:45
Sampling Method :	Not provided	Temperature at Receipt :	15 °C
Sampled By :	Not provided	Date Tested :	2022-11-15
Sample Description :	Clear, light yellow with settled solids		

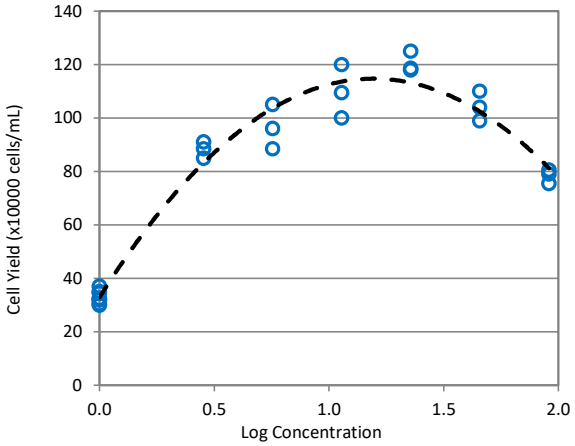
Test Method : Growth Inhibition Test Using a Freshwater Alga. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/25, 2nd ed. (March 2007).

72-HOUR TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Growth)	>90.91%	–	–

The results reported relate only to the sample tested and as received.





REFERENCE TOXICANT DATA

Toxicant :	Zinc (as Zinc Sulfate)		
Date Tested :	2022-11-08	IC25 Growth :	28.8 µg/L
Analyst(s) :	AS, VBC	95% Confidence Limits :	18.7 - 35.8 µg/L
Test Duration :	72 hours	Historical Mean IC25 :	19.0 µg/L
Statistical Method :	Non-Linear Regression (CETIS)a	Warning Limits (± 2SD) :	10.1 - 35.9 µg/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

COMMENTS

Noted Deviation(s) : The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

•All test validity criteria as specified in the test method cited above were satisfied.

Approved By : _____

Project Manager

Work Order : 250143
Sample Number : 75492

TEST ORGANISM

Test Organism :	*Pseudokirchneriella subcapitata*	Organism Batch :	Ps22-11/3
Culture Origin :	University of Waterloo, Waterloo ON	Cell Density at 0-h :	10136 cells/mL
Strain Number :	CPCC 37	Inoculum Prepared :	50 min. prior to test initiation
Inoculum Source :	In-house culture	Age (at start of test) :	4 days (in exponential growth)

•Algal growth curve is determined at least twice per year as required by the test method.
•No unusual appearance or treatment of culture prior to testing.

TEST CONDITIONS

Test Type :	Static	Volume per Replicate :	220 µL
Test Duration :	72 hours	Control Replicates :	10
Mean Temperature (± SD):	24.0°C (± 0.0)	Test Replicates :	4
Sample Pre-aeration :	None	Concentrations Tested :	10 + Control
Sample Filtration :	0.45 µm preconditioned filter	Photoperiod :	Continuous light
Volume Filtered:	≥10 mL	Light Intensity :	4020-4230 lux
Control/Dilution Water :	Millipore Milli-Q (no chemicals added)	Initial pH (100% sample) :	8.0
Enrichment Medium :	Stock 2B: EDTA reduced to 25%	pH Adjustment :	None
Test Vessel :	U-shaped polystyrene microplate	Hardness Adjustment :	None
Enumeration Method :	Manual (haemocytometer)	Test Method Deviation(s) :	See 'Comments'

CELL COUNTS AT 72-HOURS

Initiated By :	AS	Control pH (at 0 hours) :	6.5
Date Counted :	2022-11-18	Control pH (at 72 hours) :	6.5
Counted By :	RD	Control Increase Factor :	33.5 times growth

Concentration	Cell Concentration (x 10000 cells/mL) Replicate								Cell Yield (x 10000 cells/mL) Mean	Standard Deviation	CV (%)	Stimulation (% of control)	
%	1	2	3	4	7	8	9	10					
Control	31.0	36.0	33.0	35.5	31.5	33.5	38.0	33.0	32.92	2.38	7.2	–	
0.18	–	–	–	–	–	–	–	–	–	–	–	–	
0.35	–	–	–	–	–	–	–	–	–	–	–	–	
0.71	–	–	–	–	–	–	–	–	–	–	–	–	
1.42	–	–	–	–	–	–	–	–	–	–	–	–	
2.85	92.0	86.0	89.5	–	–	–	–	–	88.15	3.01	3.4	167.7	*
5.68	89.5	97.0	106.0	–	–	–	–	–	96.49	8.26	8.6	193.1	*
11.36	101.0	121.0	110.5	–	–	–	–	–	109.82	10.00	9.1	233.6	*
22.73	119.0	126.0	119.5	–	–	–	–	–	120.49	3.91	3.2	266.0	*
45.46	105.0	100.0	111.0	–	–	–	–	–	104.32	5.51	5.3	216.9	*
90.91	80.0	76.5	81.5	–	–	–	–	–	78.32	2.57	3.3	137.9	*

NOTES : *Statistically significant stimulation, according to ANOVA/Dunnett Multiple Comparison Test (CETIS)[a]

- Control replicates 5 and 6 used for pH measurement.
- The Mann-Kendall test shows that there is no inhibitory gradient (α=0.05).
- No outlying data points were detected according to Grubbs Test (CETIS)[a].

Test Data Reviewed By : EK

"–" = not counted/not required

Date : 2022-12-14

REFERENCES

[a] CETIS™, © 2000-2018. V.1.9.4.7. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

AquaTox Testing & Consulting Inc.
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Tel. (519) 763-4412
Fax. (519) 763-4419

TOXICITY TEST REPORT

Lemna minor
EPS 1/RM/37

Work Order : 250143
Sample Number : 75492

SAMPLE IDENTIFICATION

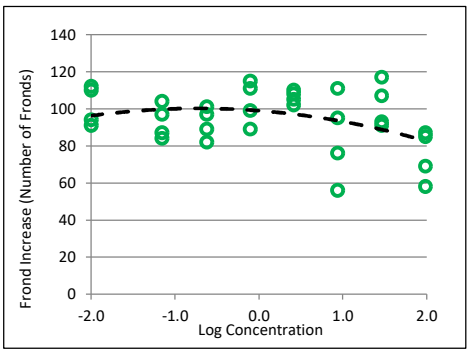
Company :	ALS Laboratory Group - Thunder Bay	Sample Date :	2022-11-09
Location :	Thunder Bay ON	Time Collected :	15:15
Job Number:	L2740058-1		
Substance :	L2740058-1 EDL1	Date Received :	2022-11-14
Sampling Method :	Not provided	Time Received :	11:45
Sampled By :	Not provided	Temperature at Receipt :	15 °C
Sample Description :	Clear, light yellow with settled solids	Date Tested :	2022-11-16

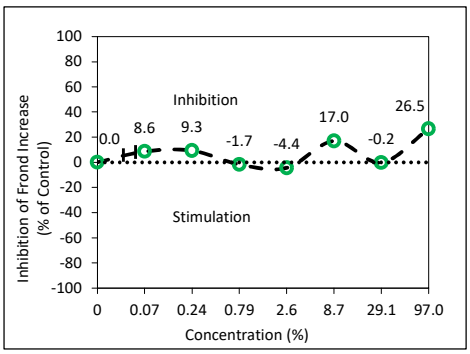
Test Method : Test for Measuring the Inhibition of Growth using the Freshwater Macrophyte, *Lemna minor*. Method Development and Application Section, Environmental Technology Centre, Environment Canada. Ottawa, Ontario. Report EPS 1/RM/37, 2nd ed. (January 2007).

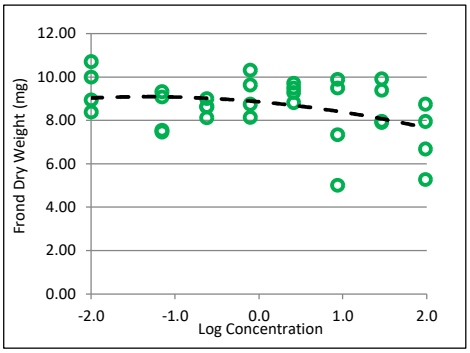
7-DAY TEST RESULTS

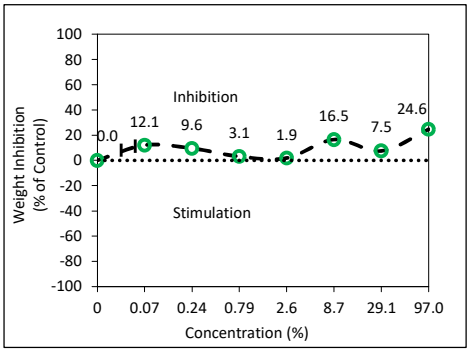
Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Frond Increase)	87.6%	8.7% – 97.1%	Linear Interplation (Toxstat)[d]
IC25 (Dry Weight)	>97.0%	–	Linear Interpolation (CETIS)[a]

The results reported relate only to the sample tested and as received.









COMMENTS

Noted Deviation: The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

•All test validity criteria as specified in the test method cited above were satisfied.

•Statistical analysis for the IC25 (Frond Increase) endpoint and IC25 (Dry Weight) endpoint could not be performed using non linear regression, since a suitable model could not be found. Therefore, test results were calculated using Linear Interpolation (Toxstat)[d] for the Frond Increase endpoint and Linear Interpolation (CETIS)[a] for the Dry Weight enpoint.

Approved By : _____

Project Manager

Work Order : 250143
Sample Number : 75492

TEST ORGANISM

Test Organism :	*Lemna minor* L., Strain 7730	Age (on Test Day 0) :	8 days
Organism Batch :	Lm22-11	Inoculum (Test Day 0) :	2 plants (3 fronds per plant)
Culture Origin :	UTCC 492	Culture Medium :	Modified Hoaglands E+
Test Organism Source :	Axenic in-house culture	Health Criteria (in APHA) :	18.2-fold frond increase in 7 days
Organism Acclimation :	18:50 hours (in APHA medium)		

No unusual appearance or treatment of culture prior to testing. Test inoculated with healthy plants.

TEST CONDITIONS

Test Type :	Static (no sub-samples required)	Sample Pre-aeration :	20 min. at ≤100 bubbles/min.
Control/Dilution Medium :	Modified APHA	pH Adjustment :	None
Source of Water :	Well Water	Hardness Adjustment :	None
Medium Preparation Water :	Reverse Osmosis	Replicates per Concentration :	4
Medium Preparation Chemicals :	Modified APHA [1]	Test Volume per Replicate :	100 mL
Nutrient Spiking of Sample :	Modified APHA [1]	Test Vessel :	200 mL glass jar
Initial pH :	7.8 (100% sample)	Depth of Test Solution :	4.0 cm
Initial temperature :	24 °C (100% sample)	Photoperiod/Light Intensity :	Continuous, 4230 - 4860 lux
Sample Filtration :	1 µm (Whatman GF/C)	Test Method Deviation(s) :	Yes (see 'COMMENTS')

[1]stocks A, B, C (10 mL/L)

REFERENCE TOXICANT DATA

Toxicant :	Potassium Chloride	IC25 (Frond Production) :	1.76 g/L
Date Tested :	2022-11-10	95% Confidence Limits :	1.15 - 2.35 g/L
Test Duration :	7 days	Statistical Method :	Linear Interpolation (CETIS)[a]
Analyst(s) :	NP	Historical Mean IC25 :	2.19 g/L
Growth Medium :	Modified APHA	Warning Limits (± 2SD) :	1.74 - 2.74 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST MONITORING

Intitiation Time :	10:30	Termination Time :	9:15
Initiated By :	PC (AS)	Terminated By :	NP (AS)

Test Day :		0	1	2	3	4	5	6	7
Date :		2022-11-16	2022-11-17	2022-11-18	2022-11-19	2022-11-20	2022-11-21	2022-11-22	2022-11-23
Temperature (° C) :		26	25	25	25	25	25	25	25
pH	Control	8.4	–	–	–	–	–	–	8.7
	0.07	8.3	–	–	–	–	–	–	8.6
	0.24	–	–	–	–	–	–	–	–
	0.79	–	–	–	–	–	–	–	–
	2.6	8.3	–	–	–	–	–	–	8.7
	8.7	–	–	–	–	–	–	–	–
	29.1	–	–	–	–	–	–	–	–
	97	8.1	–	–	–	–	–	–	8.7

"–" = not required

REFERENCES

[a] CETIS™, 2000-2022. V.2.1.4.0. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

[b]Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics,* 11:1-21.

[d] West, Inc. and D. Gulley. 1996. Toxstat Release 3.5. Western Ecosystems Technology. Cheyenne, WY, U.S.A.

Work Order : 250143
Sample Number : 75492

DAY 7 FROND COUNT DATA

Test Concentration %	Replicate	Frond Count	Frond Increase	Mean Frond Increase	Standard Deviation	Stimulation (%)	Frond/Root Appearance
Control	A	97	91	101.75	10.8	–	Fronds healthy, appearance normal in all replicates.
	B	118	112				
	C	116	110				
	D	100	94				
0.07	A	110	104	93.00	9.2	-8.6	Fronds healthy, appearance normal in all replicates.
	B	93	87				
	C	103	97				
	D	90	84				
0.24	A	88	82	92.25	8.5	-9.3	Fronds healthy, appearance normal in all replicates.
	B	107	101				
	C	95	89				
	D	103	97				
0.79	A	105	99	103.50	11.8	1.7	Fronds healthy, appearance normal in all replicates.
	B	117	111				
	C	95	89				
	D	121	115				
2.6	A	111	105	106.25	3.5	4.4	Fronds healthy, appearance normal in all replicates.
	B	114	108				
	C	108	102				
	D	116	110				
8.7	A	62	56	84.50	23.8	-17.0	Fronds healthy, appearance normal in all replicates.
	B	117	111				
	C	82	76				
	D	101	95				
29.1	A	113	107	102.00	12.3	0.2	Fronds healthy, appearance normal in all replicates.
	B	97	91				
	C	99	93				
	D	123	117				
97.0	A	64	58	74.75	13.8	-26.5	Fronds healthy, appearance normal in all replicates.
	B	91	85				
	C	75	69				
	D	93	87				

NOTES:

•No statistically significant stimulation (α=0.05) was detected by ANOVA-Dunnett Multiple Comparison Test (CETIS)[a] at any test level compared to the control.

•A 17.0-fold increase in frond number was observed in the control over the testing period.

•No outlying data points were detected according to Grubbs Test[b].

Test Data Reviewed By : EK
Date : 2022-12-15

"–" = not available/not required

Work Order : 250143
Sample Number : 75492

DAY 7 FROND WEIGHT DATA

Test Concentration %	Replicate	Dry Weight of Fronds (mg)	Treatment Mean Dry Weight (mg)	Standard Deviation	Stimulation (%)
Control	A	8.38	9.51	1.0	–
	B	9.99			
	C	10.71			
	D	8.94			
0.07	A	9.08	8.35	1.0	-12.1
	B	7.46			
	C	9.32			
	D	7.55			
0.24	A	8.63	8.59	0.4	-9.6
	B	8.62			
	C	8.12			
	D	9.00			
0.79	A	8.75	9.21	1.0	-3.1
	B	9.63			
	C	8.14			
	D	10.31			
2.6	A	9.28	9.33	0.4	-1.9
	B	9.72			
	C	8.82			
	D	9.49			
8.7	A	5.01	7.93	2.2	-16.5
	B	9.89			
	C	7.34			
	D	9.49			
29.1	A	9.39	8.79	1.0	-7.5
	B	7.90			
	C	7.96			
	D	9.91			
97.0	A	5.28	7.16	1.5	-24.6
	B	7.95			
	C	6.68			
	D	8.74			

NOTES :

•No stimulation compared to the control was observed at any test level.

•No outlying data points were detected according to Grubbs Test[b].

Test Data Reviewed By : EK
Date : 2022-12-15

"–" = not available/not required

L2740058

THUNDERBAY

ALS Environmental

Subcontract Request Form

Subcontract To:

AquaTox Testing & Consulting Inc. **ING**
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Ph: (519) 763-4412

Fathead minnow 7-day
Ceriodaphnia dubia 3-brood
Lemna minor 7-day
Freshwater alga 72-hour

NOTES: Please rereference on final report and invoice: PO# *L2740058*
ALS requires QC data to be provided with your final results.

Please see enclosed 3 sample(s) in 0 Container(s)

SAMPLE NUMBER	ANALYTICAL REQUIRED	DATE SAMPLED / DUE DATE	Priority Flag
L2740058-1 EDL1		**11/9/2022**	
Sample# 75492	Special Request Aquatox (SPECIAL REQUEST-AQT 14)	12/9/2022	
L2740058-2 SED2DIS		**11/9/2022**	
Sample# 75493	Special Request Aquatox (SPECIAL REQUEST-AQT 14)	12/9/2022	
L2740058-3 EDL2		**11/9/2022**	
Sample# 75494	Special Request Aquatox (SPECIAL REQUEST-AQT 14)	12/9/2022	

Subcontract Info Contact: Thunder Bay Login (807) 623-6463
Analysis and reporting info contact: Christine Paradis
1081 BARTON STREET
THUNDER BAY,ON P7B 5N3
Phone: (807) 623-6463 Email: christine.paradis@alsglobal.com

Please email confirmation of receipt to: **christine.paradis@alsglobal.com**

Shipped By: _____ Date Shipped: _____

Received By: VC Date Received: 2022-11-14

Verified By: _____ Date Verified: _____

Temperature: 15°C

Sample Integrity Issues: _____

EDL1 - time collected 15:15 *
SED2DIS - time collected 12:20 *
EDL2 - time collected 16:00 *
* As per pail label (VC) 2022-11-14

Aquatox workorder #250143.

AquaTox Testing & Consulting Inc.
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Tel. (519) 763-4412
Fax. (519) 763-4419

TOXICITY TEST REPORT

Ceriodaphnia dubia
EPS 1/RM/21

Work Order : 250143
Sample Number : 75493

SAMPLE IDENTIFICATION

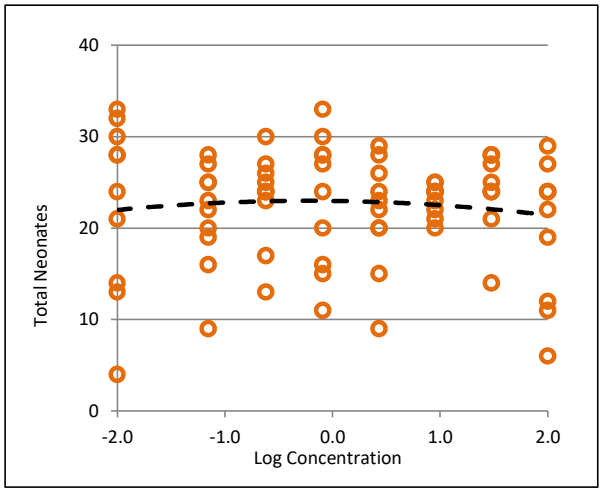
Company :	ALS Laboratory Group - Thunder Bay		
Location :	Thunder Bay ON	Sample Date :	2022-11-09
Job Number :	L2740058-2	Time Collected :	12:20
Substance :	L2740058-2 SED2DIS	Date Received :	2022-11-14
Sampling Method :	Not provided	Time Received :	11:45
Sampled By :	Not provided	Temperature at Receipt :	15 °C
Sample Description :	Clear, light yellow	Date Tested :	2022-11-15

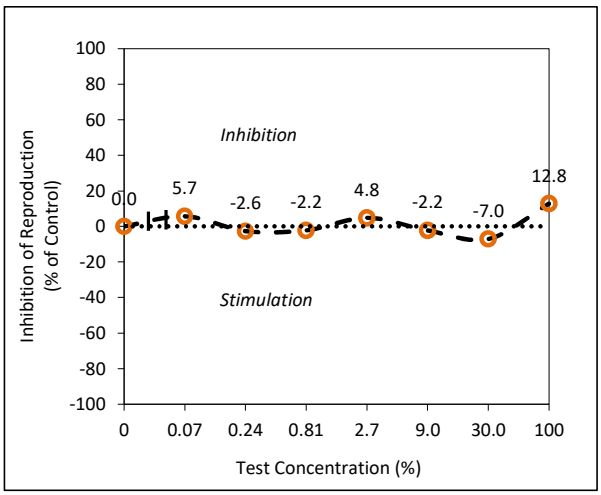
Test Method : Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia* . Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/21, 2nd ed. (February 2007).

7-DAY TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Reproduction)	>100%	–	–
LC50	>100%	–	–

The results reported relate only to the sample tested and as received.





COMMENTS

Noted Deviation: Due to a temperature system malfunction, the daily average temperature fell below the allowable range of 25 ± 1°C as per the test method. Since all validity criteria were satisfied, this deviation is not considered to have significantly altered the outcome of the test, and the test is considered to be valid. The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

•All test validity criteria as specified in the test method cited above were satisfied.

Approved By : _____

Project Manager

Work Order : 250143
Sample Number : 75493

TEST ORGANISM

Test Organism :	*Ceriodaphnia dubia*	Range of Age (at start of test) :	08:08 h - 20:08 h
Organism Batch :	Cd22-11	Mean Brood Organism Mortality :	3.3% (previous 7 days)
Organism Origin :	Single in-house mass culture	Average Total Neonates :	25.6 (first three broods)
Test Organism Origin :	Individual in-house cultures	Average Neonates :	12.5 (3rd or subsequent brood)
Ephippia in Culture :	None		

No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.

TEST CONDITIONS

Test Type :	Static renewal	Control/Dilution Water :	Well water [2]
Renewal Method :	Transferred to fresh solutions	Test Volume per Replicate :	15 mL
Renewal Frequency :	≤ 24 hours	Test Vessel :	20 mL glass vial
Sample Filtration :	None	Depth of Test Solution :	4 cm
Test Aeration :	None	Organisms per Replicate :	1
pH Adjustment :	None	Number of Replicates :	10
Hardness Adjustment :	None	Test Method Deviation(s) :	Yes (see 'Comments')

[2]no additional chemicals

REFERENCE TOXICANT DATA

Toxicant :	Sodium Chloride	Analyst(s) :	CL2, ELS, CGR, AW, XD
Date Tested :	2022-11-01	Test Duration :	6 days
IC25 (Reproduction) :	1.13 g/L	LC50 :	2.21 g/L
95% Confidence Limits :	0.64 - 1.27 g/L	95% Confidence Limits :	2.00 - 2.43 g/L
Statistical Method :	Linear Interpolation (CETIS)[a]	Statistical Method :	Spearman-Kärber (CETIS)[a]
Historical Mean IC25 :	1.06 g/L	Historical Mean LC50 :	2.02 g/L
Warning Limits (± 2SD) :	0.65 - 1.74 g/L	Warning Limits (± 2SD) :	1.58 - 2.58 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

CUMULATIVE DAILY MORTALITY DATA

		Test Concentration (%)							
Date	Test Day	**Control**	**0.07**	**0.24**	**0.81**	**2.7**	**9**	**30**	**100**
2022-11-16	1	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0
2022-11-19	4	0	0	0	0	0	0	0	10
2022-11-20	5	0	0	0	0	0	0	0	10
2022-11-21	6	10	10	0	0	0	0	0	10
2022-11-22	7	20	10	0	0	0	0	10	10
Total Mortality (%) :		**20**	**10**	**0**	**0**	**0**	**0**	**10**	**10**

REFERENCES

[a] CETIS™, © 2000-2022. V.2.1.4.0. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

[b]Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11* :1-21.

Work Order : 250143
Sample Number : 75493

SURVIVAL AND REPRODUCTION

Test Initiation Date : 2022-11-15
Initiated By : MR
Initiation Time : 14:08
Test Completion Date : 2022-11-22

Control

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)	Analyst(s)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0	MR
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0	CGR
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0	SJC (KP)
2022-11-19	4	4	4	6	5	6	6	5	6	6	6	5.4	SK
2022-11-20	5	8	0	9	12	9	6	9	8	8	5	7.4	JJ(SK)
2022-11-21	6	9	0 x	0	0	0	0	0	0	0	0	0.9	PG (PC)
2022-11-22	7	–	0	17	16	15	1	14	14	0 x	13	9	SJC (PC)
Total		**21**	**4**	**32**	**33**	**30**	**13**	**28**	**28**	**14**	**24**	**22.7 (±9.6)**	

2.7%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	4	5	6	6	5	4	2	4	4	6	4.6
2022-11-20	5	5	0	7	8	7	5	2	9	6	9	5.8
2022-11-21	6	0	9	0	0	0	0	0	0	0	0	0.9
2022-11-22	7	0	14	11	12	11	11	11	9	10	14	10.3
Total		**9**	**28**	**24**	**26**	**23**	**20**	**15**	**22**	**20**	**29**	**21.6 (±6.1)**

0.07%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	6	4	4	6	4	7	4	6	4	2	4.7
2022-11-20	5	10	9	7	10	6	6	7	9	5	7	7.6
2022-11-21	6	0	0	0	0	0	0	0	0	0	0 x	0
2022-11-22	7	12	12	5	11	9	9	9	10	14	0	9.1
Total		**28**	**25**	**16**	**27**	**19**	**22**	**20**	**25**	**23**	**9**	**21.4 (±5.7)**

9%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	4	4	6	6	3	5	5	6	4	2	4.5
2022-11-20	5	8	7	6	5	6	6	7	2	8	5	6
2022-11-21	6	0	0	1	0	0	0	1	0	0	0	0.2
2022-11-22	7	13	9	10	11	15	13	12	16	12	14	12.5
Total		**25**	**20**	**23**	**22**	**24**	**24**	**25**	**24**	**24**	**21**	**23.2 (±1.7)**

0.24%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	6	5	4	4	5	6	4	6	4	6	5
2022-11-20	5	7	0	9	7	6	10	9	7	7	8	7
2022-11-21	6	0	8	0	0	2	1	0	0	0	1	1.2
2022-11-22	7	4	11	11	12	–	13	11	13	14	12	10.1
Total		**17**	**24**	**24**	**23**	**13**	**30**	**24**	**26**	**25**	**27**	**23.3 (±4.9)**

30%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	6	2	4	3	4	6	4	5	4	5	4.3
2022-11-20	5	10	0	6	7	8	8	10	8	6	9	7.2
2022-11-21	6	12	10	0	0	0	0	0	0	0	0	2.2
2022-11-22	7	0	13	14	11	12	0 x	14	14	14	14	10.6
Total		**28**	**25**	**24**	**21**	**24**	**14**[3]	**28**	**27**	**24**	**28**	**24.3 (±4.3)**

0.81%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	6	3	4	7	6	7	2	5	5	6	5.1
2022-11-20	5	8	0	9	12	11	11	7	10	0	7	7.5
2022-11-21	6	0	7	0	0	0	0	2	0	8	0	1.7
2022-11-22	7	2	10	11	14	10	12	–	13	2	15	8.9
Total		**16**	**20**	**24**	**33**	**27**	**30**	**11**	**28**	**15**	**28**	**23.2 (±7.3)**

100%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	6	5	5	5	6	4	6	6 x	5	5	5.3
2022-11-20	5	6	7	4	6	8	5	8	0	6	5	5.5
2022-11-21	6	1	0	0	0	0	0	0	0	1	0	0.2
2022-11-22	7	11	10	2	13	15	10	13	0	0	14	8.8
Total		**24**	**22**	**11**	**24**	**29**	**19**	**27**	**6**	**12**	**24**	**19.8 (±7.6)**

NOTES : •All young produced by a test organism during its fourth and subsequent broods were discarded and not included in the above counts. The presence of two or more neonates in any test chamber, during any given day of the test, constitutes a brood.

•[3] Outlier according to Grubbs Test[b]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

x = test organism mortality

* = accidental test organism mortality

– =4th brood (see 'NOTES')

Test Data Reviewed By : KP

Date : 2022-11-30

Work Order : 250143
Sample Number : 75493

WATER CHEMISTRY DATA

			Day 0 - 1	Day 1 - 2	Day 2 - 3	Day 3 - 4	Day 4 - 5	Day 5 - 6	Day 6 - 7
	Date :		2022-11-15	2022-11-16	2022-11-17	2022-11-18	2022-11-19	2022-11-20	2022-11-21
	Sub-sample Used		1	1	1	2	2	3	3
Initial Chemistry (100 %)	Temperature (°C)		24	24	24	24	24	25	24
	Dissolved O_2 (mg/L)		9.0	8.3	8.6	8.9	8.6	8.7	8.5
	Dissolved O_2 (% Sat.)[4]		110	104	106	109	106	110	104
	pH		8.1	8.0	8.1	8.2	8.1	8.1	8.2
	Conductivity (µmhos/cm)		617	607	615	565	581	585	568
	Pre-aeration Time (min)[5]		20	20	20	20	20	20	20
	Analyst(s)	Initial	PG (VBC)	CL2 (VBC)	CL2 (VBC)	KM	ELS (SK)	ELS (SK)	CL2 (PC)
		Final	SK	CGR	SJC (KP)	SK	SK	VC	SJC (PC)
Control	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (% Sat.)[4]	Initial	101	101	100	101	96	98	100
	Dissolved O_2 (mg/L)	Initial	8.1	8.3	7.9	8.1	7.7	7.8	8.1
		Final	7.4	6.9	7.0	7.1	7.6	7.2	6.6
	pH	Initial	8.3	8.4	8.3	8.3	8.3	8.4	8.4
		Final	8.1	8.1	8.1	8.2	8.2	8.1	8.0
	Conductivity (µmhos/cm)	Initial	491	540	531	448	430	417	419
0.07 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (mg/L)	Initial	7.9	8.1	7.7	8.0	7.5	7.8	7.6
		Final	7.3	6.9	7.3	7.1	7.6	7.2	6.7
	pH	Initial	8.2	8.2	8.2	8.3	8.3	8.3	8.3
		Final	8.1	8.1	8.1	8.2	8.2	8.1	8.0
	Conductivity (µmhos/cm)	Initial	484	525	480	434	426	413	419
9 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (mg/L)	Initial	8.0	8.2	7.7	8.0	7.6	8.0	7.7
		Final	7.3	6.9	7.3	7.1	7.6	7.2	6.8
	pH	Initial	8.3	8.3	8.2	8.3	8.3	8.3	8.3
		Final	8.1	8.1	8.1	8.2	8.2	8.1	8.1
	Conductivity (µmhos/cm)	Initial	499	538	493	456	445	432	437
100 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (mg/L)	Initial	8.1	8.2	7.8	8.3	7.9	8.2	8.2
		Final	7.3	6.7	7.3	6.9	7.3	7.2	6.8
	pH	Initial	8.2	8.2	8.2	8.2	8.3	8.2	8.2
		Final	8.1	8.1	8.2	8.2	8.2	8.2	8.1
	Conductivity (µmhos/cm)	Initial	615	613	575	572	571	570	570
	Hardness (mg/L as $CaCO_3$)		320	–	–	–	–	–	–

"–" = not measured/not required
[4] adjusted for temperature and barometric pressure
[5] ≤100 bubbles/minute

Test Data Reviewed By : KP
Date : 2022-11-30

AquaTox Testing & Consulting Inc.
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Tel. (519) 763-4412
Fax. (519) 763-4419

TOXICITY TEST REPORT

Fathead Minnow
EPS 1/RM/22

Work Order : 250143
Sample Number : 75493

SAMPLE IDENTIFICATION

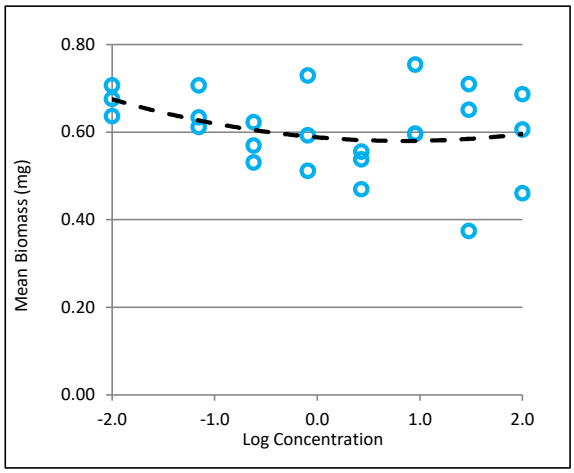
Company :	ALS Laboratory Group - Thunder Bay		
Location :	Thunder Bay ON	Sample Date :	2022-11-09
Job Number :	L2740058-2	Time Collected :	12:20
Substance :	L2740058-2 SED2DIS	Date Received :	2022-11-14
Sampling Method :	Not provided	Time Received :	11:45
Sampled By :	Not provided	Temperature at Receipt :	15 °C
Sample Description :	Clear, light yellow	Date Tested :	2022-11-15

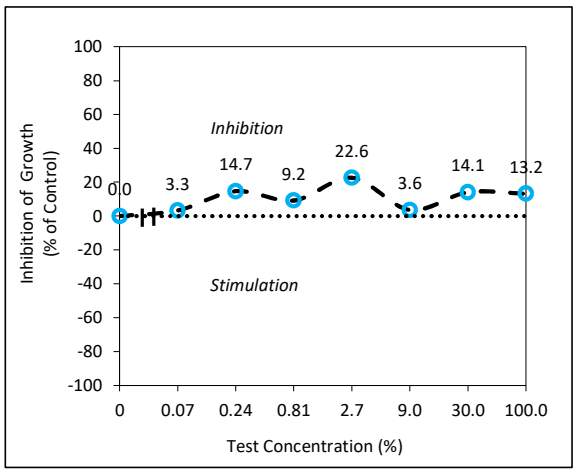
Test Method : Test of Larval Growth and Survival Using Fathead Minnows. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/22 , 2nd ed. (February 2011).

7-DAY TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Biomass)[1]	>100%	–	Linear Interpolation (CETIS)[a]
LC50	>100%	–	–

The results reported relate only to the sample tested and as received.





COMMENTS

Noted Deviation: The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

[1]as a measure of Growth

•All test validity criteria as specified in the test method cited above were satisfied.

•Statistical analysis for the IC25 (Biomass)[1] endpoint could not be conducted using Non-Linear Regression, since a suitable model could not be identified. Therefore, test results were calculated using Linear Interpolation (CETIS)[a].

Approved By : _____

Project Manager

Work Order : 250143
Sample Number : 75493

TEST ORGANISM

Test Organism :	*Pimephales promelas*	Culture Mortality/Diseased :	0 % (previous 7 days)
Organism Batch :	Fm22-11	Organism Age :	~07:00 - 23:15 h at test start
Source :	In-house culture		

•No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.
•Inflated swim bladders were confirmed in all test organisms used in this test.

TEST CONDITIONS

Test Type :	Static Renewal	Control/Dilution Water :	Well water [3]
Renewal Method :	80-85% syphoned and replaced	Test Volume / Replicate :	300 mL
Renewal Frequency :	≤ 24 hours	Test Vessel :	420 mL polystyrene beaker
Sample Filtration :	None	Depth of Test Solution :	8 cm
Test Aeration :	None	Organisms per Replicate :	10
pH Adjustment :	None	Number of Replicates :	3
Hardness Adjustment :	None	Test Method Deviation(s):	Yes (see 'Comments')

[3]no additional chemicals

REFERENCE TOXICANT DATA

Toxicant :	Potassium Chloride	Analyst(s) :	CGR, NP, XD
Date Tested :	2022-11-01	Test Duration :	7 days
IC25 (Biomass)1 :	0.98 g/L	LC50 :	1.07 g/L
95% Confidence Limits :	0.83 - 1.07 g/L	95% Confidence Limits :	1.00 - 1.14 g/L
Statistical Method :	Non-Linear Regression (CETIS)[a]	Statistical Method :	Linear Regression (MLE) (CETIS)[a]
Historical Mean IC25 :	1.01 g/L	Historical Mean LC50 :	1.14 g/L
Warning Limits (± 2SD)	0.92 - 1.10 g/L	Warning Limits (± 2SD) :	1.01 - 1.29 g/L

[1]as a measure of Growth

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

REFERENCES

[a] CETIS™, © 2000-2022. V.2.1.4.0. Comprehensive Environmental Toxicity Information System. Tidepool

Noted Deviation: The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was

[b]Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11* :1-21.

Work Order : 250143
Sample Number : 75493

CUMULATIVE DAILY CONTROL MORTALITY AND IMPAIRMENT

Date :	2022-11-15	2022-11-16	2022-11-17	2022-11-18	2022-11-19	2022-11-20	2022-11-21	2022-11-22
Mortality/Impairment :	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Standard Deviation :	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)

CUMULATIVE DAILY MORTALITY

Initiation Time : 15:15
Initiation Date : 2022-11-15
Completion Date : 2022-11-22

		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Treatment
Date :		2022-11-15		2022-11-16		2022-11-17		2022-11-18		2022-11-19		2022-11-20		2022-11-21		2022-11-22		Mean Mortality (± SD)
Analyst(s):		CN		AW		SV		JJ (VBC)		ELS (SK)		ELS(SK)		AW		ELS (PC)		
Concentration		Number	**%**	Number	**%**	Number	**%**	Number	**%**	Number	**%**	Number	**%**	Number	**%**	Number	**%**	**%**
%	**Replicate**	Dead	**Dead**	Dead	**Dead**	Dead	**Dead**	Dead	**Dead**	Dead	**Dead**	Dead	**Dead**	Dead	**Dead**	Dead	**Dead**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
Control	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
0.07	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
0.24	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
0.81	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	1	**10**	1	**10**	1	**10**	**3.33**
2.7	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±5.77)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
9	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0*	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**20.00**
30	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±34.64)
	C	0	**0**	0	**0**	0	**0**	2	**20**	3	**30**	3	**30**	5	**50**	6	**60**	
	A	0	**0**	0	**0**	1	**10**	3	**30**	3	**30**	3	**30**	3	**30**	3	**30**	**13.33**
100	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±15.28)
	C	0	**0**	0	**0**	1	**10**	1	**10**	1	**10**	1	**10**	1	**10**	1	**10**	

Aberrant behaviour or swimming impairment : 2022-11-20: 1 small fish observed in the 2.7% concentration, Replicate A (ELS).

NOTES : *One test organism was missing from the 9% concentration replicate C (JJ). The missing test organism was excluded from calculations/statistical analysis. The IC25 (Growth)[1] and LC50 endpoints were determined based on a total of 9 test organisms exposed in replicate C of the 9% concentration. (KP).

Test Data Reviewed By : KP
Date : 2022-11-30

Work Order : 250143
Sample Number : 75493

DRY WEIGHT AND BIOMASS DATA

Concentration %	Replicate	Number Exposed	Replicate Mean Dry Weight (mg)	Treatment Mean Biomass (mg)	Standard Deviation
Control	A	10	0.636	0.672	0.035
	B	10	0.706		
	C	10	0.675		
0.07	A	10	0.611	0.650	0.050
	B	10	0.633		
	C	10	0.706		
0.24	A	10	0.622	0.574	0.046
	B	10	0.569		
	C	10	0.530		
0.81	A	10	0.511	0.611	0.110
	B	10	0.592		
	C	10	0.729		
2.7	A	10	0.469	0.520	0.045
	B	10	0.555		
	C	10	0.537		
9	A	10	0.595	0.648	0.091
	B	10	0.596		
	C	9*	0.753[4]		
30	A	10	0.650	0.578	0.179
	B	10	0.709		
	C	10	0.374		
100	A	10	0.460	0.584	0.115
	B	10	0.686		
	C	10	0.605		

NOTES :

- [4]Outlier according to Grubbs Test[b]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

*One test organism missing from 9C (see 'NOTES' on page 3).

- Control average dry weight per surviving organism = 0.672 mg

Test Data Reviewed By : KP
Date : 2022-11-30

Work Order : 250143
Sample Number : 75493

WATER CHEMISTRY DATA

			Day 0 - 1 2022-11-15	Day 1 - 2 2022-11-16	Day 2 - 3 2022-11-17	Day 3 - 4 2022-11-18	Day 4 - 5 2022-11-19	Day 5 - 6 2022-11-20	Day 6 - 7 2022-11-21
Initial Chemistry (100%)	Sub-sample Used		1	1	1	2	2	3	3
	Temperature (°C)		24	24	24	24	24	25	24
	Dissolved O_2 (mg/L)		9.0	8.3	8.6	8.9	8.6	8.7	8.5
	Dissolved O_2 % Sat.[5]		110	104	106	109	106	110	104
	pH		8.1	8.0	8.1	8.2	8.1	8.1	8.2
	Conductivity (µmhos/cm)		617	607	615	565	581	585	568
	Pre-aeration Time (min)[6]		20	20	20	20	20	20	20
	Analyst(s) :	Initial	PG (VBC)	CL2 (VBC)	CL2 (VBC)	KM	ELS (SK)	ELS (SK)	CL2 (PC)
		Final	AW	ELS (VBC)	JJ	ELS (SK)	ELS (SK)	AW	ELS (PC)
Control	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 % Sat.[5]	Initial	101	101	100	101	96	98	100
	Dissolved O_2 (mg/L)	Initial	8.1	8.3	7.9	8.1	7.7	7.8	8.1
		Final	7.4	6.7	6.8	6.4	6.9	6.8	6.5
	pH	Initial	8.3	8.4	8.3	8.3	8.3	8.4	8.4
		Final	8.2	8.0	7.9	8.0	8.0	8.1	8.0
	Conductivity (µmhos/cm)	Initial	491	540	531	448	430	417	419
0.07 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 (mg/L)	Initial	7.9	8.1	7.7	8.0	7.5	7.8	7.6
		Final	7.5	6.7	6.8	6.4	6.9	6.8	6.7
	pH	Initial	8.2	8.2	8.2	8.3	8.3	8.3	8.3
		Final	8.2	8.0	8.0	8.0	8.1	8.1	8.0
	Conductivity (µmhos/cm)	Initial	484	525	480	434	426	413	419
9 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 (mg/L)	Initial	8.0	8.2	7.7	8.0	7.6	8.0	7.7
		Final	7.5	6.8	6.9	6.4	6.9	6.7	6.7
	pH	Initial	8.3	8.3	8.2	8.3	8.3	8.3	8.3
		Final	8.2	8.0	8.0	8.0	8.1	8.0	8.0
	Conductivity (µmhos/cm)	Initial	499	538	493	456	445	432	437
100 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 (mg/L)	Initial	8.1	8.2	7.8	8.3	7.9	8.2	8.2
		Final	7.7	6.8	6.7	6.3	6.8	6.6	6.7
	pH	Initial	8.2	8.2	8.2	8.2	8.3	8.2	8.2
		Final	8.2	8.1	8.1	8.1	8.1	8.1	8.1
	Conductivity (µmhos/cm)	Initial	615	613	575	572	571	570	570
	Hardness (mg/L as $CaCO_3$)		320	–	–	–	–	–	–

"–" = not measured/not required

[5] adjusted for temperature and barometric pressure

[6] ≤100 bubbles/minute

Test Data Reviewed By : KP

Date : 2022-11-30

AquaTox Testing & Consulting Inc.
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Tel. (519) 763-4412
Fax. (519) 763-4419

TOXICITY TEST REPORT

Pseudokirchneriella subcapitata
EPS 1/RM/25

Work Order : 250143
Sample Number : 75493

SAMPLE IDENTIFICATION

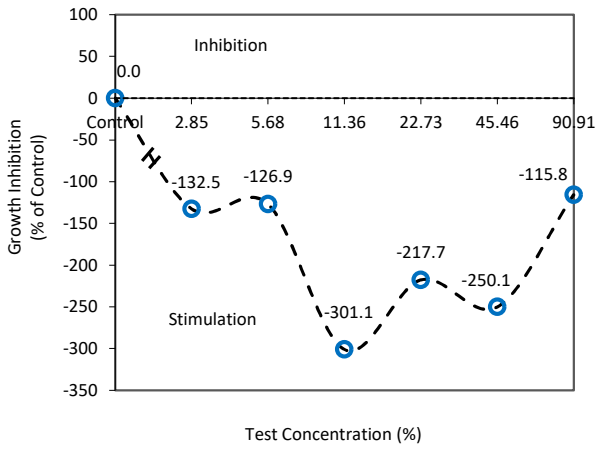
Company :	ALS Laboratory Group - Thunder Bay	Sample Date :	2022-11-09
Location :	Thunder Bay ON	Time Collected :	12:20
Job nimber:	L2740058-2	Date Received :	2022-11-14
Substance :	L2740058-2 SED2DIS	Time Received :	11:45
Sampling Method :	Not provided	Temperature at Receipt :	15 °C
Sampled By :	Not provided	Date Tested :	2022-11-15
Sample Description :	Clear, light yellow		

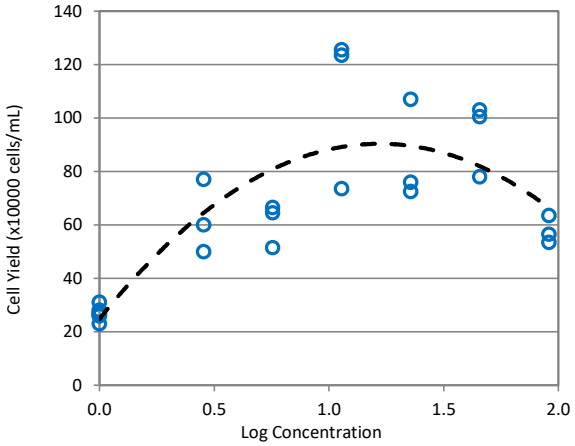
Test Method : Growth Inhibition Test Using a Freshwater Alga. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/25, 2nd ed. (March 2007).

72-HOUR TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Growth)	>90.91%	–	–

The results reported relate only to the sample tested and as received.





REFERENCE TOXICANT DATA

Toxicant :	Zinc (as Zinc Sulfate)		
Date Tested :	2022-11-08	IC25 Growth :	28.8 µg/L
Analyst(s) :	AS, VBC	95% Confidence Limits :	18.7 - 35.8 µg/L
Test Duration :	72 hours	Historical Mean IC25 :	19.0 µg/L
Statistical Method :	Non-Linear Regression (CETIS)[a]	Warning Limits (± 2SD) :	10.1 - 35.9 µg/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

COMMENTS

Noted Deviation(s) : The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

•All test validity criteria as specified in the test method cited above were satisfied.

Approved By : _____
Project Manager

Work Order : 250143
Sample Number : 75493

TEST ORGANISM

Test Organism :	*Pseudokirchneriella subcapitata*	Organism Batch :	Ps22-11/3
Culture Origin :	University of Waterloo, Waterloo ON	Cell Density at 0-h :	10136 cells/mL
Strain Number :	CPCC 37	Inoculum Prepared :	55 min. prior to test initiation
Inoculum Source :	In-house culture	Age (at start of test) :	4 days (in exponential growth)

•Algal growth curve is determined at least twice per year as required by the test method.
•No unusual appearance or treatment of culture prior to testing.

TEST CONDITIONS

Test Type :	Static	Volume per Replicate :	220 µL
Test Duration :	72 hours	Control Replicates :	10
Mean Temperature (± SD):	24.0°C (± 0.0)	Test Replicates :	4
Sample Pre-aeration :	None	Concentrations Tested :	10 + Control
Sample Filtration :	0.45 µm preconditioned filter	Photoperiod :	Continuous light
Volume Filtered:	≥10 mL	Light Intensity :	4020-4230 lux
Control/Dilution Water :	Millipore Milli-Q (no chemicals added)	Initial pH (100% sample) :	8.2
Enrichment Medium :	Stock 2B: EDTA reduced to 25%	pH Adjustment :	None
Test Vessel :	U-shaped polystyrene microplate	Hardness Adjustment :	None
Enumeration Method :	Manual (haemocytometer)	Test Method Deviation(s) :	See 'Comments'

CELL COUNTS AT 72-HOURS

Initiated By :	AS	Control pH (at 0 hours) :	6.5
Date Counted :	2022-11-18	Control pH (at 72 hours) :	6.5
Counted By :	AS	Control Increase Factor :	27.4 times growth

Concentration	Cell Concentration (x 10000 cells/mL) Replicate								Cell Yield (x 10000 cells/mL) Mean	Standard Deviation	CV (%)	Stimulation (% of control)	
%	1	2	3	4	7	8	9	10					
Control	32.0	27.0	27.5	28.5	27.5	29.0	27.0	24.0	26.80	2.25	8.4	–	
0.18	–	–	–	–	–	–	–	–	–	–	–	–	
0.35	–	–	–	–	–	–	–	–	–	–	–	–	
0.71	–	–	–	–	–	–	–	–	–	–	–	–	
1.42	–	–	–	–	–	–	–	–	–	–	–	–	
2.85	51.0	61.0	78.0	–	–	–	–	–	62.32	13.65	21.9	132.5	*
5.68	65.5	52.5	67.5	–	–	–	–	–	60.82	8.14	13.4	126.9	*
11.36	74.5	124.5	126.5	–	–	–	–	–	107.49	29.46	27.4	301.1	*
22.73	73.5	77.0	108.0	–	–	–	–	–	85.15	18.99	22.3	217.7	*
45.46	79.0	104.0	101.5	–	–	–	–	–	93.82	13.77	14.7	250.1	*
90.91	64.5	57.5	54.5	–	–	–	–	–	57.82	5.13	8.9	115.8	*

NOTES : *Statistically significant stimulation, according to ANOVA/Dunnett Multiple Comparison Test (CETIS)[a], (α=0.05) with l

- Control replicates 5 and 6 used for pH measurement.
- The Mann-Kendall test shows that there is no inhibitory gradient (α=0.05).
- No outlying data points were detected according to Grubbs Test (CETIS)[a].

Test Data Reviewed By : EK

"–" = not counted/not required

Date : 2022-12-15

REFERENCES

[a] CETIS™, © 2000-2022. V.2.1.4.0. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

[b] Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11* :1-21.

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B-11 Nicholas Beaver Road
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TOXICITY TEST REPORT

Lemna minor
EPS 1/RM/37

Work Order : 250143
Sample Number : 75493

SAMPLE IDENTIFICATION

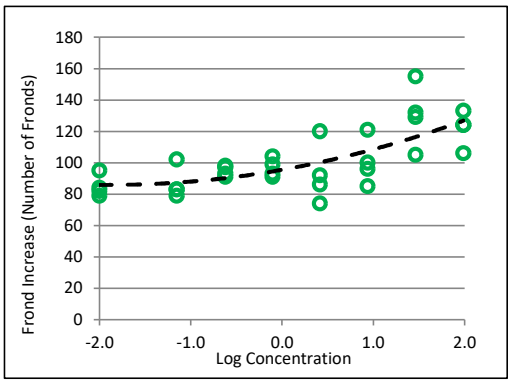
Company :	ALS Laboratory Group - Thunder Bay	Sample Date :	2022-11-09
Location :	Thunder Bay ON	Time Collected :	12:20
Job Number:	L2740058-2	Date Received :	2022-11-14
Substance :	L2740058-2 SED2DIS	Time Received :	11:45
Sampling Method :	Not provided	Temperature at Receipt :	15 °C
Sampled By :	Not provided	Date Tested :	2022-11-16
Sample Description :	Clear, light yellow		

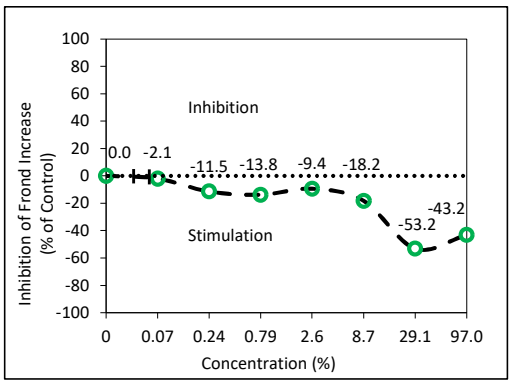
Test Method : Test for Measuring the Inhibition of Growth using the Freshwater Macrophyte, *Lemna minor*. Method Development and Application Section, Environmental Technology Centre, Environment Canada. Ottawa, Ontario. Report EPS 1/RM/37, 2nd ed. (January 2007).

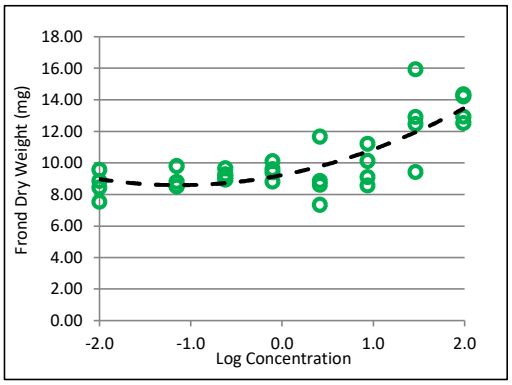
7-DAY TEST RESULTS

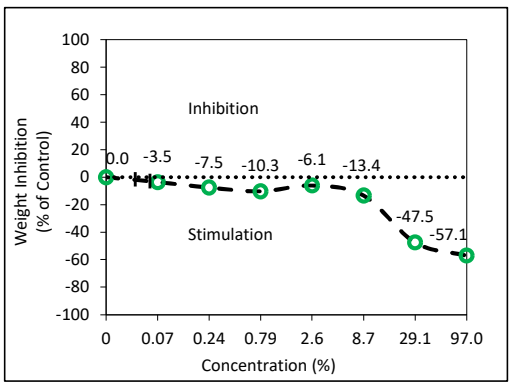
Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Frond Increase)	>97.0%	–	–
IC25 (Dry Weight)	>97.0%	–	–

The results reported relate only to the sample tested and as received.









COMMENTS

Noted Deviation: The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

•All test validity criteria as specified in the test method cited above were satisfied.

Approved By : _____

Project Manager

Work Order : 250143
Sample Number : 75493

TEST ORGANISM

Test Organism :	*Lemna minor* L., Strain 7730	Age (on Test Day 0) :	8 days
Organism Batch :	Lm22-11	Inoculum (Test Day 0) :	2 plants (3 fronds per plant)
Culture Origin :	UTCC 492	Culture Medium :	Modified Hoaglands E+
Test Organism Source :	Axenic in-house culture	Health Criteria (in APHA) :	18.2-fold frond increase in 7 days
Organism Acclimation :	18:20 hours (in APHA medium)		

No unusual appearance or treatment of culture prior to testing. Test inoculated with healthy plants.

TEST CONDITIONS

Test Type :	Static (no sub-samples required)	Sample Pre-aeration :	20 min. at ≤100 bubbles/min.
Control/Dilution Medium :	Modified APHA	pH Adjustment :	None
Source of Water :	Well Water	Hardness Adjustment :	None
Medium Preparation Water :	Reverse Osmosis	Replicates per Concentration :	4
Medium Preparation Chemicals :	Modified APHA [1]	Test Volume per Replicate :	100 mL
Nutrient Spiking of Sample :	Modified APHA [1]	Test Vessel :	200 mL glass jar
Initial pH :	8.3 (100% sample)	Depth of Test Solution :	4.0 cm
Initial temperature :	25 °C (100% sample)	Photoperiod/Light Intensity :	Continuous, 4260 - 5420 lux
Sample Filtration :	1 µm (Whatman GF/C)	Test Method Deviation(s) :	Yes (see 'COMMENTS')

[1]stocks A, B, C (10 mL/L)

REFERENCE TOXICANT DATA

Toxicant :	Potassium Chloride	IC25 (Frond Production) :	1.76 g/L
Date Tested :	2022-11-10	95% Confidence Limits :	1.15 - 2.35 g/L
Test Duration :	7 days	Statistical Method :	Linear Interpolation (CETIS)[a]
Analyst(s) :	NP	Historical Mean IC25 :	2.19 g/L
Growth Medium :	Modified APHA	Warning Limits (± 2SD) :	1.74 - 2.74 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST MONITORING

Intitiation Time :	9:50	Termination Time :	10:40
Initiated By :	NP (AS)	Terminated By :	PC (AS)

Test Day :		0	1	2	3	4	5	6	7
Date :		2022-11-16	2022-11-17	2022-11-18	2022-11-19	2022-11-20	2022-11-21	2022-11-22	2022-11-23
Temperature (° C) :		24	23	23	23	23	23	23	23
pH	Control	8.4	–	–	–	–	–	–	8.5
	0.07	8.4	–	–	–	–	–	–	8.6
	0.24	–	–	–	–	–	–	–	–
	0.79	–	–	–	–	–	–	–	–
	2.6	8.4	–	–	–	–	–	–	8.6
	8.7	–	–	–	–	–	–	–	–
	29.1	–	–	–	–	–	–	–	–
	97	8.3	–	–	–	–	–	–	9.0

"–" = not required

REFERENCES

[a] CETIS™, © 2000-2022. V.2.1.4.0. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

Work Order : 250143
Sample Number : 75493

DAY 7 FROND COUNT DATA

Test Concentration %	Replicate	Frond Count	Frond Increase	Mean Frond Increase	Standard Deviation	Stimulation (%)	Frond/Root Appearance
Control	A	85	79	85.00	7.0	–	Fronds healthy, appearance normal in all replicates.
	B	88	82				
	C	90	84				
	D	101	95				
0.07	A	89	83	86.75	10.3	2.1	Fronds healthy, appearance normal in all replicates.
	B	108	102				
	C	89	83				
	D	85	79				
0.24	A	104	98	94.75	3.3	11.5	Fronds healthy, appearance normal in all replicates.
	B	97	91				
	C	99	93				
	D	103	97				
0.79	A	97	91	96.75	5.9	13.8	Fronds healthy, appearance normal in all replicates.
	B	105	99				
	C	110	104				
	D	99	93				
2.6	A	80	74	93.00	19.5	9.4	Fronds healthy, appearance normal in all replicates.
	B	126	120				
	C	98	92				
	D	92	86				
8.7	A	91	85	100.50	15.1	18.2	Fronds healthy, appearance normal in all replicates.
	B	106	100				
	C	102	96				
	D	127	121				
29.1	A	135	129	130.25	20.5	53.2*	Fronds healthy, appearance normal in all replicates.
	B	161	155				
	C	111	105				
	D	138	132				
97.0	A	112	106	121.75	11.3	43.2*	Fronds healthy, appearance normal in all replicates.
	B	130	124				
	C	139	133				
	D	130	124				

NOTES: *Statistically significant stimulation (α=0.05) compared to the control, according to ANOVA/Dunnett Multiple Comparison Test (CETIS)[a]

•A 14.2-fold increase in frond number was observed in the control over the testing period.

•No outlying data points were detected according to Grubbs Test (CETIS)[a].

Test Data Reviewed By : EK
Date : 2022-12-15

"–" = not available/not required

Work Order : 250143
Sample Number : 75493

DAY 7 FROND WEIGHT DATA

Test Concentration %	Replicate	Dry Weight of Fronds (mg)	Treatment Mean Dry Weight (mg)	Standard Deviation	Stimulation (%)
Control	A	7.53	8.60	0.8	–
	B	8.86			
	C	8.44			
	D	9.56			
0.07	A	8.80	8.90	0.6	3.5
	B	9.79			
	C	8.51			
	D	8.51			
0.24	A	9.27	9.24	0.3	7.5
	B	9.66			
	C	9.12			
	D	8.93			
0.79	A	8.81	9.48	0.5	10.3
	B	9.40			
	C	9.61			
	D	10.10			
2.6	A	7.35	9.12	1.8	6.1
	B	11.67			
	C	8.86			
	D	8.61			
8.7	A	8.56	9.75	1.2	13.4
	B	10.12			
	C	9.10			
	D	11.21			
29.1	A	12.91	12.68	2.7	47.5*
	B	15.93			
	C	9.43			
	D	12.46			
97.0	A	12.93	13.51	0.9	57.1*
	B	14.22			
	C	14.35			
	D	12.52			

NOTES : *Statistically significant stimulation (α=0.05) compared to the control, according to ANOVA/Dunnett Multiple Comparison Test (CETIS)a

•No outlying data points were detected according to Grubbs Test (CETIS)[a].

Test Data Reviewed By : EK
Date : 2022-12-15

"–" = not available/not required

L2740058

THUNDERBAY

ALS Environmental

Subcontract Request Form

Subcontract To:

AquaTox Testing & Consulting Inc. **ING**
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Ph: (519) 763-4412

Fathead minnow 7-day
Ceriodaphnia dubia 3-brood
Lemna minor 7-day
Freshwater alga 72-hour

NOTES: Please rereference on final report and invoice: PO# *L2740058*
ALS requires QC data to be provided with your final results.

Please see enclosed 3 sample(s) in 0 Container(s)

SAMPLE NUMBER	ANALYTICAL REQUIRED	DATE SAMPLED / DUE DATE	Priority Flag
L2740058-1 EDL1		**11/9/2022**	
Sample# 75492	Special Request Aquatox (SPECIAL REQUEST-AQT 14)	12/9/2022	
L2740058-2 SED2DIS		**11/9/2022**	
Sample# 75493	Special Request Aquatox (SPECIAL REQUEST-AQT 14)	12/9/2022	
L2740058-3 EDL2		**11/9/2022**	
Sample# 75494	Special Request Aquatox (SPECIAL REQUEST-AQT 14)	12/9/2022	

Subcontract Info Contact: Thunder Bay Login (807) 623-6463
Analysis and reporting info contact: Christine Paradis
1081 BARTON STREET
THUNDER BAY,ON P7B 5N3
Phone: (807) 623-6463 Email: christine.paradis@alsglobal.com

Please email confirmation of receipt to: **christine.paradis@alsglobal.com**

Shipped By: _____ Date Shipped: _____

Received By: VC Date Received: 2022-11-14

Verified By: _____ Date Verified: _____

Temperature: 15°C

Sample Integrity Issues: _____

EDL1 - time collected 15:15 *
SED2DIS - time collected 12:20 *
EDL2 - time collected 16:00 *
* As per pail label (VC) 2022-11-14

Aquatox workorder #250143.

AquaTox Testing & Consulting Inc.
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Tel. (519) 763-4412
Fax. (519) 763-4419

TOXICITY TEST REPORT

Ceriodaphnia dubia
EPS 1/RM/21

Work Order : 250143
Sample Number : 75494

SAMPLE IDENTIFICATION

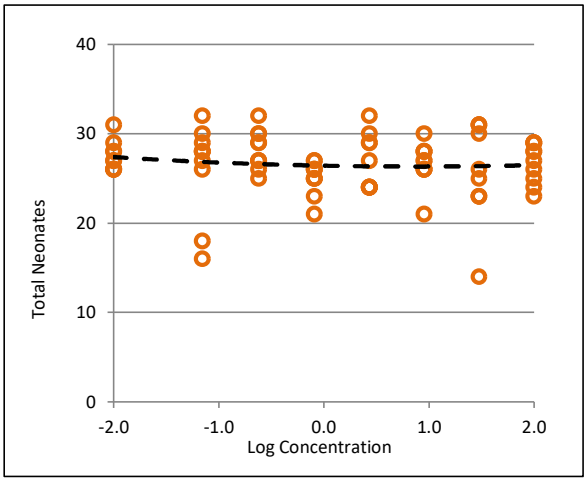
Company :	ALS Laboratory Group - Thunder Bay		
Location :	Thunder Bay ON	Sample Date :	2022-11-09
Job Number :	L2740058-3	Time Collected :	16:00
Substance :	L2740058-3 EDL2	Date Received :	2022-11-14
Sampling Method :	Not provided	Time Received :	11:45
Sampled By :	Not provided	Temperature at Receipt :	15 °C
Sample Description :	Clear, light yellow, settled solids	Date Tested :	2022-11-15

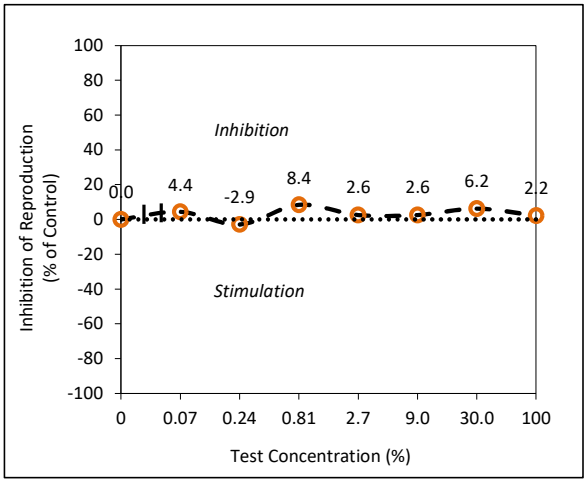
Test Method : Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia* . Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/21, 2nd ed. (February 2007).

7-DAY TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Reproduction)	>100%	–	–
LC50	>100%	–	–

The results reported relate only to the sample tested and as received.





COMMENTS

Noted Deviation: Due to a temperature system malfunction, the daily average temperature fell below the allowable range of 25 ± 1°C as per the test method. Since all validity criteria were satisfied, this deviation is not considered to have significantly altered the outcome of the test, and the test is considered to be valid. The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

•All test validity criteria as specified in the test method cited above were satisfied.

Approved By : _____

Project Manager

Work Order : 250143
Sample Number : 75494

TEST ORGANISM

Test Organism :	*Ceriodaphnia dubia*	Range of Age (at start of test) :	10:10 h - 22:10 h
Organism Batch :	Cd 22-11	Mean Brood Organism Mortality :	2% (previous 7 days)
Organism Origin :	Single in-house mass culture	Average Total Neonates :	23.3 (first three broods)
Test Organism Origin :	Individual in-house cultures	Average Neonates :	15.1 (3rd or subsequent brood)
Ephippia in Culture :	None		

No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.

TEST CONDITIONS

Test Type :	Static renewal	Control/Dilution Water :	Well water [2]
Renewal Method :	Transferred to fresh solutions	Test Volume per Replicate :	15 mL
Renewal Frequency :	≤ 24 hours	Test Vessel :	20 mL glass vial
Sample Filtration :	None	Depth of Test Solution :	4 cm
Test Aeration :	None	Organisms per Replicate :	1
pH Adjustment :	None	Number of Replicates :	10
Hardness Adjustment :	None	Test Method Deviation(s) :	Yes (see 'Comments')

[2]no additional chemicals

REFERENCE TOXICANT DATA

Toxicant :	Sodium Chloride	Analyst(s) :	CL2, ELS, CGR, AW, XD
Date Tested :	2022-11-01	Test Duration :	6 days
IC25 (Reproduction) :	1.13 g/L	LC50 :	2.21 g/L
95% Confidence Limits :	0.64 - 1.27 g/L	95% Confidence Limits :	2.00 - 2.43 g/L
Statistical Method :	Linear Interpolation (CETIS)[a]	Statistical Method :	Spearman-Kärber (CETIS)[a]
Historical Mean IC25 :	1.06 g/L	Historical Mean LC50 :	2.02 g/L
Warning Limits (± 2SD) :	0.65 - 1.74 g/L	Warning Limits (± 2SD) :	1.58 - 2.58 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

CUMULATIVE DAILY MORTALITY DATA

		Test Concentration (%)							
Date	Test Day	**Control**	**0.07**	**0.24**	**0.81**	**2.7**	**9**	**30**	**100**
2022-11-16	1	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0
2022-11-19	4	0	0	0	0	0	0	0	0
2022-11-20	5	0	0	0	0	0	0	0	0
2022-11-21	6	0	10	0	0	0	0	10	0
2022-11-22	7	0	10	0	0	0	0	10	0
Total Mortality (%) :		**0**	**10**	**0**	**0**	**0**	**0**	**10**	**0**

REFERENCES

[a] CETIS™, © 2000-2018. V.1.9.4.7. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

[b]Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11* :1-21.

Work Order : 250143
Sample Number : 75494

SURVIVAL AND REPRODUCTION

Test Initiation Date : 2022-11-15
Initiated By : CGR
Initiation Time : 16:10
Test Completion Date : 2022-11-22

Control

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)	Analyst(s)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0	MR
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0	CGR
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0	SJC (KP)
2022-11-19	4	6	6	6	6	4	7	6	5	5	5	5.6	SK
2022-11-20	5	9	10	11	10	10	11	9	9	10	10	9.9	NP(SK)
2022-11-21	6	11	12	0	10	0	0	0	0	0	12	4.5	PG (PC)
2022-11-22	7	0	0	12	0	12	13	12	12	13	0	7.4	SJC (PC)
Total		**26**	**28**	**29**	**26**	**26**	**31[3]**	**27**	**26**	**28**	**27**	**27.4 (±1.6)**	

2.7%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	6	5	4	5	5	6	6	4	6	5	5.2
2022-11-20	5	10	10	7	8	9	13	11	9	10	9	9.6
2022-11-21	6	14	0	0	11	0	0	0	0	0	10	3.5
2022-11-22	7	0	14	13	0	10	13	12	11	11	0	8.4
Total		**30**	**29**	**24**	**24**	**24**	**32**	**29**	**24**	**27**	**24**	**26.7 (±3.1)**

0.07%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	4	5	6	6	6	6	7	7	4	5	5.6
2022-11-20	5	12	11 *	9	11	10	12	10	10	10	11	10.6
2022-11-21	6	12	0	0	0	0	0 x	0	0	0	12	2.4
2022-11-22	7	0	0	12	15	12	0	13	12	12	0	7.6
Total		**28**	**16**	**27**	**32**	**28**	**18**	**30**	**29**	**26**	**28**	**26.2 (±5.1)**

9%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	4	5	4	5	4	3	5	7	4	6	4.7
2022-11-20	5	12	12	9	6	8	10	9	6	11	11	9.4
2022-11-21	6	12	0	13	10	0	0	16	0	0	11	6.2
2022-11-22	7	0	11	0	0	14	14	0	14	11	0	6.4
Total		**28**	**28**	**26**	**21[3]**	**26**	**27**	**30**	**27**	**26**	**28**	**26.7 (±2.4)**

0.24%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	6	5	3	6	6	5	5	6	5	6	5.3
2022-11-20	5	12	10	11	8	9	11	11	11	10	11	10.4
2022-11-21	6	0	10	0	0	0	0	0	0	0	13	2.3
2022-11-22	7	12	0	13	12	12	13	16	12	12	0	10.2
Total		**30**	**25**	**27**	**26**	**27**	**29**	**32**	**29**	**27**	**30**	**28.2 (±2.1)**

30%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	7	7	6	5	6	4	5	5	3	6	5.4
2022-11-20	5	9	9	10	8	11	10	7	10	9	11	9.4
2022-11-21	6	7	0	0	10	0	0 x	0	0	0	14	3.1
2022-11-22	7	–	15	14	0	14	0	11	11	13	0	7.8
Total		**23**	**31**	**30**	**23**	**31**	**14**	**23**	**26**	**25**	**31**	**25.7 (±5.4)**

0.81%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	5	5	6	6	5	5	6	3	6	4	5.1
2022-11-20	5	8	9	9	9	8	10	9	9	8	10	8.9
2022-11-21	6	0	11	0	0	0	0	0	0	0	13	2.4
2022-11-22	7	12	0	11	11	8	8	12	13	12	0	8.7
Total		**25**	**25**	**26**	**26**	**21[3]**	**23**	**27**	**25**	**26**	**27**	**25.1 (±1.9)**

100%

	Day	Replicate 1	2	3	4	5	6	7	8	9	10	Mean Young (±SD)
2022-11-16	1	0	0	0	0	0	0	0	0	0	0	0
2022-11-17	2	0	0	0	0	0	0	0	0	0	0	0
2022-11-18	3	0	0	0	0	0	0	0	0	0	0	0
2022-11-19	4	5	4	6	6	6	4	7	6	4	5	5.3
2022-11-20	5	8	11	12	7	8	9	9	10	10	11	9.5
2022-11-21	6	0	0	9	11	0	0	0	0	0	13	3.3
2022-11-22	7	10	13	0	0	12	12	13	13	14	0	8.7
Total		**23**	**28**	**27**	**24**	**26**	**25**	**29**	**29**	**28**	**29**	**26.8 (±2.2)**

NOTES : •All young produced by a test organism during its fourth and subsequent broods were discarded and not included in the above counts. The presence of two or more neonates in any test chamber, during any given day of the test, constitutes a brood.

•[3] Outlier according to Grubbs Test[b]. Outlying data points were not excluded from statistical analysis, since they could not be attributed to error.

x = test organism mortality

* = accidental test organism mortality

– =4th brood (see 'NOTES')

Test Data Reviewed By : KP

Date : 2022-11-30

Work Order : 250143
Sample Number : 75494

WATER CHEMISTRY DATA

			Day 0 - 1	Day 1 - 2	Day 2 - 3	Day 3 - 4	Day 4 - 5	Day 5 - 6	Day 6 - 7
	Date :		2022-11-15	2022-11-16	2022-11-17	2022-11-18	2022-11-19	2022-11-20	2022-11-21
	Sub-sample Used		1	1	1	2	2	3	3
Initial Chemistry (100 %)	Temperature (°C)		24	24	24	24	24	24	24
	Dissolved O_2 (mg/L)		8.8	8.4	8.4	9.2	9.0	9.6	8.9
	Dissolved O_2 (% Sat.)[4]		109	104	105	114	111	120	109
	pH		7.8	7.9	7.9	7.9	7.9	7.9	8.0
	Conductivity (µmhos/cm)		1535	1533	1528	1532	1528	1533	1525
	Pre-aeration Time (min)[5]		20	20	20	20	20	20	20
	Analyst(s)	Initial	PG (VBC)	CL2 (VBC)	CGR	KM (KP)	ELS (SK)	ELS(SK)	CL2 (PC)
		Final	SK	CGR	SJC (KP)	JJ (SK)	NP	VC	SJC (PC)
Control	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (% Sat.)[4]	Initial	101	101	100	101	96	98	100
	Dissolved O_2 (mg/L)	Initial	8.1	8.3	7.9	8.1	7.7	7.8	8.1
		Final	7.4	6.6	7.2	6.9	7.7	6.9	6.7
	pH	Initial	8.3	8.4	8.3	8.3	8.3	8.4	8.4
		Final	8.1	8.0	8.1	8.1	8.2	8.1	8.0
	Conductivity (µmhos/cm)	Initial	491	540	531	448	430	417	419
0.07 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (mg/L)	Initial	7.8	8.0	7.6	7.9	7.4	7.8	7.5
		Final	7.4	6.7	7.3	6.9	7.7	7.0	6.8
	pH	Initial	8.2	8.2	8.2	8.3	8.2	8.3	8.3
		Final	8.1	8.1	8.1	8.1	8.2	8.1	8.0
	Conductivity (µmhos/cm)	Initial	485	529	493	441	425	415	422
9 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (mg/L)	Initial	8.0	8.2	7.8	7.9	7.5	7.9	7.7
		Final	7.3	6.8	7.4	7.0	7.8	6.9	6.8
	pH	Initial	8.2	8.2	8.2	8.3	8.3	8.3	8.3
		Final	8.1	8.1	8.1	8.2	8.2	8.1	8.1
	Conductivity (µmhos/cm)	Initial	587	630	599	550	538	526	531
100 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	23	24
	Dissolved O_2 (mg/L)	Initial	8.2	8.2	7.9	8.3	8.1	8.3	8.5
		Final	7.2	6.8	7.4	7.0	7.8	6.9	6.8
	pH	Initial	8.0	8.0	8.0	8.1	8.0	8.1	8.0
		Final	8.0	8.0	8.1	8.1	8.2	8.0	8.0
	Conductivity (µmhos/cm)	Initial	1538	1541	1539	1533	1526	1531	1533
	Hardness (mg/L as $CaCO_3$)		550	–	–	–	–	–	–

"–" = not measured/not required
[4] adjusted for temperature and barometric pressure
[5] ≤100 bubbles/minute

Test Data Reviewed By : KP
Date : 2022-11-30

AquaTox Testing & Consulting Inc.
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Tel. (519) 763-4412
Fax. (519) 763-4419

TOXICITY TEST REPORT

Fathead Minnow
EPS 1/RM/22

Work Order : 250143
Sample Number : 75494

SAMPLE IDENTIFICATION

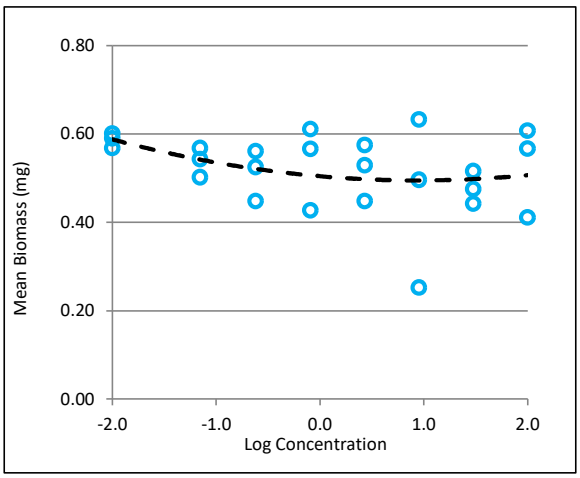
Company :	ALS Laboratory Group - Thunder Bay		
Location :	Thunder Bay ON	Sample Date :	2022-11-09
Job Number :	L2740058-3	Time Collected :	16:00
Substance :	L2740058-3 EDL2	Date Received :	2022-11-14
Sampling Method :	Not provided	Time Received :	11:45
Sampled By :	Not provided	Temperature at Receipt :	15 °C
Sample Description :	Clear, light yellow, settled solids	Date Tested :	2022-11-15

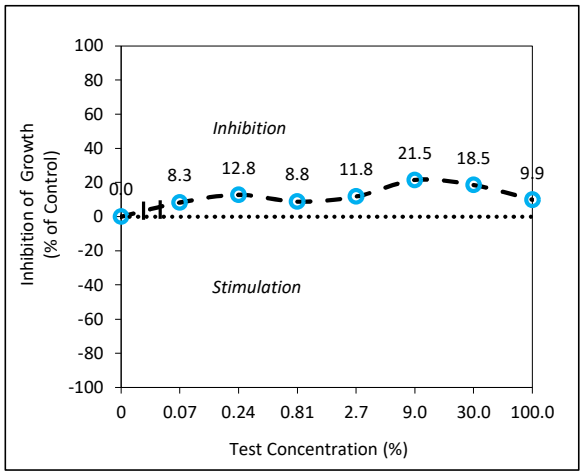
Test Method : Test of Larval Growth and Survival Using Fathead Minnows. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/22 , 2nd ed. (February 2011).

7-DAY TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Biomass)[1]	>100%	–	Linear Interpolation (CETIS)[a]
LC50	>100%	–	–

The results reported relate only to the sample tested and as received.





COMMENTS

Noted Deviation: The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

[1]as a measure of Growth

•All test validity criteria as specified in the test method cited above were satisfied.

•Statistical analysis for the IC25 (Biomass)[1] endpoint could not be conducted using Non-Linear Regression, since a suitable model could not be identified. Therefore, test results were calculated using Linear Interpolation (CETIS)[a].

Approved By : _____

Project Manager

Work Order : 250143
Sample Number : 75494

TEST ORGANISM

Test Organism :	*Pimephales promelas*	Culture Mortality/Diseased :	0 % (previous 7 days)
Organism Batch :	Fm22-11	Organism Age :	~07:00 - 23:45 h at test start
Source :	In-house culture		

•No organisms exhibiting unusual appearance, behaviour, or undergoing unusual treatment were used in the test.
•Inflated swim bladders were confirmed in all test organisms used in this test.

TEST CONDITIONS

Test Type :	Static Renewal	Control/Dilution Water :	Well water [3]
Renewal Method :	80-85% syphoned and replaced	Test Volume / Replicate :	300 mL
Renewal Frequency :	≤ 24 hours	Test Vessel :	420 mL polystyrene beaker
Sample Filtration :	None	Depth of Test Solution :	8 cm
Test Aeration :	None	Organisms per Replicate :	10
pH Adjustment :	None	Number of Replicates :	3
Hardness Adjustment :	None	Test Method Deviation(s):	Yes (see 'Comments')

[3]no additional chemicals

REFERENCE TOXICANT DATA

Toxicant :	Potassium Chloride	Analyst(s) :	CGR, NP, XD
Date Tested :	2022-11-01	Test Duration :	7 days
IC25 (Biomass)1 :	0.98 g/L	LC50 :	1.07 g/L
95% Confidence Limits :	0.83 - 1.07 g/L	95% Confidence Limits :	1.00 - 1.14 g/L
Statistical Method :	Non-Linear Regression (CETIS)[a]	Statistical Method :	Linear Regression (MLE) (CETIS)[a]
Historical Mean IC25 :	1.01 g/L	Historical Mean LC50 :	1.14 g/L
Warning Limits (± 2SD) :	0.92 - 1.10 g/L	Warning Limits (± 2SD) :	1.01 - 1.29 g/L

[1]as a measure of Growth

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

REFERENCES

[a] CETIS™, © 2000-2018. V.1.9.4.7. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

[b]Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. *Technometrics, 11* :1-21.

Work Order : 250143
Sample Number : 75494

CUMULATIVE DAILY CONTROL MORTALITY AND IMPAIRMENT

Date :	2022-11-15	2022-11-16	2022-11-17	2022-11-18	2022-11-19	2022-11-20	2022-11-21	2022-11-22
Mortality/Impairment :	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	3.33%
Standard Deviation :	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±5.8)

CUMULATIVE DAILY MORTALITY

Initiation Time : 15:45
Initiation Date : 2022-11-15
Completion Date : 2022-11-22

Date :		Day 0 2022-11-15		Day 1 2022-11-16		Day 2 2022-11-17		Day 3 2022-11-18		Day 4 2022-11-19		Day 5 2022-11-20		Day 6 2022-11-21		Day 7 2022-11-22		Treatment Mean Mortality (± SD)
Analyst(s):		AW		AW		SV		JJ (VBC)		ELS(SK)		ELS(SK)		PC		ELS (PC)		
Concentration		Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	Number Dead	**% Dead**	**%**
%	**Replicate**																	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**3.33**
Control	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	1	**10**	(±5.77)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
0.07	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
0.24	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**3.33**
0.81	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±5.77)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	1	**10**	1	**10**	1	**10**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.00**
2.7	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±0.00)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	4	**40**	5	**50**	5	**50**	5	**50**	**16.67**
9	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±28.87)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	1	**10**	1	**10**	**3.33**
30	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±5.77)
	C	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	
	A	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**10.00**
100	B	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	(±17.32)
	C	0	**0**	0	**0**	0	**0**	0	**0**	3	**30**	3	**30**	3	**30**	3	**30**	

Aberrant behaviour or swimming impairment : 2022-11-21: One fish is swimming in circles in the 30% concentration, Replicate B (PC).

Test Data Reviewed By : KP
Date : 2022-12-01

Work Order : 250143
Sample Number : 75494

DRY WEIGHT AND BIOMASS DATA

Concentration %	Replicate	Number Exposed	Replicate Mean Dry Weight (mg)	Treatment Mean Biomass (mg)	Standard Deviation
Control	A	10	0.601	0.586	0.017
	B	10	0.590		
	C	10	0.568		
0.07	A	10	0.502	0.538	0.033
	B	10	0.543		
	C	10	0.568		
0.24	A	10	0.525	0.511	0.058
	B	10	0.561		
	C	10	0.448		
0.81	A	10	0.566	0.535	0.096
	B	10	0.427		
	C	10	0.611		
2.7	A	10	0.575	0.517	0.064
	B	10	0.448		
	C	10	0.529		
9	A	10	0.252	0.460	0.193
	B	10	0.633		
	C	10	0.496		
30	A	10	0.442	0.478	0.037
	B	10	0.475		
	C	10	0.516		
100	A	10	0.567	0.528	0.104
	B	10	0.607		
	C	10	0.411		

NOTES : •No outlying data points were detected according to Grubbs Test[b].

• Control average dry weight per surviving organism = 0.608 mg

Test Data Reviewed By : KP

Date : 2022-12-01

Work Order : 250143
Sample Number : 75494

WATER CHEMISTRY DATA

			Day 0 - 1 2022-11-15	Day 1 - 2 2022-11-16	Day 2 - 3 2022-11-17	Day 3 - 4 2022-11-18	Day 4 - 5 2022-11-19	Day 5 - 6 2022-11-20	Day 6 - 7 2022-11-21
Initial Chemistry (100%)	Sub-sample Used		1	1	1	2	2	3	3
	Temperature (°C)		24	24	24	24	24	24	24
	Dissolved O_2 (mg/L)		8.8	8.4	8.4	9.2	9.0	9.6	8.9
	Dissolved O_2 % Sat.[5]		109	104	105	114	111	120	109
	pH		7.8	7.9	7.9	7.9	7.9	7.9	8.0
	Conductivity (µmhos/cm)		1535	1533	1528	1532	1528	1533	1525
	Pre-aeration Time (min)[6]		20	20	20	20	20	20	20
	Analyst(s) :	Initial	PG (VBC)	CL2 (VBC)	CGR	KM (KP)	ELS (SK)	ELS(SK)	CL2 (PC)
		Final	AW	ELS (VBC)	JJ (VBC)	ELS (SK)	SK	VC	ELS (PC)
Control	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 % Sat.[5]	Initial	101	101	100	101	96	98	100
	Dissolved O_2 (mg/L)	Initial	8.1	8.3	7.9	8.1	7.7	7.8	8.1
		Final	7.9	7.1	7.1	6.9	7.3	7.3	6.4
	pH	Initial	8.3	8.4	8.3	8.3	8.3	8.4	8.4
		Final	8.1	8.1	8.0	8.1	8.1	8.1	8.0
	Conductivity (µmhos/cm)	Initial	491	540	531	448	430	417	419
0.07 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 (mg/L)	Initial	7.8	8.0	7.6	7.9	7.4	7.8	7.5
		Final	7.8	7.1	7.1	6.8	7.2	7.3	6.7
	pH	Initial	8.2	8.2	8.2	8.3	8.2	8.3	8.3
		Final	8.2	8.1	8.0	8.1	8.1	8.1	8.1
	Conductivity (µmhos/cm)	Initial	485	529	493	441	425	415	422
9 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 (mg/L)	Initial	8.0	8.2	7.8	7.9	7.5	7.9	7.7
		Final	7.8	7.1	7.0	6.8	7.0	7.3	6.8
	pH	Initial	8.2	8.2	8.2	8.3	8.3	8.3	8.3
		Final	8.2	8.1	8.0	8.1	8.0	8.1	8.0
	Conductivity (µmhos/cm)	Initial	587	630	599	550	538	526	531
100 %	Temperature (°C)	Initial	24	24	25	24	24	24	24
		Final	24	23	23	23	23	24	25
	Dissolved O_2 (mg/L)	Initial	8.2	8.2	7.9	8.3	8.1	8.3	8.5
		Final	7.8	7.1	6.9	6.7	6.9	7.3	6.8
	pH	Initial	8.0	8.0	8.0	8.1	8.0	8.1	8.0
		Final	8.2	8.0	7.9	8.0	7.9	8.0	7.9
	Conductivity (µmhos/cm)	Initial	1538	1541	1539	1533	1526	1531	1533
	Hardness (mg/L as $CaCO_3$)		550	–	–	–	–	–	–

"–" = not measured/not required

[5] adjusted for temperature and barometric pressure

[6] ≤100 bubbles/minute

Test Data Reviewed By : KP

Date : 2022-12-01

AquaTox Testing & Consulting Inc.
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Tel. (519) 763-4412
Fax. (519) 763-4419

TOXICITY TEST REPORT

Pseudokirchneriella subcapitata
EPS 1/RM/25

Work Order : 250143
Sample Number : 75494

SAMPLE IDENTIFICATION

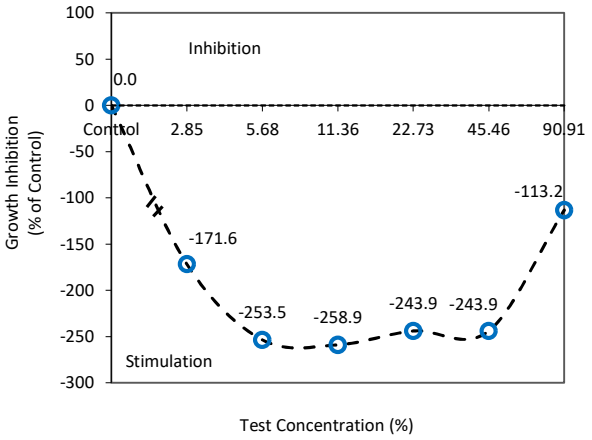
Company :	ALS Laboratory Group - Thunder Bay	Sample Date :	2022-11-09
Location :	Thunder Bay ON	Time Collected :	16:00
Job Number:	L2740058-3	Date Received :	2022-11-14
Substance :	L2740058-3 EDL2	Time Received :	11:45
Sampling Method :	Not provided	Temperature at Receipt :	15 °C
Sampled By :	Not provided	Date Tested :	2022-11-15
Sample Description :	Clear, light yellow, settled solids		

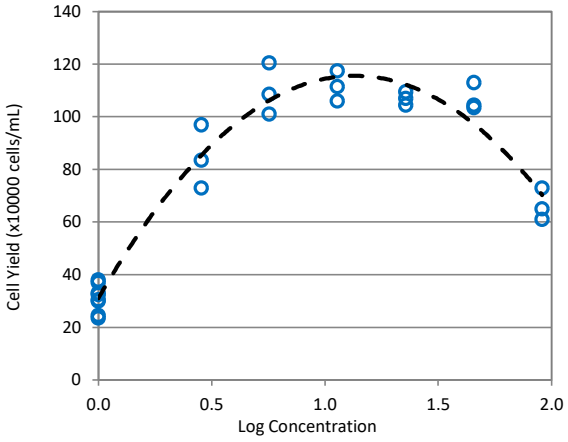
Test Method : Growth Inhibition Test Using a Freshwater Alga. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/25, 2nd ed. (March 2007).

72-HOUR TEST RESULTS

Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Growth)	>90.91%	–	–

The results reported relate only to the sample tested and as received.





REFERENCE TOXICANT DATA

Toxicant :	Zinc (as Zinc Sulfate)		
Date Tested :	2022-11-08	IC25 Growth :	28.8 µg/L
Analyst(s) :	AS, VBC	95% Confidence Limits :	18.7 - 35.8 µg/L
Test Duration :	72 hours	Historical Mean IC25 :	19.0 µg/L
Statistical Method :	Non-Linear Regression (CETIS)[a]	Warning Limits (± 2SD) :	10.1 - 35.9 µg/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

COMMENTS

The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

•All test validity criteria as specified in the test method cited above were satisfied.

Approved By : _____
Project Manager

Work Order : 250143
Sample Number : 75494

TEST ORGANISM

Test Organism :	*Pseudokirchneriella subcapitata*	Organism Batch :	Ps22-11/3
Culture Origin :	University of Waterloo, Waterloo ON	Cell Density at 0-h :	10136 cells/mL
Strain Number :	CPCC 37	Inoculum Prepared :	60 min. prior to test initiation
Inoculum Source :	In-house culture	Age (at start of test) :	4 days (in exponential growth)

•Algal growth curve is determined at least twice per year as required by the test method.
•No unusual appearance or treatment of culture prior to testing.

TEST CONDITIONS

Test Type :	Static	Volume per Replicate :	220 µL
Test Duration :	72 hours	Control Replicates :	10
Mean Temperature (± SD):	24.0°C (± 0.0)	Test Replicates :	4
Sample Pre-aeration :	None	Concentrations Tested :	10 + Control
Sample Filtration :	0.45 µm preconditioned filter	Photoperiod :	Continuous light
Volume Filtered:	≥10 mL	Light Intensity :	4020-4230 lux
Control/Dilution Water :	Millipore Milli-Q (no chemicals added)	Initial pH (100% sample) :	7.9
Enrichment Medium :	Stock 2B: EDTA reduced to 25%	pH Adjustment :	None
Test Vessel :	U-shaped polystyrene microplate	Hardness Adjustment :	None
Enumeration Method :	Manual (haemocytometer)	Test Method Deviation(s) :	See 'Comments'

CELL COUNTS AT 72-HOURS

Initiated By :	AS	Control pH (at 0 hours) :	6.5
Date Counted :	2022-11-18	Control pH (at 72 hours) :	6.5
Counted By :	NP/AS	Control Increase Factor :	31.7 times growth

Concentration	Cell Concentration (x 10000 cells/mL) Replicate								Cell Yield (x 10000 cells/mL)				
%	1	2	3	4	7	8	9	10	Mean	Standard Deviation	CV (%)	Stimulation (% of control)	
Control	31.5	34.0	31.0	33.5	39.0	24.5	25.5	38.0	31.11	5.22	16.8	–	
0.18	–	–	–	–	–	–	–	–	–	–	–	–	
0.35	–	–	–	–	–	–	–	–	–	–	–	–	
0.71	–	–	–	–	–	–	–	–	–	–	–	–	
1.42	–	–	–	–	–	–	–	–	–	–	–	–	
2.85	84.5	98.0	74.0	–	–	–	–	–	84.49	12.03	14.2	171.6	*
5.68	109.5	102.0	121.5	–	–	–	–	–	109.99	9.84	8.9	253.5	*
11.36	107.0	112.5	118.5	–	–	–	–	–	111.65	5.75	5.2	258.9	*
22.73	105.5	108.0	110.5	–	–	–	–	–	106.99	2.50	2.3	243.9	*
45.46	114.0	105.5	104.5	–	–	–	–	–	106.99	5.22	4.9	243.9	*
90.91	62.0	66.0	74.0	–	–	–	–	–	66.32	6.11	9.2	113.2	*

NOTES : *Statistically significant stimulation, according to ANOVA/Dunnett Multiple Comparison Test (CETIS)[a], (α=0.05)

- Control replicates 5 and 6 used for pH measurement.
- The Mann-Kendall test shows that there is no inhibitory gradient (α=0.05).
- No outlying data points were detected according to Grubbs Test (CETIS)[a].

Test Data Reviewed By : EK

"–" = not counted/not required

Date : 2022-12-15

REFERENCES

[a] CETIS™, © 2000-2022. V.2.1.4.0. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

AquaTox Testing & Consulting Inc.
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Tel. (519) 763-4412
Fax. (519) 763-4419

TOXICITY TEST REPORT

Lemna minor
EPS 1/RM/37

Work Order : 250143
Sample Number : 75494

SAMPLE IDENTIFICATION

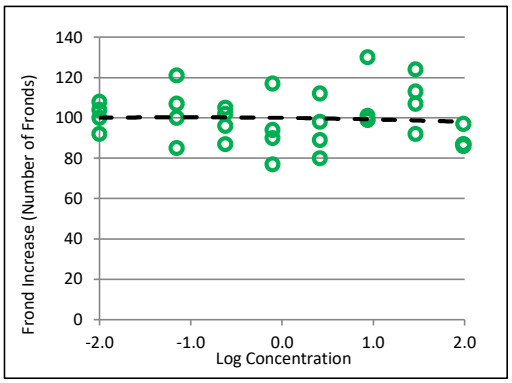
Company :	ALS Laboratory Group - Thunder Bay	Sample Date :	2022-11-09
Location :	Thunder Bay ON	Time Collected :	16:00
Job Number:	2740058-3	Date Received :	2022-11-14
Substance :	L2740058-3 EDL2	Time Received :	11:45
Sampling Method :	Not provided	Temperature at Receipt :	15 °C
Sampled By :	Not provided	Date Tested :	2022-11-16
Sample Description :	Clear, light yellow, settled solids		

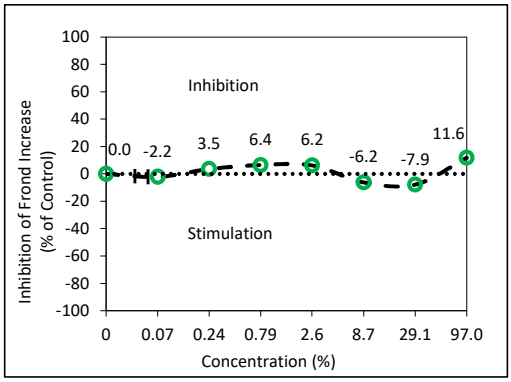
Test Method : Test for Measuring the Inhibition of Growth using the Freshwater Macrophyte, *Lemna minor*. Method Development and Application Section, Environmental Technology Centre, Environment Canada. Ottawa, Ontario. Report EPS 1/RM/37, 2nd ed. (January 2007).

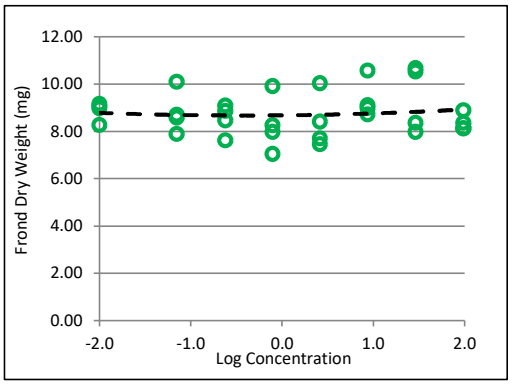
7-DAY TEST RESULTS

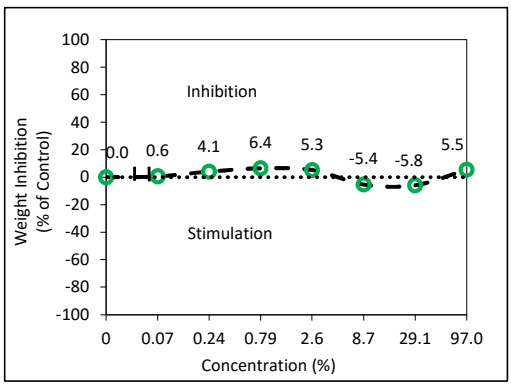
Effect	Value	95% Confidence Limits	Statistical Method
IC25 (Frond Increase)	>97.0%	–	–
IC25 (Dry Weight)	>97.0%	–	–

The results reported relate only to the sample tested and as received.









COMMENTS

The maximum sample holding time of 3 days allowed by the test method was exceeded. The sample was tested with the client's consent. There were no other unusual conditions or deviations from the test method, and the test is considered to be valid.

•All test validity criteria as specified in the test method cited above were satisfied.

Approved By : _____

Project Manager

Work Order : 250143
Sample Number : 75494

TEST ORGANISM

Test Organism :	*Lemna minor* L., Strain 7730	Age (on Test Day 0) :	8 days
Organism Batch :	Lm22-11	Inoculum (Test Day 0) :	2 plants (3 fronds per plant)
Culture Origin :	UTCC 492	Culture Medium :	Modified Hoaglands E+
Test Organism Source :	Axenic in-house culture	Health Criteria (in APHA) :	18.2-fold frond increase in 7 days
Organism Acclimation :	21:20 hours (in APHA medium)		

No unusual appearance or treatment of culture prior to testing. Test inoculated with healthy plants.

TEST CONDITIONS

Test Type :	Static (no sub-samples required)	Sample Pre-aeration :	20 min. at ≤100 bubbles/min.
Control/Dilution Medium :	Modified APHA	pH Adjustment :	None
Source of Water :	Well Water	Hardness Adjustment :	None
Medium Preparation Water :	Reverse Osmosis	Replicates per Concentration :	4
Medium Preparation Chemicals :	Modified APHA [1]	Test Volume per Replicate :	100 mL
Nutrient Spiking of Sample :	Modified APHA [1]	Test Vessel :	200 mL glass jar
Initial pH :	7.9 (100% sample)	Depth of Test Solution :	4.0 cm
Initial temperature :	23 °C (100% sample)	Photoperiod/Light Intensity :	Continuous, 4120 - 4910 lux
Sample Filtration :	1 µm (Whatman GF/C)	Test Method Deviation(s) :	Yes (see 'COMMENTS')

[1] stocks A, B, C (10 mL/L)

REFERENCE TOXICANT DATA

Toxicant :	Potassium Chloride	IC25 (Frond Production) :	1.76 g/L
Date Tested :	2022-11-10	95% Confidence Limits :	1.15 - 2.35 g/L
Test Duration :	7 days	Statistical Method :	Linear Interpolation (CETIS)[a]
Analyst(s) :	NP	Historical Mean IC25 :	2.19 g/L
Growth Medium :	Modified APHA	Warning Limits (± 2SD) :	1.74 - 2.74 g/L

The reference toxicity test was performed under the same experimental conditions as those used with the test sample.

TEST MONITORING

Intitiation Time :	13:10	Termination Time :	15:25
Initiated By :	NP/VBC	Terminated By :	VBC

Test Day :		0	1	2	3	4	5	6	7
Date :		2022-11-16	2022-11-17	2022-11-18	2022-11-19	2022-11-20	2022-11-21	2022-11-22	2022-11-23
Temperature (° C) :		24	25	25	24	24	24	25	25
pH	Control	8.4	–	–	–	–	–	–	8.6
	0.07	8.3	–	–	–	–	–	–	8.7
	0.24	–	–	–	–	–	–	–	–
	0.79	–	–	–	–	–	–	–	–
	2.6	8.3	–	–	–	–	–	–	8.6
	8.7	–	–	–	–	–	–	–	–
	29.1	–	–	–	–	–	–	–	–
	97	8.1	–	–	–	–	–	–	8.8

"–" = not required

REFERENCES

[a] CETIS™, ©2000-2022. V.2.1.4.0. Comprehensive Environmental Toxicity Information System. Tidepool Scientific Software, LLC, McKinleyville, CA 95519 [Program on disk and printed User's Guide].

Work Order : 250143
Sample Number : 75494

DAY 7 FROND COUNT DATA

Test Concentration %	Replicate	Frond Count	Frond Increase	Mean Frond Increase	Standard Deviation	Stimulation (%)	Frond/Root Appearance
Control	A	110	104	101.00	6.8	–	Fronds healthy, appearance normal in all replicates.
	B	98	92				
	C	114	108				
	D	106	100				
0.07	A	127	121	103.25	15.0	2.2	Fronds healthy, appearance normal in all replicates.
	B	91	85				
	C	106	100				
	D	113	107				
0.24	A	93	87	97.50	7.9	-3.5	Fronds healthy, appearance normal in all replicates.
	B	111	105				
	C	102	96				
	D	108	102				
0.79	A	123	117	94.50	16.7	-6.4	Fronds healthy, appearance normal in all replicates.
	B	96	90				
	C	100	94				
	D	83	77				
2.6	A	118	112	94.75	13.6	-6.2	Fronds healthy, appearance normal in all replicates.
	B	104	98				
	C	86	80				
	D	95	89				
8.7	A	105	99	107.25	15.2	6.2	Fronds healthy, appearance normal in all replicates.
	B	105	99				
	C	136	130				
	D	107	101				
29.1	A	119	113	109.00	13.3	7.9	Fronds healthy, appearance normal in all replicates.
	B	113	107				
	C	130	124				
	D	98	92				
97.0	A	93	87	89.25	5.2	-11.6	Some fronds are yellow, and root some destruction in all replicates.
	B	92	86				
	C	103	97				
	D	93	87				

NOTES:

•No statistically significant stimulation (α=0.05) was detected by ANOVA-Dunnett Multiple Comparison Test (CETIS)[a] at any test level compared to the control.

•A 16.8-fold increase in frond number was observed in the control over the testing period.

•No outlying data points were detected according to Grubbs Test (CETIS)[a].

Test Data Reviewed By : EK
Date : 2022-12-15

"–" = not available/not required

Work Order : 250143
Sample Number : 75494

DAY 7 FROND WEIGHT DATA

Test Concentration %	Replicate	Dry Weight of Fronds (mg)	Treatment Mean Dry Weight (mg)	Standard Deviation	Stimulation (%)
Control	A	9.07	8.87	0.4	–
	B	8.27			
	C	8.98			
	D	9.16			
0.07	A	10.10	8.82	0.9	-0.6
	B	7.89			
	C	8.70			
	D	8.59			
0.24	A	7.62	8.51	0.6	-4.1
	B	8.87			
	C	9.09			
	D	8.46			
0.79	A	9.91	8.30	1.2	-6.4
	B	7.98			
	C	8.26			
	D	7.05			
2.6	A	10.04	8.40	1.2	-5.3
	B	8.41			
	C	7.70			
	D	7.46			
8.7	A	8.72	9.35	0.8	5.4
	B	8.99			
	C	10.57			
	D	9.10			
29.1	A	10.68	9.39	1.4	5.8
	B	8.36			
	C	10.53			
	D	7.98			
97.0	A	8.14	8.38	0.4	-5.5
	B	8.36			
	C	8.90			
	D	8.13			

NOTES :

•No statistically significant stimulation (α=0.05) was detected by ANOVA-Dunnett Multiple Comparison Test (CETIS)[a] at any test level compared to the control.

•No outlying data points were detected according to Grubbs Test (CETIS)[a].

Test Data Reviewed By : EK
Date : 2022-12-15

"–" = not available/not required

L2740058

THUNDERBAY

ALS Environmental

Subcontract Request Form

Subcontract To:

AquaTox Testing & Consulting Inc. **ING**
B-11 Nicholas Beaver Road
Puslinch, ON N0B 2J0
Ph: (519) 763-4412

Fathead minnow 7-day
Ceriodaphnia dubia 3-brood
Lemna minor 7-day
Freshwater alga 72-hour

NOTES: Please rereference on final report and invoice: PO# *L2740058*
ALS requires QC data to be provided with your final results.

Please see enclosed 3 sample(s) in 0 Container(s)

SAMPLE NUMBER	ANALYTICAL REQUIRED	DATE SAMPLED / DUE DATE	Priority Flag
L2740058-1 EDL1		**11/9/2022**	
Sample# 75492	Special Request Aquatox (SPECIAL REQUEST-AQT 14)	12/9/2022	
L2740058-2 SED2DIS		**11/9/2022**	
Sample# 75493	Special Request Aquatox (SPECIAL REQUEST-AQT 14)	12/9/2022	
L2740058-3 EDL2		**11/9/2022**	
Sample# 75494	Special Request Aquatox (SPECIAL REQUEST-AQT 14)	12/9/2022	

Subcontract Info Contact: Thunder Bay Login (807) 623-6463
Analysis and reporting info contact: Christine Paradis
1081 BARTON STREET
THUNDER BAY,ON P7B 5N3
Phone: (807) 623-6463 Email: christine.paradis@alsglobal.com

Please email confirmation of receipt to: **christine.paradis@alsglobal.com**

Shipped By: _____ Date Shipped: _____

Received By: VC Date Received: 2022-11-14

Verified By: _____ Date Verified: _____

Temperature: 15°C

Sample Integrity Issues: _____

EDL1 - time collected 15:15 *
SED2DIS - time collected 12:20 *
EDL2 - time collected 16:00 *
* As per pail label (VC) 2022-11-14

Aquatox workorder #250143.

Chain of Custody (COC) / Analytical Request Form

www.alsglobal.com

Canada Toll Free: 1 800 668 9878

L2740058 COFC

Report To	Contact and company name below will appear on the final report
Company:	New Gold
Contact:	Garnet Comell
Phone:	
	Company address below will appear on the final report
Street:	
City/Province:	
Postal Code:	
Invoice To	Same as Report To ☐ YES ☐ NO
	Copy of Invoice with Report ☐ YES ☐ NO
Company:	
Contact:	

Reports / Recipients

Select Report Format: ☐ PDF ☐ EXCEL ☐ EDD (DIGITAL)

Merge QC/QCI Reports with COA ☐ YES ☐ NO ☐ N/A

☐ Compare Results to Criteria on Report - provide details below if box checked

Select Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Email 3

Invoice Recipients

Select Invoice Distribution: ☐ EMAIL ☐ MAIL ☐ FAX

Email 1

Email 2

Turnaround Time (TAT) Requested

☐ Routine [R] if received by 3pm M-F - no surcharges apply
☐ 4 day [P4] if received by 3pm M-F - 20% rush surcharge minimum
☐ 3 day [P3] if received by 3pm M-F - 25% rush surcharge minimum
☐ 2 day [P2] if received by 3pm M-F - 50% rush surcharge minimum
☐ 1 day [E] if received by 3pm M-F - 100% rush surcharge minimum
☐ Same day [E2] if received by 10am M-S - 200% rush surcharge.

Additional fees may apply to rush requests on weekends, statutory holidays and for non-routine tests.

Date and Time Required for all E&P TATs: dd-mmm-yy hh:mm am/pm

For all tests with rush TATs requested, please contact your AM to confirm availability.

AFFIX ALS BARCODE LABEL HERE (ALS use only)

Project Information		Oil and Gas Required Fields (client use)	
ALS Account # / Quote #:		AFE/Cost Center:	PO#
Job #:		Major/Minor Code:	Routing Code:
PO / AFE:		Requisitioner:	
LSD:		Location:	
ALS Lab Work Order # (ALS use only):	L2740058	ALS Contact:	Sampler:

Analysis Request

Indicate Filtered (F), Preserved (P) or Filtered and Preserved (F/P) below

ALS Sample # (ALS use only)	Sample Identification and/or Coordinates (This description will appear on the report)	Date (dd-mmm-yy)	Time (hh:mm)	Sample Type	NUMBER OF CONTAINERS	F/P - NG-ST-P-TB	SAMPLES ON HOLD	EXTENDED STORAGE REQUIRED	SUSPECTED HAZARD (see notes)
	EDL1	09-Nov-22	15:15	EFF	3	X			
	SED2DIS	9-Nov-22	16:00	EFF	3	X			
	EDL2	9-Nov-22	12:20	EFF	3	X			

Drinking Water (DW) Samples[1] (client use)

Are samples taken from a Regulated DW System? ☐ YES ☐ NO

Are samples for human consumption/ use? ☐ YES ☐ NO

Notes / Specify Limits for result evaluation by selecting from drop-down below (Excel COC only)

SAMPLE RECEIPT DETAILS (ALS use only)

Cooling Method: ☐ NONE ☐ ICE ☐ ICE PACKS ☐ FROZEN ☐ COOLING INITIATED

Submission Comments identified on Sample Receipt Notification: ☐ YES ☐ NO

Cooler Custody Seals Intact: ☐ YES ☐ N/A Sample Custody Seals Intact: ☐ YES ☐ N/A

INITIAL COOLER TEMPERATURES °C				FINAL COOLER TEMPERATURES °C			
14.6							

SHIPMENT RELEASE (client use)			INITIAL SHIPMENT RECEPTION (ALS use only)			FINAL SHIPMENT RECEPTION (ALS use only)		
Released by:	Date:	Time:	Received by: CP	Date: 10-Nov-22	Time: 2:45	Received by:	Date:	Time:

REFER TO BACK PAGE FOR ALS LOCATIONS AND SAMPLING INFORMATION

WHITE - LABORATORY COPY YELLOW - CLIENT COPY

AUG 2020 FRONT

Failure to complete all portions of this form may delay analysis. Please fill in this form LEGIBLY. By the use of this form the user acknowledges and agrees with the Terms and Conditions as specified on the back page of the white - report copy.

1. If any water samples are taken from a Regulated Drinking Water (DW) System, please submit using an Authorized DW COC form.

CERTIFICATE OF ANALYSIS

Work Order	: **TY2303086**	Page	: 1 of 3
Client	: **New Gold Inc. (Rainy River)**	Laboratory	: Thunder Bay - Environmental
Contact	: Garnet Cornell	Account Manager	: Christine Paradis
Address	: 24 Marr Rd. Barwick ON Canada P0W 1A0	Address	: 1081 Barton Street Thunder Bay ON Canada P7B 5N3
Telephone	: 807-234-8170	Telephone	: +1 807 623 6463
Project	: Sublethal Toxicity	Date Samples Received	: 18-Apr-2023 09:40
PO	: 4500062842	Date Analysis Commenced	: 20-Apr-2023
C-O-C number	: ----	Issue Date	: 08-May-2023 14:10
Sampler	: ----		
Site	: ----		
Quote number	: New Gold Rainy River Project - Picka Project		
No. of samples received	: 3		
No. of samples analysed	: 3		

This report supersedes any previous report(s) with this reference. Results apply to the sample(s) as submitted. This document shall not be reproduced, except in full.

This Certificate of Analysis contains the following information:

- General Comments
- Analytical Results

Additional information pertinent to this report will be found in the following separate attachments: Quality Control Report, QC Interpretive report to assist with Quality Review and Sample Receipt Notification (SRN).

Signatories

This document has been electronically signed by the authorized signatories below. Electronic signing is conducted in accordance with US FDA 21 CFR Part 11.

Signatories	*Position*	*Laboratory Department*
Daron Mooney	Account Manager	External Subcontracting, Point Edward, Ontario

General Comments

The analytical methods used by ALS are developed using internationally recognized reference methods (where available), such as those published by US EPA, APHA Standard Methods, ASTM, ISO, Environment Canada, BC MOE, and Ontario MOE. Refer to the ALS Quality Control Interpretive report (QCI) for applicable references and methodology summaries. Reference methods may incorporate modifications to improve performance.

Where a reported less than (<) result is higher than the LOR, this may be due to primary sample extract/digestate dilution and/or insufficient sample for analysis.

Where the LOR of a reported result differs from standard LOR, this may be due to high moisture content, insufficient sample (reduced weight employed) or matrix interference.

Please refer to Quality Control Interpretive report (QCI) for information regarding Holding Time compliance.

Key : CAS Number: Chemical Abstracts Services number is a unique identifier assigned to discrete substances

LOR: Limit of Reporting (detection limit).

Unit	*Description*
-	no units

<: less than.

>: greater than.

Surrogate: An analyte that is similar in behavior to target analyte(s), but that does not occur naturally in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery.

Test results reported relate only to the samples as received by the laboratory.

UNLESS OTHERWISE STATED on SRN or QCI Report, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.

Analytical Results

Sub-Matrix: Water (Matrix: Water)				Client sample ID	EDL1	SED2DIS	EDL2	----	----
				Client sampling date / time	17-Apr-2023 08:15	17-Apr-2023 08:40	17-Apr-2023 07:40	----	----
Analyte	CAS Number	Method	LOR	Unit	TY2303086-001	TY2303086-002	TY2303086-003	--------	--------
					Result	Result	Result	----	----
Bioassays									
Ceriodaphnia dubia, LC50	----	CER-LC50-7d	-	-	see attached	see attached	see attached	----	----
Fathead minnow, survival	n/a	FAT-LC50-7d	-	-	See attached	See attached	See attached	----	----
Green algae, growth	n/a	ALG-IC50-72h	-	-	See attached	See attached	See attached	----	----
Green algae, IC25	n/a	ALG-IC50-72h	-	-	See attached	See attached	See attached	----	----
Lemna minor, dry weight	n/a	LEM-IC50-7d	-	-	See attached	See attached	See attached	----	----
Lemna minor, frond count	n/a	LEM-IC50-7d	-	-	See attached	See attached	See attached	----	----

Please refer to the General Comments section for an explanation of any qualifiers detected.

QUALITY CONTROL INTERPRETIVE REPORT

Work Order	: **TY2303086**	Page	: 1 of 5
Client	: **New Gold Inc. (Rainy River)**	Laboratory	: Thunder Bay - Environmental
Contact	: Garnet Cornell	Account Manager	: Christine Paradis
Address	: 24 Marr Rd. Barwick ON Canada P0W 1A0	Address	: 1081 Barton Street Thunder Bay, Ontario Canada P7B 5N3
Telephone	: 807-234-8170	Telephone	: +1 807 623 6463
Project	: Sublethal Toxicity	Date Samples Received	: 18-Apr-2023 09:40
PO	: 4500062842	Issue Date	: 08-May-2023 14:10
C-O-C number	: ----		
Sampler	: ----		
Site	: ----		
Quote number	: New Gold Rainy River Project - Picka Project		
No. of samples received	: 3		
No. of samples analysed	: 3		

This report is automatically generated by the ALS LIMS (Laboratory Information Management System) through evaluation of Quality Control (QC) results and other QA parameters associated with this submission, and is intended to facilitate rapid data validation by auditors or reviewers. The report highlights any exceptions and outliers to ALS Data Quality Objectives, provides holding time details and exceptions, summarizes QC sample frequencies, and lists applicable methodology references and summaries.

Key

Anonymous: Refers to samples which are not part of this work order, but which formed part of the QC process lot.
CAS Number: Chemical Abstracts Service number is a unique identifier assigned to discrete substances.
DQO: Data Quality Objective.
LOR: Limit of Reporting (detection limit).
RPD: Relative Percent Difference.

Workorder Comments

Holding times are displayed as "---" if no guidance exists from CCME, Canadian provinces, or broadly recognized international references.

Summary of Outliers

Outliers : Quality Control Samples

- No Test sample Surrogate recovery outliers exist.

Outliers: Reference Material (RM) Samples

- No Reference Material (RM) Sample outliers occur.

Outliers : Analysis Holding Time Compliance (Breaches)

- No Analysis Holding Time Outliers exist.

Outliers : Frequency of Quality Control Samples

- No Quality Control Sample Frequency Outliers occur.

Analysis Holding Time Compliance

This report summarizes extraction / preparation and analysis times and compares each with ALS recommended holding times, which are selected to meet known provincial and /or federal requirements. In the absence of regulatory hold times, ALS establishes recommendations based on guidelines published by organizations such as CCME, US EPA, APHA Standard Methods, ASTM, or Environment Canada (where available). Dates and holding times reported below represent the first dates of extraction or analysis. If subsequent tests or dilutions exceeded holding times, qualifiers are added (refer to COA).

If samples are identified below as having been analyzed or extracted outside of recommended holding times, measurement uncertainties may be increased, and this should be taken into consideration when interpreting results.

Where actual sampling date is not provided on the chain of custody, the date of receipt with time at 00:00 is used for calculation purposes.

Where only the sample date without time is provided on the chain of custody, the sampling date at 00:00 is used for calculation purposes.

Matrix: **Water**

Evaluation: × = Holding time exceedance ; ✓ = Within Holding Time

Analyte Group / Container / Client Sample ID(s)	Method	Sampling Date	Extraction / Preparation				Analysis			
			Preparation Date	Holding Times Rec	Holding Times Actual	Eval	Analysis Date	Holding Times Rec	Holding Times Actual	Eval
Bioassays : Growth/IC Green Algae (P. subcapitata) 72 hour										
LDPE carboy EDL1	ALG-IC50-72h	17-Apr-2023	----	----	----		20-Apr-2023	----	----	
Bioassays : Growth/IC Green Algae (P. subcapitata) 72 hour										
LDPE carboy EDL2	ALG-IC50-72h	17-Apr-2023	----	----	----		20-Apr-2023	----	----	
Bioassays : Growth/IC Green Algae (P. subcapitata) 72 hour										
LDPE carboy SED2DIS	ALG-IC50-72h	17-Apr-2023	----	----	----		20-Apr-2023	----	----	
Bioassays : Growth/IC Lemna Minor (Duckweed) 7 days										
LDPE carboy EDL1	LEM-IC50-7d	17-Apr-2023	----	----	----		20-Apr-2023	----	----	
Bioassays : Growth/IC Lemna Minor (Duckweed) 7 days										
LDPE carboy EDL2	LEM-IC50-7d	17-Apr-2023	----	----	----		20-Apr-2023	----	----	
Bioassays : Growth/IC Lemna Minor (Duckweed) 7 days										
LDPE carboy SED2DIS	LEM-IC50-7d	17-Apr-2023	----	----	----		20-Apr-2023	----	----	
Bioassays : Survival/Growth/LC50/IC Fathead Minnow (P. promelas) 7 Days										
LDPE carboy EDL1	FAT-LC50-7d	17-Apr-2023	----	----	----		20-Apr-2023	----	----	

Matrix: **Water**

Evaluation: × = Holding time exceedance ; ✓ = Within Holding Time

Analyte Group / Container / Client Sample ID(s)	Method	Sampling Date	Extraction / Preparation				Analysis			
			Preparation Date	Holding Times Rec	Holding Times Actual	Eval	Analysis Date	Holding Times Rec	Holding Times Actual	Eval
Bioassays : Survival/Growth/LC50/IC Fathead Minnow (P. promelas) 7 Days										
LDPE carboy EDL2	FAT-LC50-7d	17-Apr-2023	----	----	----		20-Apr-2023	----	----	
Bioassays : Survival/Growth/LC50/IC Fathead Minnow (P. promelas) 7 Days										
LDPE carboy SED2DIS	FAT-LC50-7d	17-Apr-2023	----	----	----		20-Apr-2023	----	----	
Bioassays : Survival/LC50 and Reproduction (ICp) of Ceriodaphnia dubia to Effluents 7 days										
LDPE carboy EDL1	CER-LC50-7d	17-Apr-2023	----	----	----		20-Apr-2023	----	----	
Bioassays : Survival/LC50 and Reproduction (ICp) of Ceriodaphnia dubia to Effluents 7 days										
LDPE carboy EDL2	CER-LC50-7d	17-Apr-2023	----	----	----		20-Apr-2023	----	----	
Bioassays : Survival/LC50 and Reproduction (ICp) of Ceriodaphnia dubia to Effluents 7 days										
LDPE carboy SED2DIS	CER-LC50-7d	17-Apr-2023	----	----	----		20-Apr-2023	----	----	

Legend & Qualifier Definitions

Rec. HT: ALS recommended hold time (see units).

Quality Control Parameter Frequency Compliance

- **No Quality Control data available for this section.**

Methodology References and Summaries

The analytical methods used by ALS are developed using internationally recognized reference methods (where available), such as those published by US EPA, APHA Standard Methods, ASTM, ISO, Environment Canada, BC MOE, and Ontario MOE. Reference methods may incorporate modifications to improve performance (indicated by "mod").

Analytical Methods	Method / Lab	Matrix	Method Reference	Method Descriptions
Growth/IC Green Algae (P. subcapitata) 72 hour	ALG-IC50-72h Nautilus Environmental (Ontario) - 704 Mara St., Suite 122 Point Edward Ontario Canada N7V 1X4	Water	EPS1/RM/25	See attached report.
Survival/LC50 and Reproduction (ICp) of Ceriodaphnia dubia to Effluents 7 days	CER-LC50-7d Nautilus Environmental (Ontario) - 704 Mara St., Suite 122 Point Edward Ontario Canada N7V 1X4	Water	EPS1/RM/21	See attached report.
Survival/Growth/LC50/IC Fathead Minnow (P. promelas) 7 Days	FAT-LC50-7d Nautilus Environmental (Ontario) - 704 Mara St., Suite 122 Point Edward Ontario Canada N7V 1X4	Water	EPS1/RM/22	See attached report
Growth/IC Lemna Minor (Duckweed) 7 days	LEM-IC50-7d Nautilus Environmental (Ontario) - 704 Mara St., Suite 122 Point Edward Ontario Canada N7V 1X4	Water	EPS1/RM/37	See attached report.

QUALITY CONTROL REPORT

Work Order	: **TY2303086**	Page	: 1 of 2
Client	: New Gold Inc. (Rainy River)	Laboratory	: Thunder Bay - Environmental
Contact	: Garnet Cornell	Account Manager	: Christine Paradis
Address	: 24 Marr Rd. Barwick ON Canada P0W 1A0	Address	: 1081 Barton Street Thunder Bay, Ontario Canada P7B 5N3
Telephone	:	Telephone	: +1 807 623 6463
Project	: Sublethal Toxicity	Date Samples Received	: 18-Apr-2023 09:40
PO	: 4500062842	Date Analysis Commenced	: 20-Apr-2023
C-O-C number	: ----	Issue Date	: 08-May-2023 14:10
Sampler	: ---- 807-234-8170		
Site	: ----		
Quote number	: New Gold Rainy River Project - Picka Project		
No. of samples received	: 3		
No. of samples analysed	: 3		

This report supersedes any previous report(s) with this reference. Results apply to the sample(s) as submitted. This document shall not be reproduced, except in full.

This Quality Control Report contains the following information:

Signatories

This document has been electronically signed by the authorized signatories below. Electronic signing is conducted in accordance with US FDA 21 CFR Part 11.

Signatories	*Position*	*Laboratory Department*
Daron Mooney	Account Manager	Nautilus Environmental (Ontario) External Subcontracting, Point Edward, Ontario

General Comments

The ALS Quality Control (QC) report is optionally provided to ALS clients upon request. ALS test methods include comprehensive QC checks with every analysis to ensure our high standards of quality are met. Each QC result has a known or expected target value, which is compared against predetermined Data Quality Objectives (DQOs) to provide confidence in the accuracy of associated test results. This report contains detailed results for all QC results applicable to this sample submission. Please refer to the ALS Quality Control Interpretation report (QCI) for applicable method references and methodology summaries.

Key :

Anonymous = Refers to samples which are not part of this work order, but which formed part of the QC process lot.
CAS Number = Chemical Abstracts Service number is a unique identifier assigned to discrete substances.
DQO = Data Quality Objective.
LOR = Limit of Reporting (detection limit).
RPD = Relative Percent Difference
# = Indicates a QC result that did not meet the ALS DQO.

Workorder Comments

Holding times are displayed as "---" if no guidance exists from CCME, Canadian provinces, or broadly recognized international references.

May 5, 2023

ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Attention Lab:

On April 20, 2023, Nautilus Environmental Company Inc. personnel received one water sample EDL1 (TY03086_001_AB) from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected April 17, 2023

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
EDL1 (TY03086_001_AB) #8730-0032311	Fathead Minnow	7-day LC50 (95% Confidence) 7-day IC25 (95% Confidence)	Survival Biomass	>100% Volume[2] (Not Applicable) >100% Volume[2] (Not Applicable)
	Ceriodaphnia dubia	3-brood LC50 (95% Confidence) 3-brood IC25 (95% Confidence)	Survival Reproduction	>100% Volume[2] (Not Applicable) >100% Volume[2] (Not Applicable)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[2] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence) 7-day IC25 Dry Weight (95% Confidence)	Growth Growth	8.29% Volume (N/A; 54.84)% Volume 7.03% Volume (N/A; 81.67)% Volume

1 - Results relate only to the sample tested
2 - Highest concentration tested, based on test method

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 90.9%, 30.30%, 10.10%, 3.367% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted by a factor of 0.33 (i.e., 97%, 31%, 9.7% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

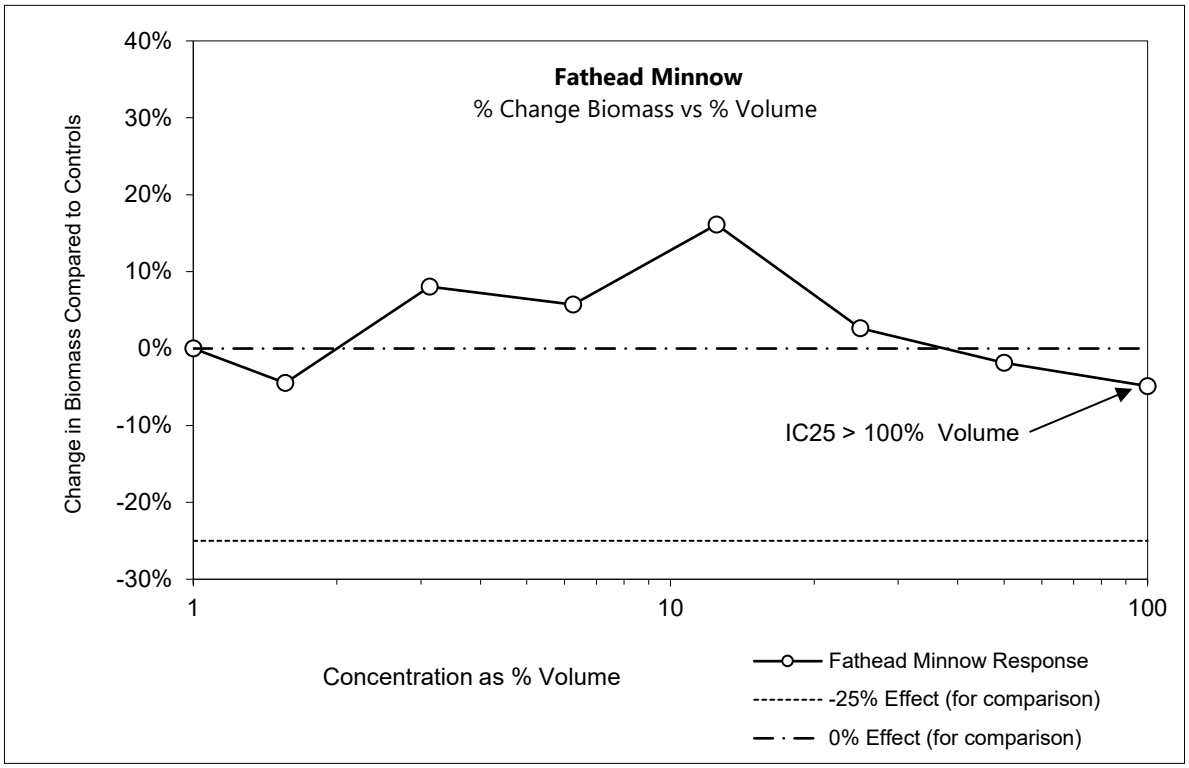
If there are any further details which you require, please do not hesitate to contact us.

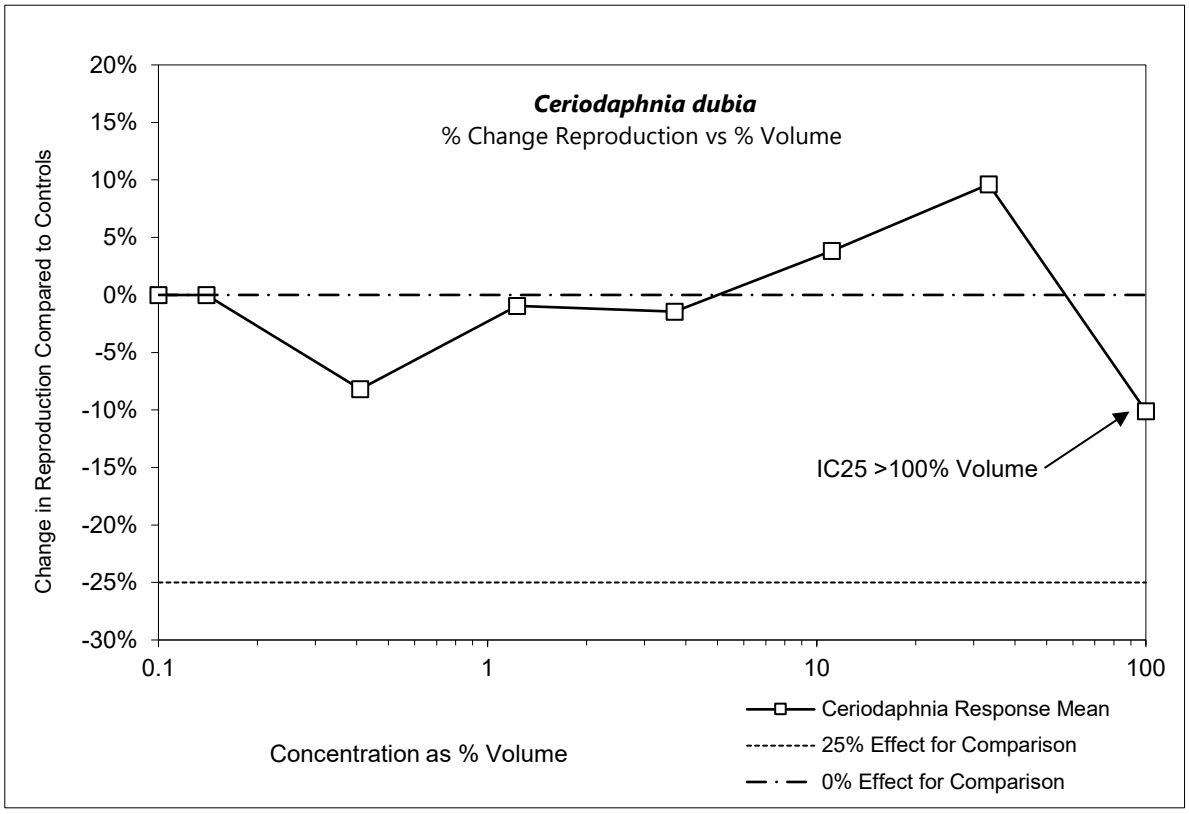
Yours very truly,
Nautilus Environmental Company Inc.

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2023\8000\8730-003\8730-0032311 FCRL

ALS Thunder Bay - EDL1 (TY03086_001_AB) - April 17, 2023





P:\Data\IC25\2023\8730-0032311

Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Water	**Source:**	EDL1 (TY03086_001_AB)
Date/Time Sampled:	April 17, 2023; 08:15	**Date/Time Received:**	April 20, 2023; 11:20
Date/Time Test Started:	April 20, 2023; 14:30	**Date Test Finished:**	April 27, 2023
Description:	Clear, Light green	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032311	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.5°C
Collected By:	Not available		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species:	Fathead Minnow (*Pimephales promelas*)		
Source:	Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)		
Culture Temp.:	22 to 26 °C	**Life Stage:**	<24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 0.7%

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032311 **Sources:** EDL1 (TY03086_001_AB)

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Test Conditions

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 1 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (3.3%)

Average Weight of Controls is ≥ 250 µg: Acceptable (536 µg)

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032311 **Sources:** EDL1 (TY03086_001_AB)

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	10	0	10	0	20	0	0
	2	0	10	0	10	0	0	0	10
	3	10	0	0	0	10	0	10	10
Mean % Mortality		3.3	6.7	0.0	6.7	3.3	6.7	3.3	6.7
S.D.		5.8	5.8	0.0	5.8	5.8	11.5	5.8	5.8
Survival/Growth Data									
Mean Dry Biomass (mg)	1	0.543	0.465	0.663	0.514	0.647	0.448	0.574	0.452
	2	0.564	0.456	0.519	0.577	0.604	0.441	0.507	0.531
	3	0.450	0.566	0.500	0.555	0.557	0.709	0.447	0.498
% Effect (+ or -)		0.0	-4.5	8.0	5.7	16.1	2.6	-1.9	-4.9
Mean Dry Biomass/ Concentration (mg)		0.519	0.496	0.561	0.549	0.603	0.533	0.509	0.494
S.D.		0.06	0.06	0.09	0.03	0.05	0.15	0.06	0.04

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032311 **Sources:** EDL1 (TY03086_001_AB)

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival		
7-day LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Biomass (Survival and Growth)		
7-day IC25 (Confidence Interval)[2]	>100% Volume (Not Applicable)	Non-linear Regression 2P Exponential Model

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 22-Apr-2023 **Reference Batch #:** Zn2301

Method of Analysis: Untrimmed Spearman-Karber α = 0%

7-Day LC50 (95% Confidence Limits): 0.72 mg/L (0.65 mg/L; 0.80 mg/L)

Historic Geometric Mean LC50: 0.65 mg/L (0.33 mg/L; 1.28 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS-Thunder Bay Sample Name: EDL1 TY 03086-001-AB Sample #: 8730-0032311

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 3.3 %

Average Weight of Controls is ≥ 250 µg: (Acceptable) Not Acceptable 536 µg

Summary of Test Results

Pre-aeration: Yes Reason: supersaturation Duration: ≤ 20 min Days: 1 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
7-day LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	or IA % Volume	
Survival / Growth Biomass		
7-day IC25	>100 % Volume	Nonlinear regression 2P Exponential model
95% Confidence Interval[2]	or IA % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 03/05/23

Results Verified: Initials AO Date 040523

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** EDL1 **Sample #:** 8730.003.2311

Day	Date 2023	Initial Variables: Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	Meters/Probes Used: D.O. / °C	pH	Cond.	Pre-aeration: yes/no	Rate (bubbles/min)	Duration (min)	Pail Sub-Sampled	Initials
0	20.04	26	7.3	7.8	1742	6/4	14/90	5/6	NO	≤100	≤ 20	1	KP
1	21.04	~~25~~ ET	7.4	9.4	1728	6/4	14/90	5/6	yes	≤100	≤ 20	1	ET
2	22.04	24	7.5	10.7	1748	6/4	14/90	5/6	yes	≤100	≤ 20	1	↓
3	23.04	24	7.5	11.2	1780	6/4	14/90	5/6	yes	≤100	≤ 20	2	↓
4	24.04	24	7.6	12.2	1753	6/4	14/90	5/6	yes	≤100	≤ 20	2	ET
5	25.04	25	7.5	11.3	1658	6/4	14/90	5/6	yes	≤100	≤ 20	3	ET
6	26.04	25	7.5	11.8	1651	6/4	14/90	5/6	yes	≤100	≤ 20	3	ET
7	27.04												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of brine shrimp days 0 to 6? (Yes)/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: Control Sample Name: EDL1 Sample #: 8730-003-2311

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04	25	8.2	7.8	246	6/4	14/90	5/6	ET
1	21-04	25	8.1	8.1	252	6/4	14/90	5/6	y
2	22-04	24	8.1	8.1	250	6/4	14/90	5/6	VS
3	23-04	25	8.1	7.6	258	6/4	14/90	5/6	KK
4	24-04	24/16	8.1/3	~~8.~~ 7.7	261	6/4	14/90	5/6	SO
5	25-04	25	~~8.~~ 7.9	8.2	237	6/4	14/90	5/6	SO
6	26-04	24	8.1	8.1	234	6/4	14/90	5/6	KP
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	7.9	7.1	6/4	14/90	y							
25	7.8	6.6	6/4	14/90	KK	0	0	0	0	0	0	KK
25	7.8	6.6	6/4	14/90	VS	0	0	0 +1 VM	0	0	0	VS
25	7.9	6.9	6/4	14/90	SO	0	0	0	0	0	0	SO
25	7.9	7.0	6/4	14/90	SO	0	0	0	0	0	0	SO
25	7.8	6.9	6/4	14/90	KP	0	0	0	0	0	0	KP
25	7.6	6.8	6/4	14/90	DS	0	0	0	0	0	0	DS
						0	0	10	0	0	0	DS

Observations:

Concentration: 1.56% v/v

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04	25	8.1	7.6	268	6/4	14/90	5/6	ET
1	21-04	24	8.1	8.1	276	6/4	14/90	5/6	y
2	22-04	24	8.1	7.8	276	6/4	14/90	5/6	VS
3	23-04	24	8.2	7.7	281	6/4	14/90	5/6	KK
4	24-04	25	8.1	7.8	285	6/4	14/90	5/6	SO
5	25-04	25	7.9	8.0	263	6/4	14/90	5/6	SO
6	26-04	24	8.1	8.1	260	6/4	14/90	5/6	KP
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	7.9	7.1	6/4	14/90	y							
25	7.8	6.6	6/4	14/90	KK	0	10	0	0	0	0	KK
25	7.8	6.7	6/4	14/90	VS	0	10	0	0	0	0	VS
25	8.0	7.1	6/4	14/90	SO	0	10	0	0	0	0	SO
25	7.9	7.0	6/4	14/90	SO	0	10	0	0	0	0	SO
25	7.8	6.9	6/4	14/90	KP	10	10	0	0	0	0	DS
25	7.6	6.4	6/4	14/90	DS	10	10	0	0	0	0	DS
						10	10	0	0	0	0	DS

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13% v/v Sample Name: EDL1 Sample #: 8730-003-2311

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	KB
						0	0	0	0	0	0	KB
						0	0	0	0	0	0	SO
						0	0	0	0	0	0	DS
						0	0	0	0	0	0	DS
						0	0	0	0	0	0	DS

Observations:

Concentration: 6.25% v/v

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	KB
						0	0	0	0	0	0	KB
						0	10	0	0	0	0	SO
						0	10	0	0	0	0	DS
						0	10	0	0	0	0	DS
						10	10	0	0	0	0	DS

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5% v/v Sample Name: EDL1 Sample #: 8730-003-2311

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20.04	25	8.0	7.5	449	6/4	14/90	5/6	ET
1	21.04	25	8.0	8.1	458	6/4	14/90	5/6	g
2	22.04	24	8.0	7.9	468	6/4	14/90	5/6	VB
3	23.04	24	8.1	7.8	475	6/4	14/90	5/6	KK
4	24.04	25	8.1	7.8	478	6/4	14/90	5/6	SO
5	25.04	25	7.9	8.0	443	6/4	14/90	5/6	SO
6	26.04	24	8.0	8.0	436	6/4	14/90	5/6	KP
7	27.04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	7.9	7.0	6/4	14/90	J							
25	~~8.1~~ 7.7 KK	~~7.3~~ 6.8 KK	6/4	14/90	KK	0	0	0	0	0	0	KK
25	7.9	6.6	6/4	14/90	VB	0	0	0	0	0	0	VB
25	7.9	6.8	6/4	14/90	SO	0	0	0	0	0	0	SO
25	7.9	6.8	6/4	14/90	SO	0	0	10	0	0	0	SO
25	7.8	6.7	6/4	14/90	KP	0	0	10	0	0	0	DS
25	7.7	6.5	6/4	14/90	DS	0	0	10	0	0	0	DS
						0	0	10	0	0	0	DS

Observations:

Concentration: 25% v/v

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	VB
						0	0	0	0	0	0	SO
						0	0	0	0	0	0	SO
						10	0	0	0	0	0	DS
						20	0	0	0	0	0	DS
						20	0	0	0	0	0	DS

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50% v/v Sample Name: EDL1 Sample #: 8730-003-2311

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	10	0	0	0	KK
						0	0	10	0	0	0	VS
						0	0	10	0	0	0	VS
						0	0	10	0	0	0	SO
						0	0	10	0	0	0	DS
						0	0	10	0	0	0	DS
						0	0	10	0	0	0	DS

Observations: _____

Concentration: 100% v/v

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04	26	7.5	7.7	1702	6/4	14/90	5/6	ET
1	21-04	26	7.5	8.4	1706	6/4	14/90	5/6	SJ
2	22-04	25	7.6	9.2	~~1763~~ 1766	6/4	14/90	5/6	VS
3	23-04	24	7.7	9.1	1773	6/4	14/90	5/6	KK
4	24-04	24	7.7	9.7	1772	6/4	14/90	5/6	SO
5	25-04	25	7.7	9.3	1655	6/4	14/90	5/6	SO
6	26-04	24	7.7	9.7	1672	6/4	14/90	5/6	KP
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	7.9	7.0	6/4	14/90	SJ							
25	8.1	7.3	6/4	14/90	KK	0	0	10	0	0	0	KK
25	7.9	6.4	6/4	14/90	VS	0	0	10	0	0	0	VS
25	8.0	6.7	6/4	14/90	SO	0	0	10	0	0	0	SO
25	8.0	6.6	6/4	14/90	SO	6	0	10	0	0	0	SO
25	7.8	5.7	6/4	14/90	KP	0	10	10	0	0	0	DS
25	7.8	6.2	6/4	14/90	DS	0	10	10	0	0	0	DS
						0	10	10	0	0	0	DS

Observations: _____

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information
Client ALS - THUNDER BAY
Sample # 8730-003-2311
Date/Time Received 20-04-23 / 1120
Sample Type WATER
100% Hardness 516

Sample Name EDL1
Sample Date/Time 17/04/23 / 0815
Arrival Temp 17.5 °C
Person Sampling N/A
Sample Description CLEAR, LIGHT GREEN

Test Information
Date/Time Started 20-04-23 / 14:30
Test started by KK
Fathead Batch # PH 0622/0122/0621
Date eggs laid 16/17/18-04-23
Culture mortality within 7 days of egg collection 0.7
Swim bladder inflated; (yes) / no KK
Age of Larvae at start of test in hours 24
Control Hardness 90
Water Bath Quadrant A
Average Temperature during Test: 25.1 °C
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes (No) (circle one)

Conc.	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	10	0.85524	0.84981	0.543	0.519
	B	10	0.84535	0.83971	0.564	
	C	9	0.83391	0.82941	0.450	
1.56	A	9	0.83887	0.83422	0.465	0.496
	B	9	0.83886	0.83430	0.456	
	C	10	0.83668	0.83102	0.566	
3.13	A	10	0.84467	0.83804	0.663	0.561
	B	10	0.84051	0.83535 ET	0.519	
	C	10	0.84418	0.83918 ET	0.500	
6.25	A	9	0.84723	0.84209	0.514	0.549
	B	9	0.84580	0.84003	0.577	
	C	10	0.84772	0.84217	0.555	
12.5	A	10	0.85778	0.85131	0.647	0.603
	B	10	0.84362	0.83758	0.604	
	C	9	0.83589	0.83032	0.557	
25	A	8	0.84027	0.83579	0.448	0.533
	B	10	0.83268	0.82827	0.441	
	C	10	0.84297	0.83588	0.709	
50	A	10	0.85389	0.84815	0.574	0.509
	B	10	0.85281	0.84774	0.507	
	C	9	0.85417	0.84970	0.447	
100	A	10	0.85020	0.84568	0.452	0.494
	B	9	0.85014	0.84483	0.531	
	C	9	0.85021	0.84523	0.498	
Initials			KP	ET	D	G

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8730-0032311 **Sample Name** EDL1

Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug) 536

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	10	0.85524	0.84981	0.543	0.543	0.536	0.519	0.032624	0.060671	6.090377
	10	10	0.84535	0.83971	0.564	0.564					
	10	9	0.83391	0.82941	0.500	0.450					
1.56	10	9	0.83887	0.83422	0.517	0.465	0.530	0.496	0.031765	0.061076	5.995975
	10	9	0.83886	0.83430	0.507	0.456					
	10	10	0.83668	0.83102	0.566	0.566					
3.13	10	10	0.84467	0.83804	0.663	0.663	0.561	0.561	0.089131	0.089131	15.89732
	10	10	0.84051	0.83532	0.519	0.519					
	10	10	0.84418	0.83918	0.500	0.500					
6.25	10	9	0.84723	0.84209	0.571	0.514	0.589	0.549	0.04578	0.031974	7.771469
	10	9	0.84580	0.84003	0.641	0.577					
	10	10	0.84772	0.84217	0.555	0.555					
12.5	10	10	0.85778	0.85131	0.647	0.647	0.623	0.603	0.021836	0.045015	3.503339
	10	10	0.84362	0.83758	0.604	0.604					
	10	9	0.83589	0.83032	0.619	0.557					
25	10	8	0.84027	0.83579	0.560	0.448	0.570	0.533	0.13428	0.152749	23.55782
	10	10	0.83268	0.82827	0.441	0.441					
	10	10	0.84297	0.83588	0.709	0.709					
50	10	10	0.85389	0.84815	0.574	0.574	0.526	0.509	0.041985	0.063532	7.983543
	10	10	0.85281	0.84774	0.507	0.507					
	10	9	0.85417	0.84970	0.497	0.447					
100	10	10	0.85020	0.84568	0.452	0.452	0.532	0.494	0.071481	0.039678	13.44182
	10	9	0.85014	0.84483	0.590	0.531					
	10	9	0.85021	0.84523	0.553	0.498					

SD: Standard Deviation
CV: Coefficient of Variation

AD 04.05.23

NAUTILUS ENVIRONMENTAL

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Water	**Source:**	EDL1 (TY03086_001_AB)
Date/Time Sampled	April 17, 2023; 08:15	**Date/Time Received:**	April 20, 2023; 11:20
Date Test Started:	April 20, 2023; 15:10	**Date Test Finished:**	April 27, 2023
Description:	Clear, Light green	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032311	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.5°C
Collected By:	Not available		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032311 **Sources:** EDL1 (TY03086_001_AB)

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 6%

Mean Number of Surviving Young Produced Within First 3 Broods: 20.3

Mean Number of Surviving Young Produced Within 7 Days of Testing: 42.4

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 1 to 6

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032311 **Sources:** EDL1 (TY03086_001_AB)

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD23-52

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (20.8 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (80% of controls)

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032311 **Sources:** EDL1 (TY03086_001_AB)

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	0	0	10	0	0	0	10
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	9	28	1	21	29	33	19	19
	2	26	14	21	28	19	12	19	24
	3	24	22	20	28	18	26	20	22
	4	16	27	19	32	17	1	22	20
	5	1	3	27	0	31	23	26	28
	6	25	9	32	9	19	26	28	26
	7	26	29	23	22	0	13	22	8
	8	29	22	18	22	26	26	29	14
	9	35	24	0	22	26	29	28	20
	10	17	30	30	22	20	27	15	6
Total Number of Live Neonates in First 3 Broods or Less		208	208	191	206	205	216	228	187
% Effect (+ or -)		0.0	0.0	-8.2	-1.0	-1.4	3.8	9.6	-10.1
Mean Number of Live Neonates in First 3 Broods or Less		20.8	20.8	19.1	20.6	20.5	21.6	22.8	18.7
SD		10.1	9.2	10.9	9.5	8.7	9.8	4.7	7.3

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Ceriodaphnia dubia **Survival and Reproduction Study -** Continued

Sample #: 8730-0032311 **Sources:** EDL1 (TY03086_001_AB)

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 22-Apr-2023 **Reference Batch #:** Zn2301

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 0.08 mg/L (0.06 mg/L; 0.11 mg/L)

Historic Geometric Mean LC50: 0.10 mg/L (0.03 mg/L; 0.32 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS - Thunder Bay Sample Name: EDL1 TY 03086-001-AB Sample #: 8730-00323/1

Conditions for Test Validity

Control Mortality is ≤ 20%: (Acceptable / Not Acceptable): 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable / Not Acceptable): 8 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable / Not Acceptable): 20.8 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: supersaturation Duration: ≤20 min Days: 1 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	> 100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
REPRODUCTION		No nonlinear regression models would fit
3-brood IC25	> 100 % Volume	IC25 IV – Linear interpolation
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 28/04/23

Results Verified: Initials AO Date 04/05/23

C *odaphnia dubia* Initial Sample Measuremen Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** EDL1 TT 03086-001- AB **Sample #:** 8730.003.23.11

Day	Date 2023	Initial Measurements				Meters / Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	°C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	20.04	26	7.3	7.8	~~1742~~ 1742 KP	6/4	14/90	5/6	NO	≤100	≤ 20	1	KP
1	21.04	25	7.4	9.4	1728	6/4	14/90	5/6	Yes	≤100	≤ 20	1	CS
2	22.04	24	7.5	10.7	1748	6/4	14/90	5/6	yes	≤100	≤ 20	1	J
3	23.04	24	7.5	11.2	1780	6/4	14/90	5/6	yes	≤100	≤ 20	2	J
4	24.04	24	7.6	12.2	1753	6/4	14/90	5/6	yes	≤100	≤ 20	2	ET
5	25.04	25	7.5	11.3	1658	6/4	14/90	5/6	yes	≤100	≤ 20	3	ET
6	26.04	25	7.5	11.8	1651	6/4	14/90	5/6	yes	≤100	≤ 20	3	ET
7	27.04									≤100	≤ 20	3	
8	28.04									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/No If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/No If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/No If yes, describe further: _____

Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily? Yes/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/No If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/No If yes, describe further: _____

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: Control **Sample Name:** EDL1 **Sample #:** 8730.002.2311

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	20.04	25	8.84	7.89	448 ~~246~~	6/4	14/90	~~5/6~~	ET	24	7.9	6.2	6/4	14/90	KP
1	21.04	26	8.3	7.9	471	6/4	14/90	5/6	"	25	7.8	6.4	6/4	14/90	"
2	22.04	25	8.3	7.8	485	6/4	14/90	5/6	KK	24	7.9	6.1	6/4	14/90	KK
3	23.04	24	8.4	7.8	472	6/4	14/90	5/6	KP	24	8.1	7.2	6/4	14/90	SO
4	24.04	24	8.4	8.2	469	6/4	14/90	5/6	JS	25	8.2	7.6	6/4	14/90	SO
5	25.04	25.4	8.3	8.0	449.4	6/4	14/90	5/6	SO	24	7.9	7.1	6/4	14/90	KP
6	26.04	24	8.3	8.2	420	6/4	14/90	5/6	KP	25	8.1	7.8	6/4	14/90	"
7	27.04														

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JS	
3	23.04	1	5	11	0	0	3	0	0	0	0	320	—	0	0	"	"
4	24.04	0	0	0	4	0	0	4	0	0	0	8	—	0	0	KK	KK
5	25.04	3	8	11	4	0	8	7	8	10	4	63	—	0	0	SO	SO
6	26.04	5	13	0	8	1	14	0	7	14	7	69	—	0	0	KK	KK
7	27.04	0	*	2	*	0	*	15	14	11	6	48	—	0	0	"	"
8	28.04																
Total Neonates		9	26	24	16	1	25	26	29	35	17	208	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.137% v/v Sample Name: EDL1 Sample #: 8730.003.2311

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20.04	25	8.3	7.4	455	6/4	14/90	5/6	ET
1	21.04	26	8.2	7.7	483	6/4	14/90	5/6	JS
2	22.04	25	8.3	7.8	490	6/4	14/90	5/6	KK
3	23.04	24	8.3	7.4	476	6/4	14/90	5/6	JD
4	24.04	24	8.3	8.0	478	6/4	14/90	5/6	DS
5	25.04	25	8.3	7.9	449	6/4	14/90	5/6	SO
6	26.04	25	8.1	8.0	439	6/4	14/90	5/6	KP
7	27.04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24	7.8	6.1	6/4	14/90	KP
25	7.9	6.0	6/4	14/90	JS
24	7.9	5.7	6/4	14/90	JD
24	8.1	7.2	6/4	14/90	SO
25	8.1	7.4	6/4	14/90	SO
24	7.9	7.0	6/4	14/90	KP
24	8.1	7.6	6/4	14/90	DS

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JS	
3	23.04	2	0	3	2	3	0	2	3	1	5	21	—	0	0	JS	JS
4	24.04	0	4	0	0	0	3	0	7	0	0	14	—	0	0	MM	MM
5	25.04	10	4	7	10	0	2	10	0	10	11	64	—	0	0	SO	SO
6	26.04	16	6	12	15	0	4	17	12	13	14	109	—	0	0	KK	KK
7	27.04	*	0	0	*	0	0	*	*	*	*	0	—	0	0	DS	DS
8	28.04																
Total Neonates		28	14	22	27	3	9	29	22	24	30	208	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survi and Reproduction Toxicity Test

Concentration: 0.410% v/v Sample Name: EDL1 Sample #: 8730.003.2311

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JS	
3	23.04	0	2	0	1	0	6	3	4	0	6	22	—	0	0	JS	JS
4	24.04	0	0	5	2	5	0	0	0	0	0	12	—	0	0	MC	MC
5	25.04	0	7	8	6	12	12	7	7	0	10	69	—	0	0	SO	SO
6	26.04	0	0	0	10	0	14	13	7	0	14	58	—	0	0	KK	KK
7	27.04	1	12	7	0	10	*	*	*	0	*	30	—	0	0	CP	CP
8	28.04																
Total Neonates		1	21	19	19	27	32	23	18	0	30	191	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 1.23% v/v Sample Name: EDL1 Sample #: 8730.003.2311

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04	0															
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	↓	
3	23.04	0	4	4	4	0 Dead	0	2	3	2	4	23	5	10	0	↓	↓
4	24.04	0	0	0	0	|	7	0	0	0	0	7	←	10	0	↓	↓
5	25.04	9	10	10	12	|	0	7	8	10	7	73	—	10	0	SO	SO
6	26.04	4	14	14	16	|	2	13	11	10	11	95	—	10	0	KK	KK
7	27.04	8	*	*	*	|	0	*	*	*	*	8	—	10	0	↓	↓
8	28.04					L											
Total Neonates		21	28	28	32	0	9	22	22	22	22	206	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 3.7% v/v Sample Name: EDL1 Sample #: 8730.003.2311

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20.04	25	8.3	7.5 ET	511	6/4	14/90	5/6	ET
1	21.04	26	8.3	7.8	528	6/4	14/90	5/6	[illegible]
2	22.04	25	8.3	7.8	545	6/4	14/90	5/6	[illegible]
3	23.04	25	8.3	7.4	529	6/4	14/90	5/6	[illegible]
4	24.04	24	8.3	8.1	532	6/4	14/90	5/6	DS
5	25.04	25	8.3	8.0	499	6/4	14/90	5/6	SO
6	26.04	25	8.2	8.0	487	6/4	14/90	5/6	KP
7	27.04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24	7.8	6.0	6/4	14/90	KP
25	7.9	6.1	6/4	14/90	[illegible]
24	7.9	5.9	6/4	14/90	[illegible]
24	8.1	7.1	6/4	14/90	SO
25	8.1	7.3	6/4	14/90	SO
24	7.9	6.9	6/4	14/90	KP
24	8.1	7.6	6/4	14/90	[illegible]

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	
3	23.04	3	0	1	0	0	3	0	4	5	0	16	—	0	0	[illegible]	[illegible]
4	24.04	0	2	0	1	0	0	0	0	0	10	13	—	0	0	[illegible]	[illegible]
5	25.04	12	8	7	7	8	7	0	10	8	0	67	—	0	0	SO	SO
6	26.04	14	9	10	0	11	9	0	12	13	0	78	—	0	0	KP	KP
7	27.04	*	*	*	9	12	*	0	*	*	10	31	—	0	0	[illegible]	[illegible]
8	28.04																
Total Neonates		29	19	18	17	31	19	0	26	26	20	205					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia 3-Brood Survi and Reproduction Toxicity Test

Concentratio. 11.11% v/v Sample Name: EDL1 Sample #: 8730.003.2311

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	y	
3	23.04	2	0	2	0	3	2	0	0	3	0	12	—	0	0	JS	JS
4	24.04	0	0	10	0	0	0	0	0	0	3	13	—	0	0	MC	MC
5	25.04	14	4	0	1	9	9	6	6	10	9	68	—	0	0	SO	SO
6	26.04	17	8	14	0	11	15	6+1	10	16	15	113	—	0	0	KK	KK
7	27.04	*	0	*	6	*	*	0	10	*	0	10	—	0	0	JS	JS
8	28.04																
Total Neonates		33	12	26	1	23	26	13	26	29	27	216	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survi and Reproduction Toxicity Test

Concentration: 33.33% v/v Sample Name: EDL1 Sample #: 8730.003.2311

Day	Date 2023	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	

Day	Date 2023	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SJ	
3	23.04	0	3	5	3	0	5	2	4	1	5	28	—	0	0	SJ	SJ
4	24.04	3	0	0	0	4	0	0	0	0	~~5~~ 0	16	—	0	0	MC	MC
5	25.04	6	7	7+1	8	8	8	10	9	12	0	76	—	0	0	SO	SO
6	26.04	10	9	0	11	13+1	15	10	16	15	1	101	—	0	0	KK	KK
7	27.04	0	*	7	*	0	*	*	*	*	0	7	—	0	0	SJ	SJ
8	28.04																
Total Neonates		19	19	20	22	26	28	22	29	28	15	228	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 100% v/v Sample Name: EDL1 Sample #: 8730.003.2311

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20.04	25	7.7	ET 7.5	1698	6/4	14/90	5/6	ET
1	21.04	26	7.6	8.6	1749	6/4	14/90	5/6	SJ
2	22.04	25	7.7	9.6	1772	6/4	14/90	5/6	KK
3	23.04	24	7.7	9.1	1770	6/4	14/90	5/6	KP
4	24.04	24	7.8	9.4	1773	6/4	14/90	5/6	DS
5	25.04	25	7.7	9.5	1665	6/4	14/90	5/6	SO
6	26.04	25	7.8	9.0	1640	6/4	14/90	5/6	KP
7	27.04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24	7.8	6.2	6/4	14/90	KP
25	8.1	7.2	6/4	14/90	SJ
24	8.0	6.8	6/4	14/90	KK
24	8.2	7.4	6/4	14/90	SO
25	8.2	7.5	6/4	14/90	SO
24	8.1	7.5	6/4	14/90	KP
24	8.2	7.9	6/4	14/90	SJ

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	SJ	
3	23.04	0	2	4	4	5	3	0	0	2	1	21	—	0	0	SJ	SJ
4	24.04	3	0	0	0	0	0	3	5	0	0	11	—	0	0	KP	KP
5	25.04	7	9	9	7	10	11	5	0	6	0	64	—	0	0	SO	SO
6	26.04	0	13	9	9	13	12	0	5	12	2	75	—	0	0	KK	KK
7	27.04	9	*	*	*	*	0	0 dead	4	*	3	16	7	10	0	DS	DS
8	28.04							V									
Total Neonates		19	24	22	20	28	26	8	14	20	6	~~187~~	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia Neonate Origin

Sample Information

Client ALS – THUNDER BAY
Sample # 8730-003-2311
Date/Time Received 20-04-23 / 11:20
Sample Type WATER
100% Hardness 510

Sample Name EDL1
Date/Time Collected 17/04/23 / 0815 Person Sampling N/A
Arrival Temp (°C) 17.5
Sample Description CLEAR, LIGHT GREEN

Test Information

Date Test Started 20-04-23 / 1510
Dilution Water Batch Number CD2352

Test Started By ET
Control Hardness 128

Template Used for Randomization 1

Individual Culture Health Data

Date Culture Started 12-04-23
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 23.1

Culture I.D. (*e.g.*, Wed Row 4) WED Row 5
Average # neos in previous 7 days (must be ≥15) 34
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 12-04-23
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 17.0

Culture I.D. (*e.g.*, Wed Row 4) WED Row 8
Average # neos in previous 7 days (must be ≥15) 31.3
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 07-04-23
% mortality in previous 7 days (must be ≤20%) 20
Average # neos in 1st 3 broods (must be ≥ 15) 22.6

Culture I.D. (*e.g.*, Wed Row 4) FRI Row 3
Average # neos in previous 7 days (must be ≥15) 63.8
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 07-04-23
% mortality in previous 7 days (must be ≤20%) 10
Average # neos in 1st 3 broods (must be ≥ 15) 17.1

Culture I.D. (*e.g.*, Wed Row 4) FRI Row 4
Average # neos in previous 7 days (must be ≥15) 60.8
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ~~05-KK~~ 14-04-23
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 21.9

Culture I.D. (*e.g.*, Wed Row 4) FRI Row 5
Average # neos in previous 7 days (must be ≥15) 21.9
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 6% (add up % mortality for all cultures used / # cultures used)
Mean Number of Young Produced within first 3 broods 20.3 (avg # 1st 3 broods for all cultures used / # cultures used)
Mean Number of Young Produced in previous 7 days 42.4 (avg # neos in prev 7 days for all cultures used / # cultures used)
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes (No) (circle one)

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
W5.3	(Y) / N	(Y) N	13	1	KK
.5	(Y) / N	(Y) N	12	2	KK
.8	(Y) / N	(Y) / N	15	3	KK
.10	(Y) / N	(Y) / N	13	4	KK
8.2	(Y) / N	(Y) / N	8	5	KK
.7	(Y) / N	(Y) / N	11	6	KK
F3.4	(Y) / N	(Y) / N	10	7	KK
4.2	(Y) / N	(Y) / N	10	8	KK
.9	(Y) / N	(Y) / N	15	9	KK
5.5	(Y) / N	(Y) / N	10	10	KK
	Y / N	Y / N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032311	**Sample Name:**	EDL1 (TY03086_001_AB)
Sample Method:	Grab	**Collected by:**	N/A
Date/Time Collected:	April 17, 2023; 08:15	**Arrival Temp.:**	17.5°C
Date/Time Received:	April 20, 2023; 11:20	**Sample Description:**	Clear, Light green
Sample Point Description:	N/A	**Sample Type:**	Water
Transportation:	Road		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species (Strain #):	*Raphidocelis subcapitata* (CPCC # 37)
Source:	Nautilus Plant Culture Unit (from CPCC)
Culture Temp.:	24 ± 2 °C
Test Culture Number:	G2(l)c
Culture Age at Test Start:	6 days old
Cell Density in the Microplate Wells at the Start of the Test:	10,454.55 cells/ml

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032311 **Sample Name:** EDL1 (TY03086_001_AB)

Test Conditions

Date/Time Test Start:	April 20, 2023; 14:20	**T=0 Control pH:**	6.2
Date/Time Test End:	April 23, 2023; 12:30-14:30	**T=72 Control pH:**	6.2
Sample pH Before Dilution:	7.0	**pH Adjustment:**	None
Test Duration:	72 hours		

Mean Test Temperature (±Standard Deviation): 25.6 (±0.7)°C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Millipore

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

***Raphidocelis subcapitata*72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032311 **Sample Name:** EDL1 (TY03086_001_AB)

Test Conditions - continued

Enumeration Technique:	Neubauer Haemacytometer
Test Vessel:	96-Well U-bottomed Polystyrene Microplate
Concentration of Test Solutions:	90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control
# Replicates/Concentration:	4 reps started; 3 reps counted of test solutions 10 control reps started, 2 used for pH measurement
Design if Specialized Procedure:	Not applicable
Method Deviations or Unusual Occurrences:	None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times:	Acceptable (19.1 times)
pH in controls did not vary by more than 1.5 units:	Acceptable (0 units)
C V for cell yields is < 20% within the control wells:	Acceptable (4.1 units)
No inhibitory trend detected across the control wells:	Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
202,045	209,545	199,545	194,545	207,045	187,045	204,545	189,545	199,233	4.1

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 CV = Coefficient of Variation = (100 x standard deviation / mean)

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032311 **Sample Name:** EDL1 (TY03086_001_AB)

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	537,045	357,045	247,045	207,045	194,545
2	509,545	374,545	244,545	209,545	224,545
3	539,545	392,045	224,545	207,045	222,045
Mean Cell Yield	528,712	374,545	238,712	207,878	213,712
Coefficient Variation[2]	3	5	5	1	8
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	184,545	184,545			
2	212,045	197,045			
3	217,045	204,545			
Mean Cell Yield	204,545	195,378			
Coefficient Variation[2]	9	5			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	Result[1]	Method of Calculation
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	No trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032311 **Sample Name:** EDL1 (TY03086_001_AB)

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: No

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2306 **Date Test Initiated:** 17-Apr-23

Method of Analysis: 2P Exponential **Algae Lot #:** G7(l)c

72-hour IC25 (95% Confidence Limits): 28.68 mg/L (21.36 mg/L; 36.59 mg/L)

Historic Geometric Mean IC25: 57.39 mg/L (27.05 mg/L; 121.80 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS Thunder Bay Sample Name EDL1 TY 03086-001-AB Sample # 8730-003231

Conditions for Test Validity

Cell increase for control is >16	Acceptable/Not acceptable	19.1 (times)
CV among controls ≤ 20	Acceptable/Not acceptable	4.1
Result of Mann-Kendall test for trend	Acceptable/Not acceptable	no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 115 000 (cells/mL)

Used: Yes/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10 454.55 (cells/mL)

Analysis Completed:	Initials:	Date: 26/04/23
Results Verified:	Initials:	Date: 04.05.23

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location:	ALS TB		
Sample #:	9730.00332311	Sample Name:	EDL 1 - TY03086-001_AB
Collection Method:	grab	Collected By:	n/a
Date/Time Collected:	17/04/23 0815	Arrival Temp.: (meter/probe)	17.5 °C (44)
Date/Time Received:	20/04/23 1120	Sample Description:	Clear, Light green
Collection Point Description:	n/a	Sample Type: ☑ Effluent ☐ Chemical ☐ Other:	
Transportation:	road	Storage:	n/a

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	AO
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # G2(1) C	AO
Culture Age at Start of Test	6 days old (must be 3 to 7 days old)	AO
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	AO
	Axenic culture? Yes/No (Circle one)	AO

Notes:

Test Conditions:

Date / Time Test Start: 20.04.23 Started By: AO 1420		Date / Time Test End: 23.04.23 Finished By: 1230 - 1430	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 7.0	pH adjustment: Y/N	pH of well D6 at T=0 h 6.0	pH of well D7 at T=72 h 6.2
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # ———		Metal mining NUT Lot # NUT2302
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation)		25.6 ± 0.7 °C	

	Day 1		Day 2	
¼ plate rotation (Initial)	AM (J)	PM (J)	AM	PM
Lights ON (Initial)	AM (J)	PM (J)	AM	PM

* For reference test, record pH of top concentration before use for series dilutions.

Notes:

72-Hour Qualitative Observations:

Condensation:	no	
Growth:	yes	
Were there any other method variations or deviations from methods? Yes/No		If yes, describe further:
Anything unusual about the test? Yes/No		
Any problems encountered? Yes/No		
Any remedial measures taken? Yes/No		

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 8730-0032311	Sample Name: EDL 1

Reference Data:

Reference Chemical Batch #	Phenol P2306	Date test started	17.04.23
Method of Analysis	Nonlinear regression 2P Exponential	Algae Lot #	G7(1)C
72-hour IC25 (95% C.I.)[3] mg/L	28.68 21.36; 36.59	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	57.39 27.05; 121.80

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	> 90.91% (N/A)	No nonlinear regression model would fit IC25 – linear interpolation
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers		
Test for Outliers	No Outliers Present ✓	Grubbs' Test for Residual Outlier Initial D
	If outliers present, indicate Concentration/Rep:	
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed.	
	No statistically significant growth stimulation.	Williams' or Dunnett's Multiple Comparison Test Initial E
	Yes, statistically significant growth stimulation at these concentrations: 90.91; 30.303; 10.101	

1) Results relate only to the sample tested.
2) Tidepool Scientific Software © 2000-2019, Comprehensive Environmental Toxicity Information System – CETIS v. ~~1.9.0.7~~ 2.1.3.5
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/No

Any outliers and justification for their removal? Yes/No

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Inoculum Preparation

Sample Name: Sample Number: 8730.003231 Date Test Start: 20.04.23
" " 12
" " 13

Cell count	0.1 µl / 0.004 µl		0.1 µl / 0.004 µl		0.1 µl / 0.004 µl	
1	27	30	28	26	12	10
2	32	50	19	21	8	13
3	41	44	25	23	13	13
4	43	47	26	22	11	12
5	44	50	-	-	-	-
Total cells	187	227	98	92	44	48
Cells/ µl *	9350	11 350	245	230	110	120
Cells/ ml	9 350 000	11 350 000	245 000	230 000	110 000	120 000
Avg. cells/ ml	10 350 000		237 500		115 000	
Time / Initials	1345 / ao		1400 / ao		1415 / ao	
Comments/ calculations	2 200 000 / 10 350 000 = 0.21		OK.		1/2 NUT OK - used	

Cell count	0.1 µl / 0.004 µl		0.1 µl / 0.004 µl		0.1 µl / 0.004 µl	
1						
2						
3						
4						
5						
Total cells						
Cells/ µl *						
Cells/ ml						
Avg. cells/ ml						
Time / Initials						
Comments/ calculations						

* Cells/ul: count 4 outside large squares (0.1 ul), add, multiply by 2.5 OR count 5 inside small squares (0.004 ul), add, multiply by 50

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)
72-Hour Growth Inhibition Test – Continued
72-Hour Quantitative Observations of Controls

Sample Name: TY03036_001 – AB Sample Number: 330-0032311 Date Test Start: 20.04.23

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D8	Well # D9	Well # D10	Well # D11
1	19	25	19	21	17	23	25	18
2	26	17	27	22	23	18	17	24
3	23	18	20	18	20	19	24	20
4	17	23	18	21	27	19	20	18
5	–	–	–	–	–	–	–	–
Initials	ao	ao	ao	ao	ao	ao	ao	ao

Cell increase for controls = 19.1	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = 4.1	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = no significant trend	Controls are invalid if there is a trend detected by the Mann-Kendall test

Mann-Kendall Trend Test for *Selenastrum capricornutum* Growth Inhibtion Test

EPS Method 1/RM/25
Version 1.1, Nov. 2000
Pollutech EnviroQuatics

Sample #: 8730-0092311
Client #: ALS TB
Date tested: 20.04.23

Instructions:

Enter control data below cells labelled D2..D5 and D8..D11.
The test of significance is completed immediately following data entry.

D2	D3	D4	D5	D8	D9	D10	D11
202045	209545	199545	194545	207045	187045	204545	189545

✓

Results:

There is no significant trend.

ao
04/05/23

Notes:

The test is a two-sided alternative using a nominal alpha value of 0.05%.
Due to the discrete nature of the test statistic, the true alpha value is 0.062%.

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: TY03086_001-AB Sample Number: 8730.00323 11 Date Test Start: 20.04.23

Theoretical Test Concentration: 100.00 %. v/v					Actual Test Concentration: 90.91 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	57	57	53		528 712 (± 16646)
2	52	56	56		Coefficient of Variation of Cell Yield
3	55	44	49		3
4	55	51	62		Average % Inhibition (-ve number = enhancement)
5	–	–	–		−165.374
Initials	ao	ao	ao		C

Theoretical Test Concentration: 33.33 %. v/v					Actual Test Concentration: 30.303 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	40	35	41		374 545 (± 17 500)
2	36	46	48		Coefficient of Variation of Cell Yield
3	35	36	40		5
4	36	37	32		Average % Inhibition (-ve number = enhancement)
5	–	–	–		−87.994
Initials	ao	ao	ao		C

Theoretical Test Concentration: 11.11 %. v/v					Actual Test Concentration: 10.101 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	21	32	23		238 712 (± 12332)
2	27	22	23		Coefficient of Variation of Cell Yield
3	26	20	26		5
4	27	27	22		Average % Inhibition (-ve number = enhancement)
5	–	–	–		−19.816
Initials	ao	ao	ao		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: TY03086_001 -AB Sample Number: 8730.00323 11 Date Test Start: 20.04.23

Theoretical Test Concentration: 3.704 %. v/v					Actual Test Concentration: 3.367 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation)
1	22	28	25		207879 (± 1443)
2	20	21	20		Coefficient of Variation of Cell Yield
3	18	19	23		1
4	27	20	19		Average % Inhibition (-ve number = enhancement)
5	–	–	–		-4.340
Initials	ao	ao	ao		C

Theoretical Test Concentration: 1.235 %. v/v					Actual Test Concentration: 1.122 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation)
1	18	23	25		213772 (± 16646)
2	27	22	19		Coefficient of Variation of Cell Yield
3	17	21	23		8
4	20	28	26		Average % Inhibition (-ve number = enhancement)
5	–	–	–		-7.267
Initials	ao	ao	ao		C

Theoretical Test Concentration: 0.412 %. v/v					Actual Test Concentration: 0.374 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation)
1	20	21	25		204545 (± 17800)
2	16	23	21		Coefficient of Variation of Cell Yield
3	22	22	19		9
4	20	23	26		Average % Inhibition (-ve number = enhancement)
5	–	–	–		-2.666
Initials	ao	ao	ao		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: TY03086_001_AB Sample Number: 8730.00323 11 Date Test Start: 20.04.23

Theoretical Test Concentration: 0.137 % v/v					Actual Test Concentration: 0.125 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	19	20	23		195 379 (± 1904)
2	21	20	24		Coefficient of Variation of Cell Yield
3	22	17	19		5
4	16	26	20		Average % Inhibition (-ve number = enhancement)
5	–	–	–		1.935
Initials	SO	SO	SO		G

Theoretical Test Concentration:			Actual Test Concentration:		
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:			Actual Test Concentration:		
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	EDL-1 Ty03086-001-AB	Sample #	8730-0032311	Date test start	20.04.23

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	20	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	115000	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2300	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	10454.5455	=	19.057065

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	19	25	19	21	17	23	25	18
	26.0	17.0	27.0	22.0	23.0	18.0	17.0	24.0
	23	18.0	20.0	18.0	20.0	19.0	24.0	20.0
	17.0	28.0	18.0	21.0	27.0	19.0	20.0	18.0
total cells	85	88	84	82	87	79	86	80
cells/ul	212.5	220	210	205	217.5	197.5	215	200
cells/ml	212500	220000	210000	205000	217500	197500	215000	200000
Cell yield = measured concentration - initial algal cell concentration								
	202045	209545	199545	194545	207045	187045	204545	189545

Mean cell yield for the control = Rc | Standard deviation | coefficient of variation

Rc 199233 | SD 8176.34873 | CV 4.1039138 **Must be ≤20**

% inhibition summary

Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	1.935
0.374	-2.666
1.122	-7.267
3.367	-4.340
10.101	-19.816
30.303	-87.994
90.910	-165.374

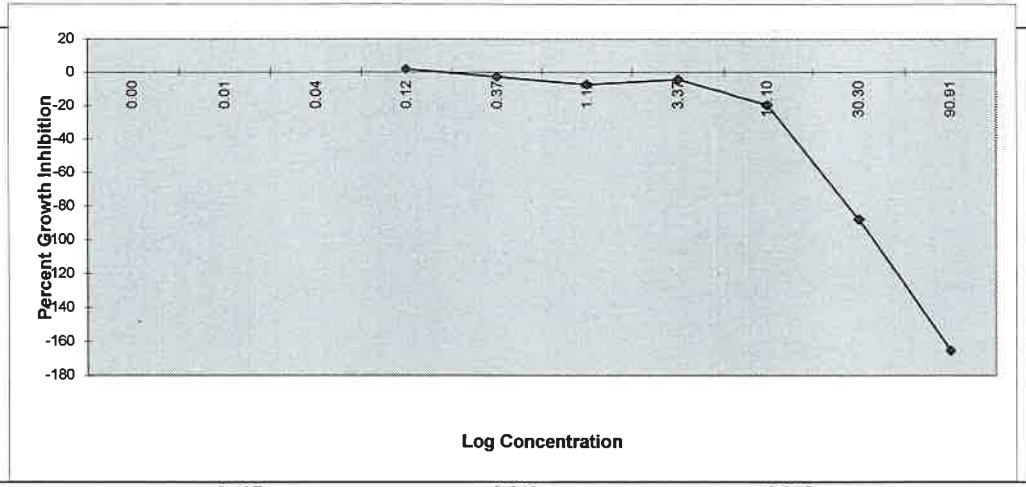
enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	57	57	53	40	35	41	21	32	23	22	28	25	18	23	25
	52.0	56.0	56.0	36.0	46.0	48.0	28.0	22.0	23.0	20.0	21.0	20.0	27.0	22.0	19.0
	55.0	44.0	49.0	35.0	36.0	40.0	26.0	20.0	26.0	18.0	19.0	23.0	17.0	21.0	23.0
	55.0	51.0	62.0	36.0	37.0	32.0	28.0	28.0	22.0	27.0	20.0	19.0	20.0	28.0	26.0
total cells	219	208	220	147	154	161	103	102	94	87	88	87	82	94	93
cells/ul	547.5	520	550	367.5	385	402.5	257.5	255	235	217.5	220	217.5	205	235	232.5
cells/ml	547500	520000	550000	367500	385000	402500	257500	255000	235000	217500	220000	217500	205000	235000	232500
Cell yield = measured concentration - initial algal cell concentration															
	537045	509545	539545	357045	374545	392045	247045	244545	224545	207045	209545	207045	194545	224545	222045
Mean Yield	528712			374545			238712			207879			213712		
STD Yield	16646			17500			12332			1443			16646		
CV Yield	3			5			5			1			8		

Average % inhibition														
for 90.910%:	-165.374		for 30.303%	-87.994		for 10.101%	-19.816		for 3.367%	-4.340		for 1.122%	-7.267	

Average % stimulation														
for 90.910%:	165.374		for 30.303%	87.994		for 10.101%	19.816		for 3.367%	4.340		for 1.122%	7.267	

CO 04/05/23



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
20	21	25	19	20	23									
16.0	23.0	21.0	21.0	20.0	24.0									
22.0	22.0	19.0	22.0	17.0	19.0									
20.0	23.0	26.0	16.0	26.0	20.0									
78	89	91	78	83	86	0	0	0	0	0	0	0	0	0
195	222.5	227.5	195	207.5	215	0	0	0	0	0	0	0	0	0
195000	222500	227500	195000	207500	215000	0	0	0	0	0	0	0	0	0
184545	212045	217045	184545	197045	204545	-10455	-10455	-10455	-10455	-10455	-10455	-10455	-10455	-10455
204545			195379			-10455			-10455			-10455		
17500			10104			0			0			0		
9			5			0			0			0		
for 0.374%	-2.666		for 0.125%	1.935		for 0.042%	105.247		for 0.014%	105.247		for 0.005%	105.247	
for 0.374%	2.666		for 0.125%	-1.935		for 0.042%	-105.247		for 0.014%	-105.247		for 0.005%	-105.247	

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
EDL1 (TY03086_001_AB)	8730-0032311	April 17, 2023	April 20, 2023	April 20, 2023	8.29% Volume (N/A; 54.84)% Volume	7.03% Volume (N/A; 81.67)% Volume	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	28-Apr-2023	**Reference Chemical:**	KCl
Frond Increase IC25:	1.97 g/L	**95% Confidence Limits:**	1.29; 2.63 g/L
Historic Geometric Mean IC25:	2.04 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.26; 3.28 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	Na_2MoO_4	0.0726
	K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$2H_2O$	0.00003
	KCl	10.1	$MgSO_4\ 7H_2O$	147	$ZnCl_2$	0.00001
					$CoCl_2\ 6H_2O$	0.00001
					$CuCl_2\ 2H_2O$	

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

C. D'Andrea

Date: May 5, 2023

Carol D'Andrea
Laboratory Supervisor

Test Material

Client Name/Location: ALS – Thunder Bay	
Sample #: 8730-003 23 11	Sample Name: EDL 1 TY 03086-001-AB
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 17/04/23 8:15	Arrival Temperature (meter/probe): 17.5 °C (44)
Date/Time Received: 20/04/23 11:20	Sample Description: clear, light green
Collection Point Description: other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: Air / Road	Storage: none

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	G
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	G
Culture Age at Start of Test:	8 days old, acclimated 23 hours in fresh test solution (mAPHA)	G
Culture Medium:	Modified Hoagland's E+ medium, Lot # M142301	G
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/No	G
	Axenic culture? Yes/No	G
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 42 in HT 1, 41 in HT 2, ____ in HT 3 at finish	G

Test Conditions and Procedures

Date / Time Test Start: 20.04.23 15:20 Started By: TY	Date / Time Test End: 27.04.23 10:40 Finished By: TY
Test Type: Static (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / No (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: ~~Modified APHA~~ (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 8.0 (13/91)	pH Adjustment: none
Test Volume and Depth: 150 mL / 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / No	If yes, describe further:
Anything unusual about the test? Yes / No	
Any problems encountered? Yes / No	
Any remedial measures taken? Yes / No	Randomization Template: A

Sample #: 8730-00323 11	Sample Name: EDL 1

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	24	25	26	25	26	25	25	26
Low	24	25	26	25	26	25	25	26
Medium	24	25	26	25	26	25	25	26
High	24	25	26	25	26	25	25	26
Initials	G	G	KK	KK	CQ	CQ	CQ	G
meter/probe	50	50	44	44	44	44	44	44
Mean Test Temperature (average of 24h high / low temperatures):					25.4 ± 0.1 °C			

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.2	8.2	8.2	7.9	G	13/91
Day 7	**Control**	**Low**	**Medium**	**High**	**Initials**	**pH meter/probe**
	8.4	9.7	9.7	9.1	CQ	13/91

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)	—							—

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 24/04/23 (4)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #):					Initials: G
4740	5540	5500	5100	5430	Mean Light Measurement: 5262
±15% Variation of Mean: 4576 - 6051					(Acceptable) / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) / No
If yes, describe further: Brown ppt / algae at the bottom of the concentrations

Reference Data

Reference Date	(Frond Increase) or Dry Weights (circle one)			
28/04/23	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
	~~2.59~~ 1.97	~~2.19~~ 1.29; ~~2.72~~ 2.63	2.074	1.276; 3.358

Sample #: 8730-003 2311	Sample Name: EDL 1

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 74	B 70	C 79	D 109	83.0

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy							Initials
							C
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV: 77.0 (17.7) 23.0							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-7.14	-2.27	-4.22	-8.12	-27.92	-28.57	-50.97
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-5.70	-2.72	-2.18	-9.43	-34.53	-28.79	-36.21

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: Yes / (Not applicable) (no stimulation) (Circle one)

Test Endpoints and Calculations:

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	8.29 (N/A; 54.84)	Nonlinear regression model fit but calculated IC25 didn't make sense ICPIN - linear interpolation
IC25 (95% C.I.)[3] If calculated without outlier[4]		
Dry Weights		
IC25 (95% C.I.)[3]	7.03 (N/A; 81.67)	Nonlinear regression models fit but calculated IC25 didn't make sense ICPIN - linear interpolation
IC25 (95% C.I.)[3] If calculated without outlier[4]		

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019. Comprehensive Environmental Toxicity Information System CETISv ~~1.9.6.7~~ 2.1.3.5
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / (No)

Any outliers and justification for their removal? Yes / (No)

Lemna minor E , 7 Observations

Client: ALS Thunder Bay
Site: EDL1
Concentration: Control
Sample number: 8730-003231 1
Observations By: C
Date Started: 17 20.04.23
Date Ended: 27.04.23

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	74	70	79	109
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X
Other Observations				

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO
N/A
Weights: YES / NO

Concentration: 0.097 T.U.
Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	75	73	83	79
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	X	✓ Smaller	✓ Smaller	✓ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X
Other Observations				

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO)
Weights: YES / (NO)

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor D.., 7 Observations

Client: ALS Thunder Bay		Sample number: 8730-003231	Date Started: 17.04.23
Site: EDL1			Date Ended: 27.04.23
Concentration: 0.29 %	Observations By: C	Concentration: 0.97 %	Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	95	83	93	54	Number of	78	93	84	64
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	✓ Smaller	✓ Smaller	✓ Smaller	✓ Smaller	Abnormally sized fronds	✓ Smaller	✓ Smaller	✓ Smaller	✓ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	algae	—		→	Other Observations				
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor L., 7 Observations

Client: ALS Thunder Bay
Site: EDL 1
Concentration: 3.1 / v/v
Sample number: 8730003231
Observations By: C
Date Started: 17.04.23
Date Ended: 27.04.23

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	70	76	75	86
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	~	~
Other Observations	brown ppt	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO
Weights: YES / NO

Concentration: 9.7 / v/v
Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	67	55	57	67
Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X
Other Observations	brown ppt	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO
Weights: YES / NO

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor D.., 7 Observations

Client: ALS Thunder Bay	Sample number: 8730-003231	Date Started: 20.04.23	
Site: EOL 1		Date Ended: 27.04.23	
Concentration: 31.1	Observations By: C	Concentration: 97.1	Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	62	58	61	63	Number of	38	41	39	57
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	√√	√√	√√	√√
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	brown ppt/algae	→	→	→	Other Observations	brown ppt at the bottom	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Weights

Client	Site	Sample number
ALS-Thunder Bay	EDL1	8730-0032311

In Oven Date/Time/ °C: 27/04/23 11:10 60° Out Oven Date/Time/°C: 28/04/23 11:10 60°

Conc. 1.1V	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	68	77.0 (17.7)	0.84570	0.84097	4.73	5.97 (1.5)
	B	64		0.83223	0.82676	5.47	
	C	73		0.84374	0.83828	5.46	
	D	103		0.85051	0.84231	8.20	
0.097	A	69	71.5 (4.4)	0.86836	0.86424	5.12	5.63 (0.5)
	B	67		0.84466	0.83941	5.25	
	C	77		0.86189	0.85558	6.31	
	D	73		0.84682	0.84100	5.82	
0.29	A	89	75.3 (18.9)	0.84987	0.84285	7.02	5.80 (1.3)
	B	77		0.85076	0.84505	5.71	
	C	87		0.84873	0.84222	6.51	
	D	48		0.84611	0.84214	3.97	
0.97	A	72	73.8 (12.4)	0.85664	0.85041	6.23	5.84 (1.0)
	B	87		0.85296	0.84597	6.99	
	C	78		0.85512	0.84976	5.36	
	D	52		0.84613	0.84137	4.76	
3.1	A	64	70.8 (6.7)	0.84875	0.84449	4.26	5.40 (1.0)
	B	70		0.84521	0.84022	4.99	
	C	69		0.84285	0.83698	5.87	
	D	80		0.85249	0.84600	6.49	
9.7	A	61	55.5 (6.4)	0.84768	0.84358	4.10	3.91 (0.8)
	B	49		0.86802	0.86431	3.71	
	C	56		0.83662	0.83373	2.89	
	D	61		0.83896	0.83404	4.92	
31	A	56	55.0 (2.4)	0.85749	0.85248	5.01	4.25 (0.8)
	B	52		0.83196	0.82889	3.09	
	C	55		0.86809	0.86339	4.70	
	D	57		0.86582	0.86163	4.19	
97	A	32	37.8 (8.9)	0.83757	0.83397	3.60	3.81 (1.1)
	B	35		0.83930	0.83567	3.63	
	C	33		0.84532	0.84260	2.72	
	D	51		0.84573	0.84046	5.27	
Initials		EB	EB	CG	CG	EB	EB

Notes:

Sample name EDL1 Date started 20/04/23 sample # 8730-0032311

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

83.0 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	74	75	95	78	70	67	62	38
	70	73	83	93	76	55	58	41
	79	83	93	84	75	57	61	39
	109	79	54	64	86	67	63	57
Total Fronds	332	310	325	319	307	246	244	175

Increase in Frond Number = Total # Fronds - 6 Starting Fronds

	68	69	89	72	64	61	56	32
	64	67	77	87	70	49	52	35
	73	77	87	78	69	51	55	33
	103	73	48	58	80	61	57	51
Total Increase	308	286	301	295	283	222	220	151
Mean Increase	77.0	71.5	75.3	73.8	70.8	55.5	55.0	37.8
SD Increase	17.7	4.4	18.9	12.2	6.7	6.4	2.2	8.9
CV Increase	23.0	6.2	25.1	16.5	9.5	11.5	3.9	23.6
% Stimulation		**-7.14**	**-2.27**	**-4.22**	**-8.12**	**-27.92**	**-28.57**	**-50.97**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.84570	0.86936	0.84987	0.85664	0.84875	0.84768	0.85749	0.83757
Pan + Plant	0.83223	0.84466	0.85076	0.85296	0.84521	0.86802	0.83196	0.83930
	0.84374	0.86189	0.84873	0.85512	0.84285	0.83662	0.86809	0.84532
	0.85051	0.84682	0.84611	0.84613	0.85249	0.83896	0.86582	0.84573
Initial Weight (g)	0.84097	0.86424	0.84285	0.85041	0.84449	0.84358	0.85248	0.83397
Pan Only	0.82676	0.83941	0.84505	0.84597	0.84022	0.86431	0.82887	0.83567
	0.83828	0.85558	0.84222	0.84976	0.83698	0.83373	0.86339	0.84260
	0.84231	0.84100	0.84214	0.84137	0.84600	0.83404	0.86163	0.84046
Plant Only (mg)	4.73	5.12	7.02	6.23	4.26	4.10	5.01	3.60
	5.47	5.25	5.71	6.99	4.99	3.71	3.09	3.63
	5.46	6.31	6.51	5.36	5.87	2.89	4.70	2.72
	8.20	5.82	3.97	4.76	6.49	4.92	4.19	5.27
Mean Dry Weight	5.965	5.625	5.803	5.835	5.403	3.905	4.248	3.805
SD Dry Weight	1.5	0.5	1.3	1.0	1.0	0.8	0.8	1.1
CV Dry Weight	25.6	9.8	23.0	16.8	18.1	21.6	19.8	28.0
% Stimulation		**-5.70**	**-2.72**	**-2.18**	**-9.43**	**-34.53**	**-28.79**	**-36.21**

For Data Transfer to CETIS

		# fronds	total mass	tare
0	1	68	0.84570	0.84097
	2	64	0.83223	0.82676
	3	73	0.84374	0.83828
	4	103	0.85051	0.84231
0.1	1	69	0.86936	0.86424
	2	67	0.84466	0.83941
	3	77	0.86189	0.85558
	4	73	0.84682	0.84100
0.3	1	89	0.84987	0.84285
	2	77	0.85076	0.84505
	3	87	0.84873	0.84222
	4	48	0.84611	0.84214
1	1	72	0.85664	0.85041
	2	87	0.85296	0.84597
	3	78	0.85512	0.84976
	4	58	0.84613	0.84137
3.1	1	64	0.84875	0.84449
	2	70	0.84521	0.84022
	3	69	0.84285	0.83698
	4	80	0.85249	0.84600
9.7	1	61	0.84768	0.84358
	2	49	0.86802	0.86431
	3	51	0.83662	0.83373
	4	61	0.83896	0.83404
31	1	56	0.85749	0.85248
	2	52	0.83196	0.82887
	3	55	0.86809	0.86339
	4	57	0.86582	0.86163
97	1	32	0.83757	0.83397
	2	35	0.83930	0.83567
	3	33	0.84532	0.84260
	4	51	0.84573	0.84046

ap 04/05/23

May 5, 2023

ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Attention Lab:

On April 20, 2023, Nautilus Environmental Company Inc. personnel received one water sample SED2DIS (TY03086_002_AB) from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected April 17, 2023

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
SED2DIS (TY03086_002_AB) #8730-0032312	Fathead Minnow	7-day LC50 (95% Confidence) 7-day IC25 (95% Confidence)	Survival Biomass	>100% Volume[2] (Not Applicable) 70.59% Volume[2] (Not Applicable)
	Ceriodaphnia dubia	3-brood LC50 (95% Confidence) 3-brood IC25 (95% Confidence)	Survival Reproduction	>100% Volume[2] (Not Applicable) >100% Volume[2] (Not Applicable)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[2] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence) 7-day IC25 Dry Weight (95% Confidence)	Growth Growth	>97% Volume (Not applicable) >97% Volume[3] (Not applicable)

1 - Results relate only to the sample tested
2 - Highest concentration tested, based on test method
3 - 25.5% inhibition at 9.7% v/v concentration

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 90.91%, 30.303%, 10.101%, 3.367% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted by a factor of 0.33 (i.e., 97%, 31%, 9.7% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

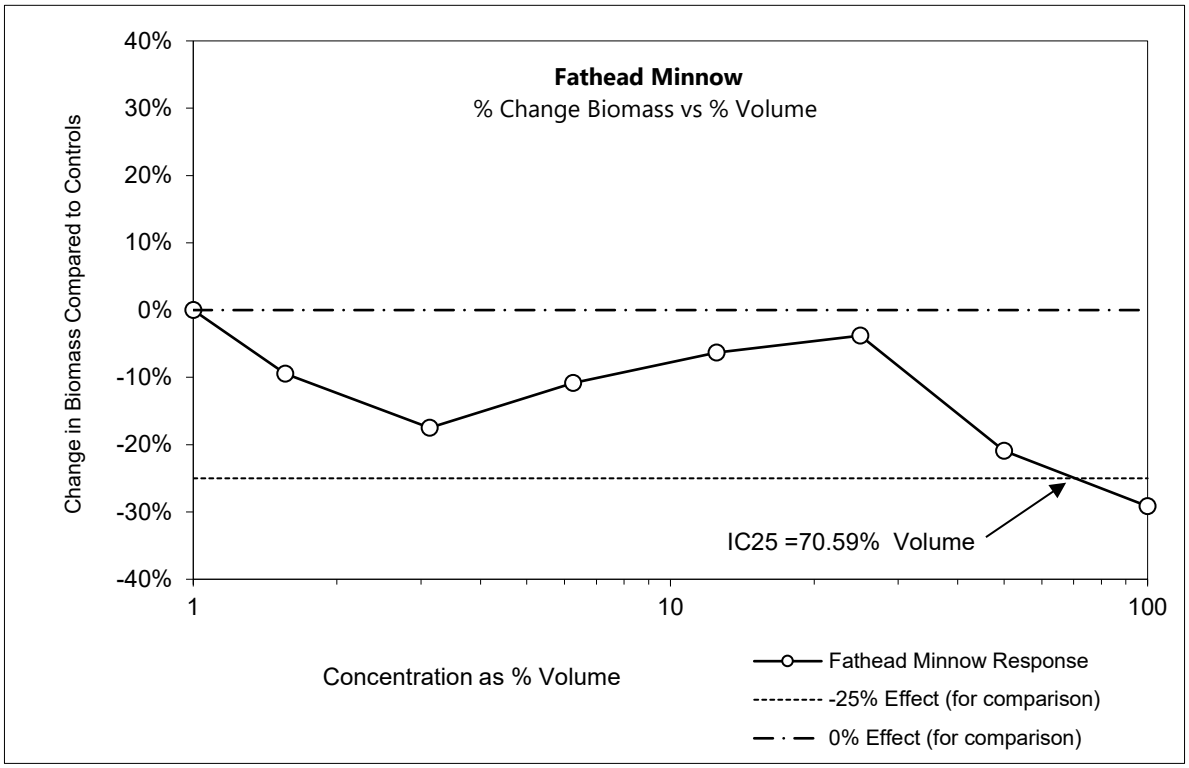
If there are any further details which you require, please do not hesitate to contact us.

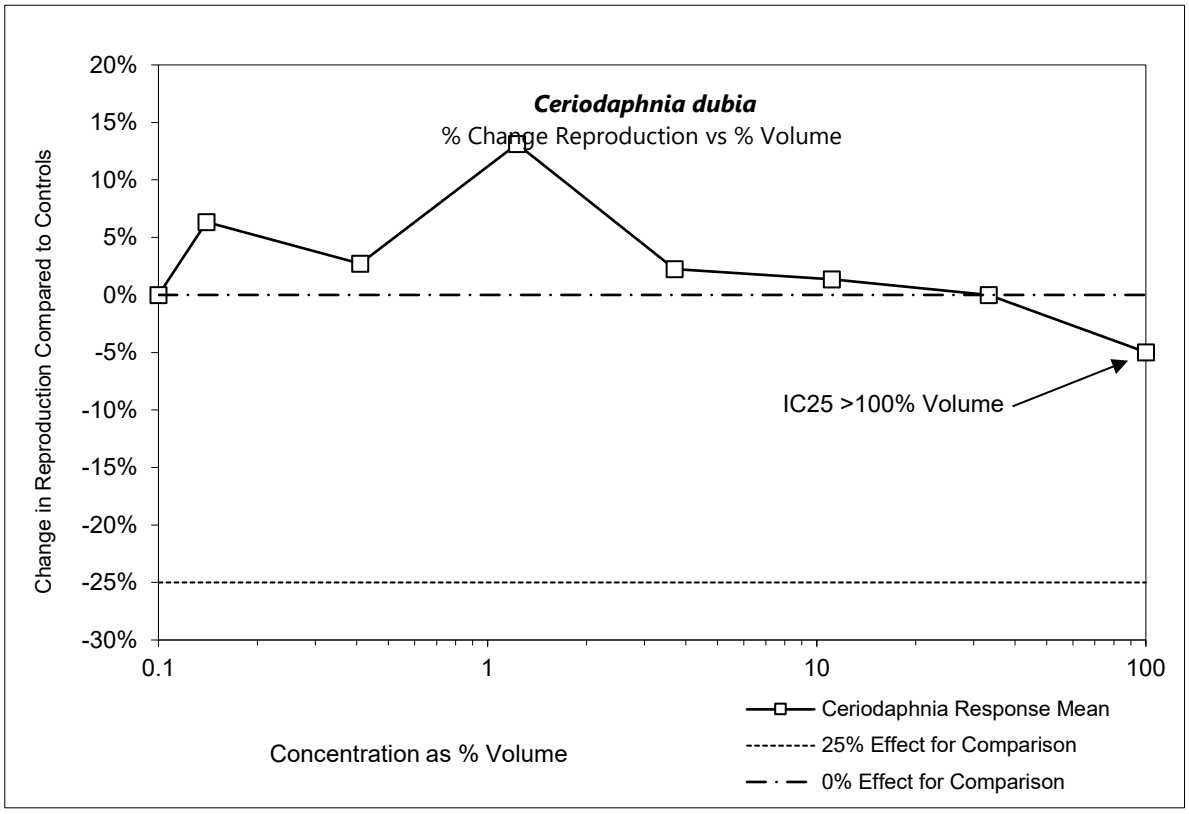
Yours very truly,
Nautilus Environmental Company Inc.

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2023\8000\8730-003\8730-0032312 FCRL

ALS Thunder Bay - SED2DIS (TY03086_002_AB) - April 17, 2023





Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Water	**Source:**	SED2DIS (TY03086_002_AB)
Date/Time Sampled:	April 17, 2023; 08:40	**Date/Time Received:**	April 20, 2023; 11:20
Date/Time Test Started:	April 20, 2023; 21:15	**Date Test Finished:**	April 27, 2023
Description:	Clear, Light yellow	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032312	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.3°C
Collected By:	Not available		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species:	Fathead Minnow (*Pimephales promelas*)		
Source:	Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)		
Culture Temp.:	22 to 26 °C	**Life Stage:**	<24-hour old larvae
Culture Mortality Within 7 Days of Egg Collection:	0.7%		

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032312 **Sources:** SED2DIS (TY03086_002_AB)

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Test Conditions

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 1 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (3.3%)

Average Weight of Controls is ≥ 250 µg: Acceptable (568 µg)

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032312 **Sources:** SED2DIS (TY03086_002_AB)

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	0	20	20	0	10	60	50
	2	0	0	10	10	10	20	20	10
	3	10	0	0	10	0	0	10	20
Mean % Mortality		3.3	0.0	10.0	13.3	3.3	10.0	30.0	26.7
S.D.		5.8	0.0	10.0	5.8	5.8	10.0	26.5	20.8
Survival/Growth Data									
Mean Dry Biomass (mg)	1	0.632	0.498	0.340	0.485	0.574	0.624	0.277	0.202
	2	0.522	0.431	0.413	0.505	0.440	0.461	0.483	0.405
	3	0.496	0.565	0.608	0.481	0.532	0.502	0.545	0.562
% Effect (+ or -)		0.0	-9.5	-17.5	-10.8	-6.3	-3.8	-20.9	-29.2
Mean Dry Biomass/ Concentration (mg)		0.550	0.498	0.454	0.490	0.515	0.529	0.435	0.390
S.D.		0.07	0.07	0.14	0.01	0.07	0.08	0.14	0.18

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032312 **Sources:** SED2DIS (TY03086_002_AB)

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 7-day LC50 (Confidence Interval)[2]	 >100% Volume (Not Applicable)	 No dose response
Biomass **(Survival and Growth)** 7-day IC25 (Confidence Interval)[2]	 70.59% Volume (Not Applicable)	 Non-linear regression models fit but calculated IC25 value didn't make sense Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 22-Apr-2023 **Reference Batch #:** Zn2301

Method of Analysis: Untrimmed Spearman-Karber α = 0%

7-Day LC50 (95% Confidence Limits): 0.72 mg/L (0.65 mg/L; 0.80 mg/L)

Historic Geometric Mean LC50: 0.65 mg/L (0.33 mg/L; 1.28 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS - Thunder Bay Sample Name: SED2 DIS TY 03086-002-AB Sample #: 8730 20032312

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 3.3 %

Average Weight of Controls is ≥ 250 µg: (Acceptable) / Not Acceptable 568 µg

Summary of Test Results

Pre-aeration: Yes Reason: supersaturation Duration: ≤ 20 min Days: 1 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL 7-day LC50 95% Confidence Interval[2]	> 100 % Volume N/A % Volume	no dose response
Survival / Growth Biomass 7-day IC25 95% Confidence Interval[2]	70.59 % Volume N/A % Volume	Nonlinear regression model fit but calculated IC25 value didn't make sense ICPIN - linear interpolation

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s):

Analysis Completed: Initials EJ Date 03/05/23

Results Verified: Initials AO Date 04/05/23

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** SED2DIS **Sample #:** 8730-003-2312

Day	Date 2023	Initial Variables				Meters/Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	D.O./°C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	20-04	26	7.6	8.1	552	6/4	14/90	5/6	NO	≤100	≤ 20	1	KP
1	21-04	25	7.7	10.0	543	6/4	14/90	5/6	yes	≤100	≤ 20	1	ET
2	22-04	25	7.7	10.7	555	6/4	14/90	5/6	yes	≤100	≤ 20	1	ET
3	23-04	25	7.7	10.7	564	6/4	14/90	5/6	yes	≤100	≤ 20	2	ET
4	24-04	24	7.7	11.6	560	6/4	14/90	5/6	yes	≤100	≤ 20	2	ET
5	25-04	25	7.7	11.8	523	6/4	14/90	5/6	yes	≤100	≤ 20	3	ET
6	26-04	25	7.8	12.1	519	6/4	14/90	5/6	yes	≤100	≤ 20	3	
7	27-04												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/(No) If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/(No) If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/(No) If yes, describe further: _____

Was test fed 100µl of brine shrimp days 0 to 6? (Yes)/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/(No) If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/(No) If yes, describe further: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: Control Sample Name: SED2D1S Sample #: 8730-003-2312

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04	25	8.2	7.8	246	6/4	14/90	5/6	ET
1	21-04	25	8.1	8.1	252	6/4	14/90	5/6	US
2	22-04	24	8.1	8.1	250	6/4	14/90	5/6	VF
3	23-04	25	8.1	7.6	258	6/4	14/90	5/6	KK
4	24-04	26	8.3	7.7	261	6/4	14/90	5/6	SO
5	25-04	24	8.0	8.2	230	6/4	14/90	5/6	SO
6	26-04	24	8.1	8.1	234	6/4	14/90	5/6	KP
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	8.0	7.1	6/4	14/90	US							
25	7.8	6.7	6/4	14/90	KK	0	0	0	0	0	0	KK
25	7.8	6.6	6/4	14/90	VF	0	0	KK 10	0	0	0	VF
25	8.0	7.1	6/4	14/90	SO	0	0	0	0	0	0	KK
25	7.9	6.9	6/4	14/90	SO	0	0	0	0	0	0	SO
25	7.9	7.0	6/4	14/90	KP	0	0	0	0	0	0	MM
25	7.7	6.8	6/4	14/90	KK	0	0	10	0	0	0	SO
						0	0	10	0	0	0	SO

Observations:

Concentration: 1.56% V/V

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04	25	8.2	7.6	246	6/4	14/90	5/6	ET
1	21-04	25	8.0	8.2	253	6/4	14/90	5/6	US
2	22-04	24	8.1	7.8	255	6/4	14/90	5/6	VF
3	23-04	24	8.1	7.8	259	6/4	14/90	5/6	KK
4	24-04	25	8.2	7.7	259	6/4	14/90	5/6	SO
5	25-04	24	8.1	8.1	235	6/4	14/90	5/6	SO
6	26-04	24	8.1	7.9	238	6/4	14/90	5/6	KP
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	8.0	7.1	6/4	14/90	US							
25	8.0	7.2	6/4	14/90	KK	0	0	0	0	0	0	KK
25	7.8	6.5	6/4	14/90	VF	0	0	KK 10	0	0	0	VF
25	8.0	7.0	6/4	14/90	SO	0	0	0	0	0	0	KK
25	7.9	6.9	6/4	14/90	SO	0	0	0	0	0	0	SO
25	7.9	6.4	6/4	14/90	KP	0	0	0	0	0	0	MM
25	7.7	6.7	6/4	14/90	KK	0	0	0	0	0	0	SO
						0	0	0	0	0	0	SO

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13% v/v Sample Name: SED2D1S Sample #: 8730.003.2312

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						10	0	0	0	0	0	KK
						10	0	KK 20	0	0	0	KK
						10	0	0	0	0	0	KK
						20	10	0	0	0	0	SO
						20	10	0	0	0	0	SO
						20	10	0	0	0	0	SO
						20	10	0	0	0	0	SO

Observations: _____

Concentration: 6.25% v/v

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	10	0	0	0	KK
						10	0	KK 20	0	0	0	KK
						10	0	10	0	0	0	KK
						20	0	10	0	0	0	SO
						20	10	10	0	0	0	SO
						20	10	10	0	0	0	SO
						20	10	10	0	0	0	SO

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5% V/V Sample Name: SED2D1S Sample #: 8730-003-2312

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04	25	8.1	7.6	279	6/4	14/90	5/6	ET
1	21-04	25	8.0	8.2	285	6/4	14/90	5/6	CJ
2	22-04	24	8.1	7.8 8.0	287	6/4	14/90	5/6	VB
3	23-04	24	8.1	7.8	294	6/4	14/90	5/6	KK
4	24-04	25	8.2	7.8	294	6/4	14/90	5/6	SO
5	25-04	24	8.1	8.1	268	6/4	14/90	5/6	SO
6	26-04	24	8.1	8.1	271	6/4	14/90	5/6	KP
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	8.0	7.1	6/4	14/90	CJ							
25	8.0	7.1	6/4	14/90	KK	0	10	0	0	0	0	KK
25	7.9	6.3	6/4	14/90	VB	0	10	0	0	0	0	VB
25	8.0	6.8	6/4	14/90	SO	0	10	0	0	0	0	KK
25	7.9	6.9	6/4	14/90	SO	0	10	0	0	0	0	SO
25	7.9	6.7	6/4	14/90	KP	0	10	0	0	0	0	ML
25	7.8	6.9	6/4	14/90	KK	0	10	0	0	0	0	SO
						0	10	0	0	0	0	SO

Observations: _____

Concentration: 25% V/V

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	KK
						1 DEAD X0	0	0	0	0	0	VB
						0	0	0	0	0	0	KK
						10	0	0	0	0	0	SO
						10	10	0	0	0	0	ML
						10	10	0	0	0	0	SO
						10	20	0	0	0	0	SO

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50% v/v Sample Name: SED2D1S Sample #: 8730.003.2312

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	0	0	0	0	KK
						0	0	0	0	0	0	S
						0	0	10	0	0	0	KK
						10	10	10	0	0	0	SO
						50	10	10	0	0	0	ML
						50	10	10	0	0	0	SO
						60	20	10	0	0	0	SO

Observations: _____

Concentration: 100% v/v

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20.04	26	7.8	7.8	538	6/4	14/90	5/6	ET
1	21.04	25	7.8	8.7	549	6/4	14/90	5/6	S
2	22.04	25	7.8	9.3	555	6/4	14/90	5/6	KK
3	23.04	24	7.8	9.0	559	6/4	14/90	5/6	KK
4	24.04	24	7.9	9.4	556	6/4	14/90	5/6	SO
5	25.04	25	7.8	9.4	521	6/4	14/90	5/6	SO
6	26.04	25	7.8	9.8	518	6/4	14/90	5/6	KP
7	27.04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	8.0	6.9	6/4	14/90	S							
25	7.9	6.2	6/4	14/90	KK	0	0	0	0	0	0	KK
25	7.9	6.3	6/4	14/90	KP	0	10	10	0	0	0	S
25	8.1	6.8	6/4	14/90	SO	0	10	10	0	0	0	KK
25	8.1	6.7	6/4	14/90	SO	10	10	20	0	0	0	SO
25	8.0	6.8	6/4	14/90	KP	50	10	20	0	0	0	ML
25	8.0	7.0	6/4	14/90	KK	50	10	20	0	0	0	SO
						50	10	20	0	0	0	SO

Observations: _____

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information
Client ALS – THUNDER BAY
Sample # 8730-003-2312
Date/Time Received 20-04-23 / 1120
Sample Type WATER
100% Hardness 212

Sample Name SED2DIS
Sample Date/Time 17/04/23 / 0840
Person Sampling N/A
Arrival Temp 17.3 °C
Sample Description CLEAR, LIGHT YELLOW

Test Information
Date/Time Started 20-04-23 / 1215
Test started by KK
Fathead Batch # FH 0621/0122/0622
Date eggs laid 16/17/18-04-23
Culture mortality within 7 days of egg collection 0.7
Swim bladder inflated; (yes) / no
Age of Larvae at start of test in hours <24
Control Hardness 90
Water Bath Quadrant B
Average Temperature during Test: 25 ± 1 °C
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / (No) (circle one)

Conc.	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	10	0.85042	0.84410	0.632	0.550
	B	10	0.84289	0.83767	0.522	
	C	9	0.85066	0.84570	0.496	
1.56	A	10	0.85564	0.85066	0.498	0.498
	B	10	0.85485	0.85054	0.431	
	C	10	0.85569	0.85004	0.565	
3.13	A	8	0.85404	0.85064	0.340	0.454
	B	9	0.85205	0.84792	0.413	
	C	10	0.85400	0.84792	0.608	
6.25	A	8	0.84712	0.84227	0.485	0.454
	B	9	0.84905	0.84400	0.505	
	C	9	0.85577	0.85096	0.481	
12.5	A	10	0.84146	0.83572	0.574	0.490
	B	9	0.82564	0.82124	0.440	
	C	10	0.85348	0.84816	0.532	
25	A	9	0.85734	0.85110	0.624	0.515
	B	8	0.85182	0.84721	0.461	
	C	10	0.85444	0.84942	0.502	
50	A	4	0.85844	0.85567	0.277	0.435
	B	8	0.84662	0.84179	0.483	
	C	9	0.85619	0.85074	0.545	
100	A	5	0.85947	0.85745	0.202	0.390
	B	9	0.85600	0.85195	0.405	
	C	8	0.85246	0.84684	0.562	
Initials		LQ	KP	KP	L	E

***Biomass** is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8730-0032312 **Sample Name** SED2DIS

Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug) 568

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	10	0.85042	0.84410	0.632	0.632	0.568	0.550	0.056995	0.072194	10.02776
	10	10	0.84289	0.83767	0.522	0.522					
	10	9	0.85066	0.84570	0.551	0.496					
1.56	10	10	0.85564	0.85066	0.498	0.498	0.498	0.498	0.067	0.067	13.45382
	10	10	0.85485	0.85054	0.431	0.431					
	10	10	0.85569	0.85004	0.565	0.565					
3.13	10	8	0.85404	0.85064	0.425	0.340	0.497	0.454	0.097358	0.138551	19.57748
	10	9	0.85205	0.84792	0.459	0.413					
	10	10	0.85400	0.84792	0.608	0.608					
6.25	10	8	0.84712	0.84227	0.606	0.485	0.567	0.490	0.036297	0.012858	6.39849
	10	9	0.84905	0.84400	0.561	0.505					
	10	9	0.85577	0.85096	0.534	0.481					
12.5	10	10	0.84146	0.83572	0.574	0.574	0.532	0.515	0.042557	0.068537	8.004965
	10	9	0.82564	0.82124	0.489	0.440					
	10	10	0.85348	0.84816	0.532	0.532					
25	10	9	0.85734	0.85110	0.693	0.624	0.591	0.529	0.096462	0.084788	16.33495
	10	8	0.85182	0.84721	0.576	0.461					
	10	10	0.85444	0.84942	0.502	0.502					
50	10	4	0.85844	0.85567	0.692	0.277	0.634	0.435	0.050727	0.1403	8.001867
	10	8	0.84662	0.84179	0.604	0.483					
	10	9	0.85619	0.85074	0.606	0.545					
100	10	5	0.85947	0.85745	0.404	0.202	0.519	0.390	0.160714	0.180489	30.97609
	10	9	0.85600	0.85195	0.450	0.405					
	10	8	0.85246	0.84684	0.702	0.562					

SD: Standard Deviation
CV: Coefficient of Variation

NAUTILUS ENVIRONMENTAL

QO 040323

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Water	**Source:**	SED2DIS (TY03086_002_AB)
Date/Time Sampled:	April 17, 2023; 08:40	**Date/Time Received:**	April 20, 2023; 11:20
Date Test Started:	April 20, 2023; 15:30	**Date Test Finished:**	April 26, 2023
Description:	Clear, Light yellow	**Days Sample Used:**	Days 0 to 5
Sample #:	8730-0032312	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.3°C
Collected By:	Not available		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032312 **Sources:** SED2DIS (TY03086_002_AB)

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 2%

Mean Number of Surviving Young Produced Within First 3 Broods: 23.1

Mean Number of Surviving Young Produced Within 7 Days of Testing: 27.3

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 1 to 5

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032312 **Sources:** SED2DIS (TY03086_002_AB)

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD23-52

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (22.1 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (80% of controls)

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032312 **Sources:** SED2DIS (TY03086_002_AB)

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	0	0	0	0	0	10	0
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	21	28	22	28	0	3	23	21
	2	27	22	20	32	29	30	23	19
	3	17	19	25	24	30	21	29	14
	4	25	22	27	25	33	30	24	4
	5	21	23	16	22	23	27	14	30
	6	28	12	31	18	21	27	0	20
	7	7	31	25	28	10	18	27	26
	8	27	24	21	26	28	12	26	24
	9	24	26	9	22	27	25	23	25
	10	24	28	31	25	25	31	32	27
Total Number of Live Neonates in First 3 Broods or Less		221	235	227	250	226	224	221	210
% Effect (+ or -)		0.0	6.3	2.7	13.1	2.3	1.4	0.0	-5.0
Mean Number of Live Neonates in First 3 Broods or Less		22.1	23.5	22.7	25.0	22.6	22.4	22.1	21.0
SD		6.3	5.4	6.8	3.9	10.2	9.1	9.1	7.5

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Ceriodaphnia dubia **Survival and Reproduction Study -** Continued

Sample #: 8730-0032312 **Sources:** SED2DIS (TY03086_002_AB)

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 22-Apr-2023 **Reference Batch #:** Zn2301

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 0.08 mg/L (0.06 mg/L; 0.11 mg/L)

Historic Geometric Mean LC50: 0.10 mg/L (0.03 mg/L; 0.32 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS-Thunder Bay Sample Name: SED2 DIS TY 03086-002-AB Sample #: 8730-0032312

Conditions for Test Validity

Control Mortality is ≤ 20%: (Acceptable) / Not Acceptable: 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable) / Not Acceptable: 8 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable) / Not Acceptable: 22.1 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: supersaturation Duration: <20 min Days: 1 to 5

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
REPRODUCTION		
3-brood IC25	>100 % Volume	No nonlinear regression models would fit IC25 - linear interpolation
95% Confidence Interval[2]	N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials TV Date 28/04/23

Results Verified: Initials AO Date 040523

C odaphnia dubia Initial Sample Measuremen Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** SED 2015 TY 03086-002-AB **Sample #:** 8730.003.2312

Day	Date 2023	Initial Measurements Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	Meters / Probes Used °C	pH	Cond.	Pre-aeration yes/no	Rate (bubbles/min)	Duration (min)	Pail Sub-Sampled	Initials
0	20·04	26	7·6	8·1	552	6/4	14/90	5/6	NO	≤100	≤ 20	1	KP
1	21·04	25	7·7	10·0	543	6/4	14/90	5/6	yes	≤100	≤ 20	1	y
2	22·04	25	7.7	10.7	555	6/4	14/90	5/6	yes	≤100	≤ 20	1	y
3	23·04	25	7.7	10.7	564	6/4	14/90	5/6	yes	≤100	≤ 20	2	y
4	24·04	24	7.7	11.6	560	6/4	14/90	5/6	yes	≤100	≤ 20	2	ET
5	25·04	25	7.7	11.8	523	6/4	14/90	5/6	yes	≤100	≤ 20	3	ET
6	26·04	~~25~~	~~7.8~~	~~12.1~~	~~519~~	~~6/4~~	~~14/90~~	~~5/6~~	~~yes~~	≤100	≤ 20	3	~~ET~~ Test came down
7	27·04									≤100	≤ 20	3	
8	28·04									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted?	Yes/No (No circled)	If yes, state mesh size: ______
Was sample pH or hardness adjusted?	Yes/No (No circled)	If yes, describe further: ______
Were alternate concentrations or dilution series used?	Yes/No (No circled)	If yes, describe further: ______
Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily?	Yes/No (Yes circled)	If no, describe further: ______
Were there any other method variations, deviations, or exclusions from method?	Yes/No (No circled)	If yes, describe further: ______
Was there anything unusual about the test, any problems encountered, or any remedial measures taken?	Yes/No (No circled)	If yes, describe further: ______

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: Control Sample Name: SED2DIS Sample #: S730-003-2312

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20.04	25	8.4	7.9	448	6/4	14/90	5/6	ET
1	21.04	26	8.3	7.9	471	6/4	14/90	5/6	y
2	22.04	25	8.3	7.8	485	6/4	14/90	5/6	KK
3	23.04	25	8.4 8.2	7.5 6	474	6/4	14/90	5/6	~~KK~~
4	24.04	24	8.4	8.2	469	6/4	14/90	5/6	DS
5	25.04	24	8.3	8.0	444	6/4	14/90	5/6	SO
6	26.04								
7	27.04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24	8.0	6.7	6/4	14/90	KP
25	8.1	6.2	6/4	14/90	y
24	8.0	6.9	6/4	14/90	KK
24	8.1	7.4	6/4	14/90	ML
25	8.2	7.5	6/4	14/90	SO
24	8.0	7.7	6/4	14/90	ML

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	y	
3	23.04	0	4	4	5	2	4	0	3	4	3	29	—	0	0	KS	KS
4	24.04	1	0	0	7	0	8	0	0	8	8	32	—	0	0	ML	ML
5	25.04	~~0~~ 4	4*	4*	6*	5*	0	7	9	0	0	42	—	0	0	KK	KK
6	26.04	16	15	5	13	13	16	0	15	12	13	118	—	0	0	ML	ML
7	27.04																
8	28.04																
Total Neonates		21	27	17	25	21	28	7	27	24	24	221	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.137% v/v Sample Name: SED2DIS Sample #: S730-003-2212

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20-04	25	8.3	7.3	452	6/4	14/90	5/6	ET
1	21-04	26	8.2	7.8	473	6/4	14/90	5/6	[illegible]
2	22-04	25	8.4	7.8	487	6/4	14/90	5/6	KK
3	23-04	24	8.3	7.4	473	6/4	14/90	5/6	[illegible]
4	24-04	24	8.2	8.2	477	6/4	14/90	5/6	DS
5	25-04	25	8.3	8.0	447	6/4	14/90	5/6	SO
6	26-04								
7	27-04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24	8.0	6.6	6/4	14/90	KP
25	8.0	5.9	6/4	14/90	[illegible]
24	8.0	6.7	6/4	14/90	KK
24	8.0	6.8	6/4	14/90	[illegible]
25	8.2	7.3	6/4	14/90	SO
24	8.0	7.5	6/4	14/90	[illegible]

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20-04																
1	21-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	
3	23-04	11	11	2	2	0	11	5	3	11	11	32	—	0	0	[illegible]	[illegible]
4	24-04	0	6	0	0	5	0	9	6	9	9+1	45	—	0	0	[illegible]	[illegible]
5	25-04	11	0	8	7	7	1	0	0	0	0	34	—	0	0	KK	KK
6	26-04	13	12	9	13	11	7	17	15	13	14	124	—	0	0	[illegible]	[illegible]
7	27-04																
8	28-04																
Total Neonates		28	22	19	22	23	12	31	24	26	28	235	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0-41% v/v Sample Name: SED2DIS Sample #: S730-003-2212

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20-04																
1	21-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	J	
3	23-04	4	3	5	3	4	5	2	4	0	4	34	—	0	0	J	J
4	24-04	0	7	8	0	0	10	7	5	4	0	41	—	0	0	MM	MM
5	25-04	6	0	0	8	4	0	0	0	5	10	33	—	0	0	KK	KK
6	26-04	12	10	12	16	8	16	16	12	0	17	119	—	0	0	MM	MM
7	27-04																
8	28-04																
Total Neonates		22	20	25	27	16	31	25	21	9	31	227	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 1.23% v/v Sample Name: SED2DIS Sample #: S730-003-2312

Day	Date 2023	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	20.04	~~25~~	~~8.3~~	~~7.2~~	~~453~~	6/14	14/10	5/6	ET
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	

Day	Date 2023	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	LS	
3	23.04	5	11	2	2	1	0	11	2	4	11	28	—	0	0	CS	LS
4	24.04	0	9	10	9	7+1	3	9	9	0	9	66	—	0	0	MC	MC
5	25.04	9	0	0	0	0	5	0	0	6	0	20	—	0	0	KK	KK
6	26.04	14	19	12	14	13	10	15	15	12	12	136	—	0	0	MC	MC
7	27.04																
8	28.04																
Total Neonates		28	32	24	25	22	18	28	26	22	25	250	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 3.7% v/v Sample Name: SED2DIS Sample #: S730-003-2312

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20-04	25	8.3	7.3	457	6/4	14/90	5/6	ET
1	21-04	26	8.3	7.8	477	6/4	14/90	5/6	CJ
2	22-04	25	8.4	7.8	489	6/4	14/90	5/6	KK
3	23-04	25	8.3	7.4	479	6/4	14/90	5/6	M
4	24-04	25	8.3	8.1	481	6/4	14/90	5/6	DS
5	25-04	25	8.3	8.0	452	6/4	14/90	5/6	SO
6	26-04								
7	27-04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24	7.9	5.8	6/4	14/90	KP
25	8.0	5.6	6/4	14/90	M
24	KK 8.9	6.3 KK	6/4	14/90	KK
24	8.1	7.4	6/4	14/90	M
25	8.1	7.3	6/4	14/90	SO
24	8.1	7.6	6/4	14/90	M

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20-04																
1	21-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	CJ	
3	23-04	0	5	1	4	3	3	4	4	5	4	33	—	0	0	CJ	CJ
4	24-04	0	0	0	11	0	0	6	8	8	0	33	—	0	0	M	M
5	25-04	0	8	10	0	8	6	0	0	0	7	39	—	0	0	KK	KK
6	26-04	0	16	19	18	12	12	0	16	14	14	121	—	0	0	M	M
7	27-04																
8	28-04																
Total Neonates		0	29	30	33	23	21	10	28	28*	25	226	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 11.11% v/v Sample Name: SED2DIS Sample #: S730-003-2312

Day	Date 2023	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	

Day	Date 2023	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	y	
3	23.04	0	3+1	2	1+1	2	2	2	5	4	3	26	—	0	0	y	y
4	24.04	0	0	0	0	10	9	7	2	8	0	36	—	0	0	m	m
5	25.04	3	9	7	12	0	0	0	5	0	9	45	—	0	0	KK	KK
6	26.04	0	17	12	16	15	16	9	0	13	19	117	—	0	0	m	m
7	27.04																
8	28.04																
Total Neonates		3	30	21	30	27	27	18	12	25	31	224	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 33.33% v/v Sample Name: SED2DIS Sample #: S730-003-2312

Day	Date 2023	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	

Day	Date 2023	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0 Dead	0	0	0	0	0	6	10	0	JJ	
3	23.04	1	1	2	1	3		2	4	5	6	31	—	10	0	JJ	JJ
4	24.04	10	10	0	5	9		0	8	6	10	58	-	10	0	MC	MC
5	25.04	0	0	10	0	2 MC		10	0	0	0	22	-	10	0	KK	KK
6	26.04	9	12	17	15	~~14~~ *		15	14	12	16	~~134~~ MC 110	-	10	0	MC	MC
7	27.04																
8	28.04																
Total Neonates		23	23	29	24	14	0	27	26	23	32	~~235~~ MC 221	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 100% v/v Sample Name: SED2DIS Sample #: S730-003-2212

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	20-04	25	7.8	7.7	548	6/4	14/90	5/6	ET	24	8.0	6.2	6/4	14/90	KP
1	21-04	26	7.8	8.8	555	6/4	14/90	5/6	y	25	8.0	6.0	6/4	14/90	y
2	22-04	25	7.9	9.6	565	6/4	14/90	5/6	KK	24	8.2	7.1	6/4	14/90	KK
3	23-04	25	7.9	8.9	565	6/4	14/90	5/6	y	24	8.3	7.6	6/4	14/90	m
4	24-04	25	7.9	9.0	563	6/4	14/90	5/6	DS	25	8.3	7.6	6/4	14/90	SO
5	25-04	25	7.9	9.7	526	6/4	14/90	5/6	SO	24	8.2	7.6	6/4	14/90	m
6	26-04														
7	27-04														

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20-04																
1	21-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	y	
3	23-04	3	0	0	3+1	5 m	0	1	4	4	3	24	—	0	0	y	y
4	24-04	9	5	6	0	6 8 m	0	5	9	8	0	50	—	0	0	m	m
5	25-04	9	0	8	0	0	10	9	0	0	8	44	—	0	0	KK	KK
6	26-04	0	14	0	0	17	10	11	11	13	16	92	—	0	0	m	m
7	27-04																
8	28-04																
Total Neonates		21	19	14	4	30	20	26	24	25	27	210					

Notes: * = ≥ 4th brood (not counted)

Ceriodaphnia dubia Neonate Origin

Sample Information

Client ALS - THUNDER BAY
Sample Name SED 2 DIS
ample # 8730-003-2312
Date/Time Collected 17/04/23 / 0840 Person Sampling N/A
Date/Time Received 20.04.23 / 11:20
Arrival Temp (°C) 17.3
Sample Type WATER
Sample Description CLEAR, LIGHT YELLOW
100% Hardness ~12

Test Information

Date Test Started 20.04.23 / 1530
Test Started By ET
Template Used for Randomization 4
Dilution Water Batch Number CD2352
Control Hardness 128

Individual Culture Health Data

Date Culture Started 14.04.23
% mortality in previous 7 days (must be ≤20%) 0 ~~31.9~~ KK
Average # neos in 1st 3 broods (must be ≥ 15) 21.9
Culture I.D. (*e.g.*, Wed Row 4) FRi Row 5
Average # neos in previous 7 days (must be ≥15) 21.9
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 14.04.23
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 19.9
Culture I.D. (*e.g.*, Wed Row 4) FRi Row 7
Average # neos in previous 7 days (must be ≥15) 20.7
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 14.04.23
% mortality in previous 7 days (must be ≤20%) 10
Average # neos in 1st 3 broods (must be ≥ 15) 23.2
Culture I.D. (*e.g.*, Wed Row 4) FRi Row 8
Average # neos in previous 7 days (must be ≥15) 20
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 12.04.23
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 25.6
Culture I.D. (*e.g.*, Wed Row 4) Xtra Row 1
Average # neos in previous 7 days (must be ≥15) 37.1
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 12.04.23
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 25.0
Culture I.D. (*e.g.*, Wed Row 4) Xtra Row 2
Average # neos in previous 7 days (must be ≥15) 37
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days ~~3.3~~ 2 KK (add up % mortality for all cultures used / # cultures used)
Mean Number of Young Produced within first 3 broods ~~21.7~~ KK (avg # 1st 3 broods for all cultures used / # cultures used)
Mean Number of Young Produced in previous 7 days ~~23.3~~ KK (avg # neos in prev 7 days for all cultures used / # cultures used)
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / (No) (circle one)

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
F 5.6	(Y) / N	(Y) / N	8	1	KK
.10	(Y) / N	(Y) / N	8	2	KK
7.3	(Y) / N	(Y) / N	8	3	KK
.7	(Y) / N	(Y) / N	12	4	KK
.10	(Y) / N	(Y) / N	12	5	KK
8.3	(Y) / N	(Y) / N	9	6	KK
X 1.1	(Y) / N	(Y) / N	13	7	KK
.3	(Y) / N	(Y) / N	12	8	KK
.7	(Y) / N	(Y) / N	14	9	KK
X 2.1	(Y) N	(Y) / N	15	10	KK
	Y / N	Y / N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032312	**Sample Name:**	SED2DIS (TY03086_002_AB)
Sample Method:	Grab	**Collected by:**	N/A
Date/Time Collected:	April 17, 2023; 08:40	**Arrival Temp.:**	17.3°C
Date/Time Received:	April 20, 2023; 11:20	**Sample Description:**	Clear, Light yellow
Sample Point Description:	N/A	**Sample Type:**	Water
Transportation:	Road		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species (Strain #):	*Raphidocelis subcapitata* (CPCC # 37)
Source:	Nautilus Plant Culture Unit (from CPCC)
Culture Temp.:	24 ± 2 °C
Test Culture Number:	G2(l)c
Culture Age at Test Start:	6 days old
Cell Density in the Microplate Wells at the Start of the Test:	10,454.55 cells/ml

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032312 **Sample Name:** SED2DIS (TY03086_002_AB)

Test Conditions

Date/Time Test Start:	April 20, 2023; 15:20	**T=0 Control pH:**	5.7
Date/Time Test End:	April 23, 2023; 14:00-16:00	**T=72 Control pH:**	6.0
Sample pH Before Dilution:	7.0	**pH Adjustment:**	None
Test Duration:	72 hours		

Mean Test Temperature (±Standard Deviation): 25.6 (±0.7) °C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Millipore

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032312 **Sample Name:** SED2DIS (TY03086_002_AB)

Test Conditions - continued

Enumeration Technique: Neubauer Haemacytometer

Test Vessel: 96-Well U-bottomed Polystyrene Microplate

Concentration of Test Solutions: 90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control

 # Replicates/Concentration: 4 reps started; 3 reps counted of test solutions
10 control reps started, 2 used for pH measurement

Design if Specialized Procedure: Not applicable

Method Deviations or Unusual Occurrences: None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times: Acceptable (18.8 times)

pH in controls did not vary by more than 1.5 units: Acceptable (0.3 units)

C V for cell yields is < 20% within the control wells: Acceptable (6.0)

No inhibitory trend detected across the control wells: Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
212,045	184,545	194,545	189,545	199,545	214,545	182,045	194,545	196,420	6.0

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 CV = Coefficient of Variation = (100 x standard deviation / mean)

***Raphidocelis subcapitata*72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032312 **Sample Name:** SED2DIS (TY03086_002_AB)

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	1,204,545	679,545	264,545	244,545	232,045
2	1,269,545	744,545	254,545	232,045	192,045
3	1,292,045	709,545	262,045	224,545	222,045
Mean Cell Yield	1,255,378	711,212	260,378	233,712	215,378
Coefficient Variation[2]	4	5	2	4	10
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	209,545	229,545			
2	219,545	212,045			
3	219,545	219,545			
Mean Cell Yield	216,212	220,378			
Coefficient Variation[2]	3	4			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	**Result[1]**	**Method of Calculation**
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	No trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032312 **Sample Name:** SED2DIS (TY03086_002_AB)

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: No

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2306 **Date Test Initiated:** 17-Apr-2023

Method of Analysis: 2P Exponential **Algae Lot #:** G7(l)c

72-hour IC25 (95% Confidence Limits): 28.68 mg/L (21.36 mg/L; 36.54 mg/L)

Historic Geometric Mean IC25: 57.39 mg/L (27.05 mg/L; 121.80 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS Thunder Bay Sample Name SED 2 DIS TY 03086 002 - AB Sample # 8730-00323 R

Conditions for Test Validity

Cell increase for control is >16	Acceptable/Not acceptable	18.48 (times)
CV among controls ≤ 20	Acceptable/Not acceptable	5.4 6.0
Result of Mann-Kendall test for trend	Acceptable/Not acceptable	no significant trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 115 000 (cells/mL)

Used: Yes/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10454.55 (cells/mL)

Analysis Completed: Initials: TV Date: 26/04/23

Results Verified: Initials: AO Date: 04/05/23

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location: ALS · TB	
Sample #: 9730 0032312	Sample Name: SED2DIS TY03080002-AB
Collection Method: grab	Collected By: n/a
Date/Time Collected: 17/04/23 @ 0740	Arrival Temp.: (meter/probe) 17.3 °C (44)
Date/Time Received: 20/04/23 @ 1120	Sample Description: clear, light yellow
Collection Point Description: n/a	Sample Type: ☑ Effluent ☐ Chemical ☐ Other:
Transportation: road	Storage: n/a

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	AO
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # G2(P)C	AO
Culture Age at Start of Test	6 days old (must be 3 to 7 days old)	AO
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	AO
	Axenic culture? Yes/No (Circle one)	AO

Notes:

Test Conditions:

Date / Time Test Start: 20.04.23 @ 1520 Started By: AO		Date / Time Test End: 23.04.23 @ 1400 – 1600 Finished By: AO	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 7.0	pH adjustment: Y/N	pH of well D6 at T=0 h 5.7 5.8	pH of well D7 at T=72 h 6.0
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # —		Metal mining NUT Lot # NUT 2302
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation) 25.6 ± 0.7 °C			

	Day 1		Day 2	
¼ plate rotation (Initial)	AM	PM	AM	PM
	J	J	✗	✓
Lights ON (Initial)	AM	PM	AM	PM
	J	J	✗	✓

* For reference test, record pH of top concentration before use for series dilutions.

Notes:

72-Hour Qualitative Observations:

Condensation: no		
Growth: yes		
Were there any other method variations or deviations from methods? Yes/No		If yes, describe further:
Anything unusual about the test? Yes/No		
Any problems encountered? Yes/No		
Any remedial measures taken? Yes/No		

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 8730-003 2312	Sample Name: SE02 DIS

Reference Data:

Reference Chemical Batch #	Phenol P 2306	Date test started	17.04.23
Method of Analysis	Nonlinear regression 2P Exponential	Algae Lot #	G 7(1)c
72-hour IC25 (95% C.I.)[3] mg/L	28.68 21.36; 36.59	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	57.39 27.05; 121.80

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	> 90.91 % (N/A)	No nonlinear regression model would fit data - linear interpolation
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers	—	
Test for Outliers	No Outliers Present	Grubbs' Test for Residual Outlier Initial C
	If outliers present, indicate Concentration/Rep: 90.91 % rep 1	
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed.	
	No statistically significant growth stimulation.	Williams' or (Dunnett's) Multiple Comparison Test Initial C
	(Yes) statistically significant growth stimulation at these concentrations: 90.91; 30.303; 10.101; 3.367	

1)Results relate only to the sample tested. ae
2) Tidepool Scientific Software © 2000-2019. Comprehensive Environmental Toxicity Information System – CETIS v. ~~1.9.6.7~~ ae 2.1.3.5
3) Empirical 95% Confidence Interval ae

Weighting techniques applied to the data? Yes/(No)

Any outliers and justification for their removal? (Yes)/No

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Inoculum Preparation

Sample Name: Sample Number: 8730.003231 Date Test Start: 20.04.23
" " 12
" " 13

Cell count	0.1 µl / 0.004 µl		0.1 µl / 0.004 µl		0.1 µl / 0.004 µl	
1	27	30	28	26	12	10
2	32	50	19	21	8	13
3	41	44	25	23	13	13
4	43	47	26	22	11	12
5	44	50	-	-	-	-
Total cells	187	227	98	92	44	48
Cells/ µl *	9350	11 350	245	230	110	120
Cells/ ml	9 350 000	11 350 000	245 000	230 000	110 000	120 000
Avg. cells/ ml	10 350 000		237 500		115 000	
Time / Initials	1345 / ao		1400 / ao		1415 / ao	
Comments/ calculations	2 200 000 / 10 350 000 = 0.21		OK		1/2 NUT OK - used	

Cell count	0.1 µl / 0.004 µl		0.1 µl / 0.004 µl		0.1 µl / 0.004 µl	
1						
2						
3						
4						
5						
Total cells						
Cells/ µl *						
Cells/ ml						
Avg. cells/ ml						
Time / Initials						
Comments/ calculations						

* Cells/ul: count 4 outside large squares (0.1 ul), add, multiply by 2.5 OR count 5 inside small squares (0.004 ul), add, multiply by 50

Nautilus Environmental Company Inc.

Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)

72-Hour Growth Inhibition Test – Continued

72-Hour Quantitative Observations of Controls

Sample Name: TY03036_002-AB Sample Number: 330-0033212 Date Test Start: 20.04.23

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D6	Well # D9	Well # D10	Well # D11
1	23	17	18	21	17	28	20	15
2	22	23	26	13	21	17	18	22
3	25	20	21	16	19	26	23	19
4	19	13	17	25	26	19	16	26
5	—	—	—	—	—	—	—	—
Initials	AO	AO	AO	AO	AO	AO	AO	AO

Cell increase for controls = 18.48	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = ~~5.4~~ 6.0	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = no significant trend	Controls are invalid if there is a trend detected by the Mann-Kendall test

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: TY03086_002 -AB Sample Number: 3730-00323 12 Date Test Start: 20-04-23

Theoretical Test Concentration: 100.00 %v/v C2				Actual Test Concentration: 90.91 %v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # ~~B3~~ C3	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	133	128	124		1255379 (± 45438)
2	121	130	131		Coefficient of Variation of Cell Yield
3	117	129	130		4
4	115	125	136		Average % Inhibition (-ve number = enhancement)
5	–	–	–		-551.568
Initials	aO	aO	aO		C

Theoretical Test Concentration: 33.33 %v/v				Actual Test Concentration: 30.303 %v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	64	72	61		711212 (± 32532)
2	76	69	83		Coefficient of Variation of Cell Yield
3	63	79	74		5
4	73	82	70		Average % Inhibition (-ve number = enhancement)
5	–	–	–		-269.134
Initials	aO	aO	aO		C

Theoretical Test Concentration: 11.11 %v/v				Actual Test Concentration: 10.101 %v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	31	26	30		260379 (± 5204)
2	21	25	27		Coefficient of Variation of Cell Yield
3	28	30	23		2
4	30	25	29		Average % Inhibition (-ve number = enhancement)
5	–	–	–		-35.142
Initials	aO	aO	aO		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: TY03086_00 2 -AB Sample Number: 8730-00323 12 Date Test Start: 20.04.23

Theoretical Test Concentration: 3.704 %. v/v				Actual Test Concentration: 3.367 %. v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation) 233712 (± 10104)
1	24	25	24		
2	30	19	21		Coefficient of Variation of Cell Yield 4
3	21	27	29		
4	27	26	20		Average % Inhibition (-ve number = enhancement) -21.301
5	–	–	–		
Initials	ao	ao	ao		C

Theoretical Test Concentration: 1.235 %. v/v				Actual Test Concentration: 1.122 %. v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation) 245379 (± 20817)
1	23	21	25		
2	23	24	20		Coefficient of Variation of Cell Yield 10
3	24	18	21		
4	27	18	27		Average % Inhibition (-ve number = enhancement) -11.786
5	–	–	–		
Initials	ao	ao	ao		D

Theoretical Test Concentration: 0.412 %. v/v				Actual Test Concentration: 0.374 %. v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation) 216212 (± 5774)
1	24	25	26		
2	21	21	22		Coefficient of Variation of Cell Yield 3
3	24	17	20		
4	19	29	24		Average % Inhibition (-ve number = enhancement) -12.219
5	–	–	–		
Initials	ao	ao	ao		E

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: TY03086_002-AB Sample Number: 8730.00323-12 Date Test Start: 20.04.23

Theoretical Test Concentration: 0.137 %. v/v				Actual Test Concentration: 0.125 %. v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	25	16	23		220379 (± 8780)
2	22	22	28		Coefficient of Variation of Cell Yield
3	30	27	21		4
4	19	24	20		Average % Inhibition (-ve number = enhancement)
5	–	–	–		-14.381
Initials	CO	CO	CO		C

Theoretical Test Concentration:			Actual Test Concentration:		
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:			Actual Test Concentration:		
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	SED2DIS Ty03086-002- Sample #	8730-0032312			Date test start	20.04.23										
Calculate initial algal cell concentration																
	Concentration of innocculum (cells/ml)	20			Use last count algae/nutrient mixture or algae only											
	Volume of algae addition (uL)	115000			Algae/nutrient mixture = 20uL, algae only 10uL											
	Cells added to each test well	2300			**Cell yield (must be >16 times in controls)**											
	Cells/ml in well at T=0	10454.5455			=	18.788043										
enter control data																
	D2	D3	D4	D5	D8	D9	D10	D11								
	23	17	18	21	18	28	20	15								
	22.0	23.0	26.0	18.0	21.0	17.0	18.0	22.0								
	25	20.0	21.0	16.0	19.0	26.0	23.0	19.0								
	19.0	18.0	17.0	25.0	26.0	19.0	16.0	26.0								
total cells	89	78	82	80	84	90	77	82								
cells/ul	222.5	195	205	200	210	225	192.5	205								
cells/ml	222500	195000	205000	200000	210000	225000	192500	205000								
Cell yield = measured concentration - initial algal cell concentration																
	212045	184545	194545	189545	199545	214545	182045	194545								
Mean cell yield for the control	= Rc		Standard deviation			coefficient of variation										
Rc	196420		SD	11858.5412		CV	6.037325	**Must be ≤20**								

% inhibition summary

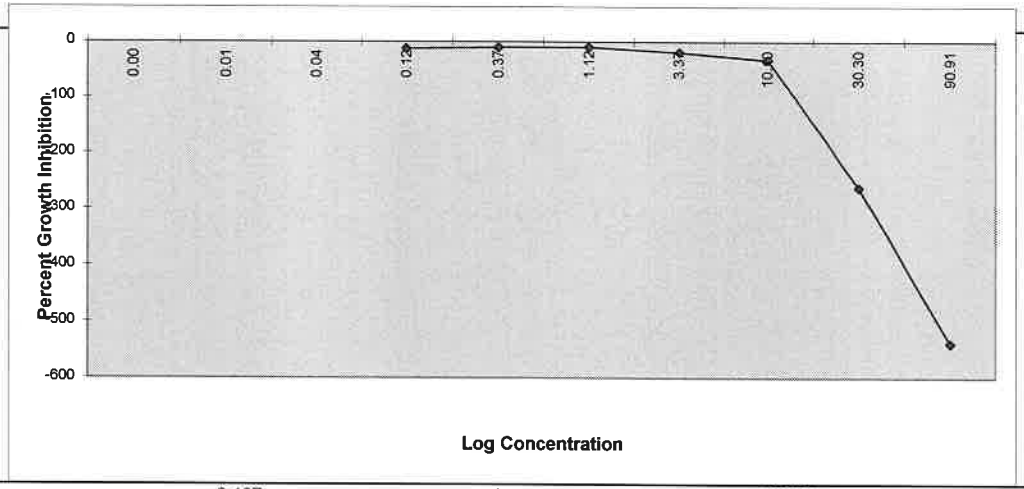
Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	-12.197
0.374	-10.076
1.122	-9.652
3.367	-18.986
10.101	-32.562
30.303	-262.087
90.910	-539.128

enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	133	128	124	64	72	61	31	26	30	24	25	24	23	21	25
	121.0	130.0	131.0	76.0	69.0	83.0	21.0	25.0	27.0	30.0	19.0	21.0	23.0	24.0	20.0
	117.0	129.0	130.0	63.0	79.0	74.0	28.0	30.0	23.0	21.0	27.0	29.0	24.0	18.0	21.0
	115.0	125.0	136.0	73.0	82.0	70.0	30.0	25.0	29.0	27.0	26.0	20.0	27.0	18.0	27.0
total cells	486	512	521	276	302	288	110	106	109	102	97	94	97	81	93
cells/ul	1215	1280	1302.5	690	755	720	275	265	272.5	255	242.5	235	242.5	202.5	232.5
cells/ml	1215000	1280000	1302500	690000	755000	720000	275000	265000	272500	255000	242500	235000	242500	202500	232500
Cell yield = measured concentration - initial algal cell concentration															
	1204545	1269545	1292045	679545	744545	709545	264545	254545	262045	244545	232045	224545	232045	192045	222045
Mean Yield	1255379			711212			260379			233712			215379		
STD Yield	45438			32532			5204			10104			20817		
CV Yield	4			5			2			4			10		

Average % inhibition											
for 90.910%:	-539.128		for 30.303%	-262.087		for 10.101%	-32.562		for 3.367%	-18.986		for 1.122%	-9.652	

Average % stimulation											
for 90.910%:	539.128		for 30.303%	262.087		for 10.101%	32.562		for 3.367%	18.986		for 1.122%	9.652	



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
24	25	26	25	16	23									
21.0	21.0	22.0	22.0	22.0	28.0									
24.0	17.0	20.0	30.0	27.0	21.0									
19.0	29.0	24.0	19.0	24.0	20.0									
88	92	92	96	89	92	0	0	0	0	0	0	0	0	0
220	230	230	240	222.5	230	0	0	0	0	0	0	0	0	0
220000	230000	230000	240000	222500	230000	0	0	0	0	0	0	0	0	0
209545	219545	219545	229545	212045	219545	-10455	-10455	-10455	-10455	-10455	-10455	-10455	-10455	-10455
216212			220379			-10455			-10455			-10455		
5774			8780			0			0			0		
3			4			0			0			0		
for 0.374%	-10.076		for 0.125%	-12.197		for 0.042%	105.323		for 0.014%	105.323		for 0.005%	105.323	
for 0.374%	10.076		for 0.125%	12.197		for 0.042%	-105.323		for 0.014%	-105.323		for 0.005%	-105.323	

Mann-Kendall Trend Test for *Selenastrum capricornutum* Growth Inhibtion Test

EPS Method 1/RM/25
Version 1.1, Nov. 2000
Pollutech EnviroQuatics

Sample #: 8730-0032312
Client #: ALS TB
Date tested: 20.04.23

Instructions:

Enter control data below cells labelled D2..D5 and D8..D11.
The test of significance is completed immediately following data entry.

D2	D3	D4	D5	D8	D9	D10	D11
212045	184545	194545	189545	199545	214545	182045	194545

Results:

There is no significant trend.

Notes:

The test is a two-sided alternative using a nominal alpha value of 0.05%.
Due to the discrete nature of the test statistic, the true alpha value is 0.062%.

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
SED2DIS (TY03086_002_AB)	8730-0032312	April 17, 2023	April 20, 2023	April 20, 2023	8.29% Volume (N/A; 54.84)% Volume	7.03% Volume (N/A; 81.67)% Volume	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	13-Apr-2023	**Reference Chemical:**	KCl
Frond Increase IC25:	2.59 g/L	**95% Confidence Limits:**	2.19; 2.82 g/L
Historic Geometric Mean IC25:	2.07 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.28; 3.35 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	Na_2MoO_4	0.0726
	K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$2H_2O$	0.00003
	KCl	10.1	$MgSO_4\ 7H_2O$	147	$ZnCl_2$	0.00001
					$CoCl_2\ 6H_2O$	0.00001
					$CuCl_2\ 2H_2O$	

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

C. D'Andrea

Date: May 5, 2023

Carol D'Andrea
Laboratory Supervisor

Test Material

Client Name/Location: ALS – Thunder Bay	
Sample #: 8730-0032312	Sample Name: SED2 DIS TY 03086-002-AB
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 17/04/23 8:40	Arrival Temperature (meter/probe): 17.3°C (44)
Date/Time Received: 20/04/23 11:20	Sample Description: clear, light yellow
Collection Point Description: River	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: MV / Road	Storage: none

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	E
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	E
Culture Age at Start of Test:	8 days old, acclimated 23 hours in fresh test solution (mAPHA)	E
Culture Medium:	Modified Hoagland's E+ medium, Lot # MM2301	E
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/No	E
	Axenic culture? Yes/No	E
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 42 in HT 1, 41 in HT 2, 40 in HT 3 at finish	E

Test Conditions and Procedures

Date / Time Test Start: 20.04.23 15:30 Started By: DV	Date / Time Test End: 27.04.23 11:35 Finished By: DV
Test Type: Static (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / No (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: Modified APHA (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 8.0 (13/91)	pH Adjustment: none
Test Volume and Depth: 150 mL / 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / No	If yes, describe further:
Anything unusual about the test? Yes / No	
Any problems encountered? Yes / No	
Any remedial measures taken? Yes / No	Randomization Template: D

Sample #: 8730-0032312	Sample Name: SED2 DIS

Test Variables, Observations, and Measurements

Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	26	25	26	25	25	25	25	26
Low	26	25	26	25	25	25	26	26
Medium	26	25	26	25	25	25	26	26
High	26	25	26	25	25	25	25	26
Initials	EV	EV	KK	KK	CG	CG	CG	EV
meter/probe	50	44	44	44	44	44	44	44
Mean Test Temperature (average of 24h high / low temperatures):	25.4 ± 0.1 °C							

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.2	8.2	8.2	8.0	EV	13/911
Day 7	Control	Low	Medium	High	Initials	pH meter/probe
	8.74	9.4	9.6	9.6	CG	13/91

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)								

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 24/04/23 (4)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 12					Initials: EV
5580	4770	5490	5190	5230	Mean Light Measurement: 5252
±15% Variation of Mean: 4465 – 6039					(Acceptable) / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) No
If yes, describe further: Haze / Brown ppt at upper concentrations.

Reference Data

Reference Date	(Frond Increase) or Dry Weights (circle one)			
28 JUN 23	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
	~~2.59~~ 1.97	~~2.19~~; ~~2.87~~ 1.29 2.63	~~2.07~~ 04	~~1.25~~; ~~3.35~~ 1.26 ; 3.28

Sample #: 8730-0032312	Sample Name: SED2 DIS

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 91	B 91	C 81	D 85	87.0

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy							Initials C
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV : 81.0 (4.9) 6.0							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-8.33	-3.40	-16.67	-7.72	-20.37	-13.89	-17.59
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control % v/v g/L	0.097	0.29	0.97	3.1	97	31	97
Mean % Stimulation	-22.60	4.90	-16.12	-9.89	-25.47	-14.00	-13.71

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: (Yes) Not applicable (no stimulation) (Circle one)
weights

Test Endpoints and Calculations:

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	>97% (N/A)	Nonlinear regression 3P log Logistic model
IC25 (95% C.I.)[3] If calculated without outlier[4]	—	—
Dry Weights		
IC25 (95% C.I.)[3]	>97% (N/A) *	Nonlinear regression 2P Exponential model
IC25 (95% C.I.)[3] If calculated without outlier[4]	—	—

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019-22 Comprehensive Environmental Toxicity Information System CETISv ~~1.9.6~~ 2.1.3.5
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

* 25.5% inhibition at 9.7% v/v

Weighting techniques applied to the data? Yes / (No)

Any outliers and justification for their removal? (Yes) / No
weights (0.097, rep2)

Lemna minor Day 7 Observations

Client: ALS Thunder Bay	Sample number: 8730-0032312	Date Started: 20.04.23	
Site: SED2 DIS		Date Ended: 27.04.23	
Concentration: control	Observations By: C	Concentration: 0.097 % v/v	Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	91	91	81	85	Number of	90	65	95	71
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X	Abnormally sized fronds	✓ Smaller	✓ Smaller	✓ Smaller	✓ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations					Other Observations	Algae bromine ppt	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

N/A

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor D_ 7 Observations

Client: ALS Thickoler Bay		Sample number: 8730-0032312	Date Started: 20.04.23
Site: SED2 DIS			Date Ended: 27.07.23
Concentration: 0.29% v/v	Observations By: C	Concentration: 0.97% v/v	Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	80	104	77	76	Number of	83	78	67	66
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	algae brown ppt	→	→	→	Other Observations	algae	→	→	→

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO (circled: NO) Weights: YES / NO (circled: YES)

Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO (circled: NO) Weights: YES / NO (circled: NO)

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor L. 7 Observations

Client: ALS Thunder Bay	Sample number: 8730-0032312	Date Started: 20.04.23	
Site: SED2 DIS		Date Ended: 27.04.23	
Concentration: 3.1 % v/v	Observations By: C	Concentration: 4.7 % v/v	Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	84	76	92	71	Number of	76	67	74	65
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	✓	Necrosis (localized dead tissue on fronds, which appears brown or white)	✓	✓	✓	✓
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√ smaller	√√ smaller	√√ smaller	√√ smaller	Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	brown ppt at the bottom	→	→	→	Other Observations	brown ppt at the bottom	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor L., 7 Observations

Client: ALS Thunder Bay	Sample number: 8730-0032312	Date Started: 20.04.23	
Site: SED2 DIS		Date Ended: 27.04.23	
Concentration: 31% v/v	Observations By: C	Concentration: 97% v/v	Observations By: C

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	91	67	77	68	Number of	74	84	65	68
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	✓ Smaller	✓ Smaller	✓ Smaller	✓ Smaller	Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	✓ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	✓	X	✓	Loss of Buoyancy	X	✓	✓	✓
Other Observations	Brown ppt →				Other Observations	algae →			
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Lemna minor Weights

Client	Site	Sample number
ALS-ThunderBay	SED2 DIS	8730.0032312
In Oven Date/Time/ °C: 27/04/23 12:15 60°	Out Oven Date/Time/°C: 28/04/23 12:15 61°	

Conc.	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	85	81.0 (4.9)	0.84743	0.84124	6.19	6.02 (0.7)
	B	85		0.86549	0.85930	6.19	
	C	75		0.86021	0.85523	4.98	
	D	79		0.84876	0.84205	6.71	
0.097	A	84	74.3 (14.5)	0.84502	0.83951	5.51	4.66 (1.9)
	B	59		0.83268	0.83054	2.14	
	C	89		0.84017	0.83364	6.53	
	D	65		0.84465	0.84020	4.45	
0.29	A	74	78.3 (13.3)	0.85337	0.84627	7.10	6.31 (1.3)
	B	98		0.85338	0.84581 CG 13	7.57	
	C	71		0.85192	0.84603	5.89	
	D	70		0.85146	0.84677	4.69	
0.97	A	77	67.5 (8.3)	0.85532	0.84922	6.10	5.05 (0.8)
	B	72		0.84992	0.84467	5.25	
	C	61		0.85542	0.85132	4.10	
	D	60		0.85216	0.84742	4.74	
3.1	A	78	74.8 (9.2)	0.84656	0.84089	5.67	5.42 (0.9)
	B	70		0.85251	0.84729	5.22	
	C	86		0.86026	0.85384	6.42	
	D	65		0.85679	0.85241	4.38	
9.7	A	70	64.5 (5.3)	0.85974	0.85514	4.60	4.49 (0.4)
	B	61		0.84968	0.84556	4.12	
	C	68		0.85949	0.85457 CG	4.92	
	D	59		0.85072	0.84642	4.30	
31	A	85	69.8 (11.1)	0.87056	0.86423	6.33	5.18 (0.9)
	B	61		0.84941	0.84508	4.33	
	C	71		0.85318	0.84771	5.47	
	D	62		0.84985	0.84528	4.57	
97	A	68	66.8 (8.4)	0.84030	0.83484 CG 6	5.44	5.19 (0.5)
	B	78		0.84383	0.83804	5.79	
	C	59		0.84495	0.84009	4.86	
	D	62		0.84035	0.83567	4.68	
Initials		CG	CG	CG	CG	CG	CG

Notes:

Sample name SED2DIS Date started 20/04/23 sample # 8730-0032312

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

87.0 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	91	90	80	83	84	76	91	74
	91	65	104	78	76	67	67	84
	81	95	77	67	92	74	77	65
	85	71	76	66	71	65	68	68
Total Fronds	348	321	337	294	323	282	303	291

Increase in Frond Number = Total # Fronds - 6 Starting Fronds

	85	84	74	77	78	70	85	68
	85	59	98	72	70	61	61	78
	75	89	71	61	86	68	71	59
	79	65	70	60	65	59	62	62
Total Increase	324	297	313	270	299	258	279	267
Mean Increase	81.0	74.3	78.3	67.5	74.8	64.5	69.8	66.8
SD Increase	4.9	14.5	13.3	8.3	9.2	5.3	11.1	8.4
CV Increase	6.0	19.5	17.0	12.4	12.3	8.3	15.9	12.6
% Stimulation		**-8.33**	**-3.40**	**-16.67**	**-7.72**	**-20.37**	**-13.89**	**-17.59**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g) **Pan + Plant**	0.84743	0.84502	0.85337	0.85532	0.84656	0.85974	0.87056	0.84030
	0.86549	0.83268	0.85338	0.84992	0.85251	0.84968	0.84941	0.84383
	0.86021	0.84017	0.85192	0.85542	0.86026	0.85949	0.85318	0.84495
	0.84876	0.84465	0.85146	0.85216	0.85679	0.85072	0.84985	0.84035
Initial Weight (g) **Pan Only**	0.84124	0.83951	0.84627	0.84922	0.84089	0.85514	0.86423	0.83486
	0.85930	0.83054	0.84581	0.84467	0.84729	0.84556	0.84508	0.83804
	0.85523	0.83364	0.84603	0.85132	0.85384	0.85457	0.84771	0.84009
	0.84205	0.84020	0.84677	0.84742	0.85241	0.84642	0.84528	0.83567
Plant Only (mg)	6.19	5.51	7.10	6.10	5.67	4.60	6.33	5.44
	6.19	2.14	7.57	5.25	5.22	4.12	4.33	5.79
	4.98	6.53	5.89	4.10	6.42	4.92	5.47	4.86
	6.71	4.45	4.69	4.74	4.38	4.30	4.57	4.68
Mean Dry Weight	6.018	4.658	6.313	5.048	5.423	4.485	5.175	5.192
SD Dry Weight	0.7	1.9	1.3	0.8	0.9	0.4	0.9	0.5
CV Dry Weight	12.2	40.4	20.5	16.7	15.7	7.8	17.6	9.9
% Stimulation		**-22.60**	**4.90**	**-16.12**	**-9.89**	**-25.47**	**-14.00**	**-13.71**

For Data Transfer to CETIS

		# fronds	total mass	tare
0	1	85	0.84743	0.84124
	2	85	0.86549	0.85930
	3	75	0.86021	0.85523
	4	79	0.84876	0.84205
0.1	1	84	0.84502	0.83951
	2	59	0.83268	0.83054
	3	89	0.84017	0.83364
	4	65	0.84465	0.84020
0.3	1	74	0.85337	0.84627
	2	98	0.85338	0.84581
	3	71	0.85192	0.84603
	4	70	0.85146	0.84677
1	1	77	0.85532	0.84922
	2	72	0.84992	0.84467
	3	61	0.85542	0.85132
	4	60	0.85216	0.84742
3.1	1	78	0.84656	0.84089
	2	70	0.85251	0.84729
	3	86	0.86026	0.85384
	4	65	0.85679	0.85241
9.7	1	70	0.85974	0.85514
	2	61	0.84968	0.84556
	3	68	0.85949	0.85457
	4	59	0.85072	0.84642
31	1	85	0.87056	0.86423
	2	61	0.84941	0.84508
	3	71	0.85318	0.84771
	4	62	0.84985	0.84528
97	1	68	0.84030	0.83486
	2	78	0.84383	0.83804
	3	59	0.84495	0.84009
	4	62	0.84035	0.83567

@ D4.05.23

May 5, 2023

ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Attention Lab:

On April 20, 2023, Nautilus Environmental Company Inc. personnel received one water sample EDL2 (TY03086_003_AB) from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected April 17, 2023

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
EDL2 (TY03086_003_AB) #8730-0032313	Fathead Minnow	7-day LC50 (95% Confidence) 7-day IC25 (95% Confidence)	Survival Biomass	>100% Volume[2] (Not Applicable) >100% Volume[2] (Not Applicable)
	Ceriodaphnia dubia	3-brood LC50 (95% Confidence) 3-brood IC25 (95% Confidence)	Survival Reproduction	>100% Volume[2] (Not Applicable) 88% Volume[2] (20.31; N/A)% Volume
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[2] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence) 7-day IC25 Dry Weight (95% Confidence)	Growth Growth	54.06% Volume (37.66; 66.65)% Volume >97% Volume (Not applicable)

1 - Results relate only to the sample tested
2 - Highest concentration tested, based on test method

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 90.9%, 30.303%, 10.101%, 3.367% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted by a factor of 0.33 (i.e., 97%, 31%, 9.7% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

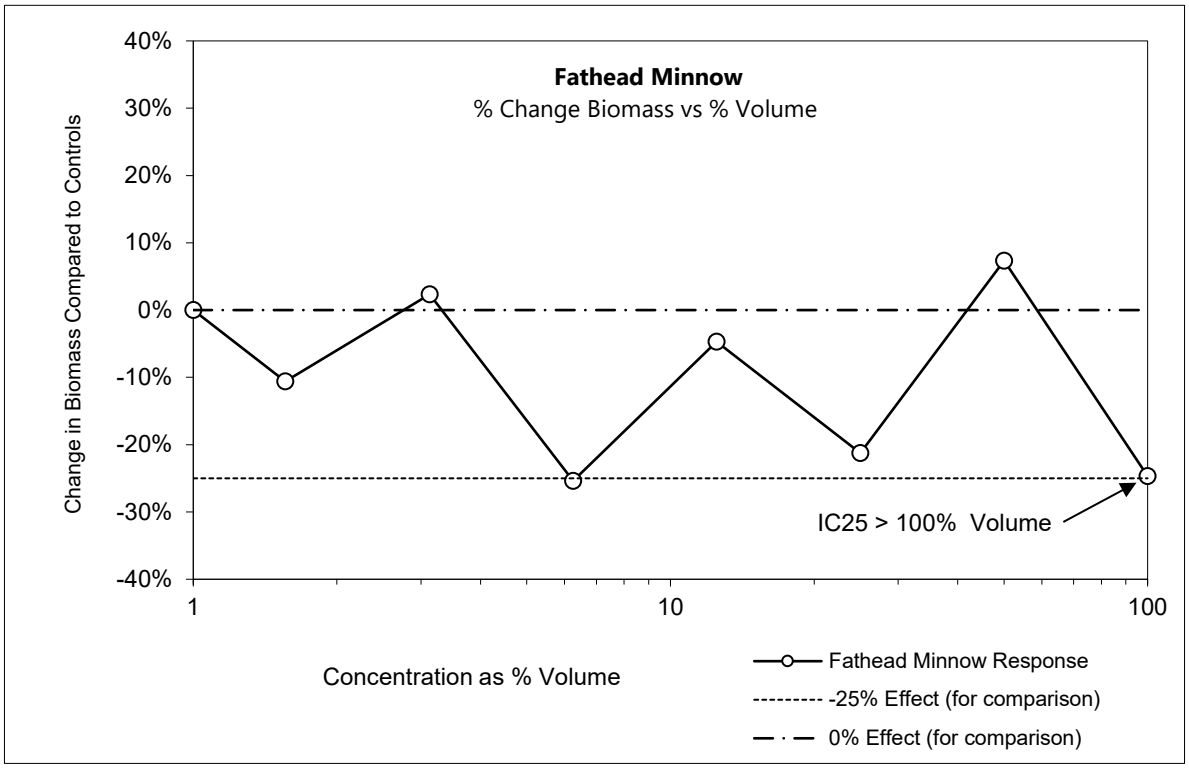
If there are any further details which you require, please do not hesitate to contact us.

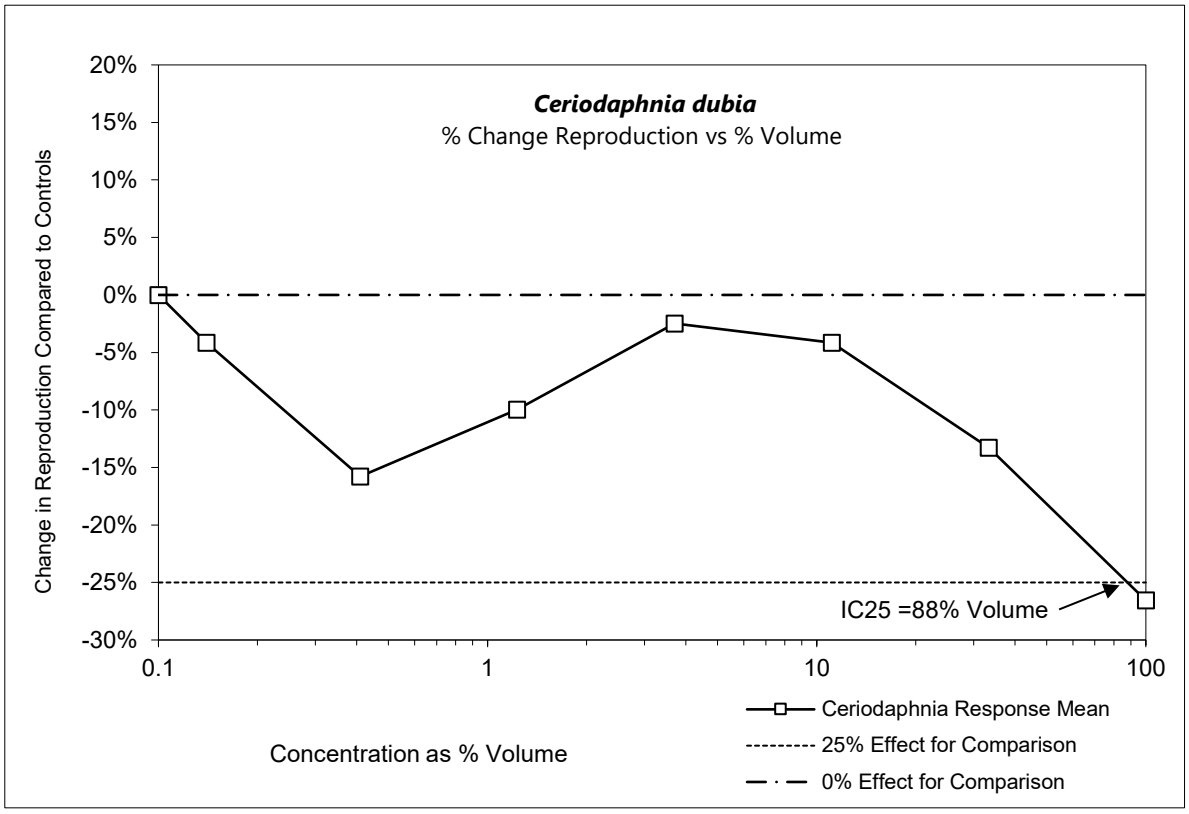
Yours very truly,
Nautilus Environmental Company Inc.

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2023\8000\8730-003\8730-0032313 FCRL

ALS Thunder Bay - EDL2 (TY03086_003_AB) - April 17, 2023





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Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Water	**Source:**	EDL2 (TY03086_003_AB)
Date/Time Sampled:	April 17, 2023; 07:40	**Date/Time Received:**	April 20, 2023; 11:20
Date/Time Test Started:	April 20, 2023; 22:30	**Date Test Finished:**	April 27, 2023
Description:	Clear, Light green	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032313	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.9°C
Collected By:	Not available		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species:	Fathead Minnow (*Pimephales promelas*)		
Source:	Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)		
Culture Temp.:	22 to 26 °C	**Life Stage:**	<24-hour old larvae
Culture Mortality Within 7 Days of Egg Collection:	0.7%		

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032313 **Sources:** EDL2 (TY03086_003_AB)

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: none

Test Conditions

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 1 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (3.3%)

Average Weight of Controls is ≥ 250 µg: Acceptable (695 µg)

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032313 **Sources:** EDL2 (TY03086_003_AB)

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	0	10	20	10	30	10	20
	2	0	20	0	20	10	0	10	30
	3	10	20	20	20	10	0	0	40
Mean % Mortality		3.3	13.3	10.0	20.0	10.0	10.0	6.7	30.0
S.D.		5.8	11.5	10.0	0.0	0.0	17.3	5.8	10.0
Survival/Growth Data									
Mean Dry Biomass	1	0.483	0.594	0.609	0.384	0.569	0.372	0.596	0.560
(mg)	2	0.785	0.545	0.711	0.547	0.729	0.521	0.822	0.453
	3	0.734	0.651	0.729	0.563	0.610	0.684	0.731	0.495
% Effect (+ or -)		0.0	-10.6	2.3	-25.4	-4.7	-21.2	7.3	-24.7
Mean Dry Biomass/ Concentration (mg)		0.667	0.597	0.683	0.498	0.636	0.526	0.716	0.503
S.D.		0.16	0.05	0.06	0.10	0.08	0.16	0.11	0.05

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032313 **Sources:** EDL2 (TY03086_003_AB)

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival		
7-day LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Biomass (Survival and Growth)		
7-day IC25 (Confidence Interval)[2]	>100% Volume (Not Applicable)	Non-linear Regression 2P Exponential Model

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 22-Apr-2023 **Reference Batch #:** Zn2301

Method of Analysis: Untrimmed Spearman-Karber α = 0%

7-Day LC50 (95% Confidence Limits): 0.72 mg/L (0.65 mg/L; 0.80 mg/L)

Historic Geometric Mean LC50: 0.65 mg/L (0.33 mg/L; 1.28 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

Client: ALS - Thunder Bay Sample Name: EDL 2 TY 03086-003-AB Sample #: 8730-003 23 13

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 3.3 %

Average Weight of Controls is ≥ 250 µg: (Acceptable) / Not Acceptable 695 µg

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 1 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL 7-day LC50 95% Confidence Interval[2]	>100 % Volume N/A % Volume	no dose response
Survival / Growth Biomass 7-day IC25 95% Confidence Interval[2]	>100 % Volume N/A % Volume	Non-linear regression 2P Exponential model

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials EV Date 03/05/23

Results Verified: Initials AO Date 04/05/23

Fathead Minnow Initial Sample Measurements Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** EDL2 **Sample #:** 8730-003-2313

Day	Date 2023	Initial Variables				Meters/Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	D.O. / °C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	20-04	26	7.3	8.1	1731	6/4	14/90	5/6	NO	≤100	≤ 20	1	KP
1	21-04	25	7.3	9.9	1713	6/4	14/90	5/6	yes	≤100	≤ 20	1	ET
2	22-04	24	7.4	10.8	1734	6/4	14/90	5/6	yes	≤100	≤ 20	1	JS
3	23-04	25	7.41	10.0	1797	6/4	14/90	5/6	yes	≤100	≤ 20	2	JS
4	24-04	25	7.5	10.9	1778	6/4	14/90	5/6	yes	≤100	≤ 20	2	ET
5	25-04	25	7.5	11.9	1650	6/4	14/90	5/6	yes	≤100	≤ 20	3	ET
6	26-04	25	7.5	12.3	1651	6/4	14/90	5/6	yes	≤100	≤ 20	3	ET
7	27-04												

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted?	Yes/(No)	If yes, state mesh size:
Was sample pH or hardness adjusted?	Yes/(No)	If yes, describe further:
Were alternate concentrations or dilution series used?	Yes/(No)	If yes, describe further:
Was test fed 100µl of brine shrimp days 0 to 6?	(Yes)/No	If no, describe further:
Were there any other method variations, deviations, or exclusions from method?	Yes/(No)	If yes, describe further:
Was there anything unusual about the test, any problems encountered, or any remedial measures taken?	Yes/(No)	If yes, describe further:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: Control Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04	~~245~~ ET	8.2	7.8	~~268~~ 246 ET	6/4	14/90	5/6	ET
1	21-04	25	8.1	8.1	252	6/4	14/90	5/6	KP
2	22-04	24	8.1	8.1	250	6/4	14/90	5/6	VB
3	23-04	25	8.1	7.6	258	6/4	14/90	5/6	KK
4	24-04	28	8.3	7.7	261	6/4	14/90	5/6	SO
5	25-04	24	8.0	8.2	230	6/4	14/90	5/6	SO
6	26-04	24	8.1	8.1	234	6/4	14/90	5/6	KP
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	8.0	7.2	6/4	14/90	ET							
25	8.0	7.3	6/4	14/90	KK	0	0	10	0	0	0	KK
25	7.9	6.6	6/4	14/90	VB	0	0	10	0	0	0	~~VB~~ KK
25	7.9	6.8	6/4	14/90	SO	0	0	10	0	0	0	KK
25	7.9	6.6	6/4	14/90	SO	0	0	10	0	0	0	CG
25	7.9	7.0	6/4	14/90	KP	0	0	10	0	0	0	KP
25	7.7	6.6	6/4	14/90	KK	0	0	10	0	0	0	SO
						0	0	10	0	0	0	KK

Observations:

Concentration: 1.56% v/v

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04	24	8.1	7.6	268	6/4	14/90	5/6	ET
1	21-04	25	8.1	8.0	276	6/4	14/90	5/6	KP
2	22-04	24	8.1	7.9	278	6/4	14/90	5/6	VB
3	23-04	24	8.2	7.7	280	6/4	14/90	5/6	KK
4	24-04	25	8.2	7.7	285	6/4	14/90	5/6	SO
5	25-04	24	8.1	8.1	257	6/4	14/90	5/6	SO
6	26-04	24	8.1	8.1	259	6/4	14/90	5/6	KP
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	7.9	7.1	6/4	14/90	ET							
25	8.0	7.3	6/4	14/90	KK	0	0	10	0	0	0	KK
25	7.9	6.7	6/4	14/90	VB	0	~~0~~ 10	~~10~~ 20	0	0	0	~~VB~~ KK
25	8.0	7.1	6/4	14/90	SO	0	10	20	0	0	0	KK
25	7.9	6.7	6/4	14/90	SO	0	10	20	0	0	0	CG
25	7.8	6.8	6/4	14/90	KP	0	10	20	0	0	0	KP
25	7.8	6.9	6/4	14/90	KK	0	20	20	0	0	0	SO
						0	20	20	0	0	0	CG

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 3.13% v/v Sample Name: EDL2 Sample #: 6730-003-2313

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	20	0	0	0	KK
						10	0	20	0	0	0	~~KK~~ KK
						10	0	20	0	0	0	KK
						10	0	20	0	0	0	CG
						10	0	20	0	0	0	MC
						10	0	20	0	0	0	SO
						10	0	20	0	0	0	CG

Observations: _____

Concentration: 6.25% v/v

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						10	0	20	0	0	0	KK
						20	0	20	0	0	0	~~KK~~ KK
						20	0	20	0	0	0	KK
						20	20	20	0	0	0	CG
						20	20	20	0	0	0	MC
						20	20	20	0	0	0	SO
						20	20	20	0	0	0	CG

Observations: _____

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 12.5% v/v Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04	25	8.0	7.6	450	6/4	14/90	5/6	ET
1	21-04	25	8.0	8.0	461	6/4	14/90	5/6	KP
2	22-04	24	8.0	7.9	461	6/4	14/90	5/6	KK
3	23-04	24	8.1	7.8	469	6/4	14/90	5/6	KK
4	24-04	25	8.1	7.7	480	6/4	14/90	5/6	SO
5	25-04	24	8.0	8.1	431	6/4	14/90	5/6	SO
6	26-04	24	8.0	8.1	437	6/4	14/90	5/6	KP
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	7.9	7.1	6/4	14/90	ET							
25	8.0	7.3	6/4	14/90	KK	0	0	10	0	0	0	KK
25	7.9	6.7	6/4	14/90	KK	0	0	10	0	0	0	KK KK
25	8.0	8.5 7.1	6/4	14/90	SO	0	0	10	0	0	0	KK
25	7.9	6.7	6/4	14/90	SO	0	10	10	0	0	0	CG
25	7.9	6.9	6/4	14/90	KP	10	10	10	0	0	0	KP
25	7.8	7.0	6/4	14/90	KK	10	10	10	0	0	0	SO
						10	10	10	0	0	0	CG

Observations:

Concentration: 25% v/v

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						10	0	0	0	0	0	KK
						10	0	0	0	0	0	KK KK
						10	0	0	0	0	0	KK
						20	0	0	0	0	0	CG
						20	0	0	0	0	0	MC
						30	0	0	0	0	0	SO
						30	0	0	0	0	0	CG

Observations:

Fathead Minnow 7-day Growth Toxicity Test

Concentration: 50% v/v Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
						0	0	10	0	0	0	KK
						0	0	10	0	0	0	KK
						0	0	10	0	0	0	KK
						0	10	0	10	0	0	CA
						10	10	0	0	0	0	ML
						10	10	0	0	0	0	SO
						10	10	0	0	0	0	CG

Observations: B LoE

Concentration: 100% v/v

Day	Date 2023	Initial Measurements °C	pH (units)	D.O. (mg/L)	Cond (µmhos)	Meter / Probe D.O. / °C	pH	cond	Initials
0	20-04	25	7.5	7.8	1695	6/4	14/90	5/6	EJ
1	21-04	25	7.5	8.6	1733	6/4	14/90	5/6	KP
2	22-04	24	7.6	9.4	1750	6/4	14/90	5/6	
3	23-04	24	7.7	8.7	1765	6/4	14/90	5/6	KK
4	24-04	24	7.7	9.2	1766	6/4	14/90	5/6	SO
5	25-04	25	7.7	9.6	1655	6/4	14/90	5/6	SO
6	26-04	24	7.7	9.6	1643	6/4	14/90	5/6	KP
7	27-04								

Final Measurements °C	pH (units)	D.O. (mg/L)	Meter / Probe D.O. / °C	pH	Initials	% Mortality / Rep A	B	C	% Atypical / Rep A	B	C	Initials
25	7.3	7.0	6/4	14/90	EJ							
25	8.0	6.9	6/4	14/90	KK	0	10	10	0	0	0	KK
25	7.9	6.6	6/4	14/90	KP	0	10	10	0	0	0	KK
25	8.0	6.9	6/4	14/90	SO	10	20	10	0	0	10 (A)	KK
25	7.9	6.7	6/4	14/90	SO	20	20	20	0	0	0	CG
25	7.9	6.5	6/4	14/90	KP	20	20	20	0	0	0	ML
25	7.9	6.7	6/4	14/90	KK	20	20	20	0	0	0	SO
						20	30	40	0	0	0	CG

Observations: (A) Kinked C swim bladder, remaining close to surface KK

FATHEAD MINNOW LARVAL WEIGHTS

Sample Information
Client ALS – THUNDER BAY
Sample # 8730-003-2313
Date/Time Received 20-04-23 / 1120
Sample Type WATER
100% Hardness 524

Sample Name EDL2
Sample Date/Time 17/04/23 / 0740
Arrival Temp 17.9 °C
Person Sampling N/A
Sample Description CLEAR, LIGHT GREEN

Test Information
Date/Time Started 20-04-23 / 22:30
Test started by KK
Fathead Batch # FH0621/0122/0622
Date eggs laid 16/17/18-04-23
Culture mortality within 7 days of egg collection 0.7
Swim bladder inflated; (yes) / no KK
Age of Larvae at start of test in hours 24
Control Hardness 90
Water Bath Quadrant C
Average Temperature during Test: 25 ± 1 °C
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / (No) (circle one)

Conc.	Rep.	# of Surviving Larvae	Final Pan Weight (g)	Initial Pan Weight (g)	Mean Dry Biomass* / Rep (mg)	Mean Dry Biomass / Concentration (mg)
Control	A	10	0.85576	0.85093	0.483	0.667
	B	10	0.86286	0.85501	0.785	
	C	9	0.85222	0.84488	0.734	
1.56	A	10	0.84811	0.84217	0.594	0.597
	B	8	0.83819	0.83274	0.545	
	C	8	0.83816	0.83165	0.651	
3.13	A	9	0.83418	0.82809	0.609	0.683
	B	10	0.83722	0.83011	0.711	
	C	8	0.84173	0.83444	0.729	
6.25	A	8	0.84266	0.83882	0.384	0.498
	B	8	0.84430	0.83883	0.547	
	C	8	0.84273	0.83710	0.563	
12.5	A	~~10~~ CG 9	0.83583	0.83014	0.569	0.636
	B	TO CG 9	0.83990	0.83261	0.729	
	C	TO CG 9	0.84781	0.84171	0.610	
25	A	7	0.84677	0.84305	0.372	0.526
	B	10	0.85334	0.84813	0.521	
	C	10	0.85836	0.85162	0.684	
50	A	9	0.84966	0.84370	0.596	0.716
	B	9	0.84748	0.83926	0.822	
	C	10	0.84~~7~~8505	0.83774	0.731	
100	A	8	0.84085	0.83525	0.560	0.503
	B	7	0.83619	0.83166	0.453	
	C	6	0.84791	0.84296	0.495	
Initials		CG	KP	KP	E	E

*Biomass is the total dry weight of fish surviving per rep divided by the number of larvae that were placed in that vessel at the start of the test (typically 10 larvae).

Sample # 8730-0032313 **Sample Name** EDL2

Validity Criteria: Mean Dry Larva Mass for Controls (must be >250ug) 695

Concentration % volume	# of Larvae at Start	# of Larvae Surviving	Final Mass (g)	Initial Mass (g)	Mean Dry Mass/Rep (mg)	Mean Dry Biomass/ Rep (mg)	Mean Larva Dry Mass/ Conc (mg)	Mean Dry Biomass/ Conc (mg)	Mass SD (mg)	Biomass SD (mg)	CV Mass
Control	10	10	0.85576	0.85093	0.483	0.483	0.695	0.667	0.183816	0.161661	26.46674
	10	10	0.86286	0.85501	0.785	0.785					
	10	9 ✓	0.85222	0.84488	0.816	0.734					
1.56	10	10	0.84811	0.84217	0.594	0.594	0.696	0.597	0.110649	0.05305	15.8902
	10	8	0.83819	0.83274	0.681	0.545					
	10	8 ✓	0.83816	0.83165	0.814	0.651					
3.13	10	9	0.83418	0.82809	0.677	0.609	0.766	0.683	0.126694	0.064715	16.53309
	10	10	0.83722	0.83011	0.711	0.711					
	10	8 ✓	0.84173	0.83444	0.911	0.729					
6.25	10	8	0.84266	0.83882	0.480	0.384	0.623	0.498	0.123813	0.09905	19.88966
	10	8	0.84430	0.83883	0.684	0.547					
	10	8 ✓	0.84273	0.83710	0.704	0.563					
12.5	10	9	0.83583	0.83014	0.632	0.569	0.707	0.636	0.092343	0.083108	13.06735
	10	9	0.83990	0.83261	0.810	0.729					
	10	9 ✓	0.84781	0.84171	0.678	0.610					
25	10	7	0.84677	0.84305	0.531	0.372	0.579	0.526	0.091247	0.156052	15.76455
	10	10	0.85334	0.84813	0.521	0.521					
	10	10 ✓	0.85836	0.85152	0.684	0.684					
50	10	9	0.84966	0.84370	0.662	0.596	0.769	0.716	0.129764	0.113712	16.87767
	10	9	0.84748	0.83926	0.913	0.822					
	10	10 ✓	0.84505	0.83774	0.731	0.731					
100	10	8	0.84085	0.83525	0.700	0.560	0.724	0.503	0.091335	0.05301	12.61444
	10	7	0.83619	0.83166	0.647	0.453					
	10	6 ✓	0.84791	0.84296	0.825	0.495					

SD: Standard Deviation
CV: Coefficient of Variation

ao 04/05/23

NAUTILUS ENVIRONMENTAL

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Water	**Source:**	EDL2 (TY03086_003_AB)
Date/Time Sampled:	April 17, 2023; 07:40	**Date/Time Received:**	April 20, 2023; 11:20
Date Test Started:	April 20, 2023; 15:50	**Date Test Finished:**	April 27, 2023
Description:	Clear, Light green	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032313	**Sample Collection:**	Grab
Transport:	Road	**Arrival Temp.:**	17.9°C
Collected By:	Not available		
Storage:	4 ± 2 °C In dark, no headspace		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032313 **Sources:** EDL2 (TY03086_003_AB)

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 0%

Mean Number of Surviving Young Produced Within First 3 Broods: 24

Mean Number of Surviving Young Produced Within 7 Days of Testing: 61.7

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 1 to 6

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032313 **Sources:** EDL2 (TY03086_003_AB)

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD23-52

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (24.1 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (100% of controls)

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032313 **Sources:** EDL2 (TY03086_003_AB)

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	0	0	0	0	0	10	0
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	27	26	10	22	24	21	26	14
	2	18	27	20	25	18	21	14	17
	3	32	19	18	24	28	27	1	23
	4	25	22	21	14	22	23	24	26
	5	26	21	13	26	21	25	29	24
	6	23	16	23	25	28	28	26	17
	7	19	29	27	17	20	19	20	20
	8	23	22	26	22	28	21	20	10
	9	29	23	17	25	23	29	22	25
	10	19	26	28	17	23	17	27	1
Total Number of Live Neonates in First 3 Broods or Less		241	231	203	217	235	231	209	177
% Effect (+ or -)		0.0	-4.1	-15.8	-10.0	-2.5	-4.1	-13.3	-26.6
Mean Number of Live Neonates in First 3 Broods or Less		24.1	23.1	20.3	21.7	23.5	23.1	20.9	17.7
SD		4.6	4.0	6.0	4.2	3.5	4.0	8.2	7.8

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Ceriodaphnia dubia **Survival and Reproduction Study -** Continued

Sample #: 8730-0032313 **Sources:** EDL2 (TY03086_003_AB)

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction 3-Brood IC25 (Confidence Interval)[2]	88% Volume (20.31; N/A)% Volume	No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: Yes, Grubb's test indicated an outlier (33.3% v/v concentration; rep. 3). No reason to remove it. Statistics include all data.
Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Zinc

Date Test Initiated: 22-Apr-2023 **Reference Batch #:** Zn2301

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 0.08 mg/L (0.06 mg/L; 0.11 mg/L)

Historic Geometric Mean LC50: 0.10 mg/L (0.03 mg/L; 0.32 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS – Thunder Bay Sample Name: EDL 2 TY 03086-003-AB Sample #: 8730-0032313

Conditions for Test Validity

Control Mortality is ≤ 20%: Acceptable / Not Acceptable: 0 %

≥ 6 Controls Produced ≥ 3 Broods: Acceptable / Not Acceptable: 10 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: Acceptable / Not Acceptable: 24.1 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 1 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	>100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
REPRODUCTION		No hourly mean reproduction models would fit
3-brood IC25	88.0 % Volume	ICPIN – Linear interpolation
95% Confidence Interval[2]	20.31; N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: (Yes) / No

Concentration(s) & Rep(s): 33.3; rep 3

Analysis Completed: Initials EO Date 28/04/23

Results Verified: Initials ad Date 04/05/23

C *odaphnia dubia* Initial Sample Measuremen Before Preparation and Use in Toxicity Test

Concentration: 100% **Sample Name:** EDL2 **Sample #:** 8730-00323 13

Day	Date 2023	Initial Measurements				Meters / Probes Used			Pre-aeration			Pail Sub-Sampled	Initials
		Temp (°C)	pH	D.O. (mg/L)	Cond (µmhos)	°C	pH	Cond.	yes/no	Rate (bubbles/min)	Duration (min)		
0	20-04	26	7.3	8.1	1731	6/4	14/90	5/6	NO	≤100	≤ 20	1	KP
1	21-04	25	7.3	9.9	1715	6/4	14/90	5/6	Yes	≤100	≤ 20	1	J
2	22-04	24	7.4	10.8	1734	6/4	14/90	5/6	Yes	≤100	≤ 20	1	J
3	23-04	25	7.4	10.0	1797	6/4	14/90	5/6	Yes	≤100	≤ 20	2	J
4	24-04	25	7.5	10.9	1778	6/4	14/90	5/6	yes	≤100	≤ 20	2	ET
5	25-04	25	7.5	11.9	1650	6/4	14/90	5/6	yes	≤100	≤ 20	3	ET
6	26-04	25	7.5	12.3	1651	6/4	14/90	5/6	yes	≤100	≤ 20	3	ET
7	27-04									≤100	≤ 20	3	
8	28-04									≤100	≤ 20	3	

Answer the following questions regarding sample treatment and test procedure:

Was sample filtered or settled and decanted? Yes/No If yes, state mesh size: _____

Was sample pH or hardness adjusted? Yes/No If yes, describe further: _____

Were alternate concentrations or dilution series used? Yes/No If yes, describe further: _____

Was test fed 100µl of YCT and 100µl of *P. subcapitata* daily? Yes/No If no, describe further: _____

Were there any other method variations, deviations, or exclusions from method? Yes/No If yes, describe further: _____

Was there anything unusual about the test, any problems encountered, or any remedial measures taken? Yes/No If yes, describe further: _____

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: Control Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials	Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
0	20.04	25	8.84	7.89	~~246~~ 448	6/4	14/90	5/6	ET	24	8.0	6.8	6/4	14/90	KP
1	21.04	26	8.3	7.9	471	6/4	14/90	5/6	CJ	25	8.1	6.4	6/4	14/90	CJ
2	22.04	25	8.3	7.78 KC	485	6/4	14/90	5/6	KC	24	7.9	5.8	6/4	14/90	KC
3	23.04	24	8.4	7.8	472	6/4	14/90	5/6	ss	24	8.1	7.1	6/4	14/90	ss
4	24.04	24	8.4	8.2	469	6/4	14/90	5/6	DS	25	8.0	7.3	6/4	14/90	SO
5	25.04	24	8.03 SO	8.20 SO	444 SO	6/4	14/90	5/6	SO	24	8.0	7.2	6/4	14/90	KC
6	26.04	24	8.3	8.2	430	6/4	14/90	5/6	KP	25	8.1	7.5	6/4	14/90	CJ
7	27.04														

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	CJ	
3	23.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ss	
4	24.04	0	4	4	6	5	6	2	4	5	4	40	—	0	0	ss	ss
5	25.04	7	7	9	11	7	8	7	7	11+1	9+1	85	—	0	0	SO	SO
6	26.04	10	0	0	0	14	0	0	12	0	0	36	—	0	0	KC	KC
7	27.04	10	7	14	8	0	9	10	0	12	5	80	—	0	0	ss	ss
8	28.04																
Total Neonates		27	18	~~23~~ 32	25	26	23	19	23	29	19	241	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.137% v/v Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20.04	25	8.4	7.4	457	6/4	14/90	5/6	ET
1	21.04	26	8.2	7.8	475	6/4	14/90	5/6	cj
2	22.04	25	8.4	7.9	488	6/4	14/90	5/6	KK
3	23.04	24	8.2	7.5	475	6/4	14/90	5/6	~~KP~~
4	24.04	24	8.2	8.0	481	6/4	14/90	5/6	DS
5	25.04	25	8.0	8.1	449	6/4	14/90	5/6	SO
6	26.04	25	8.2	8.1	436	6/4	14/90	5/6	KP
7	27.04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
25	8.0	6.9	6/4	14/90	KP
25	8.1	6.1	6/4	14/90	cj
24	7.9	5.4	6/4	14/90	KK
24	7.9	6.3	6/4	14/90	MM
25	8.0	7.3	6/4	14/90	SO
24	8.0	7.1	6/4	14/90	KK
25	8.1	7.0	6/4	14/90	DS

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	cj	
3	23.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	DS	
4	24.04	4	5	3	5	4	3	4	2	3	4	37	—	0	0	MM	MM
5	25.04	8	11	7	8	8	6	10	8	9	11	86	—	0	0	SO	SO
6	26.04	14	0	9	0	0	0	15	12	11	0	61	—	0	0	KK	KK
7	27.04	0	11	0	9	9	7	0	0	0	11	47	—	0	0	DS	DS
8	28.04																
Total Neonates		26	27	19	22	21	16	29	22	23	26	231	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 0.410% v/v Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JS	
3	23.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JS	
4	24.04	0	2	3	5	1	4	4	6	4	5	34	—	0	0	MC	MC
5	25.04	4	9	5	0	4	8+1	9	10	5	11	66	—	0	0	SO	SO
6	26.04	6	9	0	10	0	0	14	0	0	12	51	—	0	0	KK	KK
7	27.04	0	0	10	6	8	10	0	10	8	0	52	—	0	0	JS	JS
8	28.04																
Total Neonates		10	20	18	21 [19]	13	23	27	26	17	28	203	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 1.23% v/v Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Variables				Meter/Probe			Initials
		°C	pH	D.O. (mg/L)	Cond (umhos)	D.O. / °C	pH	Cond	
0	20.04								
1	21.04								
2	22.04								
3	23.04								
4	24.04								
5	25.04								
6	26.04								
7	27.04								

Final Variables			Meter/Probe		Initials
°C	pH	D.O. (mg/L)	D.O. / °C	pH	

Day	Date 2023	Neonates Per Replicate										Total	% Mortality / day		% Atypical / day	Initials	Recheck for neos = initials
		1	2	3	4	5	6	7	8	9	10		Vial	Running Total			
0	20.04																
1	21.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	
3	23.04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	[illegible]	
4	24.04	4	3	6	4	6	4	2	5	6	4	44	—	0	0	MC	MC
5	25.04	8	8	8	0	8	9	9	7	9	7	73	—	0	0	SO	SO
6	26.04	0	14	0	10	12	12	0	10	0	0	58	—	0	0	HC	HC
7	27.04	10	~~0~~*	10	0	0	0	6	0	10	6	42	—	0	0	[illegible]	[illegible]
8	28.04																
Total Neonates		22	25	24	14	26	25	17	22	25	17	217	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 3.7% v/v Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20-04	25	8.3	7.4	501	6/4	14/90	5/6	ET
1	21-04	26	8.3	7.8	519	6/4	14/90	5/6	JS
2	22-04	25	8.4	7.9	540	6/4	14/90	5/6	KK
3	23-04	25	8.2	7.5	536	6/4	14/90	5/6	MC
4	24-04	25	8.3	7.9	529	6/4	14/90	5/6	DS
5	25-04	25	8.2	8.0	502	6/4	14/90	5/6	SO
6	26-04	25	8.2	8.1	490	6/4	14/90	5/6	KP
7	27-04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
25	8.0	6.7	6/4	14/90	KP
25	8.0	5.8	6/4	14/90	JS
24	7.8	5.3	6/4	14/90	KK
24	7.9	6.3	6/4	14/90	MC
25	8.0	7.1	6/4	14/90	SO
24	8.0	7.2	6/4	14/90	KK
25	8.0	7.0	6/4	14/90	JS

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20-04																
1	21-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JS	
3	23-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JS	
4	24-04	5	2	5	4	2	7	4	5	5	3	42	—	0	0	MC	MC
5	25-04	8	7	10	8	10	7	7	9	10	8	84	—	0	0	SO	SO
6	26-04	0	9	13	10	0	14	9	14	0	12	81	—	0	0	KK	KK
7	27-04	11	0	0	0	9	0	*	0	8	0	28	—	0	0	JS	JS
8	28-04																
Total Neonates		24	18	28	22	21	28	20	28	23	283	235	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 11.11% v/v Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20-04																
1	21-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JJ	
3	23-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JJ	
4	24-04	4	3	4	5	4	5	4	4	6	2	41	—	0	0	MC	MC
5	25-04	8	10	12	10	8	9	7	8	10	9	91	—	0	0	SO	SO
6	26-04	0	0	0	0	13	14	0	0	13	0	40	-	0	0	KLC	KLC
7	27-04	9	8	11	8	0	0	8	9	0	6	589	—	0	0	JJ	JJ
8	28-04																
Total Neonates		21	21	27	23	25	28	19	21	29	17	731	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 33.33% v/v Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20-04								
1	21-04								
2	22-04								
3	23-04								
4	24-04								
5	25-04								
6	26-04								
7	27-04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20-04																
1	21-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
3	23-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
4	24-04	4	4	dead	4	6	5	5	4	4	5	42	3	10	0	ET	ET
5	25-04	8	4	|	9	10	10	10	8	9	10	78	—	10	0	ET	ET
6	26-04	14	0	|	11	13	0	0	0	9	12	59	—	10	0	ET	ET
7	27-04	0	6	|	0	0	11	5	8	0	*	30	—	10	0	ET	ET
8	28-04			↓													
Total Neonates		26	14	1	24	29	26	20	20	22	27	209	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia 3-Brood Survival and Reproduction Toxicity Test

Concentration: 100% v/v Sample Name: EDL2 Sample #: 8730-003-2313

Day	Date 2023	Initial Variables °C	pH	D.O. (mg/L)	Cond (umhos)	Meter/Probe D.O. / °C	pH	Cond	Initials
0	20-04	25	7.6	7.5	1674	6/4	14/90	5/6	ET
1	21-04	26	7.6	8.7	1747	6/4	14/90	5/6	JS
2	22-04	25	7.6	9.8	1756	6/4	14/90	5/6	KK
3	23-04	24	7.7	8.7	1770	6/4	14/90	5/6	MM
4	24-04	25	7.7	9.1	1783	6/4	14/90	5/6	DS
5	25-04	25	7.6	9.9	1675	6/4	14/90	5/6	SO
6	26-04	25	7.7	9.6	1647	6/4	14/90	5/6	KP
7	27-04								

Final Variables °C	pH	D.O. (mg/L)	Meter/Probe D.O. / °C	pH	Initials
24	8.1	6.9	6/4	14/90	KP
25	8.1	6.3	6/4	14/90	JS
24	8.0	6.2	6/4	14/90	KK
24	8.1	7.2	6/4	14/90	MM
25	8.2	7.7	6/4	14/90	SO
24	8.0	7.1	6/4	14/90	KK
25	8.1	7.5	6/4	14/90	JS

Day	Date 2023	Neonates Per Replicate 1	2	3	4	5	6	7	8	9	10	Total	% Mortality / day Vial	Running Total	% Atypical / day	Initials	Recheck for neos = initials
0	20-04																
1	21-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	ET	
2	22-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JS	
3	23-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	JS	
4	24-04	3	4	6	6	5	3	3	2	3	0	35	—	0	0	MM	MM
5	25-04	7	8	9	10	11	8	8	8	10	0	79	—	0	0	SO	SO
6	26-04	0	0	0	0	0	0	0	0	0	0	0	—	0	0	KK	KK
7	27-04	4	5	8	10	8	6	9	0	12	1	63	—	0	0	JS	JS
8	28-04																
Total Neonates		14	17	23	26	24	187	20	10	25	1	1787	Notes: * = ≥ 4th brood (not counted)				

Ceriodaphnia dubia Neonate Origin

Sample Information

Client ALS - THUNDER BAY

Sample # 8730-003 2313

Date/Time Received 20.04.23/ 11:20

Sample Type WATER

100% Hardness 384

Sample Name ~~EDL2~~ EDL2

Date/Time Collected 17/04/23 / 0740 Person Sampling N/A

Arrival Temp (°C) 17.9

Sample Description CLEAR, LIGHT GREEN

Test Information

Date Test Started 20.04.23 / 15:50

Dilution Water Batch Number CD2352

Test Started By KP

Control Hardness 128

Template Used for Randomization T4

Individual Culture Health Data

Date Culture Started 07/04/23
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 24
Culture I.D. (e.g., Wed Row 4) Friday Row 2
Average # neos in previous 7 days (must be ≥15) 61.7
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (e.g., Wed Row 4) ____
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (e.g., Wed Row 4) ____
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (e.g., Wed Row 4) ____
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started ____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) ____
Culture I.D. (e.g., Wed Row 4) ____
Average # neos in previous 7 days (must be ≥15) ____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 0 (add up % mortality for all cultures used / # cultures used)

Mean Number of Young Produced within first 3 broods 24 (avg # 1st 3 broods for all cultures used / # cultures used)

Mean Number of Young Produced in previous 7 days 61.7 (avg # neos in prev 7 days for all cultures used / # cultures used)

Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / No (circle one) — No circled

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
F 2.1	(Y) / N	(Y) / N	11	1	KK
.2	(Y) / N	(Y) / N	10	2	KK
.3	(Y) / N	(Y) / N	13	3	KK
.4	(Y) / N	(Y) / N	12	4	KK
.5	(Y) / N	(Y) / N	10	5	KK
.6	(Y) / N	(Y) / N	14	6	KK
.7	(Y) / N	(Y) / N	9	7	KK
.8	(Y) / N	(Y) / N	8	8	KK
.9	(Y) / N	(Y) / N	10	9	KK
.10	(Y) / N	(Y) / N	13	10	KK
	Y / N	Y / N			

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032313	**Sample Name:**	EDL2 (TY03086_003_AB)
Sample Method:	Grab	**Collected by:**	N/A
Date/Time Collected:	April 17, 2023; 07:40	**Arrival Temp.:**	17.9°C
Date/Time Received:	April 20, 2023; 11:20	**Sample Description:**	Clear, Light green
Sample Point Description:	N/A	**Sample Type:**	Water
Transportation:	Road		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species (Strain #): *Raphidocelis subcapitata* (CPCC # 37)

Source: Nautilus Plant Culture Unit (from CPCC)

Culture Temp.: 24 ± 2 °C

Test Culture Number: G2(l)c

Culture Age at Test Start: 6 days old

Cell Density in the Microplate Wells at the Start of the Test: 10,454.55 cells/ml

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032313 **Sample Name:** EDL2 (TY03086_003_AB)

Test Conditions

Date/Time Test Start:	April 20, 2023; 16:00	**T=0 Control pH:**	6.3
Date/Time Test End:	April 23, 2023; 16:00-18:00	**T=72 Control pH:**	6.2
Sample pH Before Dilution:	7.6	**pH Adjustment:**	None
Test Duration:	72 hours		

Mean Test Temperature (±Standard Deviation): 25.6 (±0.7) °C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Millipore

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

***Raphidocelis subcapitata*72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032313 **Sample Name:** EDL2 (TY03086_003_AB)

Test Conditions - continued

Enumeration Technique:	Neubauer Haemacytometer
Test Vessel:	96-Well U-bottomed Polystyrene Microplate
Concentration of Test Solutions:	90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control
# Replicates/Concentration:	4 reps started; 3 reps counted of test solutions 10 control reps started, 2 used for pH measurement
Design if Specialized Procedure:	Not applicable
Method Deviations or Unusual Occurrences:	None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times:	Acceptable (19.6 times)
pH in controls did not vary by more than 1.5 units:	Acceptable (0.1 units)
C V for cell yields is < 20% within the control wells:	Acceptable (4.6)
No inhibitory trend detected across the control wells:	Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
214,545	194,545	212,045	217,045	192,045	199,545	209,545	202,045	205,170	4.6

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 CV = Coefficient of Variation = (100 x standard deviation / mean)

***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032313 **Sample Name:** EDL2 (TY03086_003_AB)

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	264,545	294,545	189,545	229,545	262,045
2	217,045	247,045	192,045	214,545	249,545
3	222,045	224,545	204,545	217,045	249,545
Mean Cell Yield	234,545	255,378	195,378	220,378	253,712
Coefficient Variation[2]	11	14	4	4	3
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	244,545	204,545			
2	232,045	204,545			
3	252,045	222,045			
Mean Cell Yield	242,878	210,378			
Coefficient Variation[2]	4	5			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	**Result[1]**	**Method of Calculation**
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	No trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032313 **Sample Name:** EDL2 (TY03086_003_AB)

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: No

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2306 **Date Test Initiated:** 17-April-2023

Method of Analysis: 2P Exponential **Algae Lot #:** G7(l)c

72-hour IC25 (95% Confidence Limits): 28.68 mg/L (21.36 mg/L; 36.59 mg/L)

Historic Geometric Mean IC25: 57.39 mg/L (27.05 mg/L; 121.80 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS- Thunder Bay Sample Name EDL 2 TY 3086-003-AB Sample # 8730-00323 13

Conditions for Test Validity

Cell increase for control is >16 — Acceptable/Not acceptable 19.6 (times)

CV among controls ≤ 20 — Acceptable/Not acceptable 4.6

Result of Mann-Kendall test for trend — Acceptable/Not acceptable no significant trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 115 000 (cells/mL)

Used: Yes/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10 454.55 (cells/mL)

Analysis Completed: Initials: TV Date: 26/04/23

Results Verified: Initials: aO Date: 04/05/23

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location: ALS TB	
Sample #: 8730 · 003231 00	Sample Name: EEL-2 - TY03036-003-AB
Collection Method: grab	Collected By: nla
Date/Time Collected: 17/04/23 @ 0740	Arrival Temp.: (meter/probe) 17.9 °C (44)
Date/Time Received: 20/04/23 @ 1120	Sample Description: clear light green
Collection Point Description: nla	Sample Type: ☑ Effluent ☐ Chemical ☐ Other:
Transportation: Road	Storage: nla

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	AP
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # G2(P)C	AP
Culture Age at Start of Test	6 days old (must be 3 to 7 days old)	AP
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	AP
	Axenic culture? (Yes)/No (Circle one)	AP

Notes:

Test Conditions:

Date / Time Test Start: 20 04 23 1600 (1930) Started By: AP		Date / Time Test End: 23.04.23 @ 1600 - 1700 Finished By: AP	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 7.6	pH adjustment: Y(N)	pH of well D6 at T=0 h 6.3	pH of well D7 at T=72 h 6.2
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot #		(Metal mining) NUT Lot # NUT2302
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation) 25.6 ± 0.7 °C			

	Day 1		Day 2	
¼ plate rotation (Initial)	AM (AP)	PM (AP)	AM AP	PM AP
Lights ON (Initial)	AM AP	PM AP	AM AP	PM AP

* For reference test, record pH of top concentration before use for series dilutions.

Notes:

72-Hour Qualitative Observations:

Condensation: no		
Growth: yes		
Were there any other method variations or deviations from methods?	Yes/(No)	If yes, describe further:
Anything unusual about the test?	Yes/(No)	
Any problems encountered?	Yes/(No)	
Any remedial measures taken?	Yes/(No)	

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 8730-0032313	Sample Name: EDL 2

Reference Data:

Reference Chemical Batch #	Phenol P2306	Date test started	17.04.23
Method of Analysis	Nonlinear regression 2P Exponential	Algae Lot #	G7(l)c
72-hour IC25 (95% C.I.)[3] mg/L	28.68 21.36; 36.59	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	57.39 27.05; 121.80

Test Data:

Statistic	Result[1]	Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	> 90.91% (NA)	No nonlinear regression models valid for IC25 - linear interpolation
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers	—	
Test for Outliers	No Outliers Present ✓	Grubbs' Test for Residual Outlier Initial G
	If outliers present, indicate Concentration, Rep:	
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed.	
	No statistically significant growth stimulation.	Williams' or (Dunnett's) Multiple Comparison Test Initial G
	(Yes), statistically significant growth stimulation at these concentrations: 90.91; 30.303; 1.122; 0.374	

1)Results relate only to the sample tested.
2) Tidepool Scientific Software © 2000-~~2019~~ 2022 Comprehensive Environmental Toxicity Information System – CETIS v. ~~1.9.0.7~~ 2.1.3.5
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/(No)

Any outliers and justification for their removal? Yes/(No)

Nautilus Environmental Company Inc.

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)

72-Hour Growth Inhibition Test

Inoculum Preparation

Sample Name: Sample Number: 8730.003231 (" " 12, " " 13) Date Test Start: 20.04.23

Cell count	0.1 µl / 0.004 µl		0.1 µl / 0.004 µl		0.1 µl / 0.004 µl	
1	27	30	28	26	12	10
2	32	50	19	21	8	13
3	41	44	25	23	13	13
4	43	47	26	22	11	12
5	44	50	–	–	–	–
Total cells	187	227	98	92	44	48
Cells/ µl *	9350	11 350	245	230	110	120
Cells/ ml	9 350 000	11 350 000	245 000	230 000	110 000	120 000
Avg. cells/ ml	10 350 000		237 500		115 000	
Time / Initials	1345 / ao		1400 / ao		1415 / ao	
Comments/ calculations	2 200 000 / 10 350 000 = 0.21		OK		1/2 NUT OK - used	

Cell count	0.1 µl / 0.004 µl		0.1 µl / 0.004 µl		0.1 µl / 0.004 µl	
1						
2						
3						
4						
5						
Total cells						
Cells/ µl *						
Cells/ ml						
Avg. cells/ ml						
Time / Initials						
Comments/ calculations						

* Cells/ul: count 4 outside large squares (0.1 ul), add, multiply by 2.5 OR count 5 inside small squares (0.004 ul), add, multiply by 50

Nautilus Environmental Company Inc.

Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)

72-Hour Growth Inhibition Test – Continued

72-Hour Quantitative Observations of Controls

Sample Name: TY03036-003-AB Sample Number: 330-0032313 Date Test Start: 20.04.23

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D8	Well # D9	Well # D10	Well # D11
1	23	22	21	20	19	24	18	23
2	23	13	28	24	22	21	31	19
3	25	22	17	21	17	20	20	25
4	19	20	23	26	23	19	19	18
5	–	–	–	–	–	–	–	–
Initials	AO	AO	AO	AO	AO	AO	AO	AO

Cell increase for controls = 19.6	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = 4.6	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = No significant trend.	Controls are invalid if there is a trend detected by the Mann-Kendall test

Mann-Kendall Trend Test for *Selenastrum capricornutum* Growth Inhibtion Test

EPS Method 1/RM/25
Version 1.1, Nov. 2000
Pollutech EnviroQuatics

Sample #: 8730-0032313
Client #: ALS TB
Date tested: 20.04.23

Instructions:

Enter control data below cells labelled D2..D5 and D8..D11.
The test of significance is completed immediately following data entry.

D2	D3	D4	D5	D8	D9	D10	D11
214545	194545	212045	217045	192045	199545	209545	202045

Results:

There is no significant trend.

Notes:

The test is a two-sided alternative using a nominal alpha value of 0.05%.
Due to the discrete nature of the test statistic, the true alpha value is 0.062%.

CO 04.05.23

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: TY03036_003-AB Sample Number: 3730.00323 13 Date Test Start: 20.04.23

Theoretical Test Concentration: 100.00 %. v/v				Actual Test Concentration: 90.91 %. v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	30	27	25		234545 (± 26101)
2	27	25	22		Coefficient of Variation of Cell Yield
3	28	21	26		11
4	25	18	20		Average % Inhibition (-ve number = enhancement)
5	–	–	–		-14.317
Initials	ao	ao	ao		G

Theoretical Test Concentration: 33.33 %. v/v				Actual Test Concentration: 30.303 %. v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	35	24	26		255379 (± 35736)
2	33	21	20		Coefficient of Variation of Cell Yield
3	30	27	23		14
4	24	31	25		Average % Inhibition (-ve number = enhancement)
5	–	–	–		-24.472
Initials	ao	ao	ao		G

Theoretical Test Concentration: 11.11 %. v/v				Actual Test Concentration: 10.101 %. v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	18	24	21		195379 (± 8036)
2	17	17	19		Coefficient of Variation of Cell Yield
3	19	23	24		4
4	26	17	22		Average % Inhibition (-ve number = enhancement)
5	–	–	–		4.772
Initials	ao	ao	ao		G

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: TY03086_003_AB Sample Number: 8730.00323 13 Date Test Start: 20.04.23

Theoretical Test Concentration: 3.704 %. v/v					Actual Test Concentration: 3.367 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation) 220379 (± 8036)
1	17	19	23		
2	29	24	25		Coefficient of Variation of Cell Yield 4
3	22	25	22		
4	28	22	21		Average % Inhibition (-ve number = enhancement) -7.413
5	–	–	–		
Initials	ao	ao	ao		G

Theoretical Test Concentration: 1.235 %. v/v					Actual Test Concentration: 1.122 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation) 253712 (± 7217)
1	37	29	28		
2	22	30	25		Coefficient of Variation of Cell Yield 3
3	24	28	30		
4	26	17	21		Average % Inhibition (-ve number = enhancement) -23.659
5	–	–	–		
Initials	ao	ao	ao		O

Theoretical Test Concentration: 0.412 %. v/v					Actual Test Concentration: 0.374 %. v/v
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation) 242879 (± 10104)
1	32	24	30		
2	28	23	24		Coefficient of Variation of Cell Yield 4
3	22	24	22		
4	20	26	29		Average % Inhibition (-ve number = enhancement) -18.379
5	–	–	–		
Initials	ao	ao	ao		O

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: TY03086_003 -AB Sample Number: 8730.00323 13 Date Test Start: 20.04.23

Theoretical Test Concentration: 0.137 v/v				Actual Test Concentration: 0.125 v/v	
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # B8 C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	23	19	20		210379 (± 10104)
2	19	20	27		Coefficient of Variation of Cell Yield
3	28	26	24		5
4	16	21	22		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-2.539
Initials	CO	CO	CO		CO

Theoretical Test Concentration:				Actual Test Concentration:	
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:				Actual Test Concentration:	
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	EDL-2 Ty03086-003-AB	Sample #	8730-0032313	Date test start	20.04.23

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	20	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	115000	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2300	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	10454.5455	=	19.625

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	23	22	21	20	19	24	18	23
	23.0	18.0	28.0	24.0	22.0	21.0	31.0	19.0
	25	22.0	17.0	21.0	17.0	20.0	20.0	25.0
	19.0	20.0	23.0	26.0	23.0	19.0	19.0	18.0
total cells	90	82	89	91	81	84	88	85
cells/ul	225	205	222.5	227.5	202.5	210	220	212.5
cells/ml	225000	205000	222500	227500	202500	210000	220000	212500
Cell yield = measured concentration - initial algal cell concentration								
	214545	194545	212045	217045	192045	199545	209545	202045

Mean cell yield for the control = Rc | Standard deviation | coefficient of variation

Rc 205170 | SD 9425.45944 | CV 4.5939653 **Must be ≤20**

% inhibition summary

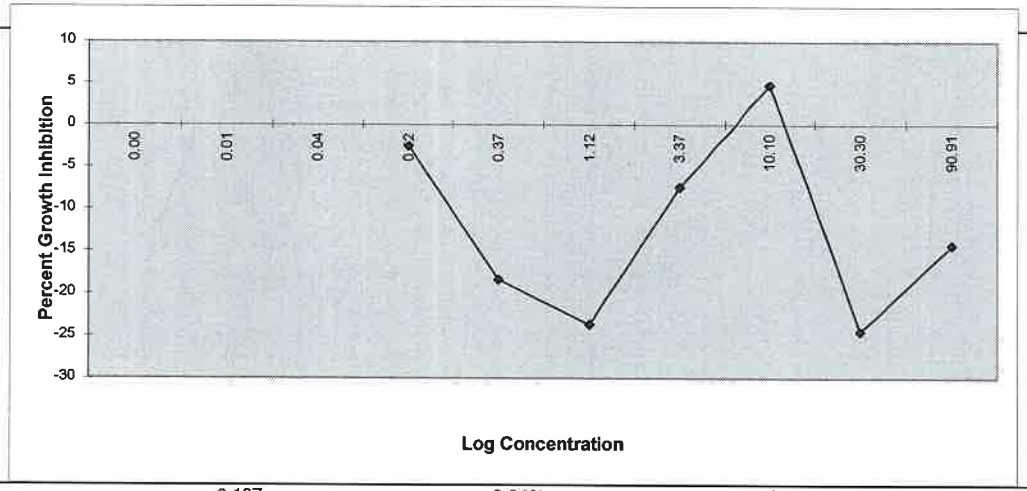
Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	-2.539
0.374	-18.379
1.122	-23.659
3.367	-7.413
10.101	4.772
30.303	-24.472
90.910	-14.317

enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	30	27	25	35	24	26	18	24	21	17	19	23	37	29	28
	27.0	25.0	22.0	33.0	21.0	20.0	17.0	17.0	19.0	29.0	24.0	25.0	22.0	30.0	25.0
	28.0	21.0	26.0	30.0	27.0	23.0	19.0	23.0	24.0	22.0	25.0	22.0	24.0	28.0	30.0
	25.0	18.0	20.0	24.0	31.0	25.0	26.0	17.0	22.0	28.0	22.0	21.0	26.0	17.0	21.0
total cells	110	91	93	122	103	94	80	81	86	96	90	91	109	104	104
cells/ul	275	227.5	232.5	305	257.5	235	200	202.5	215	240	225	227.5	272.5	260	260
cells/ml	275000	227500	232500	305000	257500	235000	200000	202500	215000	240000	225000	227500	272500	260000	260000
Cell yield = measured concentration - initial algal cell concentration															
	264545	217045	222045	294545	247045	224545	189545	192045	204545	229545	214545	217045	262045	249545	249545
Mean Yield	234545			255379			195379			220379			253712		
STD Yield	26101			35736			8036			8036			7217		
CV Yield	11			14			4			4			3		

Average % inhibition													
	for 90.910%:	-14.317		for 30.303%	-24.472		for 10.101%	4.772		for 3.367%	-7.413		for 1.122%	-23.659	

Average % stimulation													
	for 90.910%:	14.317		for 30.303%	24.472		for 10.101%	-4.772		for 3.367%	7.413		for 1.122%	23.659	



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
32	24	30	23	19	20									
28.0	23.0	24.0	19.0	20.0	27.0									
22.0	24.0	22.0	28.0	26.0	24.0									
20.0	26.0	29.0	16.0	21.0	22.0									
102	97	105	86	86	93	0	0	0	0	0	0	0	0	0
255	242.5	262.5	215	215	232.5	0	0	0	0	0	0	0	0	0
255000	242500	262500	215000	215000	232500	0	0	0	0	0	0	0	0	0
244545	232045	252045	204545	204545	222045	-10455	-10455	-10455	-10455	-10455	-10455	-10455	-10455	-10455
242879			210379			-10455			-10455			-10455		
10104			10104			0			0			0		
4			5			0			0			0		
for 0.374%	-18.379		for 0.125%	-2.539		for 0.042%	105.096		for 0.014%	105.096		for 0.005%	105.096	
for 0.374%	18.379		for 0.125%	2.539		for 0.042%	-105.096		for 0.014%	-105.096		for 0.005%	-105.096	

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
EDL2 (TY03086_003_AB)	8730-0032313	April 17, 2023	April 20, 2023	April 20, 2023	54.06% Volume (37.66; 66.65)% Volume	>97% Volume (Not applicable)	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	13-Apr-2023	**Reference Chemical:**	KCl
Frond Increase IC25:	2.59 g/L	**95% Confidence Limits:**	2.19; 2.82 g/L
Historic Geometric Mean IC25:	2.07 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.28; 3.35 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	Na_2MoO_4	0.0726
	K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$2H_2O$	0.00003
	KCl	10.1	$MgSO_4\ 7H_2O$	147	$ZnCl_2$	0.00001
					$CoCl_2\ 6H_2O$	0.00001
					$CuCl_2\ 2H_2O$	

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

C. D'Andrea

Date: May 5, 2023

Carol D'Andrea
Laboratory Supervisor

Test Material

Client Name/Location: ALS Thunder Bay	
Sample #: 8730-003 2313	Sample Name: EDL2 TY 03086-003-AB
Collection Method: Grab	Collected By: N/A
Date/Time Collected: 17/04/23 7:40	Arrival Temperature (meter/probe): 17.9 °C (44)
Date/Time Received: 20/04/23 11:20	Sample Description: Clear, light green
Collection Point Description: Other	Sample Type: ☒ Effluent ☐ Chemical ☐ Other:
Transportation: Air / Road	Storage: none

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	CB
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	CB
Culture Age at Start of Test:	8 days old, acclimated 24 hours in fresh test solution (mAPHA)	CB
Culture Medium:	Modified Hoagland's E+ medium, Lot # MH2301	CB
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/No	CB
	Axenic culture? Yes/No	CB
	Health test fronds increase ≥ 8-fold in 7 days 5 fronds at start in each health test 42 in HT 1, 41 in HT 2, 40 in HT 3 at finish	CB

Test Conditions and Procedures

Date / Time Test Start: 20.04.23 16:30 Started By: D	Date / Time Test End: 27.04.23 12:15 Finished By: D
Test Type: Static (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / No (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: Modified APHA (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 7.9 (13/91)	pH Adjustment: none
Test Volume and Depth: 150 mL | 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / No	If yes, describe further:
Anything unusual about the test? Yes / No	
Any problems encountered? Yes / No	
Any remedial measures taken? Yes / No	Randomization Template: C

Sample #: 8730-0032301B	Sample Name: EDL2

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	24	26	26	26	25	25	26	26
Low	24	26	26	26	25	25	26	26
Medium	24	26	26	26	25	25	26	26
High	24	26	26	26	25	25	25	26
Initials	EV	EV	KK	KK	CG	CG	CG	EV
meter/probe	90	44	44	44	44	44	44	44
Mean Test Temperature (average of 24h high / low temperatures):	25.4±0.1 °C							

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.2	8.2	8.2	7.9	EV	13/91
Day 7	Control	Low	Medium	High	Initials	pH meter/probe
	8.3	9.1	9.3	9.1	CG	13/91

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)	—			—				—

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 24/04/23 (4)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 12					Initials: EV
4670	4790	5510	4690	5290	Mean Light Measurement: 4990
±15% Variation of Mean: 4242 - 5742					(Acceptable) / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) / No
If yes, describe further: Algae in upper concentrations

Reference Data

Reference Date	Frond Increase or Dry Weights (circle one)			
28 Feb 23	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
	~~2.55~~ 1.97	~~2.19~~, ~~2.70~~ 1.29, 2.63	~~2.07~~ 2.04	~~1.28~~; ~~3.35~~ 1.26, 3.28

Sample #: 8730-003 23 13	Sample Name: EDL 2

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 77	B 53	C 66	D 68	66.0

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy						Initials	
						C	
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV : 60.0 (9.9) 16.5							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	7.08	11.67	16.67	30.83	15.83	6.67	-37.50
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control % v/v g/L	0.097	0.29	0.97	3.1*	9.7	31	97
Mean % Stimulation	6.46	17.76	14.56	33.51	26.02	8.42	-16.78

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: (Yes) / Not applicable (no stimulation) (Circle one)

Test Endpoints and Calculations: Fronds / Weights

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	54.06 (37.66 ; 66.65)	NO nonlinear regression models would fit
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN - linear interpolation
Dry Weights		
IC25 (95% C.I.)[3]	> 97 % (NA)	No nonlinear models would regression
IC25 (95% C.I.)[3] If calculated without outlier[4]		ICPIN - linear interpolation

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019. Comprehensive Environmental Toxicity Information System CETISv ~~1.9.6.7~~ 2.1.3.5
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / (No)

Any outliers and justification for their removal? Yes / (No)

Lemna minor L., 7 Observations

Client: ALS Thunder Bay	Sample number: 8730-0032313	Date Started: 20.04.23	
Site: EDL 2		Date Ended: 27.04.23	
Concentration: Control	Observations By: G	Concentration: 0.097 T. U.	Observations By: G

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	77	53	66	68	Number of	72	79	70	60
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	X	X	X	X	Abnormally sized fronds	✓ smaller	// smaller	✓ smaller	✓ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	✓	✓	✓
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations					Other Observations	algae			
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO N/A Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor L. 7 Observations

Client: ALS Thunder Bay	Sample number: 8730-0032313	Date Started: 20.04.23	
Site: EDL 2		Date Ended: 27.04.23	
Concentration: 0.29% v/v	Observations By: G	Concentration: 0.97% v/v	Observations By: G

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	71	54	83	84	Number of	73	64	97	70
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	✓ smaller	✓ smaller	✓ smaller	✓ smaller	Abnormally sized fronds	✓ smaller	✓ smaller	✓ smaller	✓ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	✓	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	✓	✓	X	X	Loss of Buoyancy	X	X	✓	✓
Other Observations					Other Observations				
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor L., 7 Observations

Client: ALS Thunder Bay	Sample number: 873000032313	Date Started: 20.04.23	
Site: EDL 2		Date Ended: 27.04.23	
Concentration: 3.1% v/v	Observations By: G	Concentration: 9.7% v/v	Observations By: G

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	99	68	100	71	Number of	66	84	87	65
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ Smaller	√√ Smaller	√√ Smaller	√√ Smaller	Abnormally sized fronds	√√ Smaller	√√ Smaller	√ Smaller	√ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→	Other Observations		algae	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Day 7 Observations

Client: ALS Thunder Bay	Sample number: 873070032313	Date Started: 20.04.23	
Site: EDL 2		Date Ended: 27.04.23	
Concentration: 31.1 v/v	Observations By: G	Concentration: 97.1 v/v	Observations By: G

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	66	71	69	74	Number of	42	45	41	46
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	✓	✓	✓
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	Abnormally sized fronds	X	✓ smaller	✓ smaller	✓ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	✓	Loss of Buoyancy	X	✓	✓	X
Other Observations	algae	→	→	→	Other Observations	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Weights

Client	Site	Sample number
ALS-Thunder Bay	EDL2	8730.0032313

In Oven Date/Time/ °C: 27/04/23 12:35 60° | Out Oven Date/Time/°C: 28/04/23 12:35 61°

Conc.	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	71	60.0 (9.9)	0.85964	0.85457	5.07	4.60 (0.5)
	B	47		0.85809	0.85397	4.12	
	C	60		0.85467	0.85046	4.21	
	D	62		0.85797	0.85296	5.01	
0.097	A	66	64.3 (7.8)	0.84868	0.84454	4.14	4.90 (0.7)
	B	73		0.84773	0.84289	4.84	
	C	64		0.85026	0.84538	4.88	
	D	54		0.85178	0.84604	5.74	
0.29	A	65	67.0 (14.0)	0.83370	0.82855	5.15	5.42 (1.2)
	B	48		0.86691	0.86313	3.78	
	C	77		0.82624	0.82008	6.16	
	D	78		0.86993	0.86334	6.59	
0.97	A	67	70.0 (14.5)	0.83748	0.83216	5.32	5.27 (1.2)
	B	58		0.84978	0.84557	4.21	
	C	91		0.85~~09~~18	0.84419	6.99	
	D	64		0.85070	0.84613	4.57	
3.1	A	93	78.5 (17.4)	0.84785	0.84112	6.73	6.15 (1.0)
	B	62		0.84665	0.84162	5.03	
	C	94		0.84971	0.84245	7.26	
	D	65		0.85183	0.84627	5.56	
9.7	A	60	69.5 (11.6)	0.86336	0.85868	4.68	5.80 (1.0)
	B	78		0.85183	0.84547	6.36	
	C	81		0.82499	0.81806	6.93	
	D	59		0.85373	0.84850	5.23	
31	A	60	64.0 (3.4)	0.85865	0.85387	4.78	4.99 (0.4)
	B	65		0.85039	0.84533	5.06	
	C	63		0.87103	0.86644	4.59	
	D	68		0.85289	0.84736	5.53	
97	A	36	37.5 (2.4)	0.84451	0.84051	4.00	3.83 (0.2)
	B	39		0.86584	0.86183	4.01	
	C	35		0.84787	0.84419	3.68	
	D	40		0.84720	0.84357	3.63	
Initials		CG	CG	CG	CG	CG	CG

Notes:

Sample name EDL2 Date started 20/04/23 sample # 8730-0032313

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

66.0 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	77	72	71	73	99	66	66	42
	53	79	54	64	68	84	71	45
	66	70	83	97	100	87	69	41
	68	60	84	70	71	65	74	46
Total Fronds	264	281	292	304	338	302	280	174

Increase in Frond Number = Total # Fronds - 6 Starting Fronds

	71	66	65	67	93	60	60	36
	47	73	48	58	62	78	65	39
	60	64	77	91	94	81	63	35
	62	54	78	64	65	59	68	40
Total Increase	240	257	268	280	314	278	256	150
Mean Increase	60.0	64.3	67.0	70.0	78.5	69.5	64.0	37.5
SD Increase	9.9	7.8	14.0	14.5	17.4	11.6	3.4	2.4
CV Increase	16.5	12.2	20.9	20.7	22.1	16.7	5.3	6.3
% Stimulation		**7.08**	**11.67**	**16.67**	**30.83**	**15.83**	**6.67**	**-37.50**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.85964	0.84868	0.83370	0.83748	0.84785	0.86336	0.85865	0.84451
Pan + Plant	0.85809	0.84773	0.86691	0.84978	0.84665	0.85183	0.85039	0.86584
	0.85467	0.85026	0.82624	0.85118	0.84971	0.82499	0.87103	0.84787
	0.85797	0.85178	0.86993	0.85070	0.85183	0.85373	0.85289	0.84720
Initial Weight (g)	0.85457	0.84454	0.82855	0.83216	0.84112	0.85868	0.85387	0.84051
Pan Only	0.85397	0.84289	0.86313	0.84557	0.84162	0.84547	0.84533	0.86183
	0.85046	0.84538	0.82008	0.84419	0.84245	0.81806	0.86644	0.84419
	0.85296	0.84604	0.86334	0.84613	0.84627	0.84850	0.84736	0.84357
Plant Only (mg)	5.07	4.14	5.15	5.32	6.73	4.68	4.78	4.00
	4.12	4.84	3.78	4.21	5.03	6.36	5.06	4.01
	4.21	4.88	6.16	6.99	7.26	6.93	4.59	3.68
	5.01	5.74	6.59	4.57	5.56	5.23	5.53	3.63
Mean Dry Weight	4.602	4.900	5.420	5.272	6.145	5.800	4.990	3.830
SD Dry Weight	0.5	0.7	1.2	1.2	1.0	1.0	0.4	0.2
CV Dry Weight	11.0	13.4	23.0	23.4	16.7	17.7	8.2	5.3
% Stimulation		**6.46**	**17.76**	**14.56**	**33.51**	**26.02**	**8.42**	**-16.78**

For Data Transfer to CETIS

		# fronds	total mass	tare
0	1	71	0.85964	0.85457
	2	47	0.85809	0.85397
	3	60	0.85467	0.85046
	4	62	0.85797	0.85296
0.1	1	66	0.84868	0.84454
	2	73	0.84773	0.84289
	3	64	0.85026	0.84538
	4	54	0.85178	0.84604
0.3	1	65	0.83370	0.82855
	2	48	0.86691	0.86313
	3	77	0.82624	0.82008
	4	78	0.86993	0.86334
1	1	67	0.83748	0.83216
	2	58	0.84978	0.84557
	3	91	0.85118	0.84419
	4	64	0.85070	0.84613
3.1	1	93	0.84785	0.84112
	2	62	0.84665	0.84162
	3	94	0.84971	0.84245
	4	65	0.85183	0.84627
9.7	1	60	0.86336	0.85868
	2	78	0.85183	0.84547
	3	81	0.82499	0.81806
	4	59	0.85373	0.84850
31	1	60	0.85865	0.85387
	2	65	0.85039	0.84533
	3	63	0.87103	0.86644
	4	68	0.85289	0.84736
97	1	36	0.84451	0.84051
	2	39	0.86584	0.86183
	3	35	0.84787	0.84419
	4	40	0.84720	0.84357

AO 04105123

Chain of Custody (COC) / Analytical Request Form

COC Number: 20 -

Page of

Canada Toll Free: 1 800 668 9878

www.alsglobal.com

Environmental Division
Thunder Bay
Work Order Reference
TY2303086

Telephone : +1 807 623 8463

Report To	Contact and company name below will appear on the final report
Company:	New Gold
Contact:	Garnet Cornell
Phone:	
	Company address below will appear on the final report
Street:	
City/Province:	
Postal Code:	
Invoice To	Same as Report To ☐ YES ☐ NO
	Copy of Invoice with Report ☐ YES ☐ NO
Company:	
Contact:	

Reports / Recipients

Select Report Format: ☐ PDF ☐ EXCEL ☐ EDD (DIGITAL)
Merge QC/QCI Reports with COA ☐ YES ☐ NO ☐ N/A
☐ Compare Results to Criteria on Report - provide details below if box checked
Select Distribution: ☐ EMAIL ☐ MAIL ☐ FAX
Email 1
Email 2
Email 3

Invoice Recipients

Select Invoice Distribution: ☐ EMAIL ☐ MAIL ☐ FAX
Email 1
Email 2

Turnaround Time (TAT) Requested

☐ Routine [R] if received by 3pm M-F - no surcharges apply
☐ 4 day [P4] if received by 3pm M-F - 20% rush surcharge minimum
☐ 3 day [P3] if received by 3pm M-F - 25% rush surcharge minimum
☐ 2 day [P2] if received by 3pm M-F - 50% rush surcharge minimum
☐ 1 day [E] if received by 3pm M-F - 100% rush surcharge minimum
☐ Same day [E2] if received by 10am M-S - 200% rush surcharge.
Additional fees may apply to rush requests on weekends, s
Date and Time Required for all E&P TATs:
For all tests with rush TATs requested, please contact your AM to comm

Project Information

ALS Account # / Quote #:
Job #: Sublethal Toxicity
PO / AFE:
LSD:
ALS Lab Work Order # (ALS use only): L2750068

Oil and Gas Required Fields (client use)

AFE/Cost Center: PO#
Major/Minor Code: Routing Code:
Requisitioner:
Location:
ALS Contact: Sampler:

Analysis Request

Indicate Filtered (F), Preserved (P) or Filtered and Preserved (F/P) below

ALS Sample # (ALS use only)	Sample Identification and/or Coordinates (This description will appear on the report)	Date (dd-mmm-yy)	Time (hh:mm)	Sample Type	NUMBER OF CONTAINERS	NG-ST-P-TB	F/P	SAMPLES ON HOLD	EXTENDED STORAGE REQUIRED	SUSPECTED HAZARD (see notes)
	EDL1	17-Apr-23	8:15	EFF	3	X				
	SED2DIS	17-Apr-23	8:40	EFF	3	X				
	EDL2	17-Apr-23	7:40	EFF	3	X				

Drinking Water (DW) Samples[1] (client use)

Are samples taken from a Regulated DW System?
☐ YES ☐ NO
Are samples for human consumption/ use?
☐ YES ☐ NO

Notes / Specify Limits for result evaluation by selecting from drop-down below (Excel COC only)

SAMPLE RECEIPT DETAILS (ALS use only)

Cooling Method: ☐ NONE ☐ ICE ☒ ICE PACKS ☐ FROZEN ☐ COOLING INITIATED
Submission Comments identified on Sample Receipt Notification: ☐ YES ☐ NO
Cooler Custody Seals Intact: ☐ YES ☐ N/A Sample Custody Seals Intact: ☐ YES ☐ N/A

INITIAL COOLER TEMPERATURES °C				FINAL COOLER TEMPERATURES °C			
4.2	3.4						

SHIPMENT RELEASE (client use)			INITIAL SHIPMENT RECEPTION (ALS use only)			FINAL SHIPMENT RECEPTION (ALS use only)		
Released by:	Date:	Time:	Received by:	Date:	Time:	Received by: LV	Date: 4/18/23	Time: 9:40

REFER TO BACK PAGE FOR ALS LOCATIONS AND SAMPLING INFORMATION

WHITE - LABORATORY COPY YELLOW - CLIENT COPY

AUG 2020 FRONT

Failure to complete all portions of this form may delay analysis. Please fill in this form LEGIBLY. By the use of this form the user acknowledges and agrees with the Terms and Conditions as specified on the back page of the white - report copy.

1. If any water samples are taken from a Regulated Drinking Water (DW) System, please submit using an Authorized DW COC form.

12 9

Manitoulin

2 Coolers 18 Pails

Intake and Login Verification Form

SAMPLE INTAKE		
Priority/Emergency Service Requested	YES	~~NO~~
Time Sensitive Hold Time	YES	NO

Client:	Neo Gold

SAMPLE RECEIPT INFORMATION			
Mode of Delivery:	Courier		Drop Off
COURIER	Manitoulin		
Waybill Number	350255871		
Shipment Cost		Collect?	Y/N
Temperature	9	Cooler Count	1
Cooling Method	None	Ice	Ice Packs

SAMPLE MATRIX/BOTTLE INFORMATION					
Matrix:	Water	Soil	Air	Biota	Other
DW Schedule 24 Bottles Correct?				Yes	No
DW Metals pH Check <2				Yes	No
Bottle Types: 1	Sample Count			3	
Green/white					
Orange/black					
Warm red/green/white					
Warm red/white					
Yellow/black					
Purple/white					
Light blue/white					
Others (detail) 9 Tox Pails					

Comments on Samples and Bottles:

Samples Requiring Preservation or Filtering:

ACCOUNT INFO VERIFICATION			
~~Confirmed all as accurate as per COC, Sample Remarks or PM~~			
Client	Office ✓	Work Contact ✓	Quote

RECEIPT DETAIL		
Project ✓	PO ✓	Site/LSD ✓

Recipients match CoC or Sample Remarks	~~Yes~~ ✓	No
Billing Instruction added to remarks	~~Yes~~ ✓	NA
Sample Remarks checked	~~Yes~~ ✓	
Submission Issues communicated	Yes	~~NA~~
Sample Info communicated via Remarks	Yes	~~NA~~

VERIFICATION CHECKLIST	
Sample Name entered as per CoC	✓
Sampling Date and time entered as per CoC	✓
Containers selected in order of COC	✓
Sales items from QUOTE ONLY (and/or verified as correct)	✓
Field Data/Calc Codes removed if not on COC	✓
Bottle Allocation Verified	✓
Guideline added or auto-allocated	✓
Due dates updated	✓

VALIDATION		
Validation errors or checks	Yes	~~No~~
Internal CoC created	~~Yes~~ ✓	NA

Login Comments:

Layout Staff Initials	SS09
Date and Time of Layout	18/4/23, 9:40AM
Login Staff Initials:	SS09

ALS Canada Ltd.

right solutions.
right partner.

CERTIFICATE OF ANALYSIS

Work Order	: **TY2311042**	Page	: 1 of 9
Client	: **New Gold Inc. (Rainy River)**	Laboratory	: ALS Environmental - Thunder Bay
Contact	: Garnet.Cornell@newgold.com Garnet Cornell	Account Manager	: Christine Paradis
Address	: 24 Marr Rd. Barwick ON Canada P0W 1A0	Address	: 1081 Barton Street Thunder Bay ON Canada P7B 5N3
Telephone	: 807 234 8170	Telephone	: +1 807 623 6463
Project	: Operations Discharge Monitoring	Date Samples Received	: 25-Oct-2023 09:03
PO	: 4700002620	Date Analysis Commenced	: 25-Oct-2023
C-O-C number	: ----	Issue Date	: 30-Nov-2023 13:11
Sampler	: ----		
Site	: New Gold Inc. (Rainy River)		
Quote number	: New Gold Rainy River Project - Picka Project		
No. of samples received	: 1		
No. of samples analysed	: 1		

This report supersedes any previous report(s) with this reference. Results apply to the sample(s) as submitted. This document shall not be reproduced, except in full.

This Certificate of Analysis contains the following information:

- General Comments
- Analytical Results

Additional information pertinent to this report will be found in the following separate attachments: Quality Control Report, QC Interpretive report to assist with Quality Review and Sample Receipt Notification (SRN).

Signatories

This document has been electronically signed by the authorized signatories below. Electronic signing is conducted in accordance with US FDA 21 CFR Part 11.

Signatories	*Position*	*Laboratory Department*
Cassandra Grzelewski	Team Leader - Inorganics	Inorganics, Thunder Bay, Ontario
Cassandra Grzelewski	Team Leader - Inorganics	Metals, Thunder Bay, Ontario
Greg Pokocky	Manager - Inorganics	Metals, Waterloo, Ontario
Jon Fisher	Production Manager, Environmental	Inorganics, Waterloo, Ontario
Julie Ruoho	Project Manager	External Subcontracting, Point Edward, Ontario
Julie Ruoho	Teamleader Wet Chem	Administration, Thunder Bay, Ontario
Julie Ruoho	Teamleader Wet Chem	Inorganics, Thunder Bay, Ontario
Lindsay Gung	Supervisor - Water Chemistry	Inorganics, Burnaby, British Columbia
Mingjian Yang	Account Manager	External Subcontracting, Saskatoon, Saskatchewan
Rhiannon Scheffee	Laboratory Assistant	Metals, Thunder Bay, Ontario
Shannon Veltri	Supervisor - Water Chemistry	Inorganics, Thunder Bay, Ontario
Shannon Veltri	Supervisor - Water Chemistry	Metals, Thunder Bay, Ontario
Taelur Kachur	Laboratory Analyst	Inorganics, Thunder Bay, Ontario
Taelur Kachur	Laboratory Analyst	Microbiology, Thunder Bay, Ontario

General Comments

The analytical methods used by ALS are developed using internationally recognized reference methods (where available), such as those published by US EPA, APHA Standard Methods, ASTM, ISO, Environment Canada, BC MOE, and Ontario MOE. Refer to the ALS Quality Control Interpretive report (QCI) for applicable references and methodology summaries. Reference methods may incorporate modifications to improve performance.

Where a reported less than (<) result is higher than the LOR, this may be due to primary sample extract/digestate dilution and/or insufficient sample for analysis.

Where the LOR of a reported result differs from standard LOR, this may be due to high moisture content, insufficient sample (reduced weight employed) or matrix interference.
Please refer to Quality Control Interpretive report (QCI) for information regarding Holding Time compliance.

Key : CAS Number: Chemical Abstracts Services number is a unique identifier assigned to discrete substances
LOR: Limit of Reporting (detection limit).

Unit	*Description*
-	no units
°C	degrees celsius
µS/cm	microsiemens per centimetre
Bq/L	becquerels per litre
CU	colour units (1 cu = 1 mg/l pt)
mg/L	milligrams per litre
MPN/100mL	most probable number per hundred millilitres
NTU	nephelometric turbidity units
pH units	pH units

<: less than.

>: greater than.

Surrogate: An analyte that is similar in behavior to target analyte(s), but that does not occur naturally in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery.

Test results reported relate only to the samples as received by the laboratory.

UNLESS OTHERWISE STATED on SRN or QCI Report, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.

Qualifiers

Qualifier	*Description*
< DL	*Recorded value = measured amount > LMDL (non-zero).*
< T	*A measureable trace amount: Interpret with caution.*
< W	*No measurable response (zero): < Reported Value*
DLDS	*Detection Limit Raised: Dilution required due to high Dissolved Solids / Electrical Conductivity.*

Analytical Results

Sub-Matrix: **Water (MISA)**
(Matrix: **Water**)

Analyte	CAS Number	Method/Lab	LOR	Unit	Client sample ID: SP2 DISCHARGE_EFF_20231024 EFF	----	----	----	----
					Client sampling date / time: 24-Oct-2023 08:25	----	----	----	----
					TY2311042-001	--------	--------	--------	--------
					Result	----	----	----	----
Field Tests									
Oxygen, dissolved, field	----	EF001/TY	0.01	mg/L	6.84	----	----	----	----
pH, field	----	EF001/TY	0.10	pH units	8.04	----	----	----	----
Temperature, field	----	EF001/TY	0.10	°C	10.5	----	----	----	----
Physical Tests									
Colour, true	----	E329-L/TY	2.0	CU	9.6	----	----	----	----
Conductivity	----	E100/TY	1.0	µS/cm	761	----	----	----	----
Hardness (as CaCO3), dissolved	----	EC100/TY	0.50	mg/L	304	----	----	----	----
pH	----	E108/TY	0.10	pH units	8.32	----	----	----	----
Solids, total dissolved [TDS]	----	E162/TY	10	mg/L	480	----	----	----	----
Solids, total suspended [TSS]	----	E160/TY	3.0	mg/L	2.4 < DL	----	----	----	----
Turbidity	----	E121/TY	0.10	NTU	3.92	----	----	----	----
Alkalinity, total (as CaCO3)	----	E290/TY	2.0	mg/L	144	----	----	----	----
Anions and Nutrients									
Ammonia, total (as N)	7664-41-7	E298/TY	0.0050	mg/L	0.0131 < T	----	----	----	----
Ammonia, un-ionized (as N), field	7664-41-7	EC298A/TY	0.0010	mg/L	<0.0010	----	----	----	----
Chloride	16887-00-6	E235.Cl-L/TY	0.10	mg/L	7.95	----	----	----	----
Cyanate	88402-73-7	E343/WT	0.20	mg/L	<0.20	----	----	----	----
Fluoride	16984-48-8	E235.F/TY	0.020	mg/L	<0.040 DLDS	----	----	----	----
Kjeldahl nitrogen, total [TKN]	----	E318/TY	0.050	mg/L	0.852	----	----	----	----
Nitrate (as N)	14797-55-8	E235.NO3/TY	0.020	mg/L	0.054 < T	----	----	----	----
Nitrite (as N)	14797-65-0	E235.NO2/TY	0.010	mg/L	<0.002 < W, DLDS	----	----	----	----
Phosphate, ortho-, dissolved (as P)	14265-44-2	E378-U/TY	0.0010	mg/L	<0.0010	----	----	----	----
Phosphorus, total	7723-14-0	E372-U/TY	0.0020	mg/L	0.0148 < T	----	----	----	----
Sulfate (as SO4)	14808-79-8	E235.SO4/TY	0.30	mg/L	258	----	----	----	----
Cyanides									
Thiocyanate	302-04-5	E344/VA	0.50	mg/L	<0.50	----	----	----	----
Cyanide, free	----	E339/WT	0.0020	mg/L	<0.0020	----	----	----	----

Analytical Results

Sub-Matrix: **Water (MISA)**

(Matrix: **Water**)

Analyte	CAS Number	Method/Lab	LOR	Unit	Client sample ID: SP2 DISCHARGE_EFF_20231024 EFF	----	----	----	----
					Client sampling date / time: 24-Oct-2023 08:25	----	----	----	----
					TY2311042-001	--------	--------	--------	--------
					Result	----	----	----	----
Cyanides									
Cyanide, strong acid dissociable (Total)	----	E333/WT	0.0020	mg/L	0.00064 < DL	----	----	----	----
Cyanide, weak acid dissociable	----	E336/WT	0.0020	mg/L	0.00032 < DL	----	----	----	----
Organic / Inorganic Carbon									
Carbon, dissolved organic [DOC]	----	E358-L/WT	0.50	mg/L	11.3	----	----	----	----
Carbon, total organic [TOC]	----	E355-L/WT	0.50	mg/L	11.1	----	----	----	----
Microbiological Tests									
Coliforms, Escherichia coli [E. coli]	----	E010/TY	1	MPN/100mL	10	----	----	----	----
Bioassays									
Ceriodaphnia dubia, LC50	----	CER-LC50-7d/2T	-	-	see attached	----	----	----	----
Fathead minnow, survival	n/a	FAT-LC50-7d/2T	-	-	See attached	----	----	----	----
Green algae, growth	n/a	ALG-IC50-72h/2T	-	-	See attached	----	----	----	----
Green algae, IC25	n/a	ALG-IC50-72h/2T	-	-	See attached	----	----	----	----
Lemna minor, dry weight	n/a	LEM-IC50-7d/2T	-	-	See attached	----	----	----	----
Lemna minor, frond count	n/a	LEM-IC50-7d/2T	-	-	See attached	----	----	----	----
Total Metals									
Aluminum, total	7429-90-5	E420/TY	0.0030	mg/L	0.0318	----	----	----	----
Antimony, total	7440-36-0	E420/TY	0.00010	mg/L	0.00092 < T	----	----	----	----
Arsenic, total	7440-38-2	E420/TY	0.00010	mg/L	0.00197 < T	----	----	----	----
Barium, total	7440-39-3	E420/TY	0.00010	mg/L	0.0455	----	----	----	----
Beryllium, total	7440-41-7	E420/TY	0.000020	mg/L	<0.0000001 < W	----	----	----	----
Bismuth, total	7440-69-9	E420/TY	0.000050	mg/L	<0.00001 < W	----	----	----	----
Boron, total	7440-42-8	E420/TY	0.010	mg/L	0.080	----	----	----	----
Cadmium, total	7440-43-9	E420/TY	0.0000050	mg/L	0.0000020 < DL	----	----	----	----
Calcium, total	7440-70-2	E420/TY	0.050	mg/L	69.6	----	----	----	----

Analytical Results

Sub-Matrix: **Water (MISA)**

(Matrix: **Water**)

Analyte	CAS Number	Method/Lab	LOR	Unit	Client sample ID	SP2 DISCHARGE_EFF_20231024 EFF	----	----	----	----
					Client sampling date / time	24-Oct-2023 08:25	----	----	----	----
						TY2311042-001	--------	--------	--------	--------
						Result	----	----	----	----
Total Metals										
Cesium, total	7440-46-2	E420/TY	0.000010	mg/L		0.000078	----	----	----	----
Chromium, total	7440-47-3	E420/TY	0.00050	mg/L		0.00014 <DL	----	----	----	----
Cobalt, total	7440-48-4	E420/TY	0.00010	mg/L		0.00012 <T	----	----	----	----
Copper, total	7440-50-8	E420/TY	0.00050	mg/L		0.00199 <T	----	----	----	----
Iron, total	7439-89-6	E420/TY	0.010	mg/L		0.034	----	----	----	----
Lead, total	7439-92-1	E420/TY	0.000050	mg/L		0.000047 <DL	----	----	----	----
Lithium, total	7439-93-2	E420/TY	0.0010	mg/L		0.0256 <T	----	----	----	----
Magnesium, total	7439-95-4	E420/TY	0.0050	mg/L		37.8	----	----	----	----
Manganese, total	7439-96-5	E420/TY	0.00010	mg/L		0.0311	----	----	----	----
Mercury, total	7439-97-6	E508/WT	0.0000050	mg/L		<0.000001 <W	----	----	----	----
Molybdenum, total	7439-98-7	E420/TY	0.000050	mg/L		0.00390 <T	----	----	----	----
Nickel, total	7440-02-0	E420/TY	0.00050	mg/L		0.00101 <T	----	----	----	----
Phosphorus, total	7723-14-0	E420/TY	0.050	mg/L		0.020 <DL	----	----	----	----
Potassium, total	7440-09-7	E420/TY	0.050	mg/L		6.13	----	----	----	----
Rubidium, total	7440-17-7	E420/TY	0.00020	mg/L		0.00260	----	----	----	----
Selenium, total	7782-49-2	E420/TY	0.000050	mg/L		0.000335 <T	----	----	----	----
Silicon, total	7440-21-3	E420/TY	0.10	mg/L		2.52	----	----	----	----
Silver, total	7440-22-4	E420/TY	0.000010	mg/L		0.0000010 <DL	----	----	----	----
Sodium, total	7440-23-5	E420/TY	0.050	mg/L		28.6	----	----	----	----
Strontium, total	7440-24-6	E420/TY	0.00020	mg/L		0.418	----	----	----	----
Sulfur, total	7704-34-9	E420/TY	0.50	mg/L		88.0	----	----	----	----
Tellurium, total	13494-80-9	E420/TY	0.00020	mg/L		0.000037 <DL	----	----	----	----
Thallium, total	7440-28-0	E420/TY	0.000010	mg/L		<0.000005 <W	----	----	----	----
Thorium, total	7440-29-1	E420/TY	0.00010	mg/L		<0.00001 <W	----	----	----	----
Tin, total	7440-31-5	E420/TY	0.00010	mg/L		0.000013 <DL	----	----	----	----
Titanium, total	7440-32-6	E420/TY	0.00030	mg/L		0.00064	----	----	----	----
Tungsten, total	7440-33-7	E420/TY	0.00010	mg/L		0.000024 <DL	----	----	----	----
Uranium, total	7440-61-1	E420/TY	0.000010	mg/L		0.00215 <T	----	----	----	----

Analytical Results

Sub-Matrix: **Water (MISA)**

(Matrix: **Water**)

Analyte	CAS Number	Method/Lab	LOR	Unit	Client sample ID	SP2 DISCHARGE_EFF_20231024 EFF	----	----	----	----
					Client sampling date / time	24-Oct-2023 08:25	----	----	----	----
						TY2311042-001	--------	--------	--------	--------
						Result	----	----	----	----
Total Metals										
Vanadium, total	7440-62-2	E420/TY	0.00050	mg/L		0.00084	----	----	----	----
Zinc, total	7440-66-6	E420/TY	0.0030	mg/L		0.0132	----	----	----	----
Zirconium, total	7440-67-7	E420/TY	0.00020	mg/L		0.000066 < DL	----	----	----	----
Dissolved Metals										
Aluminum, dissolved	7429-90-5	E421/TY	0.0010	mg/L		0.0024	----	----	----	----
Antimony, dissolved	7440-36-0	E421/TY	0.00010	mg/L		0.00102	----	----	----	----
Arsenic, dissolved	7440-38-2	E421/TY	0.00010	mg/L		0.00186	----	----	----	----
Barium, dissolved	7440-39-3	E421/TY	0.00010	mg/L		0.0450	----	----	----	----
Beryllium, dissolved	7440-41-7	E421/TY	0.000020	mg/L		<0.000002 < W	----	----	----	----
Bismuth, dissolved	7440-69-9	E421/TY	0.000050	mg/L		0.0000030 < DL	----	----	----	----
Boron, dissolved	7440-42-8	E421/TY	0.010	mg/L		0.075	----	----	----	----
Cadmium, dissolved	7440-43-9	E421/TY	0.0000050	mg/L		0.0000030 < DL	----	----	----	----
Calcium, dissolved	7440-70-2	E421/TY	0.050	mg/L		63.1	----	----	----	----
Cesium, dissolved	7440-46-2	E421/TY	0.000010	mg/L		0.000084	----	----	----	----
Chromium, dissolved	7440-47-3	E421/TY	0.00050	mg/L		0.000033 < DL	----	----	----	----
Cobalt, dissolved	7440-48-4	E421/TY	0.00010	mg/L		0.000075 < DL	----	----	----	----
Copper, dissolved	7440-50-8	E421/TY	0.00020	mg/L		0.00129	----	----	----	----
Iron, dissolved	7439-89-6	E421/TY	0.010	mg/L		0.0038 < DL	----	----	----	----
Lead, dissolved	7439-92-1	E421/TY	0.000050	mg/L		0.000051	----	----	----	----
Lithium, dissolved	7439-93-2	E421/TY	0.0010	mg/L		0.0239	----	----	----	----
Magnesium, dissolved	7439-95-4	E421/TY	0.0050	mg/L		35.6	----	----	----	----
Manganese, dissolved	7439-96-5	E421/TY	0.00010	mg/L		0.00072	----	----	----	----
Mercury, dissolved	7439-97-6	E509/WT	0.0000050	mg/L		<0.000001 < W	----	----	----	----
Molybdenum, dissolved	7439-98-7	E421/TY	0.000050	mg/L		0.00416	----	----	----	----
Nickel, dissolved	7440-02-0	E421/TY	0.00050	mg/L		0.00073	----	----	----	----
Phosphorus, dissolved	7723-14-0	E421/TY	0.050	mg/L		<0.005 < W	----	----	----	----
Potassium, dissolved	7440-09-7	E421/TY	0.050	mg/L		5.88	----	----	----	----
Rubidium, dissolved	7440-17-7	E421/TY	0.00020	mg/L		0.00252	----	----	----	----

Analytical Results

Sub-Matrix: **Water (MISA)**

(Matrix: **Water**)

				Client sample ID	**SP2 DISCHARGE_EFF_20231024 EFF**	**----**	**----**	**----**	**----**
				Client sampling date / time	24-Oct-2023 08:25	----	----	----	----
Analyte	*CAS Number*	*Method/Lab*	*LOR*	*Unit*	**TY2311042-001**	--------	--------	--------	--------
					Result	----	----	----	----
Dissolved Metals									
Selenium, dissolved	7782-49-2	E421/TY	0.000050	mg/L	0.000328	----	----	----	----
Silicon, dissolved	7440-21-3	E421/TY	0.050	mg/L	2.51	----	----	----	----
Silver, dissolved	7440-22-4	E421/TY	0.000010	mg/L	<0.0000005 < W	----	----	----	----
Sodium, dissolved	7440-23-5	E421/TY	0.050	mg/L	26.2	----	----	----	----
Strontium, dissolved	7440-24-6	E421/TY	0.00020	mg/L	0.407	----	----	----	----
Sulfur, dissolved	7704-34-9	E421/TY	0.50	mg/L	84.0	----	----	----	----
Tellurium, dissolved	13494-80-9	E421/TY	0.00020	mg/L	0.000018 < DL	----	----	----	----
Thallium, dissolved	7440-28-0	E421/TY	0.000010	mg/L	<0.000002 < W	----	----	----	----
Thorium, dissolved	7440-29-1	E421/TY	0.00010	mg/L	<0.00001 < W	----	----	----	----
Tin, dissolved	7440-31-5	E421/TY	0.00010	mg/L	0.0000058 < DL	----	----	----	----
Titanium, dissolved	7440-32-6	E421/TY	0.00030	mg/L	0.00018 < DL	----	----	----	----
Tungsten, dissolved	7440-33-7	E421/TY	0.00010	mg/L	<0.000002 < W	----	----	----	----
Uranium, dissolved	7440-61-1	E421/TY	0.000010	mg/L	0.00213	----	----	----	----
Vanadium, dissolved	7440-62-2	E421/TY	0.00050	mg/L	0.00058	----	----	----	----
Zinc, dissolved	7440-66-6	E421/TY	0.0010	mg/L	0.0050	----	----	----	----
Zirconium, dissolved	7440-67-7	E421/TY	0.00030	mg/L	0.000034 < DL	----	----	----	----
Dissolved mercury filtration location	----	EP509/WT	-	-	Field	----	----	----	----
Dissolved metals filtration location	----	EP421/TY	-	-	Field	----	----	----	----
Aggregate Organics									
Biochemical oxygen demand [BOD]	----	E550/TY	2.0	mg/L	<2.0	----	----	----	----
Carbonaceous biochemical oxygen demand [CBOD]	----	E555/TY	2.0	mg/L	<2.0	----	----	----	----
Chemical oxygen demand [COD]	----	E559-L/TY	10	mg/L	29	----	----	----	----
Radiological Parameters									
Radium-226	13982-63-3	Ra-226/2I	0.005	Bq/L	<0.005	----	----	----	----

Please refer to the General Comments section for an explanation of any result qualifiers detected.

Please refer to the Accreditation section for an explanation of analyte accreditations.

SASKATCHEWAN RESEARCH COUNCIL

Environmental Analytical Laboratories
143-111 Research Drive, Saskatoon, SK Canada S7N 3R2

T: 306-933-6932 **F:** 306-933-7922
Toll-free: 1-800-240-8808
E: analytical@src.sk.ca

www.src.sk.ca/analytical

SRC Group # 2023-13831

Nov 09, 2023

ALS
Thunder Bay Analytical
1081 Barton Street
Thunder Bay, ON P7B 5N3
Attn: Christine Paradis

Date Samples Received: Oct-26-2023 Client P.O.: TY2311042

All results have been reviewed and approved by a Qualified Person in accordance with the Saskatchewan Environmental Code, Corrective Action Plan Chapter, for the purposes of certifying a laboratory analysis

Results from Lab Section 4 approved by Smith-Windsor, Jenna

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* Test methods and data are validated by the laboratory's Quality Assurance Program.

* Routine methods follow recognized procedures from sources such as
    * Standard Methods for the Examination of Water and Wastewater APHA AWWA WEF
    * Environment Canada
    * US EPA
    * CANMET

* The results reported relate only to the test samples as provided by the client. Results apply to the sample as received, unless otherwise indicated.

* Data marked as "by Client" has been provided by the client and may affect the validity of results.

* Samples will be kept for 30 days after the final report is sent. Please contact the lab if you have any special requirements.

* Additional information is available upon request.

* Where applicable, unless otherwise noted, Measurement Uncertainty has not been accounted for when stating conformity to the referenced standard.

This is a final report.

SASKATCHEWAN RESEARCH COUNCIL

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SRC Group # 2023-13831

Nov 09, 2023

ALS, Thunder Bay Analytical

1081 Barton Street
Thunder Bay, ON P7B 5N3
Attn: Christine Paradis

Sample #: **2023039648**
Date Sampled: **Oct 24, 2023**
Sample Matrix: **WATER**
Description: **10/24/2023 07:25 SP2 DISCHARGE_EFF_20231024 TY2311042-001**

Client PO #: **TY2311042**
Date Received: **Oct 26, 2023**

Analyte	Units	Result	DL
Lab Section 4			
Radium-226	Bq/L	<0.005	0.005

Symbol of "<" means "less than". This indicates that it was not detected at level stated above.

The temperature of the cooler was 14.6 °C upon receipt.

SASKATCHEWAN RESEARCH COUNCIL

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SRC Group # 2023-13831

Nov 09, 2023

ALS, Thunder Bay Analytical

Analyte Methods

Name	Units	Method
Radium-226	Bq/L	Rad-105

November 28, 2023

ALS Environmental,
1081 Barton St,
Thunder Bay, ON P7B 5N3

Attention Lab:

On October 26, 2023, Nautilus Environmental Company Inc. personnel received one water sample SP2 Discharge_Eff_20231024 from ALS Environmental, Thunder Bay, ON. The following toxicity tests were performed on this sample observing Environment Canada methods:

- Fathead minnow 7-day toxicity test, according to the protocol "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS1/RM/22, February 2011.
- *Ceriodaphnia dubia* 3-brood toxicity test, according to the protocol "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007.
- Freshwater alga 72-hour toxicity test, according to the protocol "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007.
- *Lemna minor* 7-day toxicity test, according to the protocol "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Second Edition, Method Development and Applications Section, Ottawa, ON., Report EPS 1/RM/37, January 2007.

Table 1 Summary of Chronic Toxicity Results, sample collected October 24, 2023

Sample Name Sample #	Toxicity Test	Endpoint	Effect	Result[1]
SP2 Discharge_Eff _20231024 #8730-0032367	Fathead Minnow	7-day LC50 (95% Confidence) 7-day IC25 (95% Confidence)	Survival Biomass	>100% Volume[2] (Not Applicable) 40.33% Volume[2] (25.6; N/A% Volume)
	Ceriodaphnia dubia	3-brood LC50 (95% Confidence) 3-brood IC25 (95% Confidence)	Survival Reproduction	>100% Volume[2] (Not Applicable) 27.17% Volume[2] (1.14; N/A% Volume)
	Raphidocelis subcapitata	72-hour IC25 (95% Confidence)	Growth	>90.91% Volume[2] (Not applicable)
	Lemna minor	7-day IC25 Frond Number (95% Confidence) 7-day IC25 Dry Weight (95% Confidence)	Growth Growth	>97% Volume[2] (Not applicable) >97% Volume[2] (Not applicable)

1 - Results relate only to the sample tested
2 - Highest concentration tested, based on test method

Toxicity Test Endpoint Descriptions

From the data obtained in toxicity tests the following endpoints can be determined:

LC50 The estimated concentration which causes acute lethality to 50% of the test organisms.

IC25 The estimated test sample concentration which causes a 25% impairment in a quantitative biological function (*i.e.,* the concentration estimated to cause a 25% reduction in fathead minnow larvae biomass, 25% reduction in the number of *Ceriodaphnia dubia* young produced).

Chronic Toxicity Test Descriptions

Fathead Minnow Toxicity Test

The fathead minnow 7-day survival and growth toxicity test determine the effect of a sample on the ability of fathead minnow larvae to survive and grow. This toxicity test utilizes 10, <24-hour old larvae per replicate and exposes them to a dilution series of the test sample (*i.e.,* 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56% volume and a control). The endpoints of the toxicity test determine the effect of the test sample on larval survival as well as growth over the 7-day exposure. Larval biomass is considered a sublethal endpoint which combines the effects on mortality and growth. The procedure is a static replacement toxicity test where the larvae in each replicate are exposed to a fresh sample volume each day. The toxicity test utilizes a minimum of seven concentrations plus a control with 3 to 4 replicates per concentration. If there is sufficient mortality and/or impairment in growth an LC50 and/or IC25 is calculated.

Ceriodaphnia dubia Toxicity Test

The *Ceriodaphnia dubia* 3-brood survival and reproduction toxicity test determine the effect of a sample on the ability of the *C. dubia* to survive and reproduce. This toxicity test is initiated by introducing one, < 24- hour old offspring (neonate) per vessel. The neonates are exposed to a dilution series of the test sample similar to the fathead minnow toxicity test. The toxicity test procedure, like the fathead minnow toxicity test, is a static replacement toxicity test where the *C. dubia* for each replicate is transferred to a fresh sample volume each day of the toxicity test. The toxicity test utilizes a minimum of seven concentrations plus a control with ten replicates per concentration. The endpoints of the toxicity test determine the effect of the test sample on survival and reproduction. *C. dubia* reproduction is considered a chronic endpoint. During the exposure, the *C. dubia* matures and produces three broods of offspring. The number of offspring per replicate for each test concentration is tabulated at the end of the test period. If there is sufficient mortality and/or impairment in reproduction an LC50 and/or IC25 is calculated.

Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*) Toxicity Test

The *Raphidocelis subcapitata* 72-hour growth inhibition test is performed to determine the capacity of a sample to inhibit the growth of algal cells. *P. subcapitata* cells are introduced into the test in the form of an inoculum, in which the algae are 3 to 7 days old and at a concentration of 10,000 cells/mL. The test is comprised of at least 10 concentrations of 3 to 4 replicates each, diluted by a factor of 0.33 (i.e., 90.91%, 30.303%, 10.101%, 3.367% etc.). The set of controls includes 10 replicates. Into each replicate of the test, 2200 algal cells are placed. The actual concentrations are diluted by a factor of 0.9091 because of the addition of a nutrient spike (enrichment medium) and the preparation of the inoculum. After 72 hours in an incubation area, the cells in each replicate are counted, and the growth of the algae exposed to the sample is compared with growth of the controls. An IC25 is calculated to reveal if the sample caused any inhibition in the growth of the algal cells.

Lemna minor Toxicity Test

The *Lemna minor* 7-day growth inhibition test is performed to determine the capacity of a sample to inhibit plant growth. Each leaf-like structure of *L. minor* is called a frond. Three-fronded *L. minor* plants, started 7 to 10 days previous, are placed, two plants to a replicate, into test chambers. The test is comprised of at least 7 concentrations of 4 replicates each, diluted by a factor of 0.3 (*i.e.,* 97%, 31%, 9.7% etc.). The set of controls also includes 4 replicates. The actual concentrations are diluted by a factor of 0.97 because of the addition of three nutrient stock solutions (enrichment medium) to the sample prior to preparing the dilution series. After 7 days, the individual fronds of the plants in each replicate are counted, and the plant dry weight determined. The growth of the fronds exposed to the sample is compared with that of the control fronds, and an IC25 is calculated for both frond number and dry weight. This reveals if the sample caused any inhibition in the growth of the *L. minor*.

The following pages contain detailed descriptions of your fathead minnow, *Ceriodaphnia dubia, Lemna minor*, and *Raphidocelis subcapitata* toxicity tests. Bench sheets of the chronic tests have been attached for your review. Also attached is a graph of the impairment in growth/reproduction. Additional information about quality assurance/quality control procedures and results can be made available if so desired.

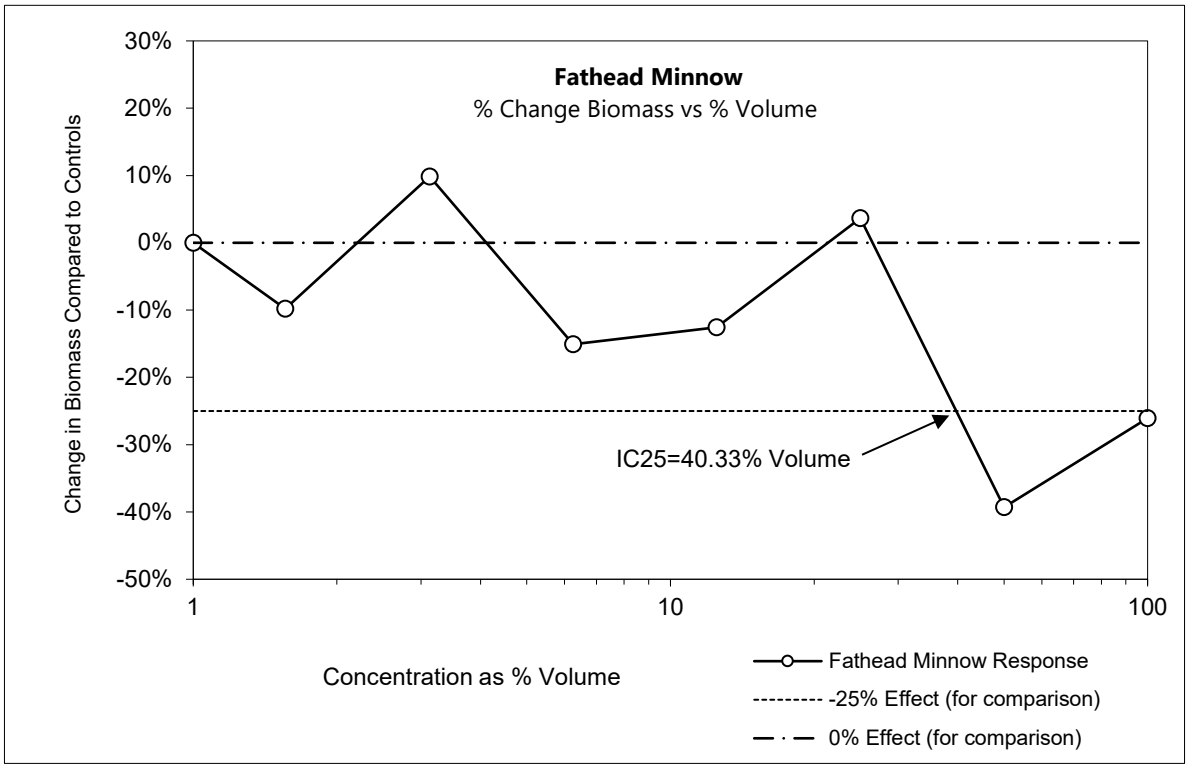
If there are any further details which you require, please do not hesitate to contact us.

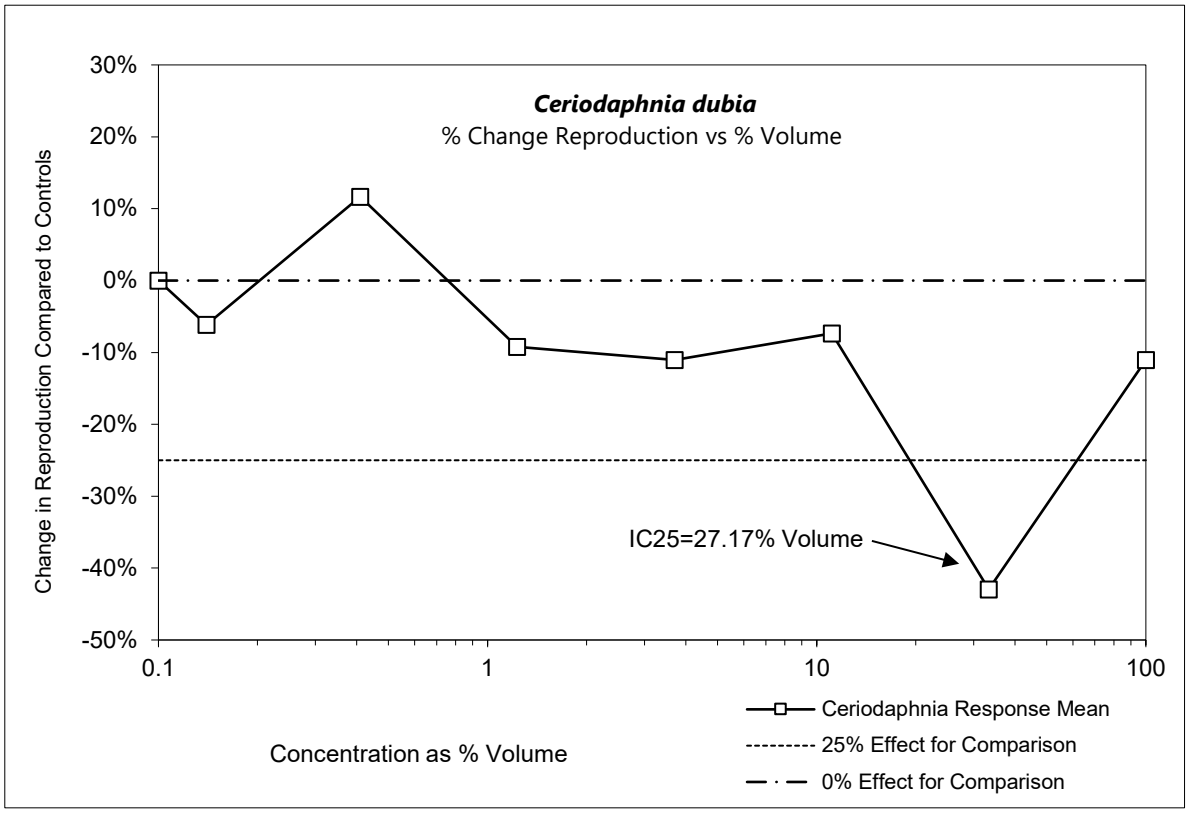
Yours very truly,
Nautilus Environmental Company Inc.

Carol D'Andrea
Laboratory Supervisor

File ID: \bioassay\2023\8000\8730-003\8730-0032367 FCRL

ALS Thunder Bay - SP2 Discharge_Eff_20231024 - October 24, 2023





Fathead Minnow Larval Survival and Growth Study

METHOD: Environment Canada, "Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/22, February 2011. Nautilus Test Method FH-GS-R14.7.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Water	**Source:**	SP2 Discharge_Eff_20231024
Date/Time Sampled:	October 24, 2023; 08:25	**Date/Time Received:**	October 26, 2023; 11:40
Date/Time Test Started:	October 26, 2023; 15:15	**Date Test Finished:**	November 2, 2023
Description:	Clear, Light Yellow	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032367	**Sample Collection:**	Other
Transport:	Road/Air	**Arrival Temp.:**	14.4°C
Collected By:	Not available		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

N/A - Not Available

Test Organisms

Species: Fathead Minnow (*Pimephales promelas*)

Source: Nautilus Fathead Minnow Culture Unit (A.B.S. Inc., Colorado)

Culture Temp.: 22 to 26 °C **Life Stage:** <24-hour old larvae

Culture Mortality Within 7 Days of Egg Collection: 0%

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032367 **Sources:** SP2 Discharge_Eff_20231024

Control and Dilution Water

Water Source: Dechlorinated municipal drinking water

Type and Quantity of Chemicals Used: None

Test Conditions

Test Volume: 533 ml/rep **Temp.:** 25 ± 1 °C

 # Organisms/rep.: 10 **Depth of solution in test vessels:** 7.9 cm

Unusual Behaviour During Test: No, see bench sheets

Reps/conc.: 3 reps/7 conc. plus a control

Pre-aerated: Yes, 100% Sample, days 0 to 6

Duration of Pre-aeration: ≤20 minutes **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Test Vessels: 1-L polypropylene cylinders

Conditions for Test Validity

Control Mortality and Atypical Behaviour is ≤ 20%: Acceptable (0%)

Average Weight of Controls is ≥ 250 µg: Acceptable (606 µg)

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032367 **Sources:** SP2 Discharge_Eff_20231024

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	1.56	3.13	6.25	12.50	25.00	50.00	100.00
Mortality Data									
Larvae % Mortality	1	0	0	0	10	0	0	50	50
	2	0	0	0	10	30	0	10	10
	3	0	30	0	10	20	10	30	0
Mean % Mortality		0.0	10.0	0.0	10.0	16.7	3.3	30.0	20.0
S.D.		0.0	17.3	0.0	0.0	15.3	5.8	20.0	26.5
Survival/Growth Data									
Mean Dry Biomass	1	0.613	0.649	0.745	0.419	0.574	0.673	0.320	0.297
(mg)	2	0.574	0.572	0.657	0.543	0.542	0.628	0.428	0.545
	3	0.630	0.418	0.594	0.581	0.473	0.582	0.355	0.501
% Effect (+ or -)		0.0	-9.8	9.9	-15.1	-12.5	3.6	-39.3	-26.1
Mean Dry Biomass/ Concentration (mg)		0.606	0.546	0.665	0.514	0.530	0.628	0.368	0.448
S.D.		0.03	0.12	0.08	0.08	0.05	0.05	0.06	0.13

S.D. = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Fathead Minnow Larval Survival and Growth Study - Continued

Sample #: 8730-0032367 **Sources:** SP2 Discharge_Eff_20231024

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival 7-day LC50 (Confidence Interval)[2]	 >100% Volume (Not Applicable)	 No dose response
Biomass **(Survival and Growth)** 7-day IC25 (Confidence Interval)[2]	 40.33% Volume (25.6; N/A% Volume)	 No nonlinear regression models fit Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

7-Day Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Phenol

Date Test Initiated: 20-Oct-2023 **Reference Batch #:** P2317

Method of Analysis: Untrimmed Spearman-Karber α = 0%

7-Day LC50 (95% Confidence Limits): 27.01 mg/L (23.04 mg/L; 31.66 mg/L)

Historic Geometric Mean LC50: 23.28 mg/L (15.81 mg/L; 34.27 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

FATHEAD MINNOW BIOASSAY SUMMMARY SHEET

lient: ALS - Thunder Bay Sample Name: SP2 Discharge EA 2023 1029 Sample #: 8730-0032367

Conditions for Test Validity TY 2311042-001

Control Mortality and Atypical Behaviour is ≤ 20%: (Acceptable) / Not Acceptable 0 %

Average Weight of Controls is ≥ 250 µg: (Acceptable) / Not Acceptable 606 µg

Summary of Test Results

Pre-aeration: Yes Reason: supersaturation Duration: ≤ 20 min Days: 0 to 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL 7-day LC50 95% Confidence Interval[2]	> 100 % Volume N/A % Volume	no dose response
Survival / Growth Biomass 7-day IC25 95% Confidence Interval[2]	40.33 % Volume 25.6 ; N/A % Volume	No nonlinear regression models would fit or print IC25 IV - linear interpolation

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s):

Analysis Completed: Initials BV Date 21/11/23

Results Verified: Initials AO Date 28.11.23

NAUTILUS ENVIRONMENTAL

Fathead minnow
TEST DATA
BENCHSHEET COPY

Sample Number : **8730-0032367**
Sample Name : **SP2-Discharge_EFF_20231**

Reference : **Test of Larval Growth and Survival Using Fathead Minnows. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/22 , 2nd ed. (February 2011).**

TEST ORGANISM

Organism Batch : FH0622/0123
Swim Bladders Inflated ? : Yes
Minimum Age (hh:mm) : ~07:00
Previous 7 d Mort/Diseased : %

Organism Age :

	Date	Time (hh:mm)
Tiles Transferred :	2023-10-25	16:30
Maximum Age :	22:45	

MORTALITY DATA

		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
Start Date :		2023-10-26	2023-10-27	2023-10-28	2023-10-29	2023-10-30	2023-10-31	2023-11-01	2023-11-02			
Analyst(s) :		DS	MB	MB	KK	DS	DS	MB	MB			
Randomized? :		Yes								**Mortality**	**Average**	**SD**
Concentration	**Replicate**									%	%	
Control (MORTALITY)	A	0	0	0	0	0	0	0	0	0	0.0	0.0
	B	0	0	0	0	0	0	0	0	0		
	C	0	0	0	0	0	0	0	0	0		
Control (IMPAIRMENT)	A	0	0	0	0	0	0	0	0	0	0.0	0.0
	B	0	0	0	0	0	0	0	0	0		
	C	0	0	0	0	0	0	0	0	0		
1.56	A	0	0	0	0	0	0	0	0	0	10.0	17.3
	B	0	0	0	0	0	0	0	0	0		
	C	0	0	1	1	2	3	3		30		
3.13	A	0	0	0	0	0	0	0	0	0	0.0	0.0
	B	0	0	0	0	0	0	0	0	0		
	C	0	0	0	0	0	0	0	0	0		
6.25	A	0	0	0	0	0	1	1		10	10.0	0.0
	B	0	0	0	0	0	1	1		10		
	C	0	0	0	0	0	1	1		10		
12.5	A	0	0	0	0	0	0	0	0	0	16.7	15.3
	B	0	0	0	0	2	2	3		30		
	C	0	0	0	0	1	2	2		20		
25	A	0	0	0	0	0	0	0	0	0	3.3	5.8
	B	0	0	0	0	0	0	0	0	0		
	C	0	0	0	0	0	0	1		10		
50	A	0	0	0	5	5	5	5		50	30.0	20.0
	B	0	0	1	1	1	1	1		10		
	C	0	0	0	2	3	3	3		30		
100	A	0	0	3	5	5	5	5		50	20.0	26.5
	B	0	0	0	1	1	1	1		10		
	C	0	0	0	0	0	0	0	0	0		

Aberrant behaviour or swimming impairment in 1.56 to 100 concentrations: 6.25 A,B & C : 1 TLOE EACH

Notes :

Reviewed/Changed By: C.D.
Date: 27/11/2023

Sample Number : **8730-0032367**
Sample Name : **SP2-Discharge_EFF_20231024**

TEST CONDITIONS

Time Start :	15:15	Sample Filtration? :	
pH Adjustment ? :	None	Filter Size :	60 µm
Hardness Adjustment ? :	None	Filtration started on :	
Test Aeration ?	None	Test Volume :	533 mL
Organisms per Replicate :	10	Test Vessel :	1000 mL polypropylene cylinder
Number of Replicates :	3	Test Solution Depth :	7.9 cm
Unit :	%	Control/Dilution Water :	Dechlorinated Municipal Water
		Additional Chemicals :	None

BIOMASS DATA

Concentration	Replicate	Pan	Dry Weight (Pan + Larvae)	Expected Fish on Pan	Actual Fish on Pan	Mean Biomass	Treatment Mean	SD	Replicate Weight	Treatment Mean Weight
		g	g			mg	mg		mg	mg
	A	0.86610	0.87223	10	10	0.613			0.613	
Control	B	0.84783	0.85357	10	10	0.574	0.606	0.029	0.574	0.606
	C	0.84422	0.85052	10	10	0.630			0.630	
	A	0.84026	0.84675	10	10	0.649				
1.56	B	0.84481	0.85053	10	10	0.572	0.546	0.118		
	C	0.84955	0.85373	7	7	0.418				
	A	0.84941	0.85686	10	10	0.745				
3.13	B	0.85056	0.85713	10	10	0.657	0.665	0.076		
	C	0.85627	0.86221	10	10	0.594				
	A	0.85675	0.86094	9	9	0.419				
6.25	B	0.85604	0.86147	9	9	0.543	0.514	0.085		
	C	0.86104	0.86685	9	9	0.581				
	A	0.85226	0.85800	10	10	0.574				
12.5	B	0.86249	0.86791	7	7	0.542	0.530	0.052		
	C	0.87081	0.87554	8	8	0.473				
	A	0.84636	0.85309	10	10	0.673				
25	B	0.86008	0.86636	10	10	0.628	0.628	0.046		
	C	0.84931	0.85513	9	9	0.582				
	A	0.84865	0.85185	5	5	0.320				
50	B	0.84341	0.84769	9	9	0.428	0.368	0.055		
	C	0.85997	0.86352	7	7	0.355				
	A	0.83954	0.84251	5	5	0.297				
100	B	0.86141	0.86686	9	9	0.545	0.448	0.132		
	C	0.84984	0.85485	10	10	0.501				

Foil		re-weighed at		Analyst(s):	ep
Foil & Larvae	0.87223	re-weighed at	0.87	Analyst(s):	SO

NOTES:

NAUTILUS ENVIRONMENTAL

Water Chemistry Data
BENCSHEET COPY

Sample Number : **8730-0032367**
Sample Name : **SP2-Discharge_EFF_20231024**

HARDNESS TITRATION

	Volume	Buret Start	Buret End	Analyst(s)	Hardness
Control (Day 0) :	50	36.5	42.0	tc	110
100% :	50	18.5	35.6	tc	342

FATHEAD MINNOW INITIAL CHEMISTRY

	Day 0 - 1	Day 1 - 2	Day 2 - 3	Day 3 - 4	Day 4 - 5	Day 5 - 6	Day 6 - 7
	2023-10-26	2023-10-27	2023-10-28	2023-10-29	2023-10-30	2023-10-31	2023-11-01
Sub-sample	1	1	1	2	2	3	3
Temperature	25	24	24	24	24	24	24
DO	10.1	10.6	11.2	11.2	11.3	10.5	11.4
pH	7.9	8.0	8.0	8.0	7.9	8.0	7.9
Conductivity	748	752	788	797	797	811	748
Pre-aeration Time (min)	≤20	≤20	≤20	≤20	≤20	≤20	≤20
Analyst(s) :	SP	SO	KK	KK	SO	SO	SO
Meter Number (Temp.) :	A	A	A	A	A	A	A
Meter Number (pH) :	A	A	A	A	A	A	A
Meter Number (Cond.) :	A	A	A	A	A	A	A

'nm' = not measured

FATHEAD MINNOW WATER CHEMISTRY DATA

Analyst(s)	Initial	SP	TC	TC	TC	SO	DS	MB
	Final	TC	TC	TC	SO	DS	MB	DS
Control								
Temperature	Initial	25.0	26.0	25.0	24.0	24.0	25.0	26.0
	Final	25.0	24.0	24.0	25.0	25.0	25.0	25.0
DO (mg/L)	Initial	7.9	7.9	8.1	8.1	8.0	7.5	7.7
	Final	7.2	7.2	6.9	7.1	6.3	6.5	6.9
pH	Initial	7.7	8.1	8.2	8.3	8.2	8.4	8.3
	Final	7.9	7.9	8.0	8.1	7.9	8.0	7.9
Conductivity	Initial	257	245	253	253	255	259	242
Hardness	Initial	110						
1.56								
Temperature	Initial	25.0	25.0	25.0	24.0	24.0	25.0	25.0
	Final	25.0	25.0	24.0	25.0	25.0	25.0	25.0
DO (mg/L)	Initial	7.8	7.8	8.0	7.9	8.0	7.5	7.6
	Final	7.2	7.1	6.9	6.9	6.4	6.8	6.7
pH	Initial	8.0	7.9	8.1	8.3	8.2	8.2	8.3
	Final	7.9	7.9	8.1	8.0	7.9	8.0	7.8
Conductivity	Initial	255	254	259	262	253	268	250
3.13								
Temperature	Initial							
	Final							
DO (mg/L)	Initial							
	Final							
pH	Initial							
	Final							
Conductivity	Initial							
6.25								
Temperature	Initial							
	Final							
DO (mg/L)	Initial							
	Final							
pH	Initial							
	Final							
Conductivity	Initial							
12.5								
Temperature	Initial	25.0	25.0	25.0	24.0	24.0	25.0	25.0
	Final	25.0	25.0	24.0	25.0	25.0	25.0	25.0
DO (mg/L)	Initial	7.9	7.9	8.2	8.0	8.0	7.5	7.7
	Final	7.2	6.9	6.8	6.9	6.2	6.8	6.9
pH	Initial	8.0	8.0	8.1	8.2	8.2	8.2	8.3
	Final	7.9	7.9	8.1	8.0	7.9	8.0	7.9
Conductivity	Initial	318	315	323	328	329	336	311
25								
Temperature	Initial							
	Final							
DO (mg/L)	Initial							
	Final							
pH	Initial							
	Final							
Conductivity	Initial							
50								
Temperature	Initial							
	Final							
DO (mg/L)	Initial							
	Final							
pH	Initial							
	Final							
Conductivity	Initial							
100								
Temperature	Initial	26.0	25.0	24.0	24.0	24.0	25.0	25.0
	Final	25.0	25.0	24.0	25.0	25.0	25.0	25.0
DO (mg/L)	Initial	8.4	8.9	10.4	9.8	9.4	8.4	9.5
	Final	6.9	6.7	6.5	6.8	6.3	6.7	6.8
pH	Initial	8.0	7.9	7.9	8.0	8.0	8.0	8.0
	Final	8.0	8.0	8.1	8.1	8.1	8.1	8.1
Conductivity	Initial	745	748	788	798	797	811	747
Hardness	Initial	342						

NOTES :

Reviewed/Changed By: C.D.
Date: 27/11/2023

Ceriodaphnia dubia Survival and Reproduction Study

METHOD: Environment Canada, "Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia",* Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/21, 2007. Nautilus Test Method CD-RS-R12.11.

Test Material

Company:	ALS Environmental, Thunder Bay, ON		
Sample Type:	Water	**Source:**	SP2 Discharge_Eff_20231024
Date/Time Sampled:	October 24, 2023; 08:25	**Date/Time Received:**	October 26, 2023; 11:40
Date Test Started:	October 26, 2023; 15:30	**Date Test Finished:**	November 2, 2023
Description:	Clear, Light Yellow	**Days Sample Used:**	Days 0 to 6
Sample #:	8730-0032367	**Sample Collection:**	Other
Transport:	Road/Air	**Arrival Temp.:**	14.4°C
Collected By:	Not available		
Storage:	4 ± 2 °C	In dark, no headspace	
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species: *Ceriodaphnia dubia* **Culture Temp.:** 25 ± 1 °C

Source: Nautilus Culture (initiated from mass culture originating from OMOE, Rexdale)

Parentage of All Organisms Originated from the Same Mass Culture: Yes

Life Stage: Neonates are less than 24 hours old, and all broods used have hatched within 12 hours of one another.

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032367 **Sources:** SP2 Discharge_Eff_20231024

Test Organisms-continued

Ephippia Present in Culture Prior to Testing: No

Mean Brood Organism Mortality Within 7 Days of Testing: 6.7%

Mean Number of Surviving Young Produced Within First 3 Broods: 19.1

Mean Number of Surviving Young Produced Within 7 Days of Testing: 26.4

Number of Young Produced by Brood Organisms in 3rd/4th Brood: see bench sheet

Control and Dilution Water

Water Source: Reconstituted/Dechlorinated Municipal Drinking Water and Distilled water

Type and Quantity of Chemicals Used: 60 mg/L $MgSO_4$, 4 mg/L KCl, 96 mg/L $NaHCO_3$, 8 ug/L B_{12}, 8 ug/L Selenium, 40 mg/L $CaSO_4$

Test Conditions

Test Volume: 15 ml/rep **Temp.:** 25 ± 1 °C

Reps/conc.: 10 reps/7conc plus a control

 # Organisms/rep.: 1 **Depth of solution in test vessels:** 4.5 cm

Test Vessel Description: 17 ml polystyrene cylinder

Unusual Behaviour During Test: No, see bench sheets

Pre-aerated: Yes, 100% Sample, days 0 to 6

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032367 **Sources:** SP2 Discharge_Eff_20231024

Test Conditions-continued

Duration of Pre-aeration: ≤ 20 min **Aeration During Test:** No

Rate and Procedure of Pre-aeration: Filtered air is dispensed through airline tubing and a disposable glass pipette; the rate should not exceed 100 bubbles per minute.

Dilution Water Batch Number: CD23-188

Conditions for Test Validity

Control Mortality is ≤ 20%	Acceptable (0%)
An Average of ≥ 15 Neonates Produced per Surviving Female in the Controls in First 3 Broods:	Acceptable (16.3 Neonates)
≥ 60% of Controls Produced ≥ 3 Broods:	Acceptable (60% of controls)

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032367 **Sources:** SP2 Discharge_Eff_20231024

Test Results

Endpoints	Rep	Concentrations (% Volume)							
		Control	0.14	0.41	1.23	3.70	11.11	33.33	100.0
Survival Data Mean % Mortality		0	0	10	0	0	0	10	0
Reproduction Data Number of Neonates per Replicate in First 3 Broods or Less	1	8	22	21	12	20	5	8	5
	2	18	25	11	19	13	3	0	12
	3	19	18	17	8	25	13	13	22
	4	21	14	21	13	18	22	0	7
	5	22	12	16	7	2	15	21	26
	6	18	9	21	27	16	21	8	0
	7	17	14	25	11	9	20	2	25
	8	8	20	27	17	16	10	12	15
	9	24	8	23	17	13	22	12	16
	10	8	11	0	17	13	20	17	17
Total Number of Live Neonates in First 3 Broods or Less		163	153	182	148	145	151	93	145
% Effect (+ or -)		0.0	-6.1	11.7	-9.2	-11.0	-7.4	-42.9	-11.0
Mean Number of Live Neonates in First 3 Broods or Less		16.3	15.3	18.2	14.8	14.5	15.1	9.3	14.5
SD		6.1	5.7	7.9	5.9	6.2	7.1	7.1	8.6

SD = Standard Deviation

Method of Analysis

LC50 and IC25:

Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

***Ceriodaphnia dubia* Survival and Reproduction Study -** Continued

Sample #: 8730-0032367 **Sources:** SP2 Discharge_Eff_20231024

Summary of Test Results

Endpoints	Result[1]	Method of Calculation
Survival		
3-Brood LC50 (Confidence Interval)[2]	>100% Volume (Not Applicable)	No dose response
Reproduction		
3-Brood IC25 (Confidence Interval)[2]	27.17% Volume (1.14; N/A% Volume)	Non-linear regression models fit but calculated IC25 value didn't make sense Linear Interpolation (ICPIN)

1 - Results relate only to the sample tested.
2 - Empirical 95% Confidence Interval

Test Method Deviation: None

Outliers and Justification for Their Removal: None

Any Transformation of Data Required: No

3-Brood Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation None **Reference Chemical:** Phenol

Date Test Initiated: 20-Oct-2023 **Reference Batch #:** P2317

Method of Analysis: Untrimmed Spearman-Karber α = 0%

3-Brood LC50 (95% Confidence Limits): 3.79 mg/L (2.88 mg/L; 4.99 mg/L)

Historic Geometric Mean LC50: 5.82 mg/L (2.91 mg/L; 11.66 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

CERIODAPHNIA DUBIA BIOASSAY SUMMARY SHEET

Client: ALS - Thunder Bay Sample Name: SP2 Discharge E/A 2023 1024 Sample #: 8730-0032367

TY 7311042-001

Conditions for Test Validity

Control Mortality is ≤ 20%: (Acceptable) / Not Acceptable: 0 %

≥ 6 Controls Produced ≥ 3 Broods: (Acceptable) / Not Acceptable: 6 Controls

An Average of ≥ 15 Neonates Produced per Surviving Females in the Controls: (Acceptable) / Not Acceptable: 16.3 Neonates

Summary of Test Results

Pre-aeration: Yes Reason: Supersaturation Duration: ≤ 20 min Days: 0 - 6

ENDPOINT	RESULT[1]	METHOD OF CALCULATION
SURVIVAL		
3-brood LC50	> 100 % Volume	no dose response
95% Confidence Interval[2]	N/A % Volume	
REPRODUCTION		
3-brood IC25	27.17 % Volume	Nonlinear regression models would fit but calculated IC25 didn't make sense ICPIN - linear interpolation
95% Confidence Interval[2]	1.14; N/A % Volume	

[1] = Results relate only to sample tested
[2] = Estimated uncertainty

Are there any outliers present: Yes / (No)

Concentration(s) & Rep(s): —

Analysis Completed: Initials BV Date 21/11/23

Results Verified: Initials AO Date 28.11.23

TY 23110412-001

Ceriodaphnia dubia Neonate Origin

Sample Information

Client AIS - Thunder Bay
Sample # 8730.00032367
Date/Time Received 26.10.23/1140
Sample Type Water
100% Hardness 342

Sample Name SPZ - Discharge - Eff - 20231024
Date/Time Collected 24.10.23 / 0825 Person Sampling —
Arrival Temp (°C) 14.4
Sample Description clear light yellow

Test Information

Date Test Started 26.10.23/1530
Dilution Water Batch Number CD 23188

Test Started By CS
Control Hardness 138

Template Used for Randomization 3

Individual Culture Health Data

Date Culture Started 18.10.23
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 19.0

Culture I.D. (*e.g.*, Wed Row 4) WED Row 1
Average # neos in previous 7 days (must be ≥15) 19.2
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 18.10.23
% mortality in previous 7 days (must be ≤20%) 0
Average # neos in 1st 3 broods (must be ≥ 15) 18.2

Culture I.D. (*e.g.*, Wed Row 4) WED Row 4
Average # neos in previous 7 days (must be ≥15) 20.2
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started 13.10.23
% mortality in previous 7 days (must be ≤20%) 20
Average # neos in 1st 3 broods (must be ≥ 15) 20.0

Culture I.D. (*e.g.*, Wed Row 4) Fri Row 8
Average # neos in previous 7 days (must be ≥15) 39.9
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started _____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) _____

Culture I.D. (*e.g.*, Wed Row 4) _____
Average # neos in previous 7 days (must be ≥15) _____
(total neos for 7 days prior of viable moms/# viable moms)

Date Culture Started _____
% mortality in previous 7 days (must be ≤20%) ____
Average # neos in 1st 3 broods (must be ≥ 15) _____

Culture I.D. (*e.g.*, Wed Row 4) _____
Average # neos in previous 7 days (must be ≥15) _____
(total neos for 7 days prior of viable moms/# viable moms)

Mean Brood Organism Mortality for previous 7 days 6.7 (add up % mortality for all cultures used / # cultures used)
Mean Number of Young Produced within first 3 broods 19.1 (avg # 1st 3 broods for all cultures used / # cultures used)
Mean Number of Young Produced in previous 7 days 26.4 (avg # neos in prev 7 days for all cultures used / # cultures used)
Is there any unusual appearance, behavior, or treatment of test organisms, before their use in the test? Yes / (No) (circle one)

Test Initiation

Brood Organism (eg. W4.6)	≥ 8 neonates in current brood	≥ 3rd brood	# neonates in 3rd/4th brood	Test columns filled	Initials
W1.7	(Y) / N	(Y) / N	11	1	CS
.8	(Y) / N	(Y) / N	10	2	CS
.10	(Y) / N	(Y) / N	10	3	CS
~~3.8~~	~~Y / N~~	~~Y / N~~			
4.4 4.4	(Y) / N	(Y) / N	8	4	CS
.8	(Y) / N	(Y) / N	12	5	CS
F 8.5	(Y) / N	(Y) / N	12	6	CS
.7	(Y) / N	(Y) / N	9	7	CS
.9	(Y) / N	(Y) / N	11	8	CS
.10	(Y) / N	(Y) / N	10	9,10	CS
	Y / N	Y / N			

NAUTILUS ENVIRONMENTAL

Ceriodaphnia dubia
Survival and Reproduction
BENCHSHEET COPY

Sample Number : **8730-0032367**
Sample Name : **SP2-Discharge_EFF_2023102**

Reference : **Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia* . Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/21, 2nd ed. (February 2007).**

TEST ORGANISM

	Date	Time (hh:mm)
First Brood Check :	2023-10-25	16:30
Last Brood Check :	2023-10-25	20:30
Minimum Age :	19:00	
Maximum Age :	23:00	

Previous 7-day Mortality : 6.7 %
3rd Brood Total :
Ephippia in Culture ? None

Mean young produced within first 3 broods : 19.1
Mean young produced in previous 7 days : 26.4

Brood Organism :	W1.7	W1.8	W1.10	W4.4	W4.8	F8.5	F8.7	F8.9	F8.10			
≥8 neonates in current brood? :	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes			
≥3rd brood ? :	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes			
Neonates in 3rd/4th brood :	11	10	10	8	12	12	9	11	10			
Test Columns Filled :	1	2	3	4	5	6	7	8	9,10			
Analyst(s) :												

Any unusual appearance, behaviour, or treatment of test organisms before their use in the test ? None

TEST CONDITIONS

Start Date :	2023-10-26	Sample Filtration? :	None	Organisms per Replicate :	1
Start Time :	15:30	Filter Size :	60 µm	Number of Replicates :	10
Completion Date :	2023-11-02	Filtration Started on :	1900-01-00	Control/Dilution Water Batch :	CD23188
Test Volume :	15 mL	pH Adjustment ?:	None	Additional Chemicals ? :	None
Test Vessel :	17 mL polystyrene cylinder	Hardness Adjustment ? :	None	Was test randomized?	Yes
Solution Depth :	4.5 cm				
Unit :	%				

SURVIVAL AND REPRODUCTION

x=adult mortality
***=accidental death**

Control		REPLICATE 1	2	3	4	5	6	7	8	9	10	Mortality (%)	Mean Young	Analyst(s)
2023-10-27	1	0	0	0	0	0	0	0	0	0	0	0.0	0.0	KP
2023-10-28	2	0	0	0	0	0	0	0	0	0	0	0.0	0.0	CS
2023-10-29	3	0	0	0	0	0	0	0	0	0	0	0.0	0.0	CS
2023-10-30	4	0	2	2	3	4	2	3	0	4	2	0.0	2.2	KP
2023-10-31	5	0	5	7	0	6	5	7	2	8	0	0.0	4.0	KP
2023-11-01	6	0	11	0	10	12	11	0	0	12	0	0.0	5.6	KP
2023-11-02	7	8	0	10	8	0	0	7	6	0	6	0.0	4.5	CS
2023-11-03	8											0.0	#####	
	Total:	8	18	19	21	22	18	17	8	24	8	0.0	**16.3**	
3rd Brood:			*	*	*	*	*			*				

0.137		REPLICATE 1	2	3	4	5	6	7	8	9	10	Mortality (%)	Mean Young
2023-10-27	1	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-28	2	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-29	3	0	0	0	0	0	0	0	3	0	0	0.0	0.3
2023-10-30	4	2	0	3	0	0	0	2	0	0	0	0.0	0.7
2023-10-31	5	10	2	5	5	2	3	2	7	2	6	0.0	4.4
2023-11-01	6	10	11	10	9	10	6	10	10	0	0	0.0	7.6
2023-11-02	7	0	12	0	0	0	0	0	0	6	5	0.0	2.3
2023-11-03	8											0.0	#####
	Total:	22	25	18	14	12	9	14	20	8	11	0.0	**15.3**
3rd Brood:													

0.41		REPLICATE 1	2	3	4	5	6	7	8	9	10	Mortality (%)	Mean Young
2023-10-27	1	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-28	2	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-29	3	0	0	0	3	0	0	4	0	0	0	0.0	0.7
2023-10-30	4	3	2	3	0	3	3	0	3	4	0	0.0	2.1
2023-10-31	5	0	9	6	6	0	8	9	11	0	0	0.0	4.9
2023-11-01	6	10	0 x	8	12	9	10	12	13	6	0	10.0	8.0
2023-11-02	7	8		0	0	4	0	0	0	13	0	10.0	2.8
2023-11-03	8											10.0	#####
	Total:	21	11 x	17	21	16	21	25	27	23	0	10.0	**18.2**

1.23		REPLICATE 1	2	3	4	5	6	7	8	9	10	Mortality (%)	Mean Young
2023-10-27	1	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-28	2	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-29	3	0	0	0	0	0	0	0	0	4	0	0.0	0.4
2023-10-30	4	3	4	4	4	0	0	4	3	0	3	0.0	2.5
2023-10-31	5	2	7	0	3	7	6	7	6	6	0	0.0	4.4
2023-11-01	6	0	8	4	6	0	12	0	8	7	6	0.0	5.1
2023-11-02	7	7	*	0	0	0	9	0	0	*	8	0.0	3.0
2023-11-03	8											0.0	#####
	Total:	12	19	8	13	7	27	11	17	17	17	0.0	**14.8**

Reviewed/Changed By: C.D.
Date: 27/11/2023

NAUTILUS ENVIRONMENTAL

Ceriodaphnia dubia
Survival and Reproduction
BENCHSHEET COPY

3.7		REPLICATE 1	2	3	4	5	6	7	8	9	10	Mortality (%)	Mean Young
2023-10-27	1	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-28	2	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-29	3	3	0	0	0	0	0	0	0	0	0	0.0	0.3
2023-10-30	4	0	0	0	4	0	0	0	3	0	3	0.0	1.0
2023-10-31	5	6	5	5	5	0	3	0	5	3	5	0.0	3.7
2023-11-01	6	11	8	10	9	0	5	5	8	10	5	0.0	7.1
2023-11-02	7	*	0	10	0	2	8	4	0	0	0	0.0	2.7
2023-11-03	8											0.0	#####
	Total:	20	13	25	18	2	16	9	16	13	13	0.0	**14.5**

11.11		REPLICATE 1	2	3	4	5	6	7	8	9	10	Mortality (%)	Mean Young
2023-10-27	1	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-28	2	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-29	3	0	0	0	4	0	0	0	0	4	5	0.0	1.3
2023-10-30	4	0	1	3	0	2	4	4	3	0	0	0.0	1.7
2023-10-31	5	0	0	0	7	2	8	7	0	9	6	0.0	3.9
2023-11-01	6	5	2	10	11	0	9	9	7	9	9	0.0	7.1
2023-11-02	7	0	0	0	0	11	0	0	0	0	*	0.0	1.2
2023-11-03	8											0.0	#####
	Total:	5	3	13	22	15	21	20	10	22	20	0.0	**15.1**

33.33		REPLICATE 1	2	3	4		5	6	7	8	9	10	Mortality (%)	Mean Young
2023-10-27	1	0	0	0	0		0	0	0	0	0	0	0.0	0.0
2023-10-28	2	0	0	0	0		0	0	0	0	0	0	0.0	0.0
2023-10-29	3	0	0	0	0		0	4	0	4	0	0	0.0	0.8
2023-10-30	4	4	0	0	0	x	3	0	0	0	3	0	10.0	1.0
2023-10-31	5	0	0	1	0		8	4	2	8	9	7	10.0	3.9
2023-11-01	6	4	0	6	0		10	0	0	0	0	10	10.0	3.0
2023-11-02	7	0	0	6	0		0	0	0	0	0	0	10.0	0.6
2023-11-03	8				0								10.0	0.0
	Total:	8	0	13	0	x	21	8	2	12	12	17	10.0	**9.3**

100		REPLICATE 1	2	3	4	5	6	7	8	9	10	Mortality (%)	Mean Young
2023-10-27	1	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-28	2	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-29	3	0	0	0	0	0	0	0	0	0	0	0.0	0.0
2023-10-30	4	0	0	2	0	0	0	4	2	0	3	0.0	1.1
2023-10-31	5	0	4	9	0	6	0	10	0	5	4	0.0	3.8
2023-11-01	6	5	0	11	7	10	0	11	6	11	10	0.0	7.1
2023-11-02	7	0	8	0	0	10	0	0	7	0	0	0.0	2.5
2023-11-03	8											0.0	#####
	Total:	5	12	22	7	26	0	25	15	16	17	0.0	**14.5**

Notes:

Reviewed/Changed By: C.D.
Date: 27/11/2023

NAUTILUS ENVIRONMENTAL

Water Chemistry Data
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Sample Number : **8730-0032367**
Sample Name : **SP2-Discharge_EFF_20231024**

***CERIODAPHNIA DUBIA* INITIAL CHEMISTRY**							
	Day 0 - 1	**Day 1 - 2**	**Day 2 - 3**	**Day 3 - 4**	**Day 4 - 5**	**Day 5 - 6**	**Day 6 - 7**
	2023-10-26	2023-10-27	2023-10-28	2023-10-29	2023-10-30	2023-10-31	2023-11-01
Sub-sample	1	1	1	2	2	3	3
Temperature	25	24	24	24	24	24	24
DO	10.1	10.6	11.2	11.2	11.3	10.5	11.4
pH	7.9	8.0	8.0	8.0	7.9	8.0	7.9
Conductivity	748	752	788	797	797	811	748
Pre-aeration Time (min)	≤20	≤20	≤20	≤20	≤20	≤20	≤20
Analyst(s) :	SP	SO	KK	KK	SO	SO	SO
Meter Number (Temp.) :	A	A	A	A	A	A	A
Meter Number (pH) :	A	A	A	A	A	A	A
Meter Number (Cond.) :	A	A	A	A	A	A	A

'nm' = not measured

***CERIODAPHNIA DUBIA* WATER CHEMISTRY DATA**								
Analyst(s)	Initial	SP	SP	TC	TC	SP	KP	SP
	Final	SP	TC	KK	SP	KP	MB	TC
Control								
Temperature	Initial	25.0	25.0	25.0	24.0	24.0	24.0	24.0
	Final	25.0	24.0	24.0	24.0	24.0	24.0	24.0
DO (mg/L)	Initial	8.1	8.1	8.0	8.1	8.0	7.7	8.2
	Final	7.1	7.0	5.4	6.9	6.6	6.5	6.7
pH	Initial	8.3	8.2	8.2	8.4	8.3	8.4	8.3
	Final	8.0	7.8	8.0	8.1	8.0	7.5	8.1
Conductivity	Initial	418	414	427	436	431	445	411
Hardness	Initial							
0.137								
Temperature	Initial	25.0	25.0	25.0	24.0	25.0	24.0	25.0
	Final	25.0	24.0	24.0	24.0	24.0	24.0	24.0
DO (mg/L)	Initial	8.4	8.3	7.7	7.7	8.6	8.2	7.8
	Final	7.1	7.0	5.4	7.1	6.5	6.4	6.7
pH	Initial	8.3	8.4	8.2	8.3	8.5	8.4	8.4
	Final	8.0	7.9	8.0	8.1	8.0	7.8	8.1
Conductivity	Initial	406	414	436	443	438	448	414
0.41								
Temperature	Initial							
	Final							
DO (mg/L)	Initial							
	Final							
pH	Initial							
	Final							
Conductivity	Initial							
1.23								
Temperature	Initial							
	Final							
DO (mg/L)	Initial							
	Final							
pH	Initial							
	Final							
Conductivity	Initial							
3.7								
Temperature	Initial	25.0	26.0	24.0	24.0	25.0	24.0	25.0
	Final	25.0	24.0	24.0	24.0	24.0	24.0	24.0
DO (mg/L)	Initial	8.8	8.6	7.9	7.9	8.8	8.4	8.4
	Final	6.8	6.1	5.7	6.9	6.7	6.7	6.5
pH	Initial	8.4	8.3	8.3	8.4	8.5	8.6	8.5
	Final	8.0	7.8	8.0	8.1	8.0	7.8	8.0
Conductivity	Initial	405	428	458	457	455	463	430
11.11								
Temperature	Initial							
	Final							
DO (mg/L)	Initial							
	Final							
pH	Initial							
	Final							
Conductivity	Initial							
33.33								
Temperature	Initial							
	Final							
DO (mg/L)	Initial							
	Final							
pH	Initial							
	Final							
Conductivity	Initial							
100								
Temperature	Initial	25.0	25.0	24.0	24.0	24.0	24.0	25.0
	Final	24.0	24.0	24.0	24.0	24.0	24.0	24.0
DO (mg/L)	Initial	8.9	9.0	9.2	9.1	9.4	8.5	8.4
	Final	7.7	7.3	6.2	7.2	6.7	6.6	6.6
pH	Initial	8.2	8.0	8.0	8.1	8.1	8.3	8.2
	Final	8.2	8.0	8.2	8.4	8.2	8.0	7.9
Conductivity	Initial	747	758	792	801	808	804	751
Hardness	Initial	342						

NOTES :

Reviewed/Changed By: C.D.
Date: 27/11/2023

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

METHOD: Environment Canada "Biological Test Method: Growth Inhibition Test Using a Freshwater Alga", Second Edition, Environmental Protection Series, Ottawa, ON. Report EPS 1/RM/25, March 2007. Nautilus Test Method PS-GI-R1.14.

Test Material

Client Name/Location: ALS Environmental, Thunder Bay, ON

Sample #:	8730-0032367	**Sample Name:**	SP2 Discharge_Eff_20231024
Sample Method:	Other	**Collected by:**	N/A
Date/Time Collected:	October 24, 2023; 08:25	**Arrival Temp.:**	14.4°C
Date/Time Received:	October 26, 2023; 11:40	**Sample Description:**	Clear, Light Yellow
Sample Point Description:	Other	**Sample Type:**	Water
Transportation:	Road/Air		
Storage:	None		
Container:	Polyethylene pails lined with polyethylene bags		

Test Organisms

Species (Strain #):	*Raphidocelis subcapitata* (CPCC # 37)
Source:	Nautilus Plant Culture Unit (from CPCC)
Culture Temp.:	24 ± 2 °C
Test Culture Number:	G4(l)a
Culture Age at Test Start:	7 days old
Cell Density in the Microplate Wells at the Start of the Test:	10,340.91 cells/ml

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032367 **Sample Name:** SP2 Discharge_Eff_20231024

Test Conditions

Date/Time Test Start:	October 27, 2023; 09:50	**T=0 Control pH:**	7.5
Date/Time Test End:	October 30, 2023; 08:30 – 10:45	**T=72 Control pH:**	7.5
Sample pH Before Dilution:	7.8	**pH Adjustment:**	None

Test Duration: 72 hours

Mean Test Temperature (±Standard Deviation): 25.1 (±0.1) °C

Pre-Aeration of Sample: None

Procedure for Sample Filtration: 50-ml subsample filtered through preconditioned 0.45-µm pore diameter membrane

Type and Source of Control/Dilution Water: Millipore

Type and Quantity of Chemicals Added to Control/Dilution Water: None

Metal Mining Effluent Nutrient Spike Used: Yes

Type and Quantity of Chemicals Added to Each Well as Nutrient Spike:	**Macronutrient**	**mg/l**	**Micronutrient**	**µg/l**
	$NaNO_3$	15.94	H_3BO_3	115.95
	$MgCl_2$ $6H_2O$	6.25	$MnCl_2$ $4H_2O$	259.76
	$CaCl_2$ $2H_2O$	2.76	$ZnCl_2$	2.05
	$MgSO_4$ $7H_2O$	9.19	$CoCl_2$ $6H_2O$	0.89
	K_2HPO_4	0.65	$CuCl_2$ $2H_2O$	0.008
	$NaHCO_3$	9.38	Na_2MoO_4 $2H_2O$	4.54
			$FeCl_3$ $6H_2O$	100
			Na_2EDTA $2H_2O$	46.9

***Raphidocelis subcapitata*72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032367 **Sample Name:** SP2 Discharge_Eff_20231024

Test Conditions - continued

Enumeration Technique: Neubauer Haemacytometer

Test Vessel: 96-Well U-bottomed Polystyrene Microplate

Concentration of Test Solutions: 90.91%; 30.30%; 10.10%; 3.37%; 1.12%; 0.374%; 0.125%; 0.042%; 0.014%; 0.005%; control

 # Replicates/Concentration: 4 reps started; 3 reps counted of test solutions
10 control reps started, 2 used for pH measurement

Design if Specialized Procedure: Not applicable

Method Deviations or Unusual Occurrences: None

Conditions for Test Validity

Algal cells in the controls increase by a factor of >16 times: Acceptable (32.8 times)

pH in controls did not vary by more than 1.5 units: Acceptable (0 units)

C V for cell yields is < 20% within the control wells: Acceptable (14.9)

No inhibitory trend detected across the control wells: Acceptable (no significant trend)

Test Results

Control 72-Hour Growth Data - Cell Yield[1] (cells/ml)									
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	CV[2]
322,159	314,659	332,159	422,159	294,659	414,659	302,159	307,159	338,722	14.9

1 Cell yield = measured algal cell concentration - initial algal cell concentration
2 CV = Coefficient of Variation = (100 x standard deviation / mean)

*Raphidocelis subcapitata**72-Hour Growth Inhibition Test

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032367 **Sample Name:** SP2 Discharge_Eff_20231024

Test Results - continued

Test Concentrations 72-Hour Growth Data - Cell Yield[1] (cells/ml)					
REP	Concentration				
	90.91%	**30.30%**	**10.10%**	**3.37%**	**1.12%**
1	454,659	849,659	909,659	604,659	477,159
2	444,659	964,659	732,159	537,159	424,659
3	577,159	777,159	717,159	427,159	509,659
Mean Cell Yield	492,159	863,826	786,326	522,992	470,492
Coefficient Variation[2]	15	11	14	17	9
REP	Concentration				
	0.374%	**0.125%**	**0.042%**	**0.014%**	**0.005%**
1	447,159	304,659			
2	599,659	349,659			
3	479,659	382,159			
Mean Cell Yield	508,826	345,492			
Coefficient Variation[2]	16	11			

1 Cell yield = measured algal cell concentration - initial algal cell concentration

2 Coefficient of Variation = (100 x standard deviation / mean)

Mean cell yield for concentrations with significant growth stimulation are shaded. Growth stimulation is determined by statistical comparison with control cell yield by pairwise comparison using Dunnett's Test.

Statistical Analysis

Statistic	Result[1]	Method of Calculation
IC25 (95% CI)[2] for Cell Yield	>90.91% Volume (Not applicable)	Linear Interpolation (ICPIN) No nonlinear regression models fit
Test for Trend in Controls	No trend	Mann-Kendall

1 - Results relate only to the sample tested.

2 - Empirical 95% Confidence Interval for the Bootstrap Estimate

***Raphidocelis subcapitata**72-Hour Growth Inhibition Test**

*(previously called *Pseudokirchneriella subcapitata*)

Sample #: 8730-0032367 **Sample Name:** SP2 Discharge_Eff_20231024

Method of Analysis

IC25 and Pairwise Comparison: Tidepool Scientific Software. ©2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v2.1.3.5.

Mann-Kendall: "Selenastrum Trend Test 2" Excel Program by B. Zadlijk

Weighting Techniques Applied to the Data: None

Outliers and Justification for Their Removal: None

Statistic Transformation of Data that Was Required: None

Reference Toxicant Results

Reference test performed under same experimental conditions as test: Yes

Test Method Deviation: None

Reference Chemical: Phenol P2316 **Date Test Initiated:** 13-Oct-2023

Method of Analysis: ICPIN-Linear interpolation **Algae Lot #:** G2(l)a

72-hour IC25 (95% Confidence Limits): 38.04 mg/L (31.27 mg/L; 44.85 mg/L)

Historic Geometric Mean IC25: 54.01 mg/L (25.13 mg/L; 116.06 mg/L)
(Historic Warning Limits) (± 2 Standard Deviations)

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Summary Sheet

Client ALS - Thunder Bay Sample Name SP2 Discharge EA - 20231024 Sample # 8730-0032367

Conditions for Test Validity

Cell increase for control is >16	(Acceptable)/Not acceptable	32.8 (times)
CV among controls ≤ 20	(Acceptable)/Not acceptable	14.9
Result of Mann-Kendall test for trend	(Acceptable)/Not acceptable	no trend

Test Organisms

Concentration of Inoculum

Algae and Nutrient spike 113 790 (cells/mL)

Used: (Yes)/No (Circle one)

Algae only — (cells/mL)

Used: Yes/No (Circle one)

Cell density in the microplate wellsat the start of the test 10340.91 (cells/mL)

Analysis Completed: Initials: EP Date: 17/11/23

Results Verified: Initials: AO Date: 28.11.23

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Test Material

Client Name/Location:	ALS – Thunder Bay	TY 2311042-001	
Sample #:	8730-00323367	Sample Name:	SP2 Discharge EFF – 20231024
Collection Method:	Other	Collected By:	AR/IA
Date/Time Collected:	24.10.23 0925	Arrival Temp.: (meter/probe)	14.4 °C (44)
Date/Time Received:	26.10.23 1140	Sample Description:	clear, light yellow
Collection Point Description:	Other	Sample Type: ☐ Effluent ☐ Chemical ☒ Other:	
Transportation:	Drv / Road	Storage:	4 ± 2

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Raphidocelis subcapitata*, U of W Clone # CPCC 37	IA
Source	Nautilus Plant Culture Unit (from CPCC), Test Culture # 6411a	IA
Culture Age at Start of Test	7 days old (must be 3 to 7 days old)	IA
Health Criteria	Any unusual appearance or treatment of known-age culture, before its use in test? Yes/No (Circle one)	IA
	Axenic culture? Yes/No (Circle one)	IA

Notes:

Test Conditions:

Date / Time Test Start: 27.10.23 9:50 Started By: IA		Date / Time Test End: 30.10.23 8:30 – 10:45 Finished By: IA	
Procedure for Sample Filtration: Through Preconditioned 0.45 µm membrane			
pH of raw sample (after filtration)* 7.8	pH adjustment: Y/N	pH of well D6 at T=0 h 7.5	pH of well D7 at T=72 h 7.5
Type of nutrient spike: (Circle one)	Regular (For references and non-mining test) NUT Lot # —		Metal mining NUT Lot # NUT 2304
Min max Temps for Days 0 to 3 (Mean Temp ± Standard Deviation)		25.1 ± 0.1 °C	

	Day 1		Day 2	
¼ plate rotation (Initial)	AM IA	PM IA	AM TC	PM MB
Lights ON (Initial)	AM IA	PM IA	AM TC	PM MB

* For reference test, record pH of top concentration before use for series dilutions.

Notes:

72-Hour Qualitative Observations:

Condensation:	NO	
Growth:	Yes	
Were there any other method variations or deviations from methods?	Yes/No	If yes, describe further:
Anything unusual about the test?	Yes/No	
Any problems encountered?	Yes/No	
Any remedial measures taken?	Yes/No	

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test

Sample #: 8730-0032367	Sample Name: SP2 Discharge E/A 20231024

Reference Data:

Reference Chemical Batch #	Phenol P2316	Date test started	13/10/23
Method of Analysis	ICP IN – linear interpolation	Algae Lot #	G 2(1)a
72-hour IC25 (95% C.I.)[3] mg/L	38.04 31.27; 44.85	Historic Geometric Mean IC25 (95% C.I.)[3] mg/L	54.01 25.13; 116.06

Test Data:

Statistic	Result[1]		Method of Calculation[2]
72-hour IC25 (95% C.I.)[3] % v/v For cell yield	>90.91% (N/A)		No nonlinear regression models would fit
72-hour IC25 (95% C.I.)[3] % v/v For cell yield If calculated without outliers			ICPIN – linear interpolation
Test for Outliers	No Outliers Present ✓		Grubbs' Test for Residual Outlier Initial E
	If outliers present, indicate Concentration/Rep:		
Test for Statistically Significant Growth Stimulation	No growth stimulation in test. Analysis not completed.		
	No statistically significant growth stimulation.		Williams' or Dunnett's Multiple Comparison Test Initial E
	Yes statistically significant growth stimulation at these concentrations: 90.91; 30.303; 10.101; 3.367; 1.122; 0.374		

1) Results relate only to the sample tested.
2) Tidepool Scientific Software © 2000-2022. Comprehensive Environmental Toxicity Information System – CETIS v. ~~1.9.6.7~~ 2.1.3.5
3) Empirical 95% Confidence Interval

Weighting techniques applied to the data? Yes/No (No circled)

Any outliers and justification for their removal? Yes/No (No circled)

Nautilus Environmental Company Inc.
Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
72-Hour Growth Inhibition Test
Inoculum Preparation

Sample Name: SP 2 Disch Eff Sample Number: 87300032367 Date Test Start: 27.10.23

Cell count	A 0.1 µl / 0.004 µl	B	A 0.1 µl / 0.004 µl	b	A 0.1 µl / 0.004 µl	B
1	32	30	21	22	12	13
2	31	30	23	23	10	10
3	38	44	24	25	10	10
4	33	33	23	23	13	13
5	33	28	—	—	—	—
Total cells	167	165	91	93	45	46
Cells/ µl *	8350	8250	227.5	232.5	112.5	115
Cells/ ml	8350 000	8250 000	227 500	232 500	112 500	115 000
Avg. cells/ ml	8300 000		230 000		113 750	
Time / Initials	9:25 / G		9:30 / G		9:45 / G	
Comments/ calculations	2200 000 cells / 8 300 000 cells/ml = 0.25 mL		ok		ok 112 in + 1/2 hs	

Cell count	0.1 µl / 0.004 µl		0.1 µl / 0.004 µl		0.1 µl / 0.004 µl	
1						
2						
3						
4						
5						
Total cells						
Cells/ µl *						
Cells/ ml						
Avg. cells/ ml						
Time / Initials						
Comments/ calculations						

* Cells/ul: count 4 outside large squares (0.1 ul), add, multiply by 2.5 OR count 5 inside small squares (0.004 ul), add, multiply by 50

Nautilus Environmental Company Inc.

Raphidocelis subcapitata (aka Psuedokirchneriella subcapitata)

72-Hour Growth Inhibition Test – Continued

72-Hour Quantitative Observations of Controls

Sample Name: SP2 Discharge Eff - 20231024 Sample Number: 8730-0032367 Date Test Start: 27.10.23

Cell count per 0.1 µl or 0.004 µl	Well # D2	Well # D3	Well # D4	Well # D5	Well # D8	Well # D9	Well # D10	Well # D11
1	34	31	36	49	31	52	28	23
2	29	36	28	30	25	28	30	31
3	35	32	40	44	41	47	31	31
4	35	31	33	50	25	43	36	42
5	—	—	—	—	—	—	—	—
Initials	EV	EV	EV	EV	EV	EV	EV	EV

I 113 750

Cell increase for controls = 32.8	Controls are invalid if cell increase is < 16
Coefficient of variation among controls = 14.9	Controls are invalid if coefficient of variation is > 20
Result of Mann-Kendall test for trend = no trend	Controls are invalid if there is a trend detected by the Mann-Kendall test

Mann-Kendall Trend Test for *Selenastrum capricornutum* Growth Inhibtion Test

EPS Method 1/RM/25
Version 1.1, Nov. 2000
Pollutech EnviroQuatics

Sample #: "8730-0032367"
Client #: "8730-003
Date tested: "27.10.23

Instructions:

Enter control data below cells labelled D2..D5 and D8..D11.
The test of significance is completed immediately following data entry.

D2	D3	D4	D5	D8	D9	D10	D11
322159	314659	332159	422159	294659	414659	302159	307159

Results:

There is no significant trend.

✓
CO 23.11.23

Notes:

The test is a two-sided alternative using a nominal alpha value of 0.05%.
Due to the discrete nature of the test statistic, the true alpha value is 0.062%.

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SP2 Discharge EH - 2023 1024 Sample Number: 8730037367 Date Test Start: 27.10.23

Theoretical Test Concentration: 100.00 % v/v					Actual Test Concentration: 90.91 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B2	Well # C2	Well # F2	Well #	Average Cell Yield (±Standard Deviation)
1	51	50	57		492159 (± 73782)
2	49	37	63		Coefficient of Variation of Cell Yield
3	42	47	55		15
4	44	48	60		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-45.299
Initials	EY	C	C		C

Theoretical Test Concentration: 33.33 % v/v					Actual Test Concentration: 30.303 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B3	Well # C3	Well # F3	Well #	Average Cell Yield (±Standard Deviation)
1	89	114	88		863826 (± 94549)
2	90	104	74		Coefficient of Variation of Cell Yield
3	84	88	72		11
4	81	84	81		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-155.025
Initials	C	C	C		C

Theoretical Test Concentration: 11.11 % v/v					Actual Test Concentration: 10.101 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B4	Well # C4	Well # F4	Well #	Average Cell Yield (±Standard Deviation)
1	91	67	68		786326 (± 107073)
2	85	69	72		Coefficient of Variation of Cell Yield
3	89	90	77		14
4	103	71	74		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-132.145
Initials	EY	C	C		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SP2 Discharge EH - 2023 1024 Sample Number: 8730 0037 367 Date Test Start: 27.10.23

Theoretical Test Concentration: 3.704 % v/v					Actual Test Concentration: 3.367 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B5	Well # C5	Well # F5	Well #	Average Cell Yield (±Standard Deviation)
1	62	49	48		522 992 (± 89 594)
2	59	55	36		Coefficient of Variation of Cell Yield
3	66	68	51		17
4	59	47	40		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-54.402
Initials	[illegible]	[illegible]	C		C

Theoretical Test Concentration: 1.235 % v/v					Actual Test Concentration: 1.122 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B6	Well # C6	Well # F6	Well #	Average Cell Yield (±Standard Deviation)
1	50	36	58		470 492 (± 42 890)
2	41	37	50		Coefficient of Variation of Cell Yield
3	56	43	41		9
4	48	58	59		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-38.902
Initials	[illegible]	[illegible]	C		C

Theoretical Test Concentration: 0.412 % v/v					Actual Test Concentration: 0.374 % v/v
Cell count per 0.1 µl or 0.004 µl	Well # B7	Well # C7	Well # F7	Well #	Average Cell Yield (±Standard Deviation)
1	42	76	49		508 826 (± 80 325)
2	44	56	35		Coefficient of Variation of Cell Yield
3	53	50	53		16
4	49	62	59		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-50.219
Initials	C	C	C		C

Raphidocelis subcapitata (aka Pseudokirchneriella subcapitata)
Growth Inhibition Test 72-Hour Quantitative Observations of Test Concentrations

Sample Name: SP2 Discharge EM - 2023 1024 Sample Number: 8730 2023 367 Date Test Start: 27.10.23

Theoretical Test Concentration: 0.137 %v/v					Actual Test Concentration: 0.125 0.12 %v/v
Cell count per 0.1 µl or 0.004 µl	Well # B8	Well # C8	Well # F8	Well #	Average Cell Yield (±Standard Deviation)
1	22	25	~~27~~ 35		345492 (± 38918)
2	30	35	~~30~~ 41		Coefficient of Variation of Cell Yield
3	44	41	~~44~~ 38		11
4	30	43	43		Average % Inhibition (-ve number = enhancement)
5	—	—	—		-1.999
Initials	C	C	C		C

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Theoretical Test Concentration:					Actual Test Concentration:
Cell count per 0.1 µl or 0.004 µl	Well #	Well #	Well #	Well #	Average Cell Yield (±Standard Deviation)
1					
2					Coefficient of Variation of Cell Yield
3					
4					Average % Inhibition (-ve number = enhancement)
5					
Initials					

Sample Name	SP2 Discharge Eff-2023 Sample #	8730-0032367	Date test start	27.10.23

Calculate initial algal cell concentration

Concentration of innocculum (cells/ml)	113750	Use last count algae/nutrient mixture or algae only	
Volume of algae addition (uL)	20	Algae/nutrient mixture = 20uL, algae only 10uL	
Cells added to each test well	2275	**Cell yield (must be >16 times in controls)**	
Cells/ml in well at T=0	10340.9091	=	32.755495

enter control data

	D2	D3	D4	D5	D8	D9	D10	D11
	34	31	36	49	31	52	28	23
	29.0	36.0	28.0	30.0	25.0	28.0	30.0	31.0
	35	32.0	40.0	44.0	41.0	47.0	31.0	31.0
	35.0	31.0	33.0	50.0	25.0	43.0	36.0	42.0
total cells	133	130	137	173	122	170	125	127
cells/ul	332.5	325	342.5	432.5	305	425	312.5	317.5
cells/ml	332500	325000	342500	432500	305000	425000	312500	317500
Cell yield = measured concentration - initial algal cell concentration								
	322159	314659	332159	422159	294659	414659	302159	307159

Mean cell yield for the control	= Rc	Standard deviation	coefficient of variation	
Rc	338722	SD 50567.0965	CV	14.928808 **Must be ≤20**

% inhibition summary

Concentrati	Average % inhibition
0.005	
0.014	
0.042	
0.125	-1.999
0.374	-50.219
1.122	-38.902
3.367	-54.402
10.101	-132.145
30.303	-155.025
90.910	-45.299

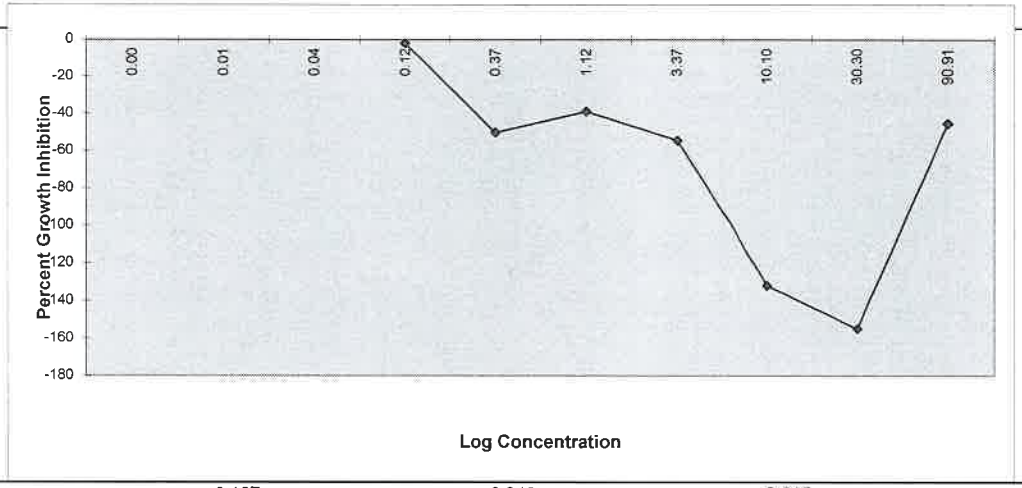
enter test data

nominal conc	100.000			33.333			11.111			3.704			1.235		
Conc.(%)	90.910			30.303			10.101			3.367			1.122		
	B2	C2	F2	B3	C3	F3	B4	C4	F4	B5	C5	F5	B6	C6	F6
	51	50	57	89	114	88	91	67	68	62	49	48	50	36	58
	49.0	37.0	63.0	90.0	104.0	74.0	85.0	69.0	72.0	59.0	55.0	36.0	41.0	37.0	50.0
	42.0	47.0	55.0	84.0	88.0	72.0	89.0	90.0	77.0	66.0	68.0	51.0	56.0	43.0	41.0
	44.0	48.0	60.0	81.0	84.0	81.0	103.0	71.0	74.0	59.0	47.0	40.0	48.0	58.0	59.0
total cells	186	182	235	344	390	315	368	297	291	246	219	175	195	174	208
cells/ul	465	455	587.5	860	975	787.5	920	742.5	727.5	615	547.5	437.5	487.5	435	520
cells/ml	465000	455000	587500	860000	975000	787500	920000	742500	727500	615000	547500	437500	487500	435000	520000
Cell yield = measured concentration - initial algal cell concentration															
	454659	444659	577159	849659	964659	777159	909659	732159	717159	604659	537159	427159	477159	424659	509659
Mean Yield	492159			863826			786326			522992			470492		
STD Yield	73782			94549			107073			89594			42890		
CV Yield	15			11			14			17			9		

Average % inhibition											
	for 90.910%:	-45.299	for 30.303%	-155.025	for 10.101%	-132.145	for 3.367%	-54.402	for 1.122%	-38.902	

Average % stimulation											
	for 90.910%:	45.299	for 30.303%	155.025	for 10.101%	132.145	for 3.367%	54.402	for 1.122%	38.902	

CO 23.11.23



0.412			0.137			0.046			0.015			0.005		
0.374			0.125			0.042			0.014			0.005		
B7	C7	F7	B8	C8	F8	B9	C9	F9	B10	C10	F10	B11	C11	F11
42	76	49	22	25	35									
44.0	56.0	35.0	30.0	35.0	41.0									
53.0	50.0	53.0	44.0	41.0	38.0									
44.0	62.0	59.0	30.0	43.0	43.0									
183	244	196	126	144	157	0	0	0	0	0	0	0	0	0
457.5	610	490	315	360	392.5	0	0	0	0	0	0	0	0	0
457500	610000	490000	315000	360000	392500	0	0	0	0	0	0	0	0	0
447159	599659	479659	304659	349659	382159	-10341	-10341	-10341	-10341	-10341	-10341	-10341	-10341	-10341
508826			345492			-10341			-10341			-10341		
80325			38918			0			0			0		
16			11			0			0			0		
for 0.374%	-50.219		for 0.125%	-1.999		for 0.042%	103.053		for 0.014%	103.053		for 0.005%	103.053	
for 0.374%	50.219		for 0.125%	1.999		for 0.042%	-103.053		for 0.014%	-103.053		for 0.005%	-103.053	

Certificate of Analysis

CHRONIC TOXICITY BIOASSAY REPORT
Lemna minor 7-Day Growth Inhibition Test

CLIENT:

ALS Environmental, 1081 Barton Street, Thunder Bay, ON P7B 5N3

TEST RESULTS:

Sample Name	Sample Number	Date Collected	Date Received	Date Tested	Frond Number 7-day IC25 % Volume[1] (95% Confidence Limits)	Dry weight 7-day IC25 % Volume[1] (95% Confidence Limits)	Method Deviations
SP2 Discharge_Eff_ 20231024	8730-0032367	October 24, 2023	October 26, 2023	October 26, 2023	>97% Volume (Not applicable)	>97% Volume (Not applicable)	No

1. Results relate only to the sample tested.
2. Highest concentration tested, based on test method

TEST PROTOCOLS:

Environment Canada, "Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*", Environmental Technology Centre, Ottawa, Ontario, Report EPS 1/RM/37, Second Edition, January 2007. (Nautilus Test Method LM-GI-R5.14)

REFERENCE/HEALTH DATA:

Reference test conducted under the same experimental conditions with same species, clone, and test medium as test: Yes
Test Method Deviations: None

Lemna minor

Date Reference Test Initiated:	13-Oct-2023	**Reference Chemical:**	KCl
Frond Increase IC25:	2.87 g/L	**95% Confidence Limits:**	2.41; 3.29 g/L
Historic Geometric Mean IC25:	2.29 g/L	**Historic Warning Limits (± 2 Standard Deviations):**	1.42; 3.68 g/L

TEST-SPECIFIC INFORMATION:

Type and Quantity of Chemicals Added to Control/Dilution Water and to Test Sample Before Start of Test:	**Substance**	**mg/l**	**Substance**	**mg/l**	**Substance**	**mg/l**
	$NaNO_3$	255	$CaCl_2\ 2H_2O$	44.1	H_3BO_3	1.86
	$NaHCO_3$	150	$MgCl_2\ 6H_2O$	121.7	Na_2MoO_4	0.0726
	K_2HPO_4	10.4	$FeCl_3\ 6H_2O$	1.6	$2H_2O$	0.00003
	KCl	10.1	$MgSO_4\ 7H_2O$	147	$ZnCl_2$	0.00001
					$CoCl_2\ 6H_2O$	0.00001
					$CuCl_2\ 2H_2O$	

Test Vessel Size, Shape, Material: 300-ml cylindrical glass tumblers
Design and Description if Specialized Procedure: None

TEST RESULTS APPROVED BY:

C. D'Andrea

Date: November 28, 2023

Carol D'Andrea
Laboratory Supervisor

Test Material

Client Name/Location: ALS- Thunder Bay	TX 231042-001
Sample #: 8730-00323 67	Sample Name: SPL Discharge_EFF_20231024
Collection Method: other	Collected By: N/A
Date/Time Collected: 24.10.23 8:25	Arrival Temperature (meter/probe): 14.4 °C (44)
Date/Time Received: 26/10/23 11:40	Sample Description: clear, light yellow
Collection Point Description: other	Sample Type: □ Effluent □ Chemical ☒ Other:
Transportation: Air / Road	Storage: N/A

Test Organisms

		Initial if Objective is Met
Species (clone #)	*Lemna minor* L. (8434)	C
Source:	Nautilus Plant Culture Unit (from CPCC, # 490)	C
Culture Age at Start of Test:	8 days old, acclimated 23 hours in fresh test solution (mAPHA)	C
Culture Medium:	Modified Hoagland's E+ medium, Lot # MH230T	C
Health Criteria:	Any unusual appearance or treatment of the test culture before use in test? Yes/(No)	C
	Axenic culture? (Yes)/No	C
	Health test fronds increase ≥ 8-fold in 7 days 3 fronds at start in each health test 33 in HT 1, 30 in HT 2, 32 in HT 3 at finish	C

Test Conditions and Procedures

Date / Time Test Start: 26.10.23 14:30 Started By: C	Date / Time Test End: 02.11.23 10:55 Finished By: C
Test Type: (Static (no renewal) or Static Renewal (circle one)	
Pre-Aeration of Sample: Time: 20 minutes, Rate: 100 bubbles / minute, Method: Filtered air is dispensed through airline tubing and a glass pipette	
Alagae Present: Yes / (No) (visual inspection)	If yes, was sample filtered though ~1µm fiber filter: Y / N
Type and Source of Control / Dilution Water: (Modified APHA (prepared with deionized municipal water) or Receiving water (filtered through ~ 0.2 µm, with additional APHA control) (circle one)	
Sample pH Before Dilution (pH metre/probe): 8.0 (14/94)	pH Adjustment: none
Test Volume and Depth: 160 mL / 4 cm	Number of Reps.: 4
Were there any other method variations or deviations from methods? Yes / (No)	If yes, describe further:
Anything unusual about the test? Yes / (No)	
Any problems encountered? Yes / (No)	
Any remedial measures taken? Yes / (No)	Randomization Template: C

Sample #: 8730-003 2367	Sample Name: SP2 Discharge - Eff - 20231024

Test Variables, Observations, and Measurements
Daily Temperatures of a representative High, Medium, Low, and Control Test Vessel

Temp. Range: 25±2°C	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	25	26	25	25	25	25	25	25
Low	25	26	26	26	25	26	26	26
Medium	25	26	26	26	25	26	26	26
High	25	26	26	26	25	26	26	26
Initials	TC	CG	TC	TC	TC	TC	TC	TC
meter/probe	52	52	52	44	44	44	44	44
Mean Test Temperature (average of 24h high / low temperatures):					25.3 ± 0.1 °C			

Measurements of pH in Representative High, Medium, Low, and Control Test Vessels at Test Start & End

Day 0	Control	Low	Medium	High	Initials	pH meter/probe
	8.2	8.2	8.2	8.0	TC	14/94
Day 7	Control	Low	Medium	High	Initials	pH meter/probe
	8.4	10.1	10.4	10.3	CG	13/93

Conductivity Reading of Representative Test Concentrations and Control Test Vessel at Test Start – Corrected To 25°C. (For Reference Test Only)

Day 0	Control	g/L	g/L	g/L	g/L	g/L	Initials	Conductivity meter/probe
(µmohs)								

Measurement of Light at Least Once During the Test

Photoperiod: Continuous Lumination					Date (day of Test): 31.10.23 (5)
Acceptable Light Fluence Range: 4000 to 5600 lux					
Light Measurement: 5 points (light metre #): 12					Initials: TC
4920	4760	5500	5530	5390	Mean Light Measurement: 5220
±15% Variation of Mean: 4437 - 6003					(Acceptable) / Not Acceptable (circle one)

Any changes in appearance of test solution during preparation or during the test: (Yes) No
If yes, describe further: Algae

Reference Data

Reference Date	(Frond Increase) or Dry Weights (circle one)			
13/10/23	IC25 (g/L)	95% C.I (g/L)	Historic IC25 (g/L)	Historic 95% C.I (g/L)
	2.87	2.41; 3.29	2.29	1.42; 3.68

Sample #: 8730-0032367	Sample Name: SP2 Discharge EFF - 20231024

Validity Criterion:

The mean number of fronds in the controls must have increased to ≥8-times original number.	Number of Fronds in Control Vessels (do not subtract 6 starting fronds)				Mean Number of Fronds (Must be ≥48 for test to be valid)
	A 76	B 68	C 60	D 72	69.0

Test Results Summary

Number and Appearance of Fronds in Each Vessel at Day 0: 2 plants, 3 fronds each in each vessel, dark green and healthy						Initials	
						U	
Number and Appearance of Fronds in Each Vessel at Day 7: See Observation Sheets							
Mean (SD) of increase in frond number in control at test end, CV : 63.0 (6.8) 10.8							
Mean % Stimulation of Fronds Number in Each Treatment:							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-0.40	4.37	7.54	-1.19	0.40	-23.41	-23.81
Mean % Stimulation for Dry Weight of Fronds in Each Treatment							
Control % v/v g/L	0.097	0.29	0.97	3.1	9.7	31	97
Mean % Stimulation	-3.16	3.35	-1.05	0.96	6.95	-11.52	-9.20

SD = Standard Deviation, CV = Coefficient of Variation
*= concentrations with significant stimulation are indicated by *. Growth stimulation is determined by statistical comparison with control growth by pairwise comparison using Dunnett's or Williams' Multiple Comparison Test.

Stimulation calculation completed: (Yes) Not applicable (no stimulation) (Circle one)

Test Endpoints and Calculations: Fronds / Weight

Statistic	Results[1]	Method of Calculation[2]
Frond Increase		
IC25 (95% C.I.)[3]	> 97 % (N/A)	ICPIN - Linear interpolation
IC25 (95% C.I.)[3] If calculated without outlier[4]	—	—
Dry Weights		
IC25 (95% C.I.)[3]	> 97 % (N/A)	ICPIN - Linear interpolation
IC25 (95% C.I.)[3] If calculated without outlier[4]	—	—

1) Results relate only to the sample tested.
2) Tidepool Scientific Software. ©2000-2019 Comprehensive Environmental Toxicity Information System CETISv ~~1.9.6.7~~ 2.1.3.5
3) Empirical 95% Confidence Interval
4) Outliers detected using Grubbs' Test for Residual Outlier

Weighting techniques applied to the data? Yes / (No)

Any outliers and justification for their removal? Yes / (No)

Lemna minor Da[illegible] Observations

Client:	ALS Thunder Bay			**Sample number:**	8730-00323 67		**Date Started:**	26.10.23	
Site:	SP2 Discharge Eff 2023/1024						**Date Ended:**	02.11.23	
Concentration:	Control		**Observations By:**	B	**Concentration:**	0.047 % v/v		**Observations By:**	B

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	76	68	60	72	**Number of**	76	63	71	65
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	X	X	X	X	**Abnormally sized fronds**	✓ smaller	✓ smaller	✓ smaller	✓ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	✓	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations					**Other Observations**	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO N/A Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Da[illegible] Observations

Client: ALS Thunder Bay	**Sample number:** 8730-00323 67	**Date Started:** 26.10.23	
Site: SP2 Discharge Eff 2023/1024		**Date Ended:** 02.11.23	
Concentration: 0.29 T. v/v	**Observations By:** [illegible]	**Concentration:** 0.97 T. v/v	**Observations By:** [illegible]

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	78	71	75	63	**Number of**	81	63	69	82
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	✓ smaller	✓ smaller	✓ smaller	✓ smaller	**Abnormally sized fronds**	✓ smaller	✓ smaller	✓ smaller	✓ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations	algae	→	→	→	**Other Observations**	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / NO Weights: YES / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Da[illegible] Observations

Client: ALS Thunder Bay	Sample number: 8730-00323 67	Date Started: 26.10.23	
Site: SP2 Discharge EM 2023/1024		Date Ended: 02.11.23	
Concentration: 3.1 % v/v	Observations By: E	Concentration: 9.7 % v/v	Observations By: E

Observations	Rep 1	Rep 2	Rep 3	Rep 4	Observations	Rep 1	Rep 2	Rep 3	Rep 4
Number of	71	74	60	68	Number of	65	73	69	70
Chlorosis (loss of pigment)	X	X	X	X	Chlorosis (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X	Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	X
Yellow fronds	X	X	X	X	Yellow fronds	X	X	X	X
Abnormally sized fronds	✓ Smaller	✓ Smaller	✓ Smaller	✓ Smaller	Abnormally sized fronds	✓ Smaller	✓ Smaller	✓ Smaller	✓ Smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	Gibbosity (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	Colony Destruction (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	Root Destruction	X	X	X	X
Loss of Buoyancy	X	X	X	X	Loss of Buoyancy	X	X	X	X
Other Observations	algae	→	→	→	Other Observations	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: (YES) / NO					Growth Stimulation (Hormesis) at this concentration? Fronds: (YES) / NO Weights: (YES) / NO				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

Y:\Masters\MASTERS BINDER\14. Lemna minor\Lemna minor Day 7 Observations Feb 2018 R1.3.doc

Lemna minor Da[illegible] Observations

Client: ALS Thunder Bay			**Sample number:** 8730-00323 67		**Date Started:** 26.10.23				
Site: SP2 Discharge Eff 2023/1024					**Date Ended:** 02.11.23				
Concentration: 31%			**Observations By:** [illegible]		**Concentration:** 97%			**Observations By:** [illegible]	
Observations	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Observations**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
Number of	56	52	59	50	**Number of**	54	54	55	53
Chlorosis (loss of pigment)	X	X	X	X	**Chlorosis** (loss of pigment)	X	X	X	X
Necrosis (localized dead tissue on fronds, which appears brown or white)	X	X	X	✓	**Necrosis** (localized dead tissue on fronds, which appears brown or white)	X	✓	✓	✓
Yellow fronds	X	X	X	X	**Yellow fronds**	X	X	X	X
Abnormally sized fronds	√√ smaller	√√ smaller	√√ smaller	√√ smaller	**Abnormally sized fronds**	√√ smaller	√√ smaller	√√ smaller	√√ smaller
Gibbosity (humped or swollen appearance)	X	X	X	X	**Gibbosity** (humped or swollen appearance)	X	X	X	X
Colony Destruction (single fronds)	X	X	X	X	**Colony Destruction** (single fronds)	X	X	X	X
Root Destruction	X	X	X	X	**Root Destruction**	X	X	X	X
Loss of Buoyancy	X	X	X	X	**Loss of Buoyancy**	X	X	X	X
Other Observations	algae	→	→	→	**Other Observations**	algae	→	→	→
Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)					Growth Stimulation (Hormesis) at this concentration? Fronds: YES / (NO) Weights: YES / (NO)				

LEGEND: X-not present √- affects < 25% of plants √√- affects 25-50% of plants √√√ - affects > 50% of plants

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Lemna minor Weights

Client	Site	Sample number
ALS – TB	SP2 Discharge	8730-0032367

In Oven Date/Time/ °C:	Out Oven Date/Time/°C:
02.11.23 11:20 63.6	09.11.23 11:20 63.6

Conc.	Rep	Frond Increase	Mean Increase (SD)	Final Pan Weight (g)	Initial Pan Weight (g)	Weight (mg)	Mean Weight (mg) (SD)
Control	A	70	63.0 (6.8)	0.83957	0.83040	9.17	7.84 (1.2)
	B	62		0.856115	0.85338	7.77	
	C	54		0.85789	0.845155	6.34	
	D	66		0.85996	0.85189	8.07	
0.097	A	70	62.8 (5.9)	0.84975	0.84177	7.98	7.59 (0.7)
	B	57		0.85718	0.84919	7.99	
	C	65		0.85508	0.84717	7.91	
	D	59		0.85200	0.84552	6.48	
0.29	A	72	65.8 (6.5)	0.86192	0.85222	9.70	8.10 (1.3)
	B	65		0.86102	0.85327	7.75	
	C	69		0.85852	0.85008	8.44	
	D	57		0.84234	0.83583	6.51	
0.97	A	75	67.8 (9.3)	0.85215	0.84292	9.23	7.76 (1.2)
	B	57		0.85758	0.85051	7.07	
	C	63		0.857700	0.85048	6.52	
	D	76		0.856085	0.85265	8.20	
3.1	A	65	62.3 (6.0)	0.86062	0.85284	7.78	7.91 (1.8)
	B	68		0.86221	0.85178	10.43	
	C	54		0.85132	0.84517	6.15	
	D	62		0.84537	0.83808	7.29	
9.7	A	59	63.3 (3.3)	0.85049	0.84241	8.08	8.38 (0.6)
	B	67		0.84894	0.83966	9.28	
	C	63		0.845119	0.84322	7.97	
	D	64		0.84920	0.84100	8.20	
31	A	50	48.3 (4.0)	0.84958	0.84226	7.32	6.94 (1.3)
	B	46		0.85577	0.84794	7.83	
	C	53		0.85175	0.84417	7.58	
	D	44		0.85437	0.84936	5.01	
97	A	48	48.0 (0.8)	0.84828	0.84086	7.42	7.13 (0.8)
	B	48		0.86112	0.85309	8.03	
	C	49		0.85888	0.85209	6.79	
	D	47		0.85188	0.84559	6.29	
Initials		EB	EB	EB	EB	EB	EB

Notes:

Sample name SP2 Discharge Eff Date started 26.10.23 sample # 8730-0032367

Number of fronds per rep at test start

2 plants, 3 fronds each = 6

Validity Criterion: Average of Fronds in Controls

69.0 (must be ≥48)

Frond Data

Conc (real % v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
	76	76	78	81	71	65	56	54
	68	63	71	63	74	73	52	54
	60	71	75	69	60	69	59	55
	72	65	63	82	68	70	50	53
Total Fronds	276	275	287	295	273	277	217	216
Increase in Frond Number = Total # Fronds - 6 Starting Fronds								
	70	70	72	75	65	59	50	48
	62	57	65	57	68	67	46	48
	54	65	69	63	54	63	53	49
	66	59	57	76	62	64	44	47
Total Increase	252	251	263	271	249	253	193	192
Mean Increase	63.0	62.8	65.8	67.8	62.3	63.3	48.3	48.0
SD Increase	6.8	5.9	6.5	9.3	6.0	3.3	4.0	0.8
CV Increase	10.8	9.4	9.9	13.7	9.7	5.2	8.4	1.7
% Stimulation		**-0.40**	**4.37**	**7.54**	**-1.19**	**0.40**	**-23.41**	**-23.81**

Weight data

Conc (real %v/v)	Control 0	0.097	0.29	0.97	3.1	9.7	31	97
Final Weight (g)	0.83957	0.84975	0.86192	0.85215	0.86062	0.85049	0.84958	0.84828
Pan + Plant	0.86115	0.85718	0.86102	0.85758	0.86221	0.84894	0.85577	0.86112
	0.85789	0.85508	0.85852	0.85700	0.85132	0.85119	0.85175	0.85888
	0.85996	0.85200	0.84234	0.86085	0.84537	0.84920	0.85437	0.85188
Initial Weight (g)	0.83040	0.84177	0.85222	0.84292	0.85284	0.84241	0.84226	0.84086
Pan Only	0.85338	0.84919	0.85327	0.85051	0.85178	0.83966	0.84794	0.85309
	0.85155	0.84717	0.85008	0.85048	0.84517	0.84322	0.84417	0.85209
	0.85189	0.84552	0.83583	0.85265	0.83808	0.84100	0.84936	0.84559
Plant Only (mg)	9.17	7.98	9.70	9.23	7.78	8.08	7.32	7.42
	7.77	7.99	7.75	7.07	10.43	9.28	7.83	8.03
	6.34	7.91	8.44	6.52	6.15	7.97	7.58	6.79
	8.07	6.48	6.51	8.20	7.29	8.20	5.01	6.29
Mean Dry Weight	7.837	7.590	8.100	7.755	7.912	8.383	6.935	7.132
SD Dry Weight	1.2	0.7	1.3	1.2	1.8	0.6	1.3	0.8
CV Dry Weight	14.9	9.8	16.4	15.6	22.9	7.2	18.7	10.6
% Stimulation		**-3.16**	**3.35**	**-1.05**	**0.96**	**6.95**	**-11.52**	**-9.00**

For Data Transfer to CETIS

	# fronds	total mass	tare
0	1	70	0.83957	0.83040
	2	62	0.86115	0.85338
	3	54	0.85789	0.85155
	4	66	0.85996	0.85189
0.1	1	70	0.84975	0.84177
	2	57	0.85718	0.84919
	3	65	0.85508	0.84717
	4	59	0.85200	0.84552
0.3	1	72	0.86192	0.85222
	2	65	0.86102	0.85327
	3	69	0.85852	0.85008
	4	57	0.84234	0.83583
1	1	75	0.85215	0.84292
	2	57	0.85758	0.85051
	3	63	0.85700	0.85048
	4	76	0.86085	0.85265
3.1	1	65	0.86062	0.85284
	2	68	0.86221	0.85178
	3	54	0.85132	0.84517
	4	62	0.84537	0.83808
9.7	1	59	0.85049	0.84241
	2	67	0.84894	0.83966
	3	63	0.85119	0.84322
	4	64	0.84920	0.84100
31	1	50	0.84958	0.84226
	2	46	0.85577	0.84794
	3	53	0.85175	0.84417
	4	44	0.85437	0.84936
97	1	48	0.84828	0.84086
	2	48	0.86112	0.85309
	3	49	0.85888	0.85209
	4	47	0.85188	0.84559

OO 23.11.23

Project Name: Rainy River
Location: Chapple
Project Number:
Project Manager:
PO Number:
Project:
Turn Around Time (days): 10 Business Days
Shipping Company:
Shipping Date: 10/24/2023 8:26:00 AM
COC Number: ALS-

Containers
Filtered
Preservatives

Environmental Division
Thunder Bay
Work Order Reference
TY2311042

Telephone : +1 807 623 6463

Sample Code	Field Dissolved Oxygen (mg/L)	Field pH (pH Units)	Field Temp (°C)	Date and Time	Matrix	NG-ODM-P-TB	NG-ST-P-TB							Number of Containers	Comments
						Discharge Monitoring	Sublethal Toxicity Pails								
						y	N								
SP2 DISCHARGE_EFF_20231024	6.84	8.04	10.51	2023-10-24 8:25	EFF	x	x							19	

Signature	Data/Time
Shipped by	2023-10-24 8:26
Received by	

MM 25 Oct 2023 @ 903 6.7°C

(1) coolers 9.9
Crozier 9.6
ice packs 10.0

Shipping Details
Method of Shipment: Courier
On Ice: yes / no
Shipped: Air/Ground
Lab Name: ALS Thunder Bay
Lab Phone:

ATTN
TY2311042

Special Instructions:

Email Invoice to: rainyriver.accounts1@newgold.com Email Report to: rainyriver.labresults@newgold.com

Intake and Login Verification Form

SAMPLE INTAKE		
Priority/Emergency Service Requested	YES	~~NO~~
Time Sensitive Hold Time	YES	~~NO~~
Client:	New Gold	

SAMPLE RECEIPT INFORMATION			
Mode of Delivery:	~~Courier~~	Drop Off	
Courier	Couriers		
Waybill Number	—		
Temperature	6.7 9.6 9.9 10.0	Cooler Count	1
Cooling Method	None	Ice	~~Ice Packs~~

SAMPLE MATRIX/BOTTLE INFORMATION					
Matrix:	~~Water~~	Soil	Air	Biota	Other
DW Schedule 24 Bottles Correct?				~~Yes~~	No
DW Metals pH Check <2				~~Yes~~	~~No~~
Regulation Circled, Works # present			~~Yes~~	~~No - Reject?~~	
# of Bottles: ~~16~~ 11	Sample Count			1	
Green/white	1WG 2 BOD				
Purple/white	3 Pres. nuts.				
Warm red/white	3 Pres. met. 1x1L Pres.				
Yellow/black	2 Hg vials				
Light blue/white	1 micro				
Orange/black					

Others (detail)
2 x Green/white sodium Hydroxide
1v CN
3x Tox Pail

Comments on Samples and Bottles:
✓

Samples Requiring Preservation or Filtering: ✓

Layout Staff Initials Date and Time of Layout	MM 23Oct23 918

ACCOUNT INFO VERIFICATION		
Priority/Emergency Service Requested	YES	~~NO~~
Confirmed all as accurate as per COC, Sample Remarks or PM		
Client ✓	Work Contact ✓	Quote ✓

RECEIPT DETAIL			
Project ✓	PO ✓	Site/LSD ✓	
Overall Description Entered		Yes	~~NA~~
Received date/time as per COC		✓	
Recipients match CoC or Sample Remarks		~~Yes~~	No
Billing Instruction added to remarks		~~Yes~~	NA
Sample Remarks/Specification Doc checked		✓	
Submission Issues communicated		Yes	~~NA~~
Sample Info communicated via Remarks		Yes	~~NA~~

VERIFICATION CHECKLIST		
Plannned Event Submission	~~Yes~~	No
Sample Name entered as per CoC	✓	
Sampling Date and time entered as per CoC	✓	
Containers selected in layout order	✓	
Sales items entered from QUOTE ONLY (and/or verified as correct)	✓	
Field Data/EC298A removed if not on COC	Yes	~~NA~~
Bottle Allocation Verified	✓	
Guideline added or auto-allocated	✓	
Due dates updated	✓	

VALIDATION		
Validation errors resolved?	~~Yes~~	No
Internal Sublet CoC created	~~Yes~~	NA

Login Comments:
✓

Login Staff Initials:	✓

CERTIFICATE OF ANALYSIS

Work Order	: **TY2311633**	Page	: 1 of 3
Client	: **New Gold Inc. (Rainy River)**	Laboratory	: ALS Environmental - Thunder Bay
Contact	: Garnet.Cornell@newgold.com Garnet Cornell	Account Manager	: Christine Paradis
Address	: 24 Marr Rd. Barwick ON Canada P0W 1A0	Address	: 1081 Barton Street Thunder Bay ON Canada P7B 5N3
Telephone	: 807 234 8170	Telephone	: +1 807 623 6463
Project	: Sublethal Toxicity	Date Samples Received	: 07-Nov-2023 10:05
PO	: 4700002620	Date Analysis Commenced	: 07-Nov-2023
C-O-C number	: ----	Issue Date	: 02-Jan-2024 16:00
Sampler	: ----		
Site	: ----		
Quote number	: New Gold Rainy River Project - Picka Project		
No. of samples received	: 2		
No. of samples analysed	: 2		

This report supersedes any previous report(s) with this reference. Results apply to the sample(s) as submitted. This document shall not be reproduced, except in full.

This Certificate of Analysis contains the following information:

- General Comments
- Analytical Results

Additional information pertinent to this report will be found in the following separate attachments: Quality Control Report, QC Interpretive report to assist with Quality Review and Sample Receipt Notification (SRN).

Signatories

This document has been electronically signed by the authorized signatories below. Electronic signing is conducted in accordance with US FDA 21 CFR Part 11.

Signatories	*Position*	*Laboratory Department*
Cassandra Grzelewski	Account Manager	External Subcontracting, Calgary, Alberta
Cassandra Grzelewski	Team Leader - Inorganics	Administration, Thunder Bay, Ontario

General Comments

The analytical methods used by ALS are developed using internationally recognized reference methods (where available), such as those published by US EPA, APHA Standard Methods, ASTM, ISO, Environment Canada, BC MOE, and Ontario MOE. Refer to the ALS Quality Control Interpretive report (QCI) for applicable references and methodology summaries. Reference methods may incorporate modifications to improve performance.

Where a reported less than (<) result is higher than the LOR, this may be due to primary sample extract/digestate dilution and/or insufficient sample for analysis.

Where the LOR of a reported result differs from standard LOR, this may be due to high moisture content, insufficient sample (reduced weight employed) or matrix interference.

Please refer to Quality Control Interpretive report (QCI) for information regarding Holding Time compliance.

Key : CAS Number: Chemical Abstracts Services number is a unique identifier assigned to discrete substances

LOR: Limit of Reporting (detection limit).

Unit	*Description*
-	no units
°C	degrees celsius
mg	milligrams
mg/L	milligrams per litre
pH units	pH units

<: less than.

>: greater than.

Surrogate: An analyte that is similar in behavior to target analyte(s), but that does not occur naturally in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery.

Test results reported relate only to the samples as received by the laboratory.

UNLESS OTHERWISE STATED on SRN or QCI Report, ALL SAMPLES WERE RECEIVED IN ACCEPTABLE CONDITION.

Analytical Results

Sub-Matrix: **Water (MISA)**
(Matrix: **Water**)

Analyte	CAS Number	Method/Lab	LOR	Unit	Client sample ID	EDL1_EFF_20231106EFF	EDL2_EFF_20231106EFF	----	----	----
					Client sampling date / time	06-Nov-2023 07:55	06-Nov-2023 09:10	----	----	----
						TY2311633-001	TY2311633-002	--------	--------	--------
						Result	Result	----	----	----
Field Tests										
Oxygen, dissolved, field	----	EF001/TY	0.01	mg/L		8.22	9.48	----	----	----
pH, field	----	EF001/TY	0.10	pH units		7.53	7.66	----	----	----
Temperature, field	----	EF001/TY	0.10	°C		4.15	5.28	----	----	----
Bioassays										
Ceriodaphnia dubia, LC50	----	CER-LC50-7d/2F	-	-		----	see attached	----	----	----
Fathead minnow, biomass	n/a	FAT-LC50-7d/2F	-	-		See attached	See attached	----	----	----
Fathead minnow, survival	n/a	FAT-LC50-7d/2F	-	-		See attached	See attached	----	----	----
Green algae, growth	n/a	ALG-SCR-72h/2F	-	-		see attached	see attached	----	----	----
Lemna minor, dry weight	n/a	LEM-SCR-7d/2F	-	mg		see attached	see attached	----	----	----
Ceriodaphnia dubia ICp	n/a	CER-LC50-7d/2F	-	-		----	see attached	----	----	----
Lemna minor, frond count	n/a	LEM-SCR-7d/2F	-	-		see attached	see attached	----	----	----

Please refer to the General Comments section for an explanation of any result qualifiers detected.

Please refer to the Accreditation section for an explanation of analyte accreditations.

Toxicity Test Results

TY2311633-001 EDL1_EFF_20231106
and
TY2311633-002 EDL2_EFF_20231106

Final Report

December 22, 2023

Submitted to: **ALS Environmental – Thunder Bay**
Thunder Bay, Ontario

10823 27 Street SE, Calgary, AB T2Z 3V9

TABLE OF CONTENTS

List of Tables

List of Appendices

SIGNATURE PAGE

Report By:
Aishah Iqbal, BSc
Biologist

Reviewed By:
Jacklyn Poole, BSc
Laboratory Manager

This report has been prepared by Nautilus Environmental Company Inc. based on data and/or samples provided by our client and the results of this study are for their sole benefit. Any reliance on the data by a third party is at the sole and exclusive risk of that party. The results presented here relate only to the samples tested.

SUMMARY

Sample and Test Type Information

Sample ID (Internal ID)	TY2311633-001 EDL1_EFF_20231106 (2324-0709-01)	TY2311633-002 EDL2_EFF_20231106 (2324-0709-02)
Sample collection date	November 6, 2023	November 6, 2023
Sample receipt date	November 8 and 9, 2023	November 8 and 9, 2023
Sample receipt temperature	11.9/15.8°C	12.2/15.7°C
Test types	72-h *Pseudokirchneriella subcapitata* growth inhibition 5 – 8 day *Ceriodaphnia dubia* survival and reproduction 7-d *Pimephales promelas* survival and biomass 7-d *Lemna minor* growth inhibition	

Summary of Results

	% v/v (95% CL)	
Endpoint	**TY2311633-001 EDL1_EFF_20231106**	**TY2311633-002 EDL2_EFF_20231106**
P. subcapitata		
Cell Yield IC25	>91	>91
C. dubia		
Survival LC50	-	>100
Reproduction IC25	-	2.1 (<1.6 - NA)
P. promelas		
Survival LC50	>100	>100
Biomass IC25	>100	>100
L. minor		
Frond Number IC25	7.2 (3.8-12.7)	6.9 (3.3 - 13.1)
Dry Weight IC25	>97	>97

LC = Lethal Concentration, IC = Inhibition Concentration, CL = Confidence Limit, NA = Not Available

1.0 INTRODUCTION

Nautilus Environmental Company Inc. conducted toxicity tests for ALS Environmental – Thunder Bay. Samples TY2311633-001 EDL1_EFF_20231106 and TY2311633-002 EDL2_EFF_20231106 were collected on November 6, 2023, and delivered to the Nautilus Environmental laboratory in Calgary, AB on November 8, 2023, and November 9, 2023. The samples were transported in six 10-L plastic containers and were received at a temperature of 11.9 and 12.2°C, respectively. Testing was initiated on November 8, and 9, 2023, for all species. Due to a technical error, the *C. dubia* test for sample TY2311633-001 EDL1_EFF_20231106 was deemed invalid and the client was notified. The samples were stored in the dark at 4 ± 2°C prior to testing. The following toxicity tests were performed on the samples:

- 72-h *Pseudokirchneriella subcapitata* growth inhibition
- *Ceriodaphnia dubia* survival and reproduction
- 7-d *Pimephales promelas* survival and biomass
- 7-d *Lemna minor* growth inhibition

This report describes the results of these toxicity tests. Copies of raw laboratory data sheets and statistical analyses for each test species are provided in Appendices A to D. The chain-of-custody form is provided in Appendix E.

2.0 METHODS

Methods for the toxicity tests are summarized in Tables 1 to 4. Testing was conducted according to procedures described by the Environment Canada protocols (2007a, 2007b, 2011, 2007c). Statistical analyses for all tests were performed using CETIS (Tidepool Scientific Software, version 2.1.5.5).

Table 1. Summary of test conditions: *Pseudokirchneriella subcapitata* growth inhibition test.

Test species	*Pseudokirchneriella subcapitata*, strain UTCC# 37
Organism source	In-house axenic culture, obtained from Canadian Phycological Culture Center
Organism age	3-to 7-day old culture in logarithmic growth phase
Test type	Static
Test duration	72 hours
Test vessel	Microplate
Test volume	220 µL
Test solution depth	11 mm
Test concentrations	10 concentrations, at least 6 enumerated, plus laboratory control
Test replicates	5 per treatment, at least 3 enumerated; 8 for control
Number of organisms	10,000 +/- 1000 cells/mL
Control/dilution water	85% deionized water; 15% City of Calgary dechlorinated tap water supplemented with nutrients
Test solution renewal	None
Test temperature	24 ± 2°C
Feeding	None
Light intensity	3600 to 4400 lux
Photoperiod	24 hours light
Aeration	None
Test measurements	pH of 91% and control at test initiation and test completion; light levels and temperature measured daily
Test protocol	Environment Canada (2007), EPS 1/RM/25
Statistical software	CETIS version 2.1.5.5
Test endpoints	Algal cell growth inhibition
Test acceptability criteria for controls	>16-fold increase in number of algal cells; CV ≤ 20%; no trend when analyzed using Mann-Kendall test
Reference toxicant	Zinc (added as $ZnSO_4$)

Table 2. Summary of test conditions: *Ceriodaphnia dubia* survival and reproduction test.

Test species	*Ceriodaphnia dubia*
Organism source	In-house culture
Organism age	<24 hour old neonates, produced within a 12-hour window
Test type	Static-renewal
Test duration	5 – 8 days
Test vessel	16 x 135 mm glass test tube
Test volume	15 mL
Test solution depth	10 cm
Test concentrations	Seven concentrations, plus laboratory control
Test replicates	10 per treatment
Number of organisms	1 per replicate
Control/dilution water	20% Perrier water and 80% deionized water supplemented with vitamin B12 (2µg/L) and Na_2SeO_4 (5µg Se/L)
Test solution renewal	Daily (100% renewal)
Test temperature	25 ± 1°C
Feeding	Daily with *Pseudokirchneriella subcapitata* and YCT
Light intensity	100 to 600 lux at water surface
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test measurements	pH, conductivity, dissolved oxygen and temperature measured daily; evaluated for survival and reproduction daily
Test protocol	Environment Canada (2007), EPS 1/RM/21
Statistical software	CETIS version 2.1.5.5
Test endpoints	Survival and reproduction
Test acceptability criteria for controls	≥80% survival; ≥15 young per surviving control producing three broods; ≥60% of controls producing three or more broods, no ephippia present
Reference toxicant	Sodium chloride (NaCl)

Table 3. Summary of test conditions: fathead minnow *(Pimephales promelas)* survival and growth test.

Test species	*Pimephales promelas*
Organism source	Aquatox Inc., Hot Springs, Arkansas
Organism age	<24 hours post hatch
Test type	Static-renewal
Test duration	7 days
Test vessel	385 mL plastic containers
Test volume	250 mL
Test solution depth	6.5 cm
Test concentrations	Seven concentrations, plus laboratory control
Test replicates	4 per treatment
Number of organisms	10 per replicate
Control/dilution water	De-chlorinated City of Calgary tap water amended with 4 mg/L KCl
Test solution renewal	Daily (80% renewal)
Test temperature	25 ± 1°C
Feeding	Twice each day with approximately 1500-2250 newly hatched brine shrimp *(Artemia nauplii)* per 10 fish.
Light intensity	100 to 500 lux
Photoperiod	16 hours light / 8 hours dark
Aeration	None
Test measurements	pH, conductivity, dissolved oxygen and temperature were measured daily; evaluated for survival daily
Test protocol	Environment Canada (2011), EPS 1/RM/22
Statistical software	CETIS version 2.1.5.5
Test endpoints	Survival and biomass
Test acceptability criteria for controls	≥80% survival, ≥0.25 mg mean dry weight
Reference toxicant	Sodium chloride (NaCl)

Table 4. Summary of test conditions: *Lemna minor* growth inhibition test.

Test species	*Lemna minor*, UTCC 492, clone 7730
Organism source	In-house axenic culture, ≥ 3 weeks in age
Culturing medium	Modified Hoagland's E+ medium
Organism age	7-to 10-day old culture
Test type	Static
Test duration	7 days
Test vessel	200- mL polyethylene plastic container
Test volume	150 mL
Test solution depth	4 cm
Test concentrations	Seven concentrations, plus laboratory control
Test replicates	4 per treatment
Number of organisms	Two 3-frond plants per replicate
Control/dilution water	Modified APHA media prepared with deionized reverse osmosis water, 255 mg/L $NaNO_3$, 150 mg/L $NaHCO_3$, 10.4 mg/L K_2HPO_4, 10.1 mg/L KCl, 44.1 mg/L $CaCl_2{\cdot}2H_2O$, 121.7 mg/L $MgCl_2{\cdot}6H_2O$, 4.149 mg/L $MnCl_2{\cdot}4H_2O$, 1.6 mg/L $FeCl_3{\cdot}6H_2O$, 147 mg/L $MgSO_4{\cdot}7H_2O$, 1.86 mg/L H_3BO_3, 0.0726 mg/L $Na_2MoO_4{\cdot}2H_2O$, 0.0327 mg/L $ZnCl_2$, 0.014 mg/L $CoCl_2{\cdot}6H2O$, 0.00015 mg/L $CuCl_2{\cdot}2H_2O$
Nutrient spike	255 mg/L $NaNO_3$, 150 mg/L $NaHCO_3$, 10.4 mg/L K_2HPO_4, 10.1 mg/L KCl, 44.1 mg/L $CaCl_2{\cdot}2H_2O$, 121.7 mg/L $MgCl_2{\cdot}6H_2O$, 4.149 mg/L $MnCl_2{\cdot}4H_2O$, 1.6 mg/L $FeCl_3{\cdot}6H_2O$, 147 mg/L $MgSO_4{\cdot}7H_2O$, 1.86 mg/L H_3BO_3, 0.0726 mg/L $Na_2MoO_4{\cdot}2H_2O$, 0.0327 mg/L $ZnCl_2$, 0.014 mg/L $CoCl_2{\cdot}6H2O$, 0.00015 mg/L $CuCl_2{\cdot}2H_2O$ added to the sample
Test solution renewal	None
Test temperature	25 ± 2°C
Feeding	None
Light intensity	4000 to 5600 lux
Photoperiod	24 hours light
Aeration	Sample pre-aerated for 20 minutes at 100 bubbles per minute
Test measurements	pH, conductivity, dissolved oxygen measured at test initiation and termination; temperature and light levels measured daily
Test protocol	Environment Canada (2007), EPS 1/RM/37
Statistical software	CETIS version 2.1.5.5
Test endpoints	Number of fronds and dry weight
Test acceptability criterion for controls	≥ 8-fold increase in number of fronds
Reference toxicant	Potassium chloride (KCl)

3.0 RESULTS

Results of the toxicity tests are summarized in Tables 5 to 7 for sample TY2311633-001 EDL1_EFF_20231106. Stimulatory effects were observed for *P. subcapitata* cell yield, resulting in an IC25 value of >91% (Table 5). Percent stimulation for algal cell growth ranged from 30.4 to 83.7%. There were no adverse effects observed on survival or reproduction of *P. promelas*, resulting in an LC50 and IC25 of >100% (Table 6). No inhibitory effects were observed on dry weight for *L. minor,* resulting in an IC25 of >97%; however, inhibitory effects on frond growth of *L. minor* were observed resulting in an IC25 of 7.6% (Table 7).

Table 5. TY2311633-001 EDL1_EFF_20231106 Results: *Pseudokirchneriella subcapitata* growth inhibition test.

Concentration (% v/v)	Cell Yield (x 10^3 cells/mL) (Mean ± SD)	Stimulation %
Laboratory Control	285.2 ± 23.8	-
2.8	384.0 ± 33.4*	34.6
5.7	372.0 ± 10.4*	30.4
11.4	416.0 ± 21.1*	45.8
22.8	472.0 ± 18.3*	65.5
45.5	524.0 ± 9.2*	83.7
91.0	470.0 ± 3.5*	64.8
Test endpoint (% v/v)		
IC25	>91	-

SD = Standard Deviation, IC = Inhibition Concentration
* = Indicates values that had statistic stimulation

Table 6. TY2311633-001 EDL1_EFF_20231106 Results: fathead minnow (*Pimephales promelas)* survival and biomass test.

Concentration (% v/v)	Survival (%) (Mean ± SD)	Biomass (mg) (Mean ± SD)
Laboratory Control	100.0 ± 0.0	0.54 ± 0.05
1.56	92.5 ± 5.0	0.53 ± 0.03
3.12	90.0 ± 8.2	0.49 ± 0.09
6.25	95.0 ± 10.0	0.48 ± 0.07
12.5	85.0 ± 10.0	0.46 ± 0.08
25	97.5 ± 5.0	0.53 ± 0.05
50	85.0 ± 12.9	0.47 ± 0.02
100	92.5 ± 9.6	0.51 ± 0.07
Test endpoint (% v/v)		
LC50	>100	--
IC25	--	>100

SD = Standard Deviation, LC = Lethal Concentration, IC = Inhibition Concentration

Table 7. TY2311633-001 EDL1_EFF_20231106 Results: *Lemna minor* growth inhibition test.

Concentration (% v/v)	Frond Growth (Increase in Frond No.) (Mean ± SD)	Stimulation %	Dry Weight (mg) (Mean ± SD)	Stimulation %
Laboratory Control	42.3 ± 3.0	-	5.8 ± 0.6	-
1.5	32.8 ± 2.8	-22.5	5.5 ± 0.3	-5.9
3.0	34.0 ± 5.2	-19.5	5.5 ± 0.4	-4.3
6.1	31.3 ± 1.9	-26.0	5.3 ± 0.2	-8.9
12.1	34.0 ± 3.9	-19.5	5.5 ± 0.5	-4.9
24.2	26.0 ± 3.7	-38.5	5.0 ± 0.5	-13.0
48.5	25.8 ± 2.5	-39.1	5.1 ± 0.3	-11.6
97.0	21.3 ± 2.8	-49.7	4.9 ± 0.6	-14.7
Test endpoint (% v/v)				
IC25 (95% CL)	7.2 (3.8-12.7)	-	>97	-

SD = Standard Deviation, IC = Inhibition Concentration, CL = Confidence Limits

Results of the toxicity tests are summarized in Tables 8 to 11 for sample TY2311633-002 EDL2_EFF_20231106. Stimulatory effects were observed for *P. subcapitata* cell yield, resulting in an IC25 value of >91% (Table 8). Percent stimulation for algal cell growth ranged from 19.7 to 67.9%. There were no adverse effects observed on survival of *C. dubia*, resulting in an LC50 of >100%; however; adverse effects were observed on reproduction of *C. dubia* resulting IC25 of 2.1% (Table 9). An abnormal dose response was observed for reproduction in the *C. dubia* test with lower reproduction observed in 3.2, 6.3, 12.5, and 25% test concentrations compared to the laboratory control, and 100 and 50% test concentrations. There were no adverse effects observed on survival or reproduction of *P. promelas*, resulting in an LC50 and IC25 of >100% (Table 10). No inhibitory effects were observed on dry weight for *L. minor,* resulting in an IC25 of >97%; however, inhibitory effects on frond growth of L. minor were observed resulting in an IC25 of 6.9% (Table 11).

Table 8. TY2311633-002 EDL2_EFF_20231106 Results: *Pseudokirchneriella subcapitata* growth inhibition test.

Concentration (% v/v)	Cell Yield (x 10^3 cells/mL) (Mean ± SD)	Stimulation %
Laboratory Control	302.5 ± 26.2	-
2.8	362.0 ± 12.5*	19.7
5.7	378.0 ± 20.8*	25.0
11.4	396.0 ± 6.0*	30.9
22.8	430.0 ± 9.2*	42.2
45.5	508.0 ± 27.1*	67.9
91.0	456.0 ± 15.9*	50.7
Test endpoint (% v/v)		
IC25	>91	-

SD = Standard Deviation, IC = Inhibition Concentration
* = Indicates values that had statistic stimulation

Table 9. TY2311633-002 EDL2_EFF_20231106 Results: *Ceriodaphnia dubia* survival and reproduction test.

Concentration (% v/v)	Survival (%)	Reproduction (Mean ± SD)
Laboratory Control	90	23.2 ± 6.4
1.56	90	18.4 ± 10.4
3.12	100	14.1 ± 6.3
6.25	90	17.0 ± 6.3
12.5	100	15.6 ± 5.9
25	90	8.8 ± 5.0
50	100	19.0 ± 8.1
100	100	21.1 ± 5.2
Test endpoint (% v/v)		
LC50	>100	--
IC25 (95% CL)	--	2.1 (<1.6 - NA)

SD = Standard Deviation, LC = Lethal Concentration, IC = Inhibition Concentration, CL=Confidence Limit, NA = Not Available

Table 10. TY2311633-002 EDL2_EFF_20231106 Results: fathead minnow (*Pimephales promelas)* survival and biomass test.

Concentration (% v/v)	Survival (%) (Mean ± SD)	Biomass (mg) (Mean ± SD)
Laboratory Control	97.5 ± 5.0	0.48 ± 0.05
1.56	90.0 ± 8.2	0.46 ± 0.03
3.12	95.0 ± 10.0	0.53 ± 0.03
6.25	97.5 ± 5.0	0.50 ± 0.07
12.5	90.0 ± 8.2	0.46 ± 0.02
25	90.0 ± 8.2	0.46 ± 0.06
50	87.5 ± 9.6	0.50 ± 0.03
100	82.5 ± 15.0	0.45 ± 0.08
Test endpoint (% v/v)		
LC50	>100	--
IC25	--	>100

SD = Standard Deviation, LC = Lethal Concentration, IC = Inhibition Concentration

Table 11. TY2311633-002 EDL2_EFF_20231106 Results: *Lemna minor* growth inhibition test.

Concentration (% v/v)	Frond Growth (Increase in Frond No.) (Mean ± SD)	Stimulation %	Dry Weight (mg) (Mean ± SD)	Stimulation %
Laboratory Control	42.8 ± 2.8	-	5.8 ± 0.3	-
1.5	35.5 ± 3.7	-17.0	5.2 ± 0.6	-9.8
3.0	33.8 ± 1.7	-21.1	5.1 ± 0.8	-12.0
6.1	37.0 ± 3.2	-13.5	5.3 ± 0.3	-8.8
12.1	27.3 ± 5.2	-36.3	4.8 ± 0.5	-17.4
24.2	27.5 ± 5.3	-35.7	5.1 ± 0.7	-11.0
48.5	24.8 ± 1.5	-42.1	5.0 ± 0.3	-13.9
97.0	25.3 ± 4.4	-40.9	4.9 ± 0.6	-15.3
Test endpoint (% v/v)				
IC25 (95% CL)	6.9 (3.3 - 13.1)	-	>97	-

SD = Standard Deviation, IC = Inhibition Concentration, CL = Confidence Limits

4.0 QA/QC

The health histories of the test organisms used in the exposures were acceptable and met the requirements of the Environment Canada protocols. The tests met all control acceptability criteria and water quality parameters remained within ranges specified in the protocols throughout the tests. There was a deviation from the test methodologies for the *P. promelas* test whereby replicate C in the 6.3% test concentration was misloaded with 12 organisms for sample TY2311633-002 EDL2_EFF_20231106. There were no other deviations from the test methodologies. Uncertainty associated with these tests is best described by the standard deviations around the means.

Results of the reference toxicant tests conducted during the testing program are summarized in Table 12. The reference toxicant tests were performed under the same conditions as those used during testing. Results for these tests fell within the acceptable range for organism performance of two standard deviations of the mean, based on historical results obtained by the laboratory with these tests. The LC50 result of the *P. promelas* reference toxicant test fell outside of two standard deviations of the historical mean; this is expected to happen 5% of the time. An investigation occurred and all testing and culturing procedures were followed appropriately. Thus, the sensitivity of the organisms used in these tests was appropriate.

Table 12. Reference toxicant test results.

Test Species	Endpoint (95% CL)	Historical Mean (2 SD Range)	CV (%)	Test Date
P. subcapitata	Growth (IC25): 29.6 (28.1-30.7) µg/L Zn	31.3 (18.6-52.7)	17.3	November 9, 2023
C. dubia	Survival (LC50): 2.0 (1.9-2.1) g NaCl/L	2.0 (1.7-2.3)	5.2	November 14, 2023
	Reproduction (IC50): 1.7 (1.6-1.8) g NaCl/L	1.7 (1.5-1.9)	4.4	
P. promelas	Survival (LC50):10.1 (7.4-11.3) g NaCl/L	6.5 (4.3-9.9)	13.8	November 9, 2023
	Biomass (IC25): 5.0 (4.0-6.0) g NaCl/L	3.7 (2.2-6.1)	16.9	
L. minor	Frond Number (IC25): 2.3 (0.9-3.5) g KCl/L	2.0 (1.0-3.9)	22.2	November 14, 2023

SD = Standard Deviation, CV = Coefficient of Variation, LC = Lethal Concentration, IC = Inhibition Concentration, CL=Confidence Limit

5.0 REFERENCES

Environment Canada. 2007a. Biological test method: growth inhibition test using the freshwater alga. EPS 1/RM/25, Second Edition, March 2007.

Environment Canada. 2007b. Biological test method: test of reproduction and survival using the cladoceran *Ceriodaphnia dubia*. EPS 1/RM/21, Second Edition, February 2007.

Environment Canada. 2007c. Biological test method: test for measuring the inhibition of growth using the freshwater macrophyte (*Lemna minor*). EPS 1/RM/37, Second Edition, January 2007.

Environment Canada. 2011. Biological test method: test of larval growth and survival using fathead minnows. EPS 1/RM/22, Second Edition, March 2011.

Tidepool Scientific Software. 2022. CETIS comprehensive environmental toxicity information system, version 2.1.5.5 Tidepool Scientific Software, McKinleyville, CA. 275 pp.

APPENDIX A – *Pseudokirchneriella subcapitata* Toxicity Test Data

NAUTILUS ENVIRONMENTAL

Algae Bench Sheet

Method: AGD Client: ALS 106 Reference: 2324-0709-01

Test log

Day	Date	Init	Time 1325	Rotate Y/N	Rotate Time*	Threshold Size (µm)	Daily Data Review
0	2023/11/08	ML/AI	~~H 95m~~	Y/N	Time*	4.00	DM
1	2023/11/09	ET	0830	Y	0800	NA	ML
2	2023/11/10	ET	0830	Y	0800	NA	AI
3	2023/11/11	EP	1030	—	0800	4.00	AM

*recommended rotation times

Sample Information

Initial pH: 7.8
Initial EC (µS/cm): 1733
Initial DO (mg/L): 8.9
Initial Temp (°C): 17

Inoculum - Physical Counts (must read between **981** and **1198** cells per 0.5 mL)		
	1	1112
	2	1190
	3	1112

Inoculum - Cell Yield	10,449

(must be 10,000 +/- 1000 cells/mL)

Nutrients used: (normal) mine

pH Measurements:

Concentration	Initial Value	Final Value
100%	7.0	-
control (well D6)	7.0	-
control (well D7)	-	7.0

***pH range: 6.5-8.5; must be <1.5 units difference between control initial and final**

Quality Assurance - Test Initiation

Algae Culture Name: TUE.Oct.20230926AG
Age of Algae (must be 3-7 days): 3 days
Test organisms appeared healthy before use: (yes)/no
Turnover Date: 2023/11/05
Start time of inoculum prep (must be <3hrs to loading): 1300
Volume filtered through the 45 µm membrane: 90mL
Appearance before filtration: C/C
Appearance after filtration: C/C
Light Levels (**must be 3600 - 4400 lux**)

Day 0:	3910	Day 2:	3960
Day 1:	3970	Day 3:	3910

Temperature (**must be 22-26°C**)

Day 0:	23	Day 2:	23
Day 1:	23	Day 3:	23

Average Temperature: 23

Quality Assurance - Test Termination

Control Fold Growth (must be >16): 28
CV of standard Control Wells (must be ≤20%): 8.3
Mann-Kendall p value (must be >0.05) if CV is >10%: —

Reviewed By: DM Date Reviewed: NOV 22 2023

NAUTILUS ENVIRONMENTAL

Algae Bench Sheet

Complete Plate Physical Counts												
		91%	46%	23%	11%	5.7%	2.8%	1.4%	0.7%	0.4%	0.2%	
A	X	X	X	X	X	X	X	X	X	X	X	X
B	X	4100	4500	4300	3700	3200	3500					X
	X	4000	4600	4100	73800 EP	3300	3600					X
	X											X
C	X	4000	4500	3900	3400	3100	3300					X
	X	4000	4400	3900	3400	3100	3400					X
	X											X
D / CTL	X	2400	2200	2500	2600	X	X	2500	2200	2900	2400	X
	X	2400	2200	2600	2700	X	X	2400	2300	2700	2500	X
	X					X	X					X
E	X	4000	4400	4000	3600	3200	3000					X
	X	4000	4400	4000	3500	3300	3000					X
	X											X
F	X											X
	X											X
	X											X
G	X											X
	X											X
	X											X
H	X	X	X	X	X	X	X	X	X	X	X	X

Only three replicates are enumerated if the CV is ≤20% within a concentration. If the CV is >20%, the additional two replicates will also be enumerated

Reviewed By: DM Date Reviewed: NOV 2 2 2023

Standard Control Well Final Cell Densities (D Row):

Column	Final Cell Density ($x10^3$ cells/mL)	Cell Yield ($x10^3$ cells/mL)
2	287	277
3	263	253
4	305	295
5	317	307
8	293	283
9	269	259
10	335	325
11	293	283
Average	295	285
SD	24	24
CV (%)	8.0	8.3
Fold Growth (x)	28	-

Test Well Final Cell Densities:

Concen. (%)	Calculated Final Cell Density ($x10^3$ cells/ mL)				
	Row				
	B	C	E	F	G
0.2	-	-	-	-	-
0.4	-	-	-	-	-
0.7	-	-	-	-	-
1.4	-	-	-	-	-
2.8	425	401	359	-	-
5.7	389	371	389	-	-
11	449	407	425	-	-
23	503	467	479	-	-
46	544	532	526	-	-
91	485	479	479	-	-

Cell Yield Results:

Concentration (%)	Cell Yield ($x10^3$ cells/ mL)							
	Row					**Mean**	SD	CV (%)
	B	C	E	F	G			
0.2	-	-	-	-	-	-	-	-
0.4	-	-	-	-	-	-	-	-
0.7	-	-	-	-	-	-	-	-
1.4	-	-	-	-	-	-	-	-
2.8	414	390	348	-	-	384	33	9
5.7	378	360	378	-	-	372	10	3
11	438	396	414	-	-	416	21	5
23	492	456	468	-	-	472	18	4
46	534	522	516	-	-	524	9	2
91	474	468	468	-	-	470	3	1

Reviewed by: DM

Date Reviewed: NOV 2 2 2022

CETIS Summary Report

Selenastrum Growth Test — **Nautilus Environmental Calgary**

Batch ID:	06-3587-8513	**Test Type:**	Cell Growth	**Analyst:**	Lab Tech		
Start Date:	08 Nov-23	**Protocol:**	EC/EPS 1/RM/25	**Diluent:**	Deionized Water		
Ending Date:	11 Nov-23	**Species:**	Pseudokirchneriella subcapitata	**Brine:**			
Test Length:	72h	**Taxon:**	Chlorophyta	**Source:**	In-House Culture	**Age:**	3

Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	48h (11.9 °C)	**Client:**	ALS Laboratories		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	PMSD	TU
12-7398-5998	Cell Density	Dunnett Multiple Comparison Test	<2.8	2.8	---	12.5%	>35.7

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	%	95% LCL	95% UCL	TU
07-1669-3398	Cell Density	Linear Interpolation (ICPIN)	IC15	>91	---	---	<1.1
			IC20	>91	---	---	<1.1
			IC25	>91	---	---	<1.1
			IC40	>91	---	---	<1.1
			IC50	>91	---	---	<1.1

Cell Density Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	8	285.2	265.4	305.1	253	325	8.404	23.77	8.33%	0.00%
2.8		3	384	301	467	348	414	19.29	33.41	8.70%	-34.62%
5.7		3	372	346.2	397.8	360	378	6	10.39	2.79%	-30.41%
11		3	416	363.7	468.3	396	438	12.17	21.07	5.07%	-45.84%
23		3	472	426.5	517.5	456	492	10.58	18.33	3.88%	-65.47%
46		3	524	501.2	546.8	516	534	5.292	9.165	1.75%	-83.70%
91		3	470	461.4	478.6	468	474	2	3.464	0.74%	-64.77%

Cell Density Detail MD5: C6C02C4A90CC2D1F5B2090DCD9152CFF

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	N	277	253	295	307	283	259	325	283
2.8		414	390	348					
5.7		378	360	378					
11		438	396	414					
23		492	456	468					
46		534	522	516					
91		474	468	468					

Selenastrum Growth Test — **Nautilus Environmental Calgary**

Analysis ID:	12-7398-5998	**Endpoint:**	Cell Density	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:07	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	23 Nov-23 16:04	**MD5 Hash:**	C6C02C4A90CC2D1F5B2090DCD9152CF	**Editor ID:**	
Batch ID:	06-3587-8513	**Test Type:**	Cell Growth	**Analyst:**	Lab Tech
Start Date:	08 Nov-23	**Protocol:**	EC/EPS 1/RM/25	**Diluent:**	Deionized Water
Ending Date:	11 Nov-23	**Species:**	Pseudokirchneriella subcapitata	**Brine:**	
Test Length:	72h	**Taxon:**	Chlorophyta	**Source:**	In-House Culture **Age:** 3
Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	48h (11.9 °C)	**Client:**	ALS Laboratories		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C < T	<2.8	2.8	---	>35.7	35.74	12.53%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Negative Control		2.8*	9	7.04	2.548	35.74	CDF	<1.0E-05	Significant Effect
		5.7*	9	6.185	2.548	35.74	CDF	1.8E-05	Significant Effect
		11*	9	9.322	2.548	35.74	CDF	<1.0E-05	Significant Effect
		23*	9	13.31	2.548	35.74	CDF	<1.0E-05	Significant Effect
		46*	9	17.02	2.548	35.74	CDF	<1.0E-05	Significant Effect
		91*	9	13.17	2.548	35.74	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	184008	30668	6	71.45	<1.0E-05	Significant Effect
Error	8155.5	429.237	19			
Total	192163		25			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Variance	Bartlett Equality of Variance Test	8.332	12.59	0.2147	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9766	0.922	0.7938	Normal Distribution

Cell Density Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	8	285.2	265.4	305.1	283	253	325	8.404	8.33%	0.00%
2.8		3	384	301	467	390	348	414	19.29	8.70%	-34.62%
5.7		3	372	346.2	397.8	378	360	378	6	2.79%	-30.41%
11		3	416	363.7	468.3	414	396	438	12.17	5.07%	-45.84%
23		3	472	426.5	517.5	468	456	492	10.58	3.88%	-65.47%
46		3	524	501.2	546.8	522	516	534	5.292	1.75%	-83.70%
91		3	470	461.4	478.6	468	468	474	2	0.74%	-64.77%

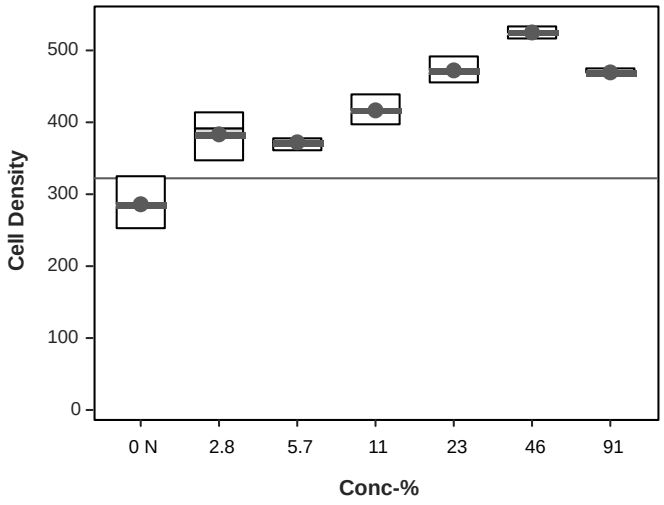
Cell Density Detail

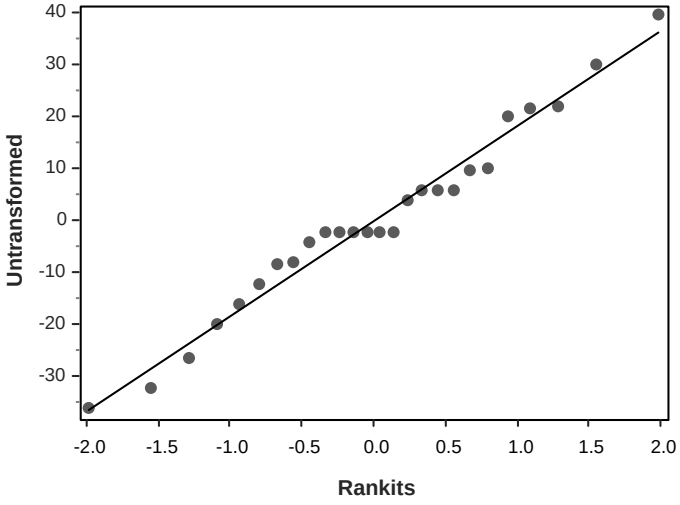
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	N	277	253	295	307	283	259	325	283
2.8		414	390	348					
5.7		378	360	378					
11		438	396	414					
23		492	456	468					
46		534	522	516					
91		474	468	468					

Selenastrum Growth Test **Nautilus Environmental Calgary**

Analysis ID:	12-7398-5998	**Endpoint:**	Cell Density	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:07	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	23 Nov-23 16:04	**MD5 Hash:**	C6C02C4A90CC2D1F5B2090DCD9152CF	**Editor ID:**	

Graphics





Selenastrum Growth Test **Nautilus Environmental Calgary**

Analysis ID:	07-1669-3398	**Endpoint:**	Cell Density	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:09	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	23 Nov-23 16:04	**MD5 Hash:**	C6C02C4A90CC2D1F5B2090DCD9152CF	**Editor ID:**	

Batch ID:	06-3587-8513	**Test Type:**	Cell Growth	**Analyst:**	Lab Tech		
Start Date:	08 Nov-23	**Protocol:**	EC/EPS 1/RM/25	**Diluent:**	Deionized Water		
Ending Date:	11 Nov-23	**Species:**	Pseudokirchneriella subcapitata	**Brine:**			
Test Length:	72h	**Taxon:**	Chlorophyta	**Source:**	In-House Culture	**Age:**	3

Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	48h (11.9 °C)	**Client:**	ALS Laboratories		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1783376	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC15	>91	---	---	<1.1	---	---
IC20	>91	---	---	<1.1	---	---
IC25	>91	---	---	<1.1	---	---
IC40	>91	---	---	<1.1	---	---
IC50	>91	---	---	<1.1	---	---

Cell Density Summary

			Calculated Variate						Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	N	8	285.2	283	253	325	8.33%	0.00%	417.6	0.00%
2.8		3	384	390	348	414	8.70%	-34.62%	417.6	0.00%
5.7		3	372	378	360	378	2.79%	-30.41%	417.6	0.00%
11		3	416	414	396	438	5.07%	-45.84%	417.6	0.00%
23		3	472	468	456	492	3.88%	-65.47%	417.6	0.00%
46		3	524	522	516	534	1.75%	-83.70%	417.6	0.00%
91		3	470	468	468	474	0.74%	-64.77%	417.6	0.00%

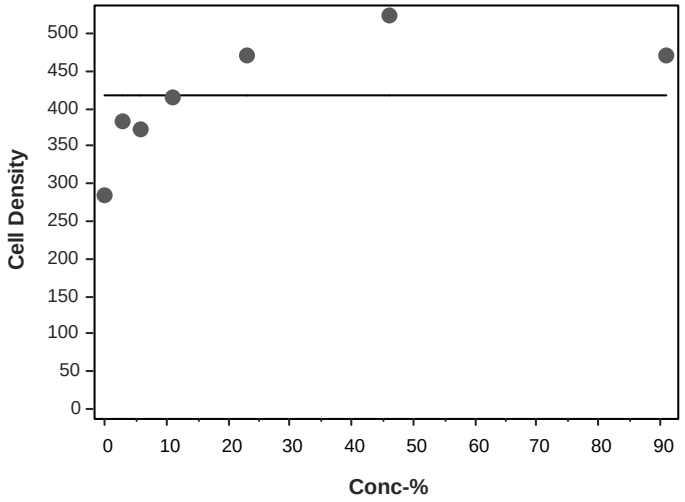
Cell Density Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	N	277	253	295	307	283	259	325	283
2.8		414	390	348					
5.7		378	360	378					
11		438	396	414					
23		492	456	468					
46		534	522	516					
91		474	468	468					

Selenastrum Growth Test **Nautilus Environmental Calgary**

Analysis ID:	07-1669-3398	**Endpoint:**	Cell Density	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:09	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	23 Nov-23 16:04	**MD5 Hash:**	C6C02C4A90CC2D1F5B2090DCD9152CF	**Editor ID:**	

Graphics



NAUTILUS ENVIRONMENTAL

Algae Bench Sheet

Method: AGD Client: ALS 106 Reference: 2324-0709-02

Test log

Day	Date	Init	Time	Rotate		Threshold Size (µm)	Daily Data Review
				Y/N	Time*		
0	2023/11/08	ML/AI	1330	Y/N	0800	4.00	DM
1	2023/11/09	ET	0830	Y	0800	NA	ML
2	2023/11/10	ET	0830	Y	0800	NA	AI
3	2023/11/11	EP	1110	—	0800	4.00	AM

*recommended rotation times

Sample Information

Initial pH:	7.9
Initial EC (µS/cm):	1728
Initial DO (mg/L):	9.0
Initial Temp (°C):	17

Inoculum - Physical Counts (must read between **981** and **1198** cells per 0.5 mL)		
	1	1112
	2	1190
	3	1112

Inoculum - Cell Yield	10,449

(must be 10,000 +/- 1000 cells/mL)

Nutrients used: (normal) mine

pH Measurements:

Concentration	Initial Value	Final Value
100%	7.5	-
control (well D6)	7.0	-
control (well D7)	-	7.0

***pH range: 6.5-8.5; must be <1.5 units difference between control initial and final**

Quality Assurance - Test Initiation	
Algae Culture Name: TU :Oct.20230926 AG	
Age of Algae (must be 3-7 days):	3 days
Test organisms appeared healthy before use: **(yes)/no**	
Turnover Date: 2023/11/05	
Start time of inoculum prep (must be <3hrs to loading): 1300	
Volume filtered through the 45 µm membrane:	100 mL
Appearance before filtration:	CLC
Appearance after filtration:	CLC
Light Levels (**must be 3600 - 4400 lux**)	
Day 0: 3840	Day 2: 3850
Day 1: 3920	Day 3: 3830
Temperature (**must be 22-26°C**)	
Day 0: 23	Day 2: 23
Day 1: 23	Day 3: 23
Average Temperature: 23	

Quality Assurance - Test Termination	
Control Fold Growth (must be >16):	30
CV of standard Control Wells (must be ≤20%):	8.6
Mann-Kendall p value (must be >0.05) if CV is >10%:	—

Reviewed By: DM Date Reviewed: NOV 22 2023

NAUTILUS ENVIRONMENTAL

Algae Bench Sheet

Complete Plate Physical Counts

		91%	46%	23%	11%	5.7%	2.8%	1.4%	0.7%	0.4%	0.2%	
A	X	X	X	X	X	X	X	X	X	X	X	X
B	X	4100	4500	3700	3800	3400	3000					X
	X	4000	4600	3700	3400	3500	3000					X
	X											X
C	X	3800	4400	3800	3400	3100	3100					X
	X	3900	4300	3700	3400	3200	3200					X
	X											X
D / CTL	X	2800	2500	2500	2900	X	X	2400	2300	2900	2600	X
	X	2700	2600	2400	3000	X	X	2300	2500	2800	2600	X
	X					X	X					X
E	X	3900	4100	3600	3500	3100	3300					X
	X	3800	4100	3600	3400	3200	3100					X
	X											X
F	X											X
	X											X
	X											X
G	X											X
	X											X
	X											X
H	X	X	X	X	X	X	X	X	X	X	X	X

Only three replicates are enumerated if the CV is ≤20% within a concentration. If the CV is >20%, the additional two replicates will also be enumerated

Reviewed By: DM Date Reviewed: NOV 2 2 2023

Standard Control Well Final Cell Densities (D Row):

Column	Final Cell Density ($x10^3$ cells/mL)	Cell Yield ($x10^3$ cells/mL)
2	329	319
3	305	295
4	293	283
5	353	343
8	281	271
9	287	277
10	341	331
11	311	301
Average	313	302
SD	26	26
CV (%)	8.4	8.6
Fold Growth (x)	30	-

Test Well Final Cell Densities:

Concen. (%)	Calculated Final Cell Density ($x10^3$ cells/ mL)				
	Row				
	B	C	E	F	G
0.2	-	-	-	-	-
0.4	-	-	-	-	-
0.7	-	-	-	-	-
1.4	-	-	-	-	-
2.8	359	377	383	-	-
5.7	413	377	377	-	-
11	401	407	413	-	-
23	443	449	431	-	-
46	544	520	491	-	-
91	485	455	461	-	-

Cell Yield Results:

Concentration (%)	Cell Yield ($x10^3$ cells/ mL)							
	Row					**Mean**	SD	CV (%)
	B	C	E	F	G			
0.2	-	-	-	-	-	-	-	-
0.4	-	-	-	-	-	-	-	-
0.7	-	-	-	-	-	-	-	-
1.4	-	-	-	-	-	-	-	-
2.8	348	366	372	-	-	362	12	3
5.7	402	366	366	-	-	378	21	5
11	390	396	402	-	-	396	6	2
23	432	438	420	-	-	430	9	2
46	534	510	480	-	-	508	27	5
91	474	444	450	-	-	456	16	3

Reviewed by: DM

Date Reviewed: NOV 2 2 2023

CETIS Summary Report

Test Code/ID: 2324-0709-02AGD / 08-7691-6831

Selenastrum Growth Test — **Nautilus Environmental Calgary**

Batch ID:	10-9161-1415	**Test Type:**	Cell Growth	**Analyst:**	Lab Tech		
Start Date:	08 Nov-23	**Protocol:**	EC/EPS 1/RM/25	**Diluent:**	Deionized Water		
Ending Date:	11 Nov-23	**Species:**	Pseudokirchneriella subcapitata	**Brine:**			
Test Length:	72h	**Taxon:**	Chlorophyta	**Source:**	In-House Culture	**Age:**	3
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**			
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories		
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110		
Sample Age:	48h (12.2 °C)	**Client:**	ALS Laboratories				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	PMSD	TU
07-0923-6032	Cell Density	Dunnett Multiple Comparison Test	<2.8	2.8	---	11.8%	>35.7

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	%	95% LCL	95% UCL	TU
14-8531-3509	Cell Density	Linear Interpolation (ICPIN)	IC15	>91	---	---	<1.1
			IC20	>91	---	---	<1.1
			IC25	>91	---	---	<1.1
			IC40	>91	---	---	<1.1
			IC50	>91	---	---	<1.1

Cell Density Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	8	302.5	280.6	324.4	271	343	9.264	26.2	8.66%	0.00%
2.8		3	362	331	393	348	372	7.211	12.49	3.45%	-19.67%
5.7		3	378	326.4	429.6	366	402	12	20.78	5.50%	-24.96%
11		3	396	381.1	410.9	390	402	3.464	6	1.52%	-30.91%
23		3	430	407.2	452.8	420	438	5.292	9.165	2.13%	-42.15%
46		3	508	440.8	575.2	480	534	15.62	27.06	5.33%	-67.93%
91		3	456	416.6	495.4	444	474	9.165	15.87	3.48%	-50.74%

Cell Density Detail MD5: 3EDF9479947648D989788E36C2918170

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	N	319	295	283	343	271	277	331	301
2.8		348	366	372					
5.7		402	366	366					
11		390	396	402					
23		432	438	420					
46		534	510	480					
91		474	444	450					

Selenastrum Growth Test — **Nautilus Environmental Calgary**

Analysis ID:	07-0923-6032	**Endpoint:**	Cell Density	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 14:29	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	23 Nov-23 16:41	**MD5 Hash:**	3EDF9479947648D989788E36C2918170	**Editor ID:**	
Batch ID:	10-9161-1415	**Test Type:**	Cell Growth	**Analyst:**	Lab Tech
Start Date:	08 Nov-23	**Protocol:**	EC/EPS 1/RM/25	**Diluent:**	Deionized Water
Ending Date:	11 Nov-23	**Species:**	Pseudokirchneriella subcapitata	**Brine:**	
Test Length:	72h	**Taxon:**	Chlorophyta	**Source:**	In-House Culture **Age:** 3
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110
Sample Age:	48h (12.2 °C)	**Client:**	ALS Laboratories		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C < T	<2.8	2.8	---	>35.7	35.82	11.84%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Negative Control		2.8*	9	4.233	2.548	35.82	CDF	0.0013	Significant Effect
		5.7*	9	5.371	2.548	35.82	CDF	0.0001	Significant Effect
		11*	9	6.652	2.548	35.82	CDF	<1.0E-05	Significant Effect
		23*	9	9.071	2.548	35.82	CDF	<1.0E-05	Significant Effect
		46*	9	14.62	2.548	35.82	CDF	<1.0E-05	Significant Effect
		91*	9	10.92	2.548	35.82	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	123132	20522	6	47.61	<1.0E-05	Significant Effect
Error	8190	431.053	19			
Total	131322		25			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Variance	Bartlett Equality of Variance Test	5.992	12.59	0.4241	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9797	0.922	0.8665	Normal Distribution

Cell Density Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	8	302.5	280.6	324.4	298	271	343	9.264	8.66%	0.00%
2.8		3	362	331	393	366	348	372	7.211	3.45%	-19.67%
5.7		3	378	326.4	429.6	366	366	402	12	5.50%	-24.96%
11		3	396	381.1	410.9	396	390	402	3.464	1.52%	-30.91%
23		3	430	407.2	452.8	432	420	438	5.292	2.13%	-42.15%
46		3	508	440.8	575.2	510	480	534	15.62	5.33%	-67.93%
91		3	456	416.6	495.4	450	444	474	9.165	3.48%	-50.74%

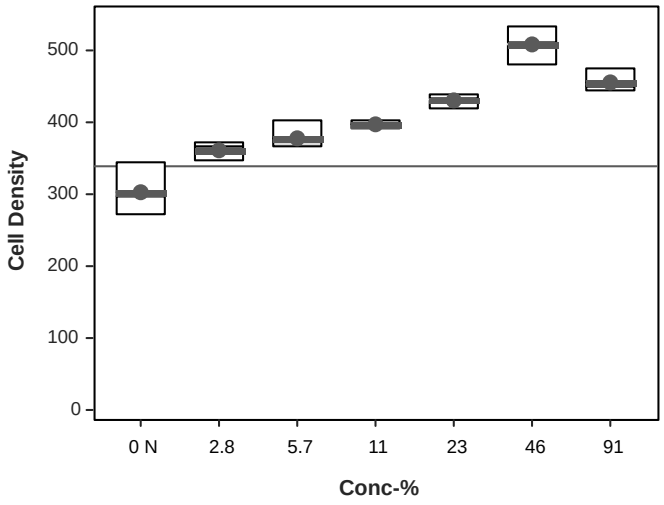
Cell Density Detail

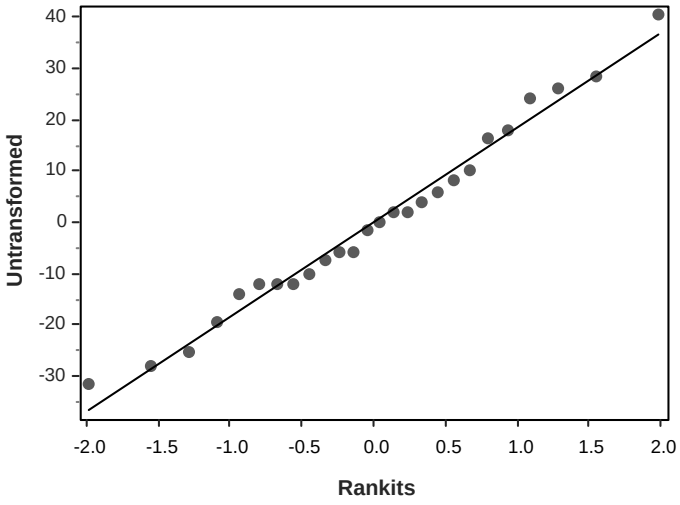
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	N	319	295	283	343	271	277	331	301
2.8		348	366	372					
5.7		402	366	366					
11		390	396	402					
23		432	438	420					
46		534	510	480					
91		474	444	450					

Selenastrum Growth Test **Nautilus Environmental Calgary**

Analysis ID:	07-0923-6032	**Endpoint:**	Cell Density	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 14:29	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	23 Nov-23 16:41	**MD5 Hash:**	3EDF9479947648D989788E36C2918170	**Editor ID:**	

Graphics





Selenastrum Growth Test — **Nautilus Environmental Calgary**

Analysis ID:	14-8531-3509	**Endpoint:**	Cell Density	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 14:30	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	23 Nov-23 16:41	**MD5 Hash:**	3EDF9479947648D989788E36C2918170	**Editor ID:**	
Batch ID:	10-9161-1415	**Test Type:**	Cell Growth	**Analyst:**	Lab Tech
Start Date:	08 Nov-23	**Protocol:**	EC/EPS 1/RM/25	**Diluent:**	Deionized Water
Ending Date:	11 Nov-23	**Species:**	Pseudokirchneriella subcapitata	**Brine:**	
Test Length:	72h	**Taxon:**	Chlorophyta	**Source:**	In-House Culture **Age:** 3
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110
Sample Age:	48h (12.2 °C)	**Client:**	ALS Laboratories		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1593275	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC15	>91	---	---	<1.1	---	---
IC20	>91	---	---	<1.1	---	---
IC25	>91	---	---	<1.1	---	---
IC40	>91	---	---	<1.1	---	---
IC50	>91	---	---	<1.1	---	---

Cell Density Summary

			Calculated Variate						Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	N	8	302.5	298	271	343	8.66%	0.00%	404.6	0.00%
2.8		3	362	366	348	372	3.45%	-19.67%	404.6	0.00%
5.7		3	378	366	366	402	5.50%	-24.96%	404.6	0.00%
11		3	396	396	390	402	1.52%	-30.91%	404.6	0.00%
23		3	430	432	420	438	2.13%	-42.15%	404.6	0.00%
46		3	508	510	480	534	5.33%	-67.93%	404.6	0.00%
91		3	456	450	444	474	3.48%	-50.74%	404.6	0.00%

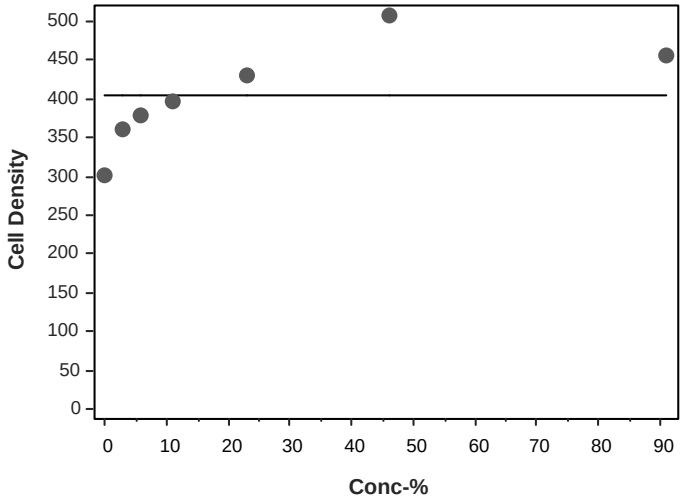
Cell Density Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	N	319	295	283	343	271	277	331	301
2.8		348	366	372					
5.7		402	366	366					
11		390	396	402					
23		432	438	420					
46		534	510	480					
91		474	444	450					

Selenastrum Growth Test **Nautilus Environmental Calgary**

Analysis ID:	14-8531-3509	**Endpoint:**	Cell Density	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 14:30	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	23 Nov-23 16:41	**MD5 Hash:**	3EDF9479947648D989788E36C2918170	**Editor ID:**	

Graphics



APPENDIX B – *Ceriodaphnia dubia* Toxicity Test Data

NAUTILUS ENVIRONMENTAL

Ceriodaphnia Bench Sheet

Method CDD Client ALS106 Reference 2324-0709-02

Test Log

Date	Day	Time	Technician	Chem. Cart Used	Subsample Fed (✓)	Daily Data Review	Subsample Used	
2023/11/08	0	1430	DM	2	✓	DM	Day 0	✓
2023/11/09	1	1030	KM	2	✓	AI	Day 1	✓
2023/11/10	2	1100	AI	2	✓	KM	Day 2	✓
2023/11/11	3	1015	BS	2	✓	DM	Day 3	✓
2023/11/12	4	0930	KM	2	✓	ML	Day 4	✓
2023/11/13	5	1100	NA	2	✓	KM	Day 5	✓
2023/11/14	6	~~0940~~	1400 AI	2	✓	KM	Day 6	✓
2023/11/15	7	0830	EP	3	XEP	ML	Day 7	—
2023/11/16	8	—	—	—	—	—	—	—

Sample Information

Initial pH: 7.9
Initial EC (µS/cm): 1728
Initial DO (mg/L): 9.0
Initial Temp (°C): 17
Filtered with 60 µm nitex screen
Yes/(No)
Sample pre-aerated/hardness/pH adjust:
Yes/(No)
*if yes, describe procedure, rate and duration

Test Specifics

Food expiration: 12/03/nH

Dilution water vessel and preparation date: 8:11/04, 1:11/~~04~~ AI 05, 7:11/08

Cup	Control Validity Day 7 Number of Young	Number of Broods
1	19	3
2	34	3
3	25	3
4	20	3
5	18	2
6	~~14~~ 0	~~2~~ 0.
7	15	2
8	28	3
9	25	3
10	31	3

Average # of Young: ~~23~~ 24

% with ≥ 3 Broods: 70%.

Control Mortality: 10%.
(must be ≤20%)

Reproduction Validity Criteria: the average number of young produced in first three broods is ≥ 15, when 60% of control organsims had 3 or more broods: **(Yes) / No**

Reviewed By: DM Date Reviewed: NOV 22 2023

NAUTILUS ENVIRONMENTAL

Ceriodaphnia Bench Sheet

Method CDD Client ALS106 Reference 2324-0709-02

Chemistry

pH (units) (range: 6.5-8.5)

Conc. (%) / Day	New Solutions CTL	1.6	3.1	6.3	13	25	50	100	Old Solutions CTL	1.6	3.1	6.3	13	25	50	100
0	7.5	7.6	7.6	7.6	7.6	7.6	7.7	7.7								
1	7.8	7.7	7.7	7.7	7.7	7.8	7.8	7.8	7.7	7.7	7.7	7.7	7.7	7.8	7.8	7.8
2	7.3	7.4	7.4	7.5	7.5	7.5	7.5	7.6	7.6	7.5	7.5	7.4	7.4	7.4	7.4	7.4
3	7.5	7.5	7.6	7.6	7.6	7.6	7.7	7.9	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.8
4	7.5	7.5	7.5	7.6	7.6	7.6	7.7	7.8	7.5	7.5	7.5	7.5	7.5	7.4	7.4	7.6
5	7.8	7.8	7.9	7.9	7.9	8.0	8.0	8.1	7.5	7.6	7.6	7.7	7.7	7.7	7.7	7.8
6	7.6	7.6	7.7	7.7	7.7	7.7	7.8	7.8	7.9	7.7	7.7	7.7	7.6	7.6	7.6	7.6
7									7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
8																

Conductance (µS/cm)

0	182	225	251	286	403	607	828	1496								
1	193	228	246	308	412	593	828	1497	193	227	251	288	404	559	809	1466
2	191	222	256	296	394	582	878	1532	241	231	251	306	414	582	814	1473
3	188	226	244	300	408	553	841	1530	189	222	252	303	395	575	860	1516
4	182	231	251	268	449	588	841	1550	206	230	248	301	405	548	875	1498
5	211	248	279	337	450	660	1031	1741	214	258	288	340	495	616	927	1683
6	217	245	274	335	443	655	954	1714	287	257	285	340	457	655	1006	1709
7									160	189	210	273	398	592	940	1474
8																

Dissolved Oxygen (mg/L) (40-100% saturation)

0	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3								
1	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	6.8	6.4	7.0	7.1	7.3	7.3	7.3	7.3
2	6.5	6.4	6.3	6.4	6.4	6.4	6.4	6.4	5.7	5.7	5.7	5.6	5.7	5.7	5.7	5.6
3	6.8	6.8	6.8	6.8	6.8	6.8	6.7	6.7	6.1	6.2	6.1	6.2	6.2	6.2	6.2	6.3
4	6.9	7.0	6.9	6.9	6.8	6.9	6.9	7.0	5.7	5.7	5.7	5.8	5.8	5.8	5.8	5.8
5	7.0	7.0	6.8	6.8	6.8	6.9	6.9	7.0	6.2	6.1	6.1	6.0	5.8	5.8	5.9	6.0
6	6.3	6.5	6.5	6.5	6.5	6.5	6.5	6.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
7									6.3	6.1	5.9	5.8	5.7	5.9	6.3	6.0
8																

Temperature 24 - 26 (°C)

0	24	24	24	24	24	24	24	24								
1	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
2	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
3	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
4	24	24	24	24	24	24	24	24	24	25	25	25	25	25	25	25
5	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
6	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
7									24	24	24	24	24	24	24	24
8																

DO Levels (40-100% saturation)* -
2.9 to 7.3 mg/L at 24°C
2.9 to 7.2 mg/L at 25°C
2.8 to 7.1 mg/L at 26°C

*corrected for altitude

Reviewed By: DM Date Reviewed: NOV 2 2 2023

NAUTILUS ENVIRONMENTAL

Ceriodaphnia Bench Sheet

Method CDD Client ALS106 Reference 2324-0709-02

Biology

(#, young produced; 0, no young; X, dead; X#, young produced-dead; —,young produced after 3rd brood)

Brood organisms produced during an organism's 4th or subsequent brood are not included in brood counts

Day	1	2	3	4	5	6	7	8
Cup	CTL							
1	0	0	2	0	8	9	—	—
2	↓	↓	6	5	0	12	17	—
3	↓	↓	↓	2	0	8	15	—
4	↓	↓	↓	2	0	7	11	—
5	↓	↓	↓	0	8	10	0	
6	↓	↓	↓	0	7	X10		
7	↓	↓	↓	0	3	12	0	
8	↓	↓	↓	5	0	11	12	—
9	↓	↓	↓	4	0	8	13	—
10	↓	↓	↓	3	0	13	15	—

1.6 %

1	0	0	0	2	0	13	16	—
2	↓	↓	↓	6	0	8	7	—
3	↓	↓	↓	0	8	3	11	—
4	↓	↓	↓	20	6	9	0	
5	↓	↓	↓	2	0	13	17	—
6	↓	↓	↓	3	0	10	18	—
7	↓	↓	↓	0	5	8	0	
8	↓	↓	↓	2	0	9	0	
9	↓	↓	↓	0	7	7	0	
10	↓	X0	↓					—

3.1 %

1	0	0	0	2	0	5	5	—
2	↓	↓	↓	5	0	7	17	—
3	↓	↓	↓	0	6	6	0	
4	↓	↓	↓	2	0	8	0	
5	↓	↓	↓	0	4	2	0	
6	↓	↓	↓	0	X0*			
7	↓	↓	↓	2	0	4	10	—
8	↓	↓	↓	0	6	7	0	
9	↓	↓	↓	2	4	9	EP0 —	—
10	↓	↓	↓	0	8	6	0	

*missing from tube, exclude from ~~mortality~~ STATS

6.3 %

1	0	0	0	4	0	5	9	—
2	↓	↓	↓	3	0	6	10	—
3	↓	↓	↓	0	0	8	0	
4	↓	↓	↓	3	0	5	14	—
5	↓	↓	↓	3	0	7	10	—
6	↓	↓	↓	0	5	0	12	
7	↓	↓	↓	5	0	X6		
8	↓	↓	↓	5	0	5	14	—
9	↓	↓	↓	3	0	4	0	
10	↓	↓	↓	5	0	5	14	—

Day	1	2	3	4	5	6	7	8
Cup	13 %							
1	0	0	0	3	0	4	18	—
2	↓	↓	↓	4	0	7	0	
3	↓	↓	↓	2	0	4	11	—
4	↓	↓	↓	3	0	4	5	—
5	↓	↓	↓	0	6	7	0	
6	↓	↓	↓	0	5	8	0	
7	↓	↓	↓	0	5	6	0	
8	↓	↓	↓	0	5	6	0	
9	↓	↓	↓	6	0	7	14	—
10	↓	↓	↓	0	7	0	9	

25 %

1	0	0	0	2	0	3	14	
2	0	0	↓	4	0	5	10	
3	X0							
4	0	0	0	2	3	6	20	—
5	↓	↓	↓	3	0	5	0	
6	↓	↓	↓	0	5	3	0	
7	↓	↓	↓	2	4	6	—	—
8	↓	↓	↓	0	6	4	0	
9	↓	↓	↓	2	0	4	0	
10	↓	↓	↓	2	0	3	0	

50 %

1	0	0	0	2	0	4	15	—
2	↓	↓	↓	4	0	0	16	
3	↓	↓	↓	3	0	0	9	
4	↓	↓	↓	0	0	2	0	
5	↓	↓	↓	2	0	3	19	—
6	↓	↓	↓	3	0	5	16	—
7	↓	↓	↓	2	0	7	14	—
8	↓	↓	↓	0	6	NS5	0	
9	↓	↓	↓	4	0	5	19	—
10	↓	↓	↓	4	0	3	18	—

100 %

1	0	0	0	4	0	4	14	—
2	↓	↓	↓	2	0	4	11	—
3	↓	↓	↓	3	0	3	15	—
4	↓	↓	↓	26	0	4	15	—
5	↓	↓	↓	5	0	0	15	—
6	↓	↓	↓	3	0	5	14	—
7	↓	↓	↓	0	7	3	0	
8	↓	↓	↓	4	0	0	14	
9	↓	↓	↓	5	0	3	18	—
10	↓	↓	↓	2	0	8	12	—

Reviewed By: DM Date Reviewed: NOV 2 2 2023

NAUTILUS ENVIRONMENTAL

Ceriodaphnia Bench Sheet

Test Organism Information

Client: ALS106

Culture history for adults used in the test for sample reference: 2324-0709-02

Day Used: 2023/11/08

Test organisms appeared healthy before use: (Yes)/No

Number of young produced per brood adult within first three broods:

Brood	row/replicate	C5	D1	D2	~~D3~~	E2	E3					
	number of young	6	7	4	\	7	5					
	number of young	10	13	7	\	14	12					
	number of young	15	12	10	\	13	11					
	number of young	14	17	12	\	15	17					

Culture(s) Used for Testing: wed B

Number of Adults Alive in Culture

7 days prior:	day used:
25	24

Notes: all cups have 1 adult

Average No. of young in first 3 broods: 29
(must be ≥15)

Culture % mortality (7-days prior to testing): 4%
(must be ≤20%)

Number of young produced by each brood organism in last complete brood is ≥8: Y
Yes (Y) or No (N)

Reviewed By: DM Date Reviewed: NOV 2 2 2023

Ceriodaphnia 7-d Survival and Reproduction Test — **Nautilus Environmental Calgary**

Batch ID:	21-3121-2308	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Lab Tech		
Start Date:	08 Nov-23	**Protocol:**	EC EPS RM/21	**Diluent:**			
Ending Date:	15 Nov-23	**Species:**	Ceriodaphnia dubia	**Brine:**			
Test Length:	7d 0h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture	**Age:**	<24
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**			
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories		
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110		
Sample Age:	48h (12.2 °C)	**Client:**	ALS Laboratories				

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	%	95% LCL	95% UCL	TU
15-1202-1924	7d Survival Rate	Linear Interpolation (ICPIN)	LC15	>100	---	---	<1
			LC20	>100	---	---	<1
			LC25	>100	---	---	<1
			LC40	>100	---	---	<1
			LC50	>100	---	---	<1
15-4687-7978	Reproduction	Linear Interpolation (ICPIN)	IC15	0.9992	0.4798	4.575	100.1
			IC20	1.518	0.6863	---	65.9
			IC25	2.128	0.9217	---	47
			IC40	>100	---	---	<1
			IC50	>100	---	---	<1

Test Acceptability

				TAC Limits			
Analysis ID	**Endpoint**	**Attribute**	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
15-1202-1924	7d Survival Rate	Control Resp	0.9	0.8	>>	Yes	Passes Criteria
15-4687-7978	Reproduction	Control Resp	23.2	15	>>	Yes	Passes Criteria

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
1.6		10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
3.1		9	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-11.11%
6.3		10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
13		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-11.11%
25		10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	0.00%
50		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-11.11%
100		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-11.11%

Reproduction Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	10	23.2	18.63	27.77	15	34	2.021	6.391	27.55%	0.00%
1.6		10	18.4	10.93	25.87	0	32	3.301	10.44	56.72%	20.69%
3.1		9	14.11	9.258	18.96	6	29	2.105	6.314	44.74%	39.18%
6.3		10	17	12.51	21.49	7	24	1.983	6.272	36.89%	26.72%
13		10	15.6	11.4	19.8	11	27	1.857	5.873	37.65%	32.76%
25		10	8.8	5.25	12.35	0	19	1.569	4.962	56.39%	62.07%
50		10	19	13.21	24.79	2	28	2.56	8.097	42.61%	18.10%
100		10	21.1	17.41	24.79	10	28	1.629	5.152	24.42%	9.05%

Ceriodaphnia 7-d Survival and Reproduction Test **Nautilus Environmental Calgary**

7d Survival Rate Detail MD5: BD8600F87CDD4CF56E142B0CF7A7AAB2

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
1.6		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000
3.1		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	
6.3		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000
13		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
50		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Reproduction Detail MD5: 7CF7B66AF17DF08934CC1B04C3DA9C95

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	19	34	25	20	18	17	15	28	25	31
1.6		31	15	22	15	32	31	13	11	14	0
3.1		12	29	12	10	6	16	13	15	14	
6.3		18	19	8	22	20	17	11	24	7	24
13		25	11	17	12	13	13	11	11	27	16
25		19	9	0	11	8	8	12	10	6	5
50		21	20	12	2	24	24	23	11	28	25
100		22	17	21	25	28	22	10	18	26	22

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1
1.6		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1
3.1		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
6.3		1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1
13		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
25		1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
50		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
100		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

CETIS Analytical Report

Test Code/ID: 2324-0709-02CDD / 07-7331-9344

Ceriodaphnia 7-d Survival and Reproduction Test **Nautilus Environmental Calgary**

Analysis ID:	15-1202-1924	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 14:40	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	24 Nov-23 14:36	**MD5 Hash:**	4EBBDDFFE443A4FCEFDBFC5FD42295E	**Editor ID:**	

Batch ID:	21-3121-2308	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Lab Tech		
Start Date:	08 Nov-23	**Protocol:**	EC EPS RM/21	**Diluent:**			
Ending Date:	15 Nov-23	**Species:**	Ceriodaphnia dubia	**Brine:**			
Test Length:	7d 0h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture	**Age:**	<24

Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110
Sample Age:	48h (12.2 °C)	**Client:**	ALS Laboratories		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1893326	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

		TAC Limits			
Attribute	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
Control Resp	0.9	0.8	>>	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
LC15	>100	---	---	<1	---	---
LC20	>100	---	---	<1	---	---
LC25	>100	---	---	<1	---	---
LC40	>100	---	---	<1	---	---
LC50	>100	---	---	<1	---	---

7d Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	**Code**	**Count**	**Mean**	**Median**	**Min**	**Max**	**CV%**	**%Effect**	**ΣA/ΣB**	**Mean**	**%Effect**
0	N	10	0.9000	1.0000	0.0000	1.0000	35.14%	0.00%	9/10	0.9500	0.00%
1.6		10	0.9000	1.0000	0.0000	1.0000	35.14%	0.00%	9/10	0.9500	0.00%
3.1		9	1.0000	1.0000	1.0000	1.0000	0.00%	-11.11%	9/9	0.9500	0.00%
6.3		10	0.9000	1.0000	0.0000	1.0000	35.14%	0.00%	9/10	0.9500	0.00%
13		10	1.0000	1.0000	1.0000	1.0000	0.00%	-11.11%	10/10	0.9500	0.00%
25		10	0.9000	1.0000	0.0000	1.0000	35.14%	0.00%	9/10	0.9500	0.00%
50		10	1.0000	1.0000	1.0000	1.0000	0.00%	-11.11%	10/10	0.9500	0.00%
100		10	1.0000	1.0000	1.0000	1.0000	0.00%	-11.11%	10/10	0.9500	0.00%

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000
1.6		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000
3.1		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	
6.3		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000
13		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
50		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

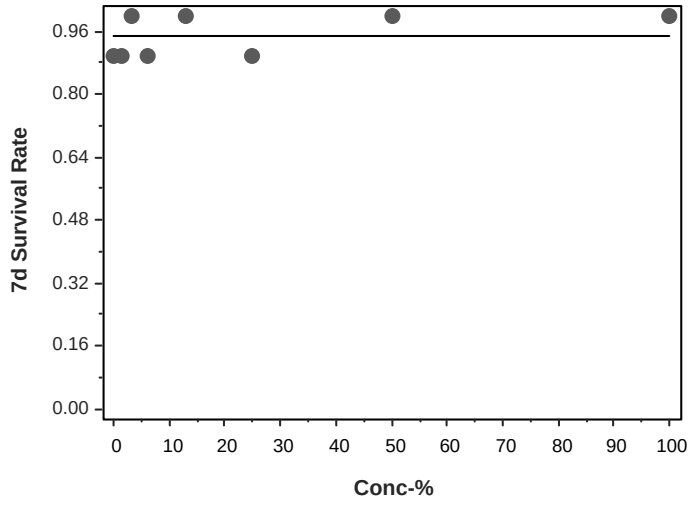
Ceriodaphnia 7-d Survival and Reproduction Test **Nautilus Environmental Calgary**

Analysis ID:	15-1202-1924	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 14:40	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	24 Nov-23 14:36	**MD5 Hash:**	4EBBDDFFE443A4FCEFDBFC5FD42295E	**Editor ID:**	

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1
1.6		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1
3.1		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
6.3		1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1
13		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
25		1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
50		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
100		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

Graphics



Ceriodaphnia 7-d Survival and Reproduction Test **Nautilus Environmental Calgary**

Analysis ID:	15-4687-7978	**Endpoint:**	Reproduction	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 14:43	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	24 Nov-23 14:36	**MD5 Hash:**	CEE7975C71DE964B70ED919BD8D3AA8	**Editor ID:**	
Batch ID:	21-3121-2308	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Lab Tech
Start Date:	08 Nov-23	**Protocol:**	EC EPS RM/21	**Diluent:**	
Ending Date:	15 Nov-23	**Species:**	Ceriodaphnia dubia	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Branchiopoda	**Source:**	In-House Culture **Age:** <24
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110
Sample Age:	48h (12.2 °C)	**Client:**	ALS Laboratories		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1717748	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

		TAC Limits			
Attribute	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
Control Resp	23.2	15	>>	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC15	0.9992	0.4798	4.575	100.1	21.9	208.4
IC20	1.518	0.6863	---	65.9	---	145.7
IC25	2.128	0.9217	---	47	---	108.5
IC40	>100	---	---	<1	---	---
IC50	>100	---	---	<1	---	---

Reproduction Summary

			Calculated Variate						Isotonic Variate	
Conc-%	**Code**	**Count**	**Mean**	**Median**	**Min**	**Max**	**CV%**	**%Effect**	**Mean**	**%Effect**
0	N	10	23.2	22.5	15	34	27.55%	0.00%	23.2	0.00%
1.6		10	18.4	15	0	32	56.72%	20.69%	18.4	20.69%
3.1		9	14.11	13	6	29	44.74%	39.18%	15.94	31.31%
6.3		10	17	18.5	7	24	36.89%	26.72%	15.94	31.31%
13		10	15.6	13	11	27	37.65%	32.76%	15.94	31.31%
25		10	8.8	8.5	0	19	56.39%	62.07%	15.94	31.31%
50		10	19	22	2	28	42.61%	18.10%	15.94	31.31%
100		10	21.1	22	10	28	24.42%	9.05%	15.94	31.31%

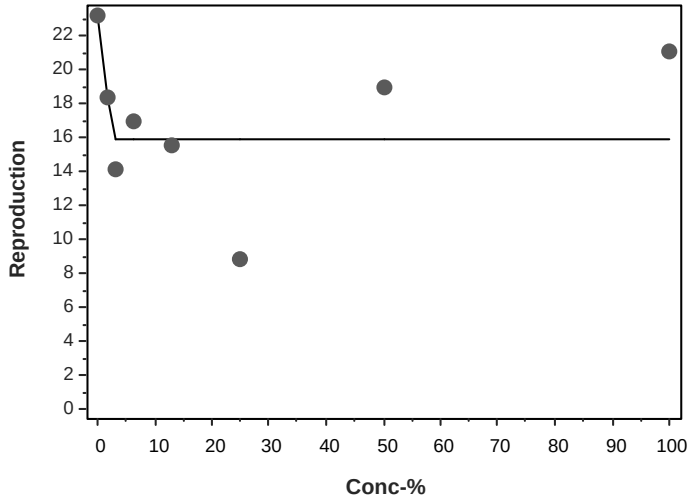
Reproduction Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	N	19	34	25	20	18	17	15	28	25	31
1.6		31	15	22	15	32	31	13	11	14	0
3.1		12	29	12	10	6	16	13	15	14	
6.3		18	19	8	22	20	17	11	24	7	24
13		25	11	17	12	13	13	11	11	27	16
25		19	9	0	11	8	8	12	10	6	5
50		21	20	12	2	24	24	23	11	28	25
100		22	17	21	25	28	22	10	18	26	22

Ceriodaphnia 7-d Survival and Reproduction Test **Nautilus Environmental Calgary**

Analysis ID:	15-4687-7978	**Endpoint:**	Reproduction	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 14:43	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	24 Nov-23 14:36	**MD5 Hash:**	CEE7975C71DE964B70ED919BD8D3AA8	**Editor ID:**	

Graphics



APPENDIX C – *Pimephales promelas* Toxicity Test Data

NAUTILUS ENVIRONMENTAL

Fathead Minnow Bench Sheet

Method FMD Client ALS 106 Reference 2324-0709-01

Test Log

Date	Day	Time	Technicians	Chem. Cart Used	Pail	Fed AM	Fed PM	Daily Data Review	Subsample Used	
2023-11-09	0	1330	BS/KCRM/ET/AB	MS 6	1	-	✓	AI	Day 0	✓
2023-11-10	1	1030	AB	6	1	✓	✓	AI	Day 1	✓
2023-11-11	2	1400	MS	6	1	✓	✓	AM	Day 2	✓
2023-11-12	3	1055	KF	6	1	✓	✓	NA	Day 3	✓
2023-11-13	4	1000	NP	6	1	✓	✓	NA	Day 4	✓
2023-11-14	5	1030	JK	6	1	✓	✓	CC	Day 5	✓
2023-11-15	6	1010	JK	6	3	✓	✓	BS	Day 6	✓
2023-11-16	7	1130	[illegible]	6	—	-	-	ZC	-	-

Sample Information

Initial pH: 7.8
Initial EC (µS/cm): 1733
Initial DO (mg/L): 8.9
Filtered with 60 µm nitex screen Yes/No
Sample pre-aerated/hardness/pH adjust: Yes/No
*if yes, describe procedure, rate and duration

Test Organisms

Fish Feeding Normally: Yes/No
Inflated Swim Bladders: Yes/No
Organism Source: AQUATOX AB DM
Batch Number: ~~2023/11/09~~ 20231105FM
Breeding Stock Mortality: <1%

Organisms upon receipt:

Mortality: <1%
Temperature (°C): 25
Dissolved Oxygen (mg/L): ~~7.7~~ 8.4
*temp. must be between 22 °C- 28°C

Biology (# of organisms alive and # of live organisms displaying atypical swimming behaviour per vessel)

conc. (%)	CTL	1.6	3.2	6.3	12	25	50	100
replicate	Day 1							
a	10	10	10	10	10	10	10	10
b			9	10	9			
c			10	9	9			
d			10	10	10			
	Day 2							
a	10	10	9	10	10	10	10	10
b	10	10	9	10	8	10	10	10
c	10	10	10	9	8	10	10	10
d	10	10	10	10	10	10	9	10
	Day 3							
a	10	10	9(1)	10	9	10	10	10
b			9	10	8		10	
c			10(1)	9	8		10	
d			10	10	10		9	
	Day 4							
a	10	10	8	10	9	10	9	10
b	10	10	9	10	8	10	10	10
c	10	10	10(1)	9	8	10	9	10
d	10	10	10	10	10	9	9	10

CTL	1.6	3.2	6.3	12	25	50	100
Day 5							
10	JK 9	8	10	8	10	9	9
10	10(1)	9	10	8	10	10	10
10	9	9(1)	8(1)	8	10	9	10
10	10	10	10	10	9	7	10
Day 6							
JK 10	9	8	10	8	10	9	9
10	10(1)	9	10	8	10	10	10
10	9	9(1)	8	8	10	8	10
10	10	10	10	10	9	7	9(1)
Day 7							
10	9	8	10	8	10	9	9
10	9	9	10	8	10	10	10
10	9	9(1)	8	8	10	8	10
10	10	10	10	10(1)	9	7	8

Comments:

Atypical Swimming Behaviour:

Unless otherwise noted, behavior is considered to be normal
Any fish that appear moribund (lethargic), display a loss of equilibrium or show atypical swimming behaviour

Scoring Convention: # alive (# atypically swimming)
e.g. 10 (4) indicates 10 alive but 4 swimming atypically in vessel
No bracketed # indicates no atypical swimming within test vessel

Reviewed By: DM Date Reviewed: NOV 2 2 2023

NAUTILUS ENVIRONMENTAL

Fathead Minnow Bench Sheet

Method FMD Client ALS 106 Reference 2324-0709-01

Chemistry

pH (units) (range: 6.5-8.5)

conc. (%) / day	New Solutions CTL	1.6	3.2	6.3	12	25	50	100	Old Solutions CTL	1.6	3.2	6.3	12	25	50	100
0	8.2	8.2	8.1	8.1	8.1	8.0	7.8	7.8	7.4	8.0	7.9	7.9	7.9	7.9	7.9	7.9
1	8.1	8.1	8.1	8.1	8.1	8.1	8.0	7.9	8.0	8.0	7.9	7.9	7.8	7.8	7.8	7.8
2	8.3	8.2	8.2	8.2	8.2	8.1	8.1	8.0	8.1	8.1	8.1	8.0	8.0	8.0	7.9	7.9
3	8.2	8.1	8.1	8.1	8.1	8.0	8.0	7.9	7.8	7.9	7.9	7.8	7.8	7.8	7.8	7.7
4	8.0	7.9	7.9	7.9	8.0	7.9	7.9	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.7	7.7
5	7.9	8.0	8.0	8.0	7.9	7.9	7.9	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.7	7.7
6	7.9	7.9	7.9	7.9	7.9	7.8	7.8	7.8	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.5
7																
8																

Conductivity (µS/cm)

conc. (%) / day	New Solutions CTL	1.6	3.2	6.3	12	25	50	100	Old Solutions CTL	1.6	3.2	6.3	12	25	50	100
0	496	468	492	545	628	799	1032	1670	435	481	503	555	639	783	1071	1643
1	440	457	476	511	593	1661	1061	1663	483	478	489	519	601	763	1078	1643
2	481	482	508	555	586	814	1126	1661	495	488	511	565	659	825	1149	1661
3	441	483	488	532	609	796	1094	1726	472	485	512	566	646	812	1141	1770
4	463	479	503	552	646	833	1145	1791	501	499	503	545	640	823	1129	1775
5	451	474	500	545	632	807	1129	1758	455	502	515	558	648	821	1147	1739
6	444	463	485	534	624	794	1125	1721	450	478	446	545	637	806	1123	1796
7																
8																

Dissolved Oxygen (mg/L) (40-100% saturation)

conc. (%) / day	New Solutions CTL	1.6	3.2	6.3	12	25	50	100	Old Solutions CTL	1.6	3.2	6.3	12	25	50	100
0	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	6.8	6.7	6.6	6.6	6.6	6.5	6.5
1	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	6.1	6.1	6.2	6.1	6.0	6.0	5.9	5.9
2	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	6.7	6.6	6.6	6.6	6.6	6.5	6.4	6.4
3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	6.4	6.5	6.6	6.5	6.5	6.5	6.5	6.5
4	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.1	6.9	6.7	6.6	6.6	6.7	6.8	6.6
5	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	6.9	6.9	6.9	6.9	7.0	7.0	7.0	7.0
6	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	5.6	6.1	6.4	6.4	6.4	6.5	6.5	6.4
7																
8																

Temperature 24 - 26 (°C)

conc. (%) / day	New Solutions CTL	1.6	3.2	6.3	12	25	50	100	Old Solutions CTL	1.6	3.2	6.3	12	25	50	100
0	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
1	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
2	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
3	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
4	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
5	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
6	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
7																
8																

DO Levels (40-100% saturation)* - 2.9 to 7.3 mg/L at 24°C 2.9 to 7.2 mg/L at 25°C 2.8 to 7.1 mg/L at 26°C

*corrected for altitude

Reviewed By: DM Date Reviewed: NOV 22 2023

NAUTILUS ENVIRONMENTAL

Organism Weights Bench Sheet

Client AES106 Sample 2324-0709 -01 Organism FHD Batch 20231109FM

	Item Weighed	Date	Initials	Balance*
Initial Weight (mg):	dried pan	2023/11/13	KT	#3
Final Weight (mg):	dried pan + organisms	2023/11/21	JK	#3

* same balance must be used for initial and final weights
* for FM/HA/CT, must use scale with 0.01 mg accuracy

Concentration

Replicate	CTL		1.6		3.2		6.3		12.5		25	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
a	401.61	406.78	390.89	395.85	399.70	403.53	402.01	406.62	405.85	409.75	404.49	409.09
b	395.82	401.29	406.03	411.65	392.05	396.68	399.00	403.25	397.51	401.81	389.72	395.11
c	392.38	397.31	398.26	403.39	398.78	404.64	402.85	407.28	403.35	407.83	403.10	408.37
d	399.26	405.26	403.96	409.60	406.02	411.12	392.65	398.38	396.80	402.49	391.06	396.71
e												

Concentration

Replicate	50		100									
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
a	405.45	409.98	399.91	404.69								
b	400.50	405.48	400.10	405.01								
c	393.52	398.21	391.46	397.58								
d	406.39	411.11	399.90	404.58								
e												

Balance Calibration Check:

	Initial	Final
first pan weighed:	CTL A	CTLA
weight of first pan:	401.61	406.78
first pan after all other pans weighed:	401.59	406.73
% difference <5%:	Yes/No	Yes/No

% difference = $\frac{\text{(initial weight - reweight)}}{\text{(initial weight + reweight) / 2)}} \times 100\%$

Test Validity Met: Yes/No/NA

Results are Logical:** Yes/No

** no negative numbers, consistent values across replicates

If "no" is circled for any parameter, notify Lab Supervisor/ QA Group to determine appropriate action

Reviewed By: DM Date Reviewed: NOV 22 2023

Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental Calgary**

Batch ID:	12-7081-1671	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech		
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/22	**Diluent:**	Dechlorinated Tap Water		
Ending Date:	16 Nov-23	**Species:**	Pimephales promelas	**Brine:**			
Test Length:	7d 0h	**Taxon:**	Actinopterygii	**Source:**	Aquatox, AR	**Age:**	<24

Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	72h (11.9 °C)	**Client:**	ALS Laboratories		

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	%	95% LCL	95% UCL	TU
17-5247-2336	7d Survival Rate	Linear Interpolation (ICPIN)	LC15	>100	---	---	<1
			LC20	>100	---	---	<1
			LC25	>100	---	---	<1
			LC40	>100	---	---	<1
			LC50	>100	---	---	<1
18-6363-9292	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	IC15	>100	---	---	<1
			IC20	>100	---	---	<1
			IC25	>100	---	---	<1
			IC40	>100	---	---	<1
			IC50	>100	---	---	<1

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
1.6		4	0.9250	0.8454	1.0050	0.9000	1.0000	0.0250	0.0500	5.41%	7.50%
3.2		4	0.9000	0.7701	1.0300	0.8000	1.0000	0.0408	0.0817	9.07%	10.00%
6.3		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	5.00%
12		4	0.8500	0.6909	1.0090	0.8000	1.0000	0.0500	0.1000	11.76%	15.00%
25		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%
50		4	0.8500	0.6446	1.0550	0.7000	1.0000	0.0646	0.1291	15.19%	15.00%
100		4	0.9250	0.7727	1.0770	0.8000	1.0000	0.0479	0.0957	10.35%	7.50%

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	0.5393	0.4658	0.6127	0.493	0.6	0.02307	0.04613	8.56%	0.00%
1.6		4	0.5337	0.4789	0.5886	0.496	0.564	0.01725	0.03449	6.46%	1.02%
3.2		4	0.4855	0.3501	0.6209	0.383	0.586	0.04254	0.08507	17.52%	9.97%
6.3		4	0.4755	0.3695	0.5815	0.425	0.573	0.03332	0.06664	14.01%	11.82%
12		4	0.4593	0.3366	0.5819	0.39	0.569	0.03854	0.07708	16.78%	14.84%
25		4	0.5315	0.4534	0.6096	0.46	0.565	0.02453	0.04906	9.23%	1.44%
50		4	0.473	0.4433	0.5027	0.453	0.498	0.009318	0.01864	3.94%	12.29%
100		4	0.5122	0.4054	0.6191	0.468	0.612	0.03358	0.06716	13.11%	5.01%

Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental Calgary**

7d Survival Rate Detail MD5: 62414796115F71A87BDBA461341A6E2A

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	1.0000	1.0000	1.0000	1.0000
1.6		0.9000	0.9000	0.9000	1.0000
3.2		0.8000	0.9000	0.9000	1.0000
6.3		1.0000	1.0000	0.8000	1.0000
12		0.8000	0.8000	0.8000	1.0000
25		1.0000	1.0000	1.0000	0.9000
50		0.9000	1.0000	0.8000	0.7000
100		0.9000	1.0000	1.0000	0.8000

Mean Dry Biomass-mg Detail MD5: F028073095FC4B208BFBF2E03F025DF7

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	0.517	0.547	0.493	0.6
1.6		0.496	0.562	0.513	0.564
3.2		0.383	0.463	0.586	0.51
6.3		0.461	0.425	0.443	0.573
12		0.39	0.43	0.448	0.569
25		0.46	0.539	0.562	0.565
50		0.453	0.498	0.469	0.472
100		0.478	0.491	0.612	0.468

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	10/10	10/10	10/10	10/10
1.6		9/10	9/10	9/10	10/10
3.2		8/10	9/10	9/10	10/10
6.3		10/10	10/10	8/10	10/10
12		8/10	8/10	8/10	10/10
25		10/10	10/10	10/10	9/10
50		9/10	10/10	8/10	7/10
100		9/10	10/10	10/10	8/10

Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental Calgary**

Analysis ID:	17-5247-2336	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:18	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	62414796115F71A87BDBA461341A6E2A	**Editor ID:**	002-704-782-4
Batch ID:	12-7081-1671	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/22	**Diluent:**	Dechlorinated Tap Water
Ending Date:	16 Nov-23	**Species:**	Pimephales promelas	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Actinopterygii	**Source:**	Aquatox, AR **Age:** <24
Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	72h (11.9 °C)	**Client:**	ALS Laboratories		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	297735	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
LC15	>100	---	---	<1	---	---
LC20	>100	---	---	<1	---	---
LC25	>100	---	---	<1	---	---
LC40	>100	---	---	<1	---	---
LC50	>100	---	---	<1	---	---

7d Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	N	4	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	40/40	1.0000	0.00%
1.6		4	0.9250	0.9000	0.9000	1.0000	5.41%	7.50%	37/40	0.9250	7.50%
3.2		4	0.9000	0.9000	0.8000	1.0000	9.07%	10.00%	36/40	0.9250	7.50%
6.3		4	0.9500	1.0000	0.8000	1.0000	10.53%	5.00%	38/40	0.9250	7.50%
12		4	0.8500	0.8000	0.8000	1.0000	11.76%	15.00%	34/40	0.9125	8.75%
25		4	0.9750	1.0000	0.9000	1.0000	5.13%	2.50%	39/40	0.9125	8.75%
50		4	0.8500	0.8500	0.7000	1.0000	15.19%	15.00%	34/40	0.8875	11.25%
100		4	0.9250	0.9500	0.8000	1.0000	10.35%	7.50%	37/40	0.8875	11.25%

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	1.0000	1.0000	1.0000	1.0000
1.6		0.9000	0.9000	0.9000	1.0000
3.2		0.8000	0.9000	0.9000	1.0000
6.3		1.0000	1.0000	0.8000	1.0000
12		0.8000	0.8000	0.8000	1.0000
25		1.0000	1.0000	1.0000	0.9000
50		0.9000	1.0000	0.8000	0.7000
100		0.9000	1.0000	1.0000	0.8000

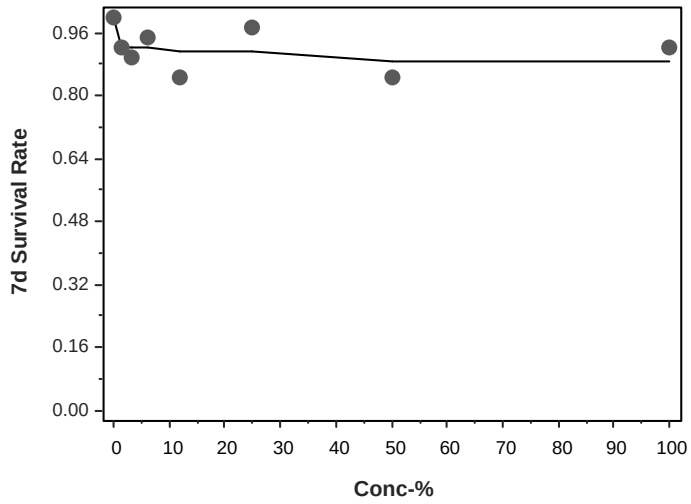
7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	10/10	10/10	10/10	10/10
1.6		9/10	9/10	9/10	10/10
3.2		8/10	9/10	9/10	10/10
6.3		10/10	10/10	8/10	10/10
12		8/10	8/10	8/10	10/10
25		10/10	10/10	10/10	9/10
50		9/10	10/10	8/10	7/10
100		9/10	10/10	10/10	8/10

Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental Calgary**

Analysis ID:	17-5247-2336	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:18	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	62414796115F71A87BDBA461341A6E2A	**Editor ID:**	002-704-782-4

Graphics



Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental Calgary**

Analysis ID:	18-6363-9292	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:20	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	F028073095FC4B208BFBF2E03F025DF7	**Editor ID:**	002-704-782-4
Batch ID:	12-7081-1671	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/22	**Diluent:**	Dechlorinated Tap Water
Ending Date:	16 Nov-23	**Species:**	Pimephales promelas	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Actinopterygii	**Source:**	Aquatox, AR **Age:** <24
Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	72h (11.9 °C)	**Client:**	ALS Laboratories		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1985551	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC15	>100	---	---	<1	---	---
IC20	>100	---	---	<1	---	---
IC25	>100	---	---	<1	---	---
IC40	>100	---	---	<1	---	---
IC50	>100	---	---	<1	---	---

Mean Dry Biomass-mg Summary

			Calculated Variate						Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	N	4	0.5392	0.532	0.493	0.6	8.56%	0.00%	0.5392	0.00%
1.6		4	0.5338	0.5375	0.496	0.564	6.46%	1.02%	0.5338	1.02%
3.2		4	0.4855	0.4865	0.383	0.586	17.52%	9.97%	0.4895	9.23%
6.3		4	0.4755	0.452	0.425	0.573	14.01%	11.82%	0.4895	9.23%
12		4	0.4592	0.439	0.39	0.569	16.78%	14.84%	0.4895	9.23%
25		4	0.5315	0.5505	0.46	0.565	9.23%	1.44%	0.4895	9.23%
50		4	0.473	0.4705	0.453	0.498	3.94%	12.29%	0.4895	9.23%
100		4	0.5122	0.4845	0.468	0.612	13.11%	5.01%	0.4895	9.23%

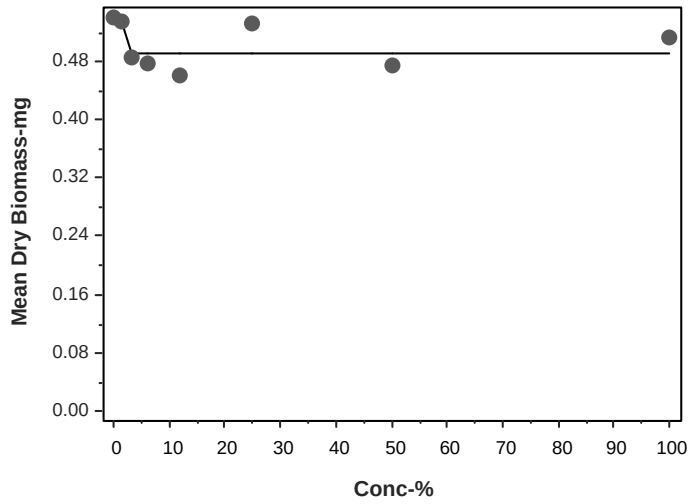
Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	0.517	0.547	0.493	0.6
1.6		0.496	0.562	0.513	0.564
3.2		0.383	0.463	0.586	0.51
6.3		0.461	0.425	0.443	0.573
12		0.39	0.43	0.448	0.569
25		0.46	0.539	0.562	0.565
50		0.453	0.498	0.469	0.472
100		0.478	0.491	0.612	0.468

Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental Calgary**

Analysis ID:	18-6363-9292	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:20	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	F028073095FC4B208BFBF2E03F025DF7	**Editor ID:**	002-704-782-4

Graphics



NAUTILUS ENVIRONMENTAL

Fathead Minnow Bench Sheet

Method FMD Client ALS 106 Reference 2324-0709-02

Test Log

Date	Day	Time	Technicians	Chem. Cart Used	Pail	Fed AM	Fed PM	Daily Data Review	Subsample Used	
2023-11-09	0	1330	BS/KCRM/ET/AB	MS 6	1	-	✓	AB	Day 0	✓
2023-11-10	1	1430	ABS	6	1	✓	✓	AB	Day 1	✓
2023-11-11	2	1415	AM	6	1	✓	✓	JC	Day 2	✓
2023-11-12	3	0935	KZ	6	1	✓	✓	NA	Day 3	✓
2023-11-13	4	0930	NP	6	1	✓	✓	NA	Day 4	✓
2023-11-14	5	1100	JK	6	1	✓	✓	CC	Day 5	✓
2023-11-15	6	1040	JK	6	3	✓	✓	BS	Day 6	✓
2023-11-16	7	1015	BS	6	-	-	-	JC	-	-

Sample Information

Initial pH: 7.9
Initial EC (µS/cm): 1728
Initial DO (mg/L): 9.0
Filtered with 60 µm nitex screen Yes/No
Sample pre-aerated/hardness/pH adjust: Yes/No
*if yes, describe procedure, rate and duration

Test Organisms

Fish Feeding Normally: Yes/No
Inflated Swim Bladders: Yes/No
Organism Source: Aquatox AB
Batch Number: ~~2023/11/08~~ 2023 11 08 FM DM
Breeding Stock Mortality: <1%

Organisms upon receipt:

Mortality: <1%
Temperature (°C): 25
Dissolved Oxygen (mg/L): 8.4
*temp. must be between 22 °C- 28°C

Biology (# of organisms alive and # of live organisms displaying atypical swimming behaviour per vessel)

Day 1

conc. (%)	CTL	1.6	3.2	6.3	12	25	50	100
a	10	10	10	10	10	10	10	10
b	↓	↓	10	~~10~~	↓	↓	↓	↓
c	↓	↓	9	DM ~~12~~	↓	↓	↓	↓
d	↓	↓	10	~~10~~	↓	↓	↓	↓

Day 2

a	10	10(1)	10	10(1)	10	10	10	10(1)
b	↓	10	10	10	↓	10	9	10
c	↓	↓	9	*12	↓	10(1)	10	9
d	↓	↓	10	10	↓	9	10	10

Day 3

a	10	10(1)	10	10(1)	10	10	10	10(1)
b	10	10	10	10	9	10(1)	9	10
c	10	10	8	12	10	10(1)	10	9(1)
d	10(1)	10	10	10	10	9	9	10

Day 4

a	10	10(1)	10	10(1)	10	9	9	9
b	10	9	10	10	9	9	9	10
c	10	9	8	12	10	10(1)	10	8
d	10(1)	10	10	10	10	9	9	10

Day 5

CTL	1.6	3.2	6.3	12	25	50	100
10	10(1)	10	~~9~~ 10	9	JK ~~10~~ 9	8	7
10	9	10	~~9~~ 10	9	8 ~~10~~ 9(1)	8	10
10	8	8	~~9~~ 12	9	10 ~~12~~ 9	10	8
9	10	10	10	10	9	9	10(1)

Day 6

10	10(1)	10	10(1)	9	JK ~~8~~ 9	8	7
10	9	10	10	9	8	8	10
10	8	8	12(1)	9	10	10	7
9	10	10	10	10(1)	9	9	9

Day 7

10	9	10	9	9	9	8	7
10	9	10	10	9	8	8	10
10	8	8	12	8	10	10	7(1)
9	10	10	10	10(1)	9	9	9

Comments:
*12 in col (6.3 C day 2) - mislead

Atypical Swimming Behaviour:

Unless otherwise noted, behavior is considered to be normal
Any fish that appear moribund (lethargic), display a loss of equilibrium or show atypical swimming behaviour

Scoring Convention: # alive (# atypically swimming)
e.g. 10 (4) indicates 10 alive but 4 swimming atypically in vessel
No bracketed # indicates no atypical swimming within test vessel

Reviewed By: DM Date Reviewed: NOV 2 2 2023

NAUTILUS ENVIRONMENTAL

Fathead Minnow Bench Sheet

Method FMD Client ALS 106 Reference 2324-0709-02

Chemistry

pH (units) (range: 6.5-8.5)

conc. (%) / day	New Solutions CTL	1.6	3.2	6.3	12	25	50	100	Old Solutions CTL	1.6	3.2	6.3	12	25	50	100
0	8.1	8.1	8.1	8.1	8.1	8.0	8.0	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.7	7.8
1	7.6	7.9	8.0	8.0	8.0	7.9	7.9	7.8	7.9	7.8	7.8	7.8	7.7	7.7	7.8	7.7
2	7.8	8.0	8.0	8.1	8.1	8.0	7.9	7.8	7.8	7.8	7.7	7.7	7.7	7.7	7.7	7.7
3	8.1	8.0	8.0	8.0	8.0	8.0	7.9	7.8	7.9	7.9	7.9	7.9	7.9	7.8	7.8	7.7
4	8.0	8.0	8.0	8.0	8.0	7.9	7.9	7.8	7.8	7.8	7.8	7.8	7.7	7.8	7.8	7.7
5	8.2	8.1	8.1	8.1	8.0	8.0	7.9	7.8	7.8	7.8	7.8	7.8	7.7	7.8	7.7	7.7
6	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.7	7.5	7.5	7.5	7.6	7.6	7.5	7.5	7.5
7																
8																

Conductivity (µS/cm)

0	451	453	458	489	557	683	959	1634	446	461	474	503	563	690	970	1586
1	446	456	466	498	573	719	1025	1638	476	468	475	513	579	721	1029	1654
2	446	461	481	526	614	781	1087	1659	444	467	484	543	618	774	1081	1665
3	456	472	495	542	626	784	1099	1572	499	505	511	560	656	831	1144	1775
4	451	472	493	542	631	816	1140	1743	473	487	511	560	651	832	1146	1776
5	464	475	494	534	620	787	1115	1628	470	498	357560		650	829	1160	1809
6	450	464	489	533	629	809	1133	1793	447	469	492	546	632	813	1134	1703
7																
8																

Dissolved Oxygen (mg/L) (40-100% saturation)

0	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	6.9	6.6	6.7	6.7	6.7	6.7
1	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	6.7	6.7	6.6	6.5	6.3	6.3	6.3	6.4
2	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.9	6.7	6.7	6.7	6.6	6.6	6.6	6.6	6.6
3	7.3	7.3	7.3	7.3	7.3	7.3	7.9	8.2	6.7	6.8	6.8	6.7	6.7	6.6	6.6	6.6
4	7.3	7.3	7.3	7.3	7.3	7.3	7.8	8.0	6.3	6.7	6.6	6.6	6.5	6.6	6.7	6.7
5	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.1	7.0	7.1	7.1	6.8	6.9	6.9	6.9
6	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	6.2	6.4	6.5	6.5	6.6	6.5	6.4	6.4
7																
8																

Temperature 24 - 26 (°C)

0	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
1	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
2	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
3	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
4	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
5	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
6	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
7																
8																

DO Levels (40-100% saturation)* - 2.9 to 7.3 mg/L at 24°C 2.9 to 7.2 mg/L at 25°C 2.8 to 7.1 mg/L at 26°C

*corrected for altitude

Reviewed By: DM Date Reviewed: NOV 2 2 2023

NAUTILUS ENVIRONMENTAL

Organism Weights Bench Sheet

Client ALS106 Sample 2324-0709-02 Organism FHM Batch 20231108FM

	Item Weighed	Date	Initials	Balance*
Initial Weight (mg):	dried pan	2023/11/13	KZ	#3
Final Weight (mg):	dried pan + organisms	2023/11/19	DK	#3

* same balance must be used for initial and final weights
* for FM/HA/CT, must use scale with 0.01 mg accuracy

Concentration

Replicate	CTL Initial	CTL Final	1.6 Initial	1.6 Final	3.2 Initial	3.2 Final	6.3 Initial	6.3 Final	12.5 Initial	12.5 Final	25 Initial	25 Final
a	421.13	426.59	421.55	426.44	423.98	429.02	419.78	424.49	420.17	424.90	423.32	427.36
b	424.39	429.22 17	424.22	428.41	422.09	427.65	426.64	431.85	419.80	424.41	422.32	426.54
c	424.21	428.56	425.73	430.14	424.83	429.93	425.94	431.05	420.99	425.32	418.77	424.13
d	419.52	424.07	424.91	429.78	421.59	427.19	419.92	425.82	419.82	424.61	423.86	428.80
e												

Concentration

Replicate	50 Initial	50 Final	100 Initial	100 Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
a	420.45	425.35	420.16	423.82								
b	423.28	427.79	420.09	425.03								
c	418.16	423.26	427.48	431.42								
d	425.87	431.19	425.16	430.46								
e												

Balance Calibration Check:

	Initial	Final
first pan weighed:	CTL A	CTL A
weight of first pan:	421.13	426.59
first pan after all other pans weighed:	421.12	426.58
% difference <5%:	Yes/No	Yes/No

Test Validity Met: Yes/No/NA

Results are Logical:** Yes/No

** no negative numbers, consistent values across replicates

$$\% \text{ difference} = \frac{(\text{initial weight} - \text{reweight})}{(\text{initial weight} + \text{reweight}) / 2)} \times 100\%$$

If "no" is circled for any parameter, notify Lab Supervisor/ QA Group to determine appropriate action

Reviewed By: D.M Date Reviewed: NOV 22 2023

Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental Calgary**

Batch ID:	21-2511-6637	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech		
Start Date:	09 Nov-23	**Protocol:**	EC EPS RM/22	**Diluent:**	Dechlorinated Tap Water		
Ending Date:	16 Nov-23	**Species:**	Pimephales promelas	**Brine:**			
Test Length:	7d 0h	**Taxon:**	Actinopterygii	**Source:**	Aquatox, AR	**Age:**	<24
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**			
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories		
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110		
Sample Age:	72h (12.2 °C)	**Client:**	ALS Laboratories				

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	%	95% LCL	95% UCL	TU
17-3462-4421	7d Survival Rate	Linear Interpolation (ICPIN)	LC15	94.95	6.614	---	1.1
			LC20	>100	---	---	<1
			LC25	>100	---	---	<1
			LC40	>100	---	---	<1
			LC50	>100	---	---	<1
07-6322-3612	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	IC15	>100	---	---	<1
			IC20	>100	---	---	<1
			IC25	>100	---	---	<1
			IC40	>100	---	---	<1
			IC50	>100	---	---	<1

Test Acceptability

				TAC Limits			
Analysis ID	**Endpoint**	**Attribute**	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
17-3462-4421	7d Survival Rate	Control Resp	0.975	0.8	>>	Yes	Passes Criteria
07-6322-3612	Mean Dry Biomass-mg	Control Resp	0.4785	0.25	>>	Yes	Passes Criteria

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
1.6		4	0.9000	0.7701	1.0300	0.8000	1.0000	0.0408	0.0817	9.07%	7.69%
3.2		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	2.56%
6.3		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
12		4	0.9000	0.7701	1.0300	0.8000	1.0000	0.0408	0.0817	9.07%	7.69%
25		4	0.9000	0.7701	1.0300	0.8000	1.0000	0.0408	0.0817	9.07%	7.69%
50		4	0.8750	0.7227	1.0270	0.8000	1.0000	0.0479	0.0957	10.94%	10.26%
100		4	0.8250	0.5863	1.0640	0.7000	1.0000	0.0750	0.1500	18.18%	15.38%

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	0.4785	0.4016	0.5554	0.435	0.546	0.02415	0.04831	10.10%	0.00%
1.6		4	0.459	0.4038	0.5142	0.419	0.489	0.01734	0.03468	7.56%	4.08%
3.2		4	0.5325	0.4854	0.5796	0.504	0.56	0.0148	0.02959	5.56%	-11.29%
6.3		4	0.502	0.3899	0.614	0.4258	0.59	0.0352	0.0704	14.02%	-4.90%
12		4	0.4615	0.429	0.494	0.433	0.479	0.01021	0.02042	4.42%	3.55%
25		4	0.464	0.3657	0.5623	0.404	0.536	0.03089	0.06177	13.31%	3.03%
50		4	0.4958	0.441	0.5505	0.451	0.532	0.01721	0.03441	6.94%	-3.61%
100		4	0.446	0.3212	0.5708	0.366	0.53	0.03923	0.07845	17.59%	6.79%

Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental Calgary**

7d Survival Rate Detail MD5: 3D9E5BB985F8EC277FA14BFB8E54D9F3

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	1.0000	1.0000	1.0000	0.9000
1.6		0.9000	0.9000	0.8000	1.0000
3.2		1.0000	1.0000	0.8000	1.0000
6.3		0.9000	1.0000	1.0000	1.0000
12		0.9000	0.9000	0.8000	1.0000
25		0.9000	0.8000	1.0000	0.9000
50		0.8000	0.8000	1.0000	0.9000
100		0.7000	1.0000	0.7000	0.9000

Mean Dry Biomass-mg Detail MD5: EFEC5E269A20F2802857482075F14AF5

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	0.546	0.478	0.435	0.455
1.6		0.489	0.419	0.441	0.487
3.2		0.504	0.556	0.51	0.56
6.3		0.471	0.521	0.4258	0.59
12		0.473	0.461	0.433	0.479
25		0.404	0.422	0.536	0.494
50		0.49	0.451	0.51	0.532
100		0.366	0.494	0.394	0.53

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	10/10	10/10	10/10	9/10
1.6		9/10	9/10	8/10	10/10
3.2		10/10	10/10	8/10	10/10
6.3		9/10	10/10	12/12	10/10
12		9/10	9/10	8/10	10/10
25		9/10	8/10	10/10	9/10
50		8/10	8/10	10/10	9/10
100		7/10	10/10	7/10	9/10

Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental Calgary**

Analysis ID:	17-3462-4421	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:15	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	3D9E5BB985F8EC277FA14BFB8E54D9F3	**Editor ID:**	002-704-782-4
Batch ID:	21-2511-6637	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech
Start Date:	09 Nov-23	**Protocol:**	EC EPS RM/22	**Diluent:**	Dechlorinated Tap Water
Ending Date:	16 Nov-23	**Species:**	Pimephales promelas	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Actinopterygii	**Source:**	Aquatox, AR **Age:** <24
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110
Sample Age:	72h (12.2 °C)	**Client:**	ALS Laboratories		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1783064	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

		TAC Limits			
Attribute	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
Control Resp	0.975	0.8	>>	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
LC15	94.95	6.614	---	1.1	---	15.1
LC20	>100	---	---	<1	---	---
LC25	>100	---	---	<1	---	---
LC40	>100	---	---	<1	---	---
LC50	>100	---	---	<1	---	---

7d Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	**Code**	**Count**	**Mean**	**Median**	**Min**	**Max**	**CV%**	**%Effect**	**ΣA/ΣB**	**Mean**	**%Effect**
0	N	4	0.9750	1.0000	0.9000	1.0000	5.13%	0.00%	39/40	0.9750	0.00%
1.6		4	0.9000	0.9000	0.8000	1.0000	9.07%	7.69%	36/40	0.9417	3.42%
3.2		4	0.9500	1.0000	0.8000	1.0000	10.53%	2.56%	38/40	0.9417	3.42%
6.3		4	0.9750	1.0000	0.9000	1.0000	5.13%	0.00%	41/42	0.9417	3.42%
12		4	0.9000	0.9000	0.8000	1.0000	9.07%	7.69%	36/40	0.9000	7.69%
25		4	0.9000	0.9000	0.8000	1.0000	9.07%	7.69%	36/40	0.9000	7.69%
50		4	0.8750	0.8500	0.8000	1.0000	10.94%	10.26%	35/40	0.8750	10.26%
100		4	0.8250	0.8000	0.7000	1.0000	18.18%	15.38%	33/40	0.8250	15.38%

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	1.0000	1.0000	1.0000	0.9000
1.6		0.9000	0.9000	0.8000	1.0000
3.2		1.0000	1.0000	0.8000	1.0000
6.3		0.9000	1.0000	1.0000	1.0000
12		0.9000	0.9000	0.8000	1.0000
25		0.9000	0.8000	1.0000	0.9000
50		0.8000	0.8000	1.0000	0.9000
100		0.7000	1.0000	0.7000	0.9000

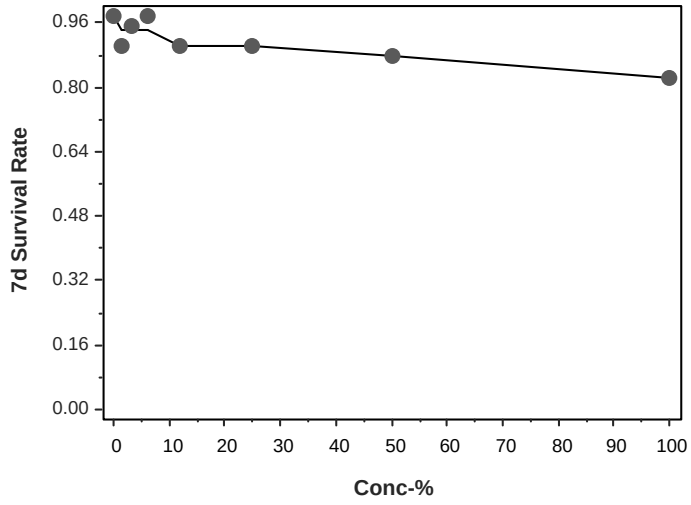
Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental Calgary**

Analysis ID:	17-3462-4421	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:15	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	3D9E5BB985F8EC277FA14BFB8E54D9F3	**Editor ID:**	002-704-782-4

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	10/10	10/10	10/10	9/10
1.6		9/10	9/10	8/10	10/10
3.2		10/10	10/10	8/10	10/10
6.3		9/10	10/10	12/12	10/10
12		9/10	9/10	8/10	10/10
25		9/10	8/10	10/10	9/10
50		8/10	8/10	10/10	9/10
100		7/10	10/10	7/10	9/10

Graphics



Fathead Minnow 7-d Larval Survival and Growth Test — **Nautilus Environmental Calgary**

Analysis ID:	07-6322-3612	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:16	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	EFEC5E269A20F2802857482075F14AF5	**Editor ID:**	002-704-782-4
Batch ID:	21-2511-6637	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Lab Tech
Start Date:	09 Nov-23	**Protocol:**	EC EPS RM/22	**Diluent:**	Dechlorinated Tap Water
Ending Date:	16 Nov-23	**Species:**	Pimephales promelas	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Actinopterygii	**Source:**	Aquatox, AR **Age:** <24
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110
Sample Age:	72h (12.2 °C)	**Client:**	ALS Laboratories		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1046362	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

		TAC Limits			
Attribute	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
Control Resp	0.4785	0.25	>>	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC15	>100	---	---	<1	---	---
IC20	>100	---	---	<1	---	---
IC25	>100	---	---	<1	---	---
IC40	>100	---	---	<1	---	---
IC50	>100	---	---	<1	---	---

Mean Dry Biomass-mg Summary

			Calculated Variate						Isotonic Variate	
Conc-%	**Code**	**Count**	**Mean**	**Median**	**Min**	**Max**	**CV%**	**%Effect**	**Mean**	**%Effect**
0	N	4	0.4785	0.4665	0.435	0.546	10.10%	0.00%	0.493	0.00%
1.6		4	0.459	0.464	0.419	0.489	7.56%	4.08%	0.493	0.00%
3.2		4	0.5325	0.533	0.504	0.56	5.56%	-11.29%	0.493	0.00%
6.3		4	0.502	0.496	0.4258	0.59	14.02%	-4.90%	0.493	0.00%
12		4	0.4615	0.467	0.433	0.479	4.42%	3.55%	0.4738	3.90%
25		4	0.464	0.458	0.404	0.536	13.31%	3.03%	0.4738	3.90%
50		4	0.4958	0.5	0.451	0.532	6.94%	-3.61%	0.4738	3.90%
100		4	0.446	0.444	0.366	0.53	17.59%	6.79%	0.446	9.53%

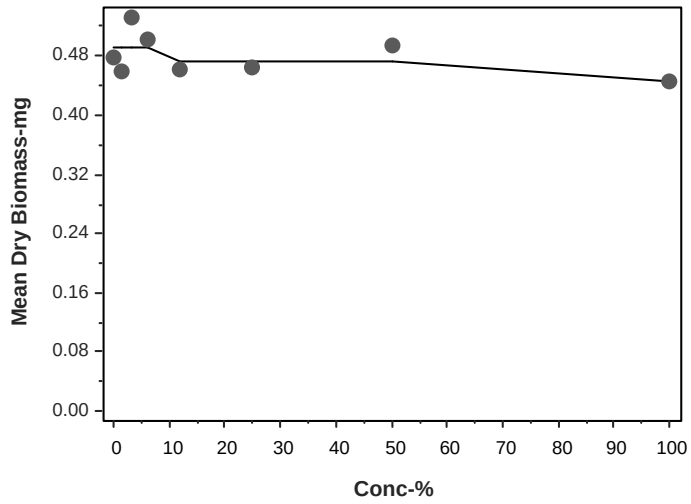
Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	0.546	0.478	0.435	0.455
1.6		0.489	0.419	0.441	0.487
3.2		0.504	0.556	0.51	0.56
6.3		0.471	0.521	0.4258	0.59
12		0.473	0.461	0.433	0.479
25		0.404	0.422	0.536	0.494
50		0.49	0.451	0.51	0.532
100		0.366	0.494	0.394	0.53

Fathead Minnow 7-d Larval Survival and Growth Test **Nautilus Environmental Calgary**

Analysis ID:	07-6322-3612	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:16	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	EFEC5E269A20F2802857482075F14AF5	**Editor ID:**	002-704-782-4

Graphics



APPENDIX D – *Lemna minor* Toxicity Test Data

NAUTILUS ENVIRONMENTAL

Lemna Test Bench Sheet

Method LMD Client ALS106 Reference 2324-0709-01

Test Log

Date	Day	Time	Initial	Rotate	Light Level* (**4000-5600**)	Temperature (°C) (range: **23-27°C**) Control	3%	24%	97%	Daily Data Review
2023-11-09	0	1240	DM	-	5000	24	→	→	→	AT
2023-11-10	1	0830	EJ	✓	4800	24	→	→	→	AL
2023-11-11	2	0810	KZ	✓	4990	24	→	→	→	AM
2023-11-12	3	0755	KZ	✓	5250	24	→	→	→	NA
2023-11-13	4	0810	PK	✓	5140	24	→	→	→	NA
2023-11-14	5	0820	PK	✓	4710	24	→	→	→	AL
2023-11-15	6	0830	EJ	✓	4570	24	→	→	→	AL
2023-11-16	7	0820	KC	—	4810	24	→	→	→	DM

*taken at centre

Sample Information

Initial pH: 7.8
Initial EC (µS/cm): 1733
Initial DO (mg/L): 8.9
Initial Temp (°C): 17

Biology (Number of Fronds)

Concentration (%) / Replicate	ctl	1.5	3.0	6.1	12.1	24.25	48.5	97
	day 0							
a	6	6	6	6	6	6	6	6
b	6	6	6	6	6	6	6	6
c	6	6	6	6	6	6	6	6
d	6	6	6	6	6	6	6	6
	day 7							
a	49	37	35	37	39	32	35	29
b	51	36	44	36	35	31	31	26
c	44	42	45	36	42	28	32	24
d	49	40	36	40	44	37	29	30

Validity Criteria: Controls must have a ≥ 8 fold increase in frond number (average of 48)

Appearance of Fronds

Concentration (%)	Day 0	Day 7
Control	Medium green, short roots, all healthy	light/med green, long roots, all healthy.
1.5	Medium green, short roots, all healthy	light/med green, long roots, green algae contamination
3.0	Medium green, short roots, all healthy	
6.1	Medium green, short roots, all healthy	
12.1	Medium green, short roots, all healthy	
24.3	Medium green, short roots, all healthy	
48.5	Medium green, short roots, all healthy	
97	Medium green, short roots, all healthy	↓ more algae as concentration increases.

Notes: Chl=chlorosis, Nec=necorsis, Yel=yellow fronds, Asf=abnormally sized fronds, Gib=gibbosity, col dest=conlony destruction, root dest=root destruction, Lob=loss of buoyancy

Reviewed By: DM Date Reviewed: NOV 22 2023

NAUTILUS ENVIRONMENTAL

Lemna Test Bench Sheet

Method LMD Client ALS106 Reference 2324-0709-01

Chem. Cart Used
Day 0: 2
Day 7: 2

Chemistry

Concentration (%)	ctl	1.5	3.0	6.1	12.1	24.25	48.5	97
New Solutions (day 0)								
pH (units) (range: 6.5-9.5)	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.0
EC (uS/cm)	762	846	889	943	1036	1217	1547	2246
DO (mg/L)	7.3	7.4	7.4	7.4	7.5	7.5	7.5	7.5
Temperature (°C) (25±2)	23	23	23	23	23	23	23	23
Old Solutions (day 7)								
pH (units) (range: 6.5-9.5)	7.8	9.2	9.4	9.5	9.4	9.5	9.5	9.1
EC (uS/cm)	960	1038	1070	1140	1259	1423	1802	2590
DO (mg/L)	7.9	12.0	13.2	13.8	13.4	15.1	17.7	16.9
Temperature (°C) (25±2)	23	23	23	23	23	23	23	23

Comments:

Quality Assurance

Organism batch (date culture was started): 20231030MoN

Age of test organisms (7-10d): 10d

Light levels of test area (4000-5600 lux) measured at sample surface: left 4970, center 5000, right 4250

Test organisms were acclimated for 18-24 hours in APHA media and appeared healthy before use: (Yes)/No

Average number of fronds in QA cups at turnover (A to C): 32

Fold Increase in culture prior to testing: 11X

Sample Treatment

supplemented with nutrients	(Y)/ N	
pH adjusted	Y / (N)	if Y, how & to what?
filtered	Y / (N)	if Y, how & what size?
preaerated	(Y) / N	if Y, how long and at what rate*?
		*must be < 100 bubbles/minute for 20 minutes

Dilution Water Treatment

pH adjusted to 8.3±0.1	(Y)/ N	
preaerated	(Y) / N	if Y, how long and at what rate*?
		*must be < 100 bubbles/minute for ≥2 hours

Other

Reviewed By: DM Date Reviewed: NOV 22 2023

NAUTILUS ENVIRONMENTAL

Organism Weights Bench Sheet

Client AIS106 Sample 2324-0709-01 Organism LMD Batch 2023/030MON

	Item Weighed	Date	Initials	Balance*
Initial Weight (mg):	dried pan	23/11/14	MS	3
Final Weight (mg):	dried pan + organisms	23/11/19	PK	Mettler #3

* same balance must be used for initial and final weights
* for FM/HA/CT, must use scale with 0.01 mg accuracy

Concentration

Replicate	CTL		1.5		3.0		6.1		12.1		24.3	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
a	800.56	805.76	777.60	782.91	851.17	856.31	803.92	809.29	805.25	810.80	806.61	811.41
b	794.78	800.72	842.69	847.97 PK	853.03	858.82	813.87	818.89	806.64	811.846	807.12	812.38
c	810.26	816.82	810.57	816.84	795.73	801.62	791.33	796.84	802.67	808.34	813.96	818.50
d	792.03	797.49 PK	842.33	847.71	808.62	813.98	802.77	807.99	813 mo	820.11	808.38	813.95
e		50							814.10			

Concentration

Replicate	48.5		97									
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
a	804.87	810.30	806.78	812.04 PK								
b	783.71	799.02	800.27	805.28 36								
c	776.75	781.71	800.93	809.94								
d	784.99	789.878	806.78	812.15 20								
e		PK		PK								

Balance Calibration Check:

	Initial	Final
first pan weighed:	CTLA	CTLA
weight of first pan:	800.56	805.76
first pan after all other pans weighed:	800.48	805.78

% difference <5%: (Yes)/No (Yes)/No

Test Validity Met: (Yes)/No/NA

Results are Logical:** (Yes)/No

** no negative numbers, consistent values across replicates

$$\% \text{ difference} = \frac{(\text{initial weight} - \text{reweight})}{(\text{initial weight} + \text{reweight}) / 2)} \times 100\%$$

If "no" is circled for any parameter, notify Lab Supervisor/ QA Group to determine appropriate action

Reviewed By: DM Date Reviewed: NOV 22 2023

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Batch ID:	15-0532-6592	**Test Type:**	Lemna Growth	**Analyst:**	Lab Tech		
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	Lemna Test Media		
Ending Date:	16 Nov-23	**Species:**	Lemna minor	**Brine:**			
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	In-House Culture	**Age:**	10

Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	72h (11.9 °C)	**Client:**	ALS Laboratories		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	PMSD	TU
10-5700-3974	Frond Count	Dunnett Multiple Comparison Test	97	>97	---	14.0%	1
01-0658-6575	Total Dry Weight-mg	Dunnett Multiple Comparison Test	97	>97	---	13.3%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	%	95% LCL	95% UCL	TU
17-0554-4857	Frond Count	NLR: 3P Log-Logistic	IC5	0.04364	---	0.4787	2291.5
			IC10	0.3442	0.03021	1.404	290.6
			IC15	1.236	0.3207	3.366	80.9
			IC20	3.237	1.323	6.842	30.9
			IC25	7.168	3.797	12.67	14
			IC40	48.69	27.48	84.32	2.1
			IC50	149.3	62.54	356.5	0.7
20-3162-0517	Total Dry Weight-mg	NLR: 3P Log-Gompertz	IC5	1.826	0.008965	14.87	54.8
			IC10	23.24	6.125	62.82	4.3
			IC15	107.5	13.71	425.1	0.9
			IC20	329.4	6.857	2538	0.3
			IC25	808.3	1.081	11520	0.1
			IC40	6148	---	886000	0
			IC50	18080	---	---	0

Frond Count Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	42.25	37.5	47	38	45	1.493	2.986	7.07%	0.00%
1.5		4	32.75	28.37	37.13	30	36	1.377	2.754	8.41%	22.49%
3		4	34	25.68	42.32	29	39	2.614	5.228	15.38%	19.53%
6.1		4	31.25	28.24	34.26	30	34	0.9465	1.893	6.06%	26.04%
12.3		4	34	27.77	40.23	29	38	1.958	3.916	11.52%	19.53%
24		4	26	20.05	31.95	22	31	1.871	3.742	14.39%	38.46%
49		4	25.75	21.77	29.73	23	29	1.25	2.5	9.71%	39.05%
97		4	21.25	16.87	25.63	18	24	1.377	2.754	12.96%	49.70%

Total Dry Weight-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	5.795	4.845	6.745	5.2	6.56	0.2985	0.5969	10.30%	0.00%
1.5		4	5.453	5.036	5.869	5.28	5.84	0.1309	0.2617	4.80%	5.91%
3		4	5.545	4.981	6.109	5.14	5.89	0.1773	0.3546	6.40%	4.31%
6.1		4	5.28	4.946	5.614	5.02	5.51	0.105	0.2099	3.98%	8.89%
12.3		4	5.513	4.715	6.31	4.82	6.01	0.2505	0.5011	9.09%	4.87%
24		4	5.043	4.309	5.776	4.54	5.57	0.2304	0.4608	9.14%	12.99%
49		4	5.122	4.648	5.597	4.79	5.43	0.1491	0.2981	5.82%	11.60%
97		4	4.945	3.93	5.96	4.01	5.42	0.3189	0.6377	12.90%	14.67%

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Frond Count Detail MD5: 8D4F3413CEB54563590D5F541DF76779

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	43	45	38	43
1.5		31	30	36	34
3		29	38	39	30
6.1		31	30	30	34
12.3		33	29	36	38
24		26	25	22	31
49		29	25	26	23
97		23	20	18	24

Total Dry Weight-mg Detail MD5: C368FA1EE7107DED6693138931FAA37D

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	5.2	5.95	6.56	5.47
1.5		5.31	5.28	5.84	5.38
3		5.14	5.79	5.89	5.36
6.1		5.37	5.02	5.51	5.22
12.3		5.55	4.82	5.67	6.01
24		4.8	5.26	4.54	5.57
49		5.43	5.31	4.96	4.79
97		5.26	5.09	4.01	5.42

Lemna Growth Inhibition Test — **Nautilus Environmental Calgary**

Analysis ID:	10-5700-3974	**Endpoint:**	Frond Count	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:32	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	8D4F3413CEB54563590D5F541DF76779	**Editor ID:**	002-704-782-4
Batch ID:	15-0532-6592	**Test Type:**	Lemna Growth	**Analyst:**	Lab Tech
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	Lemna Test Media
Ending Date:	16 Nov-23	**Species:**	Lemna minor	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	In-House Culture **Age:** 10
Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	72h (11.9 °C)	**Client:**	ALS Laboratories		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C < T	97	>97	---	1	5.905	13.98%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Negative Control		1.5	6	-3.993	2.482	5.905	CDF	1.0000	Non-Significant Effect
		3	6	-3.467	2.482	5.905	CDF	1.0000	Non-Significant Effect
		6.1	6	-4.623	2.482	5.905	CDF	1.0000	Non-Significant Effect
		12.3	6	-3.467	2.482	5.905	CDF	1.0000	Non-Significant Effect
		24	6	-6.83	2.482	5.905	CDF	1.0000	Non-Significant Effect
		49	6	-6.935	2.482	5.905	CDF	1.0000	Non-Significant Effect
		97	6	-8.826	2.482	5.905	CDF	1.0000	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1180.97	168.71	7	14.9	<1.0E-05	Significant Effect
Error	271.75	11.3229	24			
Total	1452.72		31			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Variance	Bartlett Equality of Variance Test	3.703	14.07	0.8132	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9613	0.9338	0.2986	Normal Distribution

Frond Count Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	42.25	37.5	47	43	38	45	1.493	7.07%	0.00%
1.5		4	32.75	28.37	37.13	32.5	30	36	1.377	8.41%	22.49%
3		4	34	25.68	42.32	34	29	39	2.614	15.38%	19.53%
6.1		4	31.25	28.24	34.26	30.5	30	34	0.9465	6.06%	26.04%
12.3		4	34	27.77	40.23	34.5	29	38	1.958	11.52%	19.53%
24		4	26	20.05	31.95	25.5	22	31	1.871	14.39%	38.46%
49		4	25.75	21.77	29.73	25.5	23	29	1.25	9.71%	39.05%
97		4	21.25	16.87	25.63	21.5	18	24	1.377	12.96%	49.70%

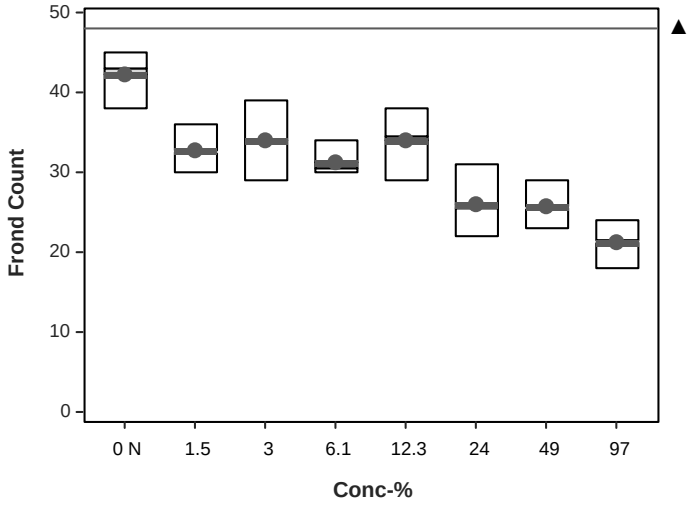
Frond Count Detail

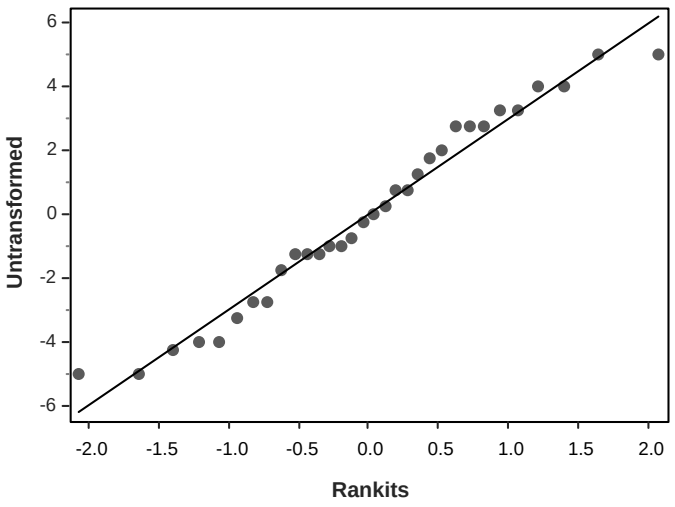
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	43	45	38	43
1.5		31	30	36	34
3		29	38	39	30
6.1		31	30	30	34
12.3		33	29	36	38
24		26	25	22	31
49		29	25	26	23
97		23	20	18	24

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	10-5700-3974	**Endpoint:**	Frond Count	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:32	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	8D4F3413CEB54563590D5F541DF76779	**Editor ID:**	002-704-782-4

Graphics





Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	17-0554-4857	**Endpoint:**	Frond Count	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:35	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	8D4F3413CEB54563590D5F541DF76779	**Editor ID:**	002-704-782-4
Batch ID:	15-0532-6592	**Test Type:**	Lemna Growth	**Analyst:**	Lab Tech
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	Lemna Test Media
Ending Date:	16 Nov-23	**Species:**	Lemna minor	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	In-House Culture **Age:** 10
Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	72h (11.9 °C)	**Client:**	ALS Laboratories		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Normal [ω=1]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
6	-40.43	87.73	91.27	0.7145	8.86%	41.81	Yes	2.054	0.1068	Non-Significant Lack-of-Fit

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC5	0.04364	---	0.4787	2291.5	208.9	---
IC10	0.3442	0.03021	1.404	290.6	71.2	3310.5
IC15	1.236	0.3207	3.366	80.9	29.7	311.9
IC20	3.237	1.323	6.842	30.9	14.6	75.6
IC25	7.168	3.797	12.67	14	7.9	26.3
IC40	48.69	27.48	84.32	2.1	1.2	3.6
IC50	149.3	62.54	356.5	0.7	0.3	1.6

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	41.81	1.812	38.1	45.51	23.08	<1.0E-05	Significant Parameter
γ	0.3618	0.07471	0.209	0.5146	4.843	3.9E-05	Significant Parameter
δ	149.3	68.13	9.959	288.7	2.191	0.0366	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	31630	10540	3	788	<1.0E-05	Significant Effect
Lack of Fit	116.3	23.26	5	2.054	0.1068	Non-Significant Lack-of-Fit
Pure Error	271.8	11.32	24			
Residual	388	13.38	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variance	Bartlett Equality of Variance Test	3.703	14.07	0.8132	Equal Variances
	Mod Levene Equality of Variance	1.458	2.423	0.2291	Equal Variances
Distribution	Anderson-Darling A2 Test	0.2653	2.492	0.7207	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9724	0.9338	0.5691	Normal Distribution

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	17-0554-4857	**Endpoint:**	Frond Count	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:35	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	8D4F3413CEB54563590D5F541DF76779	**Editor ID:**	002-704-782-4

Frond Count Summary

			Calculated Variate							
Conc-%	**Code**	**Count**	**Mean**	**Median**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
0	N	4	42.25	43	38	45	1.493	2.986	7.07%	0.00%
1.5		4	32.75	32.5	30	36	1.377	2.754	8.41%	22.49%
3		4	34	34	29	39	2.614	5.228	15.38%	19.53%
6.1		4	31.25	30.5	30	34	0.9465	1.893	6.06%	26.04%
12.3		4	34	34.5	29	38	1.958	3.916	11.52%	19.53%
24		4	26	25.5	22	31	1.871	3.742	14.39%	38.46%
49		4	25.75	25.5	23	29	1.25	2.5	9.71%	39.05%
97		4	21.25	21.5	18	24	1.377	2.754	12.96%	49.70%

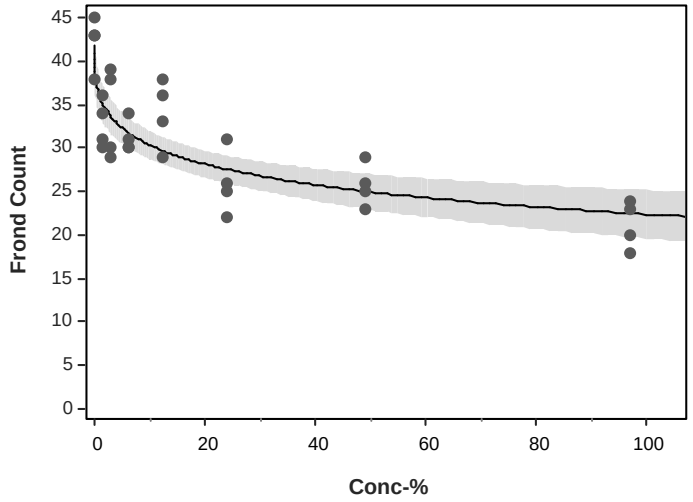
Frond Count Detail

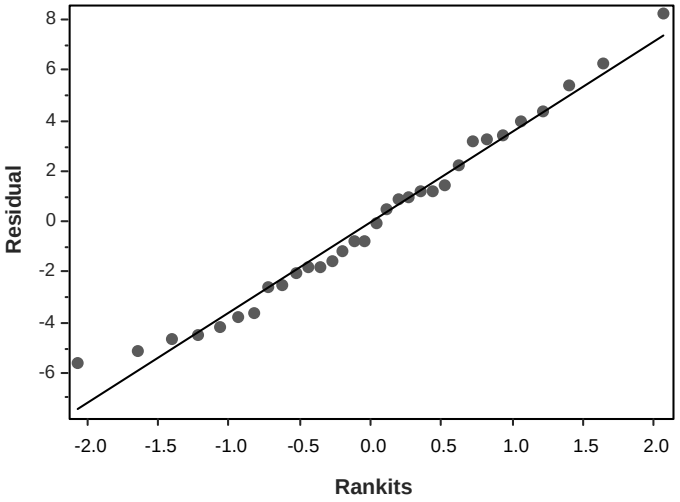
Conc-%	**Code**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
0	N	43	45	38	43
1.5		31	30	36	34
3		29	38	39	30
6.1		31	30	30	34
12.3		33	29	36	38
24		26	25	22	31
49		29	25	26	23
97		23	20	18	24

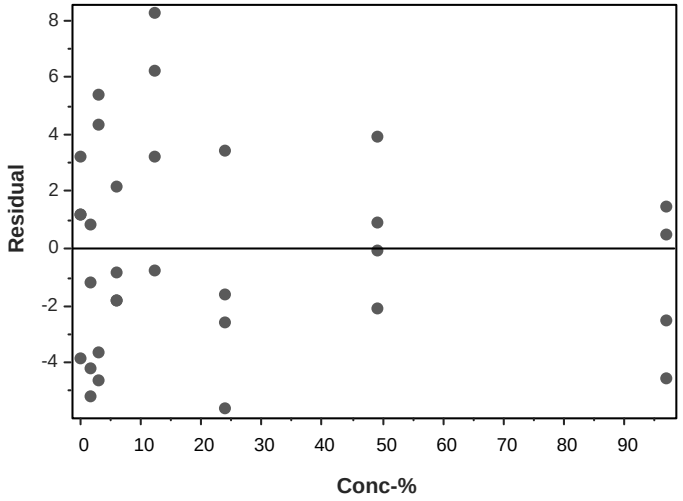
Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

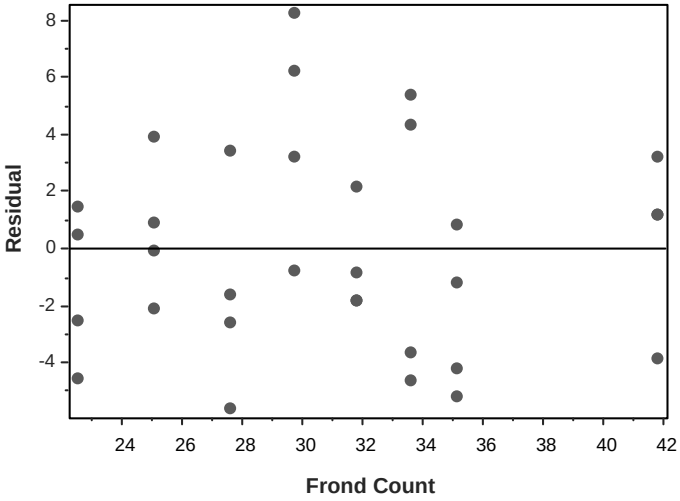
Analysis ID:	17-0554-4857	**Endpoint:**	Frond Count	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:35	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	8D4F3413CEB54563590D5F541DF76779	**Editor ID:**	002-704-782-4

Graphics Model: 3P Log-Logistic: μ=α/[1+[x/δ]^γ] Distribution: Normal [ω=1]









Lemna Growth Inhibition Test — **Nautilus Environmental Calgary**

Analysis ID:	01-0658-6575	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	13 Dec-23 17:01	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	C368FA1EE7107DED6693138931FAA37D	**Editor ID:**	002-704-782-4

Batch ID:	15-0532-6592	**Test Type:**	Lemna Growth	**Analyst:**	Lab Tech		
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	Lemna Test Media		
Ending Date:	16 Nov-23	**Species:**	Lemna minor	**Brine:**			
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	In-House Culture	**Age:**	10

Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	72h (11.9 °C)	**Client:**	ALS Laboratories		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C < T	97	>97	---	1	0.7734	13.35%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Negative Control		1.5	6	-1.099	2.482	0.7734	CDF	0.9928	Non-Significant Effect
		3	6	-0.8023	2.482	0.7734	CDF	0.9824	Non-Significant Effect
		6.1	6	-1.653	2.482	0.7734	CDF	0.9988	Non-Significant Effect
		12.3	6	-0.9066	2.482	0.7734	CDF	0.9870	Non-Significant Effect
		24	6	-2.415	2.482	0.7734	CDF	0.9999	Non-Significant Effect
		49	6	-2.158	2.482	0.7734	CDF	0.9998	Non-Significant Effect
		97	6	-2.728	2.482	0.7734	CDF	1.0000	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Outlier	Grubbs Extreme Value Test	2.411	2.938	0.3754	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.34729	0.335327	7	1.727	0.1501	Non-Significant Effect
Error	4.6608	0.1942	24			
Total	7.00809		31			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Variance	Bartlett Equality of Variance Test	5.465	14.07	0.6035	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9837	0.9338	0.8969	Normal Distribution

Total Dry Weight-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	5.795	4.845	6.745	5.71	5.2	6.56	0.2985	10.30%	0.00%
1.5		4	5.452	5.036	5.869	5.345	5.28	5.84	0.1309	4.80%	5.91%
3		4	5.545	4.981	6.109	5.575	5.14	5.89	0.1773	6.40%	4.31%
6.1		4	5.28	4.946	5.614	5.295	5.02	5.51	0.105	3.98%	8.89%
12.3		4	5.512	4.715	6.31	5.61	4.82	6.01	0.2505	9.09%	4.87%
24		4	5.042	4.309	5.776	5.03	4.54	5.57	0.2304	9.14%	12.99%
49		4	5.122	4.648	5.597	5.135	4.79	5.43	0.1491	5.82%	11.60%
97		4	4.945	3.93	5.96	5.175	4.01	5.42	0.3189	12.90%	14.67%

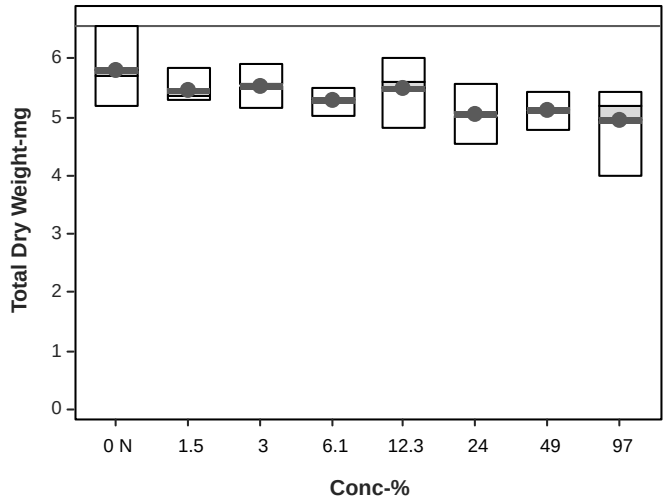
Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

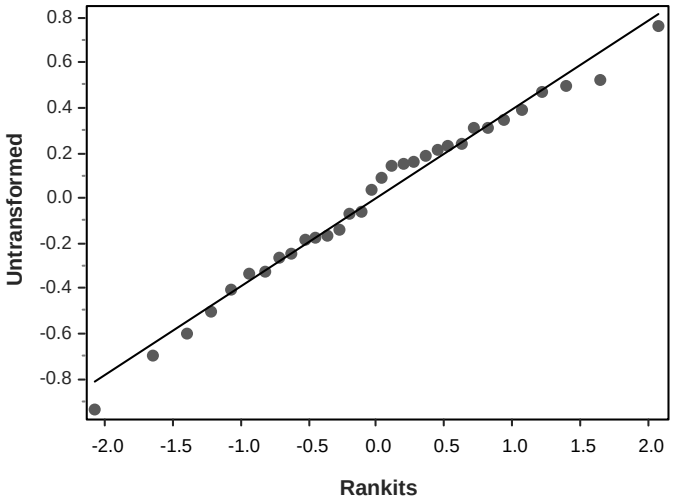
Analysis ID:	01-0658-6575	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	13 Dec-23 17:01	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	C368FA1EE7107DED6693138931FAA37D	**Editor ID:**	002-704-782-4

Total Dry Weight-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	5.2	5.95	6.56	5.47
1.5		5.31	5.28	5.84	5.38
3		5.14	5.79	5.89	5.36
6.1		5.37	5.02	5.51	5.22
12.3		5.55	4.82	5.67	6.01
24		4.8	5.26	4.54	5.57
49		5.43	5.31	4.96	4.79
97		5.26	5.09	4.01	5.42

Graphics





Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	20-3162-0517	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:30	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	C368FA1EE7107DED6693138931FAA37D	**Editor ID:**	002-704-782-4

Batch ID:	15-0532-6592	**Test Type:**	Lemna Growth	**Analyst:**	Lab Tech		
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	Lemna Test Media		
Ending Date:	16 Nov-23	**Species:**	Lemna minor	**Brine:**			
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	In-House Culture	**Age:**	10

Sample ID:	17-7121-1724	**Code:**	2324-0709-01	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-001 EDL1_EFF_2023110
Sample Age:	72h (11.9 °C)	**Client:**	ALS Laboratories		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Gompertz: μ=α·exp[log[0.5]·[x/δ]^γ]	Normal [ω=1]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
5	29.06	-51.26	-47.72	0.2308	7.28%	5.791	Yes	0.3932	0.8486	Non-Significant Lack-of-Fit

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC5	1.826	0.008965	14.87	54.8	6.7	11154.8
IC10	23.24	6.125	62.82	4.3	1.6	16.3
IC15	107.5	13.71	425.1	0.9	0.2	7.3
IC20	329.4	6.857	2538	0.3	0	14.6
IC25	808.3	1.081	11520	0.1	0	92.5
IC40	6148	---	886000	0	0	---
IC50	18080	---	---	0	---	---

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	5.791	0.206	5.37	6.212	28.11	<1.0E-05	Significant Parameter
γ	0.283	0.1664	-0.0574	0.6234	1.7	0.0998	Non-Significant Parameter
δ	18080	57500	-99530	135700	0.3143	0.7555	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	913.4	304.5	3	1751	<1.0E-05	Significant Effect
Lack of Fit	0.3818	0.07636	5	0.3932	0.8486	Non-Significant Lack-of-Fit
Pure Error	4.661	0.1942	24			
Residual	5.043	0.1739	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variance	Bartlett Equality of Variance Test	5.465	14.07	0.6035	Equal Variances
	Mod Levene Equality of Variance	0.6558	2.423	0.7063	Equal Variances
Distribution	Anderson-Darling A2 Test	0.2956	2.492	0.6252	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9815	0.9338	0.8407	Normal Distribution

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	20-3162-0517	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:30	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	C368FA1EE7107DED6693138931FAA37D	**Editor ID:**	002-704-782-4

Total Dry Weight-mg Summary

			Calculated Variate							
Conc-%	**Code**	**Count**	**Mean**	**Median**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
0	N	4	5.795	5.71	5.2	6.56	0.2985	0.5969	10.30%	0.00%
1.5		4	5.452	5.345	5.28	5.84	0.1309	0.2617	4.80%	5.91%
3		4	5.545	5.575	5.14	5.89	0.1773	0.3546	6.40%	4.31%
6.1		4	5.28	5.295	5.02	5.51	0.105	0.2099	3.98%	8.89%
12.3		4	5.512	5.61	4.82	6.01	0.2505	0.5011	9.09%	4.87%
24		4	5.042	5.03	4.54	5.57	0.2304	0.4608	9.14%	12.99%
49		4	5.122	5.135	4.79	5.43	0.1491	0.2981	5.82%	11.60%
97		4	4.945	5.175	4.01	5.42	0.3189	0.6377	12.90%	14.67%

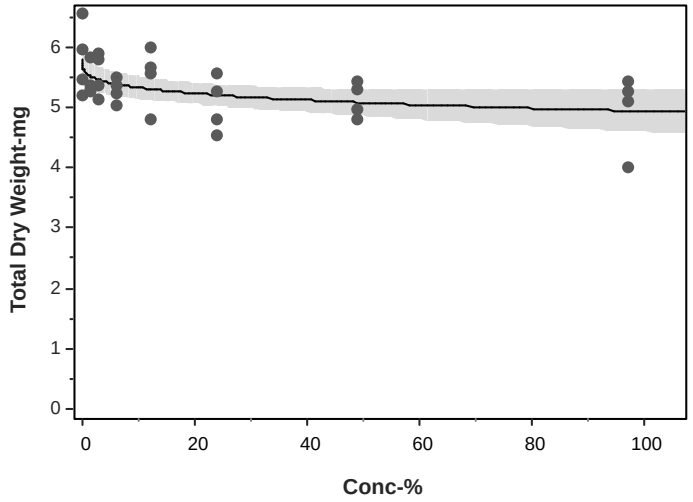
Total Dry Weight-mg Detail

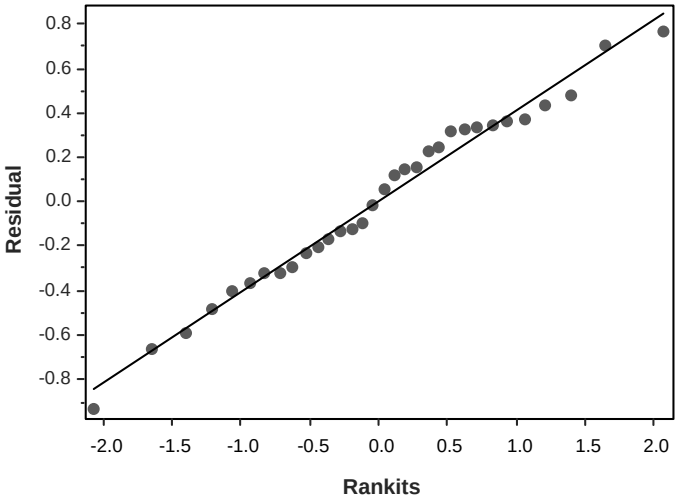
Conc-%	**Code**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
0	N	5.2	5.95	6.56	5.47
1.5		5.31	5.28	5.84	5.38
3		5.14	5.79	5.89	5.36
6.1		5.37	5.02	5.51	5.22
12.3		5.55	4.82	5.67	6.01
24		4.8	5.26	4.54	5.57
49		5.43	5.31	4.96	4.79
97		5.26	5.09	4.01	5.42

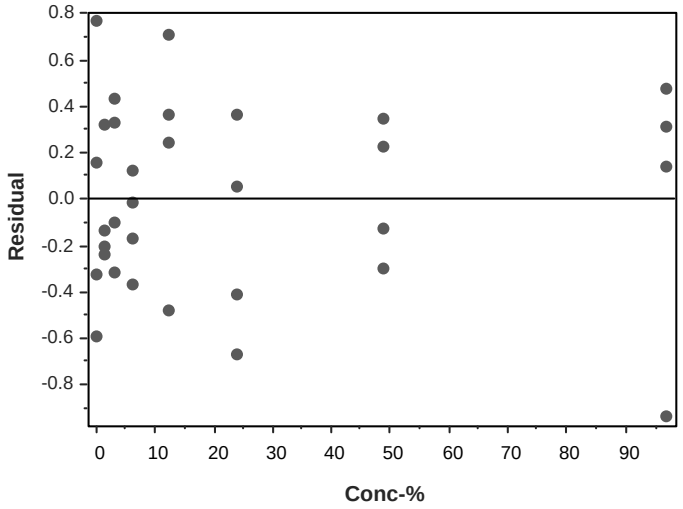
Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

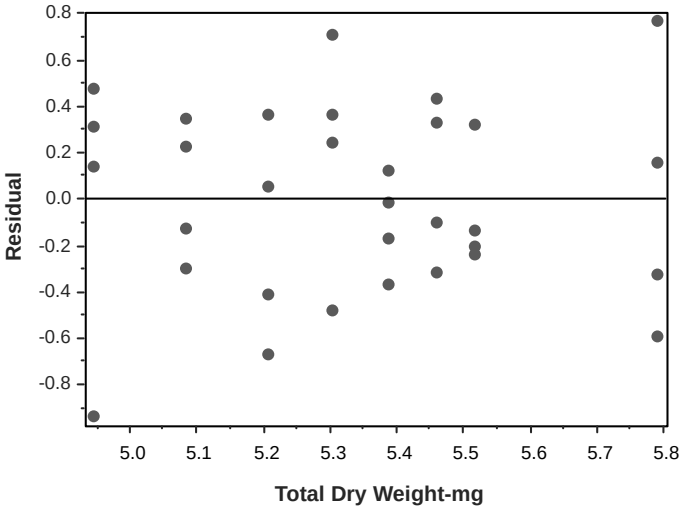
Analysis ID:	20-3162-0517	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	23 Nov-23 16:30	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	23 Nov-23 0:00	**MD5 Hash:**	C368FA1EE7107DED6693138931FAA37D	**Editor ID:**	002-704-782-4

Graphics Model: 3P Log-Gompertz: μ=α·exp[log[0.5]·[x/δ]^γ] Distribution: Normal [ω=1]









NAUTILUS ENVIRONMENTAL

Lemna Test Bench Sheet

Method LMD Client ALS106 Reference 2324-0709-02

Test Log

Date	Day	Time	Initial	Rotate	Light Level* (**4000-5600**)	Temperature (°C) (range: **23-27°C**) Control	3%	24%	97%	Daily Data Review
2023-11-09	0	1250	DM	-	4240	24	→	→	→	AI
2023-11-10	1	0830	ET	✓	4270	24	→	→	→	AI
2023-11-11	2	0805	KZ	✓	~~4220~~ 4480	24	→	→	→	AM
2023-11-12	3	0750	KZ	✓	4630	24	→	→	→	NA
2023-11-13	4	0810	DIC	✓	4760	24	→	→	→	NA
2023-11-14	5	0820	PK	✓	4380	24	→	→	→	AI
2023-11-15	6	0830	ET	✓	4200	24	→	→	→	ML
2023-11-16	7	0840	YC	-	4420	24	→	→	→	DM

*taken at centre

Sample Information

Initial pH: 7.9
Initial EC (µS/cm): 1728
Initial DO (mg/L): 9.0
Initial Temp (°C): 17

Biology (Number of Fronds)

Concentration (%) / Replicate	ctl	1.5	3.0	6.1	12.1	24.25	48.5	97
day 0								
a	6	6	6	6	6	6	6	6
b	6	6	6	6	6	6	6	6
c	6	6	6	6	6	6	6	6
d	6	6	6	6	6	6	6	6
day 7								
a	52	41	40	46	37	33	32	28
b	50	42	39	42	31	29	29	27
c	47	37	38	45	38	41	30	36
d	46	46	42	39	27	31	32	34

Validity Criteria: Controls must have a ≥ 8 fold increase in frond number (average of 48)

Appearance of Fronds

Concentration (%)	Day 0	Day 7
Control	Medium green, short roots, all healthy	med green, long roots, all healthy
1.5	Medium green, short roots, all healthy	light/med green, long roots, green algae contamination
3.0	Medium green, short roots, all healthy	
6.1	Medium green, short roots, all healthy	
12.1	Medium green, short roots, all healthy	
24.3	Medium green, short roots, all healthy	
48.5	Medium green, short roots, all healthy	
97	Medium green, short roots, all healthy	↓ more algae as concentration increases.

Notes: Chl=chlorosis, Nec=necorsis, Yel=yellow fronds, Asf=abnormally sized fronds, Gib=gibbosity, col dest=conlony destruction, root dest=root destruction, Lob=loss of buoyancy

Reviewed By: DM Date Reviewed: NOV 27 2023

NAUTILUS ENVIRONMENTAL

Lemna Test Bench Sheet

Method LMD Client ALS106 Reference 2324-0709-02

Chem. Cart Used
Day 0: 2
Day 7: 2

Chemistry

Concentration (%)	ctl	1.5	3.0	6.1	12.1	24.25	48.5	97
New Solutions (day 0)								
pH (units) (range: 6.5-9.5)	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
EC (uS/cm)	775	857	908	959	1054	1234	1560	2260
DO (mg/L)	7.3	7.4	7.4	7.4	7.5	7.5	7.5	7.5
Temperature (°C) (25±2)	23	23	23	23	23	23	23	23
Old Solutions (day 7)								
pH (units) (range: 6.5-9.5)	8.2	9.1	9.3	9.4	9.3	9.2	9.3	9.0
EC (uS/cm)	948	1029	1059	1094	1209	1414	1784	2520
DO (mg/L)	8.0	10.6	11.7	12.2	12.2	12.4	15.4	15.1
Temperature (°C) (25±2)	23	23	23	23	23	23	23	23

Comments:

Quality Assurance

Organism batch (date culture was started): 20231030 MON

Age of test organisms (7-10d): 10 d

Light levels of test area (4000-5600 lux) measured at sample surface: left 4000, center 4240, right 4180

Test organisms were acclimated for 18-24 hours in APHA media and appeared healthy before use: (Yes)/No

Average number of fronds in QA cups at turnover (A to C): 32

Fold Increase in culture prior to testing: 11 X

Sample Treatment

supplemented with nutrients	(Y) / N	
pH adjusted	Y / (N)	if Y, how & to what?
filtered	Y / (N)	if Y, how & what size?
preaerated	(Y) / N	if Y, how long and at what rate*?
		*must be < 100 bubbles/minute for 20 minutes

Dilution Water Treatment

pH adjusted to 8.3±0.1	(Y) / N	
preaerated	(Y) / N	if Y, how long and at what rate*?
		*must be < 100 bubbles/minute for ≥2 hours

Other

Reviewed By: DM Date Reviewed: NOV 2 2 2023

NAUTILUS ENVIRONMENTAL

Organism Weights Bench Sheet

Client AIS 106 Sample 2324 - 0709-02 Organism LMD Batch 20231030MON

	Item Weighed	Date	Initials	Balance*
Initial Weight (mg):	dried pan	23/11/14	MS	3
Final Weight (mg):	dried pan + organisms	23/11/19	PK	mettler #3

* same balance must be used for initial and final weights
* for FM/HA/CT, must use scale with 0.01 mg accuracy

Concentration

Replicate	CTL		0.5 1.5		3.0		6.1		12.1		24.3	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
a	808.61	814.77	796.84	801.65	824.57	829.19	782.78	788.28	799.59	804.53	783.46	788.40
b	811.36	816.95	772.72	778.21	818.23	822.93	792.73	797.77	791.86	796.33	773.05	777.58
c	814.52	820.21	821.37	826.00	791.24	796.01	779.94	785.41	793.88	799.27	792.69	798.89
d	795.76	801.41	825.42	831.31	779.96	786.20	798.59	803.63	791.29	795.57	827.87	832.73
e												

Concentration

Replicate	48.5		97									
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
a	814.63	819.75	799.97	804.32								
b	807.26	812.06	805.47	809.94								
c	784.13	788.82	854.62	860.20								
d	798.58	803.85	793.00	798.06								
e												

Balance Calibration Check:

	Initial	Final
first pan weighed:	CTLA	CTL A
weight of first pan:	808.61	814.77
first pan after all other pans weighed:	808.53	814.86
% difference <5%:	Yes/No	Yes/No

Test Validity Met: Yes/No/NA

Results are Logical:** Yes/No

** no negative numbers, consistent values across replicates

$$\% \text{ difference} = \frac{(\text{initial weight} - \text{reweight})}{(\text{initial weight} + \text{reweight}) / 2} \times 100\%$$

If "no" is circled for any parameter, notify Lab Supervisor/ QA Group to determine appropriate action

Reviewed By: DM Date Reviewed: NOV 2 2 2023

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Batch ID:	04-4355-2928	**Test Type:**	Lemna Growth	**Analyst:**	Lab Tech		
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**			
Ending Date:	16 Nov-23	**Species:**	Lemna minor	**Brine:**			
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	In-House Culture	**Age:**	10d
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**			
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories		
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110		
Sample Age:	72h (12.2 °C)	**Client:**	ALS Laboratories				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	PMSD	TU
06-4223-9053	Frond Count	Dunnett Multiple Comparison Test	97	>97	---	15.3%	1
01-3347-0336	Total Dry Weight-mg	Nemenyi-Damico-Wolfe Test	97	>97	---	---	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	%	95% LCL	95% UCL	TU
07-1904-7358	Frond Count	NLR: 3P Log-Logistic	IC5	0.03116	---	0.4692	3209.5
			IC10	0.2765	0.01318	1.403	361.6
			IC15	1.069	0.211	3.416	93.6
			IC20	2.956	1.033	7.01	33.8
			IC25	6.852	3.298	13.12	14.6
			IC40	51.92	26.47	98.85	1.9
			IC50	169.8	59.89	481.3	0.6
02-6010-9190	Total Dry Weight-mg	NLR: 3P Log-Gompertz	IC5	0.0006529	---	146.2	153151
			IC10	1.049	4.32E-05	202.6	95.3
			IC15	89.35	0.2457	4669	1.1
			IC20	2307	---	8530000	0
			IC25	31220	---	4.24E+09	0
			IC40	11260000	---	---	0
			IC50	25770000	---	---	0

Frond Count Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	42.75	38.37	47.13	40	46	1.377	2.754	6.44%	0.00%
1.5		4	35.5	29.62	41.38	31	40	1.848	3.697	10.41%	16.96%
3		4	33.75	31.03	36.47	32	36	0.8539	1.708	5.06%	21.05%
6.1		4	37	31.97	42.03	33	40	1.581	3.162	8.55%	13.45%
12.3		4	27.25	18.99	35.51	21	32	2.594	5.188	19.04%	36.26%
24		4	27.5	19.13	35.87	23	35	2.63	5.26	19.13%	35.67%
49		4	24.75	22.36	27.14	23	26	0.75	1.5	6.06%	42.11%
97		4	25.25	18.21	32.29	21	30	2.213	4.425	17.53%	40.94%

Total Dry Weight-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	5.773	5.356	6.189	5.59	6.16	0.1308	0.2616	4.53%	0.00%
1.5		4	5.205	4.269	6.141	4.63	5.89	0.294	0.588	11.30%	9.83%
3		4	5.082	3.851	6.314	4.62	6.24	0.387	0.7741	15.23%	11.95%
6.1		4	5.262	4.853	5.672	5.04	5.5	0.1286	0.2572	4.89%	8.83%
12.3		4	4.77	3.978	5.562	4.28	5.39	0.2489	0.4978	10.44%	17.37%
24		4	5.135	3.972	6.298	4.54	6.2	0.3654	0.7307	14.23%	11.04%
49		4	4.97	4.539	5.401	4.69	5.27	0.1353	0.2707	5.45%	13.90%
97		4	4.89	3.917	5.863	4.35	5.68	0.3056	0.6113	12.50%	15.29%

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Frond Count Detail MD5: 59803BC0C366F6092DBBF75EC329ED5C

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	46	44	41	40
1.5		35	36	31	40
3		34	33	32	36
6.1		40	36	39	33
12.3		31	25	32	21
24		27	23	35	25
49		26	23	24	26
97		22	21	30	28

Total Dry Weight-mg Detail MD5: 002D99855612CB936AC5A8CF1711F197

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	6.16	5.59	5.69	5.65
1.5		4.81	5.49	4.63	5.89
3		4.62	4.7	4.77	6.24
6.1		5.5	5.04	5.47	5.04
12.3		4.94	4.47	5.39	4.28
24		4.94	4.54	6.2	4.86
49		5.12	4.8	4.69	5.27
97		4.35	4.47	5.68	5.06

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	06-4223-9053	**Endpoint:**	Frond Count	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:34	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	59803BC0C366F6092DBBF75EC329ED5C	**Editor ID:**	002-704-782-4
Batch ID:	04-4355-2928	**Test Type:**	Lemna Growth	**Analyst:**	Lab Tech
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	
Ending Date:	16 Nov-23	**Species:**	Lemna minor	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	In-House Culture **Age:** 10d
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110
Sample Age:	72h (12.2 °C)	**Client:**	ALS Laboratories		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C < T	97	>97	---	1	6.525	15.26%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Negative Control		1.5	6	-2.758	2.482	6.525	CDF	1.0000	Non-Significant Effect
		3	6	-3.423	2.482	6.525	CDF	1.0000	Non-Significant Effect
		6.1	6	-2.187	2.482	6.525	CDF	0.9998	Non-Significant Effect
		12.3	6	-5.896	2.482	6.525	CDF	1.0000	Non-Significant Effect
		24	6	-5.801	2.482	6.525	CDF	1.0000	Non-Significant Effect
		49	6	-6.847	2.482	6.525	CDF	1.0000	Non-Significant Effect
		97	6	-6.657	2.482	6.525	CDF	1.0000	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1184.72	169.246	7	12.24	<1.0E-05	Significant Effect
Error	331.75	13.8229	24			
Total	1516.47		31			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Variance	Bartlett Equality of Variance Test	6.928	14.07	0.4364	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9838	0.9338	0.8983	Normal Distribution

Frond Count Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	42.75	38.37	47.13	42.5	40	46	1.377	6.44%	0.00%
1.5		4	35.5	29.62	41.38	35.5	31	40	1.848	10.41%	16.96%
3		4	33.75	31.03	36.47	33.5	32	36	0.8539	5.06%	21.05%
6.1		4	37	31.97	42.03	37.5	33	40	1.581	8.55%	13.45%
12.3		4	27.25	18.99	35.51	28	21	32	2.594	19.04%	36.26%
24		4	27.5	19.13	35.87	26	23	35	2.63	19.13%	35.67%
49		4	24.75	22.36	27.14	25	23	26	0.75	6.06%	42.11%
97		4	25.25	18.21	32.29	25	21	30	2.213	17.53%	40.94%

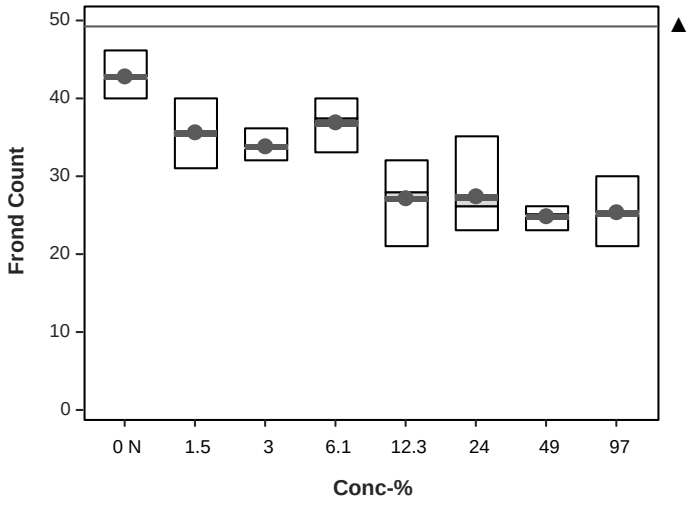
Frond Count Detail

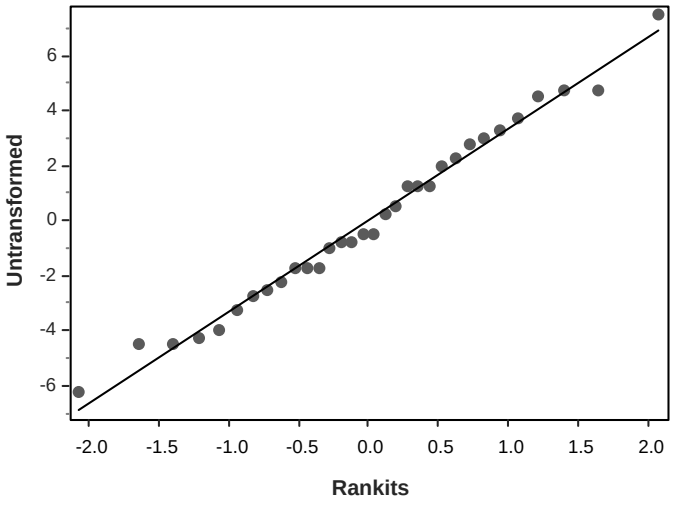
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	46	44	41	40
1.5		35	36	31	40
3		34	33	32	36
6.1		40	36	39	33
12.3		31	25	32	21
24		27	23	35	25
49		26	23	24	26
97		22	21	30	28

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	06-4223-9053	**Endpoint:**	Frond Count	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:34	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	59803BC0C366F6092DBBF75EC329ED5C	**Editor ID:**	002-704-782-4

Graphics





Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	07-1904-7358	**Endpoint:**	Frond Count	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:37	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	59803BC0C366F6092DBBF75EC329ED5C	**Editor ID:**	002-704-782-4

Batch ID:	04-4355-2928	**Test Type:**	Lemna Growth	**Analyst:**	Lab Tech		
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**			
Ending Date:	16 Nov-23	**Species:**	Lemna minor	**Brine:**			
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	In-House Culture	**Age:**	10d

Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110
Sample Age:	72h (12.2 °C)	**Client:**	ALS Laboratories		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Normal [ω=1]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
5	-43.75	94.37	97.91	0.6634	9.60%	42.89	Yes	2.11	0.0991	Non-Significant Lack-of-Fit

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC5	0.03116	---	0.4692	3209.5	213.1	---
IC10	0.2765	0.01318	1.403	361.6	71.3	7587.4
IC15	1.069	0.211	3.416	93.6	29.3	473.9
IC20	2.956	1.033	7.01	33.8	14.3	96.8
IC25	6.852	3.298	13.12	14.6	7.6	30.3
IC40	51.92	26.47	98.85	1.9	1	3.8
IC50	169.8	59.89	481.3	0.6	0.2	1.7

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	42.89	2.013	38.77	47.01	21.3	<1.0E-05	Significant Parameter
γ	0.3423	0.07892	0.1808	0.5037	4.336	0.0002	Significant Parameter
δ	169.8	91.78	-17.93	357.5	1.85	0.0745	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	33230	11080	3	672.7	<1.0E-05	Significant Effect
Lack of Fit	145.8	29.16	5	2.11	0.0991	Non-Significant Lack-of-Fit
Pure Error	331.8	13.82	24			
Residual	477.6	16.47	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variance	Bartlett Equality of Variance Test	6.928	14.07	0.4364	Equal Variances
	Mod Levene Equality of Variance	1.394	2.423	0.2530	Equal Variances
Distribution	Anderson-Darling A2 Test	0.3676	2.492	0.4353	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9734	0.9338	0.5965	Normal Distribution

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	07-1904-7358	**Endpoint:**	Frond Count	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:37	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	59803BC0C366F6092DBBF75EC329ED5C	**Editor ID:**	002-704-782-4

Frond Count Summary

			Calculated Variate							
Conc-%	**Code**	**Count**	**Mean**	**Median**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
0	N	4	42.75	42.5	40	46	1.377	2.754	6.44%	0.00%
1.5		4	35.5	35.5	31	40	1.848	3.697	10.41%	16.96%
3		4	33.75	33.5	32	36	0.8539	1.708	5.06%	21.05%
6.1		4	37	37.5	33	40	1.581	3.162	8.55%	13.45%
12.3		4	27.25	28	21	32	2.594	5.188	19.04%	36.26%
24		4	27.5	26	23	35	2.63	5.26	19.13%	35.67%
49		4	24.75	25	23	26	0.75	1.5	6.06%	42.11%
97		4	25.25	25	21	30	2.213	4.425	17.53%	40.94%

Frond Count Detail

Conc-%	**Code**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
0	N	46	44	41	40
1.5		35	36	31	40
3		34	33	32	36
6.1		40	36	39	33
12.3		31	25	32	21
24		27	23	35	25
49		26	23	24	26
97		22	21	30	28

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	07-1904-7358	**Endpoint:**	Frond Count	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:37	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	59803BC0C366F6092DBBF75EC329ED5C	**Editor ID:**	002-704-782-4

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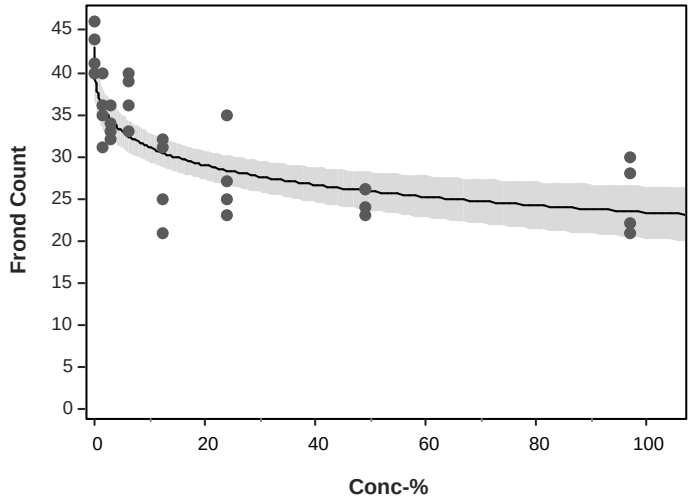
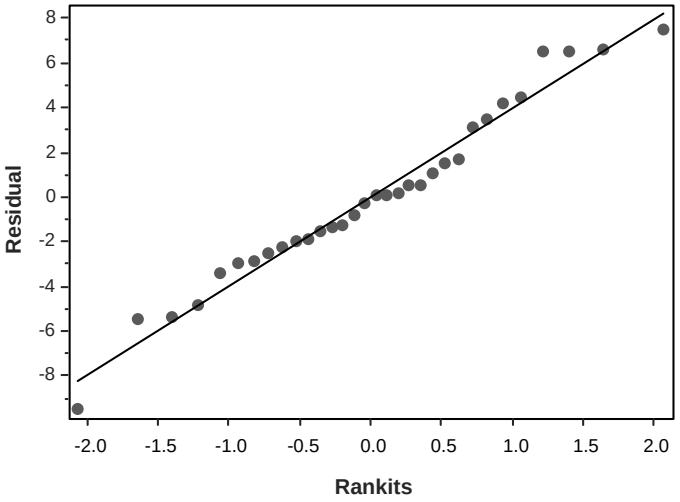
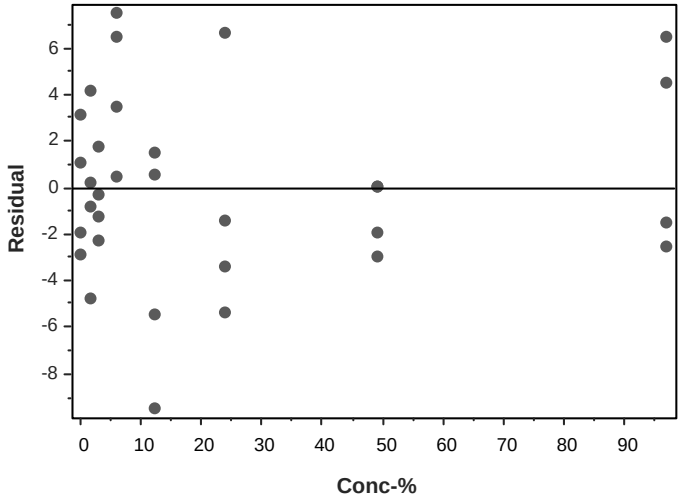
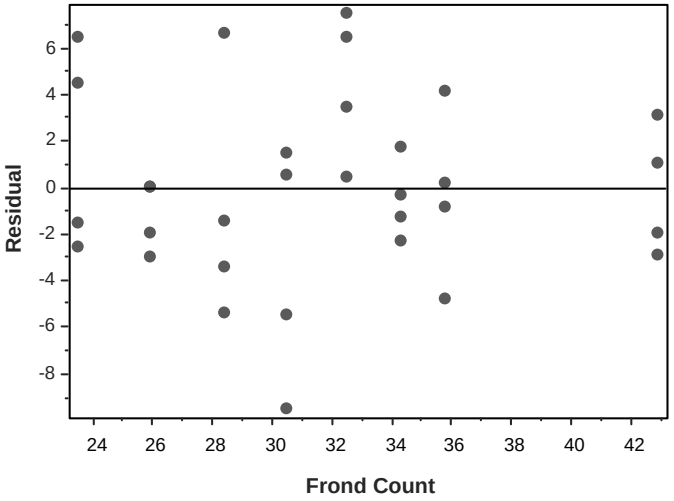








Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	01-3347-0336	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:23	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	002D99855612CB936AC5A8CF1711F197	**Editor ID:**	002-704-782-4
Batch ID:	04-4355-2928	**Test Type:**	Lemna Growth	**Analyst:**	Lab Tech
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	
Ending Date:	16 Nov-23	**Species:**	Lemna minor	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	In-House Culture **Age:** 10d
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110
Sample Age:	72h (12.2 °C)	**Client:**	ALS Laboratories		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C < T	97	>97	---	1

Nemenyi-Damico-Wolfe Test

Control	vs	Conc-%	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Negative Control		1.5	-38	62.1	3	CDF	0.9982	Non-Significant Effect
		3	-52	62.1	3	CDF	0.9998	Non-Significant Effect
		6.1	-30	62.1	3	CDF	0.9945	Non-Significant Effect
		12.3	-69	62.1	3	CDF	1.0000	Non-Significant Effect
		24	-45.5	62.1	3	CDF	0.9995	Non-Significant Effect
		49	-51	62.1	3	CDF	0.9998	Non-Significant Effect
		97	-58.5	62.1	3	CDF	0.9999	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Overall Effect	Fligner-Wolfe Omnibus Test	419		0.9957	Non-Significant Overall Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.60315	0.371878	7	1.289	0.2976	Non-Significant Effect
Error	6.92462	0.288526	24			
Total	9.52777		31			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Variance	Bartlett Equality of Variance Test	7.064	14.07	0.4223	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.91	0.9338	0.0113	Non-Normal Distribution

Total Dry Weight-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	5.772	5.356	6.189	5.67	5.59	6.16	0.1308	4.53%	0.00%
1.5		4	5.205	4.269	6.141	5.15	4.63	5.89	0.294	11.30%	9.83%
3		4	5.082	3.851	6.314	4.735	4.62	6.24	0.387	15.23%	11.95%
6.1		4	5.262	4.853	5.672	5.255	5.04	5.5	0.1286	4.89%	8.83%
12.3		4	4.77	3.978	5.562	4.705	4.28	5.39	0.2489	10.44%	17.37%
24		4	5.135	3.972	6.298	4.9	4.54	6.2	0.3654	14.23%	11.04%
49		4	4.97	4.539	5.401	4.96	4.69	5.27	0.1353	5.45%	13.90%
97		4	4.89	3.917	5.863	4.765	4.35	5.68	0.3056	12.50%	15.29%

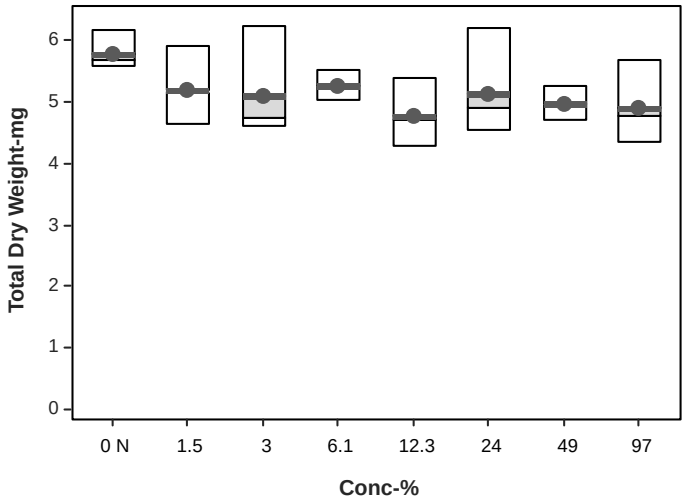
Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

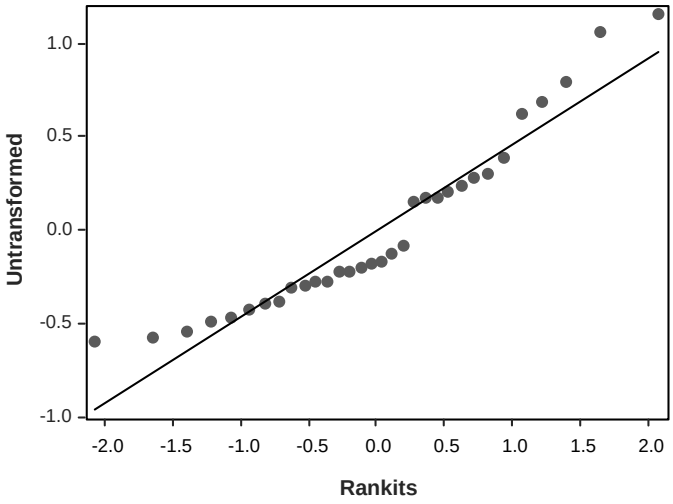
Analysis ID:	01-3347-0336	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:23	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	002D99855612CB936AC5A8CF1711F197	**Editor ID:**	002-704-782-4

Total Dry Weight-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	6.16	5.59	5.69	5.65
1.5		4.81	5.49	4.63	5.89
3		4.62	4.7	4.77	6.24
6.1		5.5	5.04	5.47	5.04
12.3		4.94	4.47	5.39	4.28
24		4.94	4.54	6.2	4.86
49		5.12	4.8	4.69	5.27
97		4.35	4.47	5.68	5.06

Graphics





Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	02-6010-9190	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:30	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	002D99855612CB936AC5A8CF1711F197	**Editor ID:**	002-704-782-4
Batch ID:	04-4355-2928	**Test Type:**	Lemna Growth	**Analyst:**	Lab Tech
Start Date:	09 Nov-23	**Protocol:**	EC/EPS 1/RM/37	**Diluent:**	
Ending Date:	16 Nov-23	**Species:**	Lemna minor	**Brine:**	
Test Length:	7d 0h	**Taxon:**	Tracheophyta	**Source:**	In-House Culture **Age:** 10d
Sample ID:	10-6191-8104	**Code:**	2324-0709-02	**Project:**	
Sample Date:	06 Nov-23	**Material:**	Water Sample	**Source:**	ALS Laboratories
Receipt Date:	08 Nov-23	**CAS (PC):**		**Station:**	TY2311633-002 EDL2_EFF_2023110
Sample Age:	72h (12.2 °C)	**Client:**	ALS Laboratories		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Gompertz: μ=α·exp[log[0.5]·[x/δ]^γ]	Normal [ω=1]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
6	22.84	-38.83	-35.29	0.1658	8.97%	5.773	Yes	0.3541	0.8745	Non-Significant Lack-of-Fit

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC5	0.0006529	---	146.2	153151.2	0.7	---
IC10	1.049	4.32E-05	202.6	95.3	0.5	2314730
IC15	89.35	0.2457	4669	1.1	0	407
IC20	2307	---	8530000	0	0	---
IC25	31220	---	4.24E+09	0	0	---
IC40	11260000	---	---	0	---	---
IC50	25770000	---	---	0	---	---

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	5.773	0.2531	5.255	6.291	22.81	<1.0E-05	Significant Parameter
γ	0.09751	0.1073	-0.122	0.3171	0.9084	0.3712	Non-Significant Parameter
δ	2.58E+08	4.53E+09	-9.0E+09	9.53E+09	0.05686	0.9550	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	846.2	282.1	3	1100	<1.0E-05	Significant Effect
Lack of Fit	0.5108	0.1022	5	0.3541	0.8745	Non-Significant Lack-of-Fit
Pure Error	6.925	0.2885	24			
Residual	7.435	0.2564	29			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variance	Bartlett Equality of Variance Test	7.064	14.07	0.4223	Equal Variances
	Mod Levene Equality of Variance	0.4989	2.423	0.8259	Equal Variances
Distribution	Anderson-Darling A2 Test	0.6945	2.492	0.0697	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9396	0.9338	0.0729	Normal Distribution

Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

Analysis ID:	02-6010-9190	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:30	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	002D99855612CB936AC5A8CF1711F197	**Editor ID:**	002-704-782-4

Total Dry Weight-mg Summary

			Calculated Variate							
Conc-%	**Code**	**Count**	**Mean**	**Median**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
0	N	4	5.772	5.67	5.59	6.16	0.1308	0.2616	4.53%	0.00%
1.5		4	5.205	5.15	4.63	5.89	0.294	0.588	11.30%	9.83%
3		4	5.082	4.735	4.62	6.24	0.387	0.7741	15.23%	11.95%
6.1		4	5.262	5.255	5.04	5.5	0.1286	0.2572	4.89%	8.83%
12.3		4	4.77	4.705	4.28	5.39	0.2489	0.4978	10.44%	17.37%
24		4	5.135	4.9	4.54	6.2	0.3654	0.7307	14.23%	11.04%
49		4	4.97	4.96	4.69	5.27	0.1353	0.2707	5.45%	13.90%
97		4	4.89	4.765	4.35	5.68	0.3056	0.6113	12.50%	15.29%

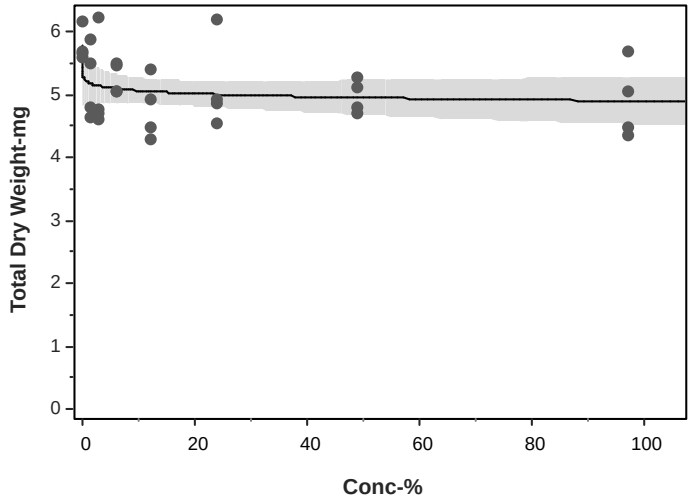
Total Dry Weight-mg Detail

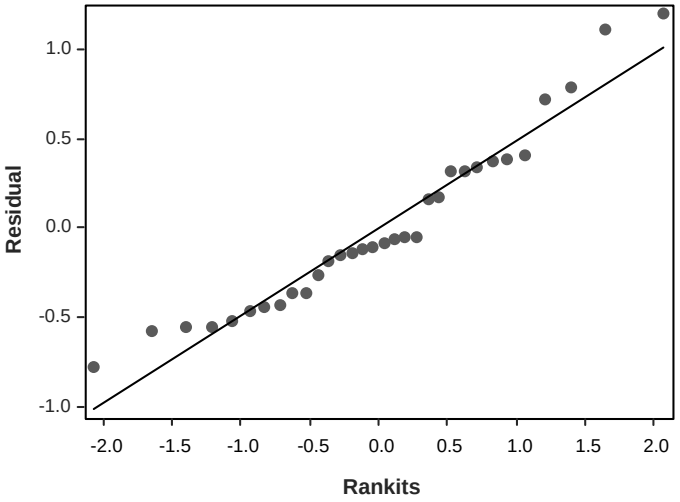
Conc-%	**Code**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
0	N	6.16	5.59	5.69	5.65
1.5		4.81	5.49	4.63	5.89
3		4.62	4.7	4.77	6.24
6.1		5.5	5.04	5.47	5.04
12.3		4.94	4.47	5.39	4.28
24		4.94	4.54	6.2	4.86
49		5.12	4.8	4.69	5.27
97		4.35	4.47	5.68	5.06

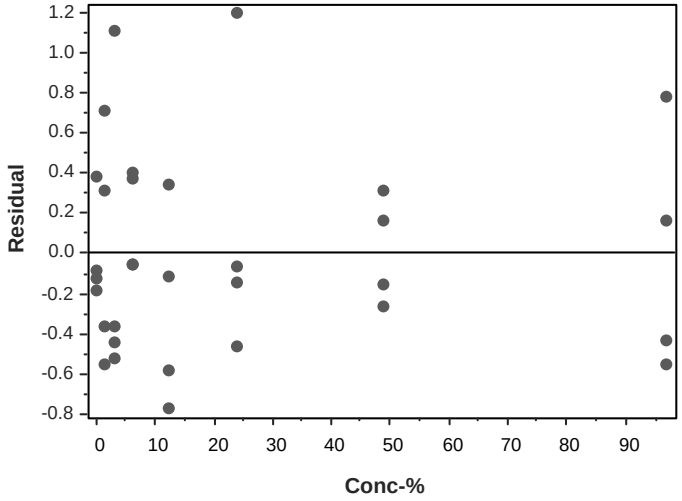
Lemna Growth Inhibition Test **Nautilus Environmental Calgary**

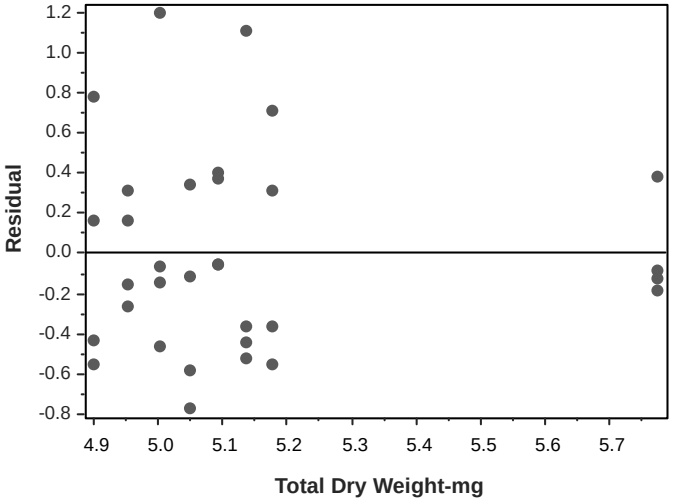
Analysis ID:	02-6010-9190	**Endpoint:**	Total Dry Weight-mg	**CETIS Version:**	CETIS v2.1.5
Analyzed:	24 Nov-23 16:30	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	24 Nov-23 0:00	**MD5 Hash:**	002D99855612CB936AC5A8CF1711F197	**Editor ID:**	002-704-782-4

Graphics Model: 3P Log-Gompertz: μ=α·exp[log[0.5]·[x/δ]^γ] Distribution: Normal [ω=1]









APPENDIX E – Chain-of-Custody Forms

ain of Custody

LS Environmental - Thunder Bay
1081 Barton Street Thunder
Bay ON Canada P7B 5N3

153186

n Lab: **Nautilus Environmental (Calgary)**

ress: 10828 27 Street SE Calgary AB Canada T2Z 3V9

Work Order Number: **TY2311633**

Original Receipt Date/Time: 07/11/2023 08:05

Instructions Received

Relinquished By
Date/Time

Received By
Date/Time
Receipt Temp

Return as Indicated: Results: NATBE.CS@alsglobal.com Invoice: NATBE.CS@alsglobal.com Electronic Data: NATBE.CS@alsglobal.com

Attention: Christine Paradis

ALS Sample ID	Client ID	Matrix	Container Type	Test Codes	Method Description	Due Date	Sampling Date and Time	Remarks
TY2311633-001	EDL1_EFF_20231106	Water	HDPE Pail	ALG-SCR-72h 11.9°C -01	Growth/Screening Green Algae (P. subcapitata) 72 hours	06-12-2023	06/11/2023 05:55	missing pail 15.8°C
TY2311633-001	EDL1_EFF_20231106	Water	HDPE Pail	CER-LC50-7d	Survival/LC50 and Reproduction (ICp) of Ceriodaphnia dubia to Effluents 7 days	06-12-2023	06/11/2023 05:55	
TY2311633-001	EDL1_EFF_20231106	Water	HDPE Pail	FAT-LC50-7d, LEM-SCR-7d	Survival/Growth/LC50/IC Fathead Minnow (P. promelas) 7 Days, Growth/Screening Lemna Minor (Duckweed) 7 days	06-12-2023	06/11/2023 05:55	
TY2311633-002	EDL2_EFF_20231106	Water	HDPE Pail	ALG-SCR-72h 12.2°C -02	Growth/Screening Green Algae (P. subcapitata) 72 hours	06-12-2023	06/11/2023 07:10	missing pail 15.7°C
TY2311633-002	EDL2_EFF_20231106	Water	HDPE Pail	CER-LC50-7d	Survival/LC50 and Reproduction (ICp) of Ceriodaphnia dubia to Effluents 7 days	06-12-2023	06/11/2023 07:10	
TY2311633-002	EDL2_EFF_20231106	Water	HDPE Pail	FAT-LC50-7d, LEM-SCR-7d	Survival/Growth/LC50/IC Fathead Minnow (P. promelas) 7 Days, Growth/Screening Lemna Minor (Duckweed) 7 days	06-12-2023	06/11/2023 07:10	

2824-0709
2023/11/08
09:10
Purolator
SC/ET
4x10L pails
NaS/NaT
Missing 2x10L pails
COC 1/2

20231111109
0850
Purolator
BS/KZ
2x10L pails
Good condition
NaS/NaT

END OF REPORT

TY2311633

Environmental Division
Thunder Bay
Work Order Reference
TY2311633

Telephone : +1 807 623 6463

Project Name: Rainy River
Location: Chapple
Project Number:
Project Manager:
PO Number:
Project:
Turn Around Time (days): 10 Business Days
Shipping Company:
Shipping Date: 11/06/2023 8:26:00 AM
COC Number: ALS-452364166

	Containers	Discharge Monitoring	Sublethal Toxicity Pails	Acute Toxicity Pails								
	Filtered	N	N	N								
	Preservatives											
Sample Code		NG-ODM-P-TB	NG-ST-P-TB	NG-AT-P-TB							Number of Containers	Comments
EDL1_EFF_20231106	8.22	7.53	4.15	2023-11-06 7:55	EFF	x	x							19	
EDL2_EFF_20231106	9.48	7.66	5.28	2023-11-06 9:10	EFF	x	x							19	
EDL1_EFF_20231103_212	8.29	7.53	5.04	2023-11-03 13:35	EFF	x		x						19	
EDL2_EFF_20231103_213	10.82	7.58	5.36	2023-11-03 15:15	EFF	x		x						19	
SP2_EFF_20231103_216	9.45	7.97	3.14	2023-11-05 14:15	EFF	x		x						19	

Signature	Data/Time
Shipped by	2023-11-06 8:26
Received by	LV 11/7/23 10:05

Shipping Details
Method of Shipment: Courier
On Ice: yes / no
Shipped: Air/Ground
Lab Name: ALS Thunder Bay
Lab Phone:

ATTN

Special Instructions:

Email Invoice to: rainyriver.accounts1@newgold.com Email Report to: rainyriver.labresults@newgold.com

9.3 10.1
10.1 11.0
10.2 9.3
} 6 pails

9.8°c
9.3°c

G. Coozier / Manitoulin
3302756359

NAUTILUS ENVIRONMENTAL

TESTING LOCATION (Please Circle)

Burnaby ( )
8664 Commerce Court
Burnaby, British Columbia, Canada
V5A 4N7
Phone 604.420.8773

Calgary (•)
10823 27 Street SE
Calgary, Alberta, Canada
T2Z 3V9
Phone 403.253.7121

Point Edward ( )
704 Mara Street, Suite 122
Point Edward, Ontario, Canada
N7V 1X4
Phone 519.339.8787

Chain of Custody

Date_____Page__of__

Report to:		**Invoice To:**	
Company	ALS Environmental	**Company**	ALS Environmental
Address	1081Barton St	**Address**	1081Barton St
City/Prov/PC	Thunder Bay ON P7B 5N3	**City/Prov/PC**	Thunder Bay ON P7B 5N3
Contact	Christine.Paradis	**Contact**	Christine.Paradis
Phone	807-623-6463	**Phone**	807-623-6463
Email	christine.paradis@alsglobal.com	**Email**	NATBE.CS@alsglobal.com
		PO No.	

Sample Collection By: **Sample Type:** Grab (•) OR Composite ( )

	SAMPLE ID	DATE (DD/MM/YY)	TIME	MATRIX	# OF CONTAINERS AND VOLUME (e.g. 1 x 20 L)	COMMENTS	ANALYSES REQUIRED: Fathead Minnow 7-day survival & growth	Ceriodaphnia dubia 3-brood reproduction & survival	Lemma minor 7-day growth	Raphidocelis subcapitata 72-hour growth	Receipt Temperature (°C)
1	EDL1_EFF_20231106	06/11/23	07:55	Water	3 x 10L		✔	✔	✔	✔	
2	EDL2_EFF_20231106	06/11/23	09:10	Water	3 x 10L		✔	✔	✔	✔	
3											
4											
5											
6											
7											
8											
9											
10											

SPECIAL INSTRUCTIONS/COMMENTS (CLIENT)

SAMPLE RECEIPT DETAILS (LABORATORY)

1. Total No. of Containers		4. Ice Present in Cooler?	Y / N
2. Courier		5. Seal Present?	Y / N
3. Good Condition?	Y / N	6. Initials Present on Seal?	Y / N

SAMPLE DESCRIPTION AND COMMENTS (LABORATORY)

RELINQUISHED BY (CLIENT)

(Printed Name) (Signature)

(Company) (Date DD/MM/YY and Time)

RECEIVED BY (LABORATORY)

(Printed Name) (Signature)

(Company) (Date DD/MM/YY and Time)

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.

Intake and Login Verification Form

SAMPLE INTAKE			
Priority/Emergency Service Requested		YES	NO
Time Sensitive Hold Time		YES	NO
Client:	New Gold		

SAMPLE RECEIPT INFORMATION			
Mode of Delivery:	Courier		Drop Off
Courier	Manitoulin		
Waybill Number	330275635 9		
Temperature	9.3, 10.1, 10.2, 10.1, 11.0, 9.3		Cooler Count 6
Cooling Method	None	Ice	Ice Packs

SAMPLE MATRIX/BOTTLE INFORMATION					
Matrix:	Water	Soil	Air	Biota	Other
DW Schedule 24 Bottles Correct?				Yes	No
DW Metals pH Check <2				Yes	No
Regulation Circled, Works # present			Yes	No - Reject?	
# of Bottles:	Sample Count			2	
Green/white					
Purple/white					
Warm red/white					
Yellow/black					
Light blue/white					
Orange/black					

Others (detail)

3x2 Box pairs

Comments on Samples and Bottles:

Samples Requiring Preservation or Filtering:

Layout Staff Initials Date and Time of Layout	NOV 07,23 10:29AM 8509

ACCOUNT INFO VERIFICATION		
Priority/Emergency Service Requested	YES	NO
Confirmed all as accurate as per COC, Sample Remarks or PM		
Client / Work Contact / Quote		

RECEIPT DETAIL		
Project / PO / Site/LSD		
Overall Description Entered	Yes	NA
Received date/time as per COC		
Recipients match CoC or Sample Remarks	Yes	No
Billing Instruction added to remarks	Yes	NA
Sample Remarks/Specification Doc checked		
Submission Issues communicated	Yes	NA
Sample Info communicated via Remarks	Yes	NA

VERIFICATION CHECKLIST		
Plannned Event Submission	Yes	No
Sample Name entered as per CoC		
Sampling Date and time entered as per CoC		
Containers selected in layout order		
Sales items entered from QUOTE ONLY (and/or verified as correct)		
Field Data/EC298A removed if not on COC	Yes	NA
Bottle Allocation Verified		
Guideline added or auto-allocated		
Due dates updated		

VALIDATION		
Validation errors resolved?	Yes	No
Internal Sublet CoC created	Yes	NA

Login Comments:

Login Staff Initials:	APS

Prepared for
New Gold Inc.
1361 Roen Road
Chapple, Ontario
Canada, P0W 1E0

Prepared by
Knight Piésold Ltd.
Suite 1400 - 750 West Pender Street
Vancouver, British Columbia
Canada, V6C 2T8

VA101-656/13-1

RAINY RIVER MINE
EFFLUENT MIXING STUDY REPORT

Rev	Description	Date
0	Issued in Final	February 7, 2024

EXECUTIVE SUMMARY

Rainy River Mine (RRM) received permits to discharge treated mine surplus water (effluent) to the Pinewood River via two separate discharge locations downstream of the Mine site. The two Effluent Discharge Locations (EDLs) are located on the Pinewood River at the Rainy River Pumphouse (EDL 1) and just downstream of the Loslo Creek confluence (EDL 2). Discharge of the treated effluent is restricted based on conditions outlined in the Amended Environmental Compliance Approval (Amended ECA) 2290-CAVKGN, issued by the Ministry of the Environment, Conservation and Parks (MECP) on April 14, 2022 (MECP, 2022). Knight Piésold Ltd. (KP) was retained by RRM to design the Effluent Mixing Structure 1 (EMS 1) and Effluent Mixing Structure 1 (EMS 2), located at EDL 1 and EDL 2, respectively. These structures were designed to enhance the mixing of the effluent with the receiving environment while complying with the requirements set in the ECA. Construction of the effluent mixing structures were completed in 2019 at EDL 1, and in 2020 at EDL 2. These structures were upgraded with different type diffusers in early 2023 to enable RRM to release effluent at greater discharge rates, while still meeting the permitted ECA criteria.

The ECA requires completing an effluent mixing study at EDL 1 and EDL 2 to evaluate the distance downstream from the diffusers required to achieve complete transverse mixing. Such study was completed in November 2023 after the most recent upgrades to the diffusers in 2023. The results from this mixing study, completed at EDL 1 and EDL 2, are presented in this report. The mixing study demonstrated that complete transverse mixing was achieved within 60 m downstream of the diffuser outlets at both EDL 1 and EDL 2. The observed effluent mixing at EDL 1 and EDL 2 was found to meet the requirement for adequate mixing with the receiving environment as required in the Amended ECA, based on the site and flow conditions tested in this study. The study has shown that the mixing structures, including the diffusers, are operating as intended, and that complete transverse mixing is achieved rapidly and before the downstream compliance points are reached.

The results from the field study were compared against the results from the analytical mixing model for the Pinewood River developed by KP, which was initially used to inform the design of the effluent mixing structures. The comparison indicates that the analytical mixing model and the observed conditions during the field mixing study are generally in good agreement. The analytical model predicted effluent mixing performance and complete transverse mixing lengths that are generally in line with the conditions observed in the field.

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ABBREVIATIONS

ECA ..... Environmental Compliance Approval
EMS 1 ..... Pinewood River Effluent Mixing Structure 1
EMS 2 ..... Pinewood River Effluent Mixing Structure 2
EDL 1 ..... Pinewood River Effluent Discharge Location 1
EDL 2 ..... Pinewood River Effluent Discharge Location 2
KP ..... Knight Piésold Ltd.
MECP ..... Ontario Ministry of the Environment, Conservation and Parks
New Gold ..... New Gold Inc. (Rainy River)
RRM ..... Rainy River Mine

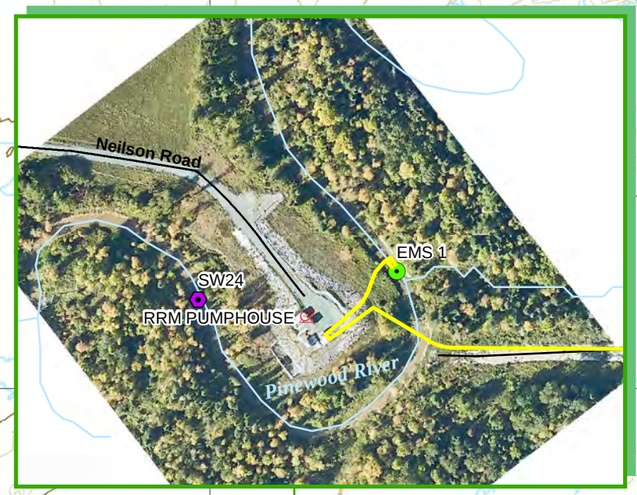
1.0 INTRODUCTION

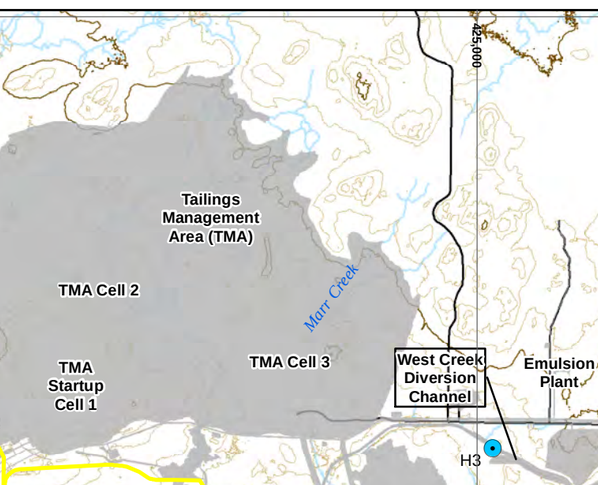
1.1 BACKGROUND

New Gold Inc. (New Gold) operates the Rainy River Mine (RRM), a gold/silver mine located in the Township of Chapple, approximately 65 kilometers (km) northwest of Fort Frances, northwestern Ontario. RRM received permits to discharge treated mine surplus water (effluent) to the Pinewood River via two separate discharge locations downstream of the Mine site. Discharge of the treated effluent, which began during the 2020 open water season, is restricted based on conditions outlined in the Amended Environmental Compliance Approval (Amended ECA) 2290-CAVKGN, issued by the Ministry of the Environment, Conservation and Parks (MECP) on April 14, 2022 (MECP, 2022). The two Effluent Discharge Locations (EDLs) are located on the Pinewood River downstream of the McCallum Creek confluence at the Rainy River Pumphouse (EDL 1) and immediately downstream of the Loslo Creek confluence (EDL 2), as shown on Figure 1.1.

Knight Piésold Ltd. (KP) was retained by RRM to design the Effluent Mixing Structure 1 (EMS 1) and EMS 2, located at EDL 1 and EDL 2, respectively. The EMS 1 and EMS 2 primarily consist of a multiport diffuser and erosion protection measures to minimize channel erosion in the vicinity of the diffusers, which were designed to enhance mixing of the effluent with the receiving environment while complying with the requirements set by the ECA. Furthermore, the multiport diffusers were designed to achieve full transverse mixing within the desired mixing zone, as discussed in the *Effluent Mixing Structure Design Report* (KP, 2019a). The design of the EMS 1 constructed at EDL 1 is presented in the *2019 Abatement Plan - Mechanical Systems Report* (KP, 2019b), while the as-built drawings are presented in the *Effluent Mixing Structure 1 (EMS 1) Construction Report* (KP, 2020a). The design and the as-built drawings for EMS 2 constructed at EDL 2 are presented in the *Long-Term Effluent Discharge Design Report* (KP, 2020b), and in the *Effluent Mixing Structure 2 (EMS 2) Construction Report* (KP, 2020c), respectively.

The multiport diffusers at both EDL 1 and EDL 2 were subsequently modified in March 2023, as presented in KP letter (Ref. No. VA23-00092) titled *Rainy River Mine – Mixing System Design Upgrades* issued on February 27, 2023 (KP, 2023a). The upgrades consisted of replacing the existing 8” in-pipe diffusers with clamp-on 8” Larflex Type 71 check valve diffusers to increase the discharge capacity of the systems, while promoting rapid mixing of the effluent with the receiving environment. Following the diffuser upgrades completed in March 2023, a mixing study was required to confirm their performance. This report presents the methodology and the results of the effluent mixing study completed in November 2023 at EDL 1 and EDL 2.





5,415,000
415,000
420,000
425,000
430,000
5,410,000
5,405,000

North Branch
Highway 617
McCallum Creek
Tailings Management Area (TMA)
TMA Cell 2
TMA Cell 3
TMA Startup Cell 1
Water Management Pond
Marr Creek
West Creek Diversion Channel
Emulsion Plant
West Creek
Log Creek
Unnamed Creek 02
H3
H4
BCR2
Constructed Wetland
Main Sub-Station
MILL
West Mine Rock Stockpile
Eosto Creek
Open Pit
Low Grade Ore Stockpile
Clark Creek
East Mine Rock Stockpile
Mine Rock Pond
Pinewood River
SW22A
EMS 2
EMS 1
SW24
RRM PUMPHOUSE
H1
H2
Unnamed Tributary 01
Camp
05PC023

LEGEND:

- EXISTING HYDROLOGY STATION
- WSC STATION
- PUMPHOUSE
- EMS EFFLUENT DISCHARGE LOCATION
- EXISTING SURFACE WATER QUALITY STATION
- ROAD
- HIGHWAY
- RIVER/STREAM
- CONTOUR 25m
- CONTOUR 5m
- MINE INFRASTRUCTURE
- TRANSMISSION LINE 230 KV
- PIPELINE

0.5 0.25 0 0.5 1 1.5 2 km

NOTES:

1. BASE MAP: PROJECT TOPOGRAPHY AND ORTHOIMAGERY.
2. COORDINATE GRID IS IN METRES.
COORDINATE SYSTEM: NAD 1983 UTM ZONE 15N.
3. THIS FIGURE IS PRODUCED AT A NOMINAL SCALE OF 1:47,500 FOR 11x17 (TABLOID) PAPER. ACTUAL SCALE MAY DIFFER ACCORDING TO CHANGES IN PRINTER SETTINGS OR PRINTED PAPER SIZE.

REV	DATE	DESCRIPTION	DESIGNED	DRAWN	REVIEWED
0	07FEB'23	ISSUED WITH REPORT	JV	KK	VM

SAVED: M:\1\101\00656\13\AGIS\Pgis\VA101-656-13-1\Fig1-1_SitePlan_rA.mxd; Feb 07, 2024 1:55 PM; dmcguire

newgold™ Rainy River

RAINY RIVER MINE
HYDROMETRIC MONITORING NETWORK

Knight Piésold CONSULTING

P/A NO. VA101-656/13
REF NO. 1
FIGURE 1.1
REV 0

1.2 SCOPE OF WORK

The Amended ECA requires that an effluent mixing study be completed at EDL 1 and EDL 2 to evaluate the distance downstream from the diffusers required to achieve complete transverse mixing, and to confirm there is adequate effluent mixing with the receiving environment by the time it reaches the downstream compliance points. KP letter (Ref. No. VA20-00733) titled *Rainy River Mine – Mixing Study Terms of Reference* (TOR) issued on May 8, 2020, outlined the proposed mixing study details, such as the proposed mixing tracer, the data collection procedures, and the general mixing study setup (KP, 2020d). After the initial installation of the diffusers, a mixing study was completed at EDL 2 in October 2020 following the methodology proposed in the TOR, with the results from the study presented in KP (2020e). A mixing study could not be completed at EDL 1 at that time due to high ambient flow conditions, as discussed in KP (2020e).

This report presents the results of a new mixing study completed in November 2023 at EDL 1 and EDL 2 following the diffuser upgrades installed in March 2023. The study was completed to evaluate the distance downstream from the diffusers required to achieve complete transverse mixing, and to confirm that the mixing performance of the EMS 1 and EMS 2 remains adequate following the 2023 diffuser upgrades.

The parameters and the proposed methodology and instrumentation for the recent study followed the procedure described in the TOR (KP, 2020), with additional consideration of requests and review comments provided by MECP issued in their memorandum New Gold Rainy River Mine – Mixing Zone Validation Terms of Reference (TOR), issued on July 8th, 2021 (MECP, 2021).

The mixing study was completed jointly by KP and Ecometrix Inc. (Ecometrix), with support from New Gold staff. KP's scope focused on assessing effluent mixing characteristics within the near-field (up to 100 m distance from the discharge points), while Ecometrix's focus was on the far-field mixing performance (beyond 100 m downstream from the discharge points). The far-field study details and results will be provided by Ecometrix and are outside the scope of this document.

2.0 EDL 1 AND EDL 2 MIXING STUDY SUMMARY

2.1 MIXING STUDY SITE VISIT

KP conducted a site visit to the RRM from November 6 to November 10, 2023 (including travel dates) to complete the proposed mixing study at EDL 1 and EDL 2. The mixing study was completed jointly by KP and Ecometrix, with support from New Gold staff. The mixing study was carried out at EDL 1 on November 8, and at EDL 2 on November 9, following the requirements specified in Condition 10(8) of the Amended ECA 2290-CAVKGN (MECP, 2022).

The ECA requires the mixing study be completed during low-flow conditions. As such, a 1:1 ambient-to-effluent discharge ratio coupled with a Pinewood River ambient flowrate of 10,000 m³/day was targeted for the study in accordance with the ECA. New Gold and KP closely monitored site weather and Pinewood River flow conditions for several months to plan the mixing study site visit at the target flow rate. However, dry conditions throughout the summer and fall prevented the Mine to discharge effluent into the Pinewood River during that period. A rapid increase in flows at the start of November 2023 provided a short window for the study to be completed before the onset of the winter season and freezing conditions. New Gold and KP made the decision to complete the study during this window despite the background flows being higher than the targeted flows, as valuable information would still be collected to inform the mixing characteristics of the EMS systems.

Table 2.1 includes details on the mixing study completed at each location, including ambient and effluent discharge rates at the time of the study, as well as the resulting ambient-to-effluent discharge ratios. Given the relatively high ambient flow conditions that were above the maximum capacity of the EMS systems, it was not possible to discharge the effluent at the targeted 1:1 ambient-to-effluent discharge ratio.

Table 2.1 Discharge Conditions During the Mixing Study

Effluent Discharge Location	Date of Study	Effluent Discharge (m³/day)	Ambient Discharge in Pinewood River (m³/s)	Ambient-to-Effluent Discharge Ratio
EDL 1	November 8, 2023	25,000	130,000	~5:1
EDL 2	November 9, 2023	22,500	46,000	~2:1

Details on the mixing study site visit are presented in a KP letter VA23-01935 titled *November 2023 Site Visit Summary Letter* (KP, 2023b). The results of the mixing study completed at EDL 1 and EDL 2 are outlined in the sections below.

The plan view and the long section of the as-built drawings for EDL 1 and EDL 2 are shown on Figure 2.1 and Figure 2.2, respectively. The dye injection locations and the transect locations where the mixing study measurements were recorded are superimposed on these figures for reference.

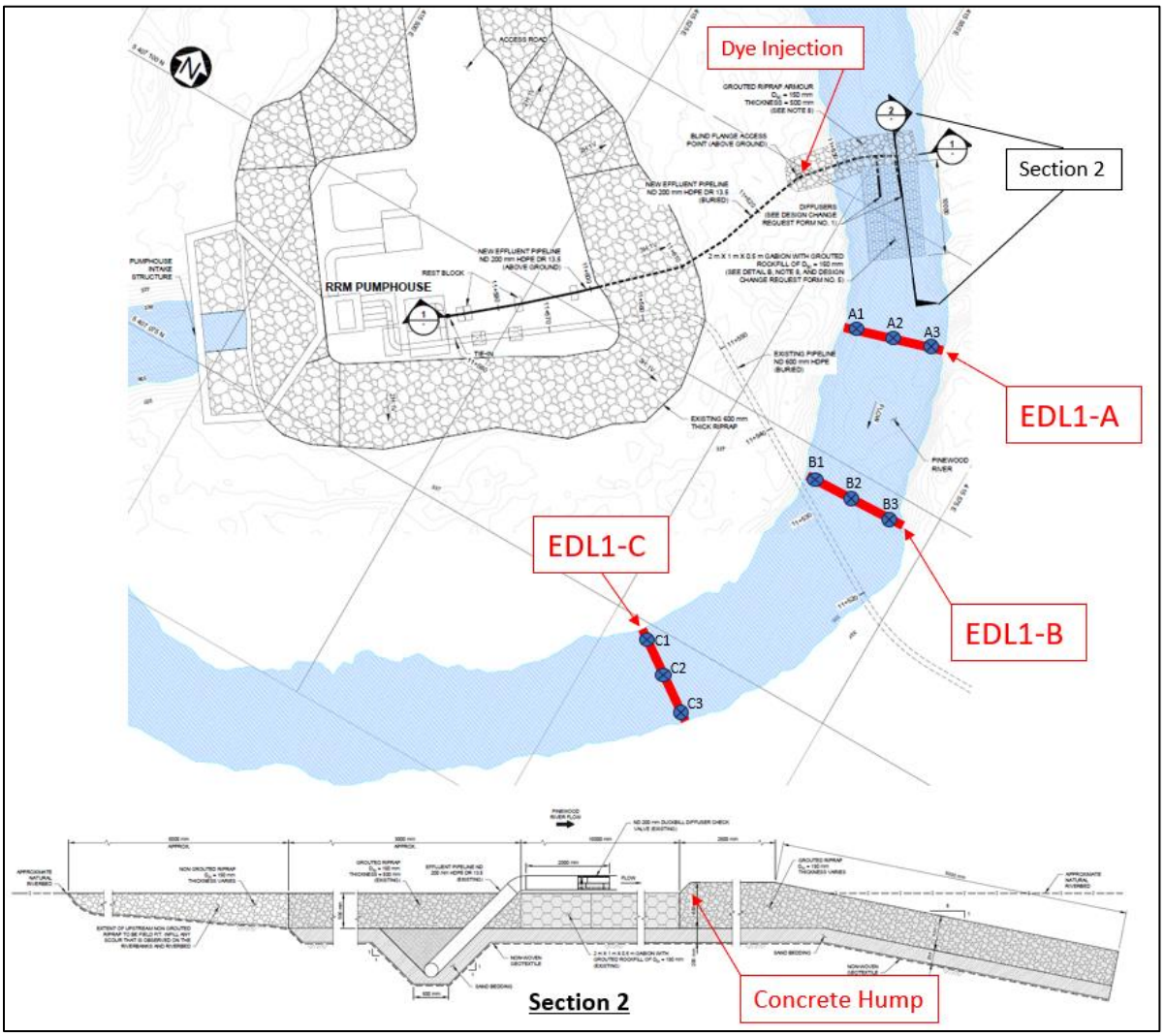


Figure 2.1 EDL 1 Plan View and Long Section of the As-Built Drawings with Mixing Study Transect Locations (Source: Drawings RR-C1100 and RR-C1106, KP, 2020a)

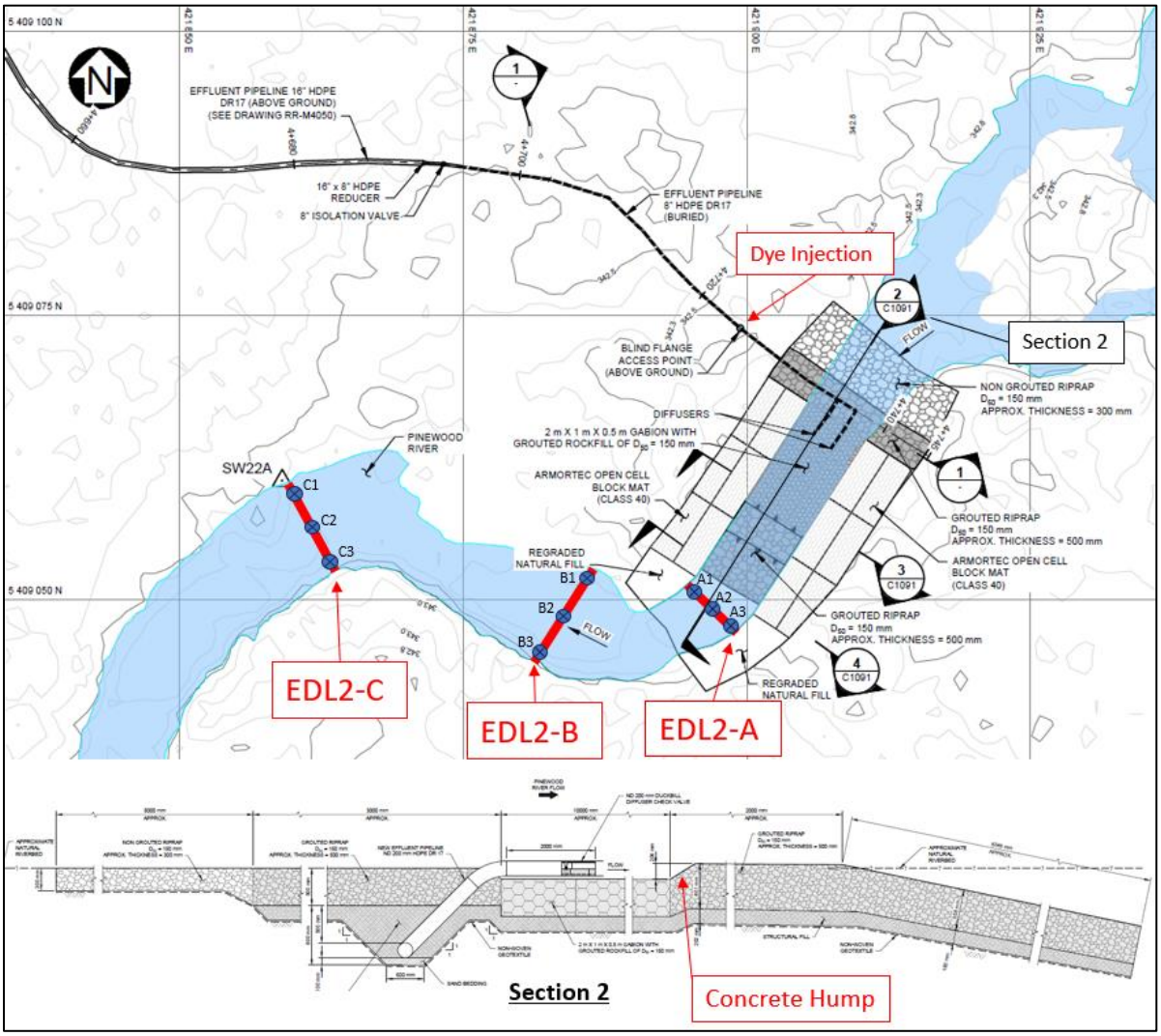


Figure 2.2 EDL 2 Plan View and Long Section of the As-Built Drawings with Mixing Study Transect Locations (Source: Drawings RR-C1090 and RR-C1091, KP, 2020c)

2.2 SITE CONDITIONS

Table 2.2 provides a summary of key climate and water temperature parameters measured during the mixing study at each EDL. The parameters were averaged over the duration of the mixing study at each site. The climate data provided by RRM were obtained from RRM's weather station, while the water temperatures were obtained from the SonTek-IQ instrument installed at the H1 hydrometric monitoring station. Water quality parameters throughout the duration of the study were provided by RRM and are shown in Appendix A.

Table 2.2 Site Conditions during the Mixing Study

Effluent Discharge Location	Average Wind Speed (km/h)	Wind Direction	Past 24-hour precipitation (mm)	Air Temperature (°C)	Water Temperature (°C)
EDL 1	13.1	N	0	3.0	3.0
EDL 2	12.7	N	0	0.1	2.8

2.3 STUDY SETUP

The mixing study was completed by injecting a constant amount of Rhodamine WT dye into the effluent discharge pipeline, while monitoring Rhodamine WT dye concentrations at various transects downstream from the discharge location (as shown on Figure 2.1 and Figure 2.2) using YSI EXO sondes. The injection setup at EDL 1 is presented on Photo 2.1. The setup at EDL 2 followed the same methodology.

Photo 2.1 Peristaltic Pump and Dye Injection Setup at EDL 1

Rhodamine WT is a fluorescent dye that is commonly used as a tracer, often used in applications such as dispersion studies or discharge measurements. Tracers mimic the movement of water molecules, and therefore a measurement of the movement of the tracer is in effect a measurement of the movement of the water (YSI Environmental, 2001). Rhodamine WT dye is one of the most common tracers in hydrology studies because it is highly soluble, does not readily absorb sediments, is non-toxic at concentrations used

for hydrology investigations, and is highly detectable even at very low concentrations (0.01 to 0.05 µg/L) (Runkel, 2015).

Fluorometers, such as the YSI EXO sondes, use the fluorescent characteristics of Rhodamine WT dye to measure its concentration in water. The measurements are in-situ, meaning that samples of the river water do not need to be taken to a laboratory to determine the extent of mixing. In-situ measurements were collected every two seconds and recorded using a handheld YSI Multi-Parameter display system. Prior to the start of the study, the YSI EXO sondes were calibrated in the lab by Hoskins Scientific before being sent to RRM.

The YSI EXO sondes were placed across the river at transects downstream of the diffusers, with one sonde placed at the channel center, and one sonde placed at each channel bank within the active flow area, for a total of three sondes per transect (Figure 2.1 and Figure 2.2). A total of three transects located at various distances downstream from the discharge points were assessed. The transect locations were selected based on visual observations of mixing conditions made during the field study.

Table 2.3 and Table 2.4 summarize the transect details at EDL 1 and EDL 2, respectively. The transect locations at EDL 1 are shown on Photo 2.2 and Photo 2.3, and at EDL 2 on Photo 2.4 and Photo 2.5.

Table 2.3 EDL 1 Transect Location Information

Transect/Sonde Name	No. of Sondes along Transect	Distance from Diffuser Outlets (m)	Location Description	Photo Reference
EDL1 - A	3	20	Immediately upstream of first channel bend	Photo 2.2
EDL1 - B	3	40	Immediately downstream of first channel bend	Photo 2.2 & Photo 2.3
EDL1 - C	3	60	Immediately upstream of a small log jam (active flow through logs with no backwatering conditions)	Photo 2.3

Table 2.4 EDL 2 Transect Location Information

Transect/Sonde Name	No. of Sondes along Transect	Distance from Diffuser Outlets (m)	Location Description	Photo Reference
EDL2 - A	3	15	Immediately upstream of first channel bend	Photo 2.4
EDL2 - B	3	25	Along first channel bend	Photo 2.4
EDL2 - C	3	60	At sampling dock downstream of first channel bend	Photo 2.5

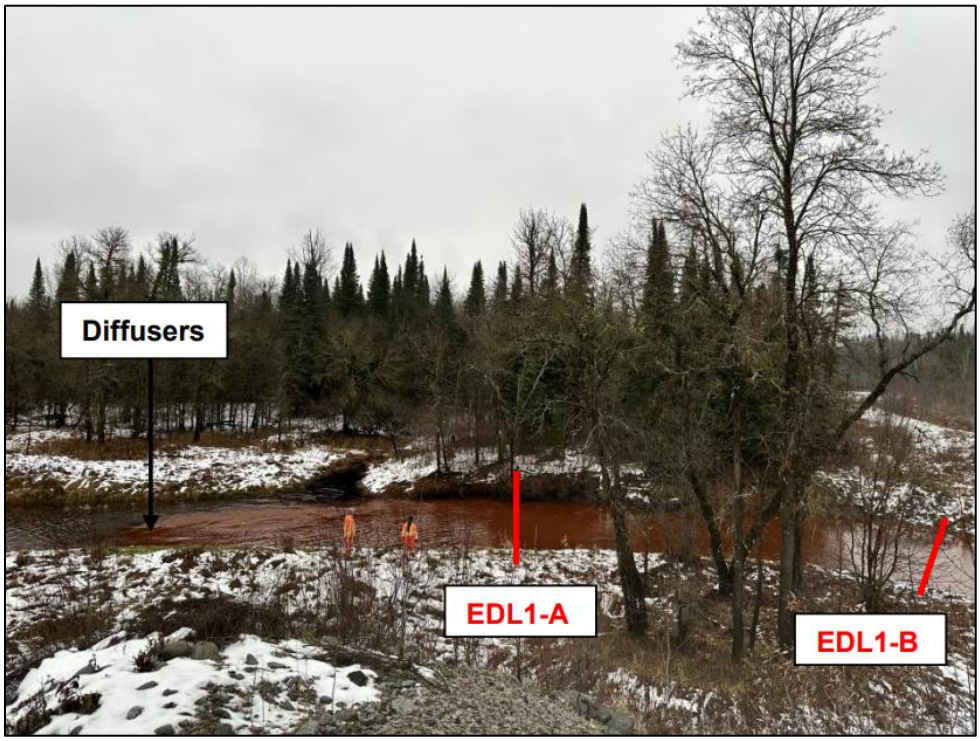


Photo 2.2 Location of transects EDL1-A and EDL1-B (looking towards left bank)

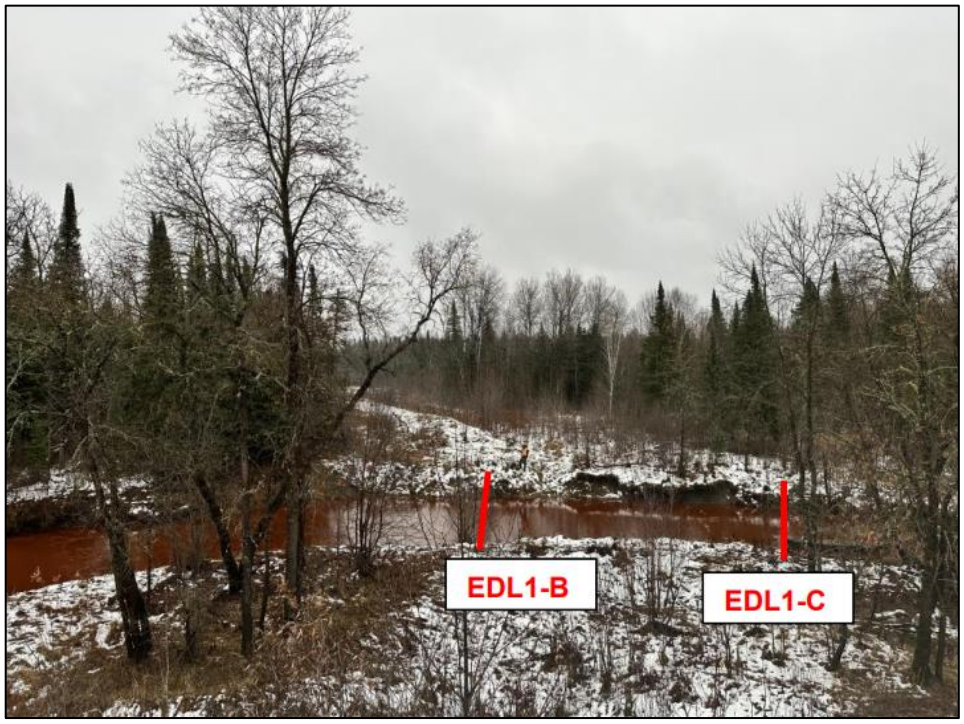


Photo 2.3 Location of transects EDL1-B and EDL1-C (looking towards left bank)

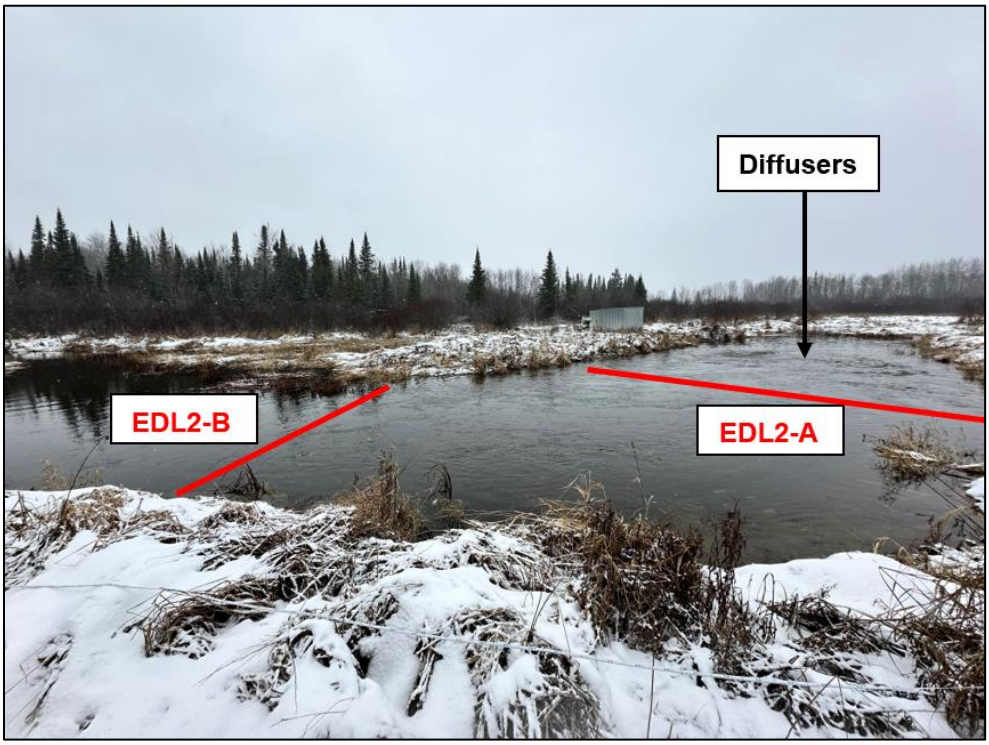


Photo 2.4 Location of transects EDL2-A and EDL2-B (looking upstream)

Photo 2.5 Location of transect EDL2-C (looking towards right bank)

The Rhodamine WT dye was injected into the effluent discharge pipeline at a constant rate and YSI EXO sonde readings were monitored until it was determined that a steady state condition in the river had been established. This steady state condition was maintained for at least 30 minutes, or long enough to ensure that the concentration of Rhodamine in the river was not notably varying in time, and that both KP and Ecometrix had collected sufficient data to meet study objectives. The injection was then stopped, and the recordings continued as dye concentrations decreased. The *November 2023 Site Visit Summary Letter* (KP, 2023b) presents a detailed description of the study setup and methodology.

3.0 MIXING STUDY RESULTS

3.1 ANALYSIS METHODOLOGY

The Rhodamine WT dye concentration time series recorded by the YSI EXO sondes at the various transects were analyzed to evaluate the effluent mixing zone within the receiving environment and the performance of the effluent mixing structure. The steady state period for each data set was visually identified and used for the analysis. A 20-second (i.e., 10 samples) moving average was applied to the data to reduce noise in the raw data. The average concentration across each transect was calculated at each time step and the deviation of each individual sonde at the corresponding transect from the mean was calculated using the following equation:

$$Percent\ Difference = \frac{Sonde\ Concentration - Mean\ Transect\ Concentration}{Mean\ Transect\ Concentration}$$

According to Fischer (1979), complete transverse mixing is achieved when the concentration everywhere across the channel transect is within 5% of the mean transect concentration. Therefore, if the percent difference calculated based on the equation above is equal or less than 5%, it can be reasonably concluded that full transverse mixing has been achieved.

The results from the mixing study at EDL 1 and EDL 2 are presented below using a combination of time series plots and box plots. The time series figures show the recorded concentrations by each of the YSI EXO sondes for the duration of the study, and the box plots summarize the typical percent difference in dye concentration observed across each transect during the selected steady state period. The mean dye concentrations calculated for each sonde during the steady state period are also presented.

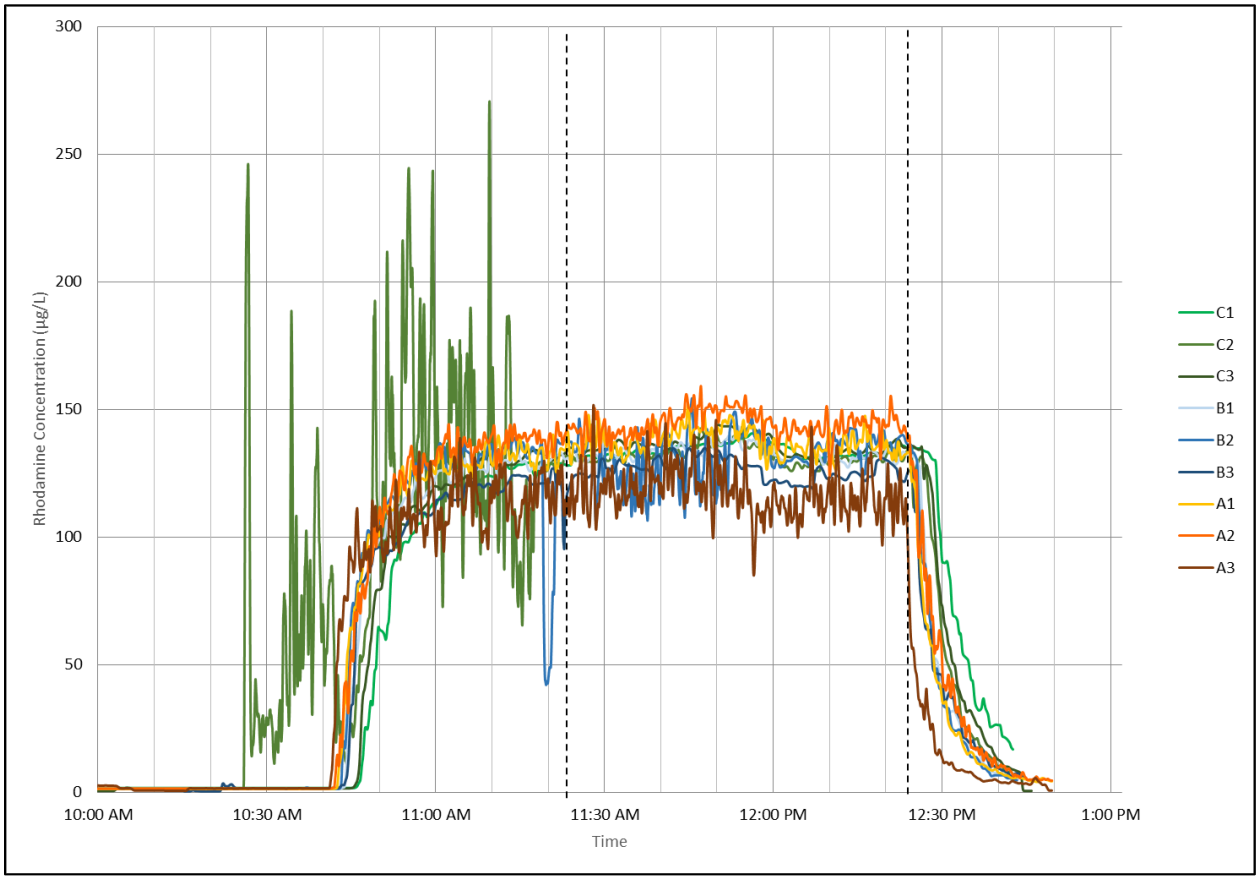
Box plots show the distribution of data, with the first and third quartiles of the data set represented by the lower and upper bound of each box, and the median represented using a line within the box. The first and third quartiles represent the values in the data set below which 25% and 75% of the measured points fall, respectively, meaning that the values encompassed by the box represent the middle 50% of the data. In addition to the 25%, 50%, and 75% data points, the box plots also indicate the typical minimum and maximum values that would be expected based on an assumed normal distribution (represented with capped lines, or “whiskers” on either side of the box), as well as any outliers if present (represented as standalone points), which fall outside of 2.698 standard deviations of the median (i.e., the most extreme 0.7% of the data).

The size and symmetry of the boxes within the box plots can therefore provide insights into the typical values, the amount of spread, and the symmetry of the data set, with the whiskers and outliers completing the visualization of the data set by presenting extreme values separately. A tightly grouped box plot with a limited range (spread) represents a relatively uniform data set, whereas a box plot with greater range or distant outliers will be associated with a data set with greater variability.

3.2 MIXING AT EDL 1

The transects at EDL 1 summarized in Table 2.3, are located 20 m (EDL1-A), 40 m (EDL1-B) and 60 m (EDL1-C) downstream of the diffuser outlets. The study setup at EDL 1 is shown on Photos 2.2 and 2.3.

The concentration time series data sets recorded by the sondes at the EDL1-A (Sondes A1, A2 & A3), EDL1-B (Sondes B1, B2 & B3) and EDL1-C (Sondes C1, C2 & C3) transects are presented on Figure 3.1.



Note(s):

1. The vertical dashed lines indicate the steady state period used for analysis.

Figure 3.1 EDL 1 Transient Rhodamine WT Dye Concentrations

The steady state period selected for analysis extends for 1 hour from 11:23 AM to 12:23 AM on November 8, 2023. During this period, the minimum, maximum and mean Rhodamine WT dye concentrations, as well as the standard deviation at each sonde are summarized in Table 3.1.

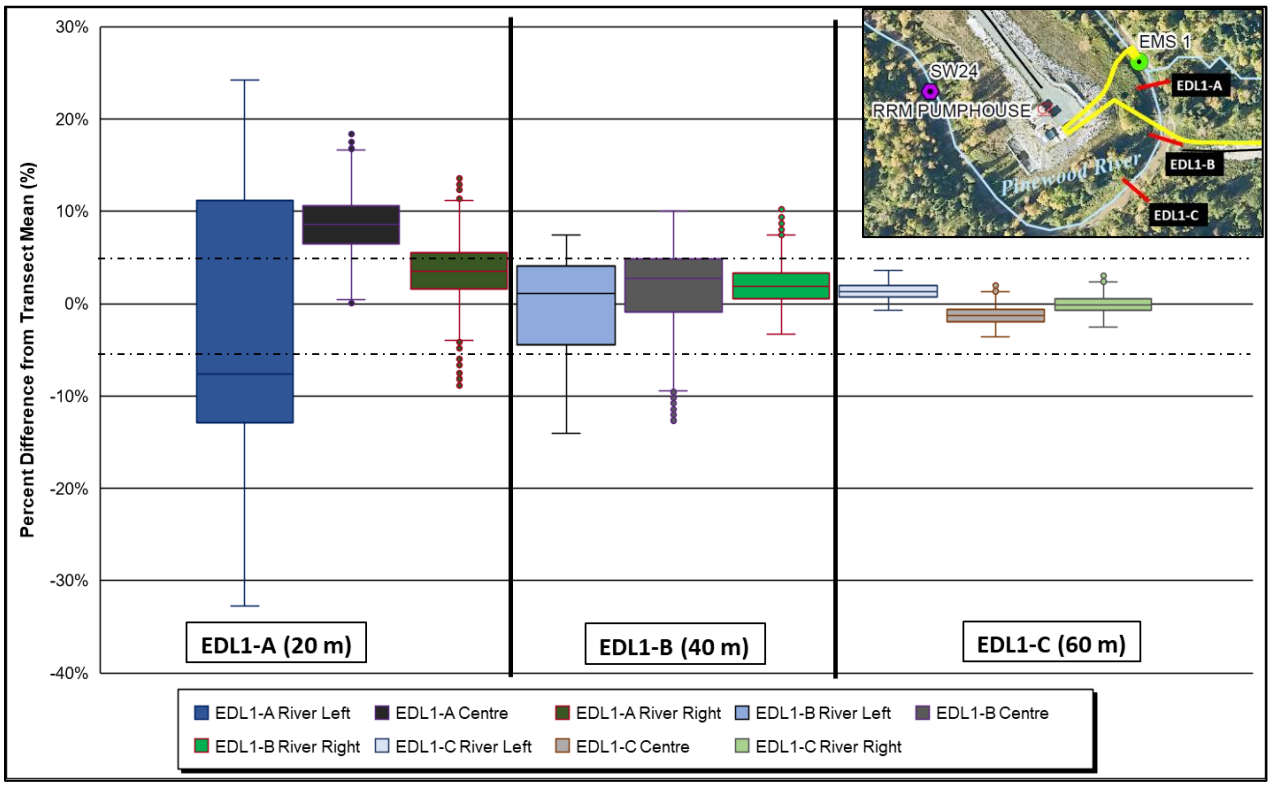
A high level of variability is experienced by sonde C2 (medium green line on Figure 3.1) in the time leading up to the start of the steady state period. This is due to the sonde becoming buried in sediment at the location where it was initially placed. After retrieving the sonde and cleaning off the sediment, the sonde was repositioned at the same location and started registering stable concentrations, after which the steady state period began for the study at 11:23 AM.

Table 3.1 Steady State Concentration Statistics at Transects for EDL 1

Transect	Sonde Label	Sonde Location	Min (µg/L)	Mean (µg/L)	Max (µg/L)	Standard Deviation
EDL1-A	A3	River Left	85	117	152	8.99
	A2	Channel Centre	124	144	159	4.91
	A1	River Right	126	138	152	4.91
EDL1-B	B3	River Left	108	126	135	4.27
	B2	Channel Centre	106	132	154	8.94
	B1	River Right	127	133	144	3.28
EDL1-C	C3	River Left	129	136	144	3.72
	C2	Channel Centre	126	132	143	3.52
	C1	River Right	128	134	141	2.73

The concentrations at EDL1-A River Left (Sonde A3) recorded the greatest variability, with the data exhibiting the highest standard deviation recorded during the study. On the other hand, EDL1-C River Right (Sonde C1) recorded the lowest standard deviation, indicating the concentrations were the least variable at this location.

The percent differences between each concentration measurement and the mean transect concentration, over the steady state period, are summarized on the box plot shown on Figure 3.2. Each set of box and whiskers represent one sonde, as labelled. Outliers that are outside of the whiskers are represented as standalone points. The ±5% envelope indicative of complete transverse mixing, as defined by Fischer (1979), is shown on the figure as dotted lines. A summary of the mean concentrations registered by each sonde and the associated percent difference from the transect mean for the steady state period is also provided in Table 3.2.



Note(s):

1. Internal line within each box indicates median values.
2. Dashed lines at ±5% present the envelope for fully mixed conditions, as defined by Fischer (1979).

Figure 3.2 EDL 1 Box and Whisker Plot Indicating Percent Difference from Transect Mean Concentration

Table 3.2 EDL 1 Steady State Mean Concentrations – Percent Difference from Transect Mean

Transect	Parameter	River Left	Channel Centre	River Right
EDL1-A	Mean Concentration (µg/L)	117	144	138
	Percent Difference from Transect Mean (%)	-11.88	8.55	3.33
EDL1-B	Mean Concentration (µg/L)	126	132	133
	Percent Difference from Transect Mean (%)	-5.06	-0.55	0.01
EDL1-C	Mean Concentration (µg/L)	136	132	134
	Percent Difference from Transect Mean (%)	2.00	-0.65	0.53

The box plots on Figure 3.2 provide a visual representation of the degree of variability, and by extension the degree of mixing, within the channel. Generally, the boxes and whiskers range more widely closest to the diffuser outlets at transect EDL1-A. This range narrows progressively as the effluent moves to transects further downstream (EDL1-B and EDL1-C), as indicated by the smaller boxes and narrower whiskers at these transects. This progression supports the reasoning that the variability in the measured dye concentration is indicative of the degree of mixing achieved between the ambient and effluent flows.

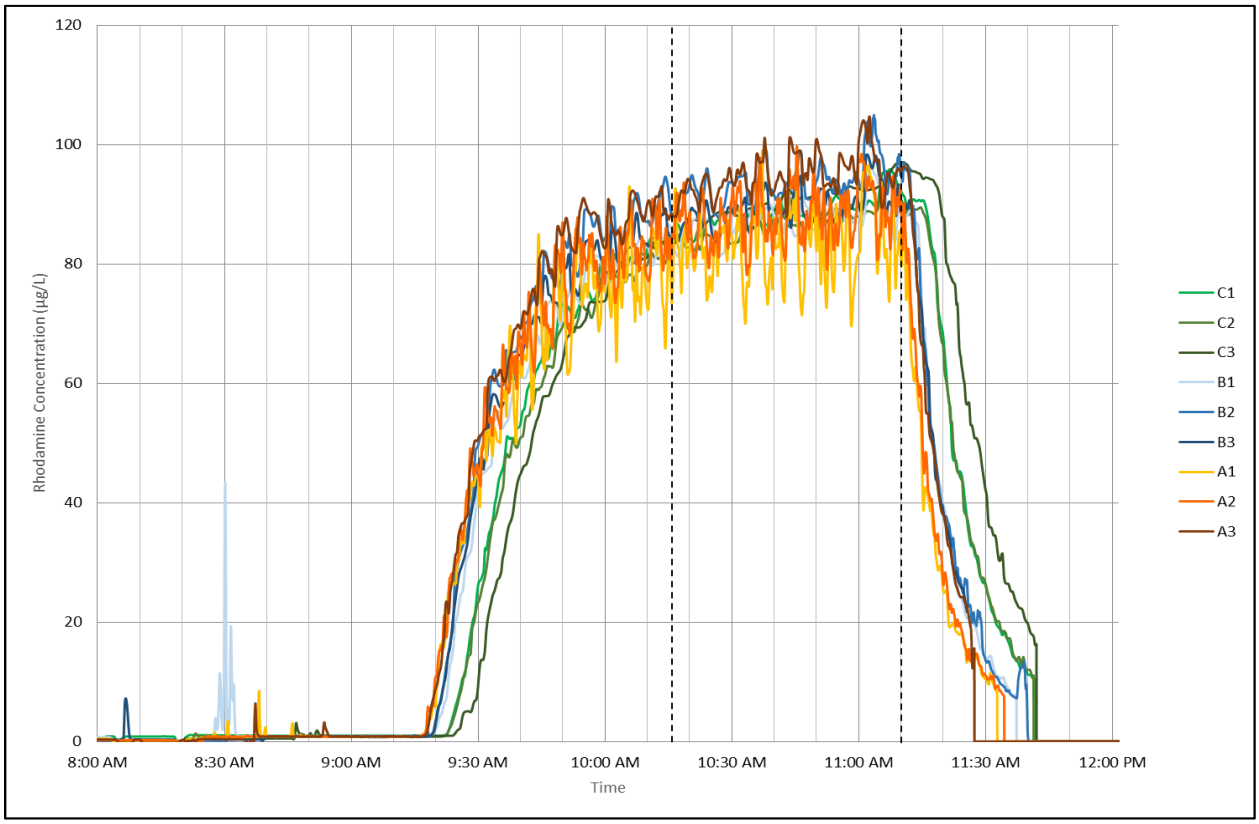
Furthermore, the variability (spread) at EDL1-C is far lower than that at EDL1-B, which in turn is lower than at EDL1-A, as indicated by the smaller boxes, respectively. This suggests that the effluent has achieved better mixing conditions as it travels downstream reaching transects farther away from the discharge location.

Fully mixed conditions have not been achieved by the time the effluent reaches transect EDL1-A located 20 m downstream of the diffusers, as indicated by the large portion of the box plot data falling outside the ±5% envelope. At ELD1-B located 40 m downstream of the diffusers, although the first and third quartiles are within the ±5% envelope, fully mixed conditions have not been completely achieved yet, as indicated by the box plot whiskers falling outside the ±5% envelope. At EDL1-C located 60 m downstream of the diffusers, the percent difference of all readings from the average transect concentrations are within the ±5% envelope, indicating that the effluent has achieved fully mixed conditions by the time it reaches this transect.

3.3 MIXING AT EDL 2

The transects at EDL 2 summarized in Table 2.4, are located 15 m (EDL2-A), 25 m (EDL2-B) and 60 m (EDL2-C) downstream of the diffuser outlets. The study setup is shown on Photos 2.4 and 2.5.

The concentration time series data sets recorded by the sondes at the EDL2-A (Sondes A1, A2 & A3), EDL2-B (Sondes B1, B2 & B3) and EDL2-C (Sondes C1, C2 & C3) transects are presented on Figure 3.3.



Note(s):

1. The vertical dashed lines indicate the steady state period used for analysis.

Figure 3.3 EDL 2 Transient Rhodamine WT Dye Concentrations

The steady state period selected for analysis extends a total duration of 54 minutes from 10:16 AM to 11:10 AM on November 9, 2023. During this period, the minimum, maximum and mean Rhodamine WT dye concentrations as well as the standard deviation at each sonde is shown in Table 3.3.

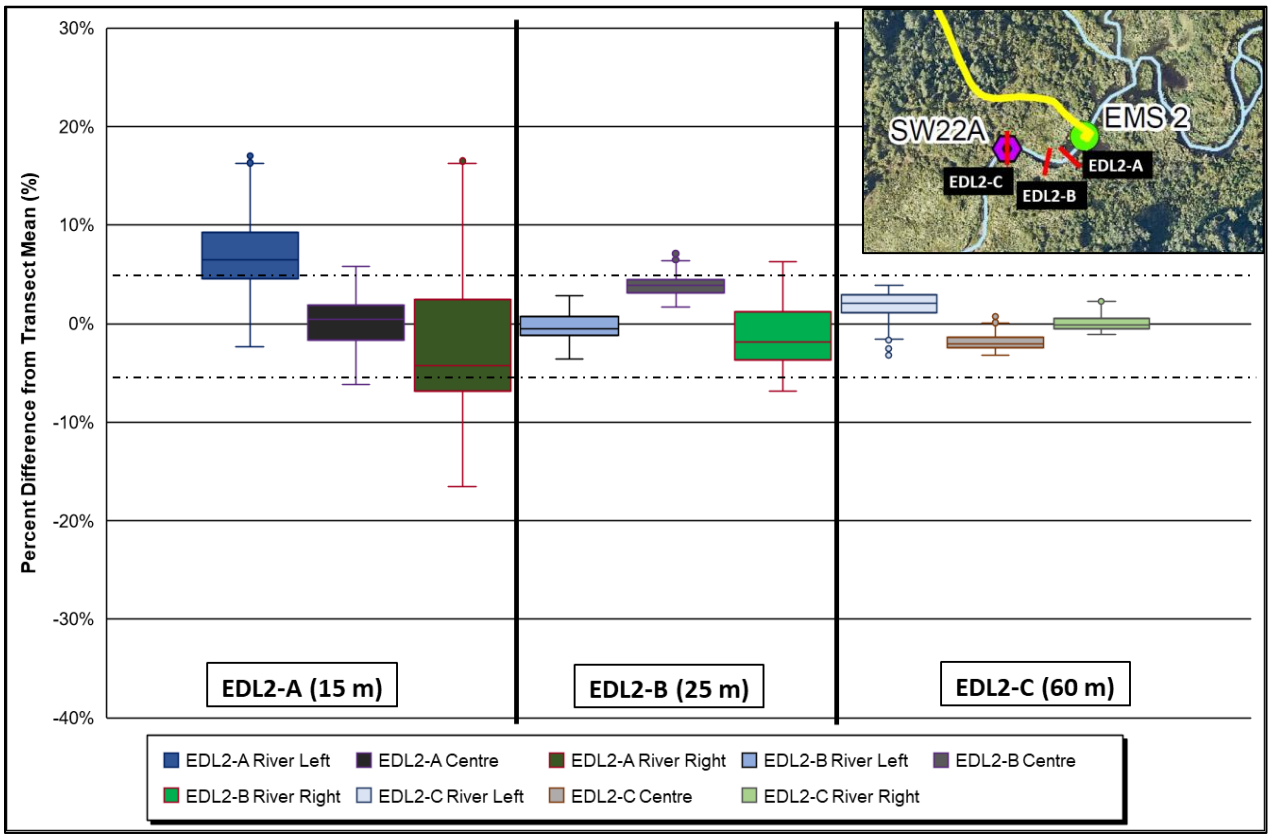
All sondes appear to experience similar levels of variability and reach steady state at approximately the same time. The concentrations measured by all sondes during the steady state period experience a slight upwards trend of increasing concentration. The increase in the average concentration between the start and end of the steady state period at all sondes is approximately 8%. This increase in concentration is proportional to the small increase in the effluent discharge rate that occurred concurrent with the steady state period. This small change in effluent discharge was not known to the field staff at the time of the mixing study, and it was only noted during data review and processing after the study. The resulting increase in concentration measured by the sondes is considered small and is not expected to affect the outcome of the mixing study.

Table 3.3 Steady State Concentration Statistics at Transects for EDL 2

Transect	Sonde Label	Sonde Location	Min (µg/L)	Mean (µg/L)	Max (µg/L)	Standard Deviation
EDL2-A	A3	River Left	88	95	105	3.45
	A2	Channel Centre	78	88	100	4.46
	A1	River Right	70	83	99	5.76
EDL2-B	B3	River Left	84	89	98	3.15
	B2	Channel Centre	88	94	105	3.43
	B1	River Right	81	88	97	3.00
EDL2-C	C3	River Left	83	90	97	2.89
	C2	Channel Centre	82	87	94	2.73
	C1	River Right	84	89	96	2.69

The concentrations at EDL2-A River Right (Sonde A1) recorded the greatest variability, with the data exhibiting the highest standard deviation recorded during the study. On the other hand, ELD2-C River Right (Sonde C1) recorded the lowest standard deviation, indicating the concentrations were the least variable at this location. Generally, the standard deviation is similar at all sondes.

The percent differences between each concentration measurement and the mean transect concentration, over the steady state period, are summarized on the box plot shown on Figure 3.4. Each set of box and whiskers represent one sonde, as labelled. Outliers that are outside of the whiskers are represented as standalone points. The ±5% envelope indicative of complete transverse mixing, as defined by Fischer (1979), is shown on the figure as dotted lines. A summary of the mean concentrations registered by each sonde and the associated percent difference from the transect mean for the steady state period is also provided in Table 3.4.



Note(s):

1. Internal line within each box indicates median values.
2. Dashed lines at ±5% present the envelope for fully mixed conditions, as defined by Fischer (1979).

Figure 3.4 EDL 2 Box and Whisker Plot Indicating Percent Difference from Transect Mean Concentration

Table 3.4 EDL 2 Steady State Mean Concentrations – Percent Difference from Transect Mean

Transect	Parameter	River Left	Channel Centre	River Right
EDL2-A	Mean Concentration (µg/L)	95	88	83
	Percent Difference from Transect Mean (%)	6.87	-0.78	-6.09
EDL2-B	Mean Concentration (µg/L)	88	94	89
	Percent Difference from Transect Mean (%)	0.45	5.33	-1.39
EDL2-C	Mean Concentration (µg/L)	89	87	90
	Percent Difference from Transect Mean (%)	1.48	-2.15	-0.22

The boxes and whiskers range more widely at transect EDL2-A closest to the diffuser outlets, as shown on Figure 3.4. The range narrows progressively as the effluent moves to transects farther downstream (EDL2-B and EDL2-C), as indicated by the smaller boxes and narrower whiskers at each of these transects. The trend is similar to what was observed at EDL 1. This progression supports the reasoning that the

variability in the measured dye concentration is indicative of the degree of mixing achieved between the ambient and effluent flows. Furthermore, the variability (spread) at EDL2-C is lower than that at EDL2-B, which in turn is lower than at EDL2-A, as indicated by the smaller boxes, respectively. This suggests that the effluent has achieved better mixing conditions as it travels downstream reaching transects farther away from the discharge location.

Fully mixed conditions have not been achieved by the time the effluent reaches transect EDL2-A located 15 m downstream of the diffusers, as indicated by the large portion of the box plot data falling outside the ±5% envelope. At EDL2-B located 25 m downstream of the diffusers, although the first and third quartiles are within the ±5% envelope, fully mixed conditions have not been completely achieved yet, as indicated by the box plot whiskers falling outside the ±5% envelope. At EDL2-C located 60 m downstream of the diffusers, the percent difference of all readings from the average transect concentrations are within the ±5% envelope, indicating that the effluent has achieved fully mixed conditions by the time it reaches this transect.

4.0 ANALYTICAL MODEL COMPARISON

4.1 ANALYTICAL MIXING MODEL RESULTS

KP completed an analytical evaluation of the performance of effluent mixing structures for the system design upgrades at EMS 1 and EMS 2 in early 2023. The design upgrades involved installation of a different diffuser type (i.e., clamp-on vs. in-pipe), which resulted in a larger diffuser opening and a higher design discharge rate. The analytical model evaluated the distance downstream from the diffusers required to achieve complete transverse mixing for the new higher range of effluent discharge rates. The results of the study were presented in a KP letter issued on February 27, 2023 (KP, 2023a). The required mixing lengths presented in that letter were estimated based on a 1:1 ambient-to-effluent discharge ratio.

The ambient-to-effluent discharge ratios at the time of the field study, however, were 2:1 at EDL 2 and 5:1 at EDL 1, as discussed in Section 2.1. The higher ambient flow conditions and the associated higher ambient flow velocities result in an increase in the required mixing lengths. As such, the analytical modelling predictions were updated based on the actual field conditions observed during the mixing study and are summarized with the updated required mixing lengths in Table 4.1.

Table 4.1 Required Mixing Lengths for the Double-Port Diffuser Systems based on Variable Ambient-to-Effluent Discharge Ratios

Effluent Discharge Location	Effluent Discharge (m^3/day)	Ambient Discharge in Pinewood River (m^3/day)	Ambient-to-Effluent Discharge Ratio	Required Mixing Length based on 1:1 Ambient-to-Effluent Ratio (m)	Updated Required Mixing Length based on Observed Mixing Study Field Conditions (m)
EDL 1	25,000	130,000	~5:1	31	38
EDL 2	22,500	46,000	~2:1	30	35

Based on the analytical evaluation, the performance of the mixing structures at EDL 1 and EDL 2 would satisfy the criteria for complete transverse mixing before the discharged effluent reached the downstream compliance points on the Pinewood River.

4.2 COMPARISON OF FIELD DATA WITH ANALYTICAL RESULTS

The field mixing study results presented in Section 3.2 and Section 3.3 are compared to the analytical mixing model results presented in Section 4.1, as summarized in Table 4.2. The field study comprised of discrete point measurements is limited by the number of transects based on the available sondes, and as such, the exact distance at which complete transverse mixing was achieved is uncertain. However, the mixing lengths observed during the field mixing study are generally in good agreement with the required mixing lengths predicted using the analytical model.

The results from the field study indicate that complete transverse mixing is achieved at some distance between the second and third transects (i.e., EDL1-B and EDL1-C at EDL 1, and EDL2-B and EDL2-C at EDL 2). It can be therefore concluded that complete transverse mixing was achieved somewhere between 40 m and 60 m downstream of the diffuser outlets at EDL 1, and between 25 m and 60 m downstream of the diffuser outlets at EDL 2. Consequently, the results of the field mixing study validate the analytical model predictions used to support the design of the mixing structures at EDL 1 and EDL 2.

Table 4.2 Comparison of Required Mixing Lengths from the Field Study and the Analytical Model

Effluent Discharge Location	Observed Mixing Length based on Field Study (m)	Predicted Mixing Length based on Analytical Model (m)
EDL 1	40 - 60	38
EDL 2	25 - 60	35

5.0 CONCLUSIONS

A mixing study was completed at EDL 1 and EDL 2 on November 8 and November 9, 2023, to confirm that the mixing structures are performing as designed, and that full transverse mixing is achieved within a reasonable short distance from the discharge points, as well as to validate the results from the analytical mixing model presented in the KP letter issued to support the diffuser upgrades (KP, 2023a).

Analytical modelling of the currently installed diffuser systems at EDL 1 and EDL 2 predicted that complete transverse mixing would be achieved within 38 m from the diffuser outlets at EDL 1, and within 35 m at EDL 2, based on the ambient and effluent discharge conditions at the time of the November 2023 mixing study. The results of the field mixing study showed that complete transverse mixing was achieved somewhere between 40 m and 60 m downstream of the discharge point at EDL 1, and between 25 m and 60 m downstream of the discharge point at EDL 2. As such, it can be concluded that the analytical mixing model and the field mixing study results are generally in good agreement. The analytical model predicts the performance of the effluent mixing structures and the required mixing lengths reasonably well, and the results are generally representative of the conditions observed in the field.

The observed near-field effluent mixing at EDL 1 and EDL 2 was found to meet the requirement for adequate mixing with the receiving environment as required in the Amended ECA (MECP, 2022), based on the site and flow conditions tested in this study. The study has shown that the diffusers at EMS 1 and EMS 2 are operating as intended, and that complete transverse mixing is achieved rapidly and before the effluent reaches the downstream compliance points.

6.0 REFERENCES

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7.0 CERTIFICATION

This report was prepared and reviewed by the undersigned.

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Prepared: 2024-02-07

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Approval that this document adheres to the Knight Piésold Quality System:

APPENDIX A

RRM Water Quality Results During Mixing Study

(Table A1)

TABLE A1

NEW GOLD INC. (RAINY RIVER MINE)
RAINY RIVER MINE

RRM WATER QUALITY RESULTS DURING MIXING STUDY

Print Feb/07/24 16:05:03

Sample						
Name	EDL2 Upstream	SW22A_SW_20231107 (D/S OF EDL2)	EDL2_EFF_20231107	SW23_SW_2023108 (U/S OF EDL1)	SW24_SW_2023108 (D/S OF EDL1)	EDL1_EFF_2023108
Sampling Date	07-11-2023 12:05	07-11-2023 12:35	07-11-2023 12:15	08-11-2023 12:05	08-11-2023 12:25	08-11-2023 12:40
ALS ID	TY2311805-001	TY2311805-002	TY2311805-003	TY2311809-001	TY2311809-002	TY2311809-003
Aggregate Organics						
Biochemical oxygen demand [BOD] mg/L	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Chemical oxygen demand [COD] mg/L	38	37	<10	58	56	11
Oil & grease (gravimetric) mg/L	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Anions and Nutrients						
Ammonia, total (as N) mg/L	0.0109	0.700	2.01	0.239	0.564	1.87
Ammonia, un-ionized (as N), field mg/L	<0.0010	0.0030	0.0285	<0.0010	0.0017	0.0260
Chloride mg/L	29.5	32.8	52.9	17.6	24.1	52.8
Fluoride mg/L	<0.020	0.069	<0.200	<0.020	0.021	<0.200
Kjeldahl nitrogen, total [TKN] mg/L	1.08	1.79	3.36	1.80	1.81	3.04
Nitrate (as N) mg/L	0.088	0.870	2.19	0.431	0.795	2.24
Nitrite (as N) mg/L	<0.010	0.012	<0.100	<0.010	<0.010	<0.100
Phosphate, ortho-, dissolved (as P) mg/L	0.0111	0.0037	<0.0010	0.0027	0.0017	<0.0010
Sulfate (as SO4) mg/L	44.4	282	704	162	263	714
Cyanides						
Cyanide, free mg/L	<0.0020	<0.0020	<0.0020	<0.0020	<0.0020	<0.0020
Cyanide, strong acid dissociable (Total) mg/L	<0.0020	<0.0020	<0.0020	<0.0020	<0.0020	<0.0020
Cyanide, weak acid dissociable mg/L	<0.0020	<0.0020	<0.0020	<0.0020	<0.0020	<0.0020
Dissolved Metals						
Aluminum, dissolved mg/L	0.0863	0.0426	0.0058	0.0289	0.0341	0.0010
Antimony, dissolved mg/L	0.00012	0.00867	0.0238	0.00280	0.00629	0.0228
Arsenic, dissolved mg/L	0.00082	0.00102	0.00128	0.00093	0.00096	0.00117
Barium, dissolved mg/L	0.0272	0.0297	0.0436	0.0318	0.0329	0.0422
Beryllium, dissolved mg/L	<0.000020	<0.000020	<0.000020	<0.000020	<0.000020	<0.000020
Bismuth, dissolved mg/L	<0.000050	<0.000050	<0.000050	<0.000050	<0.000050	<0.000050
Boron, dissolved mg/L	0.014	0.062	0.134	0.046	0.058	0.129
Cadmium, dissolved mg/L	0.0000103	0.0000124	0.0000083	0.0000052	0.0000052	0.0000062
Calcium, dissolved mg/L	57.7	97.0	182	64.3	82.0	173
Cesium, dissolved mg/L	<0.000010	0.000236	0.000659	<0.000010	0.000115	0.000631
Chromium, dissolved mg/L	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050
Cobalt, dissolved mg/L	0.00020	0.00121	0.00274	0.00058	0.00094	0.00276
Copper, dissolved mg/L	0.00173	0.00188	0.0138	0.00103	0.00103	0.00174
Dissolved mercury filtration location -	Field	Field	Field	Field	Field	Field
Dissolved metals filtration location -	Field	Field	Field	Field	Field	Field
Iron, dissolved mg/L	0.210	0.183	0.018	0.164	0.146	<0.010
Lead, dissolved mg/L	0.000075	0.000056	0.00655	<0.000050	0.000053	<0.000050
Lithium, dissolved mg/L	0.0081	0.0116	0.0189	0.0102	0.0113	0.0176
Magnesium, dissolved mg/L	23.8	22.3	26.5	20.8	21.7	25.9
Manganese, dissolved mg/L	0.0822	0.0881	0.0142	0.0395	0.0324	0.00074
Mercury, dissolved mg/L	<0.0000050	<0.0000050	<0.0000050	<0.0000050	<0.0000050	<0.0000050
Molybdenum, dissolved mg/L	0.000861	0.00599	0.0156	0.00273	0.00495	0.0154
Nickel, dissolved mg/L	0.00116	0.00198	0.00350	0.00131	0.00171	0.00325
Phosphorus, dissolved mg/L	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Potassium, dissolved mg/L	3.60	23.4	60.6	10.8	19.4	58.4
Rubidium, dissolved mg/L	0.00229	0.0148	0.0357	0.00644	0.0114	0.0341
Selenium, dissolved mg/L	0.000204	0.000459	0.00101	0.000264	0.000413	0.000993
Silicon, dissolved mg/L	3.96	3.23	2.14	3.99	3.65	2.14
Silver, dissolved mg/L	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010
Sodium, dissolved mg/L	10.1	52.4	129	27.6	45.2	125
Strontium, dissolved mg/L	0.171	0.451	1.02	0.262	0.384	0.987
Sulfur, dissolved mg/L	16.0	99.7	263	56.9	90.7	262
Tellurium, dissolved mg/L	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020
Thallium, dissolved mg/L	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010
Thorium, dissolved mg/L	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010
Tin, dissolved mg/L	<0.00010	<0.00010	0.00056	<0.00010	<0.00010	<0.00010
Titanium, dissolved mg/L	0.00375	0.00165	<0.00030	0.00108	0.00100	<0.00030
Tungsten, dissolved mg/L	<0.00010	0.00010	0.00029	<0.00010	<0.00010	0.00028
Uranium, dissolved mg/L	0.00236	0.00137	0.00146	0.000992	0.00107	0.00147
Vanadium, dissolved mg/L	0.00056	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050
Zinc, dissolved mg/L	0.0029	0.0025	0.0161	0.0011	0.0011	0.0014
Zirconium, dissolved mg/L	<0.00030	<0.00030	<0.00030	<0.00030	<0.00030	<0.00030

Sample						
Name	EDL2 Upstream	SW22A_SW_20231107 (D/S OF EDL2)	EDL2_EFF_20231107	SW23_SW_2023108 (U/S OF EDL1)	SW24_SW_2023108 (D/S OF EDL1)	EDL1_EFF_2023108
Sampling Date	07-11-2023 12:05	07-11-2023 12:35	07-11-2023 12:15	08-11-2023 12:05	08-11-2023 12:25	08-11-2023 12:40
ALS ID	TY2311805-001	TY2311805-002	TY2311805-003	TY2311809-001	TY2311809-002	TY2311809-003
Field Tests						
Oxygen, dissolved, field mg/L	8.78	8.81	7.40	9.61	8.12	8.02
pH, field pH units	7.48	7.63	8.09	7.00	7.48	8.08
Temperature, field °C	0.91	2.42	4.23	1.42	2.08	4.29
Organic / Inorganic Carbon						
Carbon, dissolved organic [DOC] mg/L	16.7	15.7	5.09	26.8	25.0	5.72
Carbon, total organic [TOC] mg/L	16.5	15.8	5.80	23.2	22.2	5.32
Physical Tests						
Acidity (as CaCO3) mg/L	<2.0	2.1	2.4	2.7	3.7	2.5
Alkalinity, total (as CaCO3) mg/L	211	167	116	134	133	120
Colour, true CU	33.1	27.4	3.8	78.7	78.0	4.7
Conductivity µS/cm	530	945	1730	638	830	1730
Hardness (as CaCO3), dissolved mg/L	242	334	564	246	294	539
pH pH units	8.18	8.07	8.08	8.04	8.03	8.09
Solids, total dissolved [TDS] mg/L	318	617	1250	421	578	1260
Solids, total suspended [TSS] mg/L	4.1	6.3	3.1	11.7	11.3	4.9
Turbidity NTU	4.51	3.71	1.37	9.61	8.47	1.73
Total Metals						
Aluminum, total mg/L	0.141	0.129	0.0164	0.302	0.298	0.0273
Antimony, total mg/L	<0.00010	0.00837	0.0236	0.00300	0.00642	0.0238
Arsenic, total mg/L	0.00080	0.00098	0.00126	0.00104	0.00114	0.00132
Barium, total mg/L	0.0265	0.0285	0.0419	0.0347	0.0358	0.0425
Beryllium, total mg/L	<0.000020	<0.000020	<0.000020	<0.000020	<0.000020	<0.000020
Bismuth, total mg/L	<0.000050	<0.000050	<0.000050	<0.000050	<0.000050	<0.000050
Boron, total mg/L	0.017	0.059	0.137	0.044	0.056	0.143
Cadmium, total mg/L	0.0000141	0.0000121	0.0000101	0.0000151	0.0000121	0.0000131
Calcium, total mg/L	59.0	94.4	174	63.5	84.3	180
Cesium, total mg/L	0.000016	0.000240	0.000657	0.000049	0.000151	0.000660
Chromium, total mg/L	0.00058	<0.00050	<0.00050	0.00293	0.00109	0.00096
Cobalt, total mg/L	0.00023	0.00124	0.00286	0.00077	0.00118	0.00294
Copper, total mg/L	0.00314	0.00277	0.0281	0.00198	0.00153	0.00243
Iron, total mg/L	0.294	0.322	0.029	0.604	0.515	0.327
Lead, total mg/L	0.000096	0.000080	0.00907	0.000238	0.000215	<0.000050
Lithium, total mg/L	0.0080	0.0100	0.0166	0.0096	0.0098	0.0178
Magnesium, total mg/L	23.7	23.2	26.7	20.9	21.6	26.3
Manganese, total mg/L	0.0824	0.0870	0.0119	0.0581	0.0527	0.0214
Mercury, total mg/L	<0.0000050	<0.0000050	<0.0000050	<0.0000050	<0.0000050	<0.0000050
Molybdenum, total mg/L	0.000848	0.00606	0.0162	0.00344	0.00516	0.0165
Nickel, total mg/L	0.00132	0.00224	0.00355	0.00182	0.00232	0.00389
Phosphorus, total mg/L	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Potassium, total mg/L	3.64	24.0	60.0	11.1	19.2	59.4
Rubidium, total mg/L	0.00238	0.0146	0.0346	0.00710	0.0118	0.0350
Selenium, total mg/L	0.000135	0.000375	0.000873	0.000242	0.000353	0.000905
Silicon, total mg/L	4.13	3.37	2.22	4.79	4.39	2.29
Silver, total mg/L	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010
Sodium, total mg/L	9.95	54.3	129	28.0	44.8	128
Strontium, total mg/L	0.171	0.444	0.999	0.284	0.402	1.02
Sulfur, total mg/L	16.0	94.8	264	58.1	93.9	263
Tellurium, total mg/L	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020
Thallium, total mg/L	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010
Thorium, total mg/L	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010
Tin, total mg/L	<0.00010	0.00018	0.00140	<0.00010	<0.00010	<0.00010
Titanium, total mg/L	0.00540	0.00449	<0.00135	0.0117	0.0116	<0.00120
Tungsten, total mg/L	<0.00010	0.00010	0.00030	<0.00010	<0.00010	0.00030
Uranium, total mg/L	0.00238	0.00137	0.00159	0.00108	0.00116	0.00152
Vanadium, total mg/L	0.00075	0.00073	<0.00050	0.00162	0.00127	<0.00050
Zinc, total mg/L	0.0057	0.0036	0.0161	0.0044	0.0031	0.0035
Zirconium, total mg/L	0.00027	0.00021	<0.00020	0.00039	0.00039	<0.00020

NOTE(S):

1. DATA PROVIDED BY RAINY RIVER MINE

Appendix B Supporting Environmental Information

Site Location: NewGold Rainy River
Your C.O.C. #: 928057-01-01

Attention: Caroline Farkas

EcoMetrix Incorporated
6800 Campobello Rd
Mississauga, ON
CANADA L5N 2L8

Report Date: 2023/05/26
Report #: R7645395
Version: 1 - Final

CERTIFICATE OF ANALYSIS

BUREAU VERITAS JOB #: C3D4190
Received: 2023/05/10, 13:49

Sample Matrix: Water
Samples Received: 9

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Analytical Method
Acidity (CaCO3) in water (1)	9	N/A	2023/05/17	ATL SOP-00205	SM 23 2310B
Alkalinity	9	N/A	2023/05/17	CAM SOP-00448	SM 23 2320 B m
Chloride by Automated Colourimetry	9	N/A	2023/05/16	CAM SOP-00463	SM 23 4500-Cl E m
Total Cyanide	3	2023/05/11	2023/05/11	CAM SOP-00457	OMOE E3015 5 m
Total Cyanide	6	2023/05/12	2023/05/16	CAM SOP-00457	OMOE E3015 5 m
Hardness (calculated as CaCO3)	9	N/A	2023/05/17	CAM SOP 00102/00408/00447	SM 2340 B
Dissolved Mercury (low level)	9	2023/05/15	2023/05/15	CAM SOP-00453	EPA 7470 m
Hardness Total (calculated as CaCO3) (2, 4)	9	N/A	2023/05/18	BBY WI-00033	Auto Calc
Elements by ICPMS Low Level (total) (2)	7	2023/05/17	2023/05/17	BBY7SOP-00003 / BBY7SOP-00002	EPA 6020b R2 m
Na, K, Ca, Mg, S by CRC ICPMS (total) (2)	9	N/A	2023/05/18	BBY WI-00033	Auto Calc
Elements by ICPMS Low Level (total) (2)	2	N/A	2023/05/18	BBY7SOP-00002	EPA 6020B R2 m
Total Ammonia-N	9	N/A	2023/05/15	CAM SOP-00441	USGS I-2522-90 m
Nitrate & Nitrite as Nitrogen in Water (5)	9	N/A	2023/05/15	CAM SOP-00440	SM 23 4500-NO3I/NO2B
pH	9	2023/05/12	2023/05/17	CAM SOP-00413	SM 4500H+ B m
Field Measured pH (6)	7	N/A	2023/05/16		Field pH Meter
Radium Isotopes by Alpha Spectrometry (3, 7)	6	N/A	2023/05/24	BQL SOP-00006 BQL SOP-00017 BQL SOP-00032	Alpha Spectrometry
Radium Isotopes by Alpha Spectrometry (3, 7)	2	N/A	2023/05/25	BQL SOP-00006 BQL SOP-00017 BQL SOP-00032	Alpha Spectrometry
Sulphate by Automated Turbidimetry	9	N/A	2023/05/16	CAM SOP-00464	SM 23 4500-SO42- E m
Total Dissolved Solids	9	2023/05/15	2023/05/16	CAM SOP-00428	SM 23 2540C m
Field Temperature (6)	7	N/A	2023/05/16		Field Thermometer
Total Phosphorus (Colourimetric)	9	2023/05/12	2023/05/12	CAM SOP-00407	SM 23 4500-P I
Total Suspended Solids	8	2023/05/12	2023/05/15	CAM SOP-00428	SM 23 2540D m
Total Suspended Solids	1	2023/05/15	2023/05/16	CAM SOP-00428	SM 23 2540D m
Un-ionized Ammonia (8)	7	2023/05/11	2023/05/16	Auto Calc.	PWQO

Reference Method suffix “m” indicates test methods incorporate validated modifications from specific reference methods to improve performance.
(1) This test was performed by Bureau Veritas Bedford, 200 Bluewater Rd Suite 105, Bedford, NS, B4B 1G9

Site Location: NewGold Rainy River
Your C.O.C. #: 928057-01-01

Attention: Caroline Farkas

EcoMetrix Incorporated
6800 Campobello Rd
Mississauga, ON
CANADA L5N 2L8

Report Date: 2023/05/26
Report #: R7645395
Version: 1 - Final

CERTIFICATE OF ANALYSIS

BUREAU VERITAS JOB #: C3D4190

Received: 2023/05/10, 13:49

(2) This test was performed by Bureau Veritas Burnaby, 4606 Canada Way , Burnaby, BC, V5G 1K5
(3) This test was performed by Bureau Veritas Kitimat, 6790 Kitimat Road, Unit 4 , Mississauga, ON, L5N 5L9
(4) "Total Hardness" was calculated from Total Ca and Mg concentrations and may be biased high (Hardness, or Dissolved Hardness, calculated from Dissolved Ca and Mg, should be used for compliance if available).
(5) Values for calculated parameters may not appear to add up due to rounding of raw data and significant figures.
(6) This is a field test, therefore, the results relate to items that were not analysed at Bureau Veritas.
(7) Radium-226 results have not been corrected for blanks.
(8) Un-ionized ammonia is calculated using the total ammonia result and field data provided by the client for pH and temperature.

Encryption Key

AUTHORIZED REPORT
RAPPORT AUTORISÉ

Bureau Veritas
26 May 2023 15:53:19

Please direct all questions regarding this Certificate of Analysis to:
Colby Coutu, Project Manager
Email: Colby.Coutu@bureauveritas.com
Phone# (905)817-5844

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This report has been generated and distributed using a secure automated process.
Bureau Veritas has procedures in place to guard against improper use of the electronic signature and have the required "signatories", as per ISO/IEC 17025, signing the reports. For Service Group specific validation, please refer to the Validation Signatures page if included, otherwise available by request. For Department specific Analyst/Supervisor validation names, please refer to the Test Summary section if included, otherwise available by request. This report is authorized by Rodney Major, General Manager responsible for Ontario Environmental laboratory operations.

Bureau Veritas Job #: C3D4190
Report Date: 2023/05/26

EcoMetrix Incorporated
Site Location: NewGold Rainy River

RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		VUA728			VUA728			VUA729		
Sampling Date		2023/05/09 09:20			2023/05/09 09:20			2023/05/09 09:35		
COC Number		928057-01-01			928057-01-01			928057-01-01		
	UNITS	**PINR-REF 1**	**RDL**	**QC Batch**	**PINR-REF 1 Lab-Dup**	**RDL**	**QC Batch**	**PINR-EXP 2**	**RDL**	**QC Batch**
Calculated Parameters										
Hardness (CaCO3)	mg/L	130	1.0	8658512				160	1.0	8658512
Total Un-ionized Ammonia	mg/L	<0.00061	0.00061	8659235				<0.00061	0.00061	8659235
Field Measurements										
Field Temperature	Celsius	9.0	N/A	ONSITE				8.8	N/A	ONSITE
Field Measured pH	pH	7.34		ONSITE				7.34		ONSITE
Inorganics										
Acidity	mg/L	5.0	5.0	8669092				<5.0	5.0	8669092
Total Ammonia-N	mg/L	<0.050	0.050	8661704				0.069	0.050	8661704
Total Dissolved Solids	mg/L	250	10	8664402				270	10	8664402
pH	pH	7.77		8662539				7.76		8662539
Total Phosphorus	mg/L	0.035	0.020	8661479				0.030	0.020	8661479
Total Suspended Solids	mg/L	10	10	8661588				10	10	8661588
Dissolved Sulphate (SO4)	mg/L	65	1.0	8661792				96	1.0	8661792
Total Cyanide (CN)	mg/L	<0.0050	0.0050	8660971				<0.0050	0.0050	8660971
Alkalinity (Total as CaCO3)	mg/L	84	1.0	8662534				88	1.0	8662534
Dissolved Chloride (Cl-)	mg/L	7.1	1.0	8661785				8.7	1.0	8661785
Nitrite (N)	mg/L	<0.010	0.010	8661729				<0.010	0.010	8661729
Nitrate (N)	mg/L	0.29	0.10	8661729				0.41	0.10	8661729
Nitrate + Nitrite (N)	mg/L	0.29	0.10	8661729				0.41	0.10	8661729
Metals										
Total Aluminum (Al)	mg/L	0.271	0.0030	8673648				0.282	0.0030	8673648
Total Antimony (Sb)	mg/L	0.000970	0.000020	8673648				0.00149	0.000020	8673648
Total Arsenic (As)	mg/L	0.000893	0.000020	8673648				0.000856	0.000020	8673648
Total Barium (Ba)	mg/L	0.0186	0.000050	8673648				0.0192	0.000050	8673648
Total Beryllium (Be)	mg/L	0.000024	0.000010	8673648				0.000023	0.000010	8673648
Total Bismuth (Bi)	mg/L	<0.000010	0.000010	8673648				<0.000010	0.000010	8673648
Total Boron (B)	mg/L	0.022	0.010	8673648				0.027	0.010	8673648
Total Cadmium (Cd)	mg/L	0.0000083	0.0000050	8673648				0.0000138	0.0000050	8673648
Total Chromium (Cr)	mg/L	0.00094	0.00010	8673648				0.00072	0.00010	8673648
Total Cobalt (Co)	mg/L	0.000361	0.000010	8673648				0.000425	0.000010	8673648
Total Copper (Cu)	mg/L	0.00139	0.00010	8673648				0.00146	0.00010	8673648
Total Iron (Fe)	mg/L	0.487	0.0050	8673648				0.440	0.0050	8673648

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate
N/A = Not Applicable

Bureau Veritas 6740 Campobello Road, Mississauga, Ontario, L5N 2L8 Tel: (905) 817-5700 Toll-Free: 800-563-6266 Fax: (905) 817-5777 www.bvna.com

RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		VUA728			VUA728			VUA729		
Sampling Date		2023/05/09 09:20			2023/05/09 09:20			2023/05/09 09:35		
COC Number		928057-01-01			928057-01-01			928057-01-01		
	UNITS	**PINR-REF 1**	**RDL**	**QC Batch**	**PINR-REF 1 Lab-Dup**	**RDL**	**QC Batch**	**PINR-EXP 2**	**RDL**	**QC Batch**
Total Lead (Pb)	mg/L	0.000212	0.000020	8673648				0.000196	0.000020	8673648
Total Lithium (Li)	mg/L	0.00505	0.00050	8673648				0.00553	0.00050	8673648
Total Manganese (Mn)	mg/L	0.0385	0.00010	8673648				0.0396	0.00010	8673648
Total Molybdenum (Mo)	mg/L	0.00165	0.000050	8673648				0.00195	0.000050	8673648
Total Nickel (Ni)	mg/L	0.00288	0.00010	8673648				0.00209	0.00010	8673648
Total Selenium (Se)	mg/L	0.000179	0.000040	8673648				0.000208	0.000040	8673648
Total Silicon (Si)	mg/L	2.22	0.050	8673648				2.14	0.050	8673648
Total Silver (Ag)	mg/L	<0.000010	0.000010	8673648				<0.000010	0.000010	8673648
Total Strontium (Sr)	mg/L	0.113	0.000050	8673648				0.144	0.000050	8673648
Total Thallium (Tl)	mg/L	0.0000061	0.0000020	8673648				0.0000062	0.0000020	8673648
Total Tin (Sn)	mg/L	<0.00020	0.00020	8673648				<0.00020	0.00020	8673648
Total Titanium (Ti)	mg/L	0.0110	0.0020	8673648				0.0091	0.0020	8673648
Total Uranium (U)	mg/L	0.000588	0.0000050	8673648				0.000661	0.0000050	8673648
Total Vanadium (V)	mg/L	0.00117	0.00020	8673648				0.00106	0.00020	8673648
Total Zinc (Zn)	mg/L	0.0033	0.0010	8673648				0.0038	0.0010	8673648
Total Zirconium (Zr)	mg/L	0.00038	0.00010	8673648				0.00035	0.00010	8673648
Total Calcium (Ca)	mg/L	33.3	0.25	8673647				38.2	0.25	8673647
Total Magnesium (Mg)	mg/L	11.1	0.25	8673647				11.5	0.25	8673647
Total Potassium (K)	mg/L	4.72	0.25	8673647				6.59	0.25	8673647
Total Sodium (Na)	mg/L	11.1	0.25	8673647				15.6	0.25	8673647
Total Sulphur (S)	mg/L	18.7	3.0	8673647				27.5	3.0	8673647
RADIONUCLIDE										
Radium-226	Bq/L	<0.010	0.010	8666867	<0.010	0.010	8666867	<0.010	0.010	8666867

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
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Bureau Veritas 6740 Campobello Road, Mississauga, Ontario, L5N 2L8 Tel: (905) 817-5700 Toll-Free: 800-563-6266 Fax: (905) 817-5777 www.bvna.com

Bureau Veritas Job #: C3D4190
Report Date: 2023/05/26

EcoMetrix Incorporated
Site Location: NewGold Rainy River

RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		VUA730			VUA730			VUA731		
Sampling Date		2023/05/09 11:45			2023/05/09 11:45			2023/05/09 16:10		
COC Number		928057-01-01			928057-01-01			928057-01-01		
	UNITS	**PINEXP**	**RDL**	**QC Batch**	**PINEXP Lab-Dup**	**RDL**	**QC Batch**	**STUC**	**RDL**	**QC Batch**
Calculated Parameters										
Hardness (CaCO3)	mg/L	200	1.0	8658512				90	1.0	8658512
Total Un-ionized Ammonia	mg/L	0.0025	0.0012	8659235				<0.0014	0.0014	8659235
Field Measurements										
Field Temperature	Celsius	11.0	N/A	ONSITE				12.6	N/A	ONSITE
Field Measured pH	pH	7.98		ONSITE				8.00		ONSITE
Inorganics										
Acidity	mg/L	<5.0	5.0	8669092				<5.0	5.0	8669092
Total Ammonia-N	mg/L	0.11	0.050	8661704				<0.050	0.050	8661704
Total Dissolved Solids	mg/L	360	10	8664402				175	10	8664402
pH	pH	7.85		8662539	7.94		8662539	7.70		8662539
Total Phosphorus	mg/L	0.034	0.020	8661479				0.046	0.020	8661479
Total Suspended Solids	mg/L	<10	10	8661588				<10	10	8661588
Dissolved Sulphate (SO4)	mg/L	120	1.0	8661792				13	1.0	8661792
Total Cyanide (CN)	mg/L	<0.0050	0.0050	8660971				<0.0050	0.0050	8658759
Alkalinity (Total as CaCO3)	mg/L	110	1.0	8662534	120	1.0	8662534	71	1.0	8662534
Dissolved Chloride (Cl-)	mg/L	15	1.0	8661785				8.7	1.0	8661785
Nitrite (N)	mg/L	<0.010	0.010	8661729				<0.010	0.010	8661729
Nitrate (N)	mg/L	0.53	0.10	8661729				<0.10	0.10	8661729
Nitrate + Nitrite (N)	mg/L	0.53	0.10	8661729				<0.10	0.10	8661729
Metals										
Total Aluminum (Al)	mg/L	0.101	0.0030	8673648				0.237	0.0030	8673648
Total Antimony (Sb)	mg/L	0.00202	0.000020	8673648				0.000052	0.000020	8673648
Total Arsenic (As)	mg/L	0.000905	0.000020	8673648				0.000751	0.000020	8673648
Total Barium (Ba)	mg/L	0.0221	0.000050	8673648				0.0148	0.000050	8673648
Total Beryllium (Be)	mg/L	0.000012	0.000010	8673648				0.000025	0.000010	8673648
Total Bismuth (Bi)	mg/L	<0.000010	0.000010	8673648				<0.000010	0.000010	8673648
Total Boron (B)	mg/L	0.036	0.010	8673648				0.014	0.010	8673648
Total Cadmium (Cd)	mg/L	0.0000088	0.0000050	8673648				0.0000164	0.0000050	8673648
Total Chromium (Cr)	mg/L	0.00035	0.00010	8673648				0.00062	0.00010	8673648
Total Cobalt (Co)	mg/L	0.000397	0.000010	8673648				0.000211	0.000010	8673648
Total Copper (Cu)	mg/L	0.00126	0.00010	8673648				0.00124	0.00010	8673648
Total Iron (Fe)	mg/L	0.275	0.0050	8673648				0.422	0.0050	8673648

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate
N/A = Not Applicable

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		VUA730			VUA730			VUA731		
Sampling Date		2023/05/09 11:45			2023/05/09 11:45			2023/05/09 16:10		
COC Number		928057-01-01			928057-01-01			928057-01-01		
	UNITS	**PINEXP**	**RDL**	**QC Batch**	**PINEXP Lab-Dup**	**RDL**	**QC Batch**	**STUC**	**RDL**	**QC Batch**
Total Lead (Pb)	mg/L	0.000092	0.000020	8673648				0.000155	0.000020	8673648
Total Lithium (Li)	mg/L	0.00729	0.00050	8673648				0.00374	0.00050	8673648
Total Manganese (Mn)	mg/L	0.0382	0.00010	8673648				0.0159	0.00010	8673648
Total Molybdenum (Mo)	mg/L	0.00262	0.000050	8673648				0.000598	0.000050	8673648
Total Nickel (Ni)	mg/L	0.00171	0.00010	8673648				0.00167	0.00010	8673648
Total Selenium (Se)	mg/L	0.000222	0.000040	8673648				0.000152	0.000040	8673648
Total Silicon (Si)	mg/L	1.45	0.050	8673648				2.39	0.050	8673648
Total Silver (Ag)	mg/L	<0.000010	0.000010	8673648				<0.000010	0.000010	8673648
Total Strontium (Sr)	mg/L	0.215	0.000050	8673648				0.0480	0.000050	8673648
Total Thallium (Tl)	mg/L	0.0000048	0.0000020	8673648				0.0000046	0.0000020	8673648
Total Tin (Sn)	mg/L	<0.00020	0.00020	8673648				<0.00020	0.00020	8673648
Total Titanium (Ti)	mg/L	0.0038	0.0020	8673648				0.0082	0.0020	8673648
Total Uranium (U)	mg/L	0.000971	0.0000050	8673648				0.000650	0.0000050	8673648
Total Vanadium (V)	mg/L	0.00068	0.00020	8673648				0.00124	0.00020	8673648
Total Zinc (Zn)	mg/L	0.0039	0.0010	8673648				0.0067	0.0010	8673648
Total Zirconium (Zr)	mg/L	0.00023	0.00010	8673648				0.00045	0.00010	8673648
Total Calcium (Ca)	mg/L	50.7	0.25	8673647				19.4	0.25	8673647
Total Magnesium (Mg)	mg/L	14.9	0.25	8673647				8.90	0.25	8673647
Total Potassium (K)	mg/L	8.54	0.25	8673647				1.62	0.25	8673647
Total Sodium (Na)	mg/L	21.5	0.25	8673647				4.37	0.25	8673647
Total Sulphur (S)	mg/L	36.4	3.0	8673647				<3.0	3.0	8673647
RADIONUCLIDE										
Radium-226	Bq/L	<0.010	0.010	8666867				<0.010	0.010	8666867

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		VUA732		VUA733		VUA734		
Sampling Date		2023/05/09 16:55		2023/05/09 17:15		2023/05/09 09:20		
COC Number		928057-01-01		928057-01-01		928057-01-01		
	UNITS	**LVR-REF 1**	**QC Batch**	**LVR-REF 2**	**QC Batch**	**DUP**	**RDL**	**QC Batch**
Calculated Parameters								
Hardness (CaCO3)	mg/L	66	8658512	80	8658512	130	1.0	8658512
Total Un-ionized Ammonia	mg/L	<0.00061	8659235	<0.00061	8659235	<0.00061	0.00061	8659235
Field Measurements								
Field Temperature	Celsius	12.2	ONSITE	11.8	ONSITE	9.0	N/A	ONSITE
Field Measured pH	pH	7.54	ONSITE	7.45	ONSITE	7.34		ONSITE
Inorganics								
Acidity	mg/L	8.0	8669092	<5.0	8669092	<5.0	5.0	8669092
Total Ammonia-N	mg/L	<0.050	8661704	<0.050	8661704	0.056	0.050	8661704
Total Dissolved Solids	mg/L	95	8664398	145	8664402	210	10	8664398
pH	pH	7.43	8662539	7.73	8662539	7.76		8662539
Total Phosphorus	mg/L	0.038	8661479	0.047	8661479	0.030	0.020	8661479
Total Suspended Solids	mg/L	<10	8661588	<10	8661588	<10	10	8661588
Dissolved Sulphate (SO4)	mg/L	12	8661792	15	8661792	65	1.0	8661792
Total Cyanide (CN)	mg/L	<0.0050	8660971	<0.0050	8660971	<0.0050	0.0050	8658759
Alkalinity (Total as CaCO3)	mg/L	52	8662534	65	8662534	85	1.0	8662534
Dissolved Chloride (Cl-)	mg/L	3.1	8661785	4.3	8661785	6.7	1.0	8661785
Nitrite (N)	mg/L	<0.010	8661729	<0.010	8661729	<0.010	0.010	8661729
Nitrate (N)	mg/L	<0.10	8661729	<0.10	8661729	0.27	0.10	8661729
Nitrate + Nitrite (N)	mg/L	<0.10	8661729	<0.10	8661729	0.27	0.10	8661729
Metals								
Total Aluminum (Al)	mg/L	0.250	8673648	0.432	8673648	0.253	0.0030	8673648
Total Antimony (Sb)	mg/L	0.000048	8673648	0.000079	8673648	0.000887	0.000020	8673648
Total Arsenic (As)	mg/L	0.000664	8673648	0.000803	8673648	0.000878	0.000020	8673648
Total Barium (Ba)	mg/L	0.0129	8673648	0.0169	8673648	0.0177	0.000050	8673648
Total Beryllium (Be)	mg/L	0.000023	8673648	0.000034	8673648	0.000020	0.000010	8673648
Total Bismuth (Bi)	mg/L	<0.000010	8673648	<0.000010	8673648	<0.000010	0.000010	8673648
Total Boron (B)	mg/L	0.014	8673648	0.015	8673648	0.021	0.010	8673648
Total Cadmium (Cd)	mg/L	0.0000088	8673648	0.0000165	8673648	0.0000122	0.0000050	8673648
Total Chromium (Cr)	mg/L	0.00067	8673648	0.00096	8673648	0.00072	0.00010	8673648
Total Cobalt (Co)	mg/L	0.000173	8673648	0.000367	8673648	0.000331	0.000010	8673648
Total Copper (Cu)	mg/L	0.00095	8673648	0.00142	8673648	0.00131	0.00010	8673648
Total Iron (Fe)	mg/L	0.455	8673648	0.704	8673648	0.446	0.0050	8673648
Total Lead (Pb)	mg/L	0.000147	8673648	0.000292	8673648	0.000188	0.000020	8673648

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
N/A = Not Applicable

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		VUA732		VUA733		VUA734		
Sampling Date		2023/05/09 16:55		2023/05/09 17:15		2023/05/09 09:20		
COC Number		928057-01-01		928057-01-01		928057-01-01		
	UNITS	**LVR-REF 1**	**QC Batch**	**LVR-REF 2**	**QC Batch**	**DUP**	**RDL**	**QC Batch**
Total Lithium (Li)	mg/L	0.00311	8673648	0.00375	8673648	0.00481	0.00050	8673648
Total Manganese (Mn)	mg/L	0.0113	8673648	0.0288	8673648	0.0361	0.00010	8673648
Total Molybdenum (Mo)	mg/L	0.000569	8673648	0.000608	8673648	0.00131	0.000050	8673648
Total Nickel (Ni)	mg/L	0.00149	8673648	0.00190	8673648	0.00156	0.00010	8673648
Total Selenium (Se)	mg/L	0.000160	8673648	0.000161	8673648	0.000172	0.000040	8673648
Total Silicon (Si)	mg/L	2.09	8673648	2.73	8673648	2.10	0.050	8673648
Total Silver (Ag)	mg/L	<0.000010	8673648	<0.000010	8673648	<0.000010	0.000010	8673648
Total Strontium (Sr)	mg/L	0.0357	8673648	0.0453	8673648	0.108	0.000050	8673648
Total Thallium (Tl)	mg/L	0.0000059	8673648	0.0000096	8673648	0.0000059	0.0000020	8673648
Total Tin (Sn)	mg/L	<0.00020	8673648	<0.00020	8673648	<0.00020	0.00020	8673648
Total Titanium (Ti)	mg/L	0.0071	8673648	0.0131	8673648	0.0084	0.0020	8673648
Total Uranium (U)	mg/L	0.000323	8673648	0.000551	8673648	0.000548	0.0000050	8673648
Total Vanadium (V)	mg/L	0.00116	8673648	0.00180	8673648	0.00106	0.00020	8673648
Total Zinc (Zn)	mg/L	0.0024	8673648	0.0038	8673648	0.0030	0.0010	8673648
Total Zirconium (Zr)	mg/L	0.00041	8673648	0.00058	8673648	0.00039	0.00010	8673648
Total Calcium (Ca)	mg/L	14.9	8673647	18.8	8673647	31.4	0.25	8673647
Total Magnesium (Mg)	mg/L	6.95	8673647	8.82	8673647	10.3	0.25	8673647
Total Potassium (K)	mg/L	2.00	8673647	2.25	8673647	4.46	0.25	8673647
Total Sodium (Na)	mg/L	2.60	8673647	3.26	8673647	10.3	0.25	8673647
Total Sulphur (S)	mg/L	<3.0	8673647	3.2	8673647	18.3	3.0	8673647
RADIONUCLIDE								
Radium-226	Bq/L	<0.010	8666867	<0.010	8666867	<0.010	0.010	8666867

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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Bureau Veritas Job #: C3D4190
Report Date: 2023/05/26

EcoMetrix Incorporated
Site Location: NewGold Rainy River

RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		VUA734			VUA735			VUA735		
Sampling Date		2023/05/09 09:20			2023/05/09 12:00			2023/05/09 12:00		
COC Number		928057-01-01			928057-01-01			928057-01-01		
	UNITS	**DUP Lab-Dup**	**RDL**	**QC Batch**	**FB**	**RDL**	**QC Batch**	**FB Lab-Dup**	**RDL**	**QC Batch**
Calculated Parameters										
Hardness (CaCO3)	mg/L				<1.0	1.0	8658512			
Inorganics										
Acidity	mg/L				<5.0	5.0	8669092			
Total Ammonia-N	mg/L				<0.050	0.050	8661704			
Total Dissolved Solids	mg/L				<10	10	8664486			
pH	pH				5.73		8662539			
Total Phosphorus	mg/L				<0.020	0.020	8661479			
Total Suspended Solids	mg/L				<10	10	8664491			
Dissolved Sulphate (SO4)	mg/L	64	1.0	8661792	<1.0	1.0	8661799			
Total Cyanide (CN)	mg/L				<0.0050	0.0050	8658759			
Alkalinity (Total as CaCO3)	mg/L				<1.0	1.0	8662534			
Dissolved Chloride (Cl-)	mg/L	6.8	1.0	8661785	<1.0	1.0	8661796			
Nitrite (N)	mg/L				<0.010	0.010	8661729			
Nitrate (N)	mg/L				<0.10	0.10	8661729			
Nitrate + Nitrite (N)	mg/L				<0.10	0.10	8661729			
Metals										
Total Aluminum (Al)	mg/L				<0.00050	0.00050	8673650	<0.00050	0.00050	8673650
Total Antimony (Sb)	mg/L				<0.000020	0.000020	8673650	<0.000020	0.000020	8673650
Total Arsenic (As)	mg/L				<0.000020	0.000020	8673650	<0.000020	0.000020	8673650
Total Barium (Ba)	mg/L				<0.000020	0.000020	8673650	<0.000020	0.000020	8673650
Total Beryllium (Be)	mg/L				<0.000010	0.000010	8673650	<0.000010	0.000010	8673650
Total Bismuth (Bi)	mg/L				<0.0000050	0.0000050	8673650	<0.0000050	0.0000050	8673650
Total Boron (B)	mg/L				<0.010	0.010	8673650	<0.010	0.010	8673650
Total Cadmium (Cd)	mg/L				<0.0000050	0.0000050	8673650	<0.0000050	0.0000050	8673650
Total Chromium (Cr)	mg/L				<0.00010	0.00010	8673650	<0.00010	0.00010	8673650
Total Cobalt (Co)	mg/L				<0.0000050	0.0000050	8673650	<0.0000050	0.0000050	8673650
Total Copper (Cu)	mg/L				<0.000050	0.000050	8673650	<0.000050	0.000050	8673650
Total Iron (Fe)	mg/L				<0.0010	0.0010	8673650	<0.0010	0.0010	8673650
Total Lead (Pb)	mg/L				<0.0000050	0.0000050	8673650	<0.0000050	0.0000050	8673650
Total Lithium (Li)	mg/L				<0.00050	0.00050	8673650	<0.00050	0.00050	8673650
Total Manganese (Mn)	mg/L				<0.000050	0.000050	8673650	<0.000050	0.000050	8673650
Total Molybdenum (Mo)	mg/L				<0.000050	0.000050	8673650	<0.000050	0.000050	8673650

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		VUA734			VUA735			VUA735		
Sampling Date		2023/05/09 09:20			2023/05/09 12:00			2023/05/09 12:00		
COC Number		928057-01-01			928057-01-01			928057-01-01		
	UNITS	**DUP Lab-Dup**	**RDL**	**QC Batch**	**FB**	**RDL**	**QC Batch**	**FB Lab-Dup**	**RDL**	**QC Batch**
Total Nickel (Ni)	mg/L				<0.000020	0.000020	8673650	<0.000020	0.000020	8673650
Total Selenium (Se)	mg/L				<0.000040	0.000040	8673650	<0.000040	0.000040	8673650
Total Silicon (Si)	mg/L				<0.050	0.050	8673650	<0.050	0.050	8673650
Total Silver (Ag)	mg/L				<0.0000050	0.0000050	8673650	<0.0000050	0.0000050	8673650
Total Strontium (Sr)	mg/L				<0.000050	0.000050	8673650	<0.000050	0.000050	8673650
Total Thallium (Tl)	mg/L				<0.0000020	0.0000020	8673650	<0.0000020	0.0000020	8673650
Total Tin (Sn)	mg/L				<0.00020	0.00020	8673650	<0.00020	0.00020	8673650
Total Titanium (Ti)	mg/L				<0.00050	0.00050	8673650	<0.00050	0.00050	8673650
Total Uranium (U)	mg/L				<0.0000020	0.0000020	8673650	<0.0000020	0.0000020	8673650
Total Vanadium (V)	mg/L				<0.00020	0.00020	8673650	<0.00020	0.00020	8673650
Total Zinc (Zn)	mg/L				0.00031	0.00010	8673650	0.00038	0.00010	8673650
Total Zirconium (Zr)	mg/L				<0.00010	0.00010	8673650	<0.00010	0.00010	8673650
Total Calcium (Ca)	mg/L				<0.050	0.050	8673647			
Total Magnesium (Mg)	mg/L				<0.050	0.050	8673647			
Total Potassium (K)	mg/L				<0.050	0.050	8673647			
Total Sodium (Na)	mg/L				<0.050	0.050	8673647			
Total Sulphur (S)	mg/L				<3.0	3.0	8673647	<0.60	0.60	8673650
RADIONUCLIDE										
Radium-226	Bq/L				<0.010	0.010	8666867			

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		VUA736		
Sampling Date		2023/05/09		
COC Number		928057-01-01		
	UNITS	**TRIP BLANK**	**RDL**	**QC Batch**
Calculated Parameters				
Hardness (CaCO3)	mg/L	<1.0	1.0	8658512
Inorganics				
Acidity	mg/L	<5.0	5.0	8669092
Total Ammonia-N	mg/L	<0.050	0.050	8661704
Total Dissolved Solids	mg/L	<10	10	8664398
pH	pH	5.75		8662539
Total Phosphorus	mg/L	<0.020	0.020	8661479
Total Suspended Solids	mg/L	<10	10	8661588
Dissolved Sulphate (SO4)	mg/L	<1.0	1.0	8661792
Total Cyanide (CN)	mg/L	<0.0050	0.0050	8660971
Alkalinity (Total as CaCO3)	mg/L	<1.0	1.0	8662534
Dissolved Chloride (Cl-)	mg/L	<1.0	1.0	8661785
Nitrite (N)	mg/L	<0.010	0.010	8661729
Nitrate (N)	mg/L	<0.10	0.10	8661729
Nitrate + Nitrite (N)	mg/L	<0.10	0.10	8661729
Metals				
Total Aluminum (Al)	mg/L	<0.00050	0.00050	8673650
Total Antimony (Sb)	mg/L	<0.000020	0.000020	8673650
Total Arsenic (As)	mg/L	<0.000020	0.000020	8673650
Total Barium (Ba)	mg/L	<0.000020	0.000020	8673650
Total Beryllium (Be)	mg/L	0.000144	0.000010	8673650
Total Bismuth (Bi)	mg/L	<0.0000050	0.0000050	8673650
Total Boron (B)	mg/L	<0.010	0.010	8673650
Total Cadmium (Cd)	mg/L	<0.0000050	0.0000050	8673650
Total Chromium (Cr)	mg/L	<0.00010	0.00010	8673650
Total Cobalt (Co)	mg/L	<0.0000050	0.0000050	8673650
Total Copper (Cu)	mg/L	<0.000050	0.000050	8673650
Total Iron (Fe)	mg/L	<0.0010	0.0010	8673650
Total Lead (Pb)	mg/L	<0.0000050	0.0000050	8673650
Total Lithium (Li)	mg/L	<0.00050	0.00050	8673650
Total Manganese (Mn)	mg/L	<0.000050	0.000050	8673650
Total Molybdenum (Mo)	mg/L	<0.000050	0.000050	8673650
Total Nickel (Ni)	mg/L	<0.000020	0.000020	8673650

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		VUA736		
Sampling Date		2023/05/09		
COC Number		928057-01-01		
	UNITS	**TRIP BLANK**	**RDL**	**QC Batch**
Total Selenium (Se)	mg/L	<0.000040	0.000040	8673650
Total Silicon (Si)	mg/L	<0.050	0.050	8673650
Total Silver (Ag)	mg/L	<0.0000050	0.0000050	8673650
Total Strontium (Sr)	mg/L	<0.000050	0.000050	8673650
Total Thallium (Tl)	mg/L	<0.0000020	0.0000020	8673650
Total Tin (Sn)	mg/L	<0.00020	0.00020	8673650
Total Titanium (Ti)	mg/L	<0.00050	0.00050	8673650
Total Uranium (U)	mg/L	<0.0000020	0.0000020	8673650
Total Vanadium (V)	mg/L	<0.00020	0.00020	8673650
Total Zinc (Zn)	mg/L	<0.00010	0.00010	8673650
Total Zirconium (Zr)	mg/L	<0.00010	0.00010	8673650
Total Calcium (Ca)	mg/L	<0.050	0.050	8673647
Total Magnesium (Mg)	mg/L	<0.050	0.050	8673647
Total Potassium (K)	mg/L	<0.050	0.050	8673647
Total Sodium (Na)	mg/L	<0.050	0.050	8673647
Total Sulphur (S)	mg/L	<3.0	3.0	8673647

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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ELEMENTS BY ATOMIC SPECTROSCOPY (WATER)

Bureau Veritas ID		VUA728	VUA729	VUA730	VUA731	VUA732	VUA733		
Sampling Date		2023/05/09 09:20	2023/05/09 09:35	2023/05/09 11:45	2023/05/09 16:10	2023/05/09 16:55	2023/05/09 17:15		
COC Number		928057-01-01	928057-01-01	928057-01-01	928057-01-01	928057-01-01	928057-01-01		
	UNITS	**PINR-REF 1**	**PINR-EXP 2**	**PINEXP**	**STUC**	**LVR-REF 1**	**LVR-REF 2**	**RDL**	**QC Batch**
Calculated Parameters									
Total Hardness (CaCO3)	mg/L	129	143	188	85.0	66.0	83.3	0.50	8668771
Metals									
Dissolved Mercury (Hg)	ug/L	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	8664950

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

Bureau Veritas ID		VUA734	VUA735	VUA736		
Sampling Date		2023/05/09 09:20	2023/05/09 12:00	2023/05/09		
COC Number		928057-01-01	928057-01-01	928057-01-01		
	UNITS	**DUP**	**FB**	**TRIP BLANK**	**RDL**	**QC Batch**
Calculated Parameters						
Total Hardness (CaCO3)	mg/L	121	<0.50	<0.50	0.50	8668771
Metals						
Dissolved Mercury (Hg)	ug/L	<0.01	<0.01	<0.01	0.01	8664950

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Package 1	6.7°C
Package 2	6.7°C

Sample VUA728 [PINR-REF 1] : Total Metals Analysis: Sample received in a container that has not been proofed by Bureau Veritas. Analysis performed with client's consent.

Sample VUA729 [PINR-EXP 2] : Total Metals Analysis: Sample received in a container that has not been proofed by Bureau Veritas. Analysis performed with client's consent.

Sample VUA730 [PINEXP] : Total Metals Analysis: Sample received in a container that has not been proofed by Bureau Veritas. Analysis performed with client's consent.

Sample VUA731 [STUC] : Total Metals Analysis: Sample received in a container that has not been proofed by Bureau Veritas. Analysis performed with client's consent.

Sample VUA732 [LVR-REF 1] : Total Metals Analysis: Sample received in a container that has not been proofed by Bureau Veritas. Analysis performed with client's consent.

Sample VUA733 [LVR-REF 2] : Total Metals Analysis: Sample received in a container that has not been proofed by Bureau Veritas. Analysis performed with client's consent.

Sample VUA734 [DUP] : Total Metals Analysis: Sample received in a container that has not been proofed by Bureau Veritas. Analysis performed with client's consent.

Sample VUA735 [FB] : Total Metals Analysis: Sample received in a container that has not been proofed by Bureau Veritas. Analysis performed with client's consent.

Results relate only to the items tested.

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QUALITY ASSURANCE REPORT

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8658759	Total Cyanide (CN)	2023/05/11	95	80 - 120	106	80 - 120	<0.0050	mg/L	5.6	20		
8660971	Total Cyanide (CN)	2023/05/16	110	80 - 120	111	80 - 120	<0.0050	mg/L	NC	20		
8661479	Total Phosphorus	2023/05/12	100	80 - 120	101	80 - 120	<0.020	mg/L	NC	20	101	80 - 120
8661588	Total Suspended Solids	2023/05/15					<10	mg/L	NC	20	95	85 - 115
8661704	Total Ammonia-N	2023/05/15	97	75 - 125	98	80 - 120	<0.050	mg/L	NC	20		
8661729	Nitrate (N)	2023/05/15	NC	80 - 120	100	80 - 120	<0.10	mg/L	2.9	20		
8661729	Nitrite (N)	2023/05/15	90	80 - 120	108	80 - 120	<0.010	mg/L	0.37	20		
8661785	Dissolved Chloride (Cl-)	2023/05/16	103	80 - 120	101	80 - 120	<1.0	mg/L	1.7	20		
8661792	Dissolved Sulphate (SO4)	2023/05/16	NC	75 - 125	100	80 - 120	<1.0	mg/L	1.8	20		
8661796	Dissolved Chloride (Cl-)	2023/05/16	NC	80 - 120	101	80 - 120	<1.0	mg/L	4.3	20		
8661799	Dissolved Sulphate (SO4)	2023/05/16	NC	75 - 125	97	80 - 120	<1.0	mg/L	0.088	20		
8662534	Alkalinity (Total as CaCO3)	2023/05/17			99	85 - 115	<1.0	mg/L	1.7	20		
8662539	pH	2023/05/17			101	98 - 103			1.2	N/A		
8664398	Total Dissolved Solids	2023/05/16					<10	mg/L	2.1	20	100	90 - 110
8664402	Total Dissolved Solids	2023/05/16					<10	mg/L	4.9	20	95	90 - 110
8664486	Total Dissolved Solids	2023/05/16					<10	mg/L	2.8	20	102	90 - 110
8664491	Total Suspended Solids	2023/05/16					<10	mg/L	NC	20	95	85 - 115
8664950	Dissolved Mercury (Hg)	2023/05/15	99	75 - 125	103	80 - 120	<0.01	ug/L	NC	20		
8666867	Radium-226	2023/05/24			104	85 - 115	<0.010	Bq/L	NC	N/A		
8669092	Acidity	2023/05/17	102	80 - 120	104	80 - 120	<5.0	mg/L	NC	25		
8673648	Total Aluminum (Al)	2023/05/17	110	80 - 120	102	80 - 120	<0.0030	mg/L				
8673648	Total Antimony (Sb)	2023/05/17	106	80 - 120	106	80 - 120	<0.000020	mg/L				
8673648	Total Arsenic (As)	2023/05/17	109	80 - 120	105	80 - 120	<0.000020	mg/L				
8673648	Total Barium (Ba)	2023/05/17	104	80 - 120	104	80 - 120	<0.000050	mg/L				
8673648	Total Beryllium (Be)	2023/05/17	106	80 - 120	102	80 - 120	<0.000010	mg/L				
8673648	Total Bismuth (Bi)	2023/05/17	102	80 - 120	102	80 - 120	<0.000010	mg/L				
8673648	Total Boron (B)	2023/05/17	109	80 - 120	102	80 - 120	<0.010	mg/L				
8673648	Total Cadmium (Cd)	2023/05/17	104	80 - 120	101	80 - 120	<0.0000050	mg/L				
8673648	Total Chromium (Cr)	2023/05/17	106	80 - 120	101	80 - 120	<0.00010	mg/L				
8673648	Total Cobalt (Co)	2023/05/17	104	80 - 120	100	80 - 120	<0.000010	mg/L				
8673648	Total Copper (Cu)	2023/05/17	104	80 - 120	97	80 - 120	<0.00010	mg/L				
8673648	Total Iron (Fe)	2023/05/17	NC	80 - 120	100	80 - 120	<0.0050	mg/L				

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QUALITY ASSURANCE REPORT(CONT'D)

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8673648	Total Lead (Pb)	2023/05/17	100	80 - 120	104	80 - 120	<0.000020	mg/L				
8673648	Total Lithium (Li)	2023/05/17	NC	80 - 120	95	80 - 120	<0.00050	mg/L				
8673648	Total Manganese (Mn)	2023/05/17	101	80 - 120	100	80 - 120	<0.00010	mg/L				
8673648	Total Molybdenum (Mo)	2023/05/17	NC	80 - 120	104	80 - 120	<0.000050	mg/L				
8673648	Total Nickel (Ni)	2023/05/17	102	80 - 120	100	80 - 120	<0.00010	mg/L				
8673648	Total Selenium (Se)	2023/05/17	86	80 - 120	104	80 - 120	<0.000040	mg/L				
8673648	Total Silicon (Si)	2023/05/17	101	80 - 120	111	80 - 120	<0.050	mg/L				
8673648	Total Silver (Ag)	2023/05/17	102	80 - 120	100	80 - 120	<0.000010	mg/L				
8673648	Total Strontium (Sr)	2023/05/17	98	80 - 120	101	80 - 120	<0.000050	mg/L				
8673648	Total Sulphur (S)	2023/05/17	97	80 - 120	102	80 - 120	<0.60	mg/L				
8673648	Total Thallium (Tl)	2023/05/17	103	80 - 120	103	80 - 120	<0.0000020	mg/L				
8673648	Total Tin (Sn)	2023/05/17	104	80 - 120	105	80 - 120	<0.00020	mg/L				
8673648	Total Titanium (Ti)	2023/05/17	105	80 - 120	103	80 - 120	<0.0020	mg/L				
8673648	Total Uranium (U)	2023/05/17	103	80 - 120	103	80 - 120	<0.0000050	mg/L				
8673648	Total Vanadium (V)	2023/05/17	108	80 - 120	102	80 - 120	<0.00020	mg/L				
8673648	Total Zinc (Zn)	2023/05/17	NC	80 - 120	102	80 - 120	<0.0010	mg/L				
8673648	Total Zirconium (Zr)	2023/05/17	93	80 - 120	103	80 - 120	<0.00010	mg/L				
8673650	Total Aluminum (Al)	2023/05/18	101	80 - 120	100	80 - 120	<0.00050	mg/L	NC	20		
8673650	Total Antimony (Sb)	2023/05/18	101	80 - 120	100	80 - 120	<0.000020	mg/L	NC	20		
8673650	Total Arsenic (As)	2023/05/18	101	80 - 120	101	80 - 120	<0.000020	mg/L	NC	20		
8673650	Total Barium (Ba)	2023/05/18	99	80 - 120	100	80 - 120	<0.000020	mg/L	NC	20		
8673650	Total Beryllium (Be)	2023/05/18	101	80 - 120	100	80 - 120	<0.000010	mg/L	NC	20		
8673650	Total Bismuth (Bi)	2023/05/18	97	80 - 120	99	80 - 120	<0.0000050	mg/L	NC	20		
8673650	Total Boron (B)	2023/05/18	100	80 - 120	102	80 - 120	<0.010	mg/L	NC	20		
8673650	Total Cadmium (Cd)	2023/05/18	99	80 - 120	99	80 - 120	<0.0000050	mg/L	NC	20		
8673650	Total Chromium (Cr)	2023/05/18	100	80 - 120	100	80 - 120	<0.00010	mg/L	NC	20		
8673650	Total Cobalt (Co)	2023/05/18	97	80 - 120	97	80 - 120	<0.0000050	mg/L	NC	20		
8673650	Total Copper (Cu)	2023/05/18	95	80 - 120	95	80 - 120	<0.000050	mg/L	NC	20		
8673650	Total Iron (Fe)	2023/05/18	101	80 - 120	104	80 - 120	<0.0010	mg/L	NC	20		
8673650	Total Lead (Pb)	2023/05/18	100	80 - 120	101	80 - 120	<0.0000050	mg/L	NC	20		
8673650	Total Lithium (Li)	2023/05/18	96	80 - 120	96	80 - 120	<0.00050	mg/L	NC	20		
8673650	Total Manganese (Mn)	2023/05/18	99	80 - 120	99	80 - 120	<0.000050	mg/L	NC	20		

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QUALITY ASSURANCE REPORT(CONT'D)

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8673650	Total Molybdenum (Mo)	2023/05/18	99	80 - 120	101	80 - 120	<0.000050	mg/L	NC	20		
8673650	Total Nickel (Ni)	2023/05/18	99	80 - 120	99	80 - 120	<0.000020	mg/L	NC	20		
8673650	Total Selenium (Se)	2023/05/18	99	80 - 120	99	80 - 120	<0.000040	mg/L	NC	20		
8673650	Total Silicon (Si)	2023/05/18	109	80 - 120	112	80 - 120	<0.050	mg/L	NC	20		
8673650	Total Silver (Ag)	2023/05/18	98	80 - 120	98	80 - 120	<0.0000050	mg/L	NC	20		
8673650	Total Strontium (Sr)	2023/05/18	100	80 - 120	99	80 - 120	<0.000050	mg/L	NC	20		
8673650	Total Sulphur (S)	2023/05/18	97	80 - 120	95	80 - 120	<0.60	mg/L	NC	20		
8673650	Total Thallium (Tl)	2023/05/18	98	80 - 120	99	80 - 120	<0.0000020	mg/L	NC	20		
8673650	Total Tin (Sn)	2023/05/18	98	80 - 120	101	80 - 120	<0.00020	mg/L	NC	20		
8673650	Total Titanium (Ti)	2023/05/18	103	80 - 120	103	80 - 120	<0.00050	mg/L	NC	20		
8673650	Total Uranium (U)	2023/05/18	100	80 - 120	101	80 - 120	<0.0000020	mg/L	NC	20		
8673650	Total Vanadium (V)	2023/05/18	99	80 - 120	100	80 - 120	<0.00020	mg/L	NC	20		
8673650	Total Zinc (Zn)	2023/05/18	102	80 - 120	104	80 - 120	<0.00010	mg/L	19	20		
8673650	Total Zirconium (Zr)	2023/05/18	101	80 - 120	100	80 - 120	<0.00010	mg/L	NC	20		

N/A = Not Applicable

Duplicate: Paired analysis of a separate portion of the same sample. Used to evaluate the variance in the measurement.

Matrix Spike: A sample to which a known amount of the analyte of interest has been added. Used to evaluate sample matrix interference.

QC Standard: A sample of known concentration prepared by an external agency under stringent conditions. Used as an independent check of method accuracy.

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method accuracy.

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

NC (Matrix Spike): The recovery in the matrix spike was not calculated. The relative difference between the concentration in the parent sample and the spike amount was too small to permit a reliable recovery calculation (matrix spike concentration was less than the native sample concentration)

NC (Duplicate RPD): The duplicate RPD was not calculated. The concentration in the sample and/or duplicate was too low to permit a reliable RPD calculation (absolute difference <= 2x RDL).

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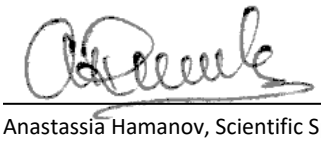
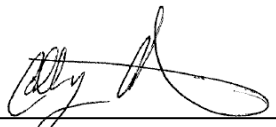
VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by:

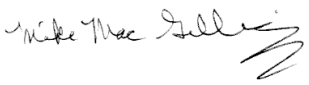


Anastassia Hamanov, Scientific Specialist



Colby Coutu, Project Manager

David Huang, BBY Scientific Specialist



Mike MacGillivray, Scientific Specialist (Inorganics)

Robert Allen, Scientific Specialist

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Colby Coutu
C3D4190

RPK ENV-1122

ssauga, Ontario Canada L5N 2L8 Tel:(905) 817-5700 Toll-free:800-563-6266 Fax:(905) 817-5777 www.bvna.com

Page of

CHAIN OF CUSTODY RECORD

Company Name:	#12046 EcoMetrix Incorporated
Attention:	Accounts Payable
Address:	6800 Campobello Rd Mississauga ON L5N 2L8
Tel:	(905) 794-2325 Fax: (905) 794-2338
Email:	accountspayable@ecometrix.ca

REPORT TO:

Company Name:	
Attention:	Caroline Farkas
Address:	
Tel:	(905) 794-2325 Ext: 244 Fax:
Email:	cfarkas@ecometrix.ca

PROJECT INFORMATION:

Quotation #:	C25356
P.O. #:	
Project:	
Project Name:	NewGold Rainy River
Site #:	
Sampled By:	

Laboratory Use Only:

Bureau Veritas Job #:	Bottle Order #:
	928057
COC #:	**Project Manager:**
C#928057-01-01	Colby Coutu

MOE REGULATED DRINKING WATER OR WATER INTENDED FOR HUMAN CONSUMPTION MUST BE SUBMITTED ON THE BUREAU VERITAS DRINKING WATER CHAIN OF CUSTODY

Regulation 153 (2011): ☐ Table 1 ☐ Res/Park ☐ Medium/Fine ☐ Table 2 ☐ Ind/Comm ☐ Coarse ☐ Table 3 ☐ Agri/Other ☐ For RSC ☐ Table ___

Other Regulations: ☐ CCME ☐ Sanitary Sewer Bylaw ☐ Reg 558 ☐ Storm Sewer Bylaw ☐ MISA Municipality ☐ PWQO ☐ Reg 406 Table ☐ Other

Special Instructions

Include Criteria on Certificate of Analysis (Y/N)? ___

Turnaround Time (TAT) Required:
Please provide advance notice for rush projects

Regular (Standard) TAT:
(will be applied if Rush TAT is not specified):
Standard TAT = 5-7 Working days for most tests.
Please note: Standard TAT for certain tests such as BOD and Dioxins/Furans are > 5 days - contact your Project Manager for details.

Job Specific Rush TAT (if applies to entire submission)
Date Required: ___ Time Required: ___
Rush Confirmation Number: ___ *(call lab for #)*

	Sample Barcode Label	Sample (Location) Identification	Date Sampled	Time Sampled	Matrix	Field Filtered (please circle): Metals / (Hg) / Cr VI	Acidity, Alkalinity and pH	Un-ionized Ammonia (incl. Total Ammonia)	Chloride, Sulphate, Nitrate and Nitrite	Low Level Dissolved Mercury	Hardness (calculated as CaCO3)	Total Cyanide	Total Phosphorus (Colourimetric)	Radium-226 by Alpha Spectrometry	Total Dissolved and Suspended Solids	Low Level Total ICPMS Metals	# of Bottles	Comments
1		PINA-REF1	23/05/09	09:20	WATER	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	7.34 pH, 9.0 °C
2		PINA-EXP2	23/05/09	09:35	WATER	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	7.34 pH, 8.8 °C
3		PINEXP	23/05/09	11:45	WATER	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	7.96 pH, 11.0 °C
4		STUC	23/05/09	16:10	WATER	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	8.00 pH, 12.6 °C
5		LVR-REF1	23/05/09	16:55	WATER	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	7.54 pH, 12.2 °C
6		LVR-REF2	23/05/09	17:15	WATER	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	7.45 pH, 11.8 °C
7		DUP	23/05/09	09:20	WATER	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	7.34 pH, 9.0 °C
8		FB	23/05/09	12:00	WATER	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	—
9		TRIP BLANK	—	—	WATER		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	8	—
10																		

Custody Seal Present Intact
Cooling Media Yes No

Rec'd In Thunder Bay

* RELINQUISHED BY: (Signature/Print)	Date: (YY/MM/DD)	Time	RECEIVED BY: (Signature/Print)	Date: (YY/MM/DD)	Time	# jars used and not submitted
Brian Kielstra	23/05/10	13:48	Bramwell Sherwe Bramwell	2023/05/10	13:49	
				2023/05/11	09:04	

Laboratory Use Only
Time Sensitive
Temperature (°C) on Recei: 10 6,7,7 6 5,7,8
Custody Seal: Present / Intact — Yes / No

* UNLESS OTHERWISE AGREED TO IN WRITING, WORK SUBMITTED ON THIS CHAIN OF CUSTODY IS SUBJECT TO BUREAU VERITAS'S STANDARD TERMS AND CONDITIONS. SIGNING OF THIS CHAIN OF CUSTODY DOCUMENT IS ACKNOWLEDGMENT AND ACCEPTANCE OF OUR TERMS WHICH ARE AVAILABLE FOR VIEWING AT WWW.BVNA.COM/ENVIRONMENTAL-LABORATORIES/RESOURCES/COC-TERMS-AND-CONDITIONS.

* IT IS THE RESPONSIBILITY OF THE RELINQUISHER TO ENSURE THE ACCURACY OF THE CHAIN OF CUSTODY RECORD. AN INCOMPLETE CHAIN OF CUSTODY MAY RESULT IN ANALYTICAL TAT DELAYS.

** SAMPLE CONTAINER, PRESERVATION, HOLD TIME AND PACKAGE INFORMATION CAN BE VIEWED AT WWW.BVNA.COM/ENVIRONMENTAL-LABORATORIES/RESOURCES/CHAIN-CUSTODY-FORMS-COCS.

SAMPLES MUST BE KEPT COOL (< 10° C) FROM TIME OF SAMPLING UNTIL DELIVERY TO BUREAU VERITAS

White: Bureau Veritas Yellow: Client

Bureau Veritas Canada (2019) Inc.

Your Project #: 22-3093
Site#: PHASE 3 EEM
Site Location: NewGold Rainy River

Attention: Joseph Tetreault

EcoMetrix Incorporated
6800 Campobello Rd
Mississauga, ON
CANADA L5N 2L8

Your C.O.C. #: 953308-01-01, 953308-02-01, 953308-04-01, 953308-03-01

Report Date: 2023/10/25
Report #: R7877971
Version: 1 - Final

CERTIFICATE OF ANALYSIS

BUREAU VERITAS JOB #: C3T6649
Received: 2023/09/25, 09:19

Sample Matrix: Sediment
Samples Received: 32

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Analytical Method
Acid Extractable Metals by ICPMS	2	2023/09/28	2023/09/28	CAM SOP-00447	EPA 6020B m
Acid Extractable Metals by ICPMS	21	2023/09/29	2023/09/29	CAM SOP-00447	EPA 6020B m
Acid Extractable Metals by ICPMS	9	2023/09/29	2023/09/30	CAM SOP-00447	EPA 6020B m
Particle size in solids (pipette&sieve) (1, 2)	15	N/A	2023/10/24	ATL SOP 00012	MSAMS'78/WREP-125R3m
Particle size in solids (pipette&sieve) (1, 2)	17	N/A	2023/10/25	ATL SOP 00012	MSAMS'78/WREP-125R3m
Total Organic Carbon in Soil	10	N/A	2023/10/02	CAM SOP-00468	BCMOE TOC Aug 2014
Total Organic Carbon in Soil	2	N/A	2023/09/28	CAM SOP-00468	BCMOE TOC Aug 2014
Total Organic Carbon in Soil	20	N/A	2023/09/29	CAM SOP-00468	BCMOE TOC Aug 2014

Sample Matrix: Water
Samples Received: 8

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Analytical Method
Acidity (CaCO3) in water (1)	8	N/A	2023/10/06	ATL SOP-00205	SM 24 2310B m
Alkalinity	1	N/A	2023/10/02	CAM SOP-00448	SM 23 2320 B m
Alkalinity	7	N/A	2023/09/30	CAM SOP-00448	SM 23 2320 B m
Chloride by Automated Colourimetry	8	N/A	2023/09/29	CAM SOP-00463	SM 23 4500-Cl E m
Total Cyanide	8	2023/09/28	2023/09/28	CAM SOP-00457	OMOE E3015 5 m
Hardness (calculated as CaCO3)	8	N/A	2023/09/29	CAM SOP 00102/00408/00447	SM 2340 B
Dissolved Mercury (low level)	8	2023/09/29	2023/09/29	CAM SOP-00453	EPA 7470 m
Hardness Total (calculated as CaCO3) (3, 5)	7	N/A	2023/10/12	BBY WI-00033	Auto Calc
Hardness Total (calculated as CaCO3) (3, 5)	1	N/A	2023/10/05	BBY WI-00033	Auto Calc
Elements by ICPMS Low Level (total) (3)	7	2023/10/12	2023/10/12	BBY7SOP-00003 / BBY7SOP-00002	EPA 6020b R2 m
Na, K, Ca, Mg, S by CRC ICPMS (total) (3)	7	N/A	2023/10/12	BBY WI-00033	Auto Calc

Your Project #: 22-3093
Site#: PHASE 3 EEM
Site Location: NewGold Rainy River

Attention: Joseph Tetreault

EcoMetrix Incorporated
6800 Campobello Rd
Mississauga, ON
CANADA L5N 2L8

Your C.O.C. #: 953308-01-01, 953308-02-01, 953308-04-01, 953308-03-01

Report Date: 2023/10/25
Report #: R7877971
Version: 1 - Final

CERTIFICATE OF ANALYSIS

BUREAU VERITAS JOB #: C3T6649
Received: 2023/09/25, 09:19

Sample Matrix: Water
Samples Received: 8

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Analytical Method
Na, K, Ca, Mg, S by CRC ICPMS (total) (3)	1	N/A	2023/10/05	BBY WI-00033	Auto Calc
Elements by ICPMS Low Level (total) (3)	1	N/A	2023/10/05	BBY7SOP-00002	EPA 6020b R2 m
Total Ammonia-N	8	N/A	2023/09/29	CAM SOP-00441	USGS I-2522-90 m
Nitrate & Nitrite as Nitrogen in Water (6)	8	N/A	2023/10/02	CAM SOP-00440	SM 23 4500-NO3I/NO2B
pH	8	2023/09/28	2023/09/30	CAM SOP-00413	SM 4500H+ B m
Field Measured pH (7)	7	N/A	2023/09/26		Field pH Meter
Radium Isotopes by Alpha Spectrometry (4, 8)	7	N/A	2023/10/20	BQL SOP-00006 BQL SOP-00017 BQL SOP-00032	Alpha Spectrometry
Sulphate by Automated Turbidimetry	8	N/A	2023/09/29	CAM SOP-00464	SM 23 4500-SO42- E m
Total Dissolved Solids	8	2023/09/28	2023/09/29	CAM SOP-00428	SM 23 2540C m
Field Temperature (7)	7	N/A	2023/09/26		Field Thermometer
Total Phosphorus (Colourimetric)	4	2023/09/28	2023/09/29	CAM SOP-00407	SM 23 4500-P I
Total Phosphorus (Colourimetric)	4	2023/09/28	2023/09/30	CAM SOP-00407	SM 23 4500-P I
Total Suspended Solids	8	2023/09/29	2023/09/29	CAM SOP-00428	SM 23 2540D m
Un-ionized Ammonia (9)	7	2023/09/26	2023/09/29	Auto Calc.	PWQO

Reference Method suffix “m” indicates test methods incorporate validated modifications from specific reference methods to improve performance.
(1) This test was performed by Bureau Veritas Bedford, 200 Bluewater Rd Suite 105, Bedford, NS, B4B 1G9
(2) Note: Graphical representation of larger fractions (PHI-4, PHI -3 and PHI -2) not applicable unless these optional parameters are specifically requested.
(3) This test was performed by Bureau Veritas Burnaby, 4606 Canada Way , Burnaby, BC, V5G 1K5
(4) This test was performed by Bureau Veritas Kitimat, 6790 Kitimat Road, Unit 4 , Mississauga, ON, L5N 5L9
(5) "Total Hardness" was calculated from Total Ca and Mg concentrations and may be biased high (Hardness, or Dissolved Hardness, calculated from Dissolved Ca and Mg, should be used for compliance if available).
(6) Values for calculated parameters may not appear to add up due to rounding of raw data and significant figures.
(7) This is a field test, therefore, the results relate to items that were not analysed at Bureau Veritas.
(8) Radium-226 results have not been corrected for blanks.
(9) Un-ionized ammonia is calculated using the total ammonia result and field data provided by the client for pH and temperature.

Bureau Veritas 6740 Campobello Road, Mississauga, Ontario, L5N 2L8 Tel: (905) 817-5700 Toll-Free: 800-563-6266 Fax: (905) 817-5777 www.bvna.com

Your Project #: 22-3093
Site#: PHASE 3 EEM
Site Location: NewGold Rainy River

Attention: Joseph Tetreault

EcoMetrix Incorporated
6800 Campobello Rd
Mississauga, ON
CANADA L5N 2L8

Your C.O.C. #: 953308-01-01, 953308-02-01, 953308-04-01, 953308-03-01

Report Date: 2023/10/25
Report #: R7877971
Version: 1 - Final

CERTIFICATE OF ANALYSIS

BUREAU VERITAS JOB #: C3T6649
Received: 2023/09/25, 09:19

Encryption Key

AUTHORIZED REPORT
RAPPORT AUTORISÉ

Bureau Veritas
25 Oct 2023 13:50:14

Please direct all questions regarding this Certificate of Analysis to:
Colby Coutu, Project Manager
Email: Colby.Coutu@bureauveritas.com
Phone# (905)817-5844

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This report has been generated and distributed using a secure automated process.
Bureau Veritas has procedures in place to guard against improper use of the electronic signature and have the required "signatories", as per ISO/IEC 17025, signing the reports. For Service Group specific validation, please refer to the Validation Signatures page if included, otherwise available by request. For Department specific Analyst/Supervisor validation names, please refer to the Test Summary section if included, otherwise available by request. This report is authorized by Rodney Major, General Manager responsible for Ontario Environmental laboratory operations.

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RESULTS OF ANALYSES OF SEDIMENT

Bureau Veritas ID		XCF508			XCF508			XCF509		
Sampling Date		2023/09/20 14:37			2023/09/20 14:37			2023/09/20 13:54		
COC Number		953308-02-01			953308-02-01			953308-02-01		
	UNITS	**STUC-1**	**RDL**	**QC Batch**	**STUC-1 Lab-Dup**	**RDL**	**QC Batch**	**STUC-2**	**RDL**	**QC Batch**
Inorganics										
Total Organic Carbon	mg/kg	25000	500	8948050	24000	500	8948050	67000	500	8948050
< -1 Phi (2 mm)	%	100 (1)	0.10	8983203				99 (1)	0.10	8983203
< 0 Phi (1 mm)	%	100 (1)	0.10	8983203				98 (1)	0.10	8983203
< +1 Phi (0.5 mm)	%	99 (1)	0.10	8983203				98 (1)	0.10	8983203
< +2 Phi (0.25 mm)	%	99 (1)	0.10	8983203				97 (1)	0.10	8983203
< +3 Phi (0.12 mm)	%	88	0.10	8983203				94	0.10	8983203
< +4 Phi (0.062 mm)	%	65	0.10	8983203				89	0.10	8983203
< +5 Phi (0.031 mm)	%	57	0.10	8983203				83	0.10	8983203
< +6 Phi (0.016 mm)	%	51	0.10	8983203				76	0.10	8983203
< +7 Phi (0.0078 mm)	%	43	0.10	8983203				65	0.10	8983203
< +8 Phi (0.0039 mm)	%	40	0.10	8983203				61	0.10	8983203
< +9 Phi (0.0020 mm)	%	35	0.10	8983203				52	0.10	8983203
Gravel	%	<0.10	0.10	8983203				0.65	0.10	8983203
Sand	%	34	0.10	8983203				11	0.10	8983203
Silt	%	26	0.10	8983203				28	0.10	8983203
Clay	%	40	0.10	8983203				61	0.10	8983203

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate
(1) PSA sample observation comment: Fraction contained organic matter.

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RESULTS OF ANALYSES OF SEDIMENT

Bureau Veritas ID		XCF509			XCF510	XCF511	XCF512	XCF513		
Sampling Date		2023/09/20 13:54			2023/09/20 13:16	2023/09/20 12:27	2023/09/19 13:12	2023/09/20 10:13		
COC Number		953308-02-01			953308-02-01	953308-02-01	953308-02-01	953308-02-01		
	UNITS	**STUC-2 Lab-Dup**	**RDL**	**QC Batch**	**STUC-3**	**STUC-4**	**STUC-5**	**PINR-EXP1**	**RDL**	**QC Batch**
Inorganics										
Total Organic Carbon	mg/kg				23000	24000	23000	12000	500	8948050
< -1 Phi (2 mm)	%	100 (1)	0.10	8983203	99 (2)	100 (1)	100 (1)	99 (2)	0.10	8983203
< 0 Phi (1 mm)	%	99 (1)	0.10	8983203	99 (1)	100 (1)	100 (1)	99 (1)	0.10	8983203
< +1 Phi (0.5 mm)	%	99 (1)	0.10	8983203	99 (1)	99 (1)	100 (1)	99 (1)	0.10	8983203
< +2 Phi (0.25 mm)	%	98 (1)	0.10	8983203	99 (1)	99 (1)	98 (1)	94 (1)	0.10	8983203
< +3 Phi (0.12 mm)	%	96	0.10	8983203	92	90	81	74	0.10	8983203
< +4 Phi (0.062 mm)	%	90	0.10	8983203	72	69	61	53	0.10	8983203
< +5 Phi (0.031 mm)	%	83	0.10	8983203	62	58	54	44	0.10	8983203
< +6 Phi (0.016 mm)	%	77	0.10	8983203	54	50	48	38	0.10	8983203
< +7 Phi (0.0078 mm)	%	66	0.10	8983203	45	40	41	32	0.10	8983203
< +8 Phi (0.0039 mm)	%	62	0.10	8983203	43	38	38	30	0.10	8983203
< +9 Phi (0.0020 mm)	%	53	0.10	8983203	37	33	33	27	0.10	8983203
Gravel	%	0.29 (3)	0.10	8983203	0.64	<0.10	<0.10	0.73	0.10	8983203
Sand	%	9.5	0.10	8983203	27	31	39	46	0.10	8983203
Silt	%	28	0.10	8983203	30	32	23	23	0.10	8983203
Clay	%	62	0.10	8983203	43	38	38	30	0.10	8983203

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate
(1) PSA sample observation comment: Fraction contained organic matter.
(2) PSA sample observation comment: Fraction contained shells and organic matter.
(3) %RPD acceptable. Duplicate values agree within 10% absolute.

Bureau Veritas 6740 Campobello Road, Mississauga, Ontario, L5N 2L8 Tel: (905) 817-5700 Toll-Free: 800-563-6266 Fax: (905) 817-5777 www.bvna.com

RESULTS OF ANALYSES OF SEDIMENT

Bureau Veritas ID		XCF514	XCF515		XCF516		XCF517	XCF564		
Sampling Date		2023/09/20 09:28	2023/09/20 08:48		2023/09/20 08:04		2023/09/19 16:50	2023/09/21 13:03		
COC Number		953308-02-01	953308-02-01		953308-02-01		953308-02-01	953308-04-01		
	UNITS	**PINR-EXP2**	**PINR-EXP3**	**QC Batch**	**PINR-EXP4**	**QC Batch**	**PINR-EXP5**	**PINR-EXP2-1**	**RDL**	**QC Batch**
Inorganics										
Total Organic Carbon	mg/kg	15000	16000	8948050	11000	8952581	17000	18000	500	8948050
< -1 Phi (2 mm)	%	100	100	8983203	100	8983203	100	100	0.10	8983203
< 0 Phi (1 mm)	%	100 (1)	100 (1)	8983203	100 (1)	8983203	100	98 (2)	0.10	8983203
< +1 Phi (0.5 mm)	%	97 (1)	99 (1)	8983203	98 (1)	8983203	99	95 (1)	0.10	8983203
< +2 Phi (0.25 mm)	%	83	95	8983203	83	8983203	91	91	0.10	8983203
< +3 Phi (0.12 mm)	%	73	74	8983203	57	8983203	66	85	0.10	8983203
< +4 Phi (0.062 mm)	%	64	54	8983203	43	8983203	51	70	0.10	8983203
< +5 Phi (0.031 mm)	%	57	44	8983203	37	8983203	45	65	0.10	8983203
< +6 Phi (0.016 mm)	%	51	38	8983203	33	8983203	39	61	0.10	8983203
< +7 Phi (0.0078 mm)	%	43	31	8983203	28	8983203	32	51	0.10	8983203
< +8 Phi (0.0039 mm)	%	40	29	8983203	27	8983203	31	48	0.10	8983203
< +9 Phi (0.0020 mm)	%	36	26	8983203	24	8983203	28	41	0.10	8983203
Gravel	%	<0.10	<0.10	8983203	<0.10	8983203	<0.10	0.47	0.10	8983203
Sand	%	36	46	8983203	57	8983203	49	30	0.10	8983203
Silt	%	24	25	8983203	16	8983203	20	22	0.10	8983203
Clay	%	40	29	8983203	27	8983203	31	48	0.10	8983203

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
(1) PSA sample observation comment: Fraction contained organic matter.
(2) PSA sample observation comment: Fraction contained rocks and organic matter.

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Bureau Veritas Job #: C3T6649
Report Date: 2023/10/25

EcoMetrix Incorporated
Client Project #: 22-3093
Site Location: NewGold Rainy River

RESULTS OF ANALYSES OF SEDIMENT

Bureau Veritas ID		XCF565	XCF566	XCF567	XCF568		XCF569		
Sampling Date		2023/09/21 12:38	2023/09/21 10:48	2023/09/21 09:47	2023/09/21 10:17		2023/09/21 14:01		
COC Number		953308-04-01	953308-04-01	953308-04-01	953308-04-01		953308-04-01		
	UNITS	**PINR-EXP2-2**	**PINR-EXP2-3**	**PINR-EXP2-4**	**PINR-EXP2-5**	**QC Batch**	**PINR-REF2-1**	**RDL**	**QC Batch**
Inorganics									
Total Organic Carbon	mg/kg	16000	10000	11000	22000	8948050	18000	500	8948050
< -1 Phi (2 mm)	%	100	98	100 (1)	100	8983203	100	0.10	8992276
< 0 Phi (1 mm)	%	99	96	99 (2)	100	8983203	100	0.10	8992276
< +1 Phi (0.5 mm)	%	98	93	98 (3)	99	8983203	99	0.10	8992276
< +2 Phi (0.25 mm)	%	97	90	95	98	8983203	98	0.10	8992276
< +3 Phi (0.12 mm)	%	93	86	86	93	8983203	82	0.10	8992276
< +4 Phi (0.062 mm)	%	83	80	57	76	8983203	54	0.10	8992276
< +5 Phi (0.031 mm)	%	76	76	44	67	8983203	45	0.10	8992276
< +6 Phi (0.016 mm)	%	70	72	36	57	8983203	40	0.10	8992276
< +7 Phi (0.0078 mm)	%	58	64	29	42	8983203	33	0.10	8992276
< +8 Phi (0.0039 mm)	%	54	61	28	39	8983203	31	0.10	8992276
< +9 Phi (0.0020 mm)	%	46	54	24	33	8983203	28	0.10	8992276
Gravel	%	<0.10	2.1	0.42	<0.10	8983203	<0.10	0.10	8992276
Sand	%	17	18	43	24	8983203	46	0.10	8992276
Silt	%	29	20	29	36	8983203	22	0.10	8992276
Clay	%	54	61	28	39	8983203	31	0.10	8992276

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
(1) PSA sample observation comment: Fraction contained shells and organic matter.
(2) PSA sample observation comment: Fraction contained rocks and organic matter.
(3) PSA sample observation comment: Fraction contained organic matter.

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RESULTS OF ANALYSES OF SEDIMENT

Bureau Veritas ID		XCF571	XCF573	XCF576	XCF578		XCF665		
Sampling Date		2023/09/21 17:05	2023/09/21 15:11	2023/09/21 15:41	2023/09/21 16:12		2023/09/22 10:33		
COC Number		953308-04-01	953308-04-01	953308-04-01	953308-04-01		953308-03-01		
	UNITS	**PINR-REF2-2**	**PINR-REF2-3**	**PINR-REF2-4**	**PINR-REF2-5**	**QC Batch**	**LVR2-REF1**	**RDL**	**QC Batch**
Inorganics									
Total Organic Carbon	mg/kg	9000	11000	11000	13000	8948050	10000	500	8946819
< -1 Phi (2 mm)	%	100	100	99 (1)	100 (2)	8992276	100 (3)	0.10	8992276
< 0 Phi (1 mm)	%	100	99	99 (1)	99 (1)	8992276	99 (1)	0.10	8992276
< +1 Phi (0.5 mm)	%	99	97	97 (3)	98 (3)	8992276	94 (1)	0.10	8992276
< +2 Phi (0.25 mm)	%	96	93	94	95	8992276	87	0.10	8992276
< +3 Phi (0.12 mm)	%	90	81	83	80	8992276	74	0.10	8992276
< +4 Phi (0.062 mm)	%	75	62	63	56	8992276	61	0.10	8992276
< +5 Phi (0.031 mm)	%	64	55	54	47	8992276	52	0.10	8992276
< +6 Phi (0.016 mm)	%	57	50	49	41	8992276	44	0.10	8992276
< +7 Phi (0.0078 mm)	%	46	42	40	33	8992276	34	0.10	8992276
< +8 Phi (0.0039 mm)	%	42	40	37	31	8992276	32	0.10	8992276
< +9 Phi (0.0020 mm)	%	35	35	32	28	8992276	28	0.10	8992276
Gravel	%	<0.10	0.32	0.50	0.39	8992276	0.15	0.10	8992276
Sand	%	25	37	36	44	8992276	38	0.10	8992276
Silt	%	33	23	26	25	8992276	30	0.10	8992276
Clay	%	42	40	37	31	8992276	32	0.10	8992276

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
(1) PSA sample observation comment: Fraction contained rocks and organic matter.
(2) PSA sample observation comment: Fraction contained shells and organic matter.
(3) PSA sample observation comment: Fraction contained organic matter.

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RESULTS OF ANALYSES OF SEDIMENT

Bureau Veritas ID		XCF665			XCF666		XCF667	XCF668		
Sampling Date		2023/09/22 10:33			2023/09/22 10:33		2023/09/22 09:45	2023/09/22 09:11		
COC Number		953308-03-01			953308-03-01		953308-03-01	953308-03-01		
	UNITS	**LVR2-REF1 Lab-Dup**	**RDL**	**QC Batch**	**LVR2-REF1 DUPLICATE**	**QC Batch**	**LVR2-REF2**	**LVR2-REF3**	**RDL**	**QC Batch**
Inorganics										
Total Organic Carbon	mg/kg				13000	8948050	8600	8300	500	8952581
< -1 Phi (2 mm)	%	100 (1)	0.10	8992276	99 (2)	8992276	90	90	0.10	8992276
< 0 Phi (1 mm)	%	98 (3)	0.10	8992276	98 (3)	8992276	86	83	0.10	8992276
< +1 Phi (0.5 mm)	%	93 (3)	0.10	8992276	94 (3)	8992276	79	75	0.10	8992276
< +2 Phi (0.25 mm)	%	86	0.10	8992276	88	8992276	69	64	0.10	8992276
< +3 Phi (0.12 mm)	%	73	0.10	8992276	72	8992276	61	55	0.10	8992276
< +4 Phi (0.062 mm)	%	61	0.10	8992276	57	8992276	49	45	0.10	8992276
< +5 Phi (0.031 mm)	%	51	0.10	8992276	44	8992276	40	37	0.10	8992276
< +6 Phi (0.016 mm)	%	43	0.10	8992276	35	8992276	33	31	0.10	8992276
< +7 Phi (0.0078 mm)	%	33	0.10	8992276	26	8992276	25	24	0.10	8992276
< +8 Phi (0.0039 mm)	%	31	0.10	8992276	24	8992276	23	22	0.10	8992276
< +9 Phi (0.0020 mm)	%	27	0.10	8992276	21	8992276	20	19	0.10	8992276
Gravel	%	0.31	0.10	8992276	0.66	8992276	9.7	10	0.10	8992276
Sand	%	39	0.10	8992276	42	8992276	41	45	0.10	8992276
Silt	%	30	0.10	8992276	33	8992276	27	24	0.10	8992276
Clay	%	31	0.10	8992276	24	8992276	23	22	0.10	8992276

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate
(1) PSA sample observation comment: Fraction contained organic matter.
(2) PSA sample observation comment: Fraction contained shells and organic matter.
(3) PSA sample observation comment: Fraction contained rocks and organic matter.

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RESULTS OF ANALYSES OF SEDIMENT

Bureau Veritas ID		XCF669	XCF670			XCF670			XCO572		
Sampling Date		2023/09/22 12:40	2023/09/22 11:13			2023/09/22 11:13			2023/09/22 16:48		
COC Number		953308-03-01	953308-03-01			953308-03-01			953308-01-01		
	UNITS	**LVR2-REF4**	**LVR2-REF5**	**RDL**	**QC Batch**	**LVR2-REF5 Lab-Dup**	**RDL**	**QC Batch**	**LVR-REF1**	**RDL**	**QC Batch**
Inorganics											
Total Organic Carbon	mg/kg	13000	14000	500	8952581	12000	500	8952581	37000	500	8946819
< -1 Phi (2 mm)	%	99	88	0.10	8992276				100 (1)	0.10	8992276
< 0 Phi (1 mm)	%	93	79	0.10	8992276				100 (1)	0.10	8992276
< +1 Phi (0.5 mm)	%	85	76	0.10	8992276				99 (1)	0.10	8992276
< +2 Phi (0.25 mm)	%	71	71	0.10	8992276				95	0.10	8992276
< +3 Phi (0.12 mm)	%	56	59	0.10	8992276				76	0.10	8992276
< +4 Phi (0.062 mm)	%	40	43	0.10	8992276				64	0.10	8992276
< +5 Phi (0.031 mm)	%	33	35	0.10	8992276				59	0.10	8992276
< +6 Phi (0.016 mm)	%	28	29	0.10	8992276				54	0.10	8992276
< +7 Phi (0.0078 mm)	%	22	23	0.10	8992276				45	0.10	8992276
< +8 Phi (0.0039 mm)	%	20	21	0.10	8992276				42	0.10	8992276
< +9 Phi (0.0020 mm)	%	17	18	0.10	8992276				37	0.10	8992276
Gravel	%	1.4	12	0.10	8992276				0.14	0.10	8992276
Sand	%	58	44	0.10	8992276				36	0.10	8992276
Silt	%	21	22	0.10	8992276				22	0.10	8992276
Clay	%	20	21	0.10	8992276				42	0.10	8992276

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate
(1) PSA sample observation comment: Fraction contained organic matter.

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RESULTS OF ANALYSES OF SEDIMENT

Bureau Veritas ID		XCF671	XCF672	XCF673	XCF674	XCF675		
Sampling Date		2023/09/22 16:00	2023/09/22 15:45	2023/09/22 15:11	2023/09/22 14:22	2023/09/22 14:22		
COC Number		953308-03-01	953308-03-01	953308-03-01	953308-03-01	953308-03-01		
	UNITS	**LVR-REF2**	**LVR-REF3**	**LVR-REF4**	**LVR-REF5**	**LVR-REF5 DUPLICATE**	**RDL**	**QC Batch**
Inorganics								
Total Organic Carbon	mg/kg	21000	58000	8800	17000	18000	500	8952581
< -1 Phi (2 mm)	%	100	98 (1)	100	100 (2)	100	0.10	8992276
< 0 Phi (1 mm)	%	100	97 (3)	99	100 (2)	99	0.10	8992276
< +1 Phi (0.5 mm)	%	100	96 (3)	98	99 (2)	98	0.10	8992276
< +2 Phi (0.25 mm)	%	99	92	91	91	82	0.10	8992276
< +3 Phi (0.12 mm)	%	93	79	70	70	59	0.10	8992276
< +4 Phi (0.062 mm)	%	84	67	57	55	49	0.10	8992276
< +5 Phi (0.031 mm)	%	75	61	51	51	45	0.10	8992276
< +6 Phi (0.016 mm)	%	67	55	46	46	40	0.10	8992276
< +7 Phi (0.0078 mm)	%	54	46	39	38	34	0.10	8992276
< +8 Phi (0.0039 mm)	%	50	42	36	36	31	0.10	8992276
< +9 Phi (0.0020 mm)	%	44	37	32	32	28	0.10	8992276
Gravel	%	<0.10	2.1	<0.10	<0.10	0.17	0.10	8992276
Sand	%	16	31	43	45	50	0.10	8992276
Silt	%	33	25	21	20	18	0.10	8992276
Clay	%	50	42	36	36	31	0.10	8992276

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
(1) PSA sample observation comment: Fraction contained shells and organic matter.
(2) PSA sample observation comment: Fraction contained organic matter.
(3) PSA sample observation comment: Fraction contained rocks and organic matter.

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ELEMENTS BY ATOMIC SPECTROSCOPY (SEDIMENT)

Bureau Veritas ID		XCF508	XCF509	XCF510	XCF511	XCF512		
Sampling Date		2023/09/20 14:37	2023/09/20 13:54	2023/09/20 13:16	2023/09/20 12:27	2023/09/19 13:12		
COC Number		953308-02-01	953308-02-01	953308-02-01	953308-02-01	953308-02-01		
	UNITS	**STUC-1**	**STUC-2**	**STUC-3**	**STUC-4**	**STUC-5**	**RDL**	**QC Batch**
Metals								
Acid Extractable Aluminum (Al)	ug/g	9300	13000	11000	8400	11000	50	8949624
Acid Extractable Antimony (Sb)	ug/g	<0.20	<0.20	<0.20	<0.20	<0.20	0.20	8949624
Acid Extractable Arsenic (As)	ug/g	1.5	3.1	1.5	1.2	1.3	1.0	8949624
Acid Extractable Barium (Ba)	ug/g	65	88	67	54	65	0.50	8949624
Acid Extractable Beryllium (Be)	ug/g	0.40	0.58	0.43	0.35	0.46	0.20	8949624
Acid Extractable Bismuth (Bi)	ug/g	<1.0	<1.0	<1.0	<1.0	<1.0	1.0	8949624
Acid Extractable Boron (B)	ug/g	6.3	8.9	6.8	6.0	7.6	5.0	8949624
Acid Extractable Cadmium (Cd)	ug/g	0.20	0.40	0.21	0.18	0.19	0.10	8949624
Acid Extractable Calcium (Ca)	ug/g	6000	7100	5300	5500	6500	50	8949624
Acid Extractable Chromium (Cr)	ug/g	20	28	23	18	23	1.0	8949624
Acid Extractable Cobalt (Co)	ug/g	8.0	10	7.9	7.5	7.7	0.10	8949624
Acid Extractable Copper (Cu)	ug/g	9.0	14	8.9	7.4	9.4	0.50	8949624
Acid Extractable Iron (Fe)	ug/g	13000	18000	14000	11000	14000	50	8949624
Acid Extractable Lead (Pb)	ug/g	6.2	9.1	6.7	5.5	6.6	1.0	8949624
Acid Extractable Magnesium (Mg)	ug/g	4500	5400	4400	4000	4800	50	8949624
Acid Extractable Manganese (Mn)	ug/g	250	370	310	210	320	1.0	8949624
Acid Extractable Molybdenum (Mo)	ug/g	<0.50	0.58	<0.50	<0.50	<0.50	0.50	8949624
Acid Extractable Nickel (Ni)	ug/g	14	19	14	12	14	0.50	8949624
Acid Extractable Phosphorus (P)	ug/g	450	570	520	430	460	50	8949624
Acid Extractable Potassium (K)	ug/g	1200	1600	1300	1100	1400	200	8949624
Acid Extractable Selenium (Se)	ug/g	<0.50	0.52	<0.50	<0.50	<0.50	0.50	8949624
Acid Extractable Silver (Ag)	ug/g	<0.20	<0.20	<0.20	<0.20	<0.20	0.20	8949624
Acid Extractable Sodium (Na)	ug/g	86	130	100	82	98	50	8949624
Acid Extractable Strontium (Sr)	ug/g	19	25	21	17	21	1.0	8949624
Acid Extractable Thallium (Tl)	ug/g	0.14	0.18	0.14	0.12	0.14	0.050	8949624
Acid Extractable Tin (Sn)	ug/g	<1.0	<1.0	<1.0	<1.0	<1.0	1.0	8949624
Acid Extractable Uranium (U)	ug/g	1.4	2.4	1.4	1.2	1.2	0.050	8949624
Acid Extractable Vanadium (V)	ug/g	27	39	30	24	31	5.0	8949624
Acid Extractable Zinc (Zn)	ug/g	59	91	63	51	61	5.0	8949624
Acid Extractable Mercury (Hg)	ug/g	<0.050	<0.050	<0.050	<0.050	<0.050	0.050	8949624

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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ELEMENTS BY ATOMIC SPECTROSCOPY (SEDIMENT)

Bureau Veritas ID		XCF513	XCF514	XCF515		XCF516		
Sampling Date		2023/09/20 10:13	2023/09/20 09:28	2023/09/20 08:48		2023/09/20 08:04		
COC Number		953308-02-01	953308-02-01	953308-02-01		953308-02-01		
	UNITS	**PINR-EXP1**	**PINR-EXP2**	**PINR-EXP3**	**QC Batch**	**PINR-EXP4**	**RDL**	**QC Batch**
Metals								
Acid Extractable Aluminum (Al)	ug/g	6700	8900	5800	8949624	5300	50	8949868
Acid Extractable Antimony (Sb)	ug/g	0.21	0.24	0.31	8949624	0.22	0.20	8949868
Acid Extractable Arsenic (As)	ug/g	1.1	1.7	1.2	8949624	1.2	1.0	8949868
Acid Extractable Barium (Ba)	ug/g	39	47	35	8949624	33	0.50	8949868
Acid Extractable Beryllium (Be)	ug/g	0.29	0.38	0.25	8949624	0.23	0.20	8949868
Acid Extractable Bismuth (Bi)	ug/g	<1.0	<1.0	<1.0	8949624	<1.0	1.0	8949868
Acid Extractable Boron (B)	ug/g	5.2	6.7	<5.0	8949624	<5.0	5.0	8949868
Acid Extractable Cadmium (Cd)	ug/g	0.10	0.13	0.11	8949624	<0.10	0.10	8949868
Acid Extractable Calcium (Ca)	ug/g	4900	8600	5900	8949624	6200	50	8949868
Acid Extractable Chromium (Cr)	ug/g	15	20	13	8949624	13	1.0	8949868
Acid Extractable Cobalt (Co)	ug/g	5.4	7.1	5.6	8949624	5.6	0.10	8949868
Acid Extractable Copper (Cu)	ug/g	9.2	12	5.9	8949624	5.6	0.50	8949868
Acid Extractable Iron (Fe)	ug/g	9800	13000	8900	8949624	8200	50	8949868
Acid Extractable Lead (Pb)	ug/g	4.3	5.8	3.7	8949624	3.8	1.0	8949868
Acid Extractable Magnesium (Mg)	ug/g	3600	5900	3900	8949624	4000	50	8949868
Acid Extractable Manganese (Mn)	ug/g	330	310	210	8949624	200	1.0	8949868
Acid Extractable Molybdenum (Mo)	ug/g	<0.50	<0.50	<0.50	8949624	<0.50	0.50	8949868
Acid Extractable Nickel (Ni)	ug/g	11	14	9.4	8949624	9.3	0.50	8949868
Acid Extractable Phosphorus (P)	ug/g	390	450	330	8949624	400	50	8949868
Acid Extractable Potassium (K)	ug/g	910	1200	830	8949624	700	200	8949868
Acid Extractable Selenium (Se)	ug/g	<0.50	<0.50	<0.50	8949624	<0.50	0.50	8949868
Acid Extractable Silver (Ag)	ug/g	<0.20	<0.20	<0.20	8949624	<0.20	0.20	8949868
Acid Extractable Sodium (Na)	ug/g	77	95	77	8949624	65	50	8949868
Acid Extractable Strontium (Sr)	ug/g	17	23	15	8949624	14	1.0	8949868
Acid Extractable Thallium (Tl)	ug/g	0.092	0.12	0.085	8949624	0.071	0.050	8949868
Acid Extractable Tin (Sn)	ug/g	<1.0	<1.0	<1.0	8949624	<1.0	1.0	8949868
Acid Extractable Uranium (U)	ug/g	0.76	0.91	0.69	8949624	0.62	0.050	8949868
Acid Extractable Vanadium (V)	ug/g	19	26	17	8949624	15	5.0	8949868
Acid Extractable Zinc (Zn)	ug/g	39	53	37	8949624	35	5.0	8949868
Acid Extractable Mercury (Hg)	ug/g	<0.050	<0.050	<0.050	8949624	<0.050	0.050	8949868

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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ELEMENTS BY ATOMIC SPECTROSCOPY (SEDIMENT)

Bureau Veritas ID		XCF517	XCF564	XCF565	XCF566	XCF567		
Sampling Date		2023/09/19 16:50	2023/09/21 13:03	2023/09/21 12:38	2023/09/21 10:48	2023/09/21 09:47		
COC Number		953308-02-01	953308-04-01	953308-04-01	953308-04-01	953308-04-01		
	UNITS	**PINR-EXP5**	**PINR-EXP2-1**	**PINR-EXP2-2**	**PINR-EXP2-3**	**PINR-EXP2-4**	**RDL**	**QC Batch**
Metals								
Acid Extractable Aluminum (Al)	ug/g	7500	9800	10000	11000	6900	50	8949624
Acid Extractable Antimony (Sb)	ug/g	<0.20	0.39	0.36	0.35	0.34	0.20	8949624
Acid Extractable Arsenic (As)	ug/g	1.5	2.5	2.5	3.2	1.5	1.0	8949624
Acid Extractable Barium (Ba)	ug/g	47	56	58	84	38	0.50	8949624
Acid Extractable Beryllium (Be)	ug/g	0.32	0.43	0.46	0.51	0.30	0.20	8949624
Acid Extractable Bismuth (Bi)	ug/g	<1.0	<1.0	<1.0	<1.0	<1.0	1.0	8949624
Acid Extractable Boron (B)	ug/g	5.9	8.9	8.7	9.6	6.3	5.0	8949624
Acid Extractable Cadmium (Cd)	ug/g	0.14	0.16	0.20	0.19	0.12	0.10	8949624
Acid Extractable Calcium (Ca)	ug/g	6400	21000	21000	24000	19000	50	8949624
Acid Extractable Chromium (Cr)	ug/g	16	22	24	25	16	1.0	8949624
Acid Extractable Cobalt (Co)	ug/g	6.2	7.8	7.8	8.8	6.0	0.10	8949624
Acid Extractable Copper (Cu)	ug/g	7.7	14	13	16	8.9	0.50	8949624
Acid Extractable Iron (Fe)	ug/g	10000	15000	15000	17000	10000	50	8949624
Acid Extractable Lead (Pb)	ug/g	4.7	6.6	7.1	7.7	4.6	1.0	8949624
Acid Extractable Magnesium (Mg)	ug/g	4400	13000	14000	15000	12000	50	8949624
Acid Extractable Manganese (Mn)	ug/g	220	390	450	400	200	1.0	8949624
Acid Extractable Molybdenum (Mo)	ug/g	<0.50	<0.50	<0.50	<0.50	0.50	0.50	8949624
Acid Extractable Nickel (Ni)	ug/g	11	19	19	22	13	0.50	8949624
Acid Extractable Phosphorus (P)	ug/g	390	410	490	470	450	50	8949624
Acid Extractable Potassium (K)	ug/g	980	1800	1800	2100	1200	200	8949624
Acid Extractable Selenium (Se)	ug/g	<0.50	<0.50	<0.50	<0.50	<0.50	0.50	8949624
Acid Extractable Silver (Ag)	ug/g	<0.20	<0.20	<0.20	<0.20	<0.20	0.20	8949624
Acid Extractable Sodium (Na)	ug/g	97	140	150	170	120	50	8949624
Acid Extractable Strontium (Sr)	ug/g	18	29	28	31	22	1.0	8949624
Acid Extractable Thallium (Tl)	ug/g	0.099	0.16	0.17	0.19	0.10	0.050	8949624
Acid Extractable Tin (Sn)	ug/g	<1.0	<1.0	<1.0	<1.0	<1.0	1.0	8949624
Acid Extractable Uranium (U)	ug/g	0.90	1.1	1.2	1.1	0.82	0.050	8949624
Acid Extractable Vanadium (V)	ug/g	22	30	32	35	22	5.0	8949624
Acid Extractable Zinc (Zn)	ug/g	44	49	54	54	36	5.0	8949624
Acid Extractable Mercury (Hg)	ug/g	<0.050	<0.050	<0.050	<0.050	<0.050	0.050	8949624

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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ELEMENTS BY ATOMIC SPECTROSCOPY (SEDIMENT)

Bureau Veritas ID		XCF568		XCF569		XCF571	XCF571		
Sampling Date		2023/09/21 10:17		2023/09/21 14:01		2023/09/21 17:05	2023/09/21 17:05		
COC Number		953308-04-01		953308-04-01		953308-04-01	953308-04-01		
	UNITS	**PINR-EXP2-5**	**QC Batch**	**PINR-REF2-1**	**QC Batch**	**PINR-REF2-2**	**PINR-REF2-2 Lab-Dup**	**RDL**	**QC Batch**
Metals									
Acid Extractable Aluminum (Al)	ug/g	6700	8949624	6200	8949648	10000	9600	50	8949624
Acid Extractable Antimony (Sb)	ug/g	0.31	8949624	<0.20	8949648	<0.20	0.22	0.20	8949624
Acid Extractable Arsenic (As)	ug/g	1.5	8949624	1.3	8949648	2.5	2.6	1.0	8949624
Acid Extractable Barium (Ba)	ug/g	46	8949624	37	8949648	64	60	0.50	8949624
Acid Extractable Beryllium (Be)	ug/g	0.28	8949624	0.27	8949648	0.47	0.41	0.20	8949624
Acid Extractable Bismuth (Bi)	ug/g	<1.0	8949624	<1.0	8949648	<1.0	<1.0	1.0	8949624
Acid Extractable Boron (B)	ug/g	5.2	8949624	5.7	8949648	10	9.2	5.0	8949624
Acid Extractable Cadmium (Cd)	ug/g	0.16	8949624	<0.10	8949648	0.13	0.14	0.10	8949624
Acid Extractable Calcium (Ca)	ug/g	7300	8949624	5300	8949648	34000	33000	50	8949624
Acid Extractable Chromium (Cr)	ug/g	15	8949624	13	8949648	24	22	1.0	8949624
Acid Extractable Cobalt (Co)	ug/g	6.6	8949624	5.5	8949648	8.3	7.8	0.10	8949624
Acid Extractable Copper (Cu)	ug/g	7.5	8949624	5.7	8949648	13	12	0.50	8949624
Acid Extractable Iron (Fe)	ug/g	10000	8949624	9400	8949648	15000	14000	50	8949624
Acid Extractable Lead (Pb)	ug/g	4.5	8949624	4.8	8949648	6.2	6.0	1.0	8949624
Acid Extractable Magnesium (Mg)	ug/g	4500	8949624	3700	8949648	19000	18000	50	8949624
Acid Extractable Manganese (Mn)	ug/g	340	8949624	160	8949648	350	330	1.0	8949624
Acid Extractable Molybdenum (Mo)	ug/g	<0.50	8949624	<0.50	8949648	<0.50	<0.50	0.50	8949624
Acid Extractable Nickel (Ni)	ug/g	11	8949624	9.7	8949648	20	19	0.50	8949624
Acid Extractable Phosphorus (P)	ug/g	420	8949624	360	8949648	460	430	50	8949624
Acid Extractable Potassium (K)	ug/g	1000	8949624	910	8949648	1900	1800	200	8949624
Acid Extractable Selenium (Se)	ug/g	<0.50	8949624	<0.50	8949648	<0.50	<0.50	0.50	8949624
Acid Extractable Silver (Ag)	ug/g	<0.20	8949624	<0.20	8949648	<0.20	<0.20	0.20	8949624
Acid Extractable Sodium (Na)	ug/g	110	8949624	80	8949648	160	160	50	8949624
Acid Extractable Strontium (Sr)	ug/g	19	8949624	14	8949648	30	30	1.0	8949624
Acid Extractable Thallium (Tl)	ug/g	0.10	8949624	0.12	8949648	0.17	0.16	0.050	8949624
Acid Extractable Tin (Sn)	ug/g	<1.0	8949624	<1.0	8949648	<1.0	<1.0	1.0	8949624
Acid Extractable Uranium (U)	ug/g	0.86	8949624	0.87	8949648	0.98	0.89	0.050	8949624
Acid Extractable Vanadium (V)	ug/g	19	8949624	21	8949648	33	32	5.0	8949624
Acid Extractable Zinc (Zn)	ug/g	47	8949624	33	8949648	44	44	5.0	8949624
Acid Extractable Mercury (Hg)	ug/g	<0.050	8949624	<0.050	8949648	<0.050	<0.050	0.050	8949624

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate

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ELEMENTS BY ATOMIC SPECTROSCOPY (SEDIMENT)

Bureau Veritas ID		XCF573	XCF576	XCF578		XCF665		
Sampling Date		2023/09/21 15:11	2023/09/21 15:41	2023/09/21 16:12		2023/09/22 10:33		
COC Number		953308-04-01	953308-04-01	953308-04-01		953308-03-01		
	UNITS	**PINR-REF2-3**	**PINR-REF2-4**	**PINR-REF2-5**	**QC Batch**	**LVR2-REF1**	**RDL**	**QC Batch**
Metals								
Acid Extractable Aluminum (Al)	ug/g	8200	7400	7700	8949624	7800	50	8947269
Acid Extractable Antimony (Sb)	ug/g	<0.20	<0.20	<0.20	8949624	<0.20	0.20	8947269
Acid Extractable Arsenic (As)	ug/g	1.8	1.8	1.6	8949624	1.5	1.0	8947269
Acid Extractable Barium (Ba)	ug/g	49	46	45	8949624	48	0.50	8947269
Acid Extractable Beryllium (Be)	ug/g	0.34	0.33	0.33	8949624	0.32	0.20	8947269
Acid Extractable Bismuth (Bi)	ug/g	<1.0	<1.0	<1.0	8949624	<1.0	1.0	8947269
Acid Extractable Boron (B)	ug/g	6.7	6.4	6.5	8949624	5.5	5.0	8947269
Acid Extractable Cadmium (Cd)	ug/g	<0.10	0.12	0.12	8949624	0.14	0.10	8947269
Acid Extractable Calcium (Ca)	ug/g	18000	18000	15000	8949624	12000	50	8947269
Acid Extractable Chromium (Cr)	ug/g	18	17	17	8949624	17	1.0	8947269
Acid Extractable Cobalt (Co)	ug/g	6.8	6.4	6.8	8949624	6.9	0.10	8947269
Acid Extractable Copper (Cu)	ug/g	8.9	8.8	8.4	8949624	9.8	0.50	8947269
Acid Extractable Iron (Fe)	ug/g	12000	12000	12000	8949624	11000	50	8947269
Acid Extractable Lead (Pb)	ug/g	5.3	4.7	4.7	8949624	7.9	1.0	8947269
Acid Extractable Magnesium (Mg)	ug/g	11000	12000	9800	8949624	7800	50	8947269
Acid Extractable Manganese (Mn)	ug/g	270	260	250	8949624	170	1.0	8947269
Acid Extractable Molybdenum (Mo)	ug/g	<0.50	<0.50	<0.50	8949624	<0.50	0.50	8947269
Acid Extractable Nickel (Ni)	ug/g	15	14	14	8949624	13	0.50	8947269
Acid Extractable Phosphorus (P)	ug/g	430	410	410	8949624	490	50	8947269
Acid Extractable Potassium (K)	ug/g	1300	1200	1200	8949624	1100	200	8947269
Acid Extractable Selenium (Se)	ug/g	<0.50	<0.50	<0.50	8949624	<0.50	0.50	8947269
Acid Extractable Silver (Ag)	ug/g	<0.20	<0.20	<0.20	8949624	<0.20	0.20	8947269
Acid Extractable Sodium (Na)	ug/g	110	110	98	8949624	84	50	8947269
Acid Extractable Strontium (Sr)	ug/g	20	20	20	8949624	18	1.0	8947269
Acid Extractable Thallium (Tl)	ug/g	0.13	0.12	0.12	8949624	0.12	0.050	8947269
Acid Extractable Tin (Sn)	ug/g	<1.0	<1.0	<1.0	8949624	2.0	1.0	8947269
Acid Extractable Uranium (U)	ug/g	0.76	0.80	0.75	8949624	0.89	0.050	8947269
Acid Extractable Vanadium (V)	ug/g	24	23	23	8949624	25	5.0	8947269
Acid Extractable Zinc (Zn)	ug/g	37	36	38	8949624	43	5.0	8947269
Acid Extractable Mercury (Hg)	ug/g	<0.050	<0.050	<0.050	8949624	<0.050	0.050	8947269

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QC Batch = Quality Control Batch

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ELEMENTS BY ATOMIC SPECTROSCOPY (SEDIMENT)

Bureau Veritas ID		XCF666		XCF667		XCF668		
Sampling Date		2023/09/22 10:33		2023/09/22 09:45		2023/09/22 09:11		
COC Number		953308-03-01		953308-03-01		953308-03-01		
	UNITS	**LVR2-REF1 DUPLICATE**	**QC Batch**	**LVR2-REF2**	**QC Batch**	**LVR2-REF3**	**RDL**	**QC Batch**
Metals								
Acid Extractable Aluminum (Al)	ug/g	6400	8949624	7900	8950272	7600	50	8949868
Acid Extractable Antimony (Sb)	ug/g	<0.20	8949624	<0.20	8950272	<0.20	0.20	8949868
Acid Extractable Arsenic (As)	ug/g	1.5	8949624	1.2	8950272	1.4	1.0	8949868
Acid Extractable Barium (Ba)	ug/g	41	8949624	48	8950272	49	0.50	8949868
Acid Extractable Beryllium (Be)	ug/g	0.30	8949624	0.37	8950272	0.37	0.20	8949868
Acid Extractable Bismuth (Bi)	ug/g	<1.0	8949624	<1.0	8950272	<1.0	1.0	8949868
Acid Extractable Boron (B)	ug/g	6.2	8949624	7.9	8950272	6.8	5.0	8949868
Acid Extractable Cadmium (Cd)	ug/g	0.12	8949624	0.14	8950272	0.13	0.10	8949868
Acid Extractable Calcium (Ca)	ug/g	12000	8949624	12000	8950272	11000	50	8949868
Acid Extractable Chromium (Cr)	ug/g	14	8949624	18	8950272	18	1.0	8949868
Acid Extractable Cobalt (Co)	ug/g	5.8	8949624	7.3	8950272	7.0	0.10	8949868
Acid Extractable Copper (Cu)	ug/g	6.4	8949624	6.7	8950272	7.1	0.50	8949868
Acid Extractable Iron (Fe)	ug/g	9400	8949624	11000	8950272	12000	50	8949868
Acid Extractable Lead (Pb)	ug/g	5.0	8949624	6.0	8950272	6.4	1.0	8949868
Acid Extractable Magnesium (Mg)	ug/g	8000	8949624	8000	8950272	7400	50	8949868
Acid Extractable Manganese (Mn)	ug/g	150	8949624	220	8950272	210	1.0	8949868
Acid Extractable Molybdenum (Mo)	ug/g	<0.50	8949624	<0.50	8950272	<0.50	0.50	8949868
Acid Extractable Nickel (Ni)	ug/g	10	8949624	12	8950272	13	0.50	8949868
Acid Extractable Phosphorus (P)	ug/g	420	8949624	480	8950272	500	50	8949868
Acid Extractable Potassium (K)	ug/g	1000	8949624	1100	8950272	1100	200	8949868
Acid Extractable Selenium (Se)	ug/g	<0.50	8949624	<0.50	8950272	<0.50	0.50	8949868
Acid Extractable Silver (Ag)	ug/g	<0.20	8949624	<0.20	8950272	<0.20	0.20	8949868
Acid Extractable Sodium (Na)	ug/g	88	8949624	93	8950272	92	50	8949868
Acid Extractable Strontium (Sr)	ug/g	16	8949624	18	8950272	18	1.0	8949868
Acid Extractable Thallium (Tl)	ug/g	0.11	8949624	0.12	8950272	0.11	0.050	8949868
Acid Extractable Tin (Sn)	ug/g	<1.0	8949624	<1.0	8950272	<1.0	1.0	8949868
Acid Extractable Uranium (U)	ug/g	0.81	8949624	0.79	8950272	0.83	0.050	8949868
Acid Extractable Vanadium (V)	ug/g	22	8949624	26	8950272	25	5.0	8949868
Acid Extractable Zinc (Zn)	ug/g	37	8949624	43	8950272	46	5.0	8949868
Acid Extractable Mercury (Hg)	ug/g	<0.050	8949624	<0.050	8950272	<0.050	0.050	8949868

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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ELEMENTS BY ATOMIC SPECTROSCOPY (SEDIMENT)

Bureau Veritas ID		XCF669		XCF670		XCO572		
Sampling Date		2023/09/22 12:40		2023/09/22 11:13		2023/09/22 16:48		
COC Number		953308-03-01		953308-03-01		953308-01-01		
	UNITS	**LVR2-REF4**	**QC Batch**	**LVR2-REF5**	**QC Batch**	**LVR-REF1**	**RDL**	**QC Batch**
Metals								
Acid Extractable Aluminum (Al)	ug/g	6300	8949868	7100	8949827	8600	50	8947269
Acid Extractable Antimony (Sb)	ug/g	<0.20	8949868	<0.20	8949827	<0.20	0.20	8947269
Acid Extractable Arsenic (As)	ug/g	1.3	8949868	1.5	8949827	1.3	1.0	8947269
Acid Extractable Barium (Ba)	ug/g	41	8949868	45	8949827	53	0.50	8947269
Acid Extractable Beryllium (Be)	ug/g	0.32	8949868	0.31	8949827	0.33	0.20	8947269
Acid Extractable Bismuth (Bi)	ug/g	<1.0	8949868	<1.0	8949827	<1.0	1.0	8947269
Acid Extractable Boron (B)	ug/g	6.6	8949868	7.0	8949827	5.9	5.0	8947269
Acid Extractable Cadmium (Cd)	ug/g	0.13	8949868	0.14	8949827	0.19	0.10	8947269
Acid Extractable Calcium (Ca)	ug/g	11000	8949868	14000	8949827	5900	50	8947269
Acid Extractable Chromium (Cr)	ug/g	15	8949868	15	8949827	21	1.0	8947269
Acid Extractable Cobalt (Co)	ug/g	6.0	8949868	5.6	8949827	6.0	0.10	8947269
Acid Extractable Copper (Cu)	ug/g	6.3	8949868	6.9	8949827	9.4	0.50	8947269
Acid Extractable Iron (Fe)	ug/g	9600	8949868	9800	8949827	12000	50	8947269
Acid Extractable Lead (Pb)	ug/g	5.1	8949868	4.9	8949827	7.7	1.0	8947269
Acid Extractable Magnesium (Mg)	ug/g	6800	8949868	8200	8949827	4200	50	8947269
Acid Extractable Manganese (Mn)	ug/g	180	8949868	200	8949827	200	1.0	8947269
Acid Extractable Molybdenum (Mo)	ug/g	<0.50	8949868	<0.50	8949827	<0.50	0.50	8947269
Acid Extractable Nickel (Ni)	ug/g	10	8949868	10	8949827	13	0.50	8947269
Acid Extractable Phosphorus (P)	ug/g	500	8949868	490	8949827	480	50	8947269
Acid Extractable Potassium (K)	ug/g	970	8949868	1100	8949827	1200	200	8947269
Acid Extractable Selenium (Se)	ug/g	<0.50	8949868	<0.50	8949827	<0.50	0.50	8947269
Acid Extractable Silver (Ag)	ug/g	<0.20	8949868	<0.20	8949827	<0.20	0.20	8947269
Acid Extractable Sodium (Na)	ug/g	72	8949868	74	8949827	77	50	8947269
Acid Extractable Strontium (Sr)	ug/g	16	8949868	16	8949827	17	1.0	8947269
Acid Extractable Thallium (Tl)	ug/g	0.096	8949868	0.088	8949827	0.14	0.050	8947269
Acid Extractable Tin (Sn)	ug/g	<1.0	8949868	<1.0	8949827	<1.0	1.0	8947269
Acid Extractable Uranium (U)	ug/g	0.84	8949868	0.79	8949827	1.3	0.050	8947269
Acid Extractable Vanadium (V)	ug/g	22	8949868	22	8949827	26	5.0	8947269
Acid Extractable Zinc (Zn)	ug/g	39	8949868	36	8949827	47	5.0	8947269
Acid Extractable Mercury (Hg)	ug/g	<0.050	8949868	<0.050	8949827	<0.050	0.050	8947269

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ELEMENTS BY ATOMIC SPECTROSCOPY (SEDIMENT)

Bureau Veritas ID		XCF671		XCF672		XCF673		
Sampling Date		2023/09/22 16:00		2023/09/22 15:45		2023/09/22 15:11		
COC Number		953308-03-01		953308-03-01		953308-03-01		
	UNITS	**LVR-REF2**	**QC Batch**	**LVR-REF3**	**QC Batch**	**LVR-REF4**	**RDL**	**QC Batch**
Metals								
Acid Extractable Aluminum (Al)	ug/g	13000	8949827	11000	8949648	7100	50	8949868
Acid Extractable Antimony (Sb)	ug/g	<0.20	8949827	<0.20	8949648	<0.20	0.20	8949868
Acid Extractable Arsenic (As)	ug/g	2.6	8949827	1.8	8949648	1.2	1.0	8949868
Acid Extractable Barium (Ba)	ug/g	74	8949827	64	8949648	38	0.50	8949868
Acid Extractable Beryllium (Be)	ug/g	0.56	8949827	0.45	8949648	0.33	0.20	8949868
Acid Extractable Bismuth (Bi)	ug/g	<1.0	8949827	<1.0	8949648	<1.0	1.0	8949868
Acid Extractable Boron (B)	ug/g	9.0	8949827	10	8949648	6.2	5.0	8949868
Acid Extractable Cadmium (Cd)	ug/g	0.12	8949827	0.22	8949648	<0.10	0.10	8949868
Acid Extractable Calcium (Ca)	ug/g	5900	8949827	6700	8949648	4500	50	8949868
Acid Extractable Chromium (Cr)	ug/g	25	8949827	23	8949648	15	1.0	8949868
Acid Extractable Cobalt (Co)	ug/g	8.5	8949827	7.6	8949648	5.3	0.10	8949868
Acid Extractable Copper (Cu)	ug/g	11	8949827	12	8949648	6.5	0.50	8949868
Acid Extractable Iron (Fe)	ug/g	17000	8949827	14000	8949648	10000	50	8949868
Acid Extractable Lead (Pb)	ug/g	8.2	8949827	7.6	8949648	6.1	1.0	8949868
Acid Extractable Magnesium (Mg)	ug/g	5100	8949827	5100	8949648	3400	50	8949868
Acid Extractable Manganese (Mn)	ug/g	280	8949827	260	8949648	150	1.0	8949868
Acid Extractable Molybdenum (Mo)	ug/g	<0.50	8949827	<0.50	8949648	<0.50	0.50	8949868
Acid Extractable Nickel (Ni)	ug/g	17	8949827	16	8949648	10	0.50	8949868
Acid Extractable Phosphorus (P)	ug/g	490	8949827	540	8949648	340	50	8949868
Acid Extractable Potassium (K)	ug/g	1500	8949827	1700	8949648	880	200	8949868
Acid Extractable Selenium (Se)	ug/g	<0.50	8949827	<0.50	8949648	<0.50	0.50	8949868
Acid Extractable Silver (Ag)	ug/g	<0.20	8949827	<0.20	8949648	<0.20	0.20	8949868
Acid Extractable Sodium (Na)	ug/g	120	8949827	150	8949648	59	50	8949868
Acid Extractable Strontium (Sr)	ug/g	22	8949827	24	8949648	14	1.0	8949868
Acid Extractable Thallium (Tl)	ug/g	0.13	8949827	0.17	8949648	0.098	0.050	8949868
Acid Extractable Tin (Sn)	ug/g	<1.0	8949827	<1.0	8949648	<1.0	1.0	8949868
Acid Extractable Uranium (U)	ug/g	1.6	8949827	1.7	8949648	0.75	0.050	8949868
Acid Extractable Vanadium (V)	ug/g	39	8949827	34	8949648	22	5.0	8949868
Acid Extractable Zinc (Zn)	ug/g	51	8949827	59	8949648	33	5.0	8949868
Acid Extractable Mercury (Hg)	ug/g	<0.050	8949827	<0.050	8949648	<0.050	0.050	8949868

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ELEMENTS BY ATOMIC SPECTROSCOPY (SEDIMENT)

Bureau Veritas ID		XCF674		XCF675		
Sampling Date		2023/09/22 14:22		2023/09/22 14:22		
COC Number		953308-03-01		953308-03-01		
	UNITS	**LVR-REF5**	**QC Batch**	**LVR-REF5 DUPLICATE**	**RDL**	**QC Batch**
Metals						
Acid Extractable Aluminum (Al)	ug/g	7300	8949827	8300	50	8949868
Acid Extractable Antimony (Sb)	ug/g	<0.20	8949827	<0.20	0.20	8949868
Acid Extractable Arsenic (As)	ug/g	1.1	8949827	1.5	1.0	8949868
Acid Extractable Barium (Ba)	ug/g	43	8949827	47	0.50	8949868
Acid Extractable Beryllium (Be)	ug/g	0.29	8949827	0.36	0.20	8949868
Acid Extractable Bismuth (Bi)	ug/g	<1.0	8949827	<1.0	1.0	8949868
Acid Extractable Boron (B)	ug/g	6.6	8949827	8.7	5.0	8949868
Acid Extractable Cadmium (Cd)	ug/g	<0.10	8949827	0.11	0.10	8949868
Acid Extractable Calcium (Ca)	ug/g	6100	8949827	7100	50	8949868
Acid Extractable Chromium (Cr)	ug/g	15	8949827	18	1.0	8949868
Acid Extractable Cobalt (Co)	ug/g	5.7	8949827	7.1	0.10	8949868
Acid Extractable Copper (Cu)	ug/g	5.7	8949827	7.4	0.50	8949868
Acid Extractable Iron (Fe)	ug/g	10000	8949827	12000	50	8949868
Acid Extractable Lead (Pb)	ug/g	4.4	8949827	7.9	1.0	8949868
Acid Extractable Magnesium (Mg)	ug/g	4300	8949827	5200	50	8949868
Acid Extractable Manganese (Mn)	ug/g	240	8949827	220	1.0	8949868
Acid Extractable Molybdenum (Mo)	ug/g	<0.50	8949827	<0.50	0.50	8949868
Acid Extractable Nickel (Ni)	ug/g	9.9	8949827	13	0.50	8949868
Acid Extractable Phosphorus (P)	ug/g	420	8949827	440	50	8949868
Acid Extractable Potassium (K)	ug/g	1100	8949827	1300	200	8949868
Acid Extractable Selenium (Se)	ug/g	<0.50	8949827	<0.50	0.50	8949868
Acid Extractable Silver (Ag)	ug/g	<0.20	8949827	<0.20	0.20	8949868
Acid Extractable Sodium (Na)	ug/g	77	8949827	86	50	8949868
Acid Extractable Strontium (Sr)	ug/g	15	8949827	18	1.0	8949868
Acid Extractable Thallium (Tl)	ug/g	0.078	8949827	0.14	0.050	8949868
Acid Extractable Tin (Sn)	ug/g	<1.0	8949827	<1.0	1.0	8949868
Acid Extractable Uranium (U)	ug/g	0.77	8949827	0.89	0.050	8949868
Acid Extractable Vanadium (V)	ug/g	22	8949827	27	5.0	8949868
Acid Extractable Zinc (Zn)	ug/g	33	8949827	43	5.0	8949868
Acid Extractable Mercury (Hg)	ug/g	<0.050	8949827	<0.050	0.050	8949868

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		XCF499			XCF500		XCF501		
Sampling Date		2023/09/22 13:20			2023/09/22 12:30		2023/09/22 09:48		
COC Number		953308-01-01			953308-01-01		953308-01-01		
	UNITS	**LVR**	**RDL**	**QC Batch**	**LVR2**	**RDL**	**PINREXP**	**RDL**	**QC Batch**
Calculated Parameters									
Hardness (CaCO3)	mg/L	160	1.0	8942148	200	1.0	290	1.0	8942148
Total Un-ionized Ammonia	mg/L	0.00099	0.00066	8941678	<0.00061	0.00061	0.00077	0.00055	8941678
Field Measurements									
Field Temperature	Celsius	15.64	N/A	ONSITE	15.09	N/A	14.88	N/A	ONSITE
Field Measured pH	pH	7.58		ONSITE	7.39		7.53		ONSITE
Inorganics									
Acidity	mg/L	6.4	5.0	8964978	5.4	5.0	11	5.0	8964978
Total Ammonia-N	mg/L	0.075	0.050	8948359	<0.050	0.050	0.069	0.050	8948359
Total Dissolved Solids	mg/L	200	10	8947549	245	10	300	10	8947549
pH	pH	8.00		8948728	7.75		7.94		8948728
Total Phosphorus	mg/L	0.13	0.020	8948144	0.47	0.10	0.098	0.020	8948135
Total Suspended Solids	mg/L	28	10	8947567	11	10	<10	10	8947567
Dissolved Sulphate (SO4)	mg/L	2.5	1.0	8949155	3.6	1.0	20	1.0	8949155
Total Cyanide (CN)	mg/L	<0.0050	0.0050	8946680	<0.0050	0.0050	<0.0050	0.0050	8946680
Alkalinity (Total as CaCO3)	mg/L	140	1.0	8948786	160	1.0	240	1.0	8948786
Dissolved Chloride (Cl-)	mg/L	15	1.0	8949151	38	1.0	24	1.0	8949151
Nitrite (N)	mg/L	<0.010	0.010	8948462	<0.010	0.010	<0.010	0.010	8948462
Nitrate (N)	mg/L	<0.10	0.10	8948462	<0.10	0.10	<0.10	0.10	8948462
Nitrate + Nitrite (N)	mg/L	<0.10	0.10	8948462	<0.10	0.10	<0.10	0.10	8948462
Metals									
Total Aluminum (Al)	mg/L	0.428	0.0030	8977677	0.0877	0.0030	0.0889	0.0030	8977677
Total Antimony (Sb)	mg/L	0.000094	0.000020	8977677	0.000066	0.000020	0.000069	0.000020	8977677
Total Arsenic (As)	mg/L	0.00251	0.000020	8977677	0.00392	0.000020	0.00130	0.000020	8977677
Total Barium (Ba)	mg/L	0.0242	0.000050	8977677	0.0225	0.000050	0.0216	0.000050	8977677
Total Beryllium (Be)	mg/L	0.000034	0.000010	8977677	0.000023	0.000010	0.000012	0.000010	8977677
Total Bismuth (Bi)	mg/L	0.000011	0.000010	8977677	<0.000010	0.000010	<0.000010	0.000010	8977677
Total Boron (B)	mg/L	0.018	0.010	8977677	0.013	0.010	0.023	0.010	8977677
Total Cadmium (Cd)	mg/L	0.0000110	0.0000050	8977677	0.0000080	0.0000050	0.0000070	0.0000050	8977677
Total Chromium (Cr)	mg/L	0.00084	0.00010	8977677	0.00028	0.00010	0.00033	0.00010	8977677
Total Cobalt (Co)	mg/L	0.000736	0.000010	8977677	0.00104	0.000010	0.000210	0.000010	8977677
Total Copper (Cu)	mg/L	0.00151	0.00010	8977677	0.00075	0.00010	0.00099	0.00010	8977677
Total Iron (Fe)	mg/L	1.34	0.0050	8977677	1.95	0.0050	0.240	0.0050	8977677
Total Lead (Pb)	mg/L	0.000771	0.000020	8977677	0.000289	0.000020	0.000093	0.000020	8977677
Total Lithium (Li)	mg/L	0.00459	0.00050	8977677	0.00464	0.00050	0.00805	0.00050	8977677

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
N/A = Not Applicable

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		XCF499			XCF500		XCF501		
Sampling Date		2023/09/22 13:20			2023/09/22 12:30		2023/09/22 09:48		
COC Number		953308-01-01			953308-01-01		953308-01-01		
	UNITS	**LVR**	**RDL**	**QC Batch**	**LVR2**	**RDL**	**PINREXP**	**RDL**	**QC Batch**
Total Manganese (Mn)	mg/L	0.287	0.00010	8977677	1.08	0.00010	0.136	0.00010	8977677
Total Molybdenum (Mo)	mg/L	0.000686	0.000050	8977677	0.000340	0.000050	0.000342	0.000050	8977677
Total Nickel (Ni)	mg/L	0.00226	0.00010	8977677	0.00172	0.00010	0.00107	0.00010	8977677
Total Phosphorus (P)	mg/L	0.144	0.0050	8977677	0.276	0.0050	0.0904	0.0050	8977677
Total Selenium (Se)	mg/L	0.000189	0.000040	8977677	0.000164	0.000040	0.000132	0.000040	8977677
Total Silicon (Si)	mg/L	2.48	0.050	8977677	2.04	0.050	3.28	0.050	8977677
Total Silver (Ag)	mg/L	<0.000010	0.000010	8977677	<0.000010	0.000010	<0.000010	0.000010	8977677
Total Strontium (Sr)	mg/L	0.0662	0.000050	8977677	0.0761	0.000050	0.133	0.000050	8977677
Total Thallium (Tl)	mg/L	0.0000080	0.0000020	8977677	0.0000020	0.0000020	0.0000020	0.0000020	8977677
Total Tin (Sn)	mg/L	<0.00020	0.00020	8977677	<0.00020	0.00020	<0.00020	0.00020	8977677
Total Titanium (Ti)	mg/L	0.0130	0.0020	8977677	0.0043	0.0020	0.0030	0.0020	8977677
Total Uranium (U)	mg/L	0.000703	0.0000050	8977677	0.000347	0.0000050	0.00111	0.0000050	8977677
Total Vanadium (V)	mg/L	0.00222	0.00020	8977677	0.00113	0.00020	0.00069	0.00020	8977677
Total Zinc (Zn)	mg/L	0.0044	0.0010	8977677	0.0015	0.0010	0.0015	0.0010	8977677
Total Zirconium (Zr)	mg/L	0.00052	0.00010	8977677	0.00046	0.00010	0.00020	0.00010	8977677
Total Calcium (Ca)	mg/L	29.3	0.25	8963706	34.5	0.25	53.9	0.25	8963706
Total Magnesium (Mg)	mg/L	12.3	0.25	8963706	14.4	0.25	21.5	0.25	8963706
Total Potassium (K)	mg/L	1.61	0.25	8963706	2.67	0.25	2.81	0.25	8963706
Total Sodium (Na)	mg/L	7.10	0.25	8963706	13.1	0.25	6.92	0.25	8963706
Total Sulphur (S)	mg/L	<3.0	3.0	8963706	<3.0	3.0	5.4	3.0	8963706
RADIONUCLIDE									
Radium-226	Bq/L	<0.010	0.010	8988102	<0.010	0.010	<0.010	0.010	8988102

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		XCF501			XCF502	XCF503		
Sampling Date		2023/09/22 09:48			2023/09/22 08:50	2023/09/22 08:35		
COC Number		953308-01-01			953308-01-01	953308-01-01		
	UNITS	**PINREXP Lab-Dup**	**RDL**	**QC Batch**	**PINREXP2**	**PINRREF2**	**RDL**	**QC Batch**
Calculated Parameters								
Hardness (CaCO3)	mg/L				190	190	1.0	8942148
Total Un-ionized Ammonia	mg/L				<0.00061	<0.00061	0.00061	8941678
Field Measurements								
Field Temperature	Celsius				14.67	14.77	N/A	ONSITE
Field Measured pH	pH				7.49	7.28		ONSITE
Inorganics								
Acidity	mg/L				5.0	7.0	5.0	8964978
Total Ammonia-N	mg/L				<0.050	<0.050	0.050	8948359
Total Dissolved Solids	mg/L				260	230	10	8947549
pH	pH				7.88	7.87		8948728
Total Phosphorus	mg/L				0.10	0.11	0.020	8948144
Total Suspended Solids	mg/L	<10	10	8947567	15	12	10	8947567
Dissolved Sulphate (SO4)	mg/L				7.9	4.0	1.0	8949155
Total Cyanide (CN)	mg/L				<0.0050	<0.0050	0.0050	8946680
Alkalinity (Total as CaCO3)	mg/L				170	170	1.0	8948786
Dissolved Chloride (Cl-)	mg/L				5.0	4.3	1.0	8949151
Nitrite (N)	mg/L	<0.010	0.010	8948462	<0.010	<0.010	0.010	8948462
Nitrate (N)	mg/L	<0.10	0.10	8948462	<0.10	<0.10	0.10	8948462
Nitrate + Nitrite (N)	mg/L	<0.10	0.10	8948462	<0.10	<0.10	0.10	8948462
Metals								
Total Aluminum (Al)	mg/L				0.789	0.747	0.0030	8977677
Total Antimony (Sb)	mg/L				0.000246	0.000150	0.000020	8977677
Total Arsenic (As)	mg/L				0.00322	0.00332	0.000020	8977677
Total Barium (Ba)	mg/L				0.0258	0.0249	0.000050	8977677
Total Beryllium (Be)	mg/L				0.000046	0.000050	0.000010	8977677
Total Bismuth (Bi)	mg/L				0.000011	0.000013	0.000010	8977677
Total Boron (B)	mg/L				0.019	0.018	0.010	8977677
Total Cadmium (Cd)	mg/L				0.0000140	0.0000110	0.0000050	8977677
Total Chromium (Cr)	mg/L				0.00135	0.00134	0.00010	8977677
Total Cobalt (Co)	mg/L				0.000856	0.000888	0.000010	8977677
Total Copper (Cu)	mg/L				0.00214	0.00480	0.00010	8977677
Total Iron (Fe)	mg/L				1.47	1.57	0.0050	8977677
Total Lead (Pb)	mg/L				0.000964	0.000928	0.000020	8977677

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate
N/A = Not Applicable

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		XCF501			XCF502	XCF503		
Sampling Date		2023/09/22 09:48			2023/09/22 08:50	2023/09/22 08:35		
COC Number		953308-01-01			953308-01-01	953308-01-01		
	UNITS	**PINREXP Lab-Dup**	**RDL**	**QC Batch**	**PINREXP2**	**PINRREF2**	**RDL**	**QC Batch**
Total Lithium (Li)	mg/L				0.00521	0.00495	0.00050	8977677
Total Manganese (Mn)	mg/L				0.553	0.701	0.00010	8977677
Total Molybdenum (Mo)	mg/L				0.000678	0.000601	0.000050	8977677
Total Nickel (Ni)	mg/L				0.00321	0.00309	0.00010	8977677
Total Phosphorus (P)	mg/L				0.113	0.108	0.0050	8977677
Total Selenium (Se)	mg/L				0.000224	0.000214	0.000040	8977677
Total Silicon (Si)	mg/L				5.91	5.49	0.050	8977677
Total Silver (Ag)	mg/L				<0.000010	<0.000010	0.000010	8977677
Total Strontium (Sr)	mg/L				0.0945	0.0893	0.000050	8977677
Total Thallium (Tl)	mg/L				0.0000110	0.0000110	0.0000020	8977677
Total Tin (Sn)	mg/L				<0.00020	<0.00020	0.00020	8977677
Total Titanium (Ti)	mg/L				0.0319	0.0258	0.0020	8977677
Total Uranium (U)	mg/L				0.000822	0.000684	0.0000050	8977677
Total Vanadium (V)	mg/L				0.00276	0.00257	0.00020	8977677
Total Zinc (Zn)	mg/L				0.0038	0.0042	0.0010	8977677
Total Zirconium (Zr)	mg/L				0.00118	0.00112	0.00010	8977677
Total Calcium (Ca)	mg/L				37.6	36.3	0.25	8963706
Total Magnesium (Mg)	mg/L				13.7	13.5	0.25	8963706
Total Potassium (K)	mg/L				2.65	2.24	0.25	8963706
Total Sodium (Na)	mg/L				4.16	3.50	0.25	8963706
Total Sulphur (S)	mg/L				<3.0	<3.0	3.0	8963706
RADIONUCLIDE								
Radium-226	Bq/L				<0.010	<0.010	0.010	8988102

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		XCF504			XCF505		
Sampling Date		2023/09/22 11:10			2023/09/22 09:48		
COC Number		953308-01-01			953308-01-01		
	UNITS	**STUCREF**	**RDL**	**QC Batch**	**DUP**	**RDL**	**QC Batch**
Calculated Parameters							
Hardness (CaCO3)	mg/L	170	1.0	8942148	290	1.0	8942148
Total Un-ionized Ammonia	mg/L	<0.00061	0.00061	8941678	<0.00055	0.00055	8941678
Field Measurements							
Field Temperature	Celsius	15.56	N/A	ONSITE	14.88	N/A	ONSITE
Field Measured pH	pH	7.55		ONSITE	7.53		ONSITE
Inorganics							
Acidity	mg/L	<5.0	5.0	8964978	8.4	5.0	8964978
Total Ammonia-N	mg/L	<0.050	0.050	8948359	<0.050	0.050	8948359
Total Dissolved Solids	mg/L	190	10	8947549	310	10	8947549
pH	pH	7.70		8948728	7.95		8948728
Total Phosphorus	mg/L	0.080	0.020	8948144	0.095	0.020	8948135
Total Suspended Solids	mg/L	<10	10	8947567	<10	10	8947567
Dissolved Sulphate (SO4)	mg/L	2.6	1.0	8949155	19	1.0	8947766
Total Cyanide (CN)	mg/L	<0.0050	0.0050	8946680	<0.0050	0.0050	8946680
Alkalinity (Total as CaCO3)	mg/L	150	1.0	8954798	240	1.0	8948786
Dissolved Chloride (Cl-)	mg/L	6.3	1.0	8949151	25	1.0	8947749
Nitrite (N)	mg/L	<0.010	0.010	8948457	<0.010	0.010	8948457
Nitrate (N)	mg/L	<0.10	0.10	8948457	<0.10	0.10	8948457
Nitrate + Nitrite (N)	mg/L	<0.10	0.10	8948457	<0.10	0.10	8948457
Metals							
Total Aluminum (Al)	mg/L	0.0909	0.0030	8977677	0.0718 (1)	0.0030	8977677
Total Antimony (Sb)	mg/L	0.000063	0.000020	8977677	0.000067	0.000020	8977677
Total Arsenic (As)	mg/L	0.00138	0.000020	8977677	0.00135	0.000020	8977677
Total Barium (Ba)	mg/L	0.0110	0.000050	8977677	0.0221	0.000050	8977677
Total Beryllium (Be)	mg/L	0.000023	0.000010	8977677	<0.000010	0.000010	8977677
Total Bismuth (Bi)	mg/L	<0.000010	0.000010	8977677	<0.000010	0.000010	8977677
Total Boron (B)	mg/L	0.016	0.010	8977677	0.024	0.010	8977677
Total Cadmium (Cd)	mg/L	0.0000070	0.0000050	8977677	0.0000060	0.0000050	8977677
Total Chromium (Cr)	mg/L	0.00028	0.00010	8977677	0.00017	0.00010	8977677
Total Cobalt (Co)	mg/L	0.000329	0.000010	8977677	0.000212	0.000010	8977677
Total Copper (Cu)	mg/L	0.00107	0.00010	8977677	0.00118	0.00010	8977677
Total Iron (Fe)	mg/L	0.396	0.0050	8977677	0.235	0.0050	8977677

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
N/A = Not Applicable
(1) Duplicate exceeds acceptance criteria due to sample non homogeneity. Reanalysis yields similar results.

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		XCF504			XCF505		
Sampling Date		2023/09/22 11:10			2023/09/22 09:48		
COC Number		953308-01-01			953308-01-01		
	UNITS	**STUCREF**	**RDL**	**QC Batch**	**DUP**	**RDL**	**QC Batch**
Total Lead (Pb)	mg/L	0.000165	0.000020	8977677	0.000085	0.000020	8977677
Total Lithium (Li)	mg/L	0.00455	0.00050	8977677	0.00808	0.00050	8977677
Total Manganese (Mn)	mg/L	0.0943	0.00010	8977677	0.138	0.00010	8977677
Total Molybdenum (Mo)	mg/L	0.000263	0.000050	8977677	0.000333	0.000050	8977677
Total Nickel (Ni)	mg/L	0.00193	0.00010	8977677	0.00110	0.00010	8977677
Total Phosphorus (P)	mg/L	0.0788	0.0050	8977677	0.0909	0.0050	8977677
Total Selenium (Se)	mg/L	0.000168	0.000040	8977677	0.000117	0.000040	8977677
Total Silicon (Si)	mg/L	2.39	0.050	8977677	3.32	0.050	8977677
Total Silver (Ag)	mg/L	<0.000010	0.000010	8977677	<0.000010	0.000010	8977677
Total Strontium (Sr)	mg/L	0.0717	0.000050	8977677	0.136	0.000050	8977677
Total Thallium (Tl)	mg/L	0.0000030	0.0000020	8977677	0.0000020	0.0000020	8977677
Total Tin (Sn)	mg/L	<0.00020	0.00020	8977677	<0.00020	0.00020	8977677
Total Titanium (Ti)	mg/L	0.0030	0.0020	8977677	0.0029	0.0020	8977677
Total Uranium (U)	mg/L	0.000498	0.0000050	8977677	0.00113	0.0000050	8977677
Total Vanadium (V)	mg/L	0.00088	0.00020	8977677	0.00066	0.00020	8977677
Total Zinc (Zn)	mg/L	0.0045	0.0010	8977677	0.0017	0.0010	8977677
Total Zirconium (Zr)	mg/L	0.00029	0.00010	8977677	0.00016	0.00010	8977677
Total Calcium (Ca)	mg/L	30.9	0.25	8963706	55.5	0.25	8963706
Total Magnesium (Mg)	mg/L	13.8	0.25	8963706	21.9	0.25	8963706
Total Potassium (K)	mg/L	1.07	0.25	8963706	2.88	0.25	8963706
Total Sodium (Na)	mg/L	4.16	0.25	8963706	7.07	0.25	8963706
Total Sulphur (S)	mg/L	<3.0	3.0	8963706	5.4	3.0	8963706
RADIONUCLIDE							
Radium-226	Bq/L	<0.010	0.010	8988102	<0.010	0.010	8988102

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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Bureau Veritas Job #: C3T6649
Report Date: 2023/10/25

EcoMetrix Incorporated
Client Project #: 22-3093
Site Location: NewGold Rainy River

RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		XCF505			XCF506			XCF506		
Sampling Date		2023/09/22 09:48			2023/09/22 09:48			2023/09/22 09:48		
COC Number		953308-01-01			953308-01-01			953308-01-01		
	UNITS	**DUP Lab-Dup**	**RDL**	**QC Batch**	**FB**	**RDL**	**QC Batch**	**FB Lab-Dup**	**RDL**	**QC Batch**
Calculated Parameters										
Hardness (CaCO3)	mg/L				<1.0	1.0	8942148			
Inorganics										
Acidity	mg/L				<5.0	5.0	8964978	<5.0	5.0	8964978
Total Ammonia-N	mg/L				<0.050	0.050	8948359			
Total Dissolved Solids	mg/L				<10	10	8947549			
pH	pH				6.38		8948728			
Total Phosphorus	mg/L				<0.020	0.020	8948144			
Total Suspended Solids	mg/L				<10	10	8947567			
Dissolved Sulphate (SO4)	mg/L				<1.0	1.0	8947766			
Total Cyanide (CN)	mg/L				<0.0050	0.0050	8946680			
Alkalinity (Total as CaCO3)	mg/L				1.5	1.0	8948786			
Dissolved Chloride (Cl-)	mg/L				<1.0	1.0	8947749			
Nitrite (N)	mg/L				<0.010	0.010	8948457			
Nitrate (N)	mg/L				<0.10	0.10	8948457			
Nitrate + Nitrite (N)	mg/L				<0.10	0.10	8948457			
Metals										
Total Aluminum (Al)	mg/L	0.0932 (1)	0.0030	8977677	0.00101	0.00050	8977678			
Total Antimony (Sb)	mg/L	0.000060	0.000020	8977677	<0.000020	0.000020	8977678			
Total Arsenic (As)	mg/L	0.00127	0.000020	8977677	<0.000020	0.000020	8977678			
Total Barium (Ba)	mg/L	0.0214	0.000050	8977677	<0.000020	0.000020	8977678			
Total Beryllium (Be)	mg/L	<0.000010	0.000010	8977677	<0.000010	0.000010	8977678			
Total Bismuth (Bi)	mg/L	<0.000010	0.000010	8977677	<0.0000050	0.0000050	8977678			
Total Boron (B)	mg/L	0.022	0.010	8977677	<0.010	0.010	8977678			
Total Cadmium (Cd)	mg/L	0.0000060	0.0000050	8977677	<0.0000050	0.0000050	8977678			
Total Chromium (Cr)	mg/L	0.00022	0.00010	8977677	<0.00010	0.00010	8977678			
Total Cobalt (Co)	mg/L	0.000211	0.000010	8977677	<0.0000050	0.0000050	8977678			
Total Copper (Cu)	mg/L	0.00115	0.00010	8977677	<0.000050	0.000050	8977678			
Total Iron (Fe)	mg/L	0.242	0.0050	8977677	<0.0010	0.0010	8977678			
Total Lead (Pb)	mg/L	0.000085	0.000020	8977677	<0.0000050	0.0000050	8977678			
Total Lithium (Li)	mg/L	0.00775	0.00050	8977677	<0.00050	0.00050	8977678			
Total Manganese (Mn)	mg/L	0.133	0.00010	8977677	<0.000050	0.000050	8977678			

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate
(1) Recovery or RPD for this parameter is outside control limits. The overall quality control for this analysis meets acceptability criteria.

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RESULTS OF ANALYSES OF WATER

Bureau Veritas ID		XCF505			XCF506			XCF506		
Sampling Date		2023/09/22 09:48			2023/09/22 09:48			2023/09/22 09:48		
COC Number		953308-01-01			953308-01-01			953308-01-01		
	UNITS	**DUP Lab-Dup**	**RDL**	**QC Batch**	**FB**	**RDL**	**QC Batch**	**FB Lab-Dup**	**RDL**	**QC Batch**
Total Molybdenum (Mo)	mg/L	0.000321	0.000050	8977677	<0.000050	0.000050	8977678			
Total Nickel (Ni)	mg/L	0.00105	0.00010	8977677	<0.000020	0.000020	8977678			
Total Phosphorus (P)	mg/L	0.0898	0.0050	8977677						
Total Selenium (Se)	mg/L	0.000119	0.000040	8977677	<0.000040	0.000040	8977678			
Total Silicon (Si)	mg/L	3.24	0.050	8977677	<0.050	0.050	8977678			
Total Silver (Ag)	mg/L	<0.000010	0.000010	8977677	<0.0000050	0.0000050	8977678			
Total Strontium (Sr)	mg/L	0.130	0.000050	8977677	<0.000050	0.000050	8977678			
Total Thallium (Tl)	mg/L	0.0000020	0.0000020	8977677	<0.0000020	0.0000020	8977678			
Total Tin (Sn)	mg/L	<0.00020	0.00020	8977677	<0.00020	0.00020	8977678			
Total Titanium (Ti)	mg/L	0.0034	0.0020	8977677	<0.00050	0.00050	8977678			
Total Uranium (U)	mg/L	0.00112	0.0000050	8977677	<0.0000020	0.0000020	8977678			
Total Vanadium (V)	mg/L	0.00070	0.00020	8977677	<0.00020	0.00020	8977678			
Total Zinc (Zn)	mg/L	0.0016	0.0010	8977677	<0.00010	0.00010	8977678			
Total Zirconium (Zr)	mg/L	0.00018	0.00010	8977677	<0.00010	0.00010	8977678			
Total Calcium (Ca)	mg/L				<0.050	0.050	8963706			
Total Magnesium (Mg)	mg/L				<0.050	0.050	8963706			
Total Potassium (K)	mg/L				<0.050	0.050	8963706			
Total Sodium (Na)	mg/L				<0.050	0.050	8963706			
Total Sulphur (S)	mg/L	5.25	0.60	8977677	<3.0	3.0	8963706			

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate

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ELEMENTS BY ATOMIC SPECTROSCOPY (WATER)

Bureau Veritas ID		XCF499			XCF499			XCF500	XCF501		
Sampling Date		2023/09/22 13:20			2023/09/22 13:20			2023/09/22 12:30	2023/09/22 09:48		
COC Number		953308-01-01			953308-01-01			953308-01-01	953308-01-01		
	UNITS	**LVR**	**RDL**	**QC Batch**	**LVR Lab-Dup**	**RDL**	**QC Batch**	**LVR2**	**PINREXP**	**RDL**	**QC Batch**
Calculated Parameters											
Total Hardness (CaCO3)	mg/L	124	0.50	8961436				146	223	0.50	8961436
Metals											
Dissolved Mercury (Hg)	ug/L	<0.01	0.01	8950677	<0.01	0.01	8950677	<0.01	<0.01	0.01	8950677

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch
Lab-Dup = Laboratory Initiated Duplicate

Bureau Veritas ID		XCF502	XCF503	XCF504	XCF505	XCF506		
Sampling Date		2023/09/22 08:50	2023/09/22 08:35	2023/09/22 11:10	2023/09/22 09:48	2023/09/22 09:48		
COC Number		953308-01-01	953308-01-01	953308-01-01	953308-01-01	953308-01-01		
	UNITS	**PINREXP2**	**PINRREF2**	**STUCREF**	**DUP**	**FB**	**RDL**	**QC Batch**
Calculated Parameters								
Total Hardness (CaCO3)	mg/L	150	146	134	229	<0.50	0.50	8961436
Metals								
Dissolved Mercury (Hg)	ug/L	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	8950677

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

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GENERAL COMMENTS

Each temperature is the average of up to three cooler temperatures taken at receipt

Package 1	3.0°C
Package 2	3.7°C
Package 3	2.3°C

Results relate only to the items tested.

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QUALITY ASSURANCE REPORT

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8946680	Total Cyanide (CN)	2023/09/28	101	80 - 120	105	80 - 120	<0.0050	mg/L	1.5	20		
8946819	Total Organic Carbon	2023/09/28					<500	mg/kg	22	35	98	75 - 125
8947269	Acid Extractable Aluminum (Al)	2023/09/28	NC	75 - 125	99	80 - 120	<50	ug/g				
8947269	Acid Extractable Antimony (Sb)	2023/09/28	104	75 - 125	107	80 - 120	<0.20	ug/g	37 (1)	30		
8947269	Acid Extractable Arsenic (As)	2023/09/28	108	75 - 125	100	80 - 120	<1.0	ug/g	4.8	30		
8947269	Acid Extractable Barium (Ba)	2023/09/28	NC	75 - 125	101	80 - 120	<0.50	ug/g	22	30		
8947269	Acid Extractable Beryllium (Be)	2023/09/28	108	75 - 125	95	80 - 120	<0.20	ug/g	5.2	30		
8947269	Acid Extractable Bismuth (Bi)	2023/09/28	96	75 - 125	90	80 - 120	<1.0	ug/g				
8947269	Acid Extractable Boron (B)	2023/09/28	94	75 - 125	91	80 - 120	<5.0	ug/g	1.3	30		
8947269	Acid Extractable Cadmium (Cd)	2023/09/28	105	75 - 125	97	80 - 120	<0.10	ug/g	15	30		
8947269	Acid Extractable Calcium (Ca)	2023/09/28	NC	75 - 125	108	80 - 120	<50	ug/g				
8947269	Acid Extractable Chromium (Cr)	2023/09/28	106	75 - 125	101	80 - 120	<1.0	ug/g	4.2	30		
8947269	Acid Extractable Cobalt (Co)	2023/09/28	104	75 - 125	102	80 - 120	<0.10	ug/g	4.4	30		
8947269	Acid Extractable Copper (Cu)	2023/09/28	NC	75 - 125	99	80 - 120	<0.50	ug/g	1.6	30		
8947269	Acid Extractable Iron (Fe)	2023/09/28	NC	75 - 125	104	80 - 120	<50	ug/g				
8947269	Acid Extractable Lead (Pb)	2023/09/28	NC	75 - 125	101	80 - 120	<1.0	ug/g	0.53	30		
8947269	Acid Extractable Magnesium (Mg)	2023/09/28	NC	75 - 125	94	80 - 120	<50	ug/g				
8947269	Acid Extractable Manganese (Mn)	2023/09/28	NC	75 - 125	102	80 - 120	<1.0	ug/g				
8947269	Acid Extractable Mercury (Hg)	2023/09/28	112	75 - 125	103	80 - 120	<0.050	ug/g	9.3	30		
8947269	Acid Extractable Molybdenum (Mo)	2023/09/28	110	75 - 125	98	80 - 120	<0.50	ug/g	19	30		
8947269	Acid Extractable Nickel (Ni)	2023/09/28	104	75 - 125	101	80 - 120	<0.50	ug/g	4.0	30		
8947269	Acid Extractable Phosphorus (P)	2023/09/28	NC	75 - 125	102	80 - 120	<50	ug/g				
8947269	Acid Extractable Potassium (K)	2023/09/28	NC	75 - 125	102	80 - 120	<200	ug/g				
8947269	Acid Extractable Selenium (Se)	2023/09/28	105	75 - 125	99	80 - 120	<0.50	ug/g	NC	30		
8947269	Acid Extractable Silver (Ag)	2023/09/28	108	75 - 125	100	80 - 120	<0.20	ug/g	NC	30		
8947269	Acid Extractable Sodium (Na)	2023/09/28	NC	75 - 125	91	80 - 120	<50	ug/g				
8947269	Acid Extractable Strontium (Sr)	2023/09/28	NC	75 - 125	104	80 - 120	<1.0	ug/g				
8947269	Acid Extractable Thallium (Tl)	2023/09/28	100	75 - 125	103	80 - 120	<0.050	ug/g	7.7	30		
8947269	Acid Extractable Tin (Sn)	2023/09/28	106	75 - 125	95	80 - 120	<1.0	ug/g				
8947269	Acid Extractable Uranium (U)	2023/09/28	102	75 - 125	101	80 - 120	<0.050	ug/g	5.5	30		
8947269	Acid Extractable Vanadium (V)	2023/09/28	NC	75 - 125	100	80 - 120	<5.0	ug/g	2.2	30		

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QUALITY ASSURANCE REPORT(CONT'D)

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8947269	Acid Extractable Zinc (Zn)	2023/09/28	NC	75 - 125	101	80 - 120	<5.0	ug/g	1.6	30		
8947549	Total Dissolved Solids	2023/09/29			100	90 - 110	<10	mg/L	0	20		
8947567	Total Suspended Solids	2023/09/29			98	85 - 115	<10	mg/L	NC	20		
8947749	Dissolved Chloride (Cl-)	2023/09/29	NC	80 - 120	91	80 - 120	<1.0	mg/L	4.0	20		
8947766	Dissolved Sulphate (SO4)	2023/09/29	NC	75 - 125	94	80 - 120	<1.0	mg/L	0.26	20		
8948050	Total Organic Carbon	2023/09/29					<500	mg/kg	4.3	35	98	75 - 125
8948135	Total Phosphorus	2023/09/29	NC	80 - 120	102	80 - 120	<0.020	mg/L	1.5	20	114	80 - 120
8948144	Total Phosphorus	2023/09/29	99	80 - 120	100	80 - 120	<0.020	mg/L	NC	20	108	80 - 120
8948359	Total Ammonia-N	2023/09/29	106	75 - 125	100	80 - 120	<0.050	mg/L				
8948457	Nitrate (N)	2023/10/02	105	80 - 120	103	80 - 120	<0.10	mg/L	6.6	20		
8948457	Nitrite (N)	2023/10/02	104	80 - 120	111	80 - 120	<0.010	mg/L	4.2	20		
8948462	Nitrate (N)	2023/10/02	114	80 - 120	91	80 - 120	<0.10	mg/L	NC	20		
8948462	Nitrite (N)	2023/10/02	125 (1)	80 - 120	111	80 - 120	<0.010	mg/L	NC	20		
8948728	pH	2023/09/30			102	98 - 103			1.3	N/A		
8948786	Alkalinity (Total as CaCO3)	2023/09/30			97	85 - 115	<1.0	mg/L	0.96	20		
8949151	Dissolved Chloride (Cl-)	2023/09/29	NC	80 - 120	92	80 - 120	<1.0	mg/L	0.80	20		
8949155	Dissolved Sulphate (SO4)	2023/09/29	NC	75 - 125	95	80 - 120	<1.0	mg/L	1.5	20		
8949624	Acid Extractable Aluminum (Al)	2023/09/29	NC	75 - 125	101	80 - 120	<50	ug/g	7.6	30		
8949624	Acid Extractable Antimony (Sb)	2023/09/29	88	75 - 125	101	80 - 120	<0.20	ug/g	8.9	30		
8949624	Acid Extractable Arsenic (As)	2023/09/29	91	75 - 125	97	80 - 120	<1.0	ug/g	1.0	30		
8949624	Acid Extractable Barium (Ba)	2023/09/29	NC	75 - 125	97	80 - 120	<0.50	ug/g	6.6	30		
8949624	Acid Extractable Beryllium (Be)	2023/09/29	84	75 - 125	95	80 - 120	<0.20	ug/g	14	30		
8949624	Acid Extractable Bismuth (Bi)	2023/09/29	90	75 - 125	96	80 - 120	<1.0	ug/g	NC	30		
8949624	Acid Extractable Boron (B)	2023/09/29	74 (2)	75 - 125	93	80 - 120	<5.0	ug/g	8.1	30		
8949624	Acid Extractable Cadmium (Cd)	2023/09/29	93	75 - 125	97	80 - 120	<0.10	ug/g	4.2	30		
8949624	Acid Extractable Calcium (Ca)	2023/09/29	NC	75 - 125	107	80 - 120	<50	ug/g	2.8	30		
8949624	Acid Extractable Chromium (Cr)	2023/09/29	82	75 - 125	92	80 - 120	<1.0	ug/g	5.1	30		
8949624	Acid Extractable Cobalt (Co)	2023/09/29	86	75 - 125	96	80 - 120	<0.10	ug/g	6.0	30		
8949624	Acid Extractable Copper (Cu)	2023/09/29	82	75 - 125	97	80 - 120	<0.50	ug/g	6.8	30		
8949624	Acid Extractable Iron (Fe)	2023/09/29	NC	75 - 125	104	80 - 120	<50	ug/g	4.7	30		
8949624	Acid Extractable Lead (Pb)	2023/09/29	88	75 - 125	96	80 - 120	<1.0	ug/g	3.7	30		

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QUALITY ASSURANCE REPORT(CONT'D)

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8949624	Acid Extractable Magnesium (Mg)	2023/09/29	NC	75 - 125	100	80 - 120	<50	ug/g	5.0	30		
8949624	Acid Extractable Manganese (Mn)	2023/09/29	NC	75 - 125	94	80 - 120	<1.0	ug/g	5.6	30		
8949624	Acid Extractable Mercury (Hg)	2023/09/29	97	75 - 125	99	80 - 120	<0.050	ug/g	NC	30		
8949624	Acid Extractable Molybdenum (Mo)	2023/09/29	91	75 - 125	96	80 - 120	<0.50	ug/g	NC	30		
8949624	Acid Extractable Nickel (Ni)	2023/09/29	81	75 - 125	95	80 - 120	<0.50	ug/g	5.4	30		
8949624	Acid Extractable Phosphorus (P)	2023/09/29	79	75 - 125	97	80 - 120	<50	ug/g	7.4	30		
8949624	Acid Extractable Potassium (K)	2023/09/29	NC	75 - 125	102	80 - 120	<200	ug/g	6.6	30		
8949624	Acid Extractable Selenium (Se)	2023/09/29	91	75 - 125	98	80 - 120	<0.50	ug/g	NC	30		
8949624	Acid Extractable Silver (Ag)	2023/09/29	96	75 - 125	102	80 - 120	<0.20	ug/g	NC	30		
8949624	Acid Extractable Sodium (Na)	2023/09/29	84	75 - 125	107	80 - 120	<50	ug/g	2.6	30		
8949624	Acid Extractable Strontium (Sr)	2023/09/29	NC	75 - 125	95	80 - 120	<1.0	ug/g	2.6	30		
8949624	Acid Extractable Thallium (Tl)	2023/09/29	93	75 - 125	98	80 - 120	<0.050	ug/g	9.6	30		
8949624	Acid Extractable Tin (Sn)	2023/09/29	94	75 - 125	87	80 - 120	<1.0	ug/g	NC	30		
8949624	Acid Extractable Uranium (U)	2023/09/29	93	75 - 125	97	80 - 120	<0.050	ug/g	8.6	30		
8949624	Acid Extractable Vanadium (V)	2023/09/29	NC	75 - 125	94	80 - 120	<5.0	ug/g	5.1	30		
8949624	Acid Extractable Zinc (Zn)	2023/09/29	NC	75 - 125	97	80 - 120	<5.0	ug/g	1.4	30		
8949648	Acid Extractable Aluminum (Al)	2023/09/29	NC	75 - 125	98	80 - 120	<50	ug/g				
8949648	Acid Extractable Antimony (Sb)	2023/09/29	90	75 - 125	101	80 - 120	<0.20	ug/g	NC	30		
8949648	Acid Extractable Arsenic (As)	2023/09/29	90	75 - 125	99	80 - 120	<1.0	ug/g	6.8	30		
8949648	Acid Extractable Barium (Ba)	2023/09/29	NC	75 - 125	98	80 - 120	<0.50	ug/g	6.3	30		
8949648	Acid Extractable Beryllium (Be)	2023/09/29	86	75 - 125	92	80 - 120	<0.20	ug/g	2.5	30		
8949648	Acid Extractable Bismuth (Bi)	2023/09/29	90	75 - 125	98	80 - 120	<1.0	ug/g				
8949648	Acid Extractable Boron (B)	2023/09/29	79	75 - 125	88	80 - 120	<5.0	ug/g	NC	30		
8949648	Acid Extractable Cadmium (Cd)	2023/09/29	92	75 - 125	98	80 - 120	<0.10	ug/g	11	30		
8949648	Acid Extractable Calcium (Ca)	2023/09/29	NC	75 - 125	111	80 - 120	<50	ug/g				
8949648	Acid Extractable Chromium (Cr)	2023/09/29	85	75 - 125	92	80 - 120	<1.0	ug/g	5.8	30		
8949648	Acid Extractable Cobalt (Co)	2023/09/29	87	75 - 125	96	80 - 120	<0.10	ug/g	7.9	30		
8949648	Acid Extractable Copper (Cu)	2023/09/29	84	75 - 125	98	80 - 120	<0.50	ug/g	5.2	30		
8949648	Acid Extractable Iron (Fe)	2023/09/29	NC	75 - 125	105	80 - 120	<50	ug/g				
8949648	Acid Extractable Lead (Pb)	2023/09/29	88	75 - 125	98	80 - 120	<1.0	ug/g	3.7	30		
8949648	Acid Extractable Magnesium (Mg)	2023/09/29	NC	75 - 125	95	80 - 120	<50	ug/g				

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			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8949648	Acid Extractable Manganese (Mn)	2023/09/29	NC	75 - 125	95	80 - 120	<1.0	ug/g				
8949648	Acid Extractable Mercury (Hg)	2023/09/29	109	75 - 125	99	80 - 120	<0.050	ug/g				
8949648	Acid Extractable Molybdenum (Mo)	2023/09/29	91	75 - 125	98	80 - 120	<0.50	ug/g	NC	30		
8949648	Acid Extractable Nickel (Ni)	2023/09/29	86	75 - 125	96	80 - 120	<0.50	ug/g	6.7	30		
8949648	Acid Extractable Phosphorus (P)	2023/09/29	90	75 - 125	96	80 - 120	<50	ug/g				
8949648	Acid Extractable Potassium (K)	2023/09/29	NC	75 - 125	97	80 - 120	<200	ug/g				
8949648	Acid Extractable Selenium (Se)	2023/09/29	91	75 - 125	100	80 - 120	<0.50	ug/g	NC	30		
8949648	Acid Extractable Silver (Ag)	2023/09/29	111	75 - 125	102	80 - 120	<0.20	ug/g	NC	30		
8949648	Acid Extractable Sodium (Na)	2023/09/29	99	75 - 125	106	80 - 120	<50	ug/g				
8949648	Acid Extractable Strontium (Sr)	2023/09/29	NC	75 - 125	97	80 - 120	<1.0	ug/g				
8949648	Acid Extractable Thallium (Tl)	2023/09/29	93	75 - 125	100	80 - 120	<0.050	ug/g	1.2	30		
8949648	Acid Extractable Tin (Sn)	2023/09/29	95	75 - 125	90	80 - 120	<1.0	ug/g				
8949648	Acid Extractable Uranium (U)	2023/09/29	92	75 - 125	99	80 - 120	<0.050	ug/g	12	30		
8949648	Acid Extractable Vanadium (V)	2023/09/29	NC	75 - 125	94	80 - 120	<5.0	ug/g	10	30		
8949648	Acid Extractable Zinc (Zn)	2023/09/29	NC	75 - 125	99	80 - 120	<5.0	ug/g	10	30		
8949827	Acid Extractable Aluminum (Al)	2023/09/30	NC	75 - 125	103	80 - 120	<50	ug/g				
8949827	Acid Extractable Antimony (Sb)	2023/09/30	117	75 - 125	109	80 - 120	<0.20	ug/g	NC	30		
8949827	Acid Extractable Arsenic (As)	2023/09/30	106	75 - 125	97	80 - 120	<1.0	ug/g	4.6	30		
8949827	Acid Extractable Barium (Ba)	2023/09/30	109	75 - 125	100	80 - 120	<0.50	ug/g	4.8	30		
8949827	Acid Extractable Beryllium (Be)	2023/09/30	102	75 - 125	97	80 - 120	<0.20	ug/g	NC	30		
8949827	Acid Extractable Bismuth (Bi)	2023/09/30	91	75 - 125	83	80 - 120	<1.0	ug/g				
8949827	Acid Extractable Boron (B)	2023/09/30	99	75 - 125	89	80 - 120	<5.0	ug/g	NC	30		
8949827	Acid Extractable Cadmium (Cd)	2023/09/30	104	75 - 125	96	80 - 120	<0.10	ug/g	NC	30		
8949827	Acid Extractable Calcium (Ca)	2023/09/30	NC	75 - 125	111	80 - 120	<50	ug/g				
8949827	Acid Extractable Chromium (Cr)	2023/09/30	105	75 - 125	96	80 - 120	<1.0	ug/g	5.0	30		
8949827	Acid Extractable Cobalt (Co)	2023/09/30	100	75 - 125	93	80 - 120	<0.10	ug/g	1.9	30		
8949827	Acid Extractable Copper (Cu)	2023/09/30	103	75 - 125	99	80 - 120	<0.50	ug/g	0.16	30		
8949827	Acid Extractable Iron (Fe)	2023/09/30	NC	75 - 125	101	80 - 120	<50	ug/g				
8949827	Acid Extractable Lead (Pb)	2023/09/30	98	75 - 125	93	80 - 120	<1.0	ug/g	9.2	30		
8949827	Acid Extractable Magnesium (Mg)	2023/09/30	NC	75 - 125	95	80 - 120	<50	ug/g				
8949827	Acid Extractable Manganese (Mn)	2023/09/30	NC	75 - 125	96	80 - 120	<1.0	ug/g				

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QUALITY ASSURANCE REPORT(CONT'D)

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8949827	Acid Extractable Mercury (Hg)	2023/09/30	98	75 - 125	95	80 - 120	<0.050	ug/g				
8949827	Acid Extractable Molybdenum (Mo)	2023/09/30	108	75 - 125	97	80 - 120	<0.50	ug/g	12	30		
8949827	Acid Extractable Nickel (Ni)	2023/09/30	97	75 - 125	93	80 - 120	<0.50	ug/g	6.3	30		
8949827	Acid Extractable Phosphorus (P)	2023/09/30	120	75 - 125	100	80 - 120	<50	ug/g				
8949827	Acid Extractable Potassium (K)	2023/09/30	NC	75 - 125	104	80 - 120	<200	ug/g				
8949827	Acid Extractable Selenium (Se)	2023/09/30	104	75 - 125	98	80 - 120	<0.50	ug/g	NC	30		
8949827	Acid Extractable Silver (Ag)	2023/09/30	104	75 - 125	96	80 - 120	<0.20	ug/g	NC	30		
8949827	Acid Extractable Sodium (Na)	2023/09/30	NC	75 - 125	94	80 - 120	<50	ug/g				
8949827	Acid Extractable Strontium (Sr)	2023/09/30	NC	75 - 125	96	80 - 120	<1.0	ug/g				
8949827	Acid Extractable Thallium (Tl)	2023/09/30	99	75 - 125	93	80 - 120	<0.050	ug/g	8.9	30		
8949827	Acid Extractable Tin (Sn)	2023/09/30	107	75 - 125	96	80 - 120	<1.0	ug/g				
8949827	Acid Extractable Uranium (U)	2023/09/30	97	75 - 125	90	80 - 120	<0.050	ug/g	1.3	30		
8949827	Acid Extractable Vanadium (V)	2023/09/30	111	75 - 125	95	80 - 120	<5.0	ug/g	17	30		
8949827	Acid Extractable Zinc (Zn)	2023/09/30	99	75 - 125	92	80 - 120	<5.0	ug/g	2.8	30		
8949868	Acid Extractable Aluminum (Al)	2023/09/30	NC	75 - 125	104	80 - 120	<50	ug/g				
8949868	Acid Extractable Antimony (Sb)	2023/09/30	96	75 - 125	101	80 - 120	<0.20	ug/g	5.9	30		
8949868	Acid Extractable Arsenic (As)	2023/09/30	99	75 - 125	100	80 - 120	<1.0	ug/g	1.6	30		
8949868	Acid Extractable Barium (Ba)	2023/09/30	NC	75 - 125	100	80 - 120	<0.50	ug/g	4.5	30		
8949868	Acid Extractable Beryllium (Be)	2023/09/30	97	75 - 125	99	80 - 120	<0.20	ug/g	5.1	30		
8949868	Acid Extractable Bismuth (Bi)	2023/09/30	95	75 - 125	99	80 - 120	<1.0	ug/g				
8949868	Acid Extractable Boron (B)	2023/09/30	92	75 - 125	101	80 - 120	<5.0	ug/g	3.2	30		
8949868	Acid Extractable Cadmium (Cd)	2023/09/30	98	75 - 125	98	80 - 120	<0.10	ug/g	5.7	30		
8949868	Acid Extractable Calcium (Ca)	2023/09/30	NC	75 - 125	111	80 - 120	<50	ug/g				
8949868	Acid Extractable Chromium (Cr)	2023/09/30	98	75 - 125	99	80 - 120	<1.0	ug/g	1.5	30		
8949868	Acid Extractable Cobalt (Co)	2023/09/30	98	75 - 125	102	80 - 120	<0.10	ug/g	0.73	30		
8949868	Acid Extractable Copper (Cu)	2023/09/30	NC	75 - 125	102	80 - 120	<0.50	ug/g	5.5	30		
8949868	Acid Extractable Iron (Fe)	2023/09/30	NC	75 - 125	104	80 - 120	<50	ug/g				
8949868	Acid Extractable Lead (Pb)	2023/09/30	94	75 - 125	99	80 - 120	<1.0	ug/g	3.4	30		
8949868	Acid Extractable Magnesium (Mg)	2023/09/30	NC	75 - 125	102	80 - 120	<50	ug/g				
8949868	Acid Extractable Manganese (Mn)	2023/09/30	NC	75 - 125	101	80 - 120	<1.0	ug/g				
8949868	Acid Extractable Mercury (Hg)	2023/09/30	99	75 - 125	103	80 - 120	<0.050	ug/g	2.2	30		

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			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8949868	Acid Extractable Molybdenum (Mo)	2023/09/30	98	75 - 125	99	80 - 120	<0.50	ug/g	NC	30		
8949868	Acid Extractable Nickel (Ni)	2023/09/30	96	75 - 125	103	80 - 120	<0.50	ug/g	2.1	30		
8949868	Acid Extractable Phosphorus (P)	2023/09/30	NC	75 - 125	99	80 - 120	<50	ug/g				
8949868	Acid Extractable Potassium (K)	2023/09/30	NC	75 - 125	102	80 - 120	<200	ug/g				
8949868	Acid Extractable Selenium (Se)	2023/09/30	97	75 - 125	101	80 - 120	<0.50	ug/g	NC	30		
8949868	Acid Extractable Silver (Ag)	2023/09/30	102	75 - 125	104	80 - 120	<0.20	ug/g	NC	30		
8949868	Acid Extractable Sodium (Na)	2023/09/30	103	75 - 125	102	80 - 120	<50	ug/g				
8949868	Acid Extractable Strontium (Sr)	2023/09/30	NC	75 - 125	101	80 - 120	<1.0	ug/g				
8949868	Acid Extractable Thallium (Tl)	2023/09/30	97	75 - 125	99	80 - 120	<0.050	ug/g	3.6	30		
8949868	Acid Extractable Tin (Sn)	2023/09/30	101	75 - 125	92	80 - 120	<1.0	ug/g				
8949868	Acid Extractable Uranium (U)	2023/09/30	97	75 - 125	98	80 - 120	<0.050	ug/g	7.9	30		
8949868	Acid Extractable Vanadium (V)	2023/09/30	NC	75 - 125	102	80 - 120	<5.0	ug/g	0.85	30		
8949868	Acid Extractable Zinc (Zn)	2023/09/30	NC	75 - 125	100	80 - 120	<5.0	ug/g	2.1	30		
8950272	Acid Extractable Aluminum (Al)	2023/09/30	NC	75 - 125	104	80 - 120	<50	ug/g				
8950272	Acid Extractable Antimony (Sb)	2023/09/30	102	75 - 125	102	80 - 120	<0.20	ug/g				
8950272	Acid Extractable Arsenic (As)	2023/09/30	100	75 - 125	101	80 - 120	<1.0	ug/g	0.74	30		
8950272	Acid Extractable Barium (Ba)	2023/09/30	NC	75 - 125	100	80 - 120	<0.50	ug/g				
8950272	Acid Extractable Beryllium (Be)	2023/09/30	104	75 - 125	101	80 - 120	<0.20	ug/g				
8950272	Acid Extractable Bismuth (Bi)	2023/09/30	100	75 - 125	102	80 - 120	<1.0	ug/g				
8950272	Acid Extractable Boron (B)	2023/09/30	102	75 - 125	103	80 - 120	<5.0	ug/g				
8950272	Acid Extractable Cadmium (Cd)	2023/09/30	101	75 - 125	101	80 - 120	<0.10	ug/g				
8950272	Acid Extractable Calcium (Ca)	2023/09/30	NC	75 - 125	114	80 - 120	<50	ug/g				
8950272	Acid Extractable Chromium (Cr)	2023/09/30	100	75 - 125	101	80 - 120	<1.0	ug/g				
8950272	Acid Extractable Cobalt (Co)	2023/09/30	102	75 - 125	103	80 - 120	<0.10	ug/g				
8950272	Acid Extractable Copper (Cu)	2023/09/30	107	75 - 125	103	80 - 120	<0.50	ug/g				
8950272	Acid Extractable Iron (Fe)	2023/09/30	NC	75 - 125	104	80 - 120	<50	ug/g				
8950272	Acid Extractable Lead (Pb)	2023/09/30	NC	75 - 125	102	80 - 120	<1.0	ug/g				
8950272	Acid Extractable Magnesium (Mg)	2023/09/30	NC	75 - 125	103	80 - 120	<50	ug/g				
8950272	Acid Extractable Manganese (Mn)	2023/09/30	NC	75 - 125	103	80 - 120	<1.0	ug/g				
8950272	Acid Extractable Mercury (Hg)	2023/09/30	105	75 - 125	104	80 - 120	<0.050	ug/g				
8950272	Acid Extractable Molybdenum (Mo)	2023/09/30	103	75 - 125	98	80 - 120	<0.50	ug/g				

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QUALITY ASSURANCE REPORT(CONT'D)

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8950272	Acid Extractable Nickel (Ni)	2023/09/30	100	75 - 125	103	80 - 120	<0.50	ug/g				
8950272	Acid Extractable Phosphorus (P)	2023/09/30	NC	75 - 125	101	80 - 120	<50	ug/g				
8950272	Acid Extractable Potassium (K)	2023/09/30	NC	75 - 125	103	80 - 120	<200	ug/g				
8950272	Acid Extractable Selenium (Se)	2023/09/30	104	75 - 125	105	80 - 120	<0.50	ug/g				
8950272	Acid Extractable Silver (Ag)	2023/09/30	106	75 - 125	106	80 - 120	<0.20	ug/g				
8950272	Acid Extractable Sodium (Na)	2023/09/30	103	75 - 125	108	80 - 120	<50	ug/g				
8950272	Acid Extractable Strontium (Sr)	2023/09/30	NC	75 - 125	99	80 - 120	<1.0	ug/g				
8950272	Acid Extractable Thallium (Tl)	2023/09/30	102	75 - 125	103	80 - 120	<0.050	ug/g				
8950272	Acid Extractable Tin (Sn)	2023/09/30	103	75 - 125	91	80 - 120	<1.0	ug/g				
8950272	Acid Extractable Uranium (U)	2023/09/30	101	75 - 125	100	80 - 120	<0.050	ug/g	0.33	30		
8950272	Acid Extractable Vanadium (V)	2023/09/30	101	75 - 125	102	80 - 120	<5.0	ug/g				
8950272	Acid Extractable Zinc (Zn)	2023/09/30	NC	75 - 125	101	80 - 120	<5.0	ug/g				
8950677	Dissolved Mercury (Hg)	2023/09/29	108	75 - 125	104	80 - 120	<0.01	ug/L	NC	20		
8952581	Total Organic Carbon	2023/10/02					<500	mg/kg	19	35	101	75 - 125
8954798	Alkalinity (Total as CaCO3)	2023/10/02			97	85 - 115	<1.0	mg/L	1.0	20		
8964978	Acidity	2023/10/06	104	80 - 120	102	80 - 120	<5.0	mg/L	NC	25		
8977677	Total Aluminum (Al)	2023/10/12	137 (3)	80 - 120	102	80 - 120	<0.0030	mg/L	26 (1)	20		
8977677	Total Antimony (Sb)	2023/10/12	104	80 - 120	104	80 - 120	<0.000020	mg/L	11	20		
8977677	Total Arsenic (As)	2023/10/12	106	80 - 120	103	80 - 120	<0.000020	mg/L	6.0	20		
8977677	Total Barium (Ba)	2023/10/12	106	80 - 120	103	80 - 120	<0.000050	mg/L	3.3	20		
8977677	Total Beryllium (Be)	2023/10/12	99	80 - 120	97	80 - 120	<0.000010	mg/L	NC	20		
8977677	Total Bismuth (Bi)	2023/10/12	97	80 - 120	100	80 - 120	<0.000010	mg/L	NC	20		
8977677	Total Boron (B)	2023/10/12	96	80 - 120	94	80 - 120	<0.010	mg/L	8.0	20		
8977677	Total Cadmium (Cd)	2023/10/12	103	80 - 120	102	80 - 120	<0.0000050	mg/L	0	20		
8977677	Total Chromium (Cr)	2023/10/12	102	80 - 120	101	80 - 120	<0.00010	mg/L	NC	20		
8977677	Total Cobalt (Co)	2023/10/12	101	80 - 120	101	80 - 120	<0.000010	mg/L	0.47	20		
8977677	Total Copper (Cu)	2023/10/12	98	80 - 120	99	80 - 120	<0.00010	mg/L	2.7	20		
8977677	Total Iron (Fe)	2023/10/12	NC	80 - 120	101	80 - 120	<0.0050	mg/L	2.8	20		
8977677	Total Lead (Pb)	2023/10/12	101	80 - 120	101	80 - 120	<0.000020	mg/L	0	20		
8977677	Total Lithium (Li)	2023/10/12	95	80 - 120	93	80 - 120	<0.00050	mg/L	4.2	20		
8977677	Total Manganese (Mn)	2023/10/12	NC	80 - 120	99	80 - 120	<0.00010	mg/L	4.0	20		

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QUALITY ASSURANCE REPORT(CONT'D)

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8977677	Total Molybdenum (Mo)	2023/10/12	107	80 - 120	103	80 - 120	<0.000050	mg/L	3.7	20		
8977677	Total Nickel (Ni)	2023/10/12	99	80 - 120	100	80 - 120	<0.00010	mg/L	5.5	20		
8977677	Total Phosphorus (P)	2023/10/12	107	80 - 120	103	80 - 120	<0.0050	mg/L	1.2	20		
8977677	Total Selenium (Se)	2023/10/12	106	80 - 120	103	80 - 120	<0.000040	mg/L	1.7	20		
8977677	Total Silicon (Si)	2023/10/12	120	80 - 120	107	80 - 120	<0.050	mg/L	2.6	20		
8977677	Total Silver (Ag)	2023/10/12	103	80 - 120	100	80 - 120	<0.000010	mg/L	NC	20		
8977677	Total Strontium (Sr)	2023/10/12	NC	80 - 120	101	80 - 120	<0.000050	mg/L	4.8	20		
8977677	Total Sulphur (S)	2023/10/12	112	80 - 120	106	80 - 120	<0.60	mg/L	3.5	20		
8977677	Total Thallium (Tl)	2023/10/12	101	80 - 120	101	80 - 120	<0.0000020	mg/L	0	20		
8977677	Total Tin (Sn)	2023/10/12	98	80 - 120	99	80 - 120	<0.00020	mg/L	NC	20		
8977677	Total Titanium (Ti)	2023/10/12	112	80 - 120	103	80 - 120	<0.0020	mg/L	18	20		
8977677	Total Uranium (U)	2023/10/12	105	80 - 120	102	80 - 120	<0.0000050	mg/L	0.80	20		
8977677	Total Vanadium (V)	2023/10/12	104	80 - 120	100	80 - 120	<0.00020	mg/L	5.7	20		
8977677	Total Zinc (Zn)	2023/10/12	100	80 - 120	101	80 - 120	<0.0010	mg/L	3.7	20		
8977677	Total Zirconium (Zr)	2023/10/12	105	80 - 120	99	80 - 120	<0.00010	mg/L	8.2	20		
8977678	Total Aluminum (Al)	2023/10/05	100	80 - 120	98	80 - 120	<0.00050	mg/L				
8977678	Total Antimony (Sb)	2023/10/05	103	80 - 120	101	80 - 120	<0.000020	mg/L				
8977678	Total Arsenic (As)	2023/10/05	104	80 - 120	100	80 - 120	<0.000020	mg/L				
8977678	Total Barium (Ba)	2023/10/05	99	80 - 120	99	80 - 120	<0.000020	mg/L				
8977678	Total Beryllium (Be)	2023/10/05	100	80 - 120	101	80 - 120	<0.000010	mg/L				
8977678	Total Bismuth (Bi)	2023/10/05	99	80 - 120	97	80 - 120	<0.0000050	mg/L				
8977678	Total Boron (B)	2023/10/05	101	80 - 120	101	80 - 120	<0.010	mg/L				
8977678	Total Cadmium (Cd)	2023/10/05	102	80 - 120	99	80 - 120	<0.0000050	mg/L				
8977678	Total Chromium (Cr)	2023/10/05	96	80 - 120	94	80 - 120	<0.00010	mg/L				
8977678	Total Cobalt (Co)	2023/10/05	97	80 - 120	95	80 - 120	<0.0000050	mg/L				
8977678	Total Copper (Cu)	2023/10/05	96	80 - 120	95	80 - 120	<0.000050	mg/L				
8977678	Total Iron (Fe)	2023/10/05	99	80 - 120	100	80 - 120	<0.0010	mg/L				
8977678	Total Lead (Pb)	2023/10/05	100	80 - 120	97	80 - 120	<0.0000050	mg/L				
8977678	Total Lithium (Li)	2023/10/05	97	80 - 120	100	80 - 120	<0.00050	mg/L				
8977678	Total Manganese (Mn)	2023/10/05	NC	80 - 120	93	80 - 120	<0.000050	mg/L				
8977678	Total Molybdenum (Mo)	2023/10/05	105	80 - 120	101	80 - 120	<0.000050	mg/L				

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QUALITY ASSURANCE REPORT(CONT'D)

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8977678	Total Nickel (Ni)	2023/10/05	98	80 - 120	98	80 - 120	<0.000020	mg/L				
8977678	Total Selenium (Se)	2023/10/05	104	80 - 120	100	80 - 120	<0.000040	mg/L				
8977678	Total Silicon (Si)	2023/10/05	NC	80 - 120	103	80 - 120	<0.050	mg/L				
8977678	Total Silver (Ag)	2023/10/05	102	80 - 120	98	80 - 120	<0.0000050	mg/L				
8977678	Total Strontium (Sr)	2023/10/05	NC	80 - 120	93	80 - 120	<0.000050	mg/L				
8977678	Total Sulphur (S)	2023/10/05	102	80 - 120	98	80 - 120	<0.60	mg/L				
8977678	Total Thallium (Tl)	2023/10/05	101	80 - 120	99	80 - 120	<0.0000020	mg/L				
8977678	Total Tin (Sn)	2023/10/05	100	80 - 120	99	80 - 120	<0.00020	mg/L				
8977678	Total Titanium (Ti)	2023/10/05	101	80 - 120	99	80 - 120	<0.00050	mg/L				
8977678	Total Uranium (U)	2023/10/05	106	80 - 120	102	80 - 120	0.0000020, RDL=0.0000020 (4)	mg/L				
8977678	Total Vanadium (V)	2023/10/05	98	80 - 120	95	80 - 120	<0.00020	mg/L				
8977678	Total Zinc (Zn)	2023/10/05	102	80 - 120	106	80 - 120	<0.00010	mg/L				
8977678	Total Zirconium (Zr)	2023/10/05	102	80 - 120	97	80 - 120	<0.00010	mg/L				
8983203	Clay	2023/10/24							1.2	35		
8983203	Gravel	2023/10/24							77 (5)	35		
8983203	Sand	2023/10/24							11	35		
8983203	Silt	2023/10/24							2.6	35		
8988102	Radium-226	2023/10/19			94	85 - 115	<0.010	Bq/L	NC	N/A		
8992276	Clay	2023/10/25							4.0	35		
8992276	Gravel	2023/10/25							NC	35		
8992276	Sand	2023/10/25							0.56	35		

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QUALITY ASSURANCE REPORT(CONT'D)

			Matrix Spike		SPIKED BLANK		Method Blank		RPD		QC Standard	
QC Batch	Parameter	Date	% Recovery	QC Limits	% Recovery	QC Limits	Value	UNITS	Value (%)	QC Limits	% Recovery	QC Limits
8992276	Silt	2023/10/25							2.9	35		

N/A = Not Applicable

Duplicate: Paired analysis of a separate portion of the same sample. Used to evaluate the variance in the measurement.

Matrix Spike: A sample to which a known amount of the analyte of interest has been added. Used to evaluate sample matrix interference.

QC Standard: A sample of known concentration prepared by an external agency under stringent conditions. Used as an independent check of method accuracy.

Spiked Blank: A blank matrix sample to which a known amount of the analyte, usually from a second source, has been added. Used to evaluate method accuracy.

Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

NC (Matrix Spike): The recovery in the matrix spike was not calculated. The relative difference between the concentration in the parent sample and the spike amount was too small to permit a reliable recovery calculation (matrix spike concentration was less than the native sample concentration)

NC (Duplicate RPD): The duplicate RPD was not calculated. The concentration in the sample and/or duplicate was too low to permit a reliable RPD calculation (absolute difference <= 2x RDL).

(1) Recovery or RPD for this parameter is outside control limits. The overall quality control for this analysis meets acceptability criteria.

(2) Matrix Spike exceeds acceptance limits, probable matrix interference

(3) Matrix spike exceeds acceptance limits due to matrix interference.

(4) Method blank exceeds acceptance limits- 2X RDL acceptable for low level metals determination.

(5) %RPD acceptable. Duplicate values agree within 10% absolute.

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VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by:

Anastassia Hamanov, Scientific Specialist

Bryon Angevine, Senior Analyst

Colby Coutu, Project Manager

David Huang, BBY Scientific Specialist

Janah Rhyno, Scientific Specialist

Steven Simpson, BSc.,MBA,C.Chem,Miss.-Kitimat, Lab Director

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25-Sep-23 09:19
Colby Coutu
C3T6649
RJM ENV-1634

IMMEDIATE

INVOICE TO:		REPORT TO:		PROJECT INFORMATION:		Laboratory Use Only:	
Company Name:	#12046 EcoMetrix Incorporated	Company Name:	EcoMetrix	Quotation #:	C25356	Bottle Order #:	953308
Attention:	Accounts Payable	Attention:	Joseph Tetreault	P.O. #:			
Address:	6800 Campobello Rd, Mississauga ON L5N 2L8	Address:		Project:	22-3093	COC #:	C#953308-01-01
Tel:	(905) 794-2325 Fax: (905) 794-2338	Tel:	(905) 794-2325 Ext: 215 Fax:	Project Name:	Phase 3 EEM	Project Manager:	Colby Coutu
Email:	accountspayable@ecometrix.ca	Email:	jtetreault@ecometrix.ca	Site #:	NewGold Rainy River		
				Sampled By:			

MOE REGULATED DRINKING WATER OR WATER INTENDED FOR HUMAN CONSUMPTION MUST BE SUBMITTED ON THE BUREAU VERITAS DRINKING WATER CHAIN OF CUSTODY

Regulation 153 (2011): ☐ Table 1 ☐ Res/Park ☐ Medium/Fine ☐ Table 2 ☐ Ind/Comm ☐ Coarse ☐ Table 3 ☐ Agri/Other ☐ For RSC ☐ Table ___

Other Regulations: ☐ CCME ☐ Sanitary Sewer Bylaw ☐ Reg 558 ☐ Storm Sewer Bylaw ☐ MISA Municipality ___ ☐ PWQO ☐ Reg 406 Table ___ ☐ Other

Special Instructions:

Include Criteria on Certificate of Analysis (Y/N)? ___

Turnaround Time (TAT) Required: Please provide advance notice for rush projects

Regular (Standard) TAT: ☒
(will be applied if Rush TAT is not specified):
Standard TAT = 5-7 Working days for most tests.
Please note: Standard TAT for certain tests such as BOD and Dioxins/Furans are > 5 days - contact your Project Manager for details.

Job Specific Rush TAT (if applies to entire submission)
Date Required: ___ Time Required: ___
Rush Confirmation Number: ___ *(call lab for #)*

ANALYSIS REQUESTED (PLEASE BE SPECIFIC)

#	Sample Barcode Label	Sample (Location) Identification	Date Sampled	Time Sampled	Matrix	Field Filtered (please circle): Metals / Hg / Cr VI	Acidity, Alkalinity and pH	Un-ionized Ammonia (incl. Total Ammonia)	Chloride, Sulphate, Nitrate and Nitrite	Low Level Dissolved Mercury	Hardness (calculated as CaCO3)	Total Cyanide	Total Phosphorus (Colourimetric)	Radium-226 by Alpha Spectrometry	Total Dissolved and Suspended Solids	Low Level Total ICPMS Metals	# of Bottles	Comments
1		LVR	22-Sep-23	13:20	Water		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	pH 7.58 temp 15.64
2		LVR2	↓	1230	↓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	pH 7.39 temp 15.09
3		PINREXP	↓	0948	↓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	pH 7.53 temp 14.88
4		PINREXP2	↓	0850	↓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	pH 7.49 temp 14.67
5		PINRREF2	↓	0835	↓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	pH 7.28 temp 14.77
6		STUCREF	↓	1110	↓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	pH 7.55 temp 15.56
7		DUP	↓	0948	↓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	9	pH 7.53 temp 14.88
8		FB	↓	0948	↓		✓	✓	✓	✓	✓	✓	✓	~~✓~~	✓	✓	8	
9																		Rec'd In Thunder Bay
10																		Custody Seal Present Intact (no) Cooling Media (Yes) No

RELINQUISHED BY: (Signature/Print)	Date: (YY/MM/DD)	Time	RECEIVED BY: (Signature/Print)	Date: (YY/MM/DD)	Time	# jars used and not submitted
[signature] / J TETREAULT	23/09/23	13:50	[signature] Therese Bramwell	2023/09/25	09:19	
			[signature] RAVNEET KAUR	23/09/26	09:31	

Laboratory Use Only				
Time Sensitive	Temperature (°C) on Recei	Custody Seal	Yes	No
	① 3,3,3 ② 4,4,3 ③ 2,3,2	Present	✓	
		Intact	✓	

* UNLESS OTHERWISE AGREED TO IN WRITING, WORK SUBMITTED ON THIS CHAIN OF CUSTODY IS SUBJECT TO BUREAU VERITAS'S STANDARD TERMS AND CONDITIONS. SIGNING OF THIS CHAIN OF CUSTODY DOCUMENT IS ACKNOWLEDGMENT AND ACCEPTANCE OF OUR TERMS WHICH ARE AVAILABLE FOR VIEWING AT WWW.BVNA.COM/ENVIRONMENTAL-LABORATORIES/RESOURCES/COC-TERMS-AND-CONDITIONS.

* IT IS THE RESPONSIBILITY OF THE RELINQUISHER TO ENSURE THE ACCURACY OF THE CHAIN OF CUSTODY RECORD. AN INCOMPLETE CHAIN OF CUSTODY MAY RESULT IN ANALYTICAL TAT DELAYS.

** SAMPLE CONTAINER, PRESERVATION, HOLD TIME AND PACKAGE INFORMATION CAN BE VIEWED AT WWW.BVNA.COM/ENVIRONMENTAL-LABORATORIES/RESOURCES/CHAIN-CUSTODY-FORMS-COCS.

SAMPLES MUST BE KEPT COOL (< 10° C) FROM TIME OF SAMPLING UNTIL DELIVERY TO BUREAU VERITAS

White: Bureau Veritas Yellow: Client

Bureau Veritas
109 & 110, 4023 Meadowbrook Drive, London, Ontario Canada N6L 1E7 Tel:(519) 652-9444 Toll-free:800-563-6266 Fax:(519) 652-8189 www.bvna.com

CHAIN OF CUSTODY RECORD

Page 2 of 4

INVOICE TO:		REPORT TO:		PROJECT INFORMATION:	
Company Name:	#12046 EcoMetrix Incorporated	Company Name:	Ecometrix	Quotation #:	C25356
Attention:	Accounts Payable	Attention:	Joseph Tetreault	P.O. #:	
Address:	6800 Campobello Rd Mississauga ON L5N 2L8	Address:		Project:	22-3093
Tel:	(905) 794-2325 Fax: (905) 794-2338	Tel:	(905) 794-2325 Ext: 215 Fax:	Project Name:	Phase 3 FEM
Email:	accountspayable@ecometrix.ca	Email:	jtetreault@ecometrix.ca	Site #:	NewGold Rainy River
				Sampled By:	

Laboratory Use Only:

Bureau Veritas Job #:	Bottle Order #:
	953308
COC #: C#953308-02-01	Project Manager: Colby Coutu

MOE REGULATED DRINKING WATER OR WATER INTENDED FOR HUMAN CONSUMPTION MUST BE SUBMITTED ON THE BUREAU VERITAS DRINKING WATER CHAIN OF CUSTODY

Regulation 153 (2011): ☐ Table 1 ☐ Res/Park ☐ Medium/Fine ☐ Table 2 ☐ Ind/Comm ☐ Coarse ☐ Table 3 ☐ Agri/Other ☐ For RSC ☐ Table ___

Other Regulations: ☐ CCME ☐ Sanitary Sewer Bylaw ☐ Reg 558 ☐ Storm Sewer Bylaw ☐ MISA Municipality ___ ☐ PWQO ☐ Reg 406 Table ___ ☐ Other

Special Instructions:

Include Criteria on Certificate of Analysis (Y/N)? ___

ANALYSIS REQUESTED (PLEASE BE SPECIFIC): Field Filtered (please circle): Metals / Hg / Cr VI; Particle Size (wet sieve); Metals; TOC

Turnaround Time (TAT) Required: Please provide advance notice for rush projects

Regular (Standard) TAT: ☐ *(will be applied if Rush TAT is not specified):* *Standard TAT = 5-7 Working days for most tests.* *Please note: Standard TAT for certain tests such as BOD and Dioxins/Furans are > 5 days - contact your Project Manager for details.*

Job Specific Rush TAT (if applies to entire submission) Date Required: ___ Time Required: ☐ Rush Confirmation Number: ___ *(call lab for #)*

	Sample Barcode Label	Sample (Location) Identification	Date Sampled	Time Sampled	Matrix	Field Filtered	Particle Size	Metals	TOC	# of Bottles	Comments
1		STUC-1	23/09/20	14:37	Sediment		✓	✓	✓	1	
2		STUC-2	23/09/20	13:54	↓		✓	✓	✓	1	
3		STUC-3	23/09/20	13:16	↓		✓	✓	✓	1	
4		STUC-4	23/09/20	12:27	↓		✓	✓	✓	1	
5		STUC-5	23/09/19	13:12	↓		✓	✓	✓	1	
6		PINR-EXP1	23/09/20	10:13	↓		✓	✓	✓	1	
7		PINR-EXP2	23/09/20	9:28	↓		✓	✓	✓	1	
8		PINR-EXP3	23/09/20	8:48	↓		✓	✓	✓	1	
9		PINR-EXP4	23/09/20	8:04	↓		✓	✓	✓	1	Rec'd In Thunder Bay
10		PINR-EXP5	23/09/19	16:50	↓		✓	✓	✓	1	

* RELINQUISHED BY: (Signature/Print)	Date: (YY/MM/DD)	Time	RECEIVED BY: (Signature/Print)	Date: (YY/MM/DD)	Time	# jars used and not submitted
[signature] / JOE TETREAULT	23/09/23	13:58	See Page 1			
			[signature] RAVNEET KAUR BRAR	2023/09/26	09:31	

Laboratory Use Only

Time Sensitive	Temperature (°C) on Recei	Custody Seal	Yes	No
		Present	✓	
		Intact	✓	

White: Bureau Veritas Yellow: Client

* UNLESS OTHERWISE AGREED TO IN WRITING, WORK SUBMITTED ON THIS CHAIN OF CUSTODY IS SUBJECT TO BUREAU VERITAS'S STANDARD TERMS AND CONDITIONS. SIGNING OF THIS CHAIN OF CUSTODY DOCUMENT IS ACKNOWLEDGMENT AND ACCEPTANCE OF OUR TERMS WHICH ARE AVAILABLE FOR VIEWING AT WWW.BVNA.COM/ENVIRONMENTAL-LABORATORIES/RESOURCES/COC-TERMS-AND-CONDITIONS.

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** SAMPLE CONTAINER, PRESERVATION, HOLD TIME AND PACKAGE INFORMATION CAN BE VIEWED AT WWW.BVNA.COM/ENVIRONMENTAL-LABORATORIES/RESOURCES/CHAIN-CUSTODY-FORMS-COCS.

SAMPLES MUST BE KEPT COOL (< 10° C) FROM TIME OF SAMPLING UNTIL DELIVERY TO BUREAU VERITAS

Temp: 8/8/7 (on ice)

Bureau Veritas Canada (2019) Inc.

RAVNEET KAUR BRAR RKR 2023/09/26 Rom. to ON-TT

Bureau Veritas
109 & 110, 4023 Meadowbrook Drive, London, Ontario Canada N6L 1E7 Tel:(519) 652-9444 Toll-free:800-563-6266 Fax:(519) 652-8189 www.bvna.com

CHAIN OF CUSTODY RECORD

Page 3 of 4

INVOICE TO:		REPORT TO:		PROJECT INFORMATION:	
Company Name:	#12046 EcoMetrix Incorporated	Company Name:	Ecometrix	Quotation #:	C25356
Attention:	Accounts Payable	Attention:	Joseph Tetreault	P.O. #:	
Address:	6800 Campobello Rd, Mississauga ON L5N 2L8	Address:		Project:	22-3093
Tel:	(905) 794-2325 Fax: (905) 794-2338	Tel:	(905) 794-2325 Ext: 215 Fax:	Project Name:	Phase 3 EEM
Email:	accountspayable@ecometrix.ca	Email:	jtetreault@ecometrix.ca	Site #:	NewGold Rainy River
				Sampled By:	

Laboratory Use Only:

Bureau Veritas Job #:	Bottle Order #:
	953308
COC #:	Project Manager:
C#953308-04-01	Colby Coutu

MOE REGULATED DRINKING WATER OR WATER INTENDED FOR HUMAN CONSUMPTION MUST BE SUBMITTED ON THE BUREAU VERITAS DRINKING WATER CHAIN OF CUSTODY

Regulation 153 (2011): ☐ Table 1 ☐ Res/Park ☐ Medium/Fine ☐ Table 2 ☐ Ind/Comm ☐ Coarse ☐ Table 3 ☐ Agri/Other ☐ For RSC ☐ Table ___

Other Regulations: ☐ CCME ☐ Sanitary Sewer Bylaw ☐ Reg 558 ☐ Storm Sewer Bylaw ☐ MISA Municipality ___ ☐ PWQO ☐ Reg 406 Table ___ ☐ Other ___

Special Instructions:

Include Criteria on Certificate of Analysis (Y/N)? ___

Turnaround Time (TAT) Required: Please provide advance notice for rush projects

Regular (Standard) TAT: ☐
(will be applied if Rush TAT is not specified):
Standard TAT = 5-7 Working days for most tests.
Please note: Standard TAT for certain tests such as BOD and Dioxins/Furans are > 5 days - contact your Project Manager for details.

Job Specific Rush TAT (if applies to entire submission)
Date Required: ___ Time Required: ___ ☐
Rush Confirmation Number: ___ *(call lab for #)*

ANALYSIS REQUESTED (PLEASE BE SPECIFIC)

	Sample Barcode Label	Sample (Location) Identification	Date Sampled	Time Sampled	Matrix	Field Filtered (please circle): Metals / Hg / Cr VI	Particle Size (Wentworth)	Metals	TOC	# of Bottles	Comments
1		PINR-EXP2-1	23/09/21	13:03	Sediment		✓	✓	✓	1	
2		PINR-EXP2-2	23/09/21	12:38	↓		✓	✓	✓	1	
3		PINR-EXP2-3	23/09/21	10:48			✓	✓	✓	1	
4		PINR-EXP2-4	23/09/21	9:47			✓	✓	✓	1	
5		PINR-EXP2-5	23/09/21	10:17			✓	✓	✓	1	
6		PINR-REF2-1	23/09/21	14:01			✓	✓	✓	1	
7		PINR-REF2-2	23/09/21	17:05			✓	✓	✓	1	
8		PINR-REF2-3	23/09/21	15:11			✓	✓	✓	1	
9		PINR-REF2-4	23/09/21	15:41			✓	✓	✓	1	Rec'd In Thunder Bay
10		PINR-REF2-5	23/09/21	16:12	↓		✓	✓	✓	1	

* RELINQUISHED BY: (Signature/Print)	Date: (YY/MM/DD)	Time	RECEIVED BY: (Signature/Print)	Date: (YY/MM/DD)	Time	# jars used and not submitted
[signature] / JOE TETREAULT	23/09/23	13:30	See Page 1			
			See Page 1			

Laboratory Use Only

Time Sensitive	Temperature (°C) on Recei	Custody Seal	Yes	No
		Present		
		Intact		

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** SAMPLE CONTAINER, PRESERVATION, HOLD TIME AND PACKAGE INFORMATION CAN BE VIEWED AT WWW.BVNA.COM/ENVIRONMENTAL-LABORATORIES/RESOURCES/CHAIN-CUSTODY-FORMS-COCS.

SAMPLES MUST BE KEPT COOL (< 10° C) FROM TIME OF SAMPLING UNTIL DELIVERY TO BUREAU VERITAS

White: Bureau Veritas Yellow: Client

Refer to ACTR

Bureau Veritas Canada (2019) Inc.

Bureau Veritas
109 & 110, 4023 Meadowbrook Drive, London, Ontario Canada N6L 1E7 Tel:(519) 652-9444 Toll-free:800-563-6266 Fax:(519) 652-8189 www.bvna.com

CHAIN OF CUSTODY RECORD

INVOICE TO:
Company Name: #12046 EcoMetrix Incorporated
Attention: Accounts Payable
Address: 6800 Campobello Rd, Mississauga ON L5N 2L8
Tel: (905) 794-2325 Fax: (905) 794-2338
Email: accountspayable@ecometrix.ca

REPORT TO:
Company Name: Ecometrix
Attention: Joseph Tetreault
Address:
Tel: (905) 794-2325 Ext: 215 Fax:
Email: jtetreault@ecometrix.ca

PROJECT INFORMATION:
Quotation #: C25356
P.O. #:
Project:
Project Name: [redacted] 22-3093
Site #: NewGold Rainy River
Sampled By:

Laboratory Use Only:
Bureau Veritas Job #:
Bottle Order #: 953308
COC #: C#953308-03-01
Project Manager: Colby Coutu

MOE REGULATED DRINKING WATER OR WATER INTENDED FOR HUMAN CONSUMPTION MUST BE SUBMITTED ON THE BUREAU VERITAS DRINKING WATER CHAIN OF CUSTODY

Regulation 153 (2011): ☐ Table 1 ☐ Res/Park ☐ Medium/Fine ☐ Table 2 ☐ Ind/Comm ☐ Coarse ☐ Table 3 ☐ Agri/Other ☐ For RSC ☐ Table ___

Other Regulations: ☐ CCME ☐ Sanitary Sewer Bylaw ☐ Reg 558 ☐ Storm Sewer Bylaw ☐ MISA Municipality ☐ PWQO ☐ Reg 406 Table ☐ Other

Special Instructions

Include Criteria on Certificate of Analysis (Y/N)? ___

Field Filtered (please circle): Metals / Hg / Cr VI

ANALYSIS REQUESTED (PLEASE BE SPECIFIC)

Turnaround Time (TAT) Required:
Please provide advance notice for rush projects

Regular (Standard) TAT: ☐
(will be applied if Rush TAT is not specified):
Standard TAT = 5-7 Working days for most tests.
Please note: Standard TAT for certain tests such as BOD and Dioxins/Furans are > 5 days - contact your Project Manager for details.

Job Specific Rush TAT (if applies to entire submission) ☐
Date Required: Time Required:
Rush Confirmation Number: *(call lab for #)*

	Sample Barcode Label	Sample (Location) Identification	Date Sampled	Time Sampled	Matrix	Particle Size (Wet+Dry)	Metals	TOC	# of Bottles	Comments
1		LVR2-REF1 / LVR2-REF1 DUPLICATE	23/09/22	10:33	Sediment	✓	✓	✓	1/1	Do not homogenize. Treat as separate samples
2		LVR2-REF2	23/09/22	9:45	↓	✓	✓	✓	1	
3		LVR2-REF3	23/09/22	9:11		✓	✓	✓	1	
4		LVR2-REF4	23/09/22	12:40		✓	✓	✓	1	
5		LVR2-REF5	23/09/22	11:13		✓	✓	✓	1	
6		LVR-REF1	23/09/22	16:48		✓	✓	✓	1	
7		LVR-REF2	23/09/22	16:00		✓	✓	✓	1	
8		LVR-REF3	23/09/22	15:45		✓	✓	✓	1	Rec'd In Thunder Bay
9		LVR-REF4	23/09/22	15:11		✓	✓	✓	1	
10		LVR-REF5 / LVR-REF5 DUPLICATE	23/09/22	14:42		✓	✓	✓	1/1	Do not homogenize Treat as separate samples

* RELINQUISHED BY: (Signature/Print)	Date: (YY/MM/DD)	Time	RECEIVED BY: (Signature/Print)	Date: (YY/MM/DD)	Time	# jars used and not submitted
JOE TETREAULT	23/09/23	15:50	See Page 1			
			See Page 1			

Laboratory Use Only
Time Sensitive | Temperature (°C) on Recei | Custody Seal Present ☐ Yes ☐ No | Intact ☐ Yes ☐ No

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** SAMPLE CONTAINER, PRESERVATION, HOLD TIME AND PACKAGE INFORMATION CAN BE VIEWED AT WWW.BVNA.COM/ENVIRONMENTAL-LABORATORIES/RESOURCES/CHAIN-CUSTODY-FORMS-COCS.

SAMPLES MUST BE KEPT COOL (< 10° C) FROM TIME OF SAMPLING UNTIL DELIVERY TO BUREAU VERITAS

White: Bureau Veritas Yellow: Client
Refer to ACTR

Bureau Veritas Canada (2019) Inc.

Sent to: **Bureau Veritas Bedford**
200 Bluewater Rd Suite 105
Bedford, NS, B4B 1G9
Tel: (902) 832-4852

BV LABS: BEDF-2023-10-120

COC # C3T6649-301-01-01

REPORT INFORMATION	
Company:	Bureau Veritas Laboratories
Address:	6740 Campobello Road, Mississauga, Ontario, L5N 2L8
Contact Name:	Colby Coutu
Email:	Colby.Coutu@bureauveritas.com, scontractor@bureauveritas.com
Phone:	(905) 817-5844
BV Labs Project #:	C3T6649
Client Invoice To:	EcoMetrix Incorporated (12046)
Client Report To:	EcoMetrix Incorporated (12046) **Incl. on Report? Yes / No**

ANALYSIS REQUESTED

Job Barcode Label

C3T6649

#	SAMPLE ID	MATRIX	DATE SAMPLED (YYYY/MM/DD)	TIME SAMPLED (HH:MM)	SAMPLER INITIALS	# CONT.	Acidity ($CaCO_3$) in water	Attachment of PS PDF to WS	Particle size in solids (pipette&sieve)								ADDITIONAL SAMPLE INFORMATION
1	XCF499-LVR	W	2023/09/22	13:20		1	X										(P: 04)
2	XCF500-LVR2	W	2023/09/22	12:30		1	X										(P: 04)
3	XCF501-PINREXP	W	2023/09/22	09:48		1	X										(P: 04)
4	XCF502-PINREXP2	W	2023/09/22	08:50		1	X										(P: 04)
5	XCF503-PINRREF2	W	2023/09/22	08:35		1	X										(P: 04)
6	XCF504-STUCREF	W	2023/09/22	11:10		1	X										(P: 04)
7	XCF505-DUP	W	2023/09/22	09:48		1	X										(P: 04)
8	XCF506-FB	W	2023/09/22	09:48		1	X										(P: 03)
9	XCF508-STUC-1	SED	2023/09/20	14:37		1		X	X								(P: 01)
10	XCF509-STUC-2	SED	2023/09/20	13:54		1		X	X								(P: 01)

SITE LOCATION:	**REGULATORY CRITERIA**	**SPECIAL INSTRUCTIONS**	**REQUIRED EDDs**	**TURNAROUND TIME**
NewGold Rainy River		Please inform Bureau Veritas immediately if you are not accredited for the requested test(s) or the hold time is approaching. **Please return a copy of this form with the report.**	National Excel (N001) EcoMetrix Excel (NX435)	☐ Rush Required **2023/10/03** Date Required *Please inform us if rush charges will be incurred.*
SITE #:				
PHASE 3 EEM				
PROJECT #:				
22-3093				
PO/AFE, TASK ORDER/SERVICE ORDER, LINE ITEM:				

COOLER ID:	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present						
Custody Seal Intact						
Cooling Media Present	✓					
				5	6	5

COOLER ID:	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present						
Custody Seal Intact	✓					
Cooling Media Present						
				4	5	4

COOLER ID:	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present						
Custody Seal Intact						
Cooling Media Present						

RECEIVING LAB USE ONLY

BV Labs Job #

C3T6649

RELINQUISHED BY: (SIGN & PRINT)	**DATE:** (YYYY/MM/DD)	**TIME:** (HH:MM)	**RECEIVED BY:** (SIGN & PRINT)	**DATE:** (YYYY/MM/DD)	**TIME:** (HH:MM)	**Samples Labelled By:**	**Labels Verified By:**
1. NIRAL PATEL	2023/10/03	13:07	1. Holly Bauma			NP 4	
2.			2.				

Sent to: **Bureau Veritas Bedford**
200 Bluewater Rd Suite 105
Bedford, NS, B4B 1G9
Tel: (902) 832-4852

BV LABS INTERLAB CHAIN OF CUSTODY RECORD

COC # C3T6649-301-02-01

REPORT INFORMATION	
Company:	Bureau Veritas Laboratories
Address:	6740 Campobello Road, Mississauga, Ontario, L5N 2L8
Contact Name:	Colby Coutu
Email:	Colby.Coutu@bureauveritas.com, scontractor@bureauveritas.com
Phone:	(905) 817-5844
BV Labs Project #:	C3T6649
Client Invoice To:	EcoMetrix Incorporated (12046)
Client Report To:	EcoMetrix Incorporated (12046)

ANALYSIS REQUESTED

Job Barcode Label

C3T6649

#	SAMPLE ID	MATRIX	DATE SAMPLED (YYYY/MM/DD)	TIME SAMPLED (HH:MM)	SAMPLER INITIALS	# CONT.	Acidity (CaCO3) in water	Attachment of PS PDF to WS	Particle size in solids (pipette&sieve)	ADDITIONAL SAMPLE INFORMATION
1	XCF510-STUC-3	SED	2023/09/20	13:16		1		X	X	(P: 01)
2	XCF511-STUC-4	SED	2023/09/20	12:27		1		X	X	(P: 01)
3	XCF512-STUC-5	SED	2023/09/19	13:12		1		X	X	(P: 01)
4	XCF513-PINR-EXP1	SED	2023/09/20	10:13		1		X	X	(P: 01)
5	XCF514-PINR-EXP2	SED	2023/09/20	09:28		1		X	X	(P: 01)
6	XCF515-PINR-EXP3	SED	2023/09/20	08:48		1		X	X	(P: 01)
7	XCF516-PINR-EXP4	SED	2023/09/20	08:04		1		X	X	(P: 01)
8	XCF517-PINR-EXP5	SED	2023/09/19	16:50		1		X	X	(P: 01)
9	XCF564-PINR-EXP2-1	SED	2023/09/21	13:03		1		X	X	(P: 01)
10	XCF565-PINR-EXP2-2	SED	2023/09/21	12:38		1		X	X	(P: 01)

Incl. on Report? Yes / No

SITE LOCATION: NewGold Rainy River

SITE #: PHASE 3 EEM

PROJECT #: 22-3093

PO/AFE, TASK ORDER/SERVICE ORDER, LINE ITEM:

REGULATORY CRITERIA

SPECIAL INSTRUCTIONS

Please inform Bureau Veritas immediately if you are not accredited for the requested test(s) or the hold time is approaching.
Please return a copy of this form with the report.

REQUIRED EDDs

National Excel (N001)
EcoMetrix Excel (NX435)

TURNAROUND TIME

☐ Rush Required

2023/10/03
Date Required

Please inform us if rush charges will be incurred.

COOLER ID:

	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present						
Custody Seal Intact						
Cooling Media Present	✓					
				5	6	5

COOLER ID:

	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present						
Custody Seal Intact						
Cooling Media Present	✓					
				4	5	4

COOLER ID:

	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present						
Custody Seal Intact						
Cooling Media Present						

RECEIVING LAB USE ONLY

BV Labs Job #

C3T6649

RELINQUISHED BY: (SIGN & PRINT)	**DATE:** (YYYY/MM/DD)	**TIME:** (HH:MM)	**RECEIVED BY:** (SIGN & PRINT)	**DATE:** (YYYY/MM/DD)	**TIME:** (HH:MM)	**Samples Labelled By:**	**Labels Verified By:**
1. See pg 1			1. Holly Bouma				
2.			2.				

Sent to: **Bureau Veritas Bedford**
200 Bluewater Rd Suite 105
Bedford, NS, B4B 1G9
Tel: (902) 832-4852

BV LABS INTERLAB CHAIN OF CUSTODY RECORD

COC # C3T6649-301-03-01

Job Barcode Label: C3T6649

REPORT INFORMATION	
Company:	Bureau Veritas Laboratories
Address:	6740 Campobello Road, Mississauga, Ontario, L5N 2L8
Contact Name:	Colby Coutu
Email:	Colby.Coutu@bureauveritas.com, scontractor@bureauveritas.com
Phone:	(905) 817-5844
BV Labs Project #:	C3T6649
Client Invoice To:	EcoMetrix Incorporated (12046)
Client Report To:	EcoMetrix Incorporated (12046) — Incl. on Report? Yes / No

ANALYSIS REQUESTED

#	SAMPLE ID	MATRIX	DATE SAMPLED (YYYY/MM/DD)	TIME SAMPLED (HH:MM)	SAMPLER INITIALS	# CONT.	Acidity (CaCO3) in water	Attachment of PS PDF to WS	Particle size in solids (pipette&sieve)	ADDITIONAL SAMPLE INFORMATION
1	XCF566-PINR-EXP2-3	SED	2023/09/21	10:48		1		X	X	(P: 01)
2	XCF567-PINR-EXP2-4	SED	2023/09/21	09:47		1		X	X	(P: 01)
3	XCF568-PINR-EXP2-5	SED	2023/09/21	10:17		1		X	X	(P: 01)
4	XCF569-PINR-REF2-1	SED	2023/09/21	14:01		1		X	X	(P: 01)
5	XCF571-PINR-REF2-2	SED	2023/09/21	17:05		1		X	X	(P: 01)
6	XCF573-PINR-REF2-3	SED	2023/09/21	15:11		1		X	X	(P: 01)
7	XCF576-PINR-REF2-4	SED	2023/09/21	15:41		1		X	X	(P: 01)
8	XCF578-PINR-REF2-5	SED	2023/09/21	16:12		1		X	X	(P: 01)
9	XCF665-LVR2-REF1	SED	2023/09/22	10:33		1		X	X	(P: 01)
10	XCF666-LVR2-REF1 DUPLICATE	SED	2023/09/22	10:33		1		X	X	(P: 01)

SITE LOCATION: NewGold Rainy River
SITE #: PHASE 3 EEM
PROJECT #: 22-3093
PO/AFE, TASK ORDER/SERVICE ORDER, LINE ITEM:

REGULATORY CRITERIA

SPECIAL INSTRUCTIONS
Please inform Bureau Veritas immediately if you are not accredited for the requested test(s) or the hold time is approaching.
Please return a copy of this form with the report.

REQUIRED EDDs
National Excel (N001)
EcoMetrix Excel (NX435)

TURNAROUND TIME
☐ Rush Required
2023/10/03
Date Required
Please inform us if rush charges will be incurred.

COOLER ID:	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present				5	6	5
Custody Seal Intact						
Cooling Media Present	✓					

COOLER ID:	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present				4	5	4
Custody Seal Intact						
Cooling Media Present	✓					

COOLER ID:	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present						
Custody Seal Intact						
Cooling Media Present						

RECEIVING LAB USE ONLY
BV Labs Job #: **C3T6649**

RELINQUISHED BY: (SIGN & PRINT)	DATE: (YYYY/MM/DD)	TIME: (HH:MM)	RECEIVED BY: (SIGN & PRINT)	DATE: (YYYY/MM/DD)	TIME: (HH:MM)
1. See pg 1			1. [signature] Dolly Bauma		
2.			2.		

Samples Labelled By:	Labels Verified By:

Sent to: **Bureau Veritas Bedford**
200 Bluewater Rd Suite 105
Bedford, NS, B4B 1G9
Tel: (902) 832-4852

BV LABS INTERLAB CHAIN OF CUSTODY RECORD

COC # C3T6649-301-04-01

REPORT INFORMATION	
Company:	Bureau Veritas Laboratories
Address:	6740 Campobello Road, Mississauga, Ontario, L5N 2L8
Contact Name:	Colby Coutu
Email:	Colby.Coutu@bureauveritas.com, scontractor@bureauveritas.com
Phone:	(905) 817-5844
BV Labs Project #:	C3T6649
Client Invoice To:	EcoMetrix Incorporated (12046)
Client Report To:	EcoMetrix Incorporated (12046)

Incl. on Report? Yes / No

#	SAMPLE ID	MATRIX	DATE SAMPLED (YYYY/MM/DD)	TIME SAMPLED (HH:MM)	SAMPLER INITIALS	# CONT.	Acidity (CaCO3) in water	Attachment of PS PDF to WS	Particle size in solids (pipette&sieve)	ADDITIONAL SAMPLE INFORMATION
1	XCF667-LVR2-REF2	SED	2023/09/22	09:45		1		X	X	(P: 01)
2	XCF668-LVR2-REF3	SED	2023/09/22	09:11		1		X	X	(P: 01)
3	XCF669-LVR2-REF4	SED	2023/09/22	12:40		1		X	X	(P: 01)
4	XCF670-LVR2-REF5	SED	2023/09/22	11:13		1		X	X	(P: 01)
5	XCF671-LVR-REF2	SED	2023/09/22	16:00		1		X	X	(P: 01)
6	XCF672-LVR-REF3	SED	2023/09/22	15:45		1		X	X	(P: 01)
7	XCF673-LVR-REF4	SED	2023/09/22	15:11		1		X	X	(P: 01)
8	XCF674-LVR-REF5	SED	2023/09/22	14:22		1		X	X	(P: 01)
9	XCF675-LVR-REF5 DUPLICATE	SED	2023/09/22	14:22		1		X	X	(P: 01)
10	XCO572-LVR-REF1	SED	2023/09/22	16:48		1		X	X	(P: 01)

Job Barcode Label

C3T6649

SITE LOCATION: NewGold Rainy River
SITE #: PHASE 3 EEM
PROJECT #: 22-3093
PO/AFE, TASK ORDER/SERVICE ORDER, LINE ITEM:

REGULATORY CRITERIA

SPECIAL INSTRUCTIONS
Please inform Bureau Veritas immediately if you are not accredited for the requested test(s) or the hold time is approaching.
Please return a copy of this form with the report.

REQUIRED EDDs
National Excel (N001)
EcoMetrix Excel (NX435)

TURNAROUND TIME
☐ Rush Required
2023/10/03
Date Required
Please inform us if rush charges will be incurred.

COOLER ID:

	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present						
Custody Seal Intact						
Cooling Media Present	✓					
				5	6	5

COOLER ID:

	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present						
Custody Seal Intact						
Cooling Media Present	✓					
				4	5	4

COOLER ID:

	YES	NO	Temp: (°C)	1	2	3
Custody Seal Present						
Custody Seal Intact						
Cooling Media Present						

RECEIVING LAB USE ONLY
BV Labs Job #
C3T6649

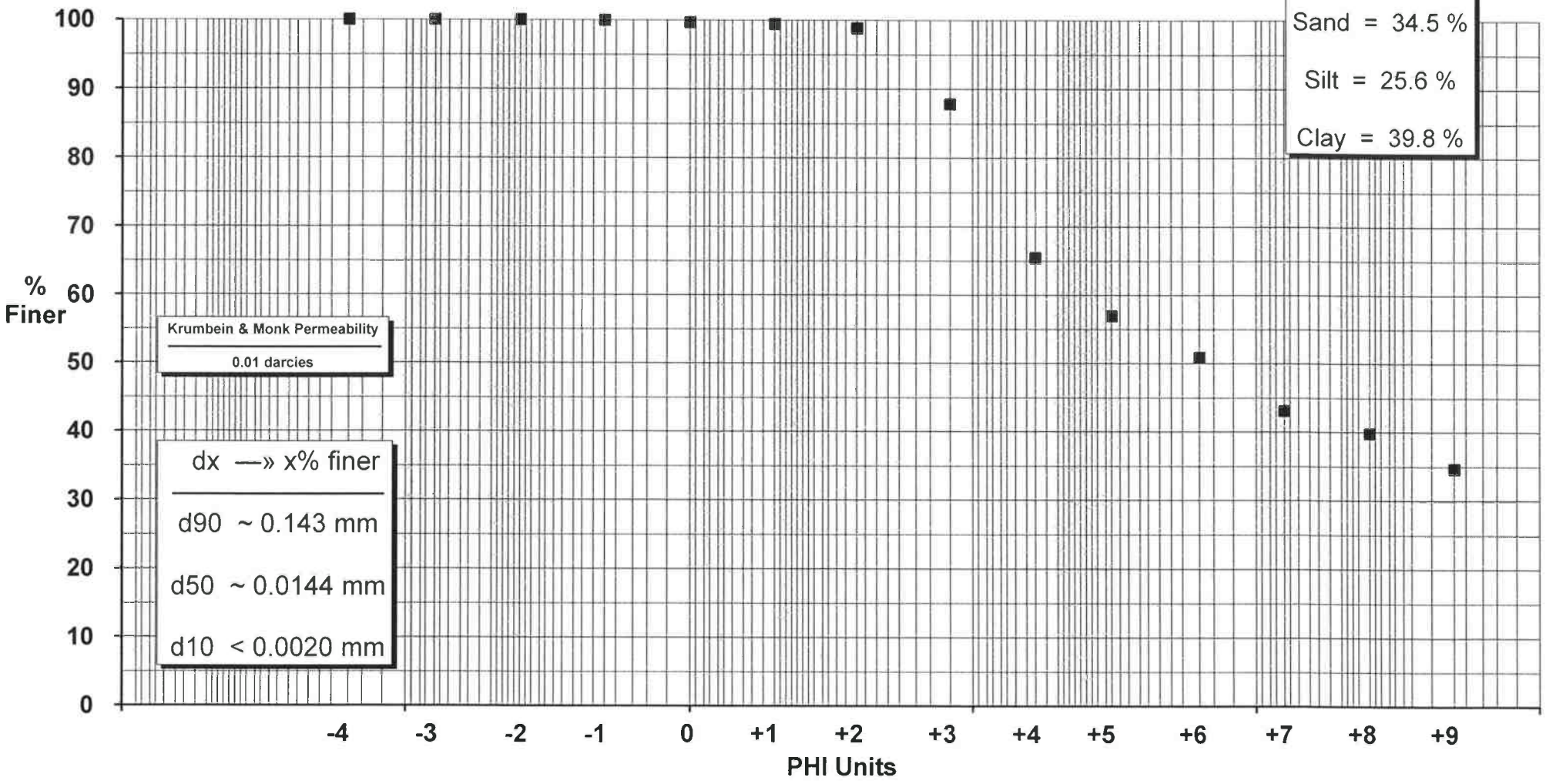
RELINQUISHED BY: (SIGN & PRINT)	DATE: (YYYY/MM/DD)	TIME: (HH:MM)	RECEIVED BY: (SIGN & PRINT)	DATE: (YYYY/MM/DD)	TIME: (HH:MM)	Samples Labelled By:	Labels Verified By:
1. See pg 1			1. Kelly Bauma				
2.			2.				

BV Labs ID: XCF508-01

STUC-1

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
28.7 %	37.3 %

Wentworth	
Gravel	0.1 %
Sand	34.5 %
Silt	25.6 %
Clay	39.8 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer
d90 ~ 0.143 mm
d50 ~ 0.0144 mm
d10 < 0.0020 mm

BAN

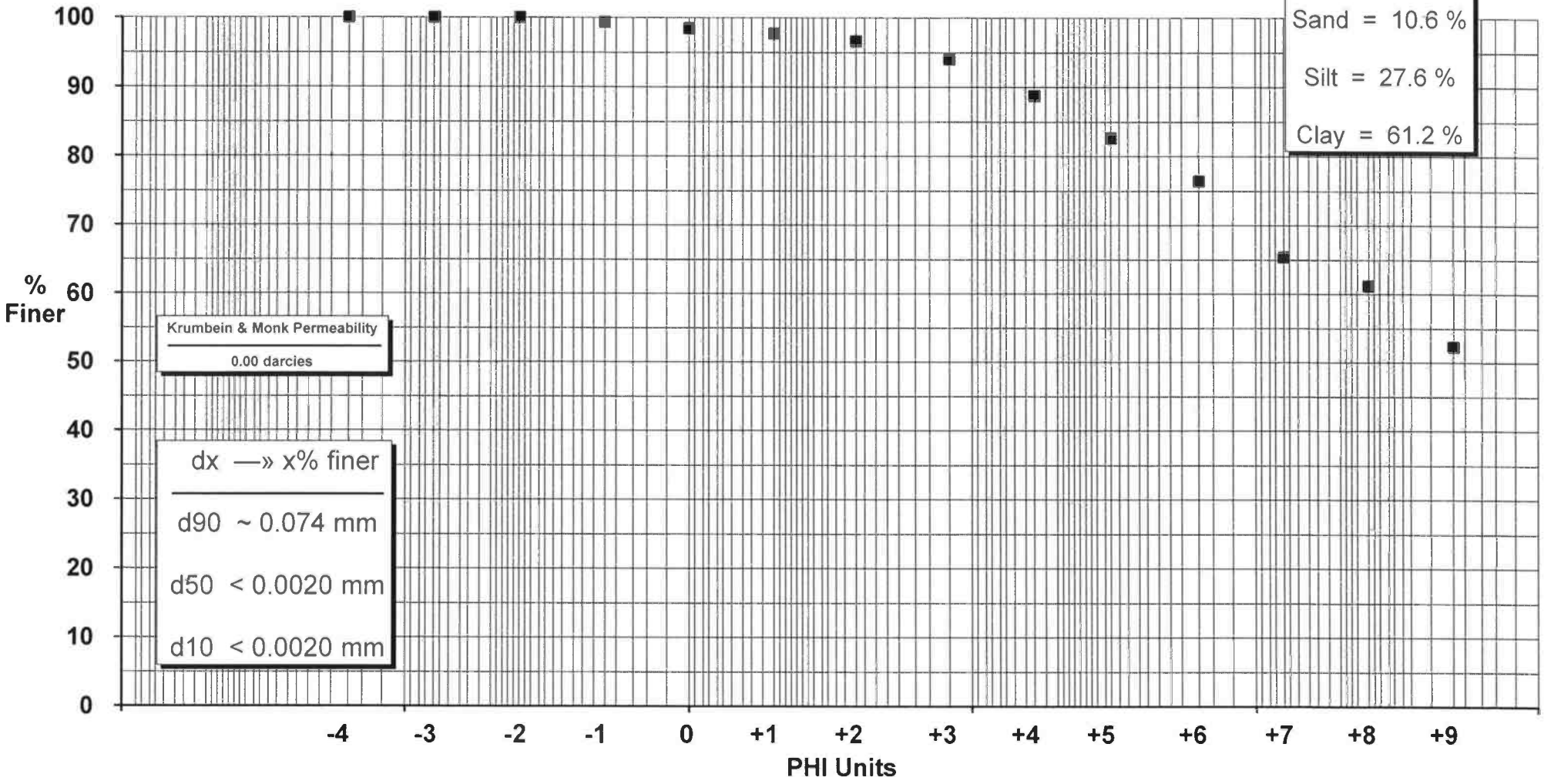
Approved

BV Labs ID: XCF509-01

STUC-2

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
9.9 %	13.2 %

Wentworth	
Gravel	0.6 %
Sand	10.6 %
Silt	27.6 %
Clay	61.2 %



Krumbein & Monk Permeability
0.00 darcies

dx —» x% finer
d90 ~ 0.074 mm
d50 < 0.0020 mm
d10 < 0.0020 mm

BAN

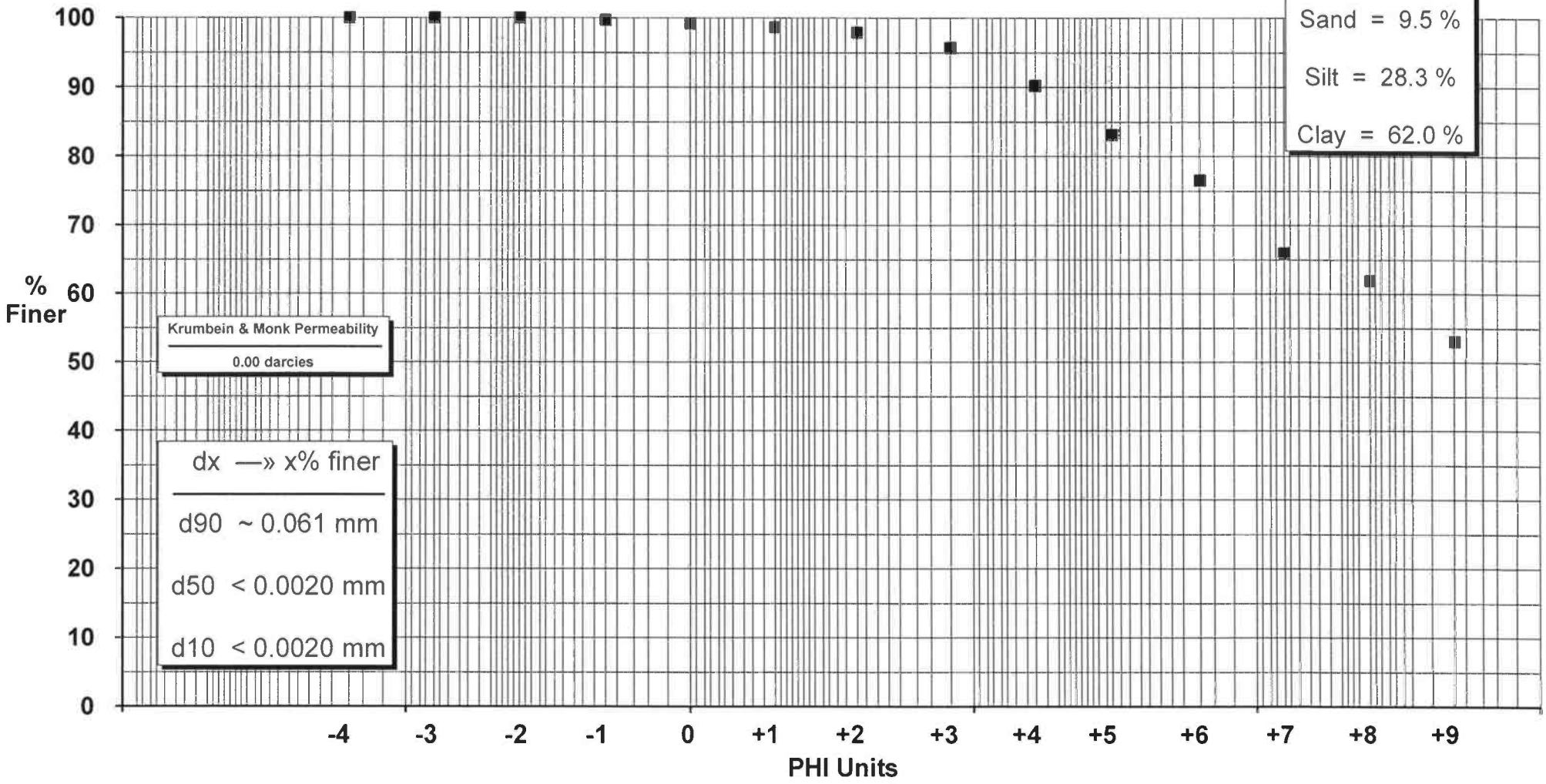
Approved

BV Labs ID: XCF509-01:D1

STUC-2

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
8.3 %	12.1 %

Wentworth	
Gravel	0.3 %
Sand	9.5 %
Silt	28.3 %
Clay	62.0 %



BAN

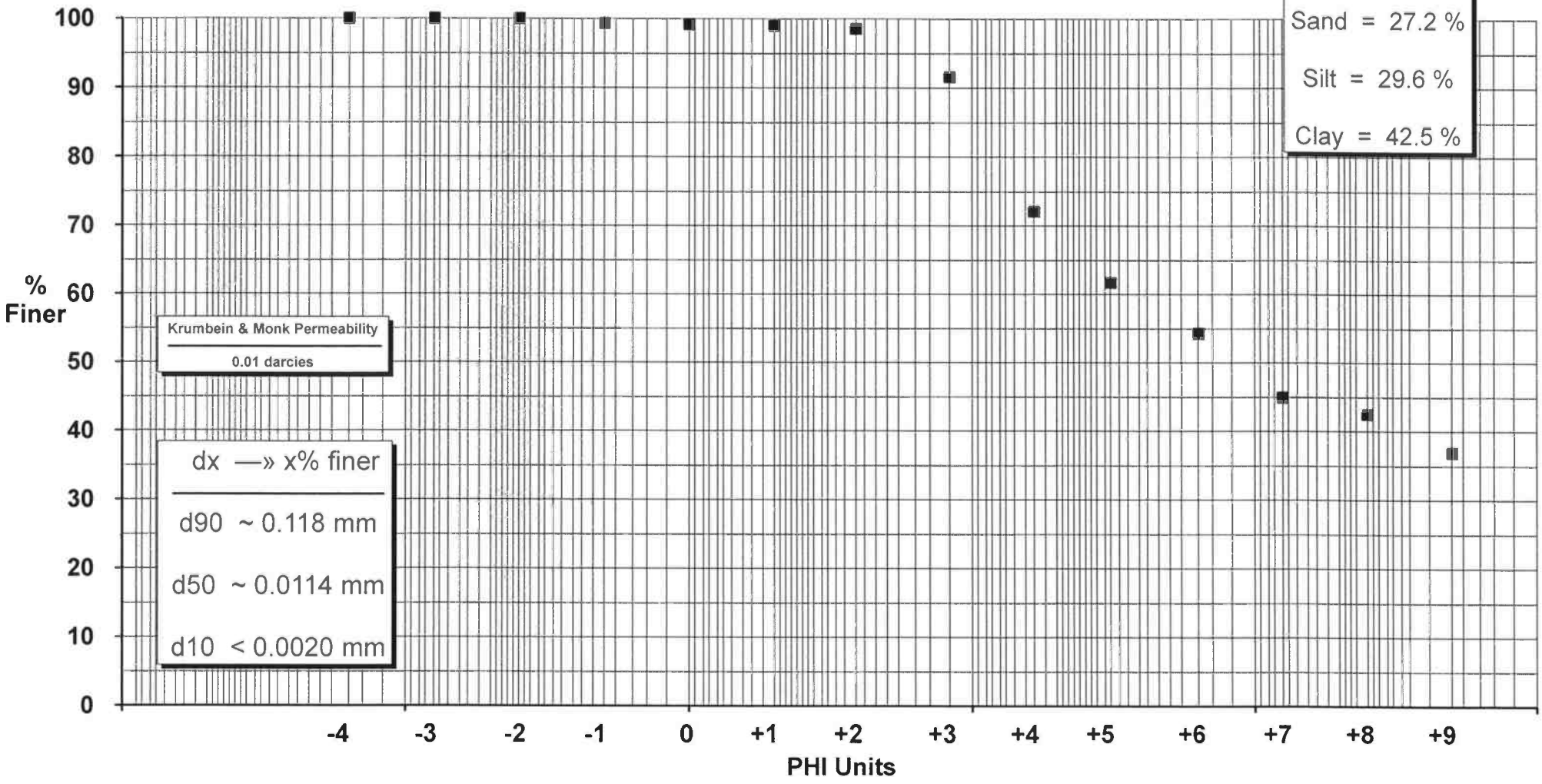
Approved

BV Labs ID: XCF510-01

STUC-3

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
22.7 %	31.2 %

Wentworth	
Gravel	0.6 %
Sand	27.2 %
Silt	29.6 %
Clay	42.5 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer

d90 ~ 0.118 mm

d50 ~ 0.0114 mm

d10 < 0.0020 mm

BAN

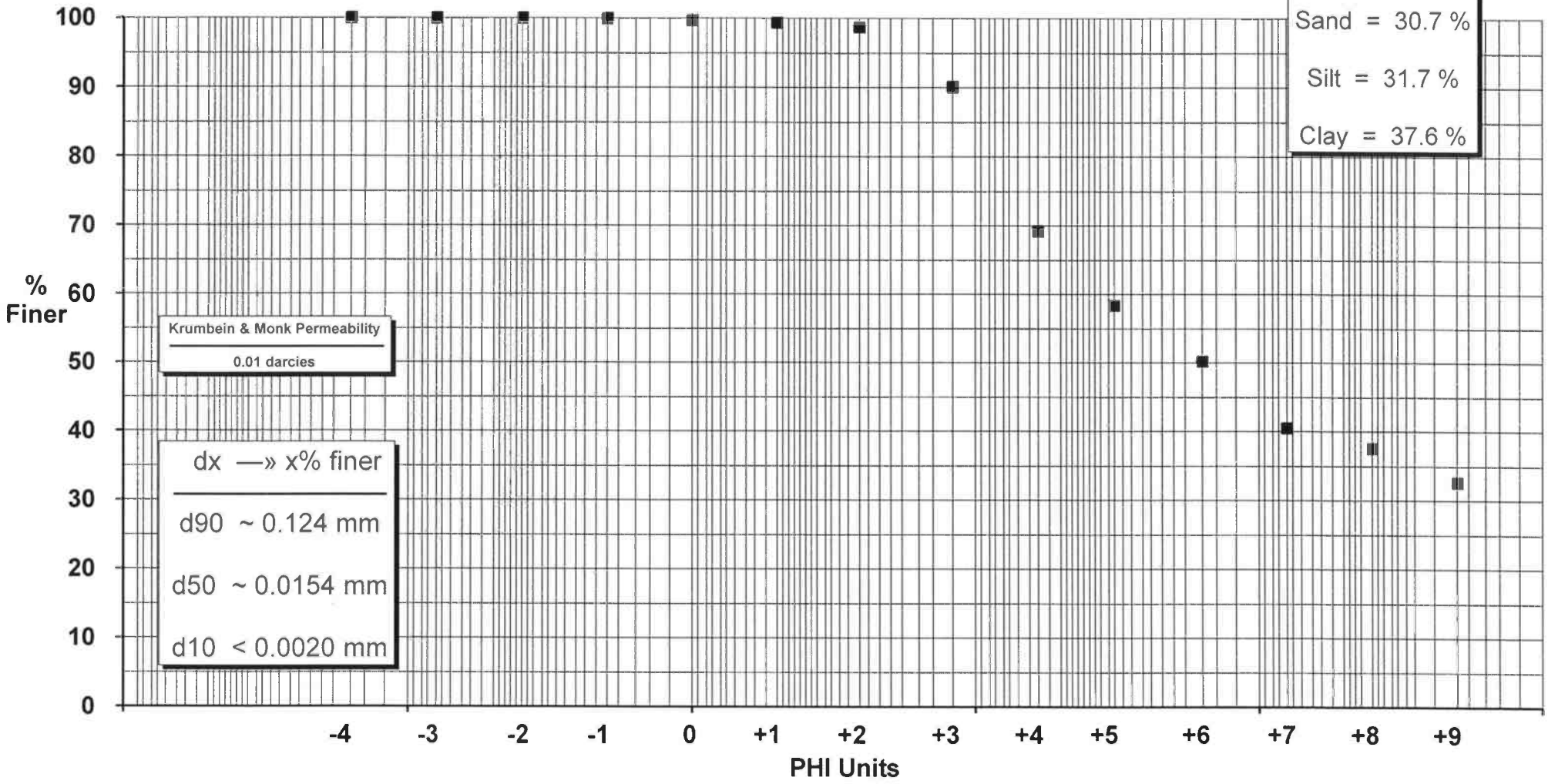
Approved

BV Labs ID: XCF511-01

STUC-4

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
25.3 %	34.3 %

Wentworth	
Gravel	0.0 %
Sand	30.7 %
Silt	31.7 %
Clay	37.6 %



Krumbein & Monk Permeability: 0.01 darcies

dx —» x% finer	
d90	~ 0.124 mm
d50	~ 0.0154 mm
d10	< 0.0020 mm

BAN

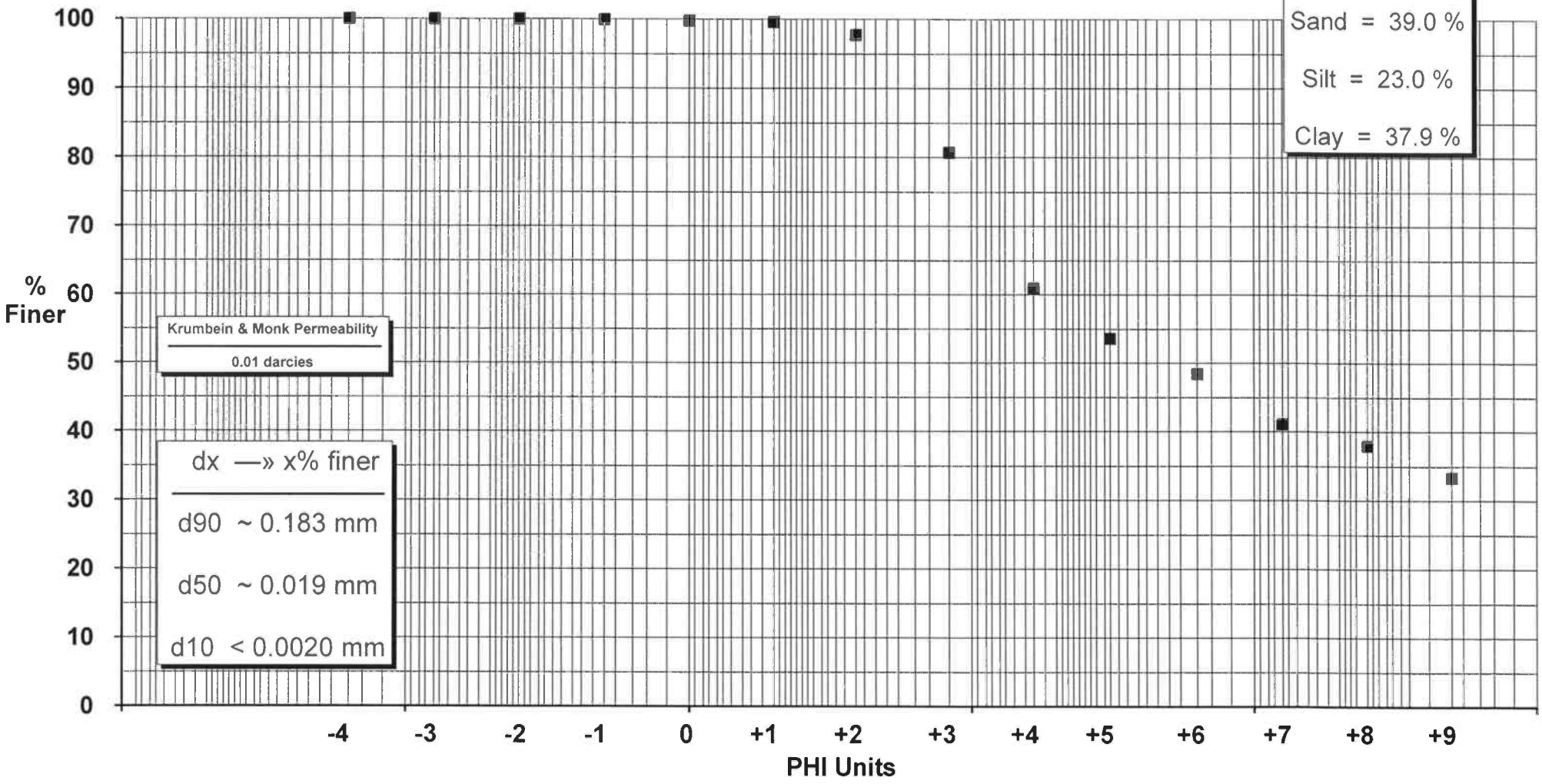
Approved

BV Labs ID: XCF512-01

STUC-5

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
33.9 %	41.4 %

Wentworth	
Gravel	0.1 %
Sand	39.0 %
Silt	23.0 %
Clay	37.9 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer

d90 ~ 0.183 mm

d50 ~ 0.019 mm

d10 < 0.0020 mm

BAN

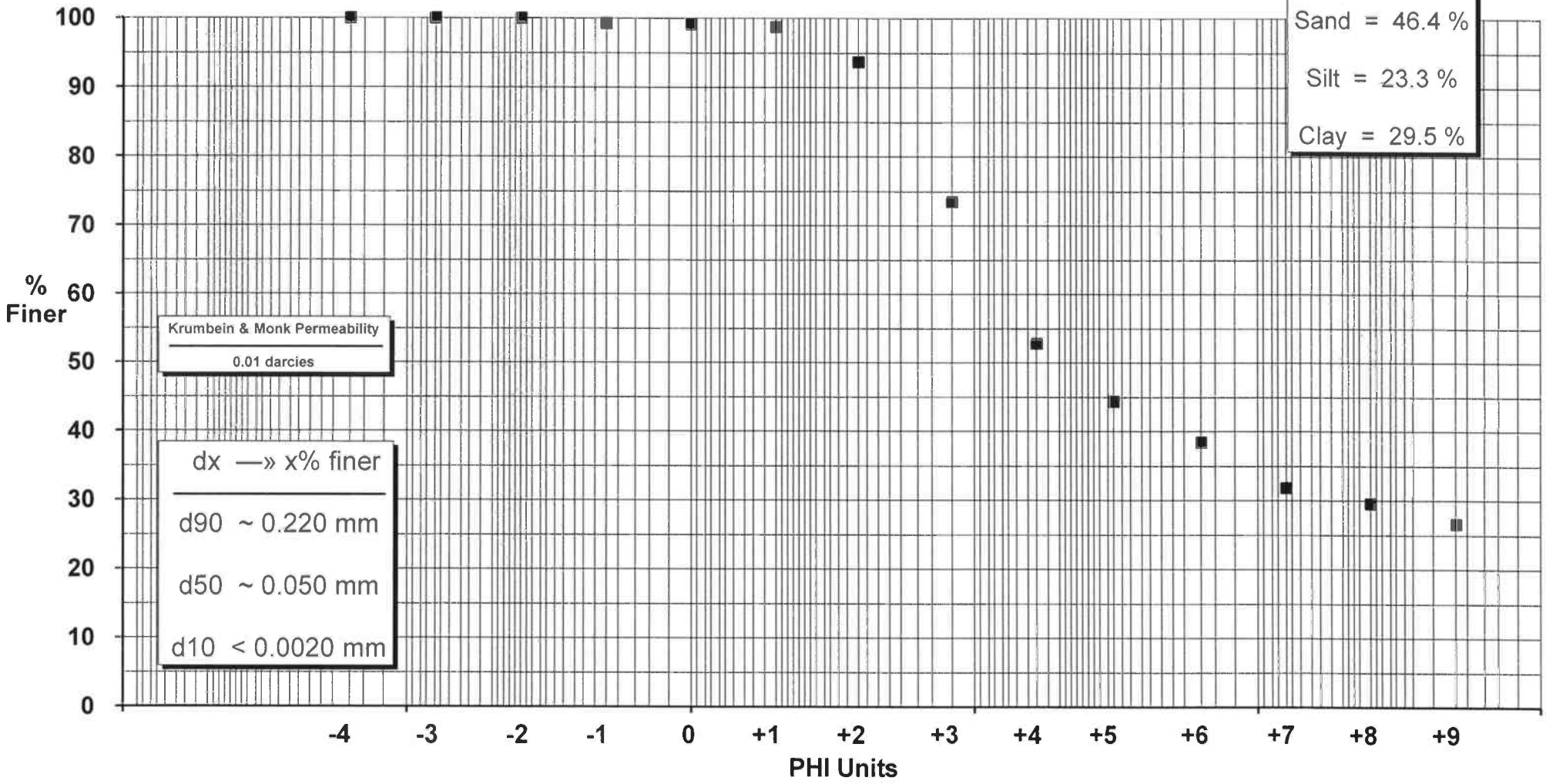
Approved

BV Labs ID: XCF513-01

PINR-EXP1

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
41.7 %	49.9 %

Wentworth	
Gravel	0.7 %
Sand	46.4 %
Silt	23.3 %
Clay	29.5 %



Krumbein & Monk Permeability
0.01 darcies

dx —» x% finer	
d90	~ 0.220 mm
d50	~ 0.050 mm
d10	< 0.0020 mm

BAN

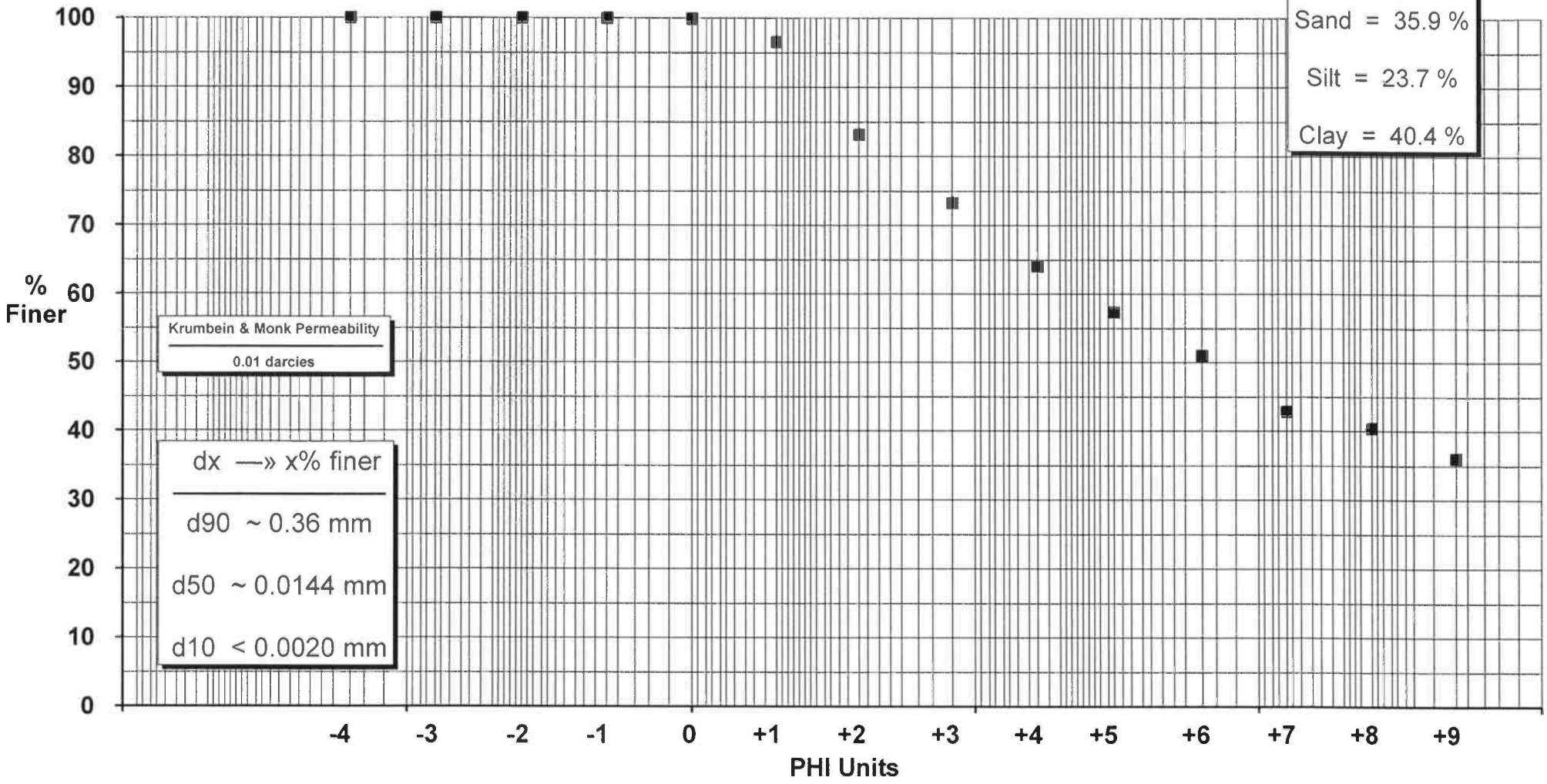
Approved

BV Labs ID: XCF514-01

PINR-EXP2

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
33.5 %	38.1 %

Wentworth	
Gravel	0.0 %
Sand	35.9 %
Silt	23.7 %
Clay	40.4 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer

d90 ~ 0.36 mm

d50 ~ 0.0144 mm

d10 < 0.0020 mm

BAN

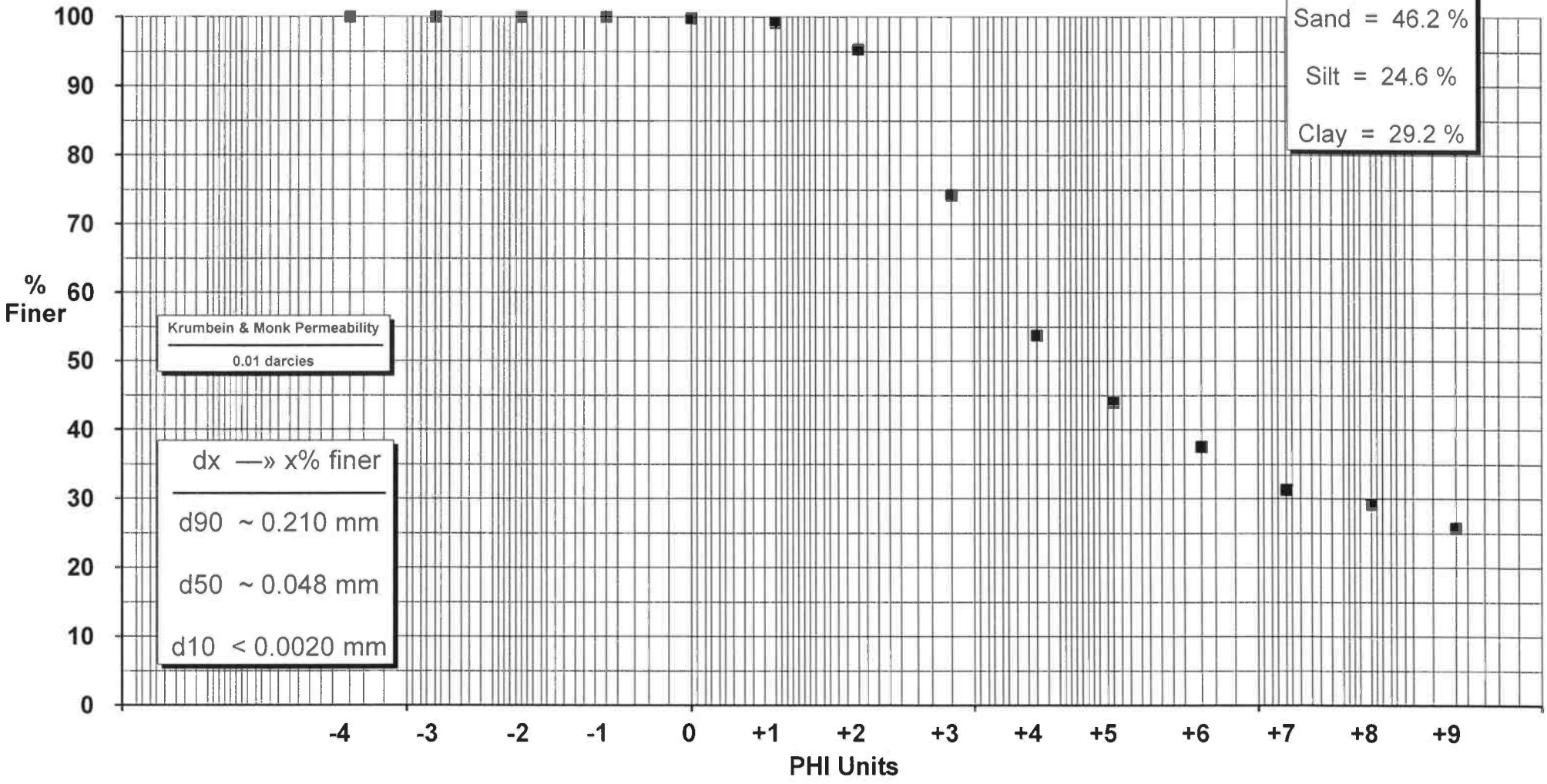
Approved

BV Labs ID: XCF515-01

PINR-EXP3

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
40.8 %	49.4 %

Wentworth	
Gravel	0.0 %
Sand	46.2 %
Silt	24.6 %
Clay	29.2 %



Krumbein & Monk Permeability

0.01 darcies

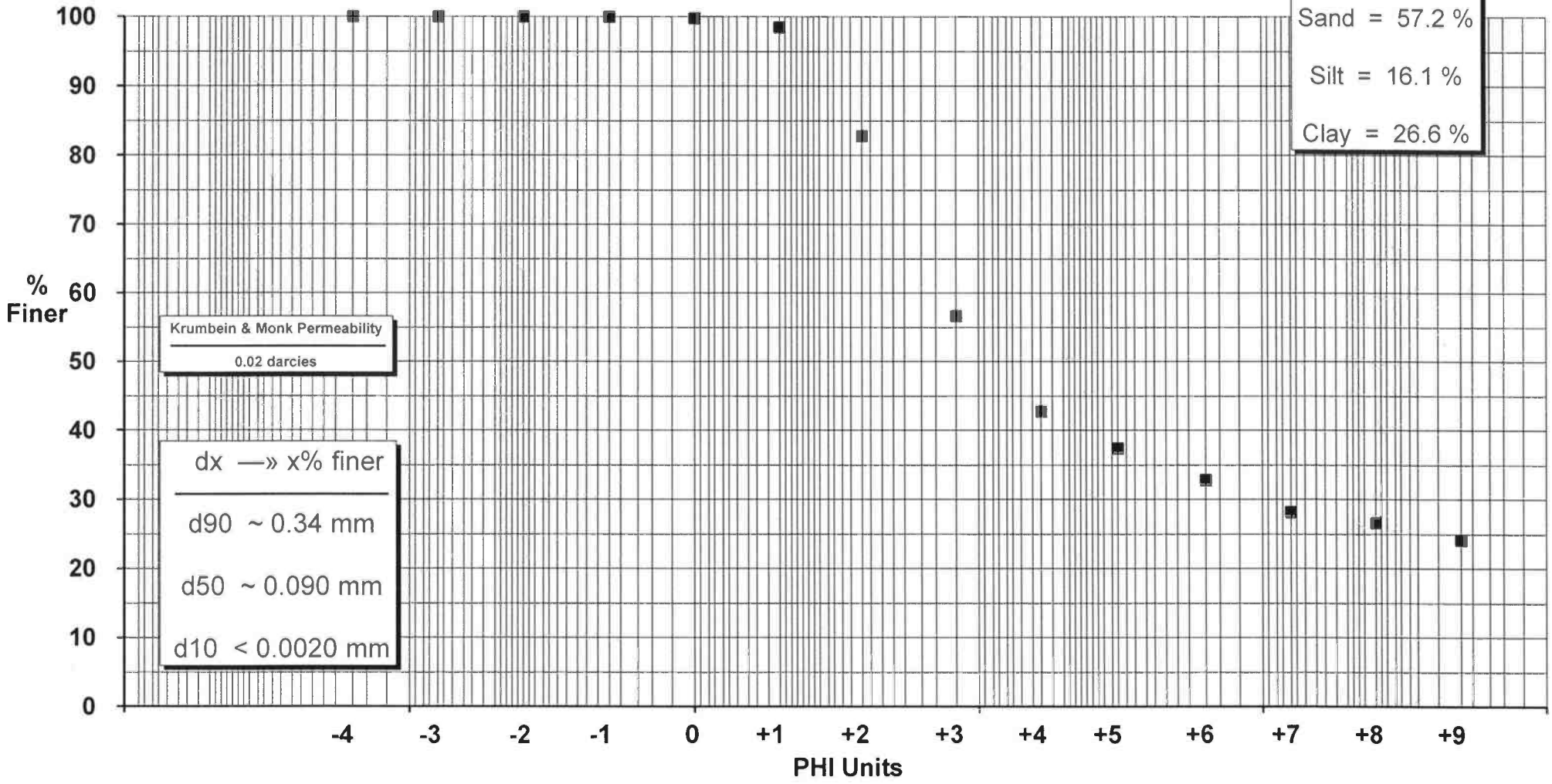
dx —» x% finer	
d90	~ 0.210 mm
d50	~ 0.048 mm
d10	< 0.0020 mm

BAN

Approved

BV Labs ID: XCF516-01

PINR-EXP4



BAN

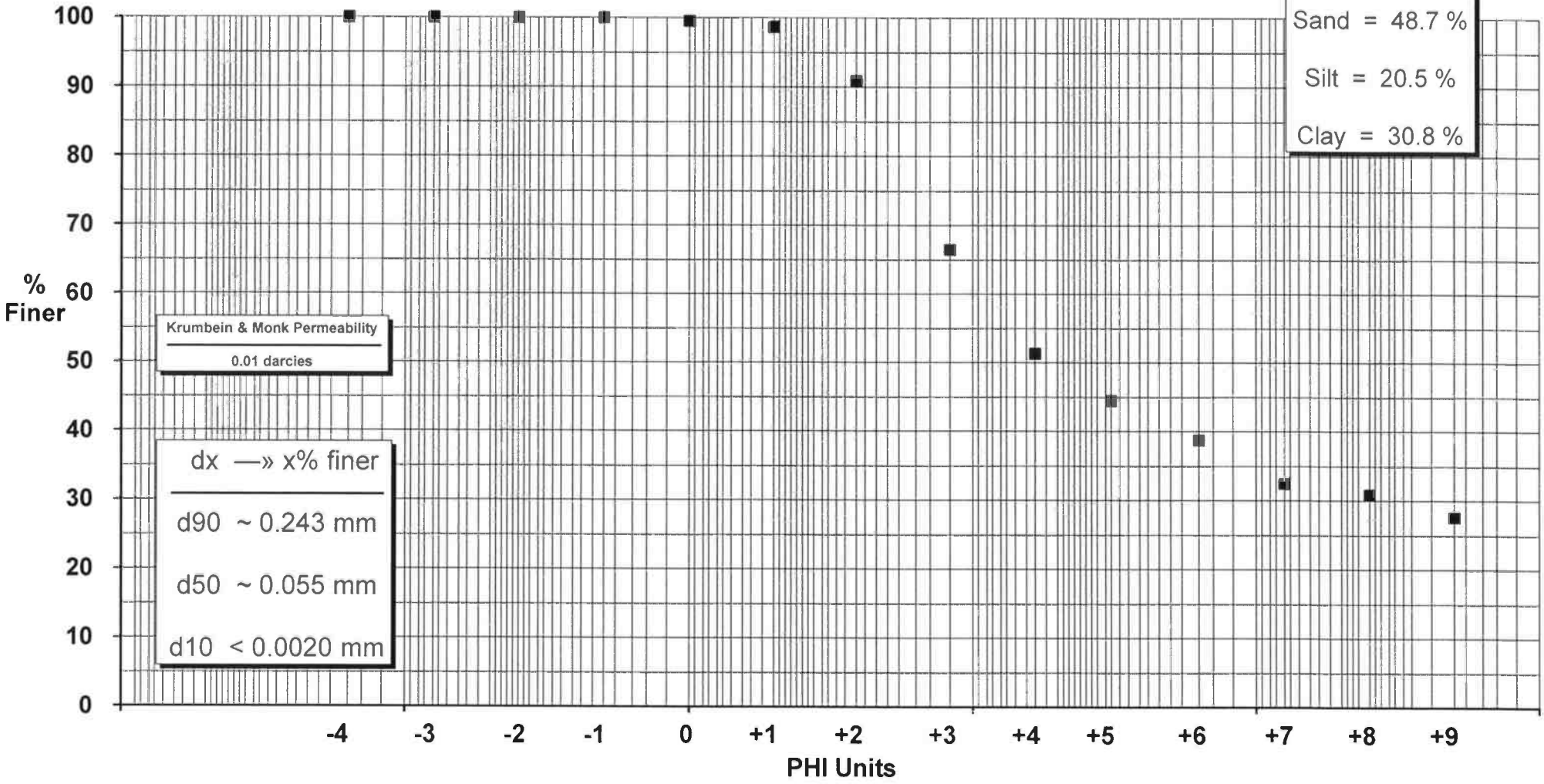
Approved

BV Labs ID: XCF517-01

PINR-EXP5

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
44.7 %	50.9 %

Wentworth	
Gravel	0.0 %
Sand	48.7 %
Silt	20.5 %
Clay	30.8 %



Krumbein & Monk Permeability

0.01 darcies

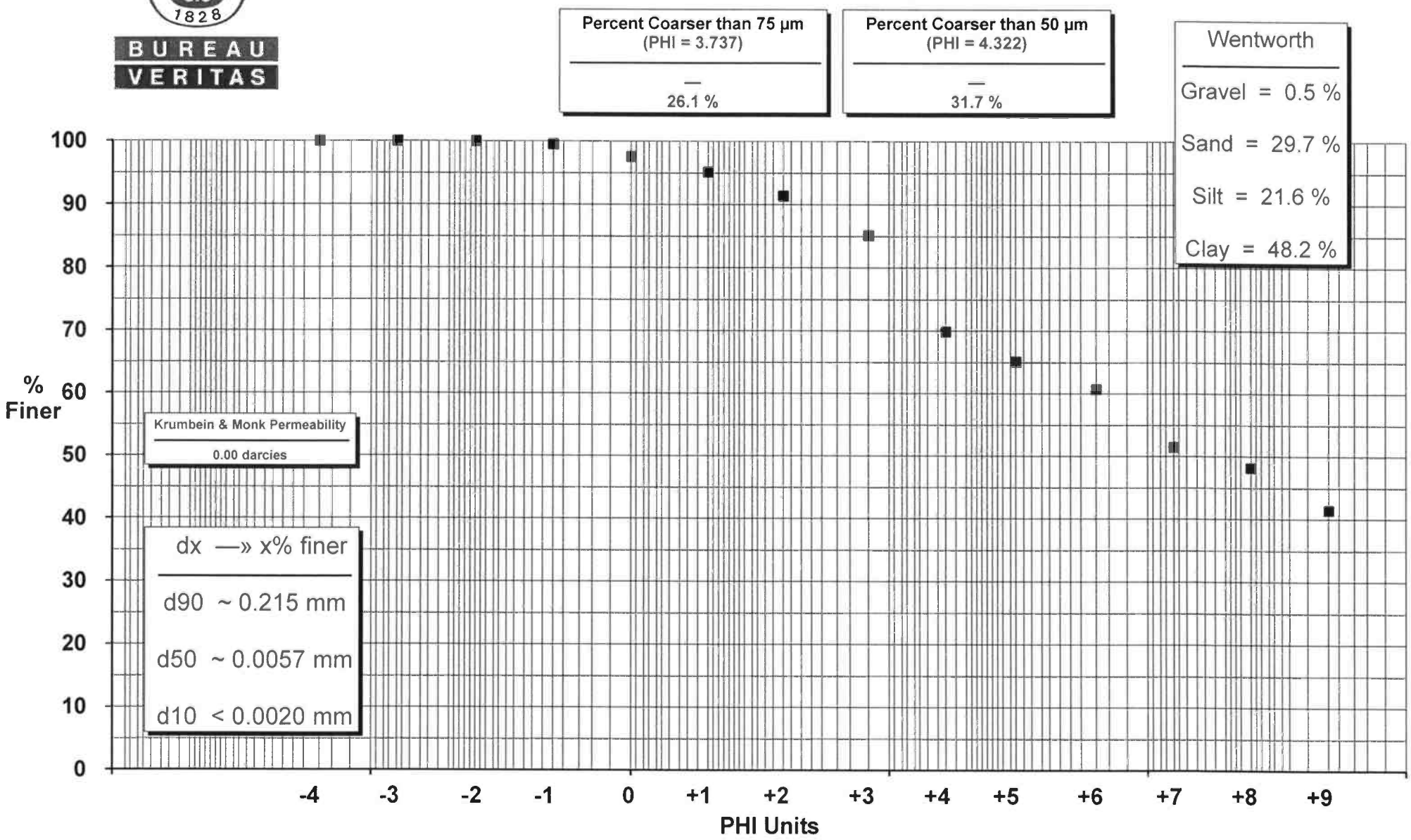
dx —» x% finer	
d90	~ 0.243 mm
d50	~ 0.055 mm
d10	< 0.0020 mm

BAN

Approved

BV Labs ID: XCF564-01

PINR-EXP2-1



BAN

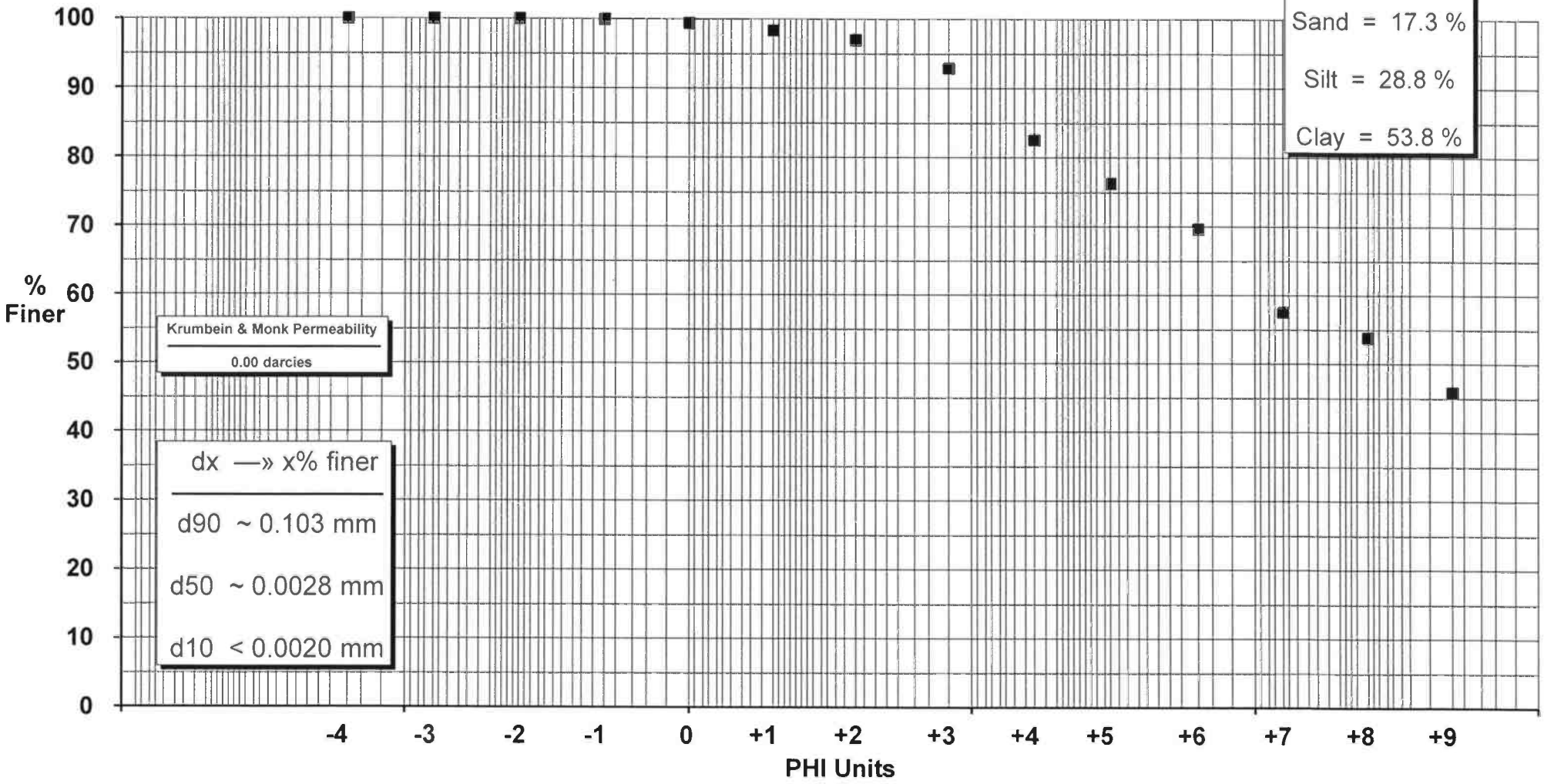
Approved

BV Labs ID: XCF565-01

PINR-EXP2-2

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
14.7 %	19.5 %

Wentworth	
Gravel	0.1 %
Sand	17.3 %
Silt	28.8 %
Clay	53.8 %



Krumbein & Monk Permeability: 0.00 darcies

dx —» x% finer	
d90	~ 0.103 mm
d50	~ 0.0028 mm
d10	< 0.0020 mm

BAN

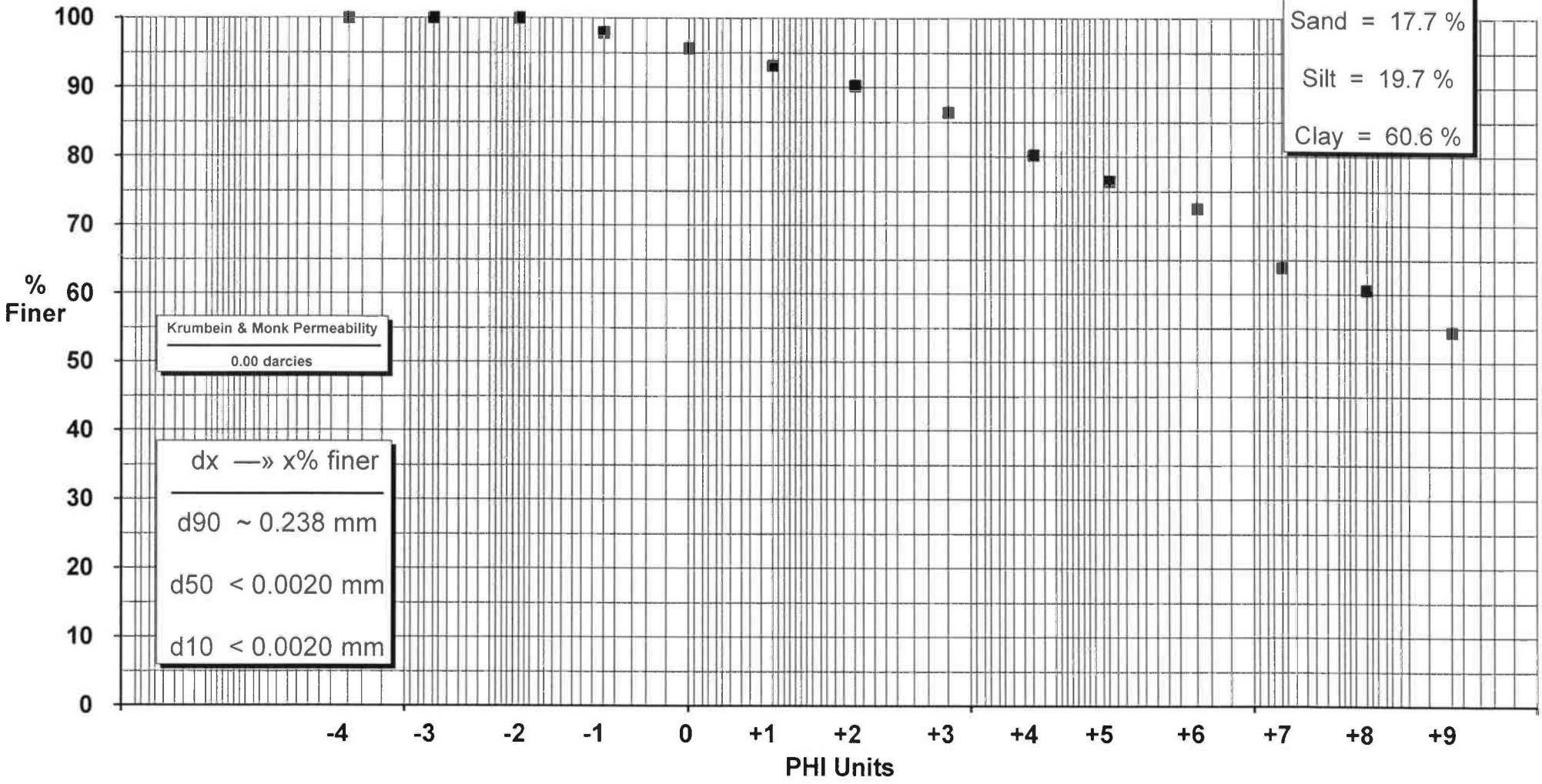
Approved

BV Labs ID: XCF566-01

PINR-EXP2-3

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
18.1 %	21.0 %

Wentworth	
Gravel	2.1 %
Sand	17.7 %
Silt	19.7 %
Clay	60.6 %



Krumbein & Monk Permeability: 0.00 darcies

dx —» x% finer	
d90	~ 0.238 mm
d50	< 0.0020 mm
d10	< 0.0020 mm

BAN

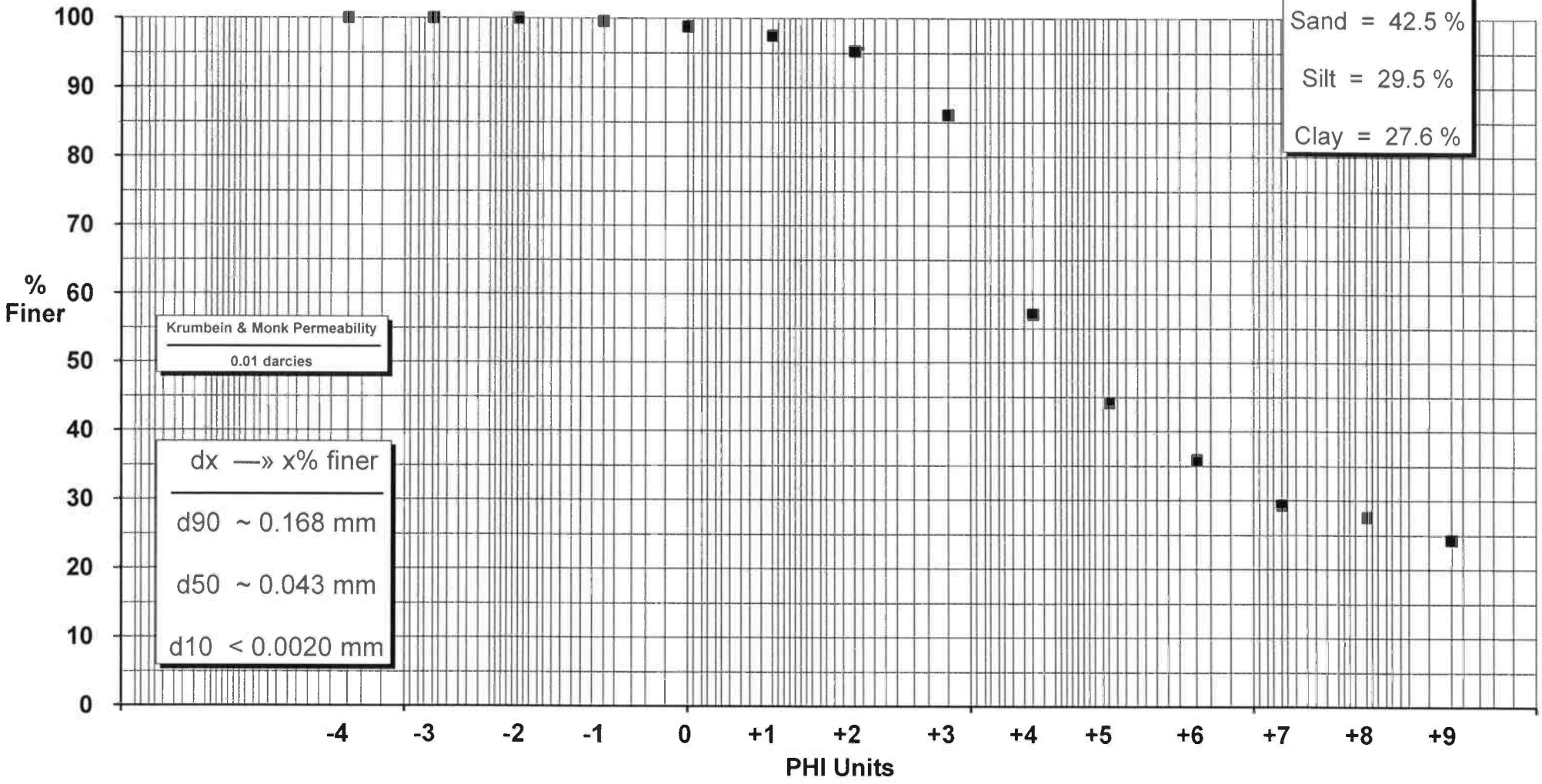
Approved

BV Labs ID: XCF567-01

PINR-EXP2-4

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
35.3 %	47.1 %

Wentworth	
Gravel	0.4 %
Sand	42.5 %
Silt	29.5 %
Clay	27.6 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer	
d90	~ 0.168 mm
d50	~ 0.043 mm
d10	< 0.0020 mm

BAN

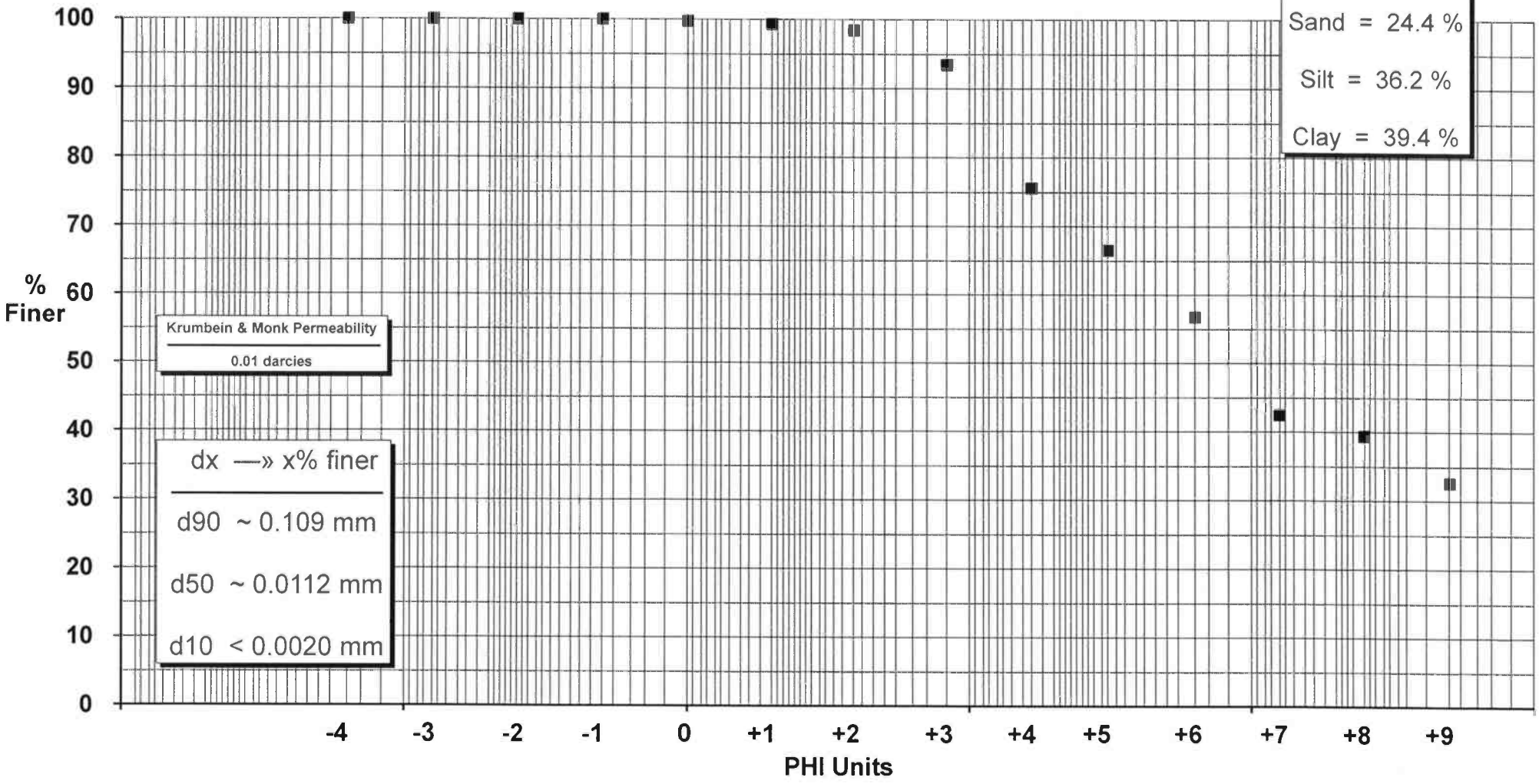
Approved

BV Labs ID: XCF568-01

PINR-EXP2-5

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
19.7 %	27.3 %

Wentworth	
Gravel	0.0 %
Sand	24.4 %
Silt	36.2 %
Clay	39.4 %



Krumbein & Monk Permeability
0.01 darcies

dx —» x% finer
d90 ~ 0.109 mm
d50 ~ 0.0112 mm
d10 < 0.0020 mm

BAN

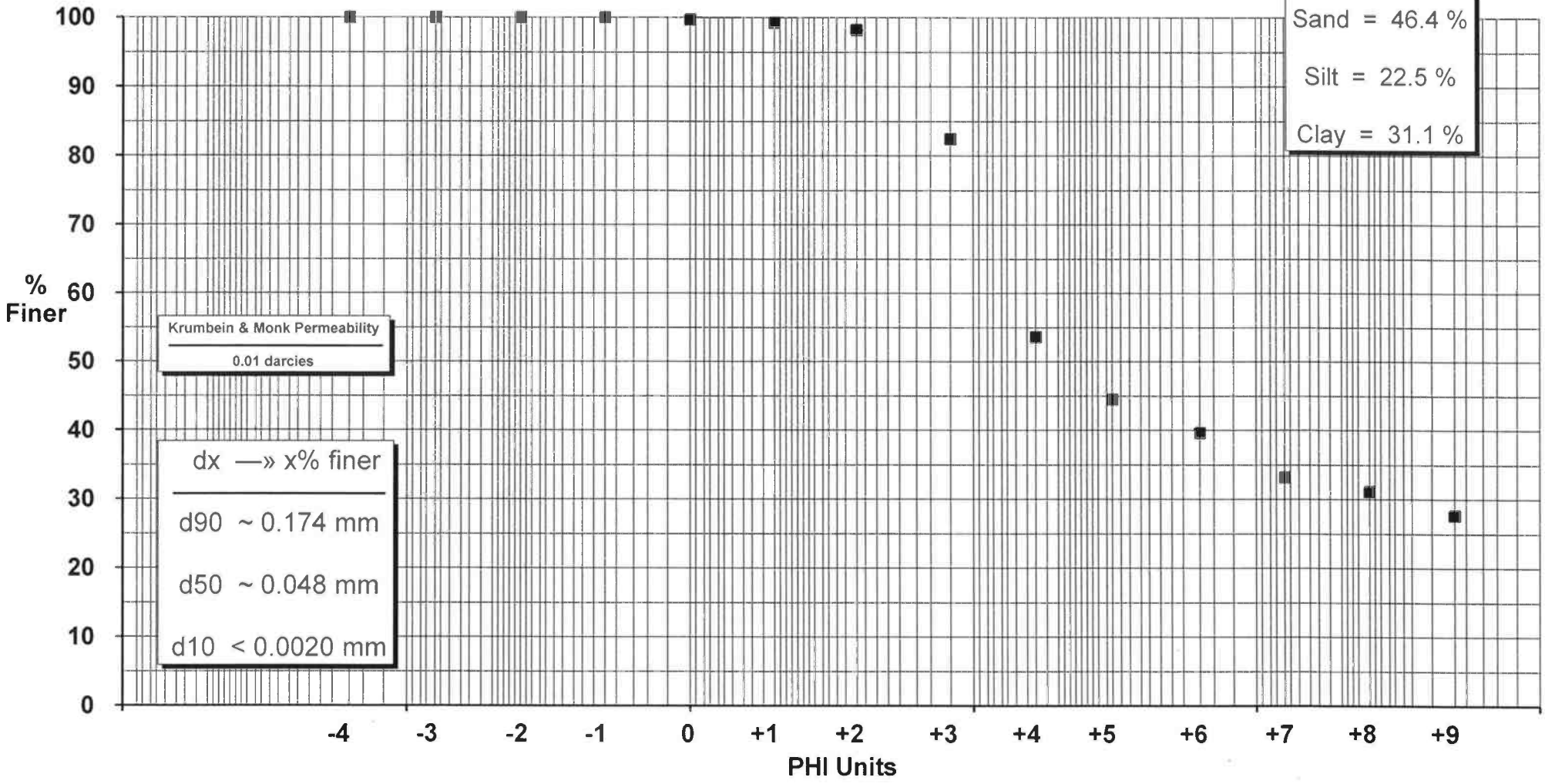
Approved

BV Labs ID: XCF569-01

PINR-REF2-1

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
38.8 %	49.3 %

Wentworth	
Gravel	0.0 %
Sand	46.4 %
Silt	22.5 %
Clay	31.1 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer	
d90	~ 0.174 mm
d50	~ 0.048 mm
d10	< 0.0020 mm

BAN

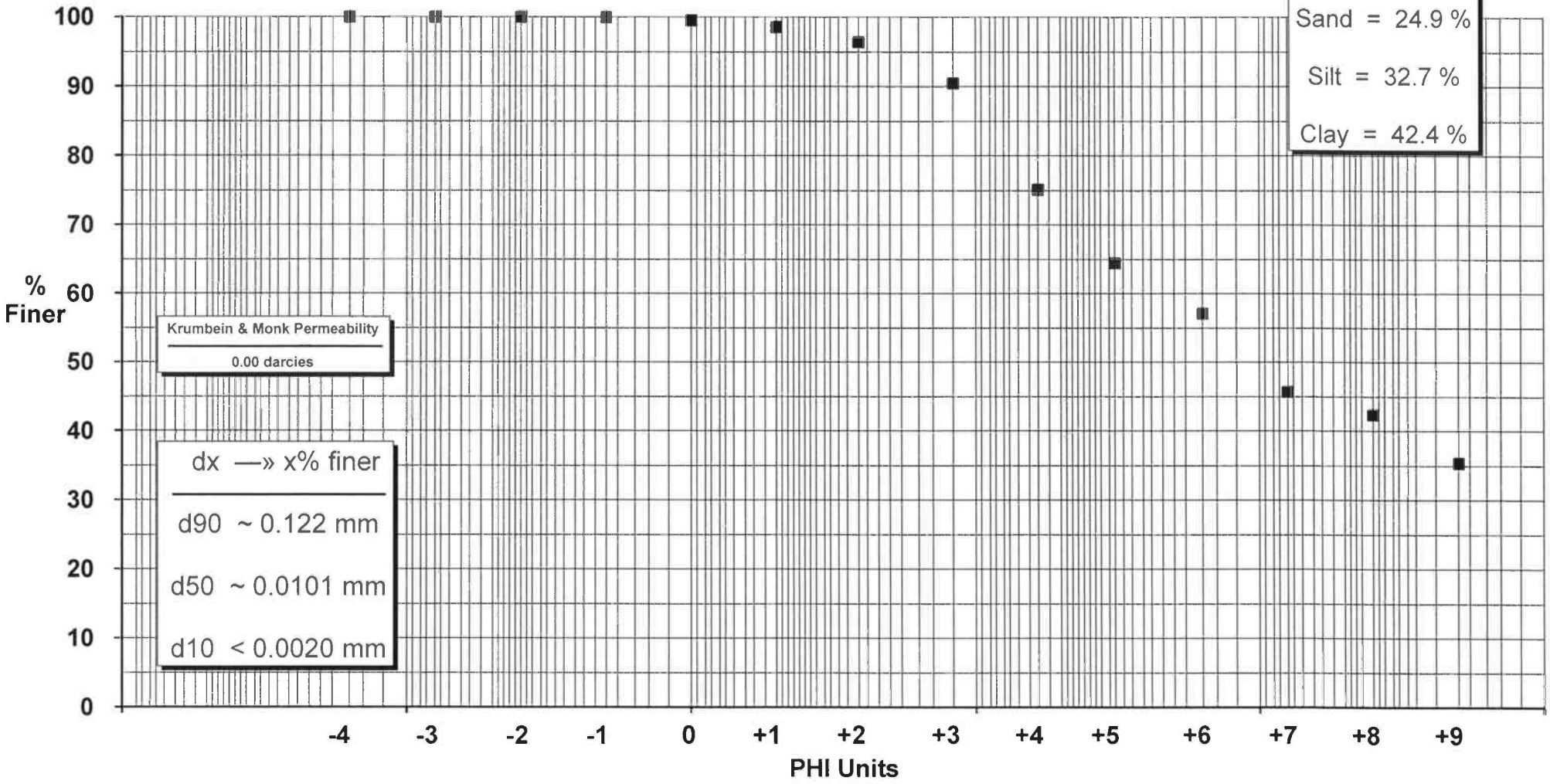
Approved

BV Labs ID: XCF571-01

PINR-REF2-2

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
20.9 %	28.3 %

Wentworth	
Gravel	0.0 %
Sand	24.9 %
Silt	32.7 %
Clay	42.4 %



Krumbein & Monk Permeability
0.00 darcies

dx —» x% finer	
d90	~ 0.122 mm
d50	~ 0.0101 mm
d10	< 0.0020 mm

BAN

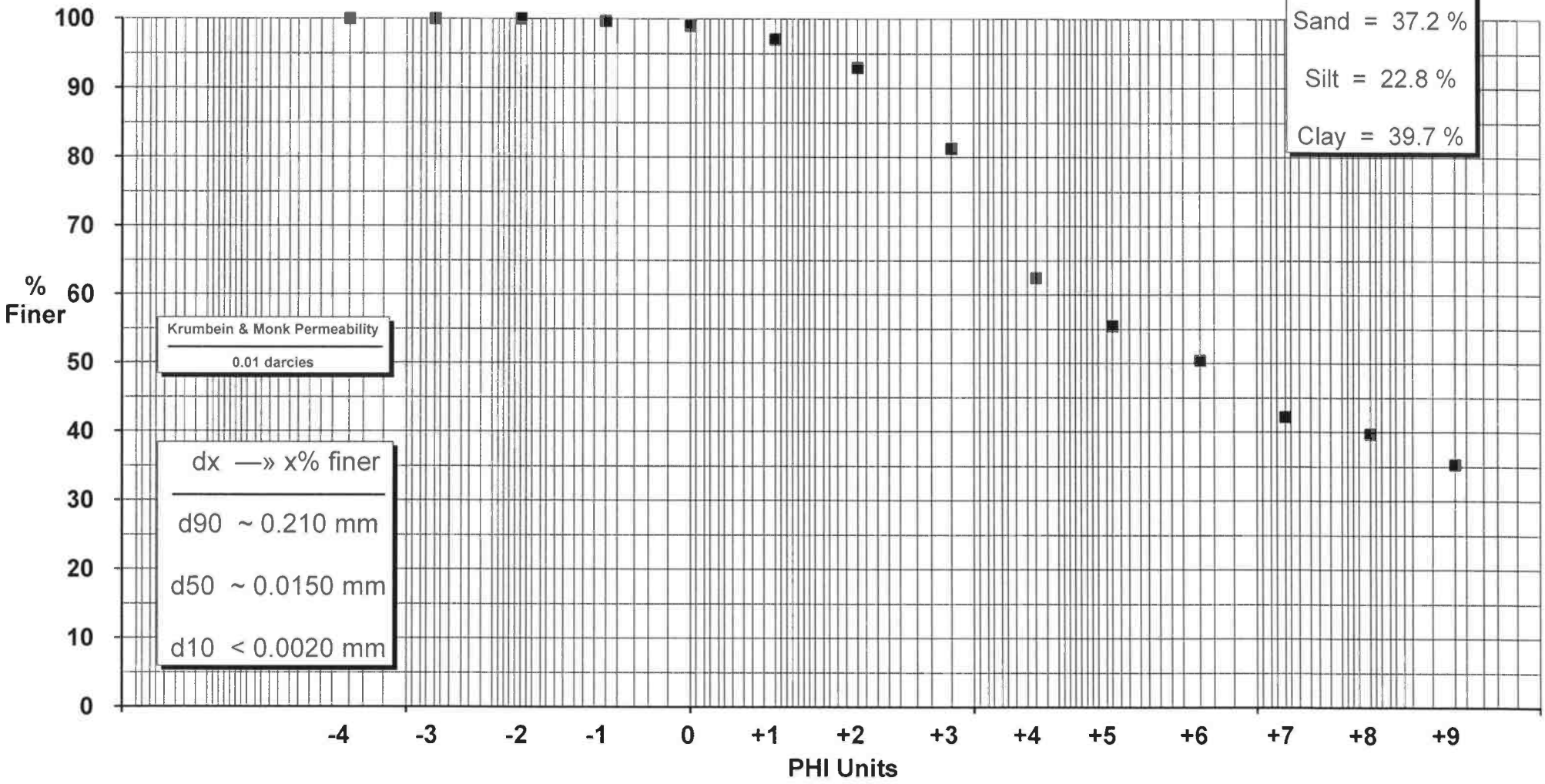
Approved

BV Labs ID: XCF573-01

PINR-REF2-3

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
32.6 %	39.8 %

Wentworth	
Gravel	0.3 %
Sand	37.2 %
Silt	22.8 %
Clay	39.7 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer	
d90	~ 0.210 mm
d50	~ 0.0150 mm
d10	< 0.0020 mm

BAN

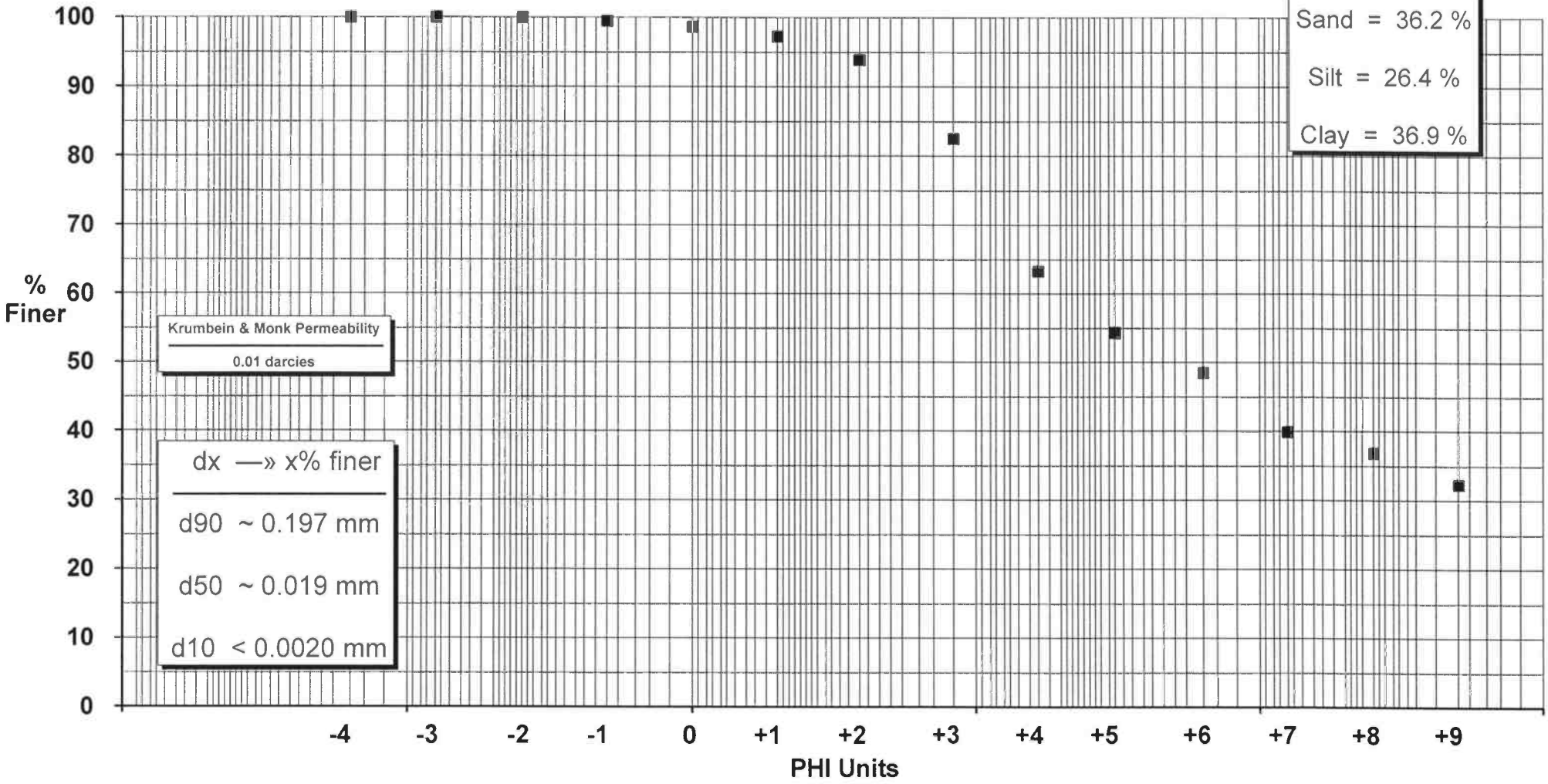
Approved

BV Labs ID: XCF576-01

PINR-REF2-4

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
—	—
31.6 %	39.6 %

Wentworth
Gravel = 0.5 %
Sand = 36.2 %
Silt = 26.4 %
Clay = 36.9 %



Krumbein & Monk Permeability
0.01 darcies

dx —» x% finer
d90 ~ 0.197 mm
d50 ~ 0.019 mm
d10 < 0.0020 mm

BAN

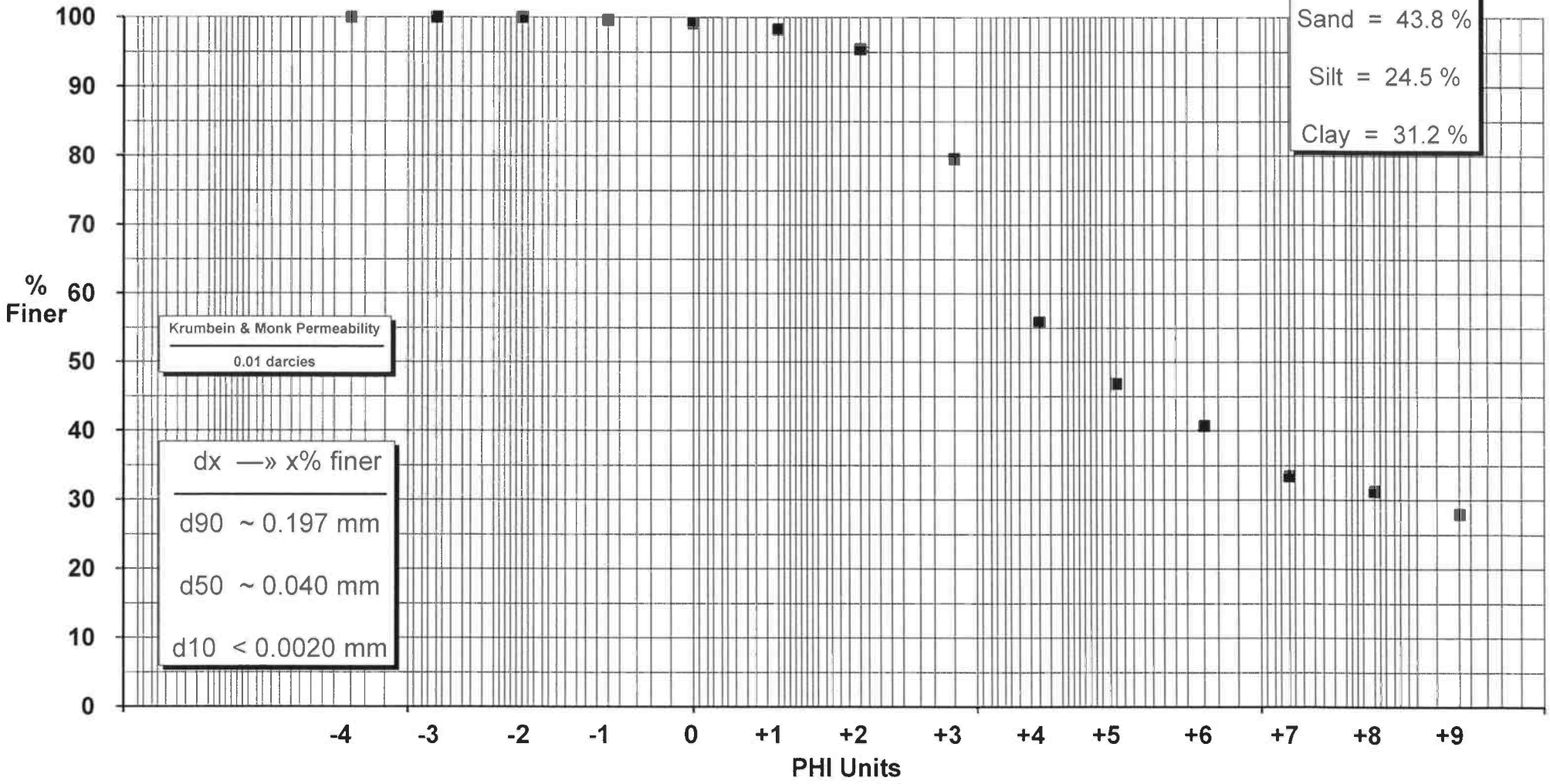
Approved

BV Labs ID: XCF578-01

PINR-REF2-5

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
37.9 %	47.1 %

Wentworth	
Gravel	0.4 %
Sand	43.8 %
Silt	24.5 %
Clay	31.2 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer	
d90	~ 0.197 mm
d50	~ 0.040 mm
d10	< 0.0020 mm

BAN

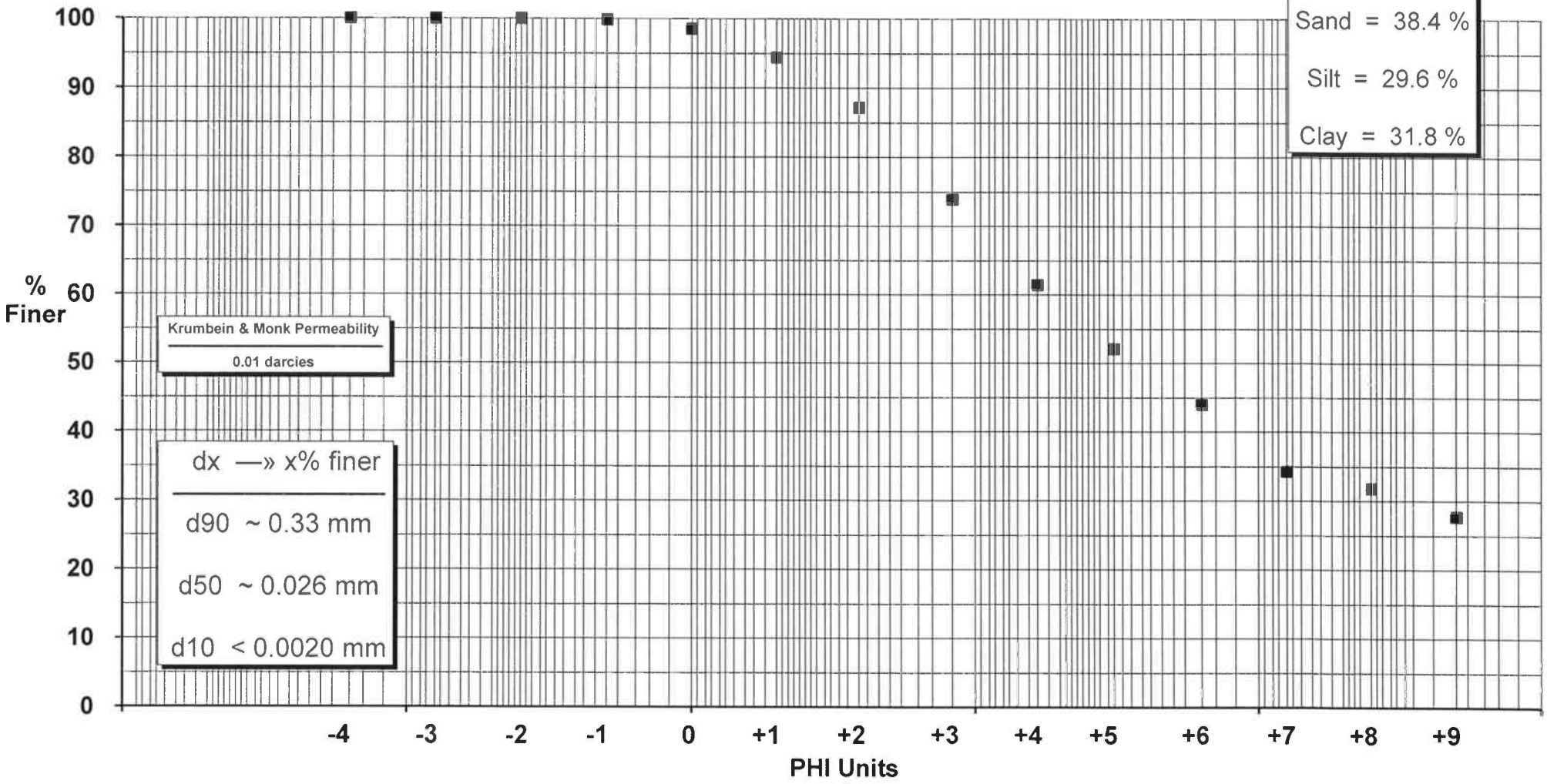
Approved

BV Labs ID: XCF665-01

LVR2-REF1

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
35.3 %	41.6 %

Wentworth	
Gravel	0.1 %
Sand	38.4 %
Silt	29.6 %
Clay	31.8 %



Krumbein & Monk Permeability: 0.01 darcies

dx —» x% finer	
d90	~ 0.33 mm
d50	~ 0.026 mm
d10	< 0.0020 mm

BAN

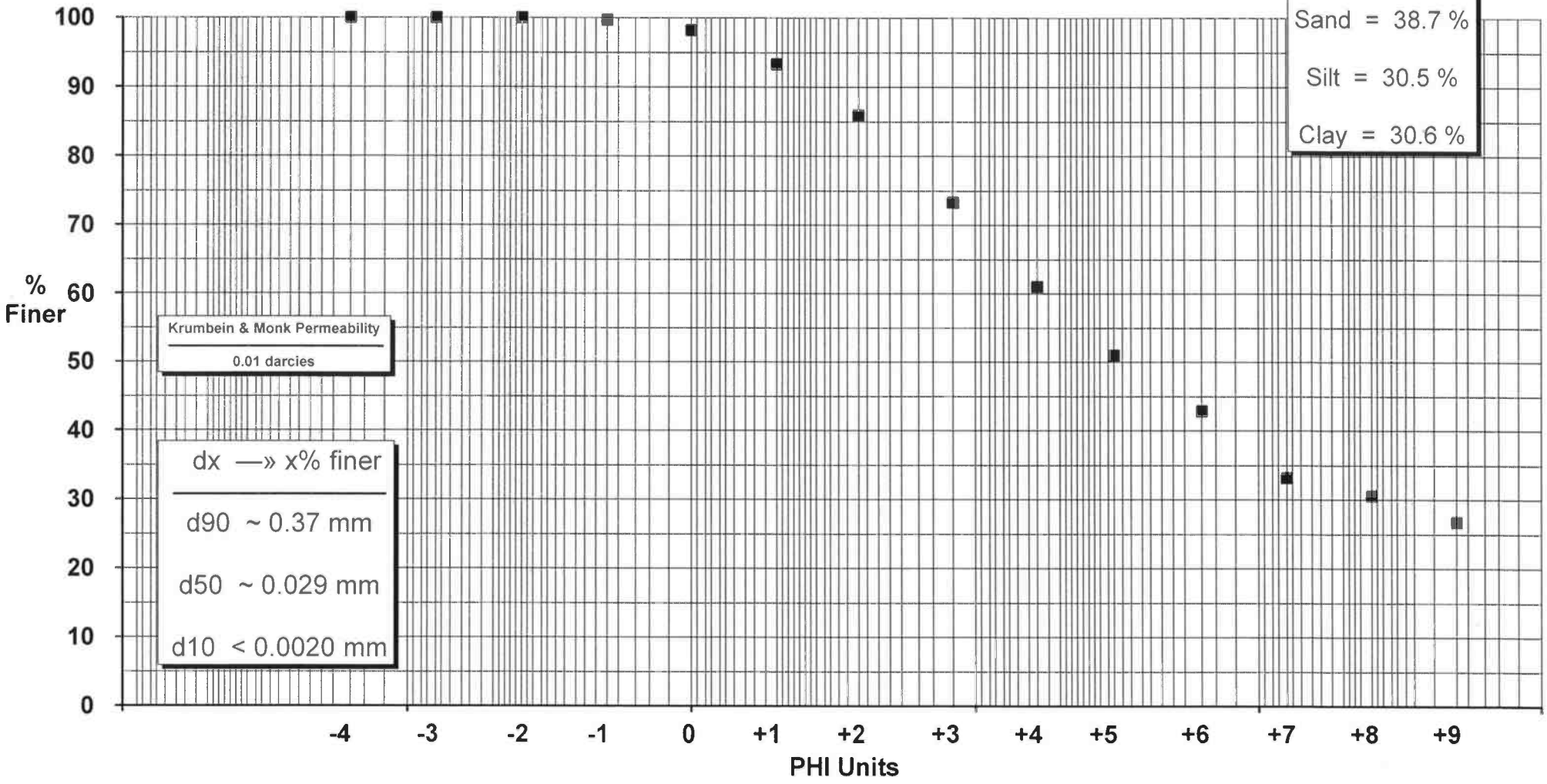
Approved

BV Labs ID: XCF665-01:D1

LVR2-REF1

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
35.7 %	42.2 %

Wentworth	
Gravel	0.3 %
Sand	38.7 %
Silt	30.5 %
Clay	30.6 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer	
d90	~ 0.37 mm
d50	~ 0.029 mm
d10	< 0.0020 mm

BAN

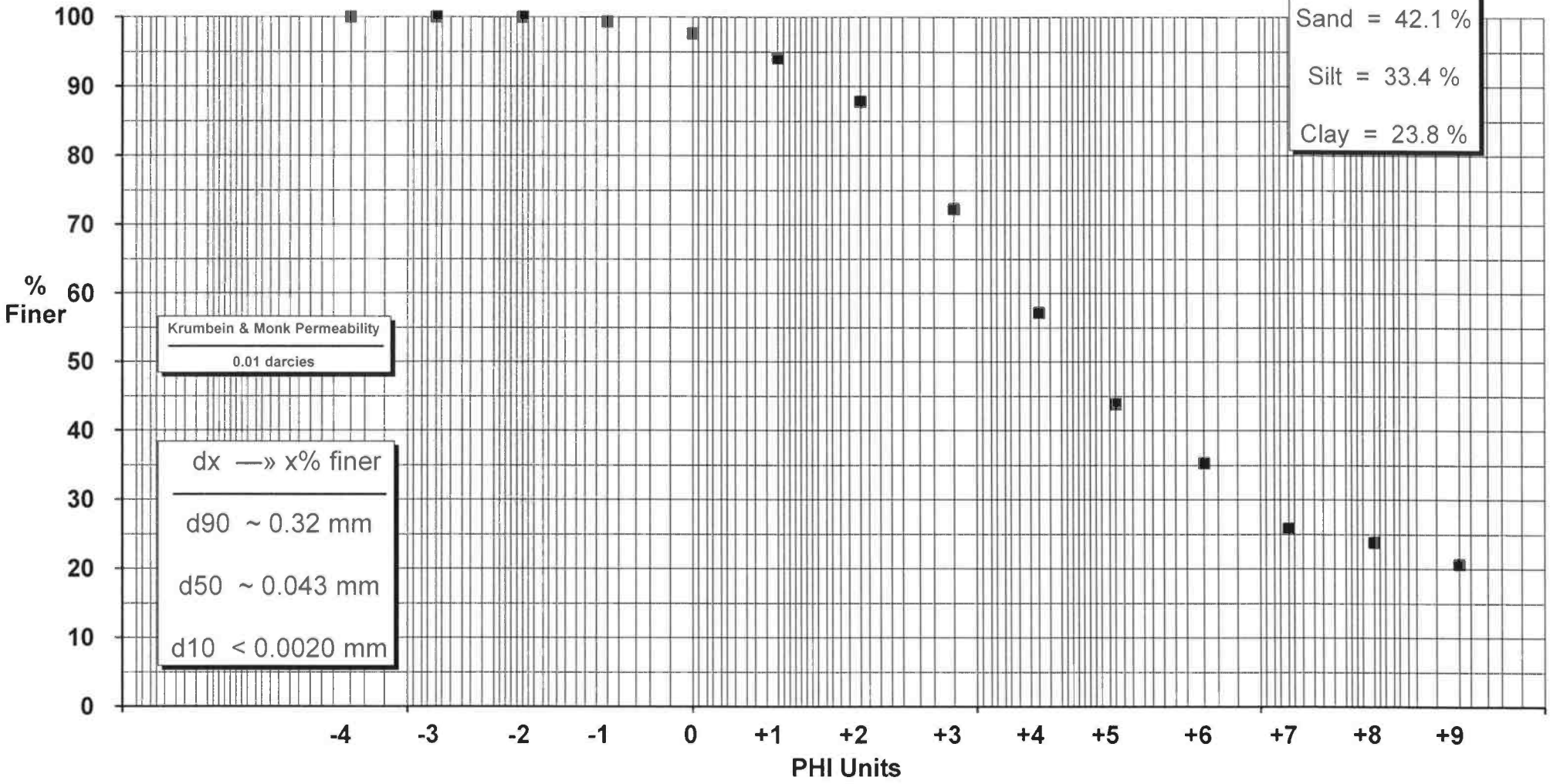
Approved

BV Labs ID: XCF666-01

LVR2-REF1 DUPLICATE

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
38.8 %	47.1 %

Wentworth	
Gravel	0.7 %
Sand	42.1 %
Silt	33.4 %
Clay	23.8 %



Krumbein & Monk Permeability
0.01 darcies

dx —» x% finer	
d90	~ 0.32 mm
d50	~ 0.043 mm
d10	< 0.0020 mm

BAN

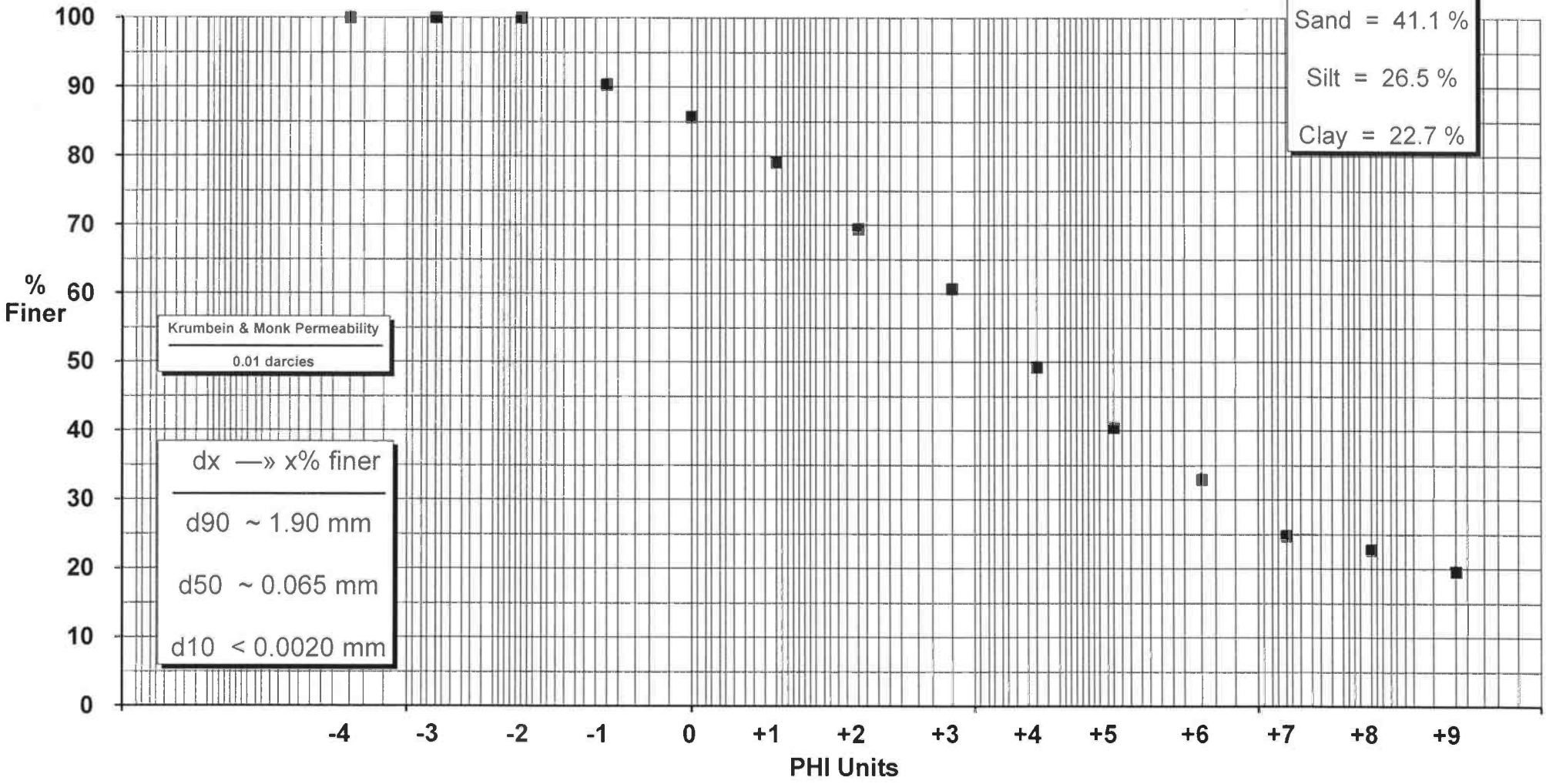
Approved

BV Labs ID: XCF667-01

LVR2-REF2

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
47.7 %	53.6 %

Wentworth	
Gravel	9.7 %
Sand	41.1 %
Silt	26.5 %
Clay	22.7 %



Krumbein & Monk Permeability
0.01 darcies

dx —» x% finer	
d90	~ 1.90 mm
d50	~ 0.065 mm
d10	< 0.0020 mm

BAN

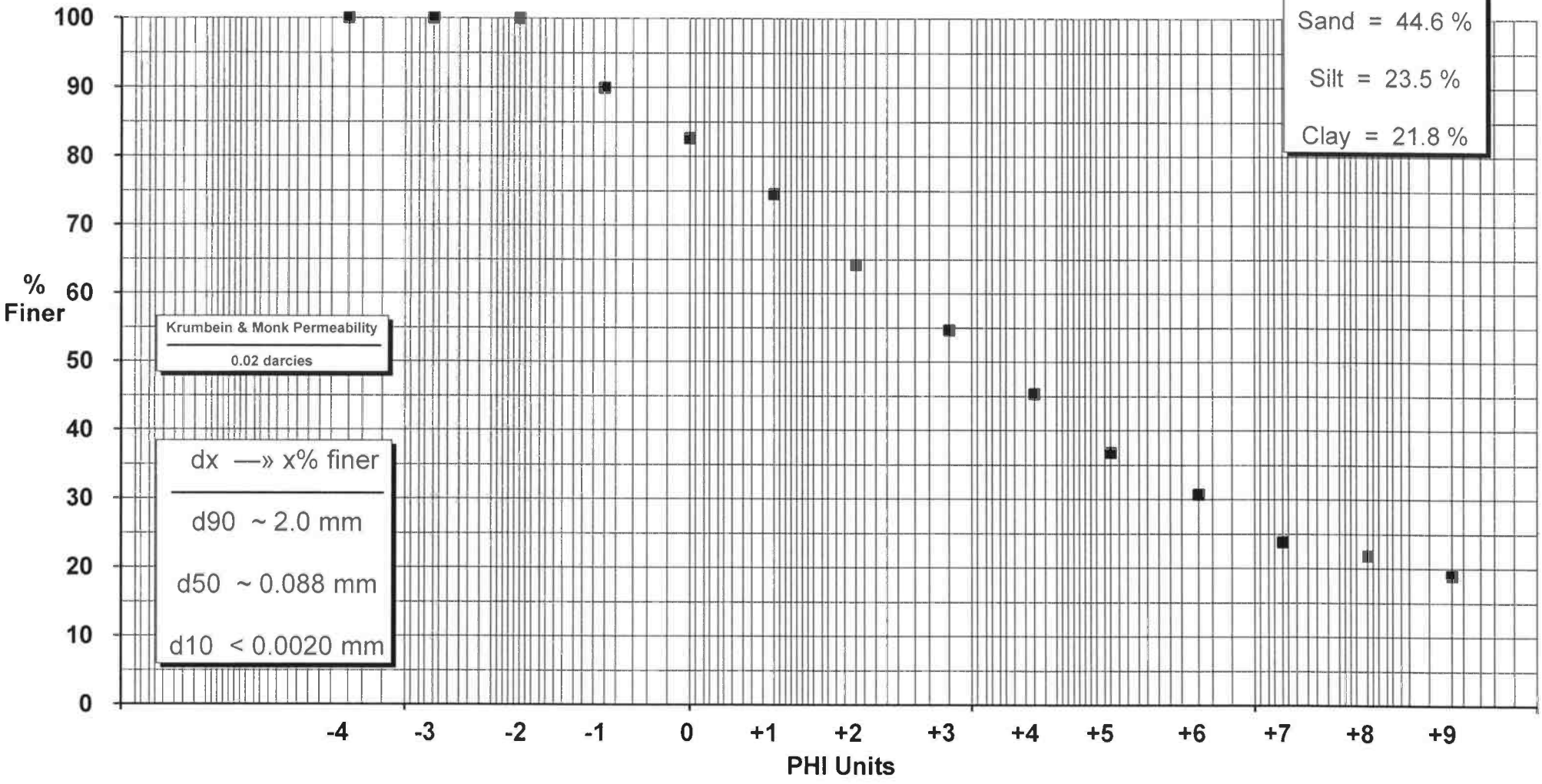
Approved

BV Labs ID: XCF668-01

LVR2-REF3

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
52.2 %	57.4 %

Wentworth	
Gravel	10.1 %
Sand	44.6 %
Silt	23.5 %
Clay	21.8 %



Krumbein & Monk Permeability
0.02 darcies

dx —» x% finer	
d90	~ 2.0 mm
d50	~ 0.088 mm
d10	< 0.0020 mm

BAN

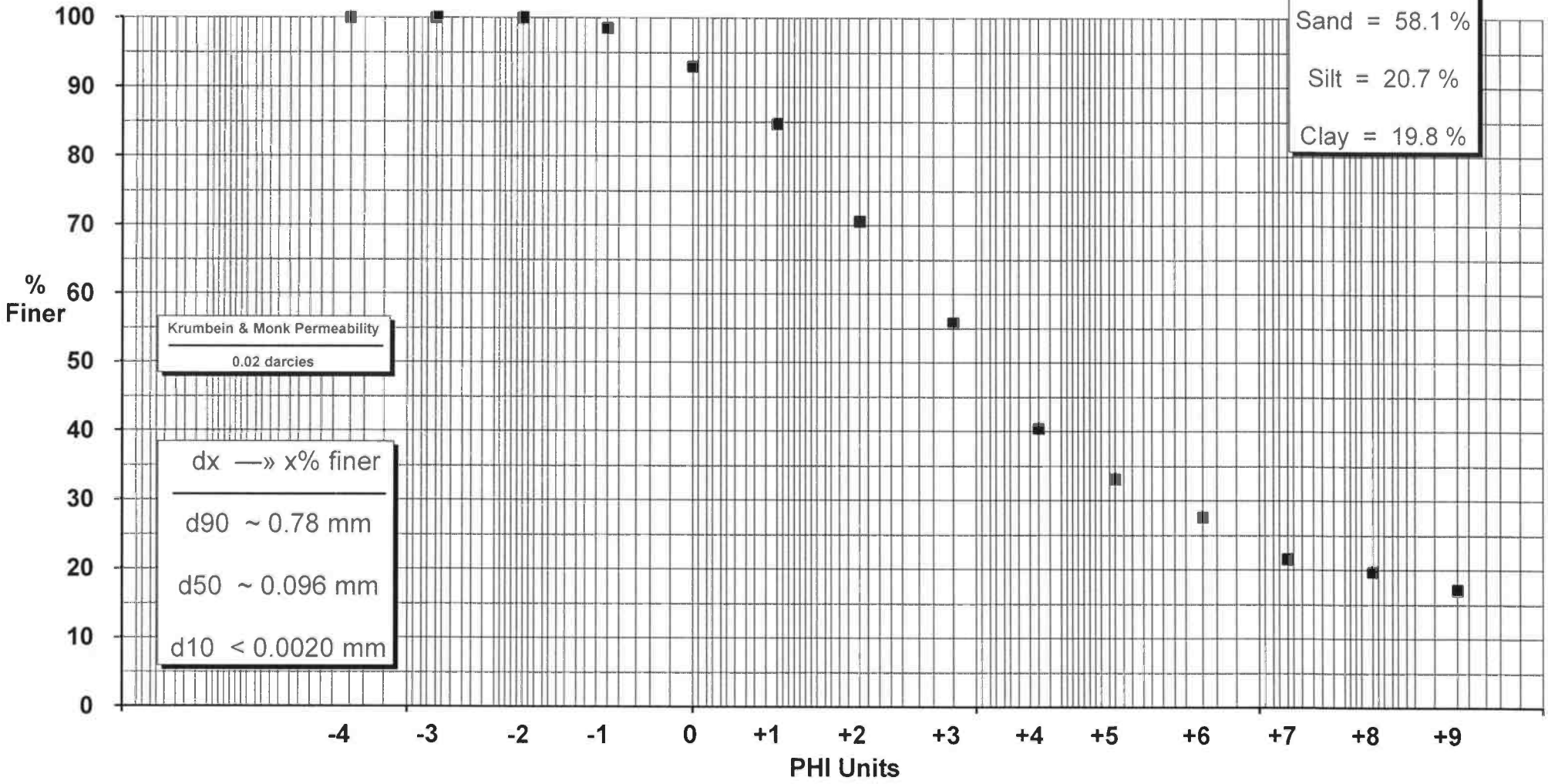
Approved

BV Labs ID: XCF669-01

LVR2-REF4

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
55.5 %	61.9 %

Wentworth	
Gravel	1.4 %
Sand	58.1 %
Silt	20.7 %
Clay	19.8 %



Krumbein & Monk Permeability: 0.02 darcies

dx —» x% finer	
d90	~ 0.78 mm
d50	~ 0.096 mm
d10	< 0.0020 mm

BAN

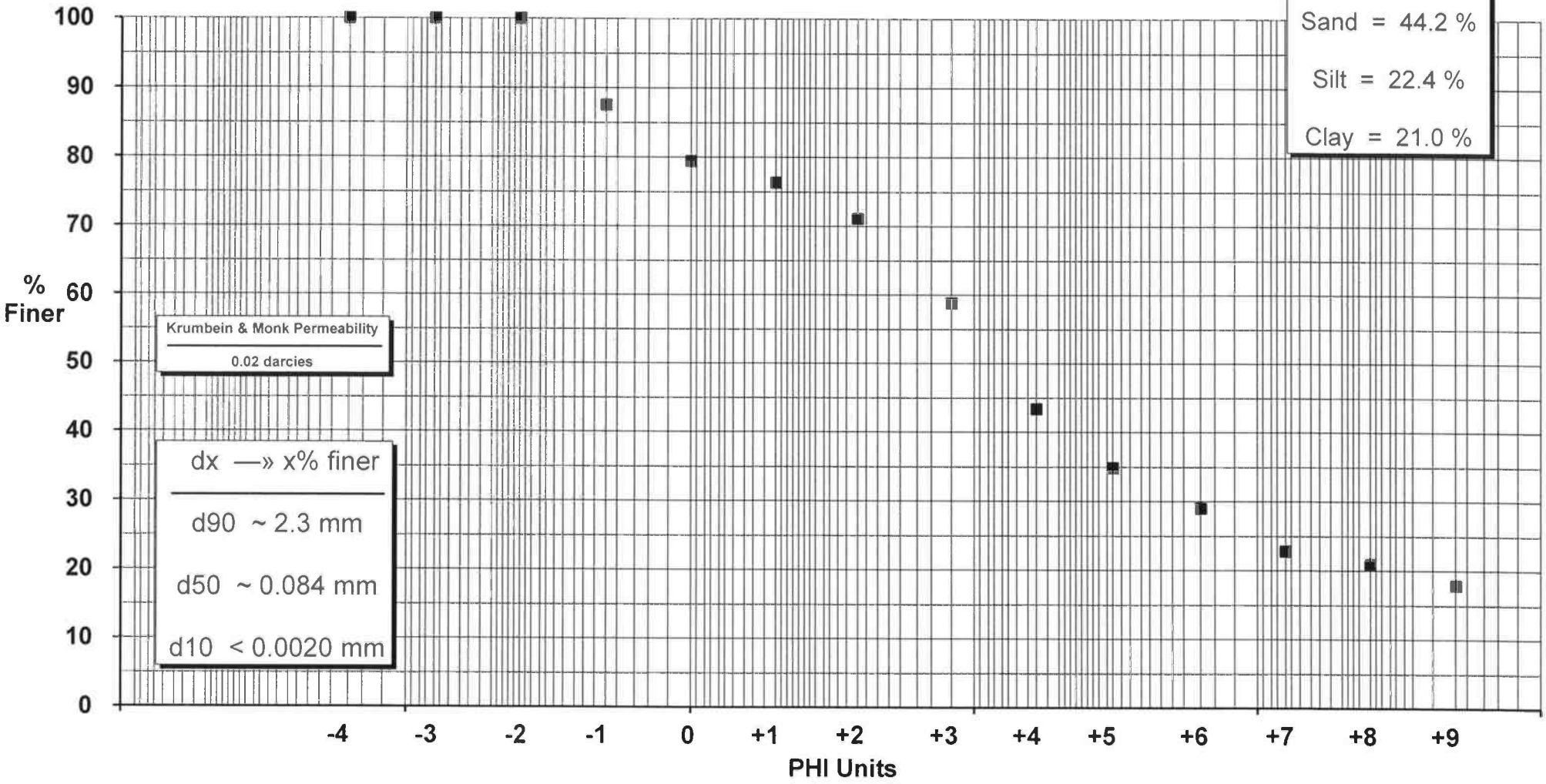
Approved

BV Labs ID: XCF670-01

LVR2-REF5

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
52.6 %	59.4 %

Wentworth	
Gravel	12.4 %
Sand	44.2 %
Silt	22.4 %
Clay	21.0 %



Krumbein & Monk Permeability: 0.02 darcies

dx —» x% finer	
d90	~ 2.3 mm
d50	~ 0.084 mm
d10	< 0.0020 mm

BAN

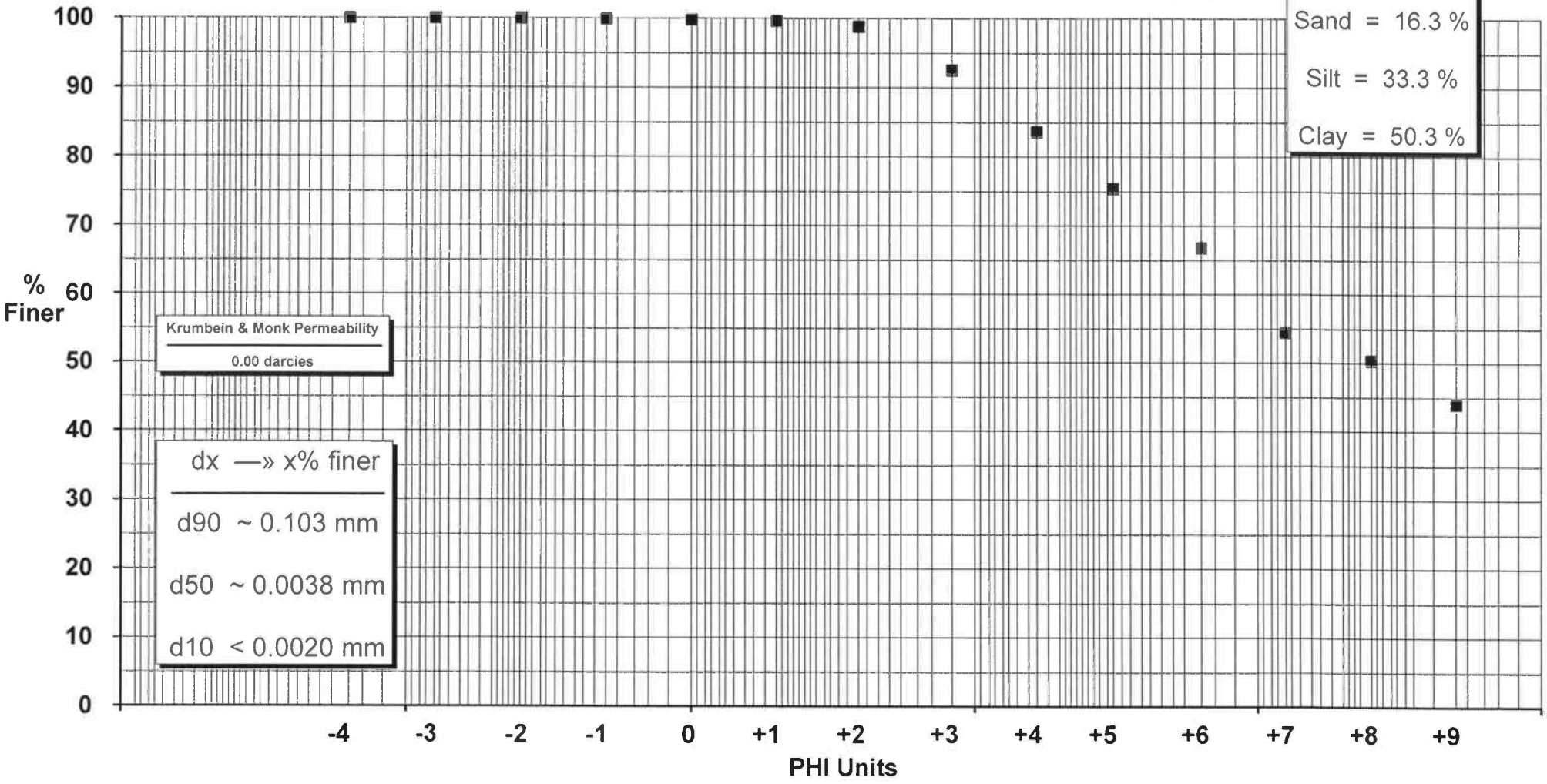
Approved

BV Labs ID: XCF671-01

LVR-REF2

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
—	—
14.0 %	19.0 %

Wentworth	
Gravel	0.1 %
Sand	16.3 %
Silt	33.3 %
Clay	50.3 %



Krumbein & Monk Permeability

0.00 darcies

dx —» x% finer	
d90	~ 0.103 mm
d50	~ 0.0038 mm
d10	< 0.0020 mm

BAN

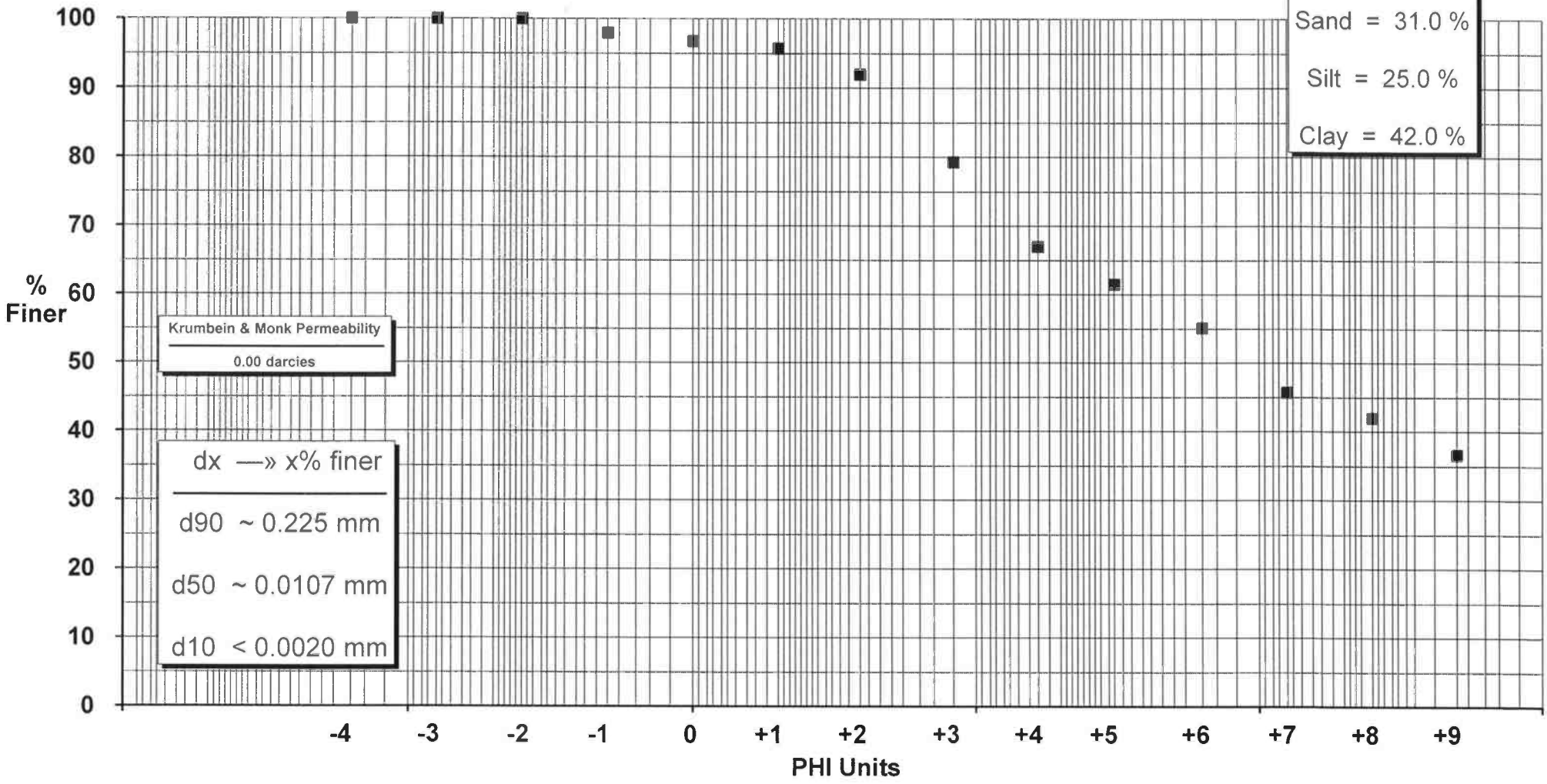
Approved

BV Labs ID: XCF672-01

LVR-REF3

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
—	—
29.8 %	34.8 %

Wentworth	
Gravel	2.1 %
Sand	31.0 %
Silt	25.0 %
Clay	42.0 %



Krumbein & Monk Permeability
0.00 darcies

dx —» x% finer
d90 ~ 0.225 mm
d50 ~ 0.0107 mm
d10 < 0.0020 mm

BAN

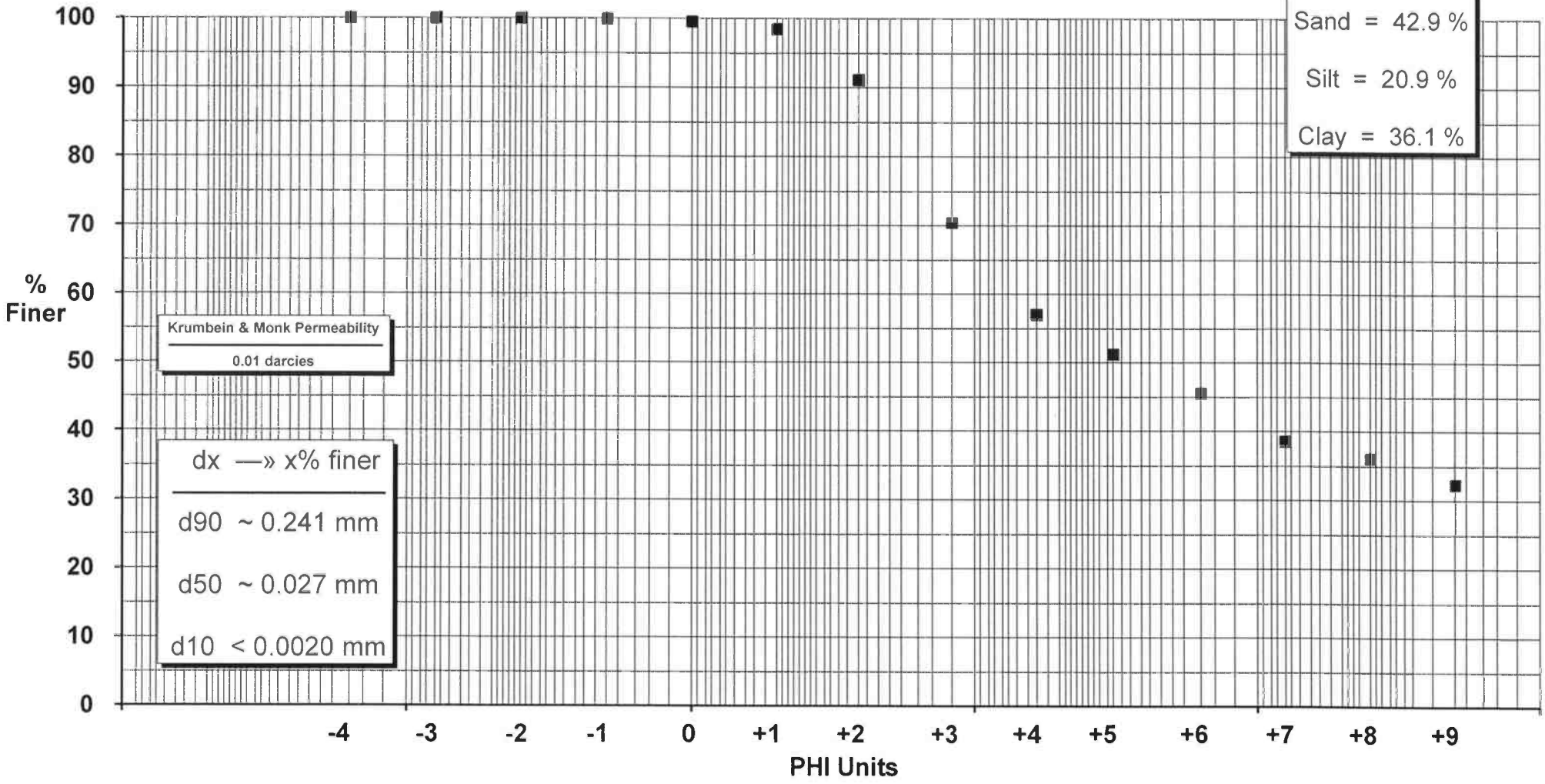
Approved

BV Labs ID: XCF673-01

LVR-REF4

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
39.5 %	44.9 %

Wentworth	
Gravel	0.1 %
Sand	42.9 %
Silt	20.9 %
Clay	36.1 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer	
d90	~ 0.241 mm
d50	~ 0.027 mm
d10	< 0.0020 mm

BAN

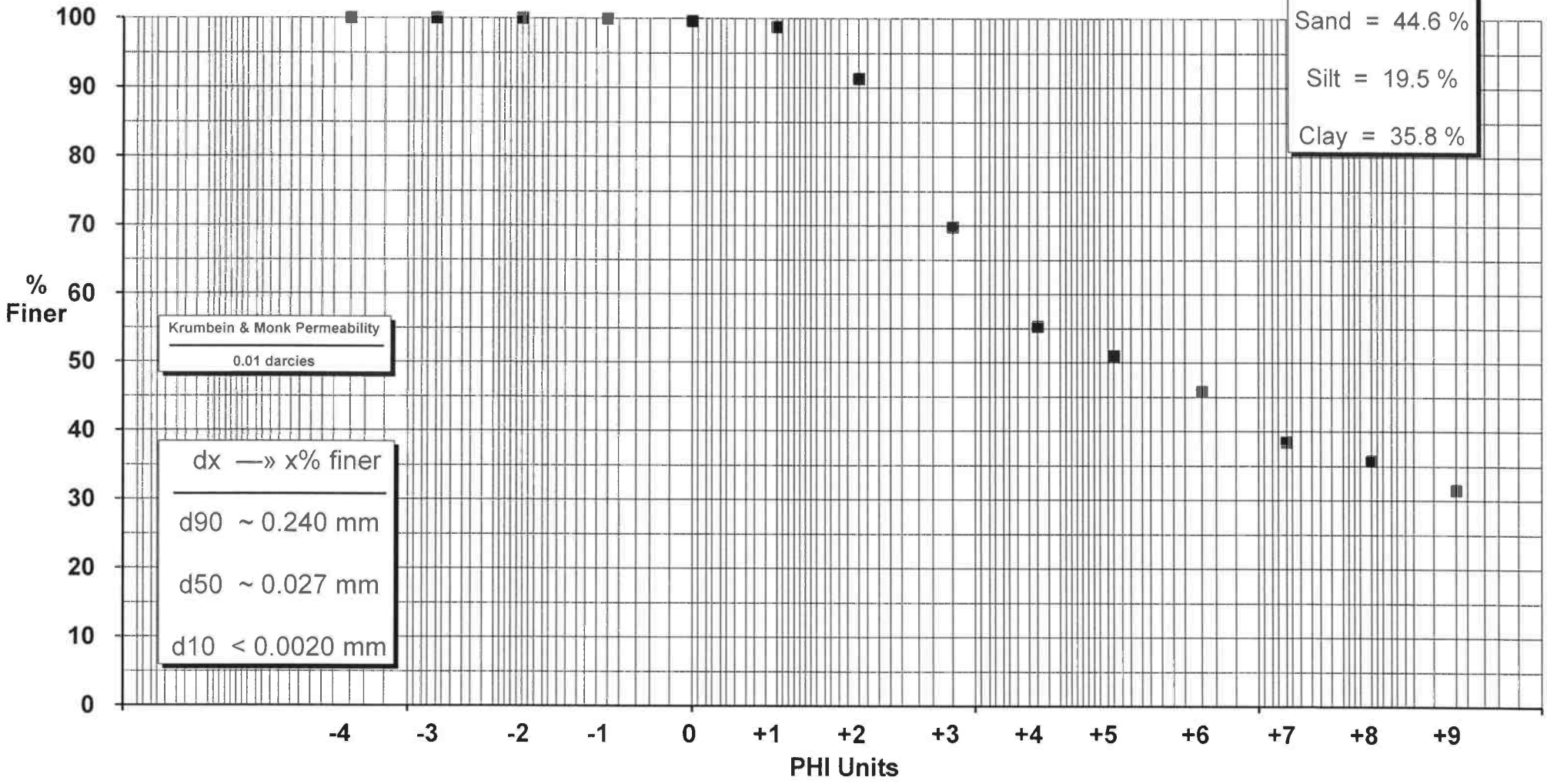
Approved

BV Labs ID: XCF674-01

LVR-REF5

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
40.9 %	46.1 %

Wentworth	
Gravel	0.1 %
Sand	44.6 %
Silt	19.5 %
Clay	35.8 %



Krumbein & Monk Permeability
0.01 darcies

dx —» x% finer	
d90	~ 0.240 mm
d50	~ 0.027 mm
d10	< 0.0020 mm

BAN

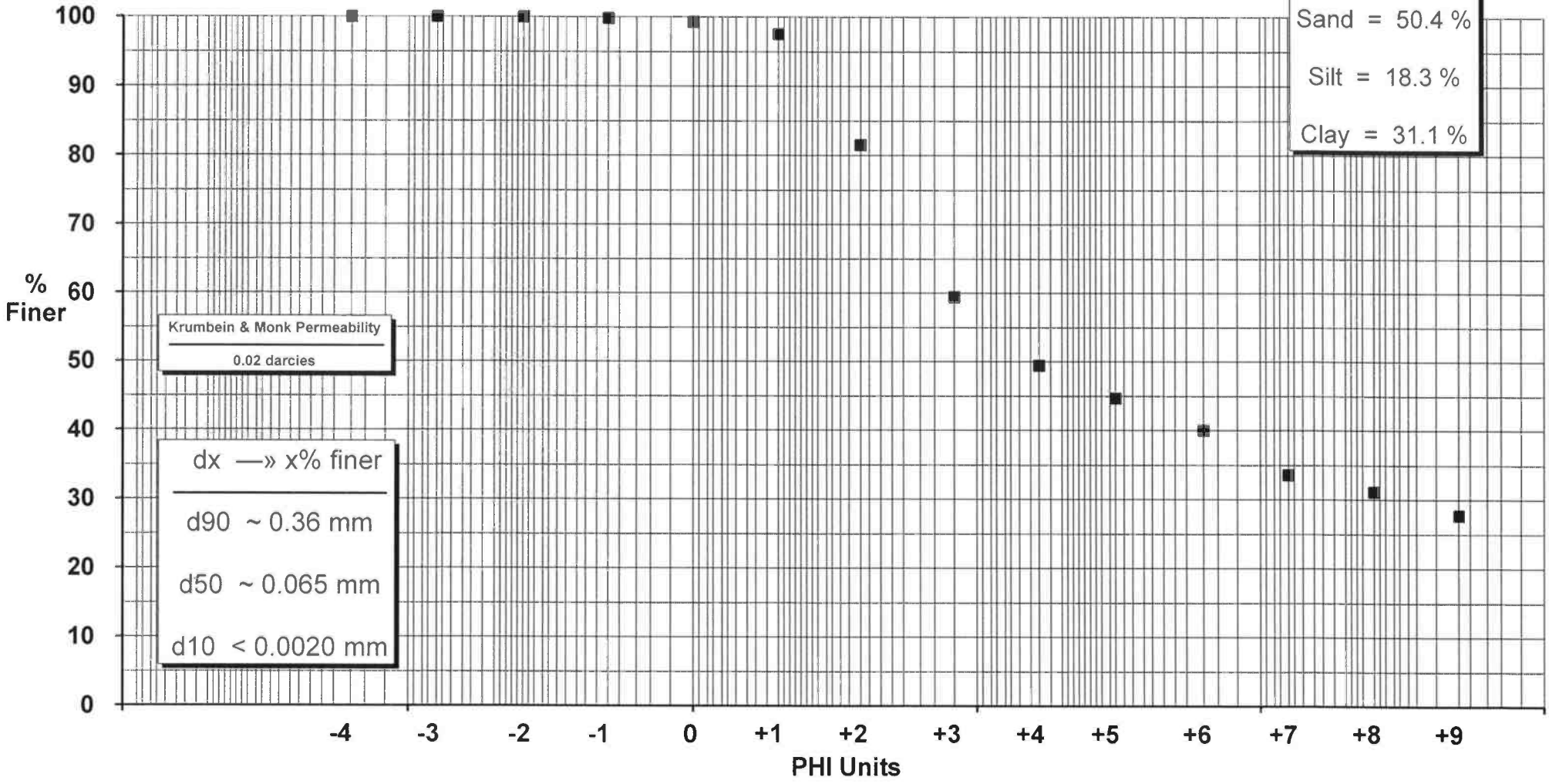
Approved

BV Labs ID: XCF675-01

LVR-REF5 DUPLICATE

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
—	—
48.0 %	52.1 %

Wentworth	
Gravel	0.2 %
Sand	50.4 %
Silt	18.3 %
Clay	31.1 %



Krumbein & Monk Permeability
0.02 darcies

dx —» x% finer
d90 ~ 0.36 mm
d50 ~ 0.065 mm
d10 < 0.0020 mm

BAN

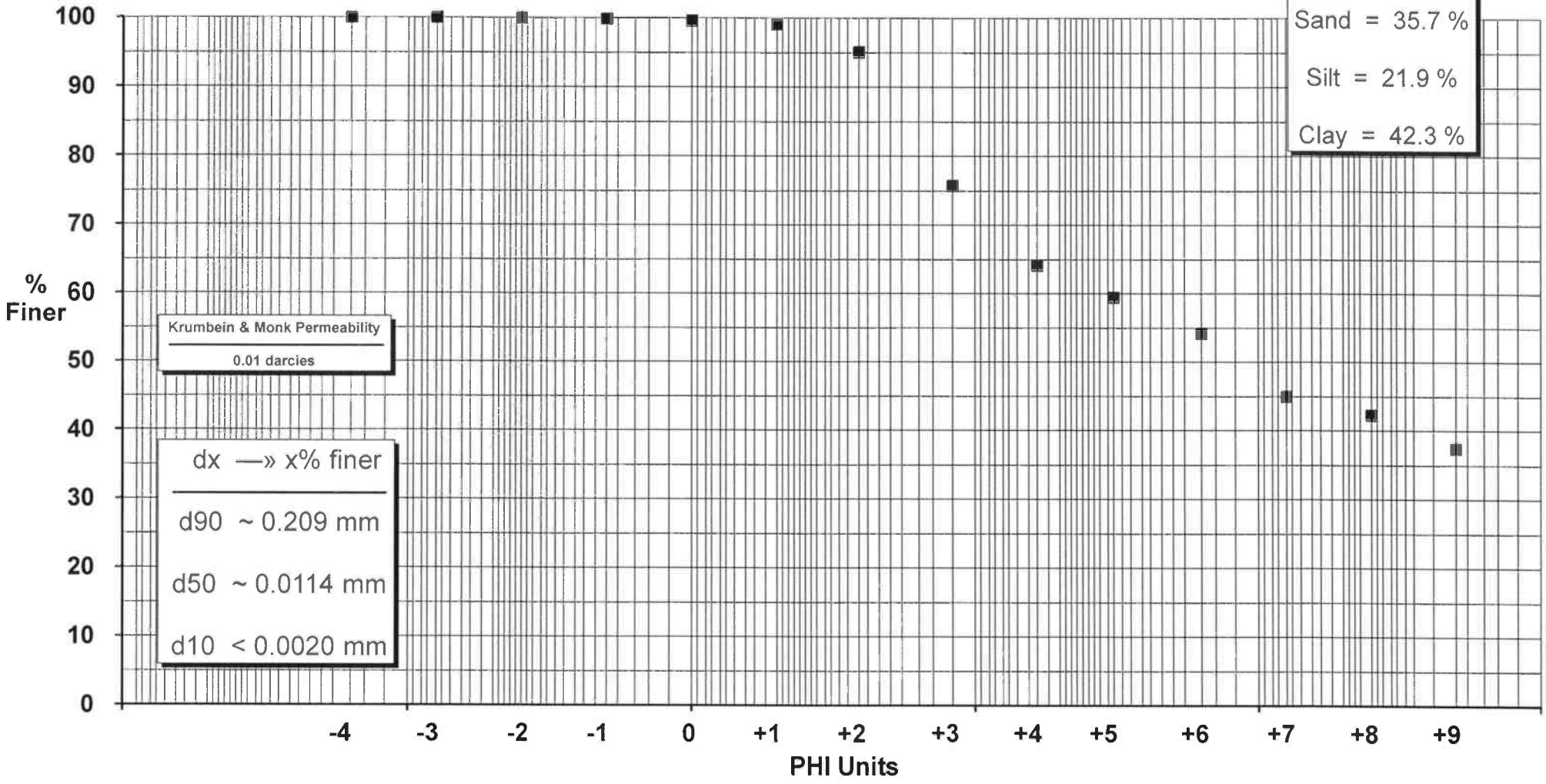
Approved

BV Labs ID: XCO572-01

LVR-REF1

Percent Coarser than 75 µm (PHI = 3.737)	Percent Coarser than 50 µm (PHI = 4.322)
32.8 %	37.4 %

Wentworth	
Gravel	0.1 %
Sand	35.7 %
Silt	21.9 %
Clay	42.3 %



Krumbein & Monk Permeability

0.01 darcies

dx —» x% finer	
d90	~ 0.209 mm
d50	~ 0.0114 mm
d10	< 0.0020 mm

BAN

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Appendix C Benthic Invertebrate Survey

Table C-1: Raw benthic invertebrate counts for the Rainy River Mine Phase 3.

Stations		PINR-REF2					PINR-EXP					PINR-EXP2					LVR-REF					LVR2-REF					STUC-REF				
Taxa	Station Numbers	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
ROUNDWORMS																															
P. Nemata		4	1	-	-	-	-	16	288	96	24	32	4	4	-	-	16	-	8	48	32	4	-	-	-	-	96	32	112	160	792
ANNELIDS																															
P. Annelida																															
	WORMS																														
	Cl. Oligochaeta																														
		F. Enchytraeidae	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	4	-	-	-	-	-	-	-
		F. Naididae																													
	S.F. Naidinae																														
		Dero digitata	-	-	-	-	-	-	-	-	-	64	-	-	-	-	16	-	-	80	123	-	-	4	-	-	293	-	192	32	18
		Dero nivea	8	-	-	-	-	-	-	-	16	-	48	20	-	-	4	8	-	8	-	-	-	-	12	-	-	244	64	-	-	-
		Haemonais waldvogeli	-	-	-	-	-	-	PINR	-	-	-	-	-	-	-	-	-	-	-	27	-	-	-	-	-	-	-	16	32	-	18
		Nais variabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27	-	-	-	-	-	-	-	-	-	-	-
		Nais	-	-	-	-	-	-	-	-	16	-	16	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-
		Slavina appendiculata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27	-	-	-	-	-	-	49	-	-	-	18
	S.F. Pristininae																														
		Pristina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	49	-	-	-	-
	S.F. Tubificinae																														
		Aulodrilus pluriseta	-	-	-	-	-	8	-	-	-	-	48	7	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Aulodrilus pigueti	-	-	-	-	-	-	-	32	-	-	32	-	-	-	-	-	-	-	-	82	-	-	-	-	-	-	-	-	-	18
		Limnodrilus hoffmeisteri	4	-	-	-	-	-	-	-	-	-	-	-	8	8	8	-	8	-	27	-	4	-	-	-	-	49	-	16	-	-
		Limnodrilus udekemianus	20	44	32	64	44	8	8	-	64	8	80	100	26	104	16	16	8	8	107	82	28	8	24	8	32	98	16	16	64	36
		immatures with hair chaetae	-	8	-	-	-	28	68	128	64	24	16	13	-	8	-	-	-	-	53	165	-	4	-	-	-	-	16	48	32	89
		immatures without hair chaetae	-	5	12	-	8	4	-	-	-	-	16	-	18	8	12	16	56	24	212	412	-	8	8	8	8	146	-	16	-	179
	LEECHES																														
	Cl. Hirudinea																														
		F. Glossiphoniidae																													
		Glossiphonia complanata	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ARTHROPODS																															
P. Arthropoda																															
	MITES																														
	Cl. Arachnida																														
	Subcl. Acari																														

Stations		PINR-REF2					PINR-EXP					PINR-EXP2					LVR-REF					LVR2-REF					STUC-REF				
Taxa	Station Numbers	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
O. Trombidiformes																															
F. Hygrobatidae																															
Hygrobates		-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HARPACTICOIDS																															
O. Harpacticoida		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	96	-	-	-	-
SEED SHRIMPS																															
Cl. Ostracoda		-	-	-	-	-	-	8	64	16	8	-	4	-	-	4	-	-	8	48	-	-	4	-	-	-	64	32	-	64	96
WATER SCUDS																															
O. Amphipoda																															
F. Hyalellidae																															
Hyalella		-	3	-	-	4	48	40	32	-	-	-	4	-	-	-	-	-	-	32	16	-	-	4	-	-	-	48	16	48	8
SPRINGTAILS																															
Cl. Entognatha																															
O. Collembola		-	-	-	16	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
INSECTS																															
Cl. Insecta																															
BEETLES																															
O. Coleoptera																															
F. Chrysomelidae																															
Donacia		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-
F. Dytiscidae																															
Liodessus		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-
F. Elmidae																															
Dubiraphia larvae		8	7	12	32	8	144	48	96	96	104	48	52	6	8	12	-	-	-	-	-	-	8	-	-	-	-	-	-	16	8
F. Haliplidae																															
Haliplus		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32	-	-	-	-
Peltodytes		-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. Hydrophilidae																															
Tropisternus		-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MAYFLIES																															
O. Ephemeroptera																															
immature		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-
F. Baetidae																															
Callibaetis		-	-	-	-	-	-	4	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-
F. Caenidae																															
Caenis		8	19	8	-	4	36	20	64	32	8	16	8	2	-	-	40	-	8	128	80	-	-	4	-	-	-	-	-	16	16
F. Ephemeridae																															
Hexagenia		12	15	64	-	3	3	2	1	-	2	-	27	6	4	16	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1

Stations		PINR-REF2					PINR-EXP					PINR-EXP2					LVR-REF					LVR2-REF					STUC-REF				
Taxa	Station Numbers	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
F. Heptageniidae																															
Stenacron		4	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. Leptophlebiidae																															
Leptophlebia		-	15	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	8
O. Megaloptera																															
ALDERFLIES																															
F. Sialidae																															
Sialis		-	1	-	-	4	-	-	-	-	-	-	4	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O. Odonata																															
DAMSELFLIES																															
F. Coenagrionidae																															
immature		-	-	-	-	-	-	-	-	32	-	-	-	-	-	-	-	-	-	-	32	-	-	-	-	-	-	16	-	-	-
DRAGONFLIES																															
F. Corduliidae																															
Epitheca		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Somatochlora		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-
F. Libellulidae																															
Leucorrhinia		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Libellula		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	1	-
CADDISFLIES																															
O. Trichoptera																															
F. Dipseudopsidae																															
Phylocentropus		-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. Leptoceridae																															
Leptocerus		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	-	-	-	-
Triaenodes		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-
F. Limnephilidae																															
immature		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-
F. Phryganeidae																															
Ptilostomis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-	-
immature		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-
F. Polycentropodidae																															
Polycentropus		-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TRUE FLIES																															
O. Diptera																															
BITING-MIDGE																															
F. Ceratopogonidae																															
Bezzia		-	-	-	-	-	4	-	64	16	8	-	-	-	-	-	-	24	-	48	32	-	-	-	-	-	-	16	-	-	8
Culicoides		-	3	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Stations				PINR-REF2					PINR-EXP					PINR-EXP2					LVR-REF					LVR2-REF					STUC-REF				
Taxa			Station Numbers	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
			Dasyhelea	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	64	16	-	-	-
			Mallochohelea	20	15	44	64	4	88	92	480	208	104	16	84	22	104	76	-	-	-	32	-	4	8	-	-	-	-	-	80	176	56
			Probezzia	-	1	-	-	-	-	4	-	16	32	-	12	-	-	12	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-
			Serromyia	-	-	-	-	-	92	16	-	-	-	-	8	-	-	-	-	-	-	112	-	-	8	-	-	-	-	-	272	208	32
			Sphaeromias	-	-	-	-	-	4	-	-	32	-	-	-	-	-	-	-	-	-	16	-	4	-	-	-	8	-	-	16	-	-
	PHANTOM MIDGE																																
		F. Chaoboridae																															
			Chaoborus flavicans	-	-	-	-	-	-	-	32	-	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32	16	-	16	-
			Chaoborus punctipennis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	8	8	-	-	-	-	-	-	-	-	-	-	-	-
	MIDGES																																
		F. Chironomidae																															
	S.F. Chironominae																																
			Chironomus	-	-	-	16	4	-	-	-	-	8	16	4	52	80	28	8	8	16	-	48	88	264	136	112	96	288	192	272	-	-
			Cladopelma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	16	-	-
			Dicrotendipes	-	-	-	-	-	4	4	-	16	-	-	-	-	-	-	-	-	8	-	16	-	8	16	-	-	96	288	32	48	16
			Einfeldia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	-	-
			Endochironomus	-	-	-	-	-	4	-	-	48	-	-	-	-	-	-	-	-	8	-	96	-	-	-	-	-	32	-	16	16	16
			Glyptotendipes	-	-	-	-	-	4	-	-	-	-	-	-	-	-	4	16	-	8	-	64	-	4	-	-	-	-	16	-	-	-
			Hyporhygma	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Microtendipes	-	-	-	16	-	4	-	32	-	-	-	-	-	-	4	24	-	-	-	128	-	-	-	-	-	-	-	-	-	-
			Parachironomus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	16	16	-	-
			Paratanytarsus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-
			Phaenopsectra	-	2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Polypedilum halterale	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	192	-	-	-	-	-	-	-	-	-	-	-	-
			Polypedilum scalaenum	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Polypedilum sordens	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-	-
			Polypedilum	-	2	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	64	-	-	4	-	-	-	-	-	-	-	-
			Pseudochironomus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	32	-	-	-	-
			Stenochironomus	-	-	-	-	4	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Stictochironomus	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Tanytarsus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	8	-	48	-	-	-	-	-	160	80	-	-	16
			Tribelos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-
			Zavreliella	-	-	-	-	-	-	-	32	16	-	-	-	-	-	-	16	-	-	112	48	-	-	-	-	-	128	-	64	16	-
	S.F. Orthocladiinae																																
			Cricotopus	-	-	-	-	-	8	-	-	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
			Cricotopus/Orthocladius	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Epoicocladius	4	7	8	-	-	16	4	-	-	-	-	8	4	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-
			Parakiefferiella	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Psectrocladius	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Stations		PINR-REF2					PINR-EXP					PINR-EXP2					LVR-REF					LVR2-REF					STUC-REF				
Taxa	Station Numbers	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
indeterminate		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-
S.F. Tanypodinae																															
Ablabesmyia		-	5	8	-	4	-	-	-	32	-	-	16	-	-	-	-	8	-	-	16	4	4	-	-	-	-	16	-	-	-
Clinotanypus		-	-	-	-	-	-	4	-	16	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Guttipelopia		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	16	-	-	-	-	-	-	-	-	-	-	-
Labrudinia		-	-	-	-	-	-	-	32	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Larsia		-	3	8	-	8	4	-	-	-	-	-	8	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Natarsia		-	6	-	-	-	-	-	-	-	-	16		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Procladius		8	16	20	32	-	36	28	96	40	120	-	52	2	24	4	-	-	-	80	64	24	120	12	8	8	64	16	336	304	128
indeterminate		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	16	-	-
F. Culicidae																															
Anopheles		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-
Mansonia perturbans		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-
F. Tabanidae													-																		
Chrysops		-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-
F. Limoniidae																															
Pilaria		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-
immature		-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MOLLUSCS																															
P. Mollusca																															
SNAILS																															
Cl. Gastropoda																															
F. Ancylidae																															
Ferrissia		-	27	20	-	44	-	4	-	64	8	-	-	2	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-
F. Hydrobiidae																															
Amnicola		-	-	-	-	-	-	-	160	64	40	-	-	-	-	-	8	24	16	144	32	-	-	-	-	-	-	-	-	-	-
Probythinella emarginata		4	-	12	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	4	16	-	-	16	-	-	-	-	-
immature		-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. Physidae																															
Physa		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	-
F. Planorbidae																															
Armiger crista		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gyraulus		-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	16	-	40	48	96	-	-	-	-	-	64	16	-	48	8
Helisoma anceps		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32	-	-	-	-	-	-	-	-	-	-	-
Promenetus exacuous		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
immature		-	-	-	-	-	-	-	64	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. Valvatidae																															
Valvata tricarinata		-	-	-	-	-	-	4	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Stations				PINR-REF2					PINR-EXP					PINR-EXP2					LVR-REF					LVR2-REF					STUC-REF				
Taxa			Station Numbers	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
			Valvata	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		F. Viviparidae																																
			Campeloma decisum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	-
	CLAMS																																
	Cl. Bivalvia																																
		F. Sphaeriidae																																
			Pisidium (Cyclocalyx)	4	1	-	-	-	12	40	-	48	64	16	-	4	2	-	-	-	-	-	-	12	8	20	-	8	-	-	-	-	32
			Sphaerium transversum	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Sphaerium (Amesoda) simile	1	1	-	-	3	3	-	-	2	-	-	-	2	1	-	-	-	-	16	-	1	-	1	-	-	-	-	-	3	1
			Sphaerium (Amesoda) striatinum	7	7	8	-	4	2	-	-	-	-	1	4	3	-	4	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-
			Sphaerium (Herringtonium) rhomboideum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	16	-	-	-	-	-	-	-	-	-	-
			Sphaerium (Musculium) immature	-	-	-	-	-	4	4	96	-	40	-	-	-	-	-	32	-	-	32	32	-	-	-	-	-	352	64	128	80	176
			immature	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**16**	-	**8**	-	-	-	-	-	-	-	-	-
		F. Unionidae																																
			Anodontoides ferussacianus	-	1	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL NUMBER OF ORGANISMS				132	223	268	241	170	584	430	1857	1131	658	481	455	165	375	232	272	152	208	1745	1956	193	500	277	136	192	2528	1057	1760	1477	1802
TOTAL NUMBER OF TAXA [a]				16	30	15	8	18	28	23	20	29	21	16	24	17	14	16	17	9	18	28	34	13	18	19	4	8	22	23	21	24	26

Note: [a] Bold entries excluded from taxa count

Table C-2: Calculation of subsampling error for benthic macroinvertebrate samples from the Rainy River Mine Phase 3 EEM.

Station	Whole Organisms	Number of Organisms in Fraction 1	Number of Organisms in Fraction 2	Number of Organisms in Fraction 3	Number of Organisms in Fraction 4	Number of Organisms in Fraction 5	Number of Organisms in Fraction 6	Number of Organisms in Fraction 7	Number of Organisms in Fraction 8	Actual Density*	Precision % range		Accuracy	
													min	max
PINR-REF2-2	4	21	24	26	26	28	29	29	36	219	0.0	41.7	2.3	31.5
PINR-REF2-2	4	50	52	55	62	-	-	-	-	219	3.8	19.4	0.5	13.2
PINR-EXP-2	-	50	57	-	-	-	-	-	-	107	12.3	-	-	-

Notes:
* whole large organisms excluded in calculations
min = minimum absolute % error
max = maximum absolute % error

Table C-3: Percent recovery of benthic macroinvertebrates samples from the Rainy River Mine Phase 3 EEM.

Station	Number of Organisms Recovered (initial sort)	Number of Organisms Recovered (re-sort)	Percent Recovery
LVR-Ref-4	104	109	95.40%
LVR2-REF-3	63	69	91.30%
PINR-Exp-5	80	82	97.60%
		Average % Recovery	**94.80%**

Table C-4: Sample fractions sorted for the benthic macroinvertebrates from the Rainy River Mine Phase 3 EEM.

Station	Fraction Sorted (500 um)	Station	Fraction Sorted (500 um)	Station	Fraction Sorted (500 um)
PINR-REF2-1	1/4	PINR-EXP2-1	1/16	LVR2-REF-1	1/4
PINR-REF2-2	Whole[a, b]	PINR-EXP2-2	1/4	LVR2-REF-2	1/4
PINR-REF2-3	1/4	PINR-EXP2-3	1/2	LVR2-REF-3	1/4
PINR-REF2-4	1/16	PINR-EXP2-4	1/8	LVR2-REF-4	1/8
PINR-REF2-5	1/4	PINR-EXP2-5	1/4	LVR2-REF-5	1/8
PINR-EXP-1	1/4	LVR-REF-1	1/8	STUC-REF-1	1/32
PINR-EXP-2	1/4[c]	LVR-REF-2	1/8	STUC-REF-2	1/16
PINR-EXP-3	1/32	LVR-REF-3	1/8	STUC-REF-3	1/16
PINR-EXP-4	1/16	LVR-REF-4	1/16	STUC-REF-4	1/16
PINR-EXP-5	1/8	LVR-REF-5	1/16	STUC-REF-5	1/8

Notes:

[a] eight eighths sorted for subsampling error calculations.

[b] four quarters sorted for subsampling error calculations.

[c] two eighths sorted for subsampling error calculations.

QA/QC Notes:

Pupae were not counted toward total number of taxa unless they were the sole representative of their taxa group.

Immatures were not counted toward total number of taxa unless they were the sole representative of their taxa group.

The exceptions to this rule are immature Tubificidae with and without hairs. Immature oligocheates are counted as taxa as the probability of the immature being a unique taxa is high.

Indeterminates are unique taxa that could not be identified further for whatever reason, e.g., (small, damaged).

Reported fractions averaged 4 hours to sort due to high quantities of organic matter.

ZEAS has shown that subsampling precision and accuracy are density dependent (Zaranko and Keene 2005). Specifically, small absolute differences between subsampled fractions become increasingly large, when expressed as a percentage of total organisms, as organism densities decline. Therefore, the probability of meeting precision and accuracy criteria is reduced in samples with low organism densities (i.e., <150 organisms/subsample).

Zaranko, D.T. and J. Keene. 2005. Are the costs to meet environmental effects monitoring (EEM) benthic sample precision and accuracy criteria justified? In Dixon, D.G., S. Munro and A.J. Niimi (eds). Proceedings of the 32nd Annual Aquatic Toxicity Workshop: October 3 to 5, 2005, Waterloo, Ontario. Can. Tech. Rep. Fish. Aquat. Sci: 2617. 120p.

Densities expressed per sampled area.

Appendix D FISH SURVEY

Table D-1: Raw counts for fish species (minnow traps) for the Rainy River Mine Phase 3 EEM

Site Code	GearID	Northing	Easting	Depth (m)	Set Date	Set Time	Lift Date	Lift Time	Effort (# of traps)	Hours	Total Effort	Notes	Blackside Darter	Brassy Minnow	Brook Stickleback	Brown Bullhead	Central Mudminnow	Common Shiner	Creek Chub	Fathead Minnow	Finescale Dace	Golden Shiner	Johnny Darter	Northern Pike	Northern Redbelly Dace	Pearl Dace	Rock Bass	White Sucker
LVR	LVRMT1	48.66557	-93.67031	0.5	2023-05-01	15:55	2023-05-02	10:00	2	18.1	36.2	NA	0	123	227	0	4	8	14	22	0	0	0	0	23	4	0	1
LVR	LVRMT1	48.66557	-93.67031	0.75	2023-05-02	11:00	2023-05-03	07:50	2	20.8	41.7	NA	0	225	501	0	2	1	16	16	0	0	0	0	82	6	0	1
LVR	LVRMT1	48.66557	-93.67031	0.3	2023-05-04	11:35	2023-05-05	10:30	3	22.9	68.8	proportionalize based on previous 2 days of LVRMT1 catches	0	126	263	0	21	3	11	14	0	0	0	0	38	4	0	1
LVR	LVRMT1	48.66557	-93.67031	0.3	2023-05-05	10:45	2023-05-06	07:45	3	21.0	63.0	proportionalized the last 57 counts	0	19	30	0	23	0	11	0	0	4	0	0	15	0	0	0
LVR	LVRMT1	48.66557	-93.67031	0.3	2023-05-06	08:00	2023-05-07	07:50	3	23.8	71.5	NA	0	0	29	0	24	0	0	0	0	1	0	0	15	0	0	0
LVR	LVRMT2	48.66541	-93.67061	0.5	2023-05-02	11:00	2023-05-03	08:40	5	21.7	108.3	NA	0	418	570	0	4	8	102	18	0	1	0	0	78	39	0	10
LVR	LVRMT2	48.66541	-93.67061	0.75	2023-05-03	10:30	2023-05-04	09:10	2	22.7	45.3	proporationalized based on previous LVRMT2 counts	0	116	160	0	0	2	28	5	0	1	0	0	22	11	0	3
LVR	LVRMT3	48.66517	-93.67103	0.3	2023-05-03	10:40	2023-05-04	09:15	1	22.6	22.6	proporationalized based on previous LVRMT2 counts	0	125	171	0	0	2	31	5	0	1	0	0	24	12	0	3
LVR	LVRMT4	48.66520	-93.67101	0.5	2023-05-03	10:40	2023-05-04	10:50	1	24.2	24.2	proporationalized based on previous LVRMT2 counts	0	119	163	0	0	2	29	5	0	1	0	0	22	11	0	3
LVR	LVRMT5	48.66535	-93.67053	0.75	2023-05-03	10:50	2023-05-04	11:00	4	24.2	96.7	proporationalized based on previous LVRMT2 counts	0	470	641	0	3	9	115	20	0	1	0	0	88	44	0	11
LVR	LVRMT5	48.66535	-93.67053	0.5	2023-05-04	11:30	2023-05-05	09:45	3	22.3	66.8	proporationalized based on previous LVRMT2 counts	0	424	579	0	2	8	103	18	0	1	0	0	79	40	0	10
LVR	LVRMT5	48.66535	-93.67053	0.3	2023-05-05	10:00	2023-05-06	08:00	3	22.0	66.0	proporationalized based on previous LVRMT2 counts	0	23	32	0	8	1	6	1	0	0	0	0	4	2	0	1
LVR2	LVR2MT1	48.61954	-93.63026	0.75	2023-05-01	16:45	2023-05-02	11:15	2	18.5	37.0	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT1	48.61954	-93.63026	0.5	2023-05-02	11:15	2023-05-03	11:45	1	24.5	24.5	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT2	48.61957	-93.63029	0.5	2023-05-02	11:15	2023-05-03	11:50	1	24.6	24.6	NA	0	0	4	1	0	0	0	0	0	0	0	1	0	0	0	0
LVR2	LVR2MT3	48.61981	-93.63114	0.5	2023-05-02	11:20	2023-05-03	11:35	1	24.3	24.3	NA	0	0	7	0	1	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT4	48.61967	-93.63060	0.5	2023-05-02	11:25	2023-05-03	11:40	1	24.3	24.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT5	48.61936	-93.63023	0.5	2023-05-02	11:30	2023-05-03	11:45	1	24.3	24.3	NA	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT6	48.61928	-93.63011	0.5	2023-05-02	11:45	2023-05-03	11:45	2	24.0	48.0	NA	0	0	15	0	2	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT6	48.61928	-93.63011	0.5	2023-05-03	11:55	2023-05-04	12:00	2	24.1	48.2	NA	0	1	155	0	1	0	1	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT6	48.61928	-93.63011	0.5	2023-05-04	13:15	2023-05-05	11:00	5	21.8	108.8	NA	0	5	233	0	0	0	1	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT6	48.61928	-93.63011	0.5	2023-05-05	11:05	2023-05-06	08:30	5	21.4	107.1	NA	0	3	34	0	0	0	1	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT7	48.61919	-93.62919	0.5	2023-05-03	12:00	2023-05-04	12:15	1	24.3	24.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT8	48.61919	-93.62943	0.5	2023-05-03	12:00	2023-05-04	12:00	1	24.0	24.0	NA	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT9	48.61913	-93.62938	0.5	2023-05-03	12:05	2023-05-04	12:05	1	24.0	24.0	NA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT10	48.61896	-93.62923	0.5	2023-05-03	12:10	2023-05-04	12:10	1	24.0	24.0	NA	0	10	79	0	0	0	0	0	0	0	0	0	2	0	0	0
LVR2	LVR2MT11	48.61995	-93.63014	0.3	2023-05-04	13:20	2023-05-05	11:30	3	22.2	66.5	NA	0	0	47	0	2	0	1	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT11	48.61995	-93.63014	0.3	2023-05-05	11:45	2023-05-06	08:40	3	20.9	62.8	NA	0	0	26	0	2	0	0	0	0	0	0	1	1	0	0	0
LVR2	LVR2MT12	48.61864	-93.62952	0.3	2023-05-06	09:15	2023-05-07	08:15	3	23.0	69.0	NA	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT13	48.62024	-93.63245	0.5	2023-05-06	09:40	2023-05-07	09:20	1	23.7	23.7	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT14	48.62102	-93.63313	0.5	2023-05-06	09:45	2023-05-07	09:20	1	23.6	23.6	NA	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT15	48.62128	-93.63377	0.5	2023-05-06	09:50	2023-05-07	09:25	3	23.6	70.8	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT16	48.62035	-93.63238	0.5	2023-05-06	10:00	2023-05-07	09:10	1	23.2	23.2	NA	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT17	48.61981	-93.63208	0.5	2023-05-06	10:05	2023-05-07	09:10	2	23.1	46.2	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT18	48.62186	-93.63452	0.5	2023-05-07	09:40	2023-05-08	08:45	2	23.1	46.2	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT19	48.62170	-93.63532	0.5	2023-05-07	09:45	2023-05-08	08:50	2	23.1	46.2	NA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT20	48.61952	-93.63001	0.5	2023-05-07	10:25	2023-05-08	09:15	2	22.8	45.7	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT5	48.61936	-93.63023	0.5	2023-05-07	10:15	2023-05-08	09:05	2	22.8	45.7	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2MT1	48.61954	-93.63026	0.5	2023-05-07	10:20	2023-05-08	09:10	2	22.8	45.7	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT1	48.72122	-93.96077	0.5	2023-05-01	18:00	2023-05-02	13:45	1	19.8	19.8	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT1	48.72122	-93.96077	0.5	2023-05-02	13:45	2023-05-03	13:50	2	24.1	48.2	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT1	48.72122	-93.96077	0.5	2023-05-03	13:50	2023-05-05	07:30	2	41.7	83.3	NA	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT1	48.72122	-93.96077	0.5	2023-05-05	07:35	2023-05-06	11:00	2	27.4	54.8	NA	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT1	48.72122	-93.96077	0.5	2023-05-06	12:20	2023-05-07	11:25	2	23.1	46.2	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT1	48.72122	-93.96077	0.5	2023-05-07	11:30	2023-05-08	12:00	2	24.5	49.0	NA	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT2	48.72131	-93.95937	0.6	2023-05-02	14:05	2023-05-03	14:30	2	24.4	48.8	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT3	48.72139	-93.95950	0.5	2023-05-02	14:10	2023-05-03	14:25	1	24.3	24.3	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT4	48.72128	-93.95972	0.75	2023-05-02	14:15	2023-05-03	14:20	2	24.1	48.2	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT5	48.72134	-93.95976	0.75	2023-05-02	14:20	2023-05-03	14:15	2	23.9	47.8	NA	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT5	48.72134	-93.95976	0.75	2023-05-03	14:15	2023-05-05	08:30	2	42.3	84.5	NA	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT5	48.72134	-93.95976	0.75	2023-05-05	08:35	2023-05-06	11:25	2	26.8	53.7	NA	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0

Site Code	GearID	Northing	Easting	Depth (m)	Set Date	Set Time	Lift Date	Lift Time	Effort (# of traps)	Hours	Total Effort	Notes	Blackside Darter	Brassy Minnow	Brook Stickleback	Brown Bullhead	Central Mudminnow	Common Shiner	Creek Chub	Fathead Minnow	Finescale Dace	Golden Shiner	Johnny Darter	Northern Pike	Northern Redbelly Dace	Pearl Dace	Rock Bass	White Sucker
STUC	STCMT5	48.72134	-93.95976	0.75	2023-05-06	11:30	2023-05-07	11:30	2	24.0	48.0	RELEASED 1 MALE CMM	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0
STUC	STCMT5	48.72134	-93.95976	0.75	2023-05-07	11:30	2023-05-08	11:40	2	24.2	48.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT6	48.72123	-93.96039	0.75	2023-05-02	14:25	2023-05-03	14:10	1	23.8	23.8	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT7	48.72193	-93.95751	0.75	2023-05-03	14:40	2023-05-05	07:55	3	41.3	123.8	NA	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT7	48.72193	-93.95751	0.75	2023-05-05	08:00	2023-05-06	12:05	3	28.1	84.3	NA	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT8	48.72177	-93.95798	0.5	2023-05-03	14:50	2023-05-05	08:05	2	41.3	82.5	NA	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT8	48.72177	-93.95798	0.5	2023-05-05	08:10	2023-05-06	11:55	2	27.8	55.5	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT8	48.72177	-93.95798	0.5	2023-05-06	12:00	2023-05-07	11:55	2	23.9	47.8	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT9	48.72167	-93.95855	0.75	2023-05-03	14:55	2023-05-05	08:10	2	41.3	82.5	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT10	48.72167	-93.95900	0.75	2023-05-03	14:55	2023-05-05	08:15	2	41.3	82.7	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT11	48.72188	-93.95958	0.5	2023-05-03	15:05	2023-05-05	08:20	2	41.3	82.5	NA	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT11	48.72188	-93.95958	0.5	2023-05-05	08:25	2023-05-06	11:40	6	27.3	163.5	NA	0	0	1	0	18	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT11	48.72188	-93.95958	0.5	2023-05-06	11:45	2023-05-07	11:45	4	24.0	96.0	NA	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT11	48.72188	-93.95958	0.5	2023-05-07	11:50	2023-05-08	12:30	4	24.7	98.7	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT12	48.72051	-93.96090	1.25	2023-05-05	08:40	2023-05-06	11:15	1	26.6	26.6	TARGETING BSBs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT13	48.72183	-93.95741	0.5	2023-05-06	12:05	2023-05-07	12:00	2	23.9	47.8	1 CMM RELEASED	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT13	48.72183	-93.95741	0.5	2023-05-07	12:05	2023-05-08	12:20	2	24.3	48.5	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT14	48.72230	-93.95729	0.3	2023-05-06	12:10	2023-05-07	12:10	3	24.0	72.0	NA	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0
STUC	STCMT14	48.72230	-93.95729	0.3	2023-05-07	12:10	2023-05-08	12:15	3	24.1	72.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT15	48.72138	-93.95929	1	2023-05-06	12:15	2023-05-07	12:15	2	24.0	48.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT16	48.72247	-93.95715	0.5	2023-05-07	12:20	2023-05-08	12:10	4	23.8	95.3	NA	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
STUC	STCMT17	48.72151	-93.96023	0.5	2023-05-07	12:30	2023-05-08	12:35	2	24.1	48.2	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT1	48.82963	-94.06465	0.75	2023-05-04	16:30	2023-05-05	16:45	1	24.3	24.3	NA	0	3	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT1	48.82963	-94.06465	0.75	2023-05-05	17:15	2023-05-06	13:25	3	20.2	60.5	NA	0	2	2	10	5	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT1	48.82963	-94.06465	0.75	2023-05-06	13:45	2023-05-07	15:35	3	25.8	77.5	NA	0	15	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT1	48.82963	-94.06465	0.75	2023-05-07	15:35	2023-05-08	14:30	5	22.9	114.6	NA	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT2	48.82963	-94.06465	0.5	2023-05-04	16:30	2023-05-05	16:50	1	24.3	24.3	NA	0	55	6	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT2	48.82963	-94.06465	0.5	2023-05-05	16:55	2023-05-06	13:25	1	20.5	20.5	NA	0	12	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT2	48.82963	-94.06465	0.5	2023-05-06	13:45	2023-05-07	15:15	3	25.5	76.5	NA	0	2	2	3	0	1	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT2	48.82963	-94.06465	0.5	2023-05-07	15:30	2023-05-08	14:35	2	23.1	46.2	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT3	48.82954	-94.06482	0.5	2023-05-04	16:30	2023-05-05	16:55	1	24.4	24.4	NA	0	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT3	48.82954	-94.06482	0.5	2023-05-05	17:10	2023-05-06	13:30	1	20.3	20.3	NA	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT4	48.82951	-94.06488	0.75	2023-05-04	16:30	2023-05-05	18:40	1	26.2	26.2	NA	0	4	4	0	2	0	1	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT4	48.82951	-94.06488	0.75	2023-05-05	17:10	2023-05-06	13:30	1	20.3	20.3	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT5	48.82946	-94.06494	0.75	2023-05-04	16:35	2023-05-05	17:05	1	24.5	24.5	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT6	48.82932	-94.06498	0.75	2023-05-04	16:40	2023-05-05	17:10	1	24.5	24.5	NA	0	1	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT7	48.82921	-94.06498	0.75	2023-05-04	16:40	2023-05-05	17:15	1	24.6	24.6	NA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT7	48.82921	-94.06498	0.75	2023-05-05	17:30	2023-05-06	13:30	1	20.0	20.0	NA	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT8	48.82911	-94.06501	0.5	2023-05-04	16:40	2023-05-05	17:20	1	24.7	24.7	NA	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT9	48.82905	-94.06509	0.75	2023-05-04	16:40	2023-05-05	17:20	1	24.7	24.7	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT10	48.82906	94.06523	0.5	2023-05-05	17:25	2023-05-06	13:35	2	20.2	40.3	NA	0	1	2	19	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT10	48.82906	94.06523	0.5	2023-05-06	13:40	2023-05-07	14:50	2	25.2	50.3	NA	0	0	5	20	0	0	0	0	0	0	0	1	0	0	0	0	0
PINR-EXP	PINEXPMT10	48.82906	94.06523	0.5	2023-05-07	14:55	2023-05-08	15:00	2	24.1	48.2	NA	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT11	48.82956	-94.06433	0.5	2023-05-07	13:35	2023-05-08	14:15	3	24.7	74.0	NA	0	5	27	2	4	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT11	48.82956	-94.06433	0.5	2023-05-08	14:15	2023-05-09	12:30	8	22.3	178.0	NA	0	1	22	9	17	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT12	48.82895	-94.06599	0.5	2023-05-08	14:50	2023-05-09	12:10	5	21.3	106.7	NA	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT13	48.82882	-94.06586	0.5	2023-05-08	14:55	2023-05-09	12:15	3	21.3	64.0	NA	0	0	7	4	11	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT14	48.82891	-94.06561	0.5	2023-05-08	15:00	2023-05-09	12:20	2	21.3	42.7	NA	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP	PINEXPMT15	48.82901	-94.06535	0.5	2023-05-08	15:00	2023-05-09	12:25	2	21.4	42.8	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT1	48.81181	-94.15078	0.5	2023-05-02	10:45	2023-05-03	08:40	1	21.9	21.9	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT2	48.81187	-94.15100	1	2023-05-02	10:50	2023-05-03	08:40	1	21.8	21.8	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT3	48.81200	-94.15095	1	2023-05-02	10:50	2023-05-03	08:45	1	21.9	21.9	NA	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT4	48.81237	-94.15133	0.5	2023-05-02	10:55	2023-05-03	09:10	1	22.3	22.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT5	48.81237	-94.15133	0.25	2023-05-02	11:00	2023-05-03	09:10	1	22.2	22.2	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT6	48.81237	-94.15133	0.25	2023-05-02	11:00	2023-05-03	09:10	1	22.2	22.2	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT7	48.81261	-94.15131	0.5	2023-05-02	11:05	2023-05-03	09:15	1	22.2	22.2	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT8	48.81295	-94.15106	1	2023-05-02	11:10	2023-05-03	09:20	1	22.2	22.2	NA	0	0	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT9	48.81295	-94.15106	0.75	2023-05-02	11:10	2023-05-03	09:20	1	22.2	22.2	NA	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT10	48.81295	-94.15106	0.75	2023-05-02	11:15	2023-05-03	09:20	1	22.1	22.1	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT10	48.81295	-94.15106	0.5	2023-05-03	09:25	2023-05-04	08:40	1	23.3	23.3	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT11	48.81295	-94.15106	0.5	2023-05-03	09:25	2023-05-04	08:40	1	23.3	23.3	NA	0	1	8	0	9	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT12	48.81295	-94.15106	1	2023-05-03	09:30	2023-05-04	08:50	1	23.3	23.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT13	48.81336	-94.15025	0.75	2023-05-03	09:35	2023-05-04	09:00	1	23.4	23.4	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT14	48.81336	-94.15025	1	2023-05-03	09:35	2023-05-04	09:00	1	23.4	23.4	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Site Code	GearID	Northing	Easting	Depth (m)	Set Date	Set Time	Lift Date	Lift Time	Effort (# of traps)	Hours	Total Effort	Notes	Blackside Darter	Brassy Minnow	Brook Stickleback	Brown Bullhead	Central Mudminnow	Common Shiner	Creek Chub	Fathead Minnow	Finescale Dace	Golden Shiner	Johnny Darter	Northern Pike	Northern Redbelly Dace	Pearl Dace	Rock Bass	White Sucker
PINR-REF2	PINRREFMT15	48.81336	-94.15025	1	2023-05-03	09:40	2023-05-04	09:00	1	23.3	23.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT16	48.81348	-94.14994	1.5	2023-05-03	09:45	2023-05-04	09:00	1	23.3	23.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT17	48.81348	-94.14994	1	2023-05-03	09:45	2023-05-04	09:00	1	23.3	23.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT18	48.81355	-94.14958	0.5	2023-05-03	09:50	2023-05-04	09:10	1	23.3	23.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT19	48.81355	-94.14958	0.75	2023-05-03	09:50	2023-05-04	09:10	1	23.3	23.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT20	48.81272	-94.15116	0.5	2023-05-04	08:35	2023-05-05	09:40	1	25.1	25.1	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT21	48.81272	-94.15116	1	2023-05-04	08:40	2023-05-05	09:40	1	25.0	25.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT22	48.81272	-94.15116	0.75	2023-05-04	08:40	2023-05-05	09:30	1	24.8	24.8	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT23	48.81272	-94.15116	0.5	2023-05-04	08:40	2023-05-05	09:30	1	24.8	24.8	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT24	48.81272	-94.15116	0.5	2023-05-04	08:50	2023-05-05	09:30	1	24.7	24.7	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT25	48.81357	-94.14949	1	2023-05-04	08:55	2023-05-05	09:25	1	24.5	24.5	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT26	48.81357	-94.14949	0.75	2023-05-04	09:00	2023-05-05	09:25	1	24.4	24.4	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT27	48.81357	-94.14949	0.5	2023-05-04	09:00	2023-05-05	09:20	1	24.3	24.3	NA	0	16	34	0	17	0	3	0	0	0	0	0	2	0	0	0
PINR-REF2	PINRREFMT28	48.81357	-94.14949	1	2023-05-04	09:05	2023-05-05	09:15	1	24.2	24.2	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT29	48.81357	-94.14949	0.5	2023-05-04	09:10	2023-05-05	09:10	1	24.0	24.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT30	48.81357	-94.14949	1	2023-05-04	09:15	2023-05-05	09:10	1	23.9	23.9	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT31	48.81357	-94.14949	0.5	2023-05-04	09:15	2023-05-05	09:10	1	23.9	23.9	NA	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT32	48.81357	-94.14949	0.25	2023-05-04	09:20	2023-05-05	09:05	1	23.8	23.8	NA	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT33	48.81357	-94.14949	0.25	2023-05-04	09:20	2023-05-05	09:05	1	23.8	23.8	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT34	48.81357	-94.14949	1	2023-05-04	09:25	2023-05-05	09:00	1	23.6	23.6	NA	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT35	48.81291	-94.14847	0.5	2023-05-04	09:25	2023-05-05	08:50	1	23.4	23.4	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT36	48.81291	-94.14847	0.75	2023-05-04	09:30	2023-05-05	08:50	1	23.3	23.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT37	48.81291	-94.14847	0.5	2023-05-04	09:30	2023-05-05	08:45	1	23.3	23.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT38	48.81291	-94.14847	1	2023-05-04	09:35	2023-05-05	08:45	1	23.2	23.2	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT39	48.81291	-94.14847	1	2023-05-04	09:35	2023-05-05	08:45	1	23.2	23.2	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT40	48.81295	-94.15106	NA	2023-05-05	10:20	2023-05-06	08:00	1	21.7	21.7	co-ordinates are just centre of the area not each trap- used pt 185	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT41	48.81295	-94.15106	NA	2023-05-05	10:25	2023-05-06	08:05	4	21.7	86.7	NA	0	68	23	0	12	0	8	0	0	0	0	0	3	3	0	1
PINR-REF2	PINRREFMT42	48.81295	-94.15106	NA	2023-05-05	10:30	2023-05-06	08:25	2	21.9	43.8	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT43	48.81295	-94.15106	NA	2023-05-05	10:35	2023-05-06	08:35	1	22.0	22.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT44	48.81295	-94.15106	NA	2023-05-05	10:40	2023-05-06	08:40	4	22.0	88.0	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT45	48.81295	-94.15106	NA	2023-05-05	10:45	2023-05-06	08:55	2	22.2	44.3	NA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT46	48.81295	-94.15106	NA	2023-05-05	10:55	2023-05-06	09:00	3	22.1	66.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT47	48.81295	-94.15106	NA	2023-05-06	08:15	2023-05-07	08:45	3	24.5	73.5	NA	0	33	8	0	2	0	2	2	0	0	0	0	1	2	0	2
PINR-REF2	PINRREFMT48	48.81295	-94.15106	NA	2023-05-06	08:35	2023-05-07	08:30	1	23.9	23.9	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT49	48.81295	-94.15106	NA	2023-05-06	08:40	2023-05-07	08:30	1	23.8	23.8	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT50	48.81295	-94.15106	NA	2023-05-06	08:50	2023-05-07	08:30	2	23.7	47.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT51	48.81295	-94.15106	NA	2023-05-06	08:55	2023-05-07	08:30	1	23.6	23.6	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT52	48.81295	-94.15106	NA	2023-05-06	09:20	2023-05-07	08:25	8	23.1	184.7	NA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT53	48.81295	-94.15106	NA	2023-05-06	09:25	2023-05-07	09:00	1	23.6	23.6	NA	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
PINR-REF2	PINRREFMT54	48.81295	-94.15106	NA	2023-05-08	08:50	2023-05-09	10:15	3	25.4	76.3	fieldbook - hard to distinguish set time	0	16	12	1	29	0	1	4	0	0	0	0	0	2	0	0
PINR-EXP2	PINREXP2MT1	48.81136	-94.15002	1	2023-05-02	11:30	2023-05-03	08:45	1	21.3	21.3	NA	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT2	48.81136	-94.15002	1	2023-05-02	11:30	2023-05-03	08:45	1	21.3	21.3	NA	0	2	1	0	4	3	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT3	48.81136	-94.15002	1	2023-05-02	11:30	2023-05-03	08:45	1	21.3	21.3	NA	0	0	8	0	1	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT4	48.81101	-94.15010	0.5	2023-05-02	11:35	2023-05-03	09:55	1	22.3	22.3	NA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT5	48.81101	-94.15010	0.5	2023-05-02	11:35	2023-05-03	10:00	1	22.4	22.4	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT6	48.81081	-94.15054	0.25	2023-05-02	11:40	2023-05-03	10:05	1	22.4	22.4	NA	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT7	48.81081	-94.15054	0.25	2023-05-02	11:45	2023-05-03	10:05	1	22.3	22.3	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT8	48.81165	-94.15304	0.5	2023-05-02	11:50	2023-05-03	10:15	1	22.4	22.4	NA	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT9	48.81165	-94.15304	0.5	2023-05-02	11:50	2023-05-03	10:15	1	22.4	22.4	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT10	48.81165	-94.15304	0.25	2023-05-02	11:50	2023-05-03	10:15	1	22.4	22.4	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT11	48.81136	-94.15002	1	2023-05-03	09:00	2023-05-04	09:05	1	24.1	24.1	NA	0	0	0	0	0	4	0	0	0	0	0	0	3	0	0	1
PINR-EXP2	PINREXP2MT12	48.81136	-94.15002	1	2023-05-03	09:00	2023-05-04	09:05	1	24.1	24.1	NA	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT13	48.81136	-94.15002	1	2023-05-03	09:00	2023-05-04	09:05	1	24.1	24.1	NA	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT14	48.81082	-94.15103	0.25	2023-05-03	10:10	2023-05-04	10:10	1	24.0	24.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT15	48.81082	-94.15103	1	2023-05-03	10:10	2023-05-04	10:10	1	24.0	24.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT16	48.81082	-94.15103	0.5	2023-05-03	10:10	2023-05-04	10:10	1	24.0	24.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT17	48.81082	-94.15103	0.75	2023-05-03	10:10	2023-05-04	10:10	1	24.0	24.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT18	48.81139	-94.15897	0.5	2023-05-03	10:20	2023-05-04	10:35	1	24.3	24.3	NA	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
PINR-EXP2	PINREXP2MT19	48.81139	-94.15897	1	2023-05-03	10:30	2023-05-04	10:40	1	24.2	24.2	NA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Site Code	GearID	Northing	Easting	Depth (m)	Set Date	Set Time	Lift Date	Lift Time	Effort (# of traps)	Hours	Total Effort	Notes	Blackside Darter	Brassy Minnow	Brook Stickleback	Brown Bullhead	Central Mudminnow	Common Shiner	Creek Chub	Fathead Minnow	Finescale Dace	Golden Shiner	Johnny Darter	Northern Pike	Northern Redbelly Dace	Pearl Dace	Rock Bass	White Sucker
PINR-EXP2	PINREXP2MT20	48.81139	-94.15897	1	2023-05-03	10:30	2023-05-04	10:55	1	24.4	24.4	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT21	48.81110	-94.15135	1	2023-05-04	10:30	2023-05-05	10:30	1	24.0	24.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT22	48.81110	-94.15135	0.25	2023-05-04	10:30	2023-05-05	10:30	1	24.0	24.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT23	48.81110	-94.15135	0.25	2023-05-04	10:35	2023-05-05	10:30	1	23.9	23.9	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT24	48.81110	-94.15135	0.5	2023-05-04	10:35	2023-05-05	10:35	1	24.0	24.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT25	48.81081	-94.15054	0.5	2023-05-04	10:40	2023-05-05	10:40	1	24.0	24.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT26	48.81081	-94.15054	1	2023-05-04	10:40	2023-05-05	10:40	1	24.0	24.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT27	48.81081	-94.15054	1	2023-05-04	10:50	2023-05-05	10:45	1	23.9	23.9	NA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT28	48.81139	-94.15897	0.5	2023-05-04	11:10	2023-05-05	08:20	3	21.2	63.5	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT29	48.79791	-94.18465	1	2023-05-04	12:00	2023-05-05	08:30	4	20.5	82.0	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT30	48.79791	-94.18465	1.5	2023-05-04	12:10	2023-05-05	09:00	3	20.8	62.5	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT31	48.81165	-94.15304	NA	2023-05-05	10:30	2023-05-06	09:45	3	23.3	69.8	used pt 192	0	1	3	0	1	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT32	48.81165	-94.15304	NA	2023-05-05	10:30	2023-05-06	10:15	2	23.8	47.5	used pt 192	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT33	48.81165	-94.15304	NA	2023-05-05	10:30	2023-05-06	10:20	1	23.8	23.8	used pt 192	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT34	48.81165	-94.15304	NA	2023-05-05	10:35	2023-05-06	10:21	3	23.8	71.3	used pt 192	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT35	48.81165	-94.15304	NA	2023-05-05	10:40	2023-05-06	10:25	1	23.8	23.8	used pt 192	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT36	48.81139	-94.15897	NA	2023-05-05	10:40	2023-05-06	10:30	1	23.8	23.8	used pt 193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT37	48.81139	-94.15897	NA	2023-05-05	10:45	2023-05-06	10:50	2	24.1	48.2	used pt 193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT38	48.81139	-94.15897	NA	2023-05-05	08:20	2023-05-06	11:20	2	27.0	54.0	used pt 193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT39	48.81139	-94.15897	NA	2023-05-05	08:30	2023-05-06	11:25	3	26.9	80.8	used pt 193	0	0	2	1	3	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT40	48.81139	-94.15897	NA	2023-05-05	09:00	2023-05-06	11:35	1	26.6	26.6	used pt 193	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT41	48.79856	-94.18374	NA	2023-05-06	09:45	2023-05-07	09:30	3	23.8	71.3	used pt 199	0	0	11	0	4	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT42	48.79856	-94.18374	NA	2023-05-06	11:55	2023-05-07	09:40	5	21.8	108.8	used pt 199	0	1	0	0	0	0	0	0	2	0	0	0	0	0	1	0
PINR-EXP2	PINREXP2MT43	48.79856	-94.18374	NA	2023-05-06	10:00	2023-05-07	09:45	2	23.8	47.5	used pt 199	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT44	48.79856	-94.18374	NA	2023-05-06	10:10	2023-05-07	10:00	5	23.8	119.2	used pt 199	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT45	48.79856	-94.18374	NA	2023-05-06	10:20	2023-05-07	10:10	1	23.8	23.8	used pt 199	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT46	48.79856	-94.18374	NA	2023-05-06	10:25	2023-05-07	10:15	1	23.8	23.8	used pt 199	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT47	48.79926	-94.18243	NA	2023-05-07	08:40	2023-05-08	08:25	3	23.8	71.3	used pt 200 from CF	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT48	48.79926	-94.18243	NA	2023-05-07	08:55	2023-05-08	08:40	5	23.8	118.8	used pt 200 from CF	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
PINR-EXP2	PINREXP2MT49	48.79926	-94.18243	NA	2023-05-07	08:50	2023-05-08	08:45	6	23.9	143.5	used pt 200 from CF	0	0	3	0	0	0	0	0	0	0	1	0	0	0	0	0
PINR-EXP2	PINREXP2MT50	48.79822	-94.18522	NA	2023-05-07	09:40	2023-05-08	09:10	3	23.5	70.5	used pt 201 from CF	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT51	48.79822	-94.18522	NA	2023-05-07	10:00	2023-05-08	09:30	8	23.5	188.0	used pt 201 from CF	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2MT52	48.79886	-94.18273	NA	2023-05-07	10:20	2023-05-08	09:40	3	23.3	70.0	used pt 202 from CF	0	7	7	0	11	0	0	3	0	0	0	0	3	0	0	2
PINR-EXP2	PINREXP2MT53	48.81136	-94.15002	NA	2023-05-08	09:45	2023-05-09	09:45	6	24.0	144.0	NA	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0

Table D-2: Raw counts for fish species (electrofishing) for the Rainy River Mine Phase 3 EEM

SiteCode	GearID	Northing	Easting	Date	Seconds	Temperature (°C)	Sampling Area Dimensions	Sampling Area (m2)	Blackside Darter	Brassy Minnow	Brook Stickleback	Brown Bullhead	Central Mudminnow	Common Shiner	Creek Chub	Fathead Minnow	Finescale Dace	Golden Shiner	Johnny Darter	Northern Pike	Northern Redbelly Dace	Pearl Dace	Rock Bass	White Sucker
LVR	LVREF1	48.66557	-93.67031	2023-05-04	1431	11	15x5, 15x3	120	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0
LVR2	LVR2EF1	48.61956	-93.63029	2023-05-04	200	10.5	15x3	45	0	0	3	0	2	0	0	0	0	0	0	1	0	0	0	0
PINR-EXP	PINEXPEF1	48.82905	-94.06509	2023-05-07	1418	11	100x3	300	0	0	1	0	10	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2EF1	48.81165	-94.15304	2023-05-06	834	9	100x2	200	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0
PINR-EXP2	PINREXP2EF2	48.81165	-94.15304	2023-05-06	746	9	100x2	200	0	0	0	0	2	5	2	0	0	0	0	0	0	0	0	4
PINR-EXP2	PINREXP2EF3	48.81165	-94.15304	2023-05-06	1021	9	100x2	200	0	0	0	0	6	0	1	0	0	0	0	0	0	0	0	2

Table D-3: Raw counts for fish species (seine) for the Rainy River Mine Phase 3 EEM

SiteCode	GearID	Northing	Easting	Date	Sampling Area (m2)	Blackside Darter	Brassy Minnow	Brook Stickleback	Brown Bullhead	Central Mudminnow	Common Shiner	Creek Chub	Fathead Minnow	Finescale Dace	Golden Shiner	Johnny Darter	Northern Pike	Northern Redbelly Dace	Pearl Dace	Rock Bass	White Sucker
PINR-EXP2	PINREXP2S1	48.81165	-94.15304	2023-05-05	225	0	20	0	0	2	182	56	16	0	0	0	0	0	3	0	1

Table D-4: Raw fish meristics for the Rainy River Mine Phase 3 EEM

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
PINR-EXP2-CMM-1	M	4.9	1	1.224	0.019	0.01				
PINR-EXP2-CMM-2	M	6.9	1	2.796	0.042	0.058				
PINR-EXP2-CMM-3	M	7.6	1	4.128	0.109	0.102				
PINR-EXP2-CMM-4	M	8	2	4.988	0.106	0.084				
PINR-EXP2-CMM-5	M	9.2	2	8.52	0.161	0.302				
PINR-EXP2-CMM-6	F	9.1	2	9.079	0.224	1.926	919	0.0021		
PINR-EXP2-CMM-7	F	11.4	2	16.706	0.452	3.187	1948	0.00164		
PINR-EXP2-CMM-8	M	4.7	1	1.154	0.033	0.018				
PINR-EXP2-CMM-9	M	8.4	3	5.19	0.145	0.159				
PINR-EXP2-CMM-10	M	6.7	1	2.697	0.079	0.101				
PINR-EXP2-CMM-11	M	5.6	1	1.818	0.038	0.015				
PINR-EXP2-CMM-12	M	9.1	2	7.323	0.161	0.061				
PINR-EXP2-CMM-13	I	4.9	1	1.312						
PINR-EXP2-CMM-14	I	5.5	1	1.444						
PINR-EXP2-CMM-15	I	4.6	2	1.004						
PINR-EXP2-CMM-16	I	4.8	1	1.103						
PINR-EXP2-CMM-17	I	4.9	2	1.274						
PINR-EXP2-CMM-18	I	4.6	1	1						
PINR-EXP2-CMM-19	I	4.3	1	0.85						
PINR-EXP2-CMM-20	I	4.9	1	1.131						
PINR-EXP2-CMM-21	I	4.4	2	0.864						
PINR-EXP2-CMM-22	I	4.7	1	1.184						
PINR-EXP2-CMM-23	M	4.9	1	1.217	0.026	0.01				
PINR-EXP2-CMM-24	M	7.4	2	3.945	0.075	0.112				
PINR-EXP2-CMM-25	M	6.2	1	2.619	0.065	0.062				
PINR-EXP2-CMM-26	M	7.5	2	3.94	0.074	0.162				
PINR-EXP2-CMM-27	F	7.9	2	4.901	0.167	0.787	600	0.00131		
PINR-EXP2-CMM-28	I	4.8	1	1.113						
PINR-EXP2-CMM-29	M	5	1	1.352	0.02	0.016				
PINR-EXP2 - BSB-1	F	5.3	1	1.14	0.048	0.044	770	0.00006		
PINR-EXP2 - BSB-2	M	4.6	1	0.947	0.029	0.004				
PINR-EXP2 - BSB-3	M	4.8	1	0.834	0.034	0.005				
PINR-EXP2 - BSB-4	M	4.5	1	0.787	0.038	0.005				
PINR-EXP2 - BSB-5	M	5.4	1	1.444	0.057	0.005				
PINR-EXP2 - BSB-6	F	4.7	1	1.015	0.074	0.053	628	0.00008		
PINR-EXP2 - BSB-7	M	5.3	1	1.346	0.073	0.005			Nematodes	
PINR-EXP2 - BSB-8	M	5.1	1	1.203	0.043	0.006				
PINR-EXP2 - BSB-9	M	5.1	1	1.15	0.05	0.007				

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
PINR-EXP2 - BSB-10	F	5.5	1	1.338	0.098	0.062	988	0.00006		
PINR-EXP2 - BSB-11	M	5.4	1	1.387	0.047	0.005				
PINR-EXP2 - BSB-12	M	5.8	2	1.745	0.067	0.009			Nematodes	
PINR-EXP2 - BSB-13	F	5.2	2	1.134	0.057	0.07	1048	0.00007		
PINR-EXP2 - BSB-14	M	5.8	1	1.628	0.057	0.012				
PINR-EXP2 - BSB-15	M	5.5	1	1.4	0.047	0.003				
PINR-EXP2 - BSB-16	F	5.2	1	1.12	0.056	0.048	865	0.00006		
PINR-EXP2 - BSB-17	M	5.4	1	1.349	0.068	0.002			Nematodes	
PINR-EXP2 - BSB-18	M	5.4	1	1.198	0.041	0.007				
PINR-EXP2 - BSB-19	F	4.9	1	0.787	0.013	0.027	588	0.00005		
PINR-EXP2 - BSB-20	M	4.9	1	0.868	0.025	0.001				
PINR-EXP2 - BSB-21	M	5.1	1	1.088	0.027	0.004				
PINR-EXP2 - BSB-22	M	4.1	1	0.712	0.016	0.001				
PINR-EXP2 - BSB-23	M	5.9	1	1.838	0.078	0.007				
PINR-EXP2 - BSB-24	M	5.7	1	1.656	0.049	0.009				
PINR-EXP2 - BSB-25	F	4.6	1	0.951	0.049	0.04	674	0.00006		
PINR-EXP2 - BSB-26	M	4.9	1	1.037	0.045	0.005				
PINR-EXP2 - BSB-27	M	4.6	1	1.011	0.036	0.004				
PINR-EXP2 - BSB-28	F	5.3	3	1.386	0.094	0.071	1304	0.00005		
PINR-EXP2 - BSB-29	F	4.8	1	1.13	0.062	0.06	930	0.00006		
PINR-EXP2 - BSB-30	F	5.1	1	1.341	0.076	0.098	918	0.00011		
PINR-EXP2 - BSB-31	F	4.4	1	0.839	0.059	0.037	586	0.00006		
PINR-EXP2 - BSB-32	M	4.7	1	0.988	0.036	0.006				
PINR-EXP2 - BSB-33	F	4.3	1	0.965	0.089	0.192	438	0.00044		
PINR-EXP2 - BSB-34	M	4.4	1	0.931	0.041	0.006				
PINR-EXP2 - BSB-35	M	5.2	1	1.291	0.054	0.008				
PINR-EXP2 - BSB-36	F	5.4	1	1.253	0.072	0.051	1044	0.00005		
PINR-EXP2 - BSB-37	F	4	1	0.631	0.038	0.028	309	0.00009		
PINR-EXP2 - BSB-38	M	4.5	1	0.833	0.024	0.006				
PINR-EXP2 - BSB-39	M	5.4	1	1.365	0.074	0.006				
PINR-EXP2 - BSB-40	F	4.5	1	0.626	0.009	0.028	618	0.00005		
PINR-EXP2 - BSB-41	F	4.5	1	0.617	0.013	0.021	735	0.00003		
PINR-EXP2 - BSB-42	F	4.7	1	0.932	0.058	0.06	800	0.00008		
PINR-EXP2 - BSB-43	F	4.8	1	1.085	0.117	0.125	966	0.00013	Nematodes	
PINR-EXP2 - BSB-44	F	5.4	1	1.336	0.076	0.099	818	0.00012		
PINR-REF1-CMM-1	M	7.7	1	5.127	0.117	0.096				
PINR-REF1-CMM-2	M	8.1	1	5.64	0.122	0.132				
PINR-REF1-CMM-3	M	7	1	3.444	0.087	0.049				
PINR-REF1-CMM-4	M	8.3	2	5.407	0.098	0.132				
PINR-REF1-CMM-5	M	9.6	2	9.101	0.201	0.253				

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
PINR-REF1-CMM-6	M	8.1	2	5.499	0.09	0.156				
PINR-REF1-CMM-7	M	8.1	1	5.259	0.106	0.132				
PINR-REF1-CMM-8	M	9.8	3	10.28	0.347	0.395				
PINR-REF1-CMM-9	M	9.4	3	8.888	0.177	0.255				
PINR-REF1-CMM-10	M	5.2	2	1.56	0.022	0.021				
PINR-REF1-CMM-11	F	7.1	1	3.411	0.122	0.214	241	0.00089		
PINR-REF1-CMM-12	M	8.5	1	6.934	0.167	0.197				
PINR-REF1-CMM-13	M	8.5	1	6.366	0.173	0.165				
PINR-REF1-CMM-14	F	11.1	3	14.699	0.43	2.829	1912	0.00148		
PINR-REF1-CMM-15	M	9.5	2	8.686	0.184	0.295				
PINR-REF1-CMM-16	M	9.3	2	8.108	0.195	0.151				
PINR-REF1-CMM-17	M	8.6	2	6.7	0.103	0.159				
PINR-REF1-CMM-18	M	9.1	2	7.578	0.091	0.211				
PINR-REF1-CMM-19	M	8.8	2	6.375	0.153	0.151				
PINR-REF1-CMM-20	F	11.4	2	14.937	0.509	2.504	1749	0.00143	Nematodes	
PINR-REF1-CMM-21	M	7.4	2	4.228	0.085	0.083				
PINR-REF1-CMM-22	M	10	2	10.075	0.176	0.219				
PINR-REF1-CMM-23	M	8.8	3	6.525	0.153	0.154				
PINR-REF1-CMM-24	F	10.5	2	11.569	0.257	1.937	1243	0.00156		
PINR-REF1-CMM-25	I	4.5	1	0.911						
PINR-REF1-CMM-26	M	5.4	2	1.564	0.023	0.013				
PINR-REF1-CMM-27	M	7	1	3.371	0.057	0.105				
PINR-REF1-CMM-28	M	5.5	1	1.328	0.031	0.022				
PINR-REF1-CMM-29	M	7.1	1	3.469	0.066	0.089				
PINR-REF1-CMM-30	I	5.2	2	1.225						
PINR-REF1-CMM-31	M	6.9	1	2.884	0.05	0.066				
PINR-REF1-CMM-32	I	4.9	1	1.072						
PINR-REF1-CMM-33	M	10.6	3	12.693	0.184	0.344				
PINR-REF1-CMM-34	M	8.7	2	7.254	0.171	0.181				
PINR-REF1-CMM-35	M	9.5	2	9.269	0.118	0.392			Liver cyst	
PINR-REF1-CMM-36	I	6.4	1	2.447						
PINR-REF1-CMM-37	I	4.7	2	0.982						
PINR-REF1-CMM-38	F	8.7	2	5.896	0.227	0.73	621	0.00118		
PINR-REF1-CMM-39	F	9.7	1	9.537	0.181	2.021	1307	0.00155		
PINR-REF1-CMM-40	F	12.3	2	21.536	0.694	3.81	1544	0.00247		
PINR-REF1-CMM-41	F	7.8	2	4.506	0.097	0.642	370	0.00174		
PINR-REF1-CMM-42	F	9.8	1	10.181	0.302	2.018	1241	0.00163		
PINR-REF1-CMM-43	F	8.9	1	7.803	0.224	1.287	973	0.00132		
PINR-REF1-CMM-44	F	8.1	1	5.585	0.146	0.822	419	0.00196		
PINR-REF1-CMM-45	F	8.4	1	5.374	0.131	0.795	581	0.00137		

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
PINR-REF1-BSB-1	M	5.2	1	1.071	0.034	0.006				
PINR-REF1-BSB-2	F	5.1	1	1.121	0.06	0.047	1004	0.00005		
PINR-REF1-BSB-3	M	5.1	1	1.44	0.05	0.004				
PINR-REF1-BSB-4	M	5.9	1	1.807	0.065	0.009				
PINR-REF1-BSB-5	M	5.8	1	1.592	0.053	0.008				
PINR-REF1-BSB-6	F	4.9	1	0.996	0.04	0.046	1140	0.00004		
PINR-REF1-BSB-7	F	4.8	1	0.923	0.042	0.036	825	0.0004		
PINR-REF1-BSB-8	F	4.7	1	0.827	0.038	0.027	716	0.0004		
PINR-REF1-BSB-9	M	4.6	1	0.856	0.03	0.004				
PINR-REF1-BSB-10	M	4.9	1	1.134	0.036	0.005				
PINR-REF1-BSB-11	M	5.9	1	1.718	0.081	0.008				
PINR-REF1-BSB-12	M	5.9	1	1.645	0.053	0.009				
PINR-REF1-BSB-13	M	4.9	1	1.042	0.04	0.009				
PINR-REF1-BSB-14	M	5.2	1	1.233	0.043	0.005				
PINR-REF1-BSB-15	F	5.2	1	1.165	0.062	0.054	995	0.00005		
PINR-REF1-BSB-16	M	4.7	1	0.841	0.027	0.004				
PINR-REF1-BSB-17	M	4.9	1	1.057	0.027	0.005				
PINR-REF1-BSB-18	M	5.3	1	1.127	0.041	0.006				
PINR-REF1-BSB-19	M	5.4	1	1.351	0.055	0.006				
PINR-REF1-BSB-20	F	5.2	1	1.17	0.044	0.053	1298	0.00004		
PINR-REF1-BSB-21	M	4.9	1	0.95	0.032	0.005				
PINR-REF1-BSB-22	M	4.4	1	0.705	0.025	0.004				
PINR-REF1-BSB-23	M	4.8	1	0.959	0.09	0.005				
PINR-REF1-BSB-24	M	4.9	1	1.023	0.055	0.006				
PINR-REF1-BSB-25	F	5.1	1	1.012	0.041	0.046	992	0.00005		
PINR-REF1-BSB-26	M	5.5	1	1.419	0.054	0.01				
PINR-REF1-BSB-27	F	5	1	1.018	0.035	0.006	NA	NA		
PINR-REF1-BSB-28	M	5.6	1	1.566	0.04	0.009				
PINR-REF1-BSB-29	F	4.7	1	0.822	0.031	0.034	936	0.00004		
PINR-REF1-BSB-30	M	5	1	1.136	0.035	0.005				
PINR-REF1-BSB-31	M	5	1	1.001	0.038	0.006				
PINR-REF1-BSB-32	M	5.2	1	1.138	0.048	0.007				
PINR-REF1-BSB-33	F	4.7	1	0.911	0.059	0.047	949	0.00005		
PINR-REF1-BSB-34	F	5.5	1	1.285	0.076	0.054	1073	0.00005		
PINR-REF1-BSB-35	F	5	1	1.006	0.047	0.045	872	0.00005		
PINR-REF1-BSB-36	M	5.4	1	1.309	0.057	0.006				
PINR-REF1-BSB-37	F	5.4	1	1.161	0.058	0.05	1250	0.00004		
PINR-REF1-BSB-38	F	4.9	1	0.939	0.038	0.037	978	0.00004		
PINR-REF1-BSB-39	M	4.8	1	0.934	0.027	0.001				
PINR-REF1-BSB-40	F	5.2	1	1.134	0.05	0.045	880	0.00005		

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
PINR-REF1-BSB-41	F	4.9	1	0.857	0.029	0.047	1174	0.00004		
PINR-REF1-BSB-42	F	4.7	1	0.807	0.028	0.045	720	0.00006		
PINR-REF1-BSB-43	F	4.9	1	0.89	0.029	0.041	1016	0.00004		
PINR-REF1-BSB-44	F	4.8	1	0.914	0.042	0.034	773	0.00004		
PINR-REF1-BSB-45	F	4.3	1	0.619	0.028	0.029	638	0.00005		
PINR-REF1-BSB-46	F	4.6	1	0.74	0.037	0.034	894	0.00004		
PINR-REF1-BSB-47	F	4.6	1	0.94	0.073	0.057	770	0.00007		
PINR-REF1-BSB-48	F	5.4	1	1.252	0.054	0.046	1067	0.00004		
PINR-REF1-BSB-49	F	4.9	1	1.021	0.055	0.048	782	0.00006		
PINR-REF1-BSB-50	F	4.4	1	0.754	0.051	0.071	810	0.00009		
PINR-REF1-BSB-51	F	4.7	1	0.829	0.035	0.034	632	0.00005		
PINEXP-CMM-1	M	9.2	1	8.28	0.119	0.236				
PINEXP-CMM-2	M	7.9	1	4.893	0.088	0.029				
PINEXP-CMM-3	M	9.1	1	8.231	0.16	0.207				
PINEXP-CMM-4	F	10.9	3	16.179	0.106	0.161	undiff	undiff		
PINEXP-CMM-5	F	8.4	1	6.048	0.338	3.827	NA	NA		
PINEXP-CMM-6	M	4.6	2	0.927	0.016	0.008				
PINEXP-CMM-7	M	11	4	14.255	0.219	0.156				
PINEXP-CMM-8	M	9.8	2	9.953	0.153	0.206				
PINEXP-CMM-9	M	8.3	2	5.544	0.105	0.182				
PINEXP-CMM-10	F	10.9	1	13.243	0.282	2.484	1131	0.0022		
PINEXP-CMM-11	M	8.8	2	7.337	0.151	0.084				
PINEXP-CMM-12	M	8.5	2	6.667	0.14	0.043				
PINEXP-CMM-13	I	5.7	2	1.827						
PINEXP-CMM-14	M	4.6	2	1.027	0.018	0.006				
PINEXP-CMM-15	I	4.3	2	0.895						
PINEXP-CMM-16	F	9	2	6.979	0.182	0.955	561	0.0017		
PINEXP-CMM-17	F	10.7	2	14.011	0.379	3.044	1698	0.00179		
PINEXP-CMM-18	M	11.1	2	14.925	0.242	0.543				
PINEXP-CMM-19	F	12.1	2	18.096	0.415	0.216	undiff	undiff		Could be spent
PINEXP-CMM-20	F	11.3	2	15.874	0.456	0.22	undiff	undiff		Could be spent
PINEXP-CMM-21	F	10.4	2	11.635	0.28	0.189	undiff	undiff		Could be spent
PINEXP-CMM-22	F	9.6	1	9.368	0.275	0.14	undiff	undiff		Could be spent
PINEXP-CMM-23	M	10.8	2	13.245	0.246	0.267				
PINEXP-CMM-24	M	9.2	2	9.274	0.187	0.112				
PINEXP-CMM-25	M	10.3	2	12.096	0.267	0.066				
PINEXP-CMM-26	I	5	2	1.314						
PINEXP-CMM-27	M	4.6	2	1.067	0.016	0.011				
PINEXP-CMM-28	I	5.9	2	2.5						
PINEXP-CMM-29	F	11.8	2	13.874	0.286	0.305	undiff	undiff		

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
PINEXP-CMM-30	F	10.2	2	12.586	0.299	3.188	1507	0.00212		
PINEXP-CMM-31	M	6.2	1	2.385	0.039	0.028				
PINEXP-CMM-32	M	5.4	1	1.615	0.034	0.018				
PINEXP-CMM-33	M	5.2	2	1.487	0.023	0.012				
PINEXP-CMM-34	I	4.9	1	1.278						
PINEXP-CMM-35	M	8.5	2	6.779	0.1	0.262				
PINEXP-CMM-36	M	7.9	2	5.494	0.114	0.136				
PINEXP-CMM-37	M	9.1	2	7.593	0.139	0.067				
PINEXP-CMM-38	M	8.7	2	7.486	0.091	0.244				
PINEXP-CMM-39	M	9.5	2	9.591	0.099	0.38				
PINEXP-CMM-40	M	11	2	16.282	0.312	0.619				
PINEXP-CMM-41	M	9.3	2	8.214	0.168	0.067				
PINEXP-CMM-42	M	10.9	2	15.068	0.267	0.401				
PINEXP-CMM-43	M	10.3	2	11.983	0.284	0.383				
PINEXP-CMM-44	F	10.6	2	12.035	0.542	0.192	undiff	undiff		Spent
PINEXP-CMM-45	F	9.6	2	9.038	0.291	0.139	undiff	undiff		Spent
PINEXP-CMM-46	I	5.9	2	2.199						
PINEXP-CMM-47	M	5.3	2	1.55	0.029	0.015				
PINEXP-CMM-48	M	5.8	2	2.048	0.035	0.014				
PINEXP-CMM-49	M	5.9	2	1.984	0.036	0.013				
PINEXP-CMM-50	M	6	2	2.206	0.056	0.02				
PINEXP-CMM-51	M	5	2	1.386	0.028	0.026				
PINEXP-CMM-52	M	4.8	2	1.147	0.024	0.012				
PINEXP-CMM-53	M	4.8	2	1.277	0.04	0.011				
PINEXP-CMM-54	M	4.7	2	1.085	0.025	0.012				
PINEXP-BSB-1	F	5.7	1	1.492	0.062	0.073	1580	0.00005		
PINEXP-BSB-2	M	5.3	1	1.197	0.035	0.006				
PINEXP-BSB-3	M	4.8	1	0.87	0.04	0.003			Nematodes	
PINEXP-BSB-4	M	5.5	1	1.499	0.064	0.007				
PINEXP-BSB-5	F	5.3	1	1.426	0.089	0.145	1231	0.00012		
PINEXP-BSB-6	M	5.6	1	1.716	0.076	0.007			Nematodes	
PINEXP-BSB-7	M	5.9	1	2	0.064	0.011				
PINEXP-BSB-8	M	5.4	1	1.444	0.035	0.006				
PINEXP-BSB-9	M	4.8	1	1.145	0.036	0.004				
PINEXP-BSB-10	F	5.3	1	1.238	0.065	0.055	996	0.00006		
PINEXP-BSB-11	F	5.8	1	1.665	0.088	0.161	1242	0.00013		
PINEXP-BSB-12	M	5	1	1.154	0.033	0.008				
PINEXP-BSB-13	M	5.1	1	1.273	0.048	0.007				
PINEXP-BSB-14	F	4.6	1	1.043	0.066	0.174	1098	0.00016		
PINEXP-BSB-15	M	5.5	1	1.521	0.049	0.007				

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
PINEXP-BSB-16	M	5.1	1	1.99	0.092	0.007			Nematodes	
PINEXP-BSB-17	M	5.5	1	1.63	0.068	0.007				
PINEXP-BSB-18	M	5.2	1	1.362	0.038	0.014				
PINEXP-BSB-19	M	6.1	1	1.906	0.074	0.006			Liver cyst	
PINEXP-BSB-20	M	5.2	1	1.342	0.027	0.008				
PINEXP-BSB-21	F	5.6	1	1.374	0.065	0.08	1336	0.00006		
PINEXP-BSB-22	F	5.3	2	1.28	0.071	0.042	1022	0.00004		
PINEXP-BSB-23	F	5	1	1.56	0.072	0.07	1673	0.00004		
PINEXP-BSB-24	F	5.4	1	1.065	0.09	0.046	1103	0.00004		
PINEXP-BSB-25	F	4.8	1	0.923	0.043	0.045	982	0.00005		
PINEXP-BSB-26	F	5	1	1.118	0.049	0.069	1185	0.00006		
PINEXP-BSB-27	M	4.6	1	0.958	0.047	0.006				
PINEXP-BSB-28	F	4.5	1	0.728	0.031	0.025	836	0.00003	Nematodes	
PINEXP-BSB-29	F	5.6	1	1.916	0.112	0.366	1727	0.00021		
PINEXP-BSB-30	M	5.6	1	1.548	0.044	0.008				
PINEXP-BSB-31	F	4.9	2	0.78	0.025	0.033	1372	0.00002		
PINEXP-BSB-32	F	6.2	1	1.836	0.152	0.083	1635	0.00005		
PINEXP-BSB-33	M	5	1	1.027	0.019	0.004				
PINEXP-BSB-34	M	5.5	1	1.443	0.031	0.004				
PINEXP-BSB-35	M	5.4	1	1.6	0.031	0.005				
PINEXP-BSB-36	M	5.3	1	1.497	0.056	0.007				
PINEXP-BSB-37	M	6.3	2	2.321	0.084	0.013				
PINEXP-BSB-38	F	4.3	1	0.807	0.037	0.059	671	0.00009		
PINEXP-BSB-39	M	5.5	1	1.57	0.047	0.009				
PINEXP-BSB-40	F	4.5	1	0.887	0.074	0.059	998	0.00006		
PINEXP-BSB-41	F	4.3	1	0.903	0.067	0.044	681	0.00006	Nematodes	
PINEXP-BSB-42	M	5.1	2	1.394	0.052	0.008				
PINEXP-BSB-43	M	4.5	1	1.146	0.054	0.005			Nematodes	
PINEXP-BSB-44	F	4.6	1	0.947	0.063	0.079	1053	0.00008		
PINEXP-BSB-45	F	5.2	1	1.569	0.108	0.228	1648	0.00014	Nematodes	
PINEXP-BSB-46	F	5.4	1	1.576	0.107	0.223	1204	0.00019		
PINEXP-BSB-47	F	4.4	1	1.026	0.078	0.234	876	0.00027		
PINEXP-BSB-48	F	4.9	1	1.141	0.102	0.113	1363	0.00008		
PINEXP-BSB-49	F	5	1	1.337	0.093	0.157	956	0.00016	Liver cyst	
PINEXP-BSB-50	F	5.3	1	1.524	0.122	0.347	1690	0.00021		
PINEXP-BSB-51	F	6.1	1	2.327	0.19	0.541	1444	0.00037	Liver cyst	
PINEXP-BSB-52	F	4.7	1	1.029	0.092	0.112	953	0.00012		
PINEXP-BSB-53	F	5.5	1	1.617	0.149	0.137	1264	0.00011		
STUC-CMM-1	F	9.5	2	9.533	0.297	1.74	970	0.00179		
STUC-CMM-2	F	9.9	2	10.746	0.343	2.017	1096	0.00184		

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
STUC-CMM-3	F	9.6	2	10.77	0.313	1.946	797	0.00221		Needed to add 0.182 to gonad weight due to scale error
STUC-CMM-4	M	8	2	5.519	0.108	0.074				
STUC-CMM-5	F	9.8	1	11.092	0.38	1.842	1549	0.00119		
STUC-CMM-6	IMM	5.9	1	1.946						
STUC-CMM-7	IMM	4.7	2	0.937						
STUC-CMM-8	M	10.5	2	11.876	0.179	1.91				
STUC-CMM-9	M	9	1	7.56	0.117	0.275				
STUC-CMM-10	M	8.4	1	5.915	0.104	0.068				
STUC-CMM-11	M	9.2	1	7.68	0.146	0.261				
STUC-CMM-12	M	8.6	1	5.704	0.07	0.093				
STUC-CMM-13	F	8.5	1	6.235	0.156	1.025	684	0.0015		Sample originally called LVR-CMM-13 but changed to STUC-CMM-13 by gonad laboratory after forensic look at data and sample checking.
STUC-CMM-14	M	8.4	1	5.19	0.101	0.058				
STUC-CMM-15	M	8	1	5.268	0.297	0.211				
STUC-CMM-16	M	9	1	7.374	0.12	0.173				
STUC-CMM-17	F	8.9	1	7.601	0.228	1.138	465	0.00245		
STUC-CMM-18	M	7.8	1	4.66	0.068	0.069				
STUC-CMM-19	M	7.7	1	4.492	0.082	0.154				
STUC-CMM-20	M	7.9	1	4.02	0.053	0.49				
STUC-CMM-21	F	8.2	2	5.216	0.119	0.79	368	0.00215		
STUC-CMM-22	M	7.4	2	3.977	0.07	0.014				
STUC-CMM-23	F	7.3	1	4.241	0.092	0.662	303	0.00218		
STUC-CMM-24	M	5.4	2	1.52	0.036	0.009				
STUC-CMM-25	M	5.1	1	1.286	0.032	0.009				
STUC-CMM-26	M	5.3	1	1.441	0.029	0.013				
STUC-CMM-27	M	5.1	1	1.365	0.029	0.019				
STUC-CMM-28	M	5.1	1	1.249	0.03	0.017				
STUC-CMM-29	M	5	1	1.425	0.022	0.024				
STUC-CMM-30	M	5.2	1	1.271	0.025					
STUC-CMM-31	IMM	4.6	1	1.027						
STUC-CMM-32	M	8.1	1	5.172	0.076	0.045				
STUC-CMM-33	M	8	1	4.954	0.076	0.052				
STUC-CMM-34	M	8.3	1	5.145	0.063	0.129				
STUC-CMM-35	F	9.9	2	9.064	0.248	0.143	undiff	undiff		
STUC-CMM-36	M	10.9	3	14.292	0.375	0.243				
STUC-CMM-37	F	11.7	2	19.511	0.366	5.368	2960	0.00181		
STUC-CMM-38	M	9.2	1	7.644	0.133	0.288				
STUC-CMM-39	F	10.3	1	12.656	0.4	2.98	1523	0.00196		
STUC-CMM-40	M	8.5	2	6.143	0.113	0.069				

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
STUC-CMM-41	F	8.8	2	6.852	0.168	1.248	512	0.00244		
STUC-CMM-42	M	8	2	5.191	0.09	0.149				
STUC-CMM-43	M	9.3	2	8.211	0.217	0.082				
STUC-CMM-44	M	8.3	2	6.016	0.086	0.256				
STUC-CMM-45	M	10.6	3	11.896	0.196	0.188				
STUC-CMM-46	M	8.8	2	6.777	0.101	0.187				
STUC-CMM-47	M	8.1	1	5.434	0.092	0.247				
STUC-CMM-48	M	8.7	1	6.186	0.113	0.09				
STUC-CMM-49	M	11.1	3	14.269	0.257	0.352				
STUC-CMM-50	M	8.2	1	5.321	0.065	0.133				
STUC-CMM-51	M	9.8	2	9.671	0.103	0.068				
STUC-CMM-52	M	9.5	1	8.928	0.124	0.133				
STUC-CMM-53	F	8	2	5.717	0.16	0.491	401	0.00122		
STUC-CMM-54	F	8.9	2	7.531	0.231	1.234	800	0.00154		
STUC-CMM-55	F	12.8	2	24.904	0.926	5.784	2946	0.00196		
STUC-CMM-56	F	10	2	10.51	0.307	0.158	undiff	undiff		Spent
STUC-BSB-1	F	4.3	1	0.797	0.059	0.091	341	0.00027	Nematodes	
STUC-BSB-2	F	4.5	1	0.625	0.031	0.03	767	0.00004		
LVR2-CMM-1	F	11.2	2	14.987	0.407	3.14	1665	0.00189		
LVR2-CMM-2	I	4.7	1	0.94						
LVR2-CMM-3	M	6.1	1	2.021	0.038	0.048				
LVR2-CMM-4	M	6.7	1	2.515	0.046	0.061				
LVR2-CMM-5	M	9.4	4	8.393	0.176	0.339				
LVR2-CMM-6	I	4.7	1	0.914						
LVR2-CMM-7	I	4.2	2	0.738						
LVR2-CMM-8	I	4.3	1	0.8						
LVR2-CMM-9	I	4.4	1	0.779						
LVR2-CMM-10	I	4.1	1	0.821						
LVR2-CMM-11	I	4.7	1	0.966						
LVR2-CMM-12	I	4.8	2	1.088						
LVR2-CMM-13	I	3.8	1	0.472						
LVR2-CMM-14	I	5.5	1	1.522						
LVR2-CMM-15	I	5.8	1	1.784						
LVR2-CMM-16	M	6.5	1	2.792	0.072	0.037				
LVR2-CMM-17	F	11.1	2	13.836	0.451	2.068	2135	0.00097		
LVR2-CMM-18	M	11.4	2	16.709	0.403	0.293				
LVR2-CMM-19	M	8.9	2	6.431	0.126	0.223				
LVR2-CMM-20	M	6.1	1	2.003	0.051	0.03				
LVR2-BSB-1	M	4.7	1	0.895	0.044	0.004				
LVR2-BSB-2	M	4.7	2	1.03	0.046	0.004				

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
LVR2-BSB-3	M	4.6	2	0.763	0.023	0.004				
LVR2-BSB-4	F	4.7	1	0.89	0.042	0.039	624	0.00006		
LVR2-BSB-5	F	5	2	0.956	0.057	0.043	591	0.00007		
LVR2-BSB-6	F	5.2	1	1.132	0.05	0.049	1103	0.00004		
LVR2-BSB-7	F	4.9	2	1.043	0.066	0.051	748	0.00007		
LVR2-BSB-8	M	4.7	2	0.883	0.038	0.004				
LVR2-BSB-9	F	5.3	1	1.138	0.059	0.049	982	0.00005		
LVR2-BSB-10	M	5.3	1	1.207	0.049	0.005				
LVR2-BSB-11	M	5.6	1	1.464	0.061	0.009				
LVR2-BSB-12	M	5.4	1	1.171	0.045	0.006				
LVR2-BSB-13	M	5	1	0.9	0.03	0.006				
LVR2-BSB-14	M	4.8	1	1.003	0.037	0.005				
LVR2-BSB-15	M	4.8	1	0.94	0.035	0.006				
LVR2-BSB-16	F	5.1	1	1.074	0.067	0.043	727	0.00006		
LVR2-BSB-17	M	5.1	1	0.988	0.036	0.005				
LVR2-BSB-18	F	4.8	1	1.023	0.067	0.046	907	0.00005		
LVR2-BSB-19	M	5	1	1.168	0.071	0.005				
LVR2-BSB-20	F	4.8	2	1.019	0.064	0.037	722	0.00005		
LVR2-BSB-21	M	4	0	0.511	0.017	0.002				
LVR2-BSB-22	F	4.7	1	0.769	0.038	0.032	610	0.00005		
LVR2-BSB-23	M	4.6	1	0.94	0.033	0.007				
LVR2-BSB-24	M	4.9	1	1.02	0.034	0.006				
LVR2-BSB-25	M	5.1	1	0.876	0.024	0.003				
LVR2-BSB-26	F	4.8	1	0.918	0.046	0.029	594	0.00005		
LVR2-BSB-27	M	5	1	1.1	0.056	0.006				
LVR2-BSB-28	M	5.9	1	1.59	0.057	0.01				
LVR2-BSB-29	M	5.2	1	1.292	0.061	0.005				
LVR2-BSB-30	M	5.3	1	1.33	0.062	0.007				
LVR2-BSB-31	M	5.6	1	1.489	0.076	0.005				
LVR2-BSB-32	M	5.5	1	1.45	0.056	0.007				
LVR2-BSB-33	M	5.8	1	1.424	0.054	0.008				
LVR2-BSB-34	M	5.5	1	1.495	0.069	0.005				
LVR2-BSB-35	M	5.3	1	1.345	0.046	0.008				
LVR2-BSB-36	F	5.5	1	1.296	0.087	0.052	1048	0.00005		
LVR2-BSB-37	F	5.2	1	1.067	0.059	0.055	940	0.00006		
LVR2-BSB-38	F	5.3	1	1.306	0.091	0.059	819	0.00007		
LVR2-BSB-39	F	5.1	1	1.079	0.051	0.042	712	0.00006		
LVR2-BSB-40	F	4.7	1	0.984	0.074	0.032	625	0.00005	Nematodes	
LVR2-BSB-41	F	5.6	1	1.675	0.134	0.081	1328	0.00006		
LVR2-BSB-42	F	4.7	1	0.867	0.036	0.034	674	0.00005		

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
LVR2-BSB-43	F	4.7	1	0.85	0.046	0.039	802	0.00005		
LVR2-BSB-44	F	4.8	1	0.932	0.056	0.038	670	0.00006		
LVR2-BSB-45	F	4.6	1	0.828	0.039	0.037	584	0.00006		
LVR2-BSB-46	F	4.9	1	0.888	0.041	0.034	728	0.00005		
LVR2-BSB-47	F	5.2	1	0.959	0.054	0.037	724	0.00005		
LVR2-BSB-48	F	4.8	1	0.964	0.046	0.035	878	0.00004		
LVR2-BSB-49	F	5	1	0.904	0.04	0.032	645	0.00005		
LVR2-BSB-50	F	4.9	1	0.912	0.043	0.039	645	0.00006		
LVR-CMM-1	F	11.1	2	15.996	0.411	2.418	1595	0.00152		
LVR-CMM-2	M	8.8	2	7.715	0.166	0.196				
LVR-CMM-3	M	8	1	5.216	0.139	0.112				
LVR-CMM-4	F	6.9	1	3.303	0.118	0.18	331	0.00054		
LVR-CMM-5	M	6.5	1	2.714	0.049	0.06				
LVR-CMM-6	M	7	2	3.69	0.094	0.031				
LVR-CMM-7	F	8.7	1	6.263	0.197	1.058	565	0.00187		
LVR-CMM-8	M	8.6	3	6.891	0.187	0.142				
LVR-CMM-9	M	8.2	3	5.791	0.132	0.143				
LVR-CMM-10	F	10.7	3	14.102	0.391	2.242	1517	0.00148		
LVR-CMM-11	I	4.3	1	0.924						
LVR-CMM-12	F	7.6	2	5.328	0.162	0.829	408	0.00203		
LVR-CMM-13	M	7.4	2	4.067	0.066	0.057				
LVR-CMM-14	M	8.3	3	7.814	0.194	0.225			Missing top caudal; deformed tail	
LVR-CMM-15	M	8.7	2	7.238	0.22	0.165				
LVR-CMM-16	M	7.9	1	5.516	0.095	0.128				
LVR-CMM-17	M	7.9	2	5.38	0.142	0.092			Nematodes	
LVR-CMM-18	F	8.1	1	7.332	0.258	1.347	885	0.00152		
LVR-CMM-19	M	7	2	3.68	0.082	0.101				
LVR-CMM-20	M	6.4	2	2.708	0.073	0.101				
LVR-CMM-21	F	7.1	2	4.025	0.134	0.366	292	0.00125		
LVR-CMM-22	M	7.6	2	4.652	0.114	0.161				
LVR-CMM-23	M	8.2	3	5.97	0.147	0.14				
LVR-CMM-24	M	8.2	1	5.683	0.16	0.17				
LVR-CMM-25	F	8.9	2	7.637	0.283	1.07	773	0.00138		
LVR-CMM-26	M	9.3	3	8.605	0.213	0.321				
LVR-CMM-27	F	9.6	3	8.434	0.351	1.224	882	0.00139		
LVR-CMM-28	I	6.8	1	2.873						
LVR-CMM-29	I	4.7	2	0.969						
LVR-CMM-30	I	4.9	1	1.178						
LVR-CMM-31	I	4.3	2	0.832						
LVR-CMM-32	I	4.9	2	1.26						

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
LVR-CMM-33	I	4.2	1	0.859						
LVR-CMM-34	M	8.6	3	6.029	0.104	0.093				
LVR-CMM-35	F	8.7	3	7.159	0.227	1.323	695	0.0019		
LVR-CMM-36	F	9.7	3	11.762	0.371	1.62	748	0.00217	Deformed spine	
LVR-CMM-37	F	7.8	1	5.033	0.166	0.708	437	0.00162		
LVR-CMM-38	F	10.6	3	14.447	0.317	2.833	1326	0.00214		
LVR-CMM-39	F	9.7	3	9.823	0.224	1.813	1525	0.00119		
LVR-CMM-40	F	10.8	3	13.555	0.37	2.158	1314	0.00164		
LVR-CMM-41	F	7.7	1	4.626	0.148	0.902	502	0.0018		
LVR-CMM-42	M	6.4	2	2.618	0.069	0.065				
LVR-CMM-43	F	10.2	3	10.903	0.349	1.655	975	0.0017		
LVR-CMM-44	F	8.4	3	6.124	0.192	0.912	475	0.00192		
LVR-CMM-45	F	10.1	3	13.545	0.372	2.111	882	0.00239		
LVR-CMM-46	F	8.1	2	5.556	0.173	0.832	454	0.00183		
LVR-CMM-47	M	8	2	5.001	0.089	0.163				
LVR-CMM-48	M	9	2	7.535	0.186	0.189				
LVR-CMM-49	M	6	1	1.943	0.04	0.023				
LVR-CMM-50	M	5.9	1	2.313	0.019	0.027				
LVR-CMM-51	M	5.8	1	1.978	0.068	0.034				
LVR-CMM-52	I	4.9	2	1.068						
LVR-CMM-53	M	8	1	4.435	0.904	0.137				
LVR-CMM-54	M	8.1	1	5.207	0.135	0.19				
LVR-CMM-55	M	7.1	2	4.243	0.076	0.153				
LVR-CMM-56	M	9.8	4	9.795	0.165	0.323				
LVR-CMM-57	M	8.1	2	5.108	0.104	0.073				
LVR-CMM-58	M	10.4	3	10.313	0.196	0.146				
LVR-CMM-59	M	7.9	2	4.643	0.069	0.153				
LVR-CMM-60	M	7.7	2	4.447	0.09	0.043				
LVR-CMM-61	M	7	2	3.342	0.06	0.073				
LVR-CMM-62	M	5	2	1.243	0.03	0.018				
LVR-CMM-63	M	4.9	1	1.143	0.022	0.012				
LVR-BSB-1	M	6.3	2	2.346	0.126	0.012				
LVR-BSB-2	M	6.1	2	2.269	0.088	0.009				
LVR-BSB-3	M	6.2	2	2.459	0.096	0.009				
LVR-BSB-4	M	5.8	1	1.823	0.055	0.01				
LVR-BSB-5	M	6.4	2	2.27	0.063	0.011				
LVR-BSB-6	M	6.5	2	2.379	0.104	0.011			Tapeworm	
LVR-BSB-7	M	6.1	1	2.235	0.109	0.013			Tapeworm	
LVR-BSB-8	M	5.7	1	1.83	0.075	0.009				
LVR-BSB-9	M	6	2	1.929	0.069	0.009				

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
LVR-BSB-10	M	5.4	1	1.456	0.075	0.007				
LVR-BSB-11	M	5.9	1	1.721	0.054	0.01				
LVR-BSB-12	M	5.5	1	1.639	0.066	0.006			Parasites	
LVR-BSB-13	M	6.5	2	2.369	0.069	0.009				
LVR-BSB-14	M	5.7	1	1.61	0.061	0.008				
LVR-BSB-15	M	6.2	2	1.957	0.092	0.013			Parasites	
LVR-BSB-16	M	5.7	1	1.815	0.065	0.007				
LVR-BSB-17	M	6.3	1	2.224	0.11	0.014			Parasites	
LVR-BSB-18	M	5.7	1	1.622	0.078	0.006			Parasites	
LVR-BSB-19	F	6.1	2	1.829	0.087	0.063	1444	0.00004		
LVR-BSB-20	M	5.9	1	1.657	0.078	0.008				
LVR-BSB-21	M	5.9	1	1.634	0.058	0.011				
LVR-BSB-22	M	5.4	1	1.297	0.058	0.004				
LVR-BSB-23	F	5.4	1	1.283	0.068	0.044	887	0.00005		
LVR-BSB-24	M	5.7	1	1.532	0.057	0.007				
LVR-BSB-25	F	6	1	1.757	0.109	0.083	1635	0.00005		
LVR-BSB-26	M	5.1	1	1.129	0.046	0.004				
LVR-BSB-27	M	5.2	1	1.092	0.031	0.005				
LVR-BSB-28	M	5.4	1	1.234	0.036	0.006				
LVR-BSB-29	F	5.9	1	1.622	0.11	0.007	1440	0.00001	Parasites	
LVR-BSB-30	F	5.6	1	1.483	0.087	0.063	1348	0.00005		
LVR-BSB-31	F	4.7	1	0.898	0.029	0.041	1224	0.00003		
LVR-BSB-32	F	4.7	1	0.821	0.04	0.036	757	0.00005		
LVR-BSB-33	F	6	2	1.791	0.107	0.086	2164	0.00004		
LVR-BSB-34	F	6.1	2	1.531	0.064	0.058	1315	0.00004		
LVR-BSB-35	F	5.6	1	1.347	0.086	0.05	1251	0.00004		
LVR-BSB-36	F	4.9	1	0.926	0.039	0.035	1288	0.00003		
LVR-BSB-37	F	6.2	2	1.917	0.135	0.071	1399	0.00005		
LVR-BSB-38	F	6.1	2	1.997	0.136	0.097	1600	0.00006		
LVR-BSB-39	F	5.3	1	1.363	0.09	0.064	938	0.00007		
LVR-BSB-40	F	5.2	1	1.128	0.05	0.04	1333	0.00003		
LVR-BSB-41	F	5.3	1	1.218	0.063	0.047	1248	0.00004		
LVR-BSB-42	F	5.4	1	1.432	0.098	0.05	1046	0.00005		
LVR-BSB-43	F	5.2	1	1.111	0.08	0.054	1116	0.00005	Liver cyst	
LVR-BSB-44	F	6.5	3	1.89	0.18	0.084	1456	0.00006		
LVR-BSB-45	F	5.3	1	1.143	0.081	0.04	1146	0.00003	Parasites	
LVR-BSB-46	F	6.6	2	2.191	0.129	0.081	1246	0.00007		
LVR-BSB-47	F	5.1	1	1.208	0.058	0.048	1255	0.00004		
LVR-BSB-48	F	5.2	1	1.2	0.094	0.063	1636	0.00004		
LVR-BSB-49	F	5.1	1	1.266	0.079	0.083	1395	0.00006		

Fish ID	Sex	Total Length (cm)	Age (years)	Body Weight (g)	Liver Weight (g)	Gonad Weight (g)	Total Fecundity	Mean Egg Weight (g)	Abnormalities	Comments
LVR-BSB-50	F	5.2	1	1.082	0.056	0.034	1040	0.00003		
LVR-BSB-51	F	6.8	1	2.199	0.126	0.105	1963	0.00005	Parasites	
LVR-BSB-52	F	6.1	1	1.602	0.077	0.138	1339	0.0001	Nematodes	
LVR-BSB-53	F	5.2	1	1.182	0.054	0.039	699	0.00006		
LVR-BSB-54	F	4.7	1	0.854	0.031	0.029	618	0.00005		
LVR-BSB-55	F	4.3	1	0.684	0.031	0.029	826	0.00004		
LVR-BSB-56	F	5	1	0.989	0.036	0.043	939	0.00005		
LVR-BSB-57	F	5.5	2	1.5	0.095	0.047	964	0.00005	Nematodes	
LVR-BSB-58	F	4.7	1	1.093	0.107	0.189	1152	0.00016		
LVR-BSB-59	F	4.7	1	0.963	0.083	0.113	876	0.00013	Nematodes	
LVR-BSB-60	F	4.4	1	0.902	0.081	0.055	937	0.00006		

Table D-5: QA/QC data for fecundity measures for Phase 3 EEM.

Sample ID	Recounted			Reported			% Difference		
	Subsample			Subsample			Subsample		
	1	2	3	1	2	3	1	2	3
PINR-EXP2-27	167	133	141	168	134	142	0.6	0.7	0.7
PINEXP-BSB-46	216	195	235	212	197	226	1.9	1.0	4.0
STUC-CMM-3	147	129	140	146	127	140	0.7	1.6	0.0
STUC-CMM-37	281	233	166	284	232	166	1.1	0.4	0.0
LVR2-BSB-5	157	158	104	149	149	111	5.4	6.0	6.3
LVR2-BSB-37	164	143	145	162	137	135	1.2	4.4	7.4
LVR2-BSB-47	172	153	133	188	137	136	8.5	11.7	2.2
PINR-EXP2-BSB-29	152	154	144	165	143	150	7.9	7.7	4.0
PINR-EXP2-BSB-42	108	109	112	125	117	112	13.6	6.8	0.0
PINR-REF1-CMM-24	152	166	139	158	135	143	3.8	23.0	2.8
PINR-REF1-CMM-41	373	--	* whole sample	370	* whole sample	--	0.8	--	--
PINR-REF1-BSB-37	167	133	142	159	141	151	5.0	5.7	6.0
PINR-REF1-BSB-48	178	118	123	176	125	128	1.1	5.6	3.9
LVR-BSB-30	200	150	143	181	172	159	10.5	12.8	10.1
PINEXP-CMM-30	123	116	158	126	117	158	2.4	0.9	0.0
PINEXP-BSB-51	104	275	227	106	287	240	1.9	4.2	5.4
LVR-CMM-25	124	157	152	124	160	152	0.0	1.9	0.0
LVR-CMM-39	189	176	159	188	176	158	0.5	0.0	0.6
LVR-BSB-50	154	161	125	155	150	127	0.6	7.3	1.6
LVR-BSB-54	131	118	111	120	115	112	9.2	2.6	0.9

Mean % Difference	Minimum % Difference	Maximum % Difference
4.1	**0**	**23.0**

Table D-6: Statistical comparison post-hoc power analysis of EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF) for female Brook Stickleback.

Effect Indicator	Comparisons			Sample Size		Model	Mean Squared Error	Magnitude of Difference[b] (%)	Minimum Detectable Effect Size (%)		Minimum Sample Size (% Increase / Decrease)				
											i=5%	i=10%	i=25%	i=50%	i=100%
	Model #	Parameter	Covariate	LVR	PINR-EXP			PINR-EXP - LVR	Increase	Decrease	d=5%	d=9%	d=20	d=33%	d=50%
Survival	1	Rank of Age (years)	--	35	28	KW	--	0	--	--	--	--	--	--	--
Energy Use	2	Body Weight (g)	Total Length (cm)	35	28	ANCOVA	0.0246	10.4	9.4	-9.4	44	22	10	5	3
	3	Log_{10} Liver Weight (g)	Log_{10} Body Weight (g)	35	28	ANCOVA	0.0137	8.7	22.7	-18.5	96	50	22	13	8
	4	Log_{10} Mean Egg Weight (g)	Log_{10} Body Weight (g) min	35	28	ANCOVA	0.0641	-5.3	55.5	-35.7	206	106	46	26	16
			Log_{10} Body Weight (g) max					272.9							
	5	Log_{10} Mean Egg Weight (g)	Log_{10} Body Weight (g) min	34	24	ANCOVA	0.0369	3.2	41.9	-29.5	156	81	35	20	12
			Log_{10} Body Weight (g) max					159.3							
Energy Use	6	Body Weight (g)	--	35	28	ANOVA	0.1563	-4.7	22.1	-22.1	101	51	21	11	6
	7	Total Length (cm)	--	35	28	ANOVA	0.3464	-5.8	8.2	-8.2	38	20	9	5	3
	8	Body Weight (g); Age 1	--	26	24	ANOVA	0.0109	9.4	150.7	-150.7	604	303	122	61	31
	9	Total Length (cm); Age 1	--	27	26	ANOVA	0.2961	-2.0	8.7	-8.7	37	19	8	5	3
	10	Log10 Gonad Weight (g)	Log_{10} Body Weight (g) min	35	28	ANCOVA	0.0566	1.7	51.4	-34.0	193	100	43	24	15
			Log_{10} Body Weight (g) max					268.0							
	11	Log10 Gonad Weight (g)	Log_{10} Body Weight (g) min	34	25	ANCOVA	0.0342	-4.7	39.6	-28.4	151	78	34	19	12
			Log_{10} Body Weight (g) max					183.1							
	12	Total Fecundity (#)	Body Weight (g)	35	28	ANCOVA	54803.6239	1.5	14.7	-14.7	67	34	14	8	4

Notes:
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions
1. Non-parametric employed due to not meeting parametric assumptions
2. Parallel model run because R2 for parallel model is also > 0.8 and is less than 0.02 (i.e. 2 percentage points) less than R2 for interaction model.
6. Model violated ANCOVA assumptions. Non-parametric approach considered but this obscured an obvious interaction in the data. Removed Cook's outliers PINEXP-BSB-31, PINEXP-BSB-32, PINEXP-BSB-47, PINEXP-BSB-51, LVR-BSB-29 in attempt to better meet ANCOVA assumptions.
8 and 9. Could only examine for Age 1 fish. Statistical comparisons could not be performed due to limited sample size for Age 2 and Age 3 fish across sites.
10 and 11. Model 10 includes outliers and Model 11 (final model) does not. Model 11 violated ANCOVA assumptions. Non-parametric approach considered but this obscured an obvious interaction in the data. Removed Cook's outliers PINEXP-BSB-32, PINEXP-BSB-47, PINEXP-BSB-51, and LVR-BSB-29 in attempt to better meet ANCOVA assumptions.

Table D-7: Statistical comparison post-hoc power analysis of EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF) for male Brook Stickleback.

Effect Indicator	Comparisons			Sample Size		Model	Mean Squared Error	Magnitude of Difference (%)		Minimum Detectable Effect Size (%)		Minimum Sample Size (% Increase / Decrease)				
												i=5%	i=10%	i=25%	i=50%	i=100%
	Model #	Parameter	Covariate	LVR	PINR-EXP			PINR-EXP to LVR	PINR-EXP to STUC	Increase	Decrease	d=5%	d=9%	d=20	d=33%	d=50%
Survival	1	Rank of Age	--	25	25	KW	--	0	--	NA	NA	--	--	--	--	--
Energy Storage	2	Body Weight	Total Length	25	25	ANCOVA	0.0024	6.4	--	10.1	-9.2	41	21	10	6	4
	3	Body Weight	Total Length	25	22	ANCOVA	0.0012	5.8	--	7.3	-6.8	29	15	7	5	3
	4	Liver Weight	Body Weight	25	25	ANCOVA	0.0002	-11.9	--	18.3	-18.3	74	38	16	8	5
Energy Use	5	Body Weight	--	25	25	ANOVA	0.1450	-19.7	--	17.9	-17.9	73	37	16	8	5
	6	Total Length	--	25	25	ANOVA	0.1693	-9.4	--	6.0	-6.0	25	13	6	4	2
	7	Body Weight; Age-1	--	17	23	ANOVA	0.0995	-11.9	--	18.8	-18.8	68	35	15	8	5
	8	Total Length; Age-1	--	17	23	ANOVA	0.1277	-6.9	--	6.1	-6.1	23	12	5	3	2
	9	Log10 Gonad Weight	Log_{10} Body Weight	25	25	ANCOVA	0.0122	1.3	--	24.4	-19.6	91	47	21	12	7

Notes:
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions
1. Non-parametric employed due to not meeting parametric assumptions
2 and 3. Model 10 includes outliers and Model 11 (final model) does not.
3. Removed Cook's outliers PINEXP-BSB-3, PINEXP-BSB-16, PINEXP-BSB-43 to better meet ANCOVA assumptions. Parallel model run because R2 for parallel model is also > 0.8 and is less than 0.02 (i.e. 2 percentage points) less than R2 for interaction model.
7 and 8. Could only examine for Age 1 fish. Statistical comparisons could not be performed due to limited sample size for Age 2 and Age 3 fish across sites.

Table D-8: Statistical comparison post-hoc power analysis of EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF, STUC-REF) for female Central Mudminnow.

Effect Indicator	Comparisons			Sample Size			Model	Mean Squared Error	Magnitude of Difference (%)		Minimum Detectable Effect Size (%)[f]		Minimum Sample Size (% Increase / Decrease)				
	Model #	Parameter	Covariate	LVR-REF	STUC-REF	PINR-EXP			PINR-EXP to LVR PINR-EXP to LVR	PINR-EXP to STUC PINR-EXP to STUC			i=5%	i=10%	i=25%	i=50%	i=100%
											Increase	d=5%	d=9%	d=20	d=33%	d=50%	d=50%
Survival	1	Rank of Age	--	20	16	13	KW	--	-20.0	0.0	NA	NA	NA	NA	NA	NA	NA
Energy Storage	2	Log_{10} Body Weight	Log_{10} Total Length	20	16	13	ANCOVA	0.0017	-7.8	-5.4	10.7	-9.6	10.7	-9.6	34	18	8
	3	Log_{10} Liver Weight	Log_{10} Body Weight; min	14	11	11	ANCOVA	0.0060	13.3	52.1	25.9	-20.6	25.9	-20.6	64	33	15
									-3.7	-18.4			25.9	-20.6	64	33	15
	4	Mean Egg Weight	Body Weight	20	14	4	ANCOVA	0.0000	14.7	3.1	29.7	-29.7	29.7	-29.7	83	42	17
Energy Use	5	Log_{10} Body Weight	--	20	16	13	ANOVA	0.0358	47.9	28.3	60.1	-37.6	60.1	-37.6	154	79	35
	6	Total Length	--	20	16	13	ANOVA	1.6076	15.5	9.6	15.2	-15.2	15.2	-15.2	49	25	11
	7	Body Weight; Age 1	--	5	5	3	ANOVA	8.3482	79.9	14.2	142.5	-142.5	142.5	-142.5	188	94	38
	8	Log_{10} Body Weight; Age 2	--	5	11	9	ANOVA	0.0347	80.1	25.2	99.6	-49.9	99.6	-49.9	152	78	34
	9	Total Length; Age 1	--	5	5	3	ANOVA	1.0311	22.9	7.5	33.9	-33.9	33.9	-33.9	46	23	10
	10	Total Length; Age 2	--	5	11	9	ANOVA	1.7345	24.2	9.0	24.8	-24.8	24.8	-24.8	54	28	12
	11	Log10 Gonad Weight	Body Weight; min	20	14	4	ANCOVA	0.0757	-5.5	-9.5	32.0	-32.0	32.0	-32.0	89	45	19
									35.6	0.4			15.0	-15.0	42	22	9
	12	Total Fecundity	Body Weight	9	7	4	ANCOVA	83970.6931	9.8	2.9	54.4	-54.4	54.4	-54.4	101	51	21

Notes:
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions
1. Non-parametric employed due to not meeting parametric assumptions
3. Used covariate overlap range to meet parametric ANCOVA assumptions.
7-10. Could only examine for Age 1 and Age 2 fish. Statistical comparisons could not be performed due to limited sample size for other aged fish across sites.

Table D-9: Statistical comparison post-hoc power analysis of EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF, STUC-REF) for male Central Mudminnow.

Effect Indicator	Comparisons			Sample Size			Model	Mean Squared Error	Magnitude of Difference (%)		Minimum Detectable Effect Size (%)		Minimum Sample Size (% Increase / Decrease)				
	Model #	Parameter	Covariate	LVR-REF	STUC-REF	PINR-EXP			PINR-EXP to LVR-REF	PINR-EXP to STUC-REF	Increase	Decrease	i=5%	i=10%	i=25%	i=50%	i=100%
													d=5%	d=9%	d=20	d=33%	d=50%
Survival	1	Rank of Age	--	35	37	35	KW	--	0.0	100.0	--	--	--	--	--	--	--
Energy Storage	2	Rank of Body Weight	Rank of Total Length	35	37	35	Non-parametric ANCOVA	24.6191	0.7	7.4	6.4	-6.4	32	17	7	4	3
	3	Log10 Liver Weight	Log10 Body Weight	33	33	23	ANCOVA	0.0242	-22.2	2.9	32.4	-24.4	127	66	29	16	10
	4	Log10 Liver Weight	Log10 Body Weight	30	32	23	ANCOVA	0.0078	-18.8	6.7	17.7	-15.1	73	38	17	10	6
Energy Use	5	Rank of Body Weight	--	35	37	35	KW	--	33.3	22.7	--	--	--	--	--	--	--
	6	Rank of Total Length	--	35	37	35	KW	--	7.6	2.4	--	--	--	--	--	--	--
	7	Rank of Body Weight; Age 1	--	10	24	5	KW	--	36.9	-5.6	--	--	--	--	--	--	--
	8	Rank of Body Weight; Age 2	--	17	10	29	KW	--	49.9	9.7	--	--	--	--	--	--	--
	9	Rank of Total Length; Age 1	--	10	24	5	KW	--	9.7	-2.5	--	--	--	--	--	--	--
	10	Rank of Total Length; Age 2	--	17	10	29	KW	--	11.8	1.2	--	--	--	--	--	--	--
	11	Rank of Gonad Weight	Rank of Body Weight	35	37	35	Non-parametric ANCOVA	403.9333	-22.0	-17.7	24.0	-24.0	117	59	24	13	7

Notes:
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions.
1 and 5-11. Non-parametric employed due to not meeting parametric assumptions.
3 and 4. Model 3 includes outliers and Model 4 (final model) does not. Removed Cook's outliers STUC-CMM-15, LVR-CMM-50, LVR-CMM-51, and LVR-CMM-53 to better meet ANCOVA assumptions.
3. Used covariate overlap range to meet parametric ANCOVA assumptions.
7-10. Could only examine for Age 1 and Age 2 fish. Statistical comparisons could not be performed due to limited sample size for other aged fish across sites.

Table D-10: Statistical comparison post-hoc power analysis of EDL1 discharge exposure (PINR-EXP2) to reference (LVR2-REF, PINR-REF2) for female Brook Stickleback.

Effect Indicator	Comparisons			Sample Size			Model	Mean Squared Error	Magnitude of Difference (%)		Individual Comparisons (e.g., Tukey HSD p-value or KW)			Minimum Detectable Effect Size (%)[f]		Minimum Sample Size (% Increase / Decrease)				
	Model #	Parameter	Covariate	LVR2-REF	PINR-REF2	PINR-EXP2			PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	LVR2-REF to PINR-REF2	Increase	Decrease	i=5%	i=10%	i=5%	i=10%	i=5%
																d=5%	d=9%	d=20	d=33%	d=50%
Survival	1	Rank of Age	--	25	26	19	KW	--	0.0	15.8	--	--	--	--	--	--	--	--	--	--
Energy Storage	2	Log10 Body Weight	Log10 Total Length	25	26	19	ANCOVA	0.002	5.6	7.7	0.1981	0.0498	0.7762	9.5	-8.7	37	19	9	5	4
	3	Log10 Liver Weight	Log10 Body Weight	25	26	19	ANCOVA	0.015	-18.6	-16.8	--	--	--	28.6	-22.2	100	52	23	13	8
									4.8	44.2						100	52	23	13	8
	4	Log10 Liver Weight	Log10 Body Weight	25	26	15	ANCOVA	0.008	40.5	50.9	--	--	--	20.5	-17.0	73	38	17	10	6
									-7.7	26.9						73	38	17	10	6
	5	Mean Egg Weight	Body Weight	25	26	19	ANCOVA	344.536	33.0	67.0	--	--	--	47.1	-47.1	183	92	38	19	10
	6	Log10 Mean Egg Weight	Log10 Body Weight	25	22	17	ANCOVA	0.010	27.8	44.3	0.00294	<0.001	0.16791	23.6	-19.1	81	42	19	11	7
Energy Use	7	Log10 Body Weight	--	25	26	19	ANOVA	0.008	-0.3	5.2	--	--	--	19.5	-16.3	71	37	17	10	6
	8	Log10 Body Weight	--	24	25	16	ANOVA	0.004	11.3	13.0	0.1981	0.0373	0.9339	15.1	-13.1	54	28	13	8	5
	9	Log10 Total Length	--	25	26	19	ANOVA	0.001	-2.2	-0.9	--	--	--	6.2	-5.8	25	13	6	4	3
	10	Log10 Total Length	--	25	26	16	ANOVA	0.001	-1.0	0.3	--	--	--	5.7	-5.4	22	12	6	4	3
	11	Body Weight; Age 1	--	22	26	17	ANOVA	0.041	-1.4	4.2	--	--	--	18.3	-18.3	69	35	15	8	5
	12	Body Weight; Age 1	--	21	26	14	ANOVA	0.027	10.0	12.6	--	--	--	15.8	-15.8	58	29	12	7	4
	13	Total Length; Age 1	--	22	26	17	ANOVA	0.112	-3.1	-1.6				6.2	-6.2	24	13	6	3	2
	14	Log10 Gonad Weight	Log10 Body Weight	22	23	12	ANCOVA	0.027	39.5	40.7				45.3	-31.2	133	69	30	17	10
	15	Log10 Gonad Weight	Log10 Body Weight	22	22	9	ANCOVA	0.006	32.4	22.7	0.0012	0.02	0.36636	21.0	-17.3	66	34	15	9	6
	16	Total Fecundity (#)	Body Weight	25	26	19	ANCOVA	18134.349	0.4	-19.6	0.9966	<0.0001	<0.0001	15.6	-15.6	61	31	13	7	4

Notes:
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions
3 and 4. Model 3 is Model 4 including outliers. In Model 4, PINR-EXP2 - BSB-19, PINR-EXP2 - BSB-37, PINR-EXP2 - BSB-40, and PINR-EXP2 - BSB-41 Cook's outliers removed to better meen ANCOVA assumptions.
5 and 6. Model 5 is Model 6 including outliers. In Model 6, PINR-EXP2 - BSB-33, PINR-EXP2 - BSB-41, PINR-REF1-BSB-7, PINR-REF1-BSB-8, and PINR-REF1-BSB-45 Cook's outliers removed to better meet ANCOVA assumptions.
7 and 8. Model 7 is Model 8 including outliers. PINR-EXP2 - BSB-33, PINR-EXP2 - BSB-41, PINR-REF1-BSB-7, PINR-REF1-BSB-8, and PINR-REF1-BSB-45 Cook's outliers removed to better meet ANOVA assumptions.
9 and 10. Model 9 is Model 10 including outliers. PINR-EXP2 - BSB-10, PINR-EXP2 - BSB-33, and PINR-EXP2 - BSB-37 Cook's outliers removed to better meet ANOVA assumptions.
11 and 12. Age 1 only as limited sample size across all sites to examine using all ages or Ages 2 and 3 separately. Model 13 is Model 14 with outliers. Cook's outliers PINR-EXP2 - BSB-37, PINR-EXP2 - BSB-40, PINR-EXP2 - BSB-41, and LVR2-BSB-41 removed to better meet ANOVA assumptions.
14 and 15. Model 14 is Model 15 including outliers. PINR-EXP2 - BSB-19, PINR-EXP2 - BSB-33, PINR-EXP2 - BSB-36, and PINR-REF1-BSB-27 Cook's outliers removed to better meet ANCOVA assumptions.

Table D-11: Statistical comparison post-hoc power analysis of EDL1 discharge exposure (PINR-EXP2) to reference (LVR2-REF, PINR-REF2) for male Brook Stickleback.

Effect Indicator	Comparisons			Sample Size			Model	Mean Squared Error	Magnitude of Difference (%)		Individual Comparisons (e.g., Tukey HSD p-value or KW)			Minimum Detectable Effect Size (%)[f]		Minimum Sample Size (% Increase / Decrease				
	Model #	Parameter	Covariate	LVR2-REF	PINR-REF2	PINR-EXP2			PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	LVR2-REF to PINR-REF2	Increase	Decrease	i=5%	i=10%	i=5%	i=10%	i=5%
																d=5%	d=9%	d=20	d=33%	d=50%
Survival	1	Rank of Age	NA	24	25	25	KW	--	0.0	0.0	--	--	--	--	--	NA	NA	NA	NA	NA
Energy Storage	2	Log10 Body Weight	Log10 Total Length	24	25	25	ANCOVA	0.001	5.5	3.4	0.0868	0.3556	0.7149	7.7	-7.2	31	17	8	5	3
	3	Log10 Body Weight	Log10 Total Length	23	25	24	ANCOVA	0.001	3.7	2.8	0.273	0.452	0.926	7.2	-6.7	29	16	7	5	3
	4	Log10 Liver Weight	Log10 Body Weight	24	25	25	ANCOVA	0.010	-7.7	1.2	--	--	--	21.4	-17.6	80	42	18	11	7
	5	Log10 Liver Weight	Log10 Body Weight	24	24	23	ANCOVA	0.006	-7.4	5.7	0.996	0.452	<0.0001	17.6	-15.0	66	34	15	9	6
Energy Use	6	Body Weight	--	24	25	25	ANOVA	0.082	3.9	-0.1	--	--	--	21.4	-21.4	86	44	18	10	5
	7	Total Length	--	24	25	25	ANOVA	0.187	-0.8	-1.3	--	--	--	7.3	-7.3	30	16	7	4	3
	8	Body Weight; Age 1	--	21	25	24	ANOVA	0.078	-1.3	-1.9	--	--	--	20.8	-20.8	81	41	17	9	5
	9	Total Length; Age 1	--	21	25	24	ANOVA	0.179	-2.7	-1.9	--	--	--	7.2	-7.2	29	15	7	4	3
	10	Log10 Gonad Weight	Log10 Body Weight	24	25	25	ANCOVA	0.029	-16.3	-13.6	--	--	--	40.4	-28.8	139	72	31	18	11
	11	Log10 Gonad Weight	Log10 Body Weight	24	24	22	ANCOVA	0.011	-0.8	-5.0	--	--	--	24.0	-19.3	86	45	20	11	7

Notes:
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions
2 and 3. Model 2 is Model 3 including outliers. PINR-EXP2 -BSB-34 and LVR2-BSB-21 Cook's outliers removed to better meet ANCOVA assumptions.
4 and 5. Model 4 is Model 5 including outliers. PINR-EXP2-BSB-4, PINR-EXP2-BSB-22, and PINR-REF2-BSB-23 Cook's outliers removed to better meet ANCOVA assumptions.
8 and 9. Could only examine Age 1 fish. Statistical comparisons could not be performed including Age 2+ fish due to limited sample size across sites.
10 and 11. Model 10 is Model 11 including outliers. PINR-EXP2 - BSB-17 PINR-EXP2 - BSB-20 PINR-EXP2 - BSB-22 PINR-REF2-BSB-39 Cook's outliers removed to better meet ANCOVA assumptions.

Table D-12: Statistical comparison post-hoc power analysis of EDL1 discharge exposure (PINR-EXP2) to reference (LVR2-REF, PINR-REF2) for male Central Mudminnow.

Effect Indicator	Comparisons			Sample Size			Model	Mean Squared Error	Magnitude of Difference (%)		Individual Comparisons (e.g., Tukey HSD p-value or KW)			Minimum Detectable Effect Size (%)[f]		Minimum Sample Size (% Increase / Decrease				
	Model #	Parameter	Covariate	LVR2-REF	PINR-REF2	PINR-EXP2			PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	PINR-EXP2 to LVR2-REF	PINR-EXP2 to PINR-REF2	LVR2-REF to PINR-REF2	Increase	Decrease	i=5%	i=10%	i=5%	i=10%	i=5%
																d=5%	d=9%	d=20	d=33%	d=50%
Survival	1	Rank of Age	--	7	28	15	KW	--	0.0	-50.0	--	--	--	--	--	--	--	--	--	--
Energy Storage	2	Log10 Body Weight	Log10 Total Length	7	28	15	ANCOVA	0.001	3.6	-1.4	--	--	--	9.3	-8.5	30	16	8	5	3
	3	Log10 Liver Weight	Log10 Body Weight	7	28	15	ANCOVA	0.010	-0.6	10.9	--	--	--	27.7	-21.7	82	42	19	11	7
Energy Use	4	Rank of Body Weight	--	7	28	15	KW	--	0.1	-56.1	0.418	0.002	0.364	--	--	--	--	--	--	--
	5	Total Length	--	7	28	15	ANOVA	2.364	-9.3	-17.3	--	--	--	20.9	-20.9	68	35	15	8	5
	6	Log10 Body Weight; Age 1	--	4	10	9	ANOVA	0.038	-17.1	-52.1	0.960	0.004	0.046	113.7	-53.2	158	81	35	20	12
	7	Body Weight; Age 2	--	--	14	5	ANOVA	6.455	--	-12.0	--	--	--	--	--	--	--	--	--	--
	8	Total Length; Age 1	--	4	10	9	ANOVA	0.847	0.6	-21.6	--	--	--	24.7	-24.7	51	26	11	6	4
	9	Rank of Total Length; Age 2	--	--	14	5	KW	--	--	-8.6	--	--	--	--	--	--	--	--	--	--
	10	Log10 Gonad Weight	Log10 Body Weight	7	28	15	ANCOVA	0.026	-5.8	-1.8	--	--	--	48.3	-32.6	131	67	29	17	10

Notes:
Parameters were transformed (i.e., log10) when needing to meet ANOVA/ANCOVA assumptions
4. Violation of ANOVA assumptions so non-parametric applied with individual comparisons being made using KW model.
7 and 9. Could only compare PINR-EXP2 to PINR-REF2 due to low sample size in LVR2-RE

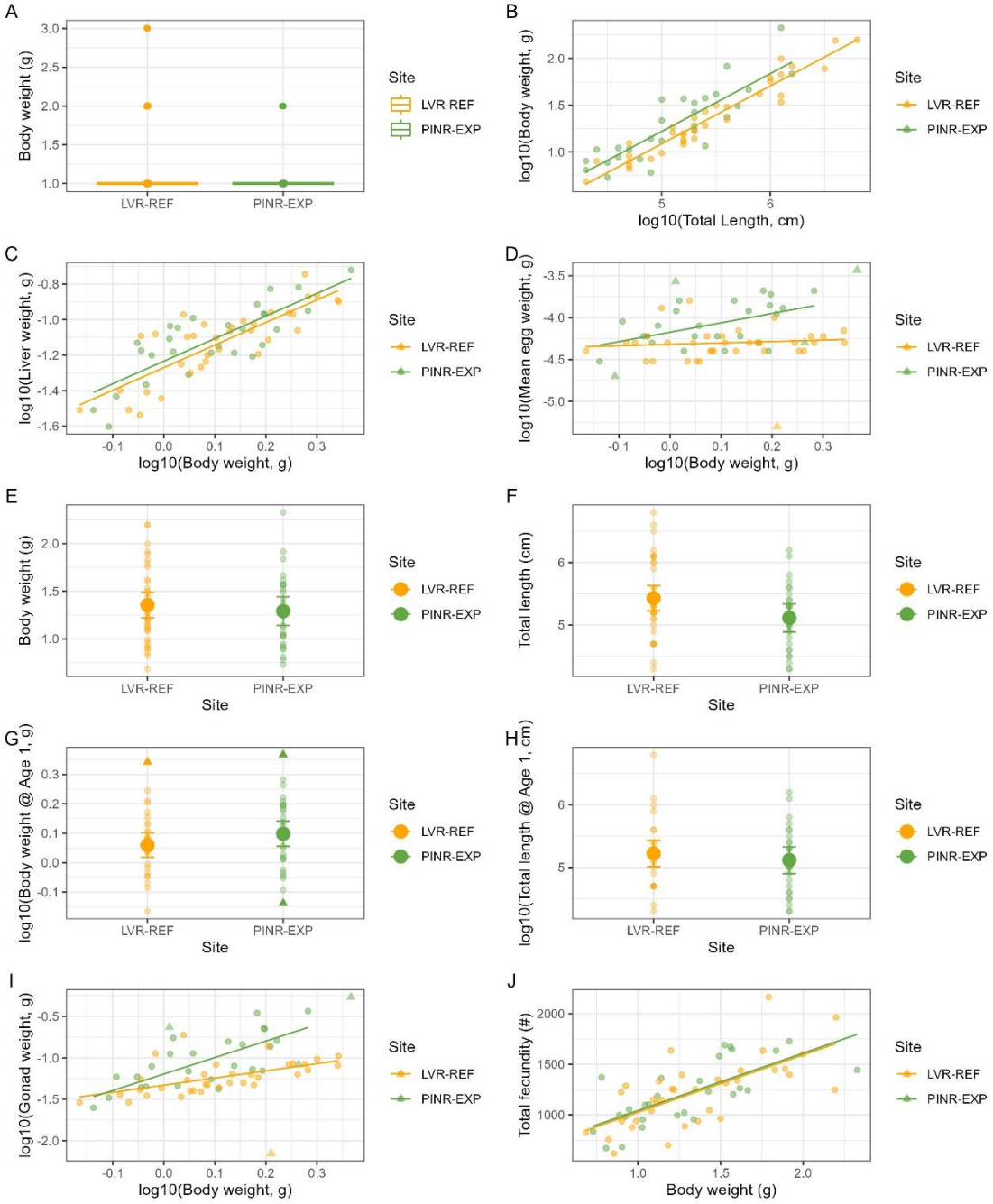

Figure D-1: Visualizations for statistical comparisons for EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF) for female Brook Stickleback.

Notes: STUC-REF comparisons not possible due to limited sample size. Boxplots are non-parametric models, plots with circle and 95% confidence interval are ANOVA models, and plots with linear relationships are ANCOVA models. Triangles are excluded outliers.

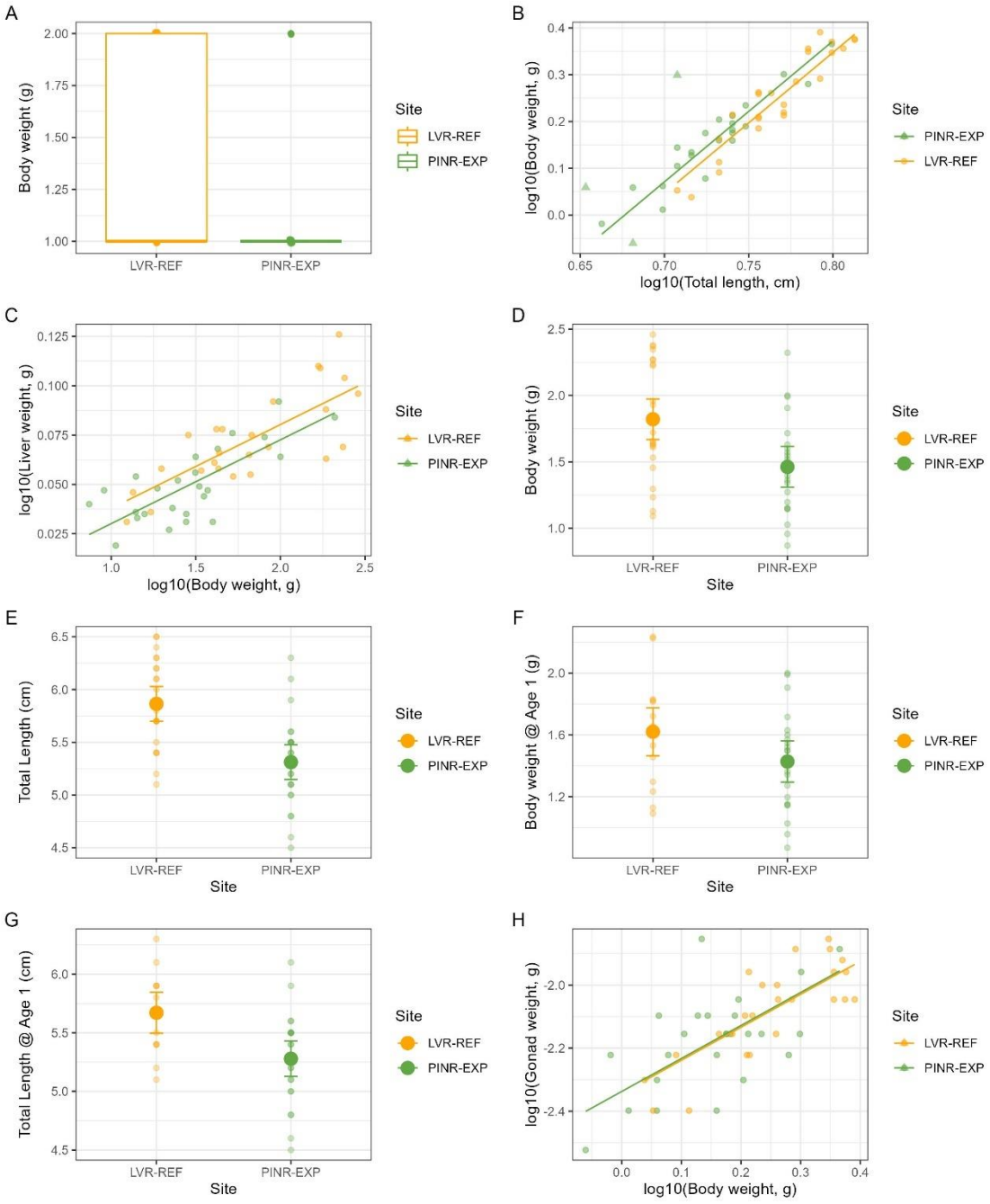

Figure D-2: Visualizations for statistical comparisons for EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF) for male Brook Stickleback.

Notes: STUC-REF comparisons not possible due to limited sample size. Boxplots are non-parametric models, plots with circle and 95% confidence interval are ANOVA models, and plots with linear relationships are ANCOVA models. Triangles are excluded outliers.

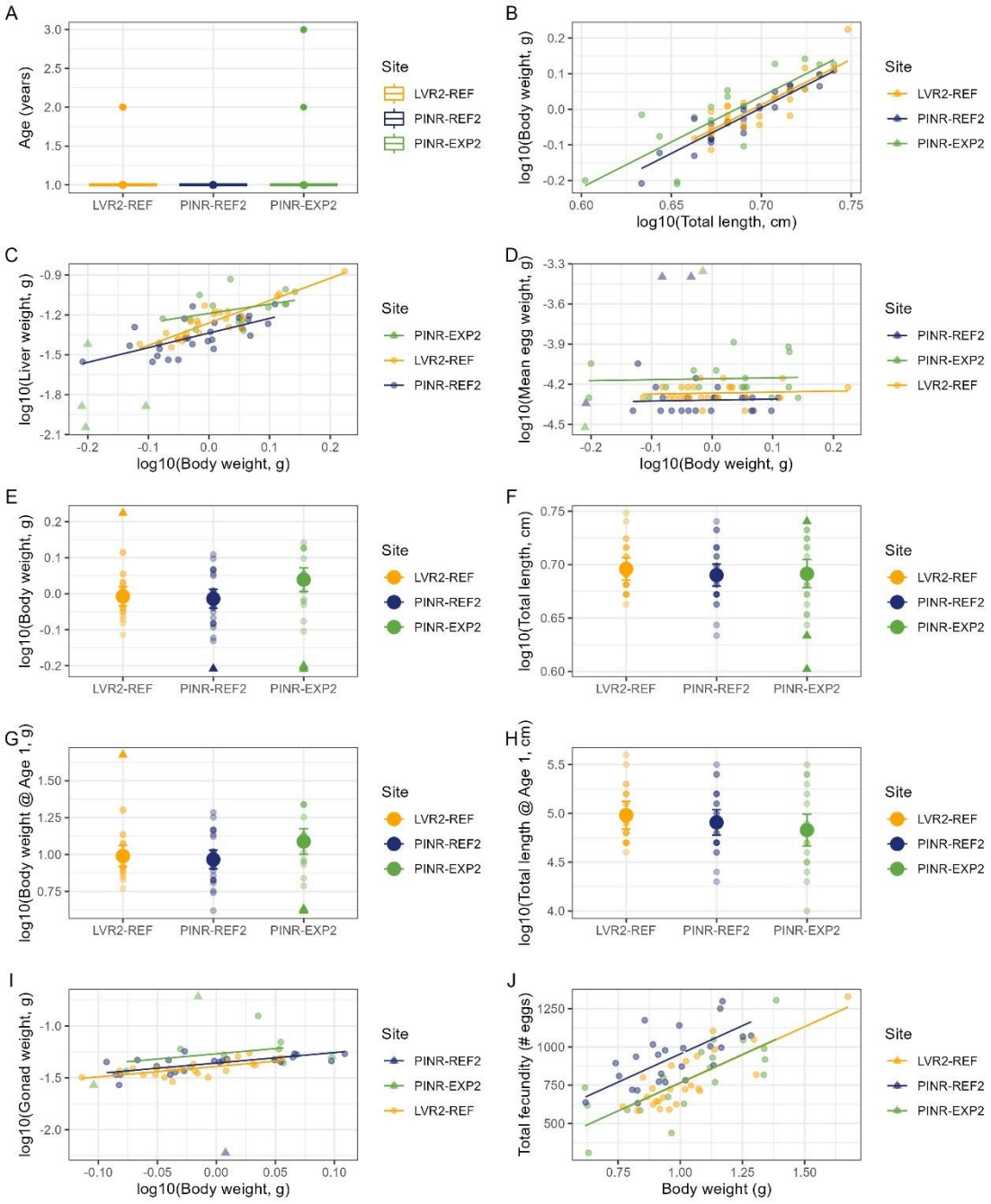

Figure D-3: Visualizations for statistical comparisons for EDL1 discharge exposure (PINR-EXP2) to reference (LVR2-REF, PINR-REF2) for female Brook Stickleback.

Notes: Boxplots are non-parametric models, plots with circle and 95% confidence interval are ANOVA models, and plots with linear relationships are ANCOVA models. Triangles are excluded outliers.

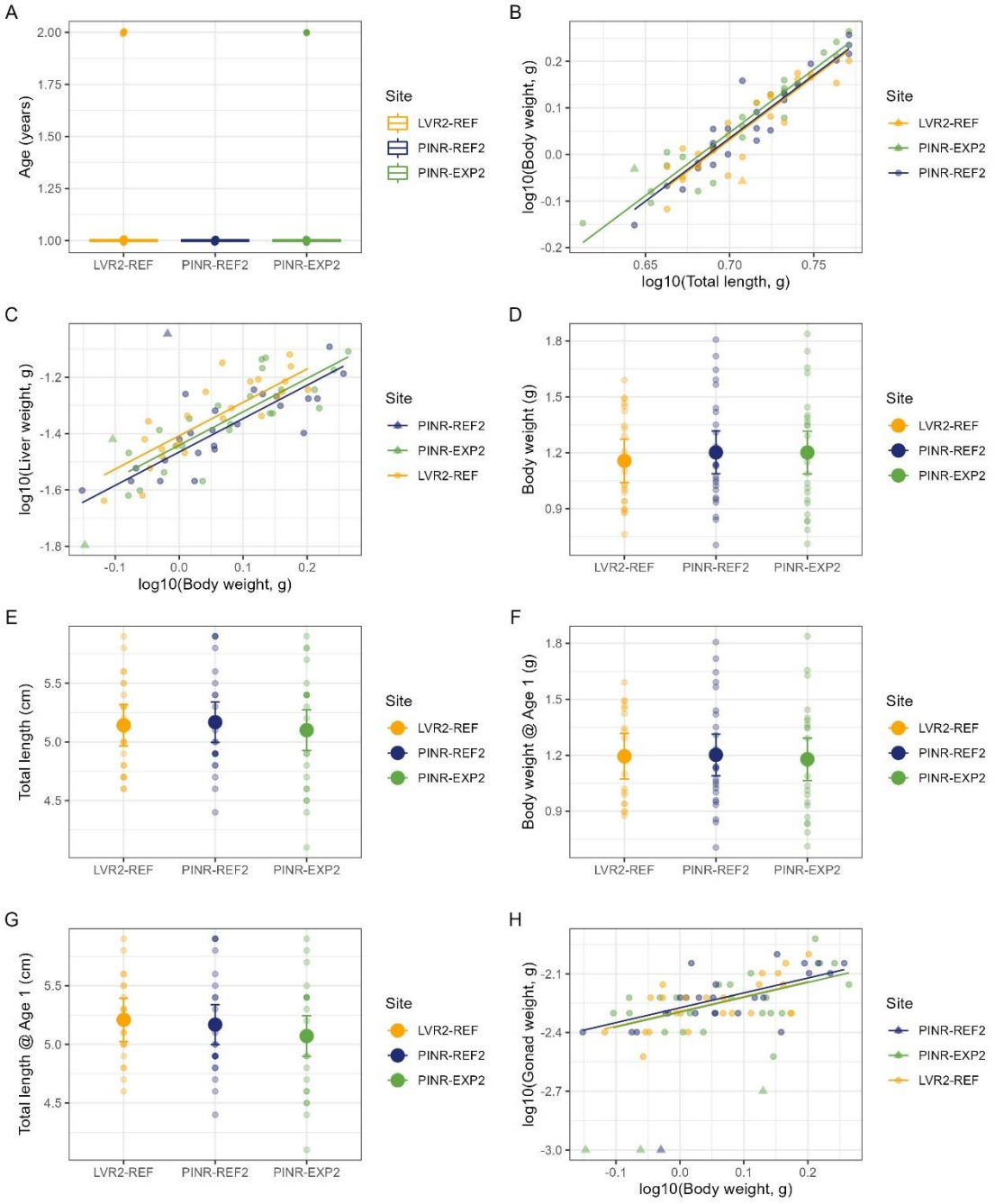

Figure D-4: Visualizations for statistical comparisons for EDL1 discharge exposure (PINR-EXP2) to reference (LVR2-REF, PINR-REF2) for male Brook Stickleback.

Notes: Boxplots are non-parametric models, plots with circle and 95% confidence interval are ANOVA models, and plots with linear relationships are ANCOVA models. Triangles are excluded outliers.

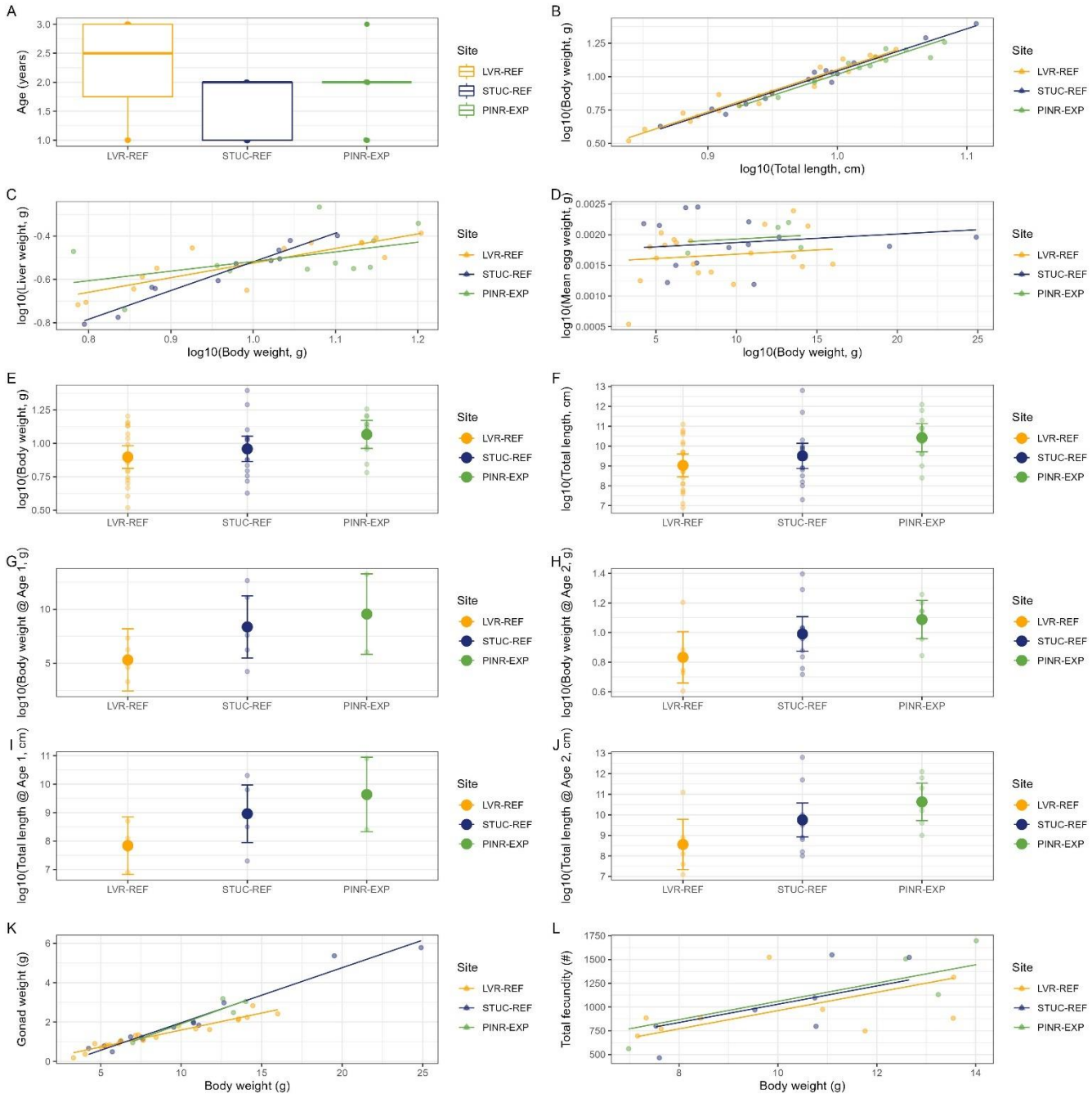

Figure D-5: Visualizations for statistical comparisons for EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF, STUC-REF) for female Central Mudminnow.

Notes: Boxplots are non-parametric models, plots with circle and 95% confidence interval are ANOVA models, and plots with linear relationships are ANCOVA models. Triangles are excluded outliers.

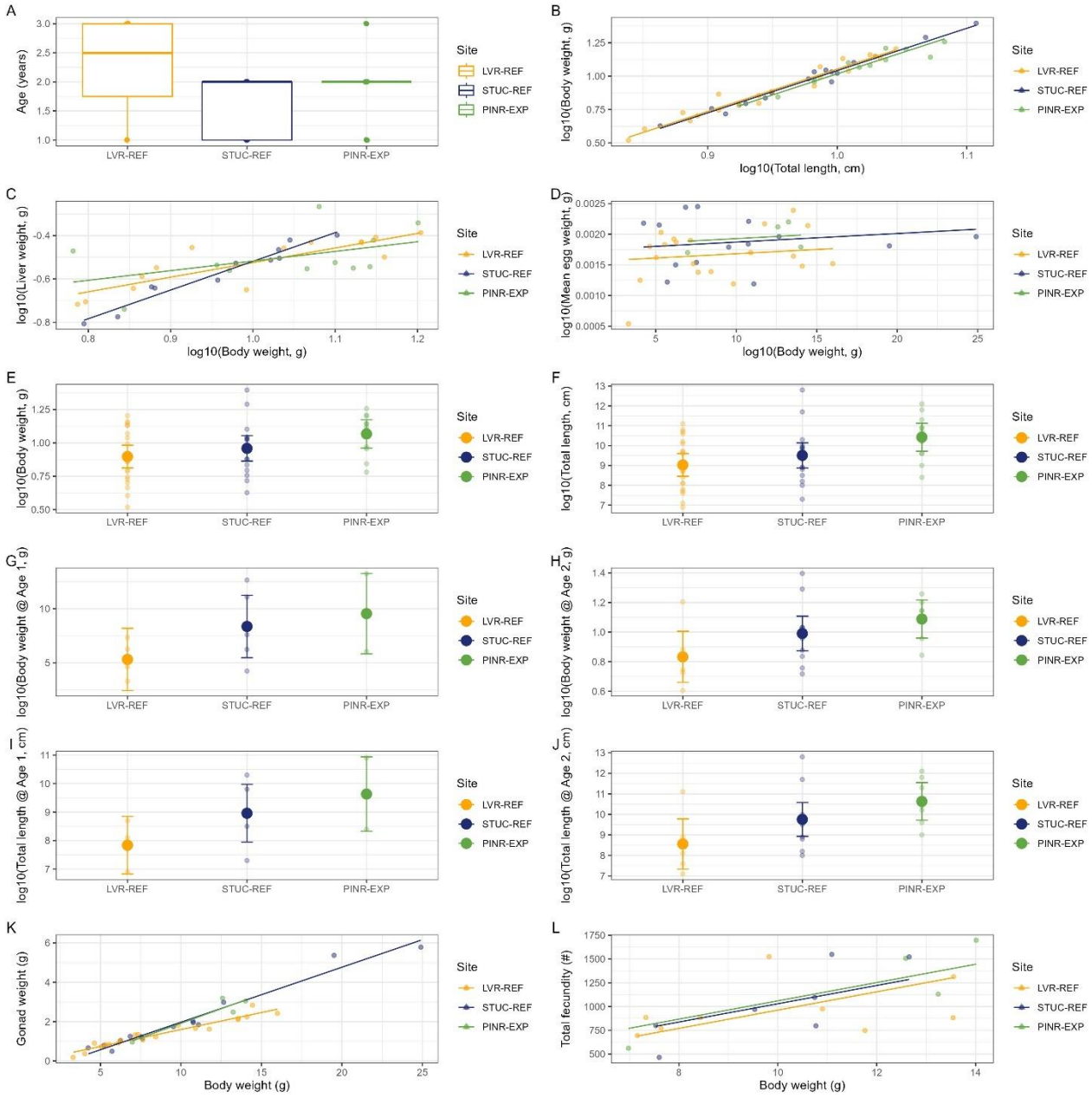

Figure D-6: Visualizations for statistical comparisons for EDL2 discharge exposure (PINR-EXP) to reference (LVR-REF, STUC-REF) for male Central Mudminnow.

Notes: Boxplots are non-parametric models, plots with circle and 95% confidence interval are ANOVA models, and plots with linear relationships are ANCOVA models. Triangles are excluded outliers.

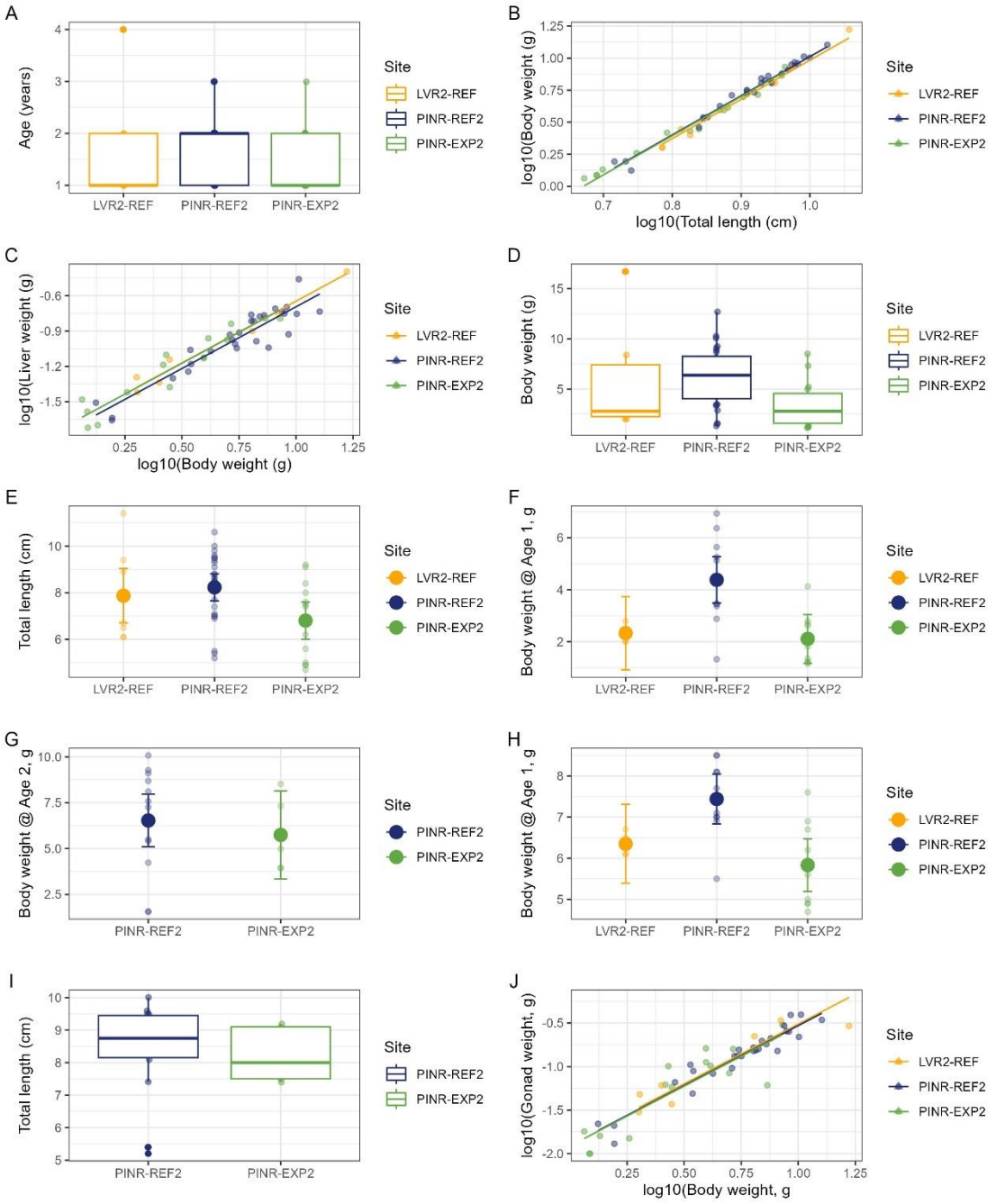

Figure D-7: Visualizations for statistical comparisons for EDL1 discharge exposure (PINR-EXP2) to reference (LVR2-REF, PINR-REF2) for male Central Mudminnow.

Notes: Boxplots are non-parametric models, plots with circle and 95% confidence interval are ANOVA models, and plots with linear relationships are ANCOVA models. Triangles are excluded outliers.