TECHNICAL RESPONSES FOLLOWING 24 AUGUST 2020 WQ-MOP WORKING GROUP MEETING

This technical response was developed to address a question raised during the 26 August 2020 WQ-MOP working group meeting and to provide supporting information related to the study design of the chronic toxicity testing program. This response is broken down into two components as follows:

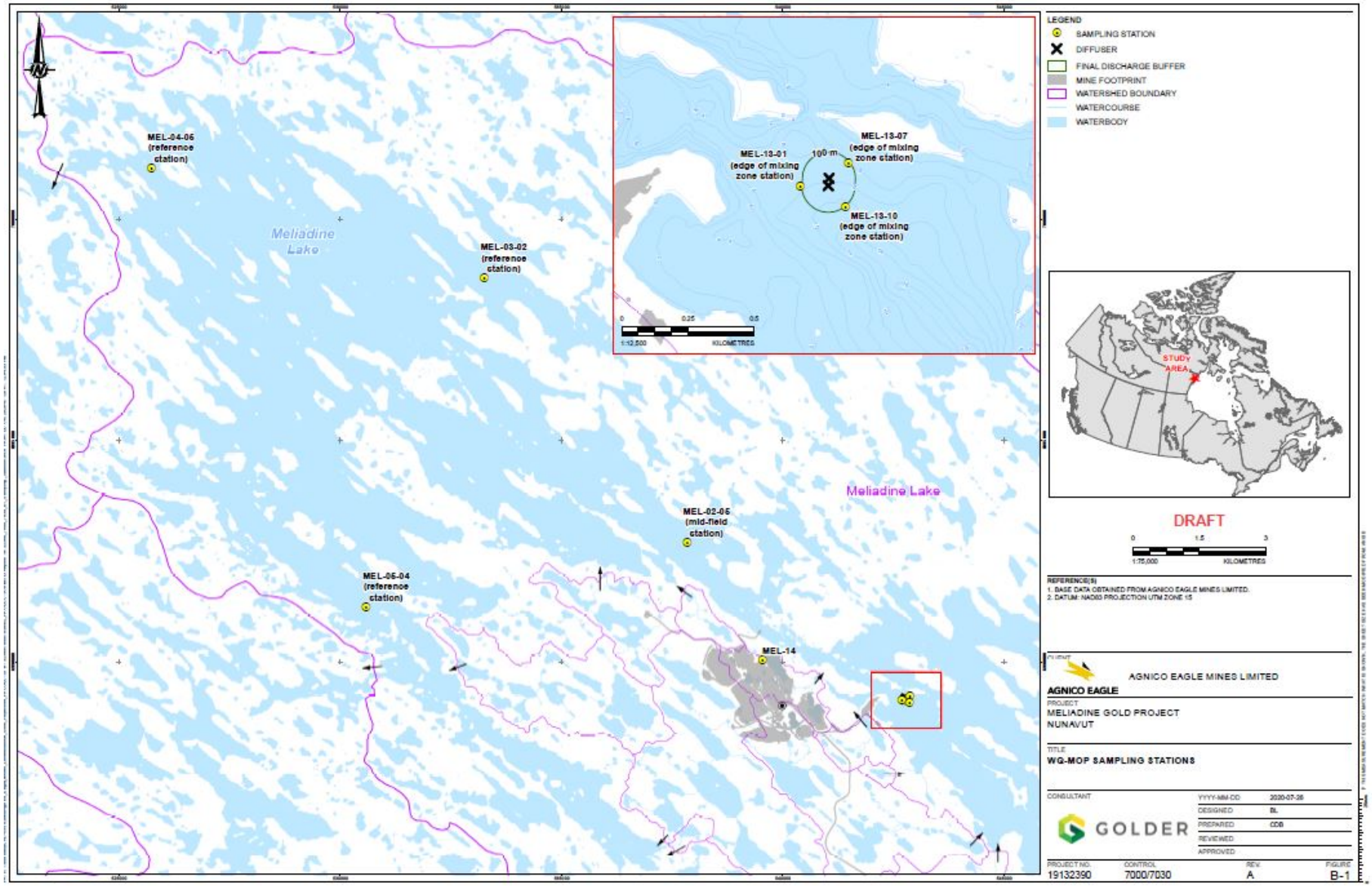
- **Response 1:** A response to a question posed by Anne Wilson from Environment and Climate Change Canada regarding the chronic toxicity test figures presented during the 26 August 2020 presentation (see attachment 1). The question raised is related to the purpose of the red dotted lines in the chronic toxicity test result figures.
- **Response 2:** To support discussions with the working group moving forward, Golder and Agnico Eagle felt that it would be helpful to provide a description of the study design for the chronic toxicity test component of the WQ-MOP, including descriptions of the measures employed to address the non-standard elements of the testing (i.e., low conductivity of receiving environment samples).

RESPONSE 1

The red dotted lines presented in the results figures represent the following:

- For figures comparing organism response in the three reference stations (MEL-03-02, MEL-04-05, and MEL-05-04) to the response observed in the full strength (100% volume/volume) samples from the mid-field (MEL-02-05) and the three edge of mixing zone stations (MEL-13-01, MEL-13-07, and MEL-13-10; see Figure 1), the red dotted lines depict the maximum and minimum response observed in the various replicates measured at the three reference stations. The whiskers coming out from the mean response at each reference station depict the range (maximum and minimum) in response observed in each of the test replicates for that sample, and the red dotted line provides an envelop of the combined range in response (maximum and minimum) observed at the three reference stations. This was added to the figures to allow the observers to visually compare if the range in response at the mid-field and edge of mixing zone stations fell within the same range as the reference stations.
- Similarly, in figures that depict a dose response relationship (i.e., full strength sample tested at multiple dilutions), the red dotted lines depict the maximum and minimum response observed in the site control (synthetic water control designed to match background water quality [low hardness] – see description in Response 2). This was added to allow the observer to compare the dose response relationship to organism response in clean laboratory water designed to mimic the non-standard low conductivity of the receiving environment.

Figure 1: WQ-MOP Sampling Stations



RESPONSE 2

A component of the WQ-MOP monitoring program involves chronic toxicity testing of monthly receiving environment samples from Meliadine Lake, as well as monthly chronic toxicity testing of the MEL-14 discharge. The goal of the testing is to assess the potential for chronic effects to aquatic receptors at, and beyond, the edge of the mixing zone (i.e., a 100 m radius surrounding the diffuser in Meliadine Lake). As outlined in the WQ-MOP Rev2 (Golder 2020), chronic effects are not anticipated at the edge of the mixing zone based on earlier chronic toxicity tests of pit water and predicted exposure concentrations. Nevertheless, four chronic toxicity test species were identified to monitor conditions in the receiving environment during the required monthly toxicity testing. These tests include:

- 21-day *Daphnia magna* (freshwater crustacean) survival, growth, and reproduction test
- 14-day *Hyalella azteca* (benthic invertebrate) survival and growth test
- 7-day *Lemna minor* (Duckweed; aquatic macrophyte) survival and growth test
- 7-day Fathead Minnow (freshwater fish) survival and growth test

The low hardness receiving environment of Meliadine Lake (ranging between approximately 20 and 40 mg/L hardness; 2019 AEMP) poses a challenge for conducting chronic toxicity testing, as the organisms used in the selected tests are typically cultured in higher hardness waters (i.e., 80–110 mg/L for *D. magna*; ~140 mg/L *H. azteca*; ~100 mg/L for *L. minor*, 130–140 mg/L for Fathead Minnow). This was identified as a project risk during conversations with Bureau Veritas Laboratories (BV Labs; chronic toxicity laboratory), as the transfer of organisms from the higher hardness culture waters to the lower hardness test waters could elicit osmotic stress to the organisms and, therefore, bias the results of the test. During conversations with the laboratory, it was concluded that potential for osmotic stress would be less of a concern for Fathead Minnows and Duckweed, as these species tend to have a larger range of tolerance to different water types. However, hardness concentrations in the Meliadine Lake receiving environment were considered to be on the lower end of the tolerance range for the two invertebrate species (*D. magna* and *H. azteca*). To reduce the potential for a confounding effect of osmotic stress for these organisms, it was considered necessary to acclimate organism cultures prior to testing. Acclimation has not been considered necessary for the MEL-14 discharge testing, as the higher conductivity and hardness in the discharge relative to receiving environment samples has facilitated the ability to conduct testing following more standard test protocols.

The chronic toxicity testing for Meliadine Lake is further complicated by the fact that the primary contaminant of concern being investigated in the MEL-14 discharge is TDS, requiring consideration of the influence of dilution water on the concentrations and ratios of major ions. Toxicity associated with TDS is typically caused by osmotic stress and is influenced by the specific ratios of the component major ions (i.e., calcium, magnesium, sodium, potassium, chloride, sulphate, and alkalinity). Chronic toxicity tests are commonly performed using dilution series tests on the discharge being investigated and, therefore, ionic concentrations tend to be greater than control/dilution water used in the tests. The standard control/dilution water used during testing is typically the same water that the organisms are cultured in. However, for tests conducted in receiving environment samples (Meliadine Lake), the ambient TDS is low relative to the culture media, such that standard dilution waters may increase TDS in receiving environment samples at higher dilutions. Therefore, a site-specific test design was required to:

1. Control for the low hardness conditions in Meliadine Lake and assess normal organism response in lower hardness waters

2. Select relevant references to compare against organism responses in exposure areas

3. Set-up the test design so that the test acceptability (e.g., organism health and validity of the tests) can be properly assessed, while also accounting for the non-standard (low hardness) exposure conditions of site media

To address these site-specific complications, a modified test design was developed and applied during the receiving environment chronic toxicity testing associated with each of the four test species. Additional controls were implemented so that organism responses resulting from low conductivity waters of the receiving environment, rather than an adverse toxicological response to TDS, can be discerned. The following represents the various components of the modified chronic toxicity test design:

- **Controls**—Three types of control water are used during the testing:
  - **Laboratory Control**—standard culture water used for each species during regular testing at the laboratory. This control is used to assess test validity per standard protocol requirements; it is intended to facilitate comparison of organism response to a normal performance range for cultured organisms in non-contaminated media.
  - **Soft Water Control**—standard culture water used for each species during regular testing is diluted down to a hardness of ~40 mg/L, while keeping ionic ratios intact. This control is used to assess organism response in low hardness waters, but at typical ratios of major ions used during standard testing. This control serves as a baseline for the receiving environment tests because endpoints such as growth or reproduction could be lower than the laboratory control in lower ionic strength waters, due to suboptimal exposure conditions for the cultured organisms. This control is compared to the response in the laboratory control to assess for potential differences in organism performance that was independent of the influence of the discharge.
  - **Site Control**—synthetic dilution water control. The site control is a synthetic water recipe developed based on ionic ratios reported in the 2019 AEMP [Azimuth 2020] and based on the pooled reference conditions in Meliadine Lake. The difference between the soft water control and the site control is that the former used a standard recipe of ions used for organism culturing, whereas the latter is customized to ambient site conditions. The site control is used to evaluate organism response in clean test water using ionic ratios that are representative of Meliadine Lake reference sites, as identified during the most recent AEMP. This water is also used as the dilution water in the dilution series tests outlined below, as this provides a more realistic assessment of how the discharge is expected to be diluted within the receiving environment. The site control is also used to assess how well organisms respond to the synthetic dilution water. Results are compared to the soft water control to assess how organisms respond to water with a similar hardness (i.e., soft water control), but with ionic ratios that more closely resemble Meliadine Lake conditions.
- **Meliadine Lake Receiving Environment Monitoring Samples**—Two types of tests are conducted using receiving environment samples during the discharge event:

- **Full strength tests**—full strength tests (sometimes called "pass/fail" tests) are performed with samples of undiluted Meliadine Lake water, including samples from the mid-field station MEL-02-05 and the three reference stations (MEL-03-02, MEL-04-05, and MEL-05-04). The reference station results are compared statistically to the mid-field results, as well as to the dilution series test results (next bullet) to investigate whether significant differences are apparent, and whether these differences could be related to the influence of the discharge.
- **Dilution series tests**—Meliadine Lake edge of mixing zone stations (MEL-13-01, MEL-13-07, and MEL-13-10) are tested using a standard volumetric dilution series (e.g., 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, and 1.56% volume/volume sample). Due to the larger test set-up for these dilution series (i.e., greater number of test vessels), dedicated controls are specified for each station to control for subtle temperature or light differences in the test chambers that may influence survival, growth, or reproduction endpoints in the tests. The chronic toxicity test results in the 100% undiluted edge of mixing zone samples are compared statistically to the results in the reference stations (MEL-03-02, MEL-04-05, and MEL-05-04) to assess whether edge of mixing zone stations show statistically significant reductions in survival, growth, or reproduction. The statistical assessment includes comparison to each individual reference station, as well as the pooled average of the reference station results. Where statistical differences are identified, the dilution series test design facilitates the investigation of any concentration-response relationships observed along the dilution series, which are expected to facilitate the calculation of relevant IC/EC_X values (inhibitory / effect concentrations influencing X% of the population). This information will be useful for confirming: 1) whether effects are apparent and not simply reflective of confounding factors (e.g., subtle temperature, light, or feeding differences); and 2) determining at what level of dilution the observed effects decrease to ambient levels.

As a result, chronic toxicity test results from receiving environment stations are assessed using the following tiered approach:

1. Compare results of the undiluted edge of mixing zone and mid-field stations to the range in response observed at the reference stations—There is natural variability in sub-lethal endpoints such as growth and reproduction and, therefore, it is necessary to evaluate the range in response observed in reference water relative to the range observed at exposure sites.
2. Evaluate the dose response relationship observed along the dilution series for edge of mixing zone stations—It is important to also consider the pattern of response as a function of dilution to determine whether the pattern suggests that a higher percentage of site water causes a larger decrease in organism performance.
3. Assess the response in the laboratory controls to determine the potential confounding influence of low hardness—The controls, both standard negative control and low hardness controls, are not compared directly to organism response in site water, as the lab water is not necessarily consistent in character as the receiving environment (e.g., micronutrients, DOC, etc.). These controls are instead used to assess test validity. In the case of the site water control (also the dilution water), the results are included as a treatment along the dilution series test design (e.g., 0% sample [site control], 1.56% sample, etc.).

Finally, as discussed above, based on commitments related to monthly chronic toxicity testing of the MEL-14 discharge arising from responses to comments from ECCC and KivIA (Agnico Eagle 2020) and

discussions through the WMWG, MEL-14 has been tested using the same suite of chronic toxicity tests starting during the second round of monthly events. This supplemental testing has involved chronic toxicity testing of the full-strength discharge plus volumetric dilutions. Due to the higher conductivity of the sample, the dilution series testing with MEL-14 follows more standard test protocol (e.g., non-acclimated standard test organisms), with the notable exception that the dilution water used during the testing is the Site Control water described above. This was performed for comparability to receiving environment assimilation in Meliadine Lake. The additional testing with MEL-14 effluent is useful for validation of the interim target of 1,000 mg/L at the edge of mixing zone, as the discharge dilution series testing is expected to encompass exposures above and below the proposed target of 1,000 mg/L calculated TDS.

REFERENCES

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