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June 25, 2014

EC file: 5100 000 034 004
MVEIRB file: EA1314-02

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RE: EA1314-02 - De Beers Canada Inc. - Snap Lake Mine - Response to New Evidence

DeBeers presented recent toxicity testing results, in support of proposed water quality objectives, at the Snap Lake Mine Environmental Assessment Public Hearings held June 5-6, 2014. This was new evidence regarding toxicity testing results which parties had not seen before the hearings. Consequently, the Mackenzie Valley Environmental Impact Review Board provided additional time to allow parties to review the new information.

Test results and lab sheets were reviewed by Environment Canada's (EC's) Biological Assessment and Standardization Section experts, and the following comments are provided for the Board's consideration:

- The proposed Site Specific Water Quality Objective (SSWQO) of 684 mg/L was based on the lowest IC20 (20% inhibition concentration) from a single *Daphnia magna* 21 day toxicity test from 2013.

  Further testing with *Daphnia magna* was done to evaluate effects on survival and reproduction - lab sheets are provided for Tests 3, 4 and 5 with the Technical Memorandum dated June 10, 2014 from Golder Associates (Golder). An additional test was run on the copepod *Cyclops vernalis* for effects on survival and growth (length) and lab data are included for this test.

  With respect to the *Daphnia magna* tests (3, 4 and 5), EC has concerns with the model used. A closer look at the statistical analysis of Test 5 showed that the IC20 of 733 mg/L was based on the 3 parameter (3P) Log-Gompertz Model. The dose-response curve for the data was non-monotonic, meaning the response did not consistently decrease with increasing total disolved solids (TDS) concentration and there was a slightly less inhibitory effect at the third test concentration of 629 mg/L.

  If the interpretation is meant to be conservative, one could consider the measured concentration of 310 mg/L to be the IC20 because this is the first point in the dose-response curve when a 20% effect size is reached. This isn't calculated by the models because all the models available assume monotonicity.

The Daphnia test uses a standardized lab method, although not EC's. It appears that EC's statistical guidance (EPS 1/RM/46) was followed for model selection using the lowest residual mean square error. However, the model chosen is not necessarily the best one for comparing the fit between models with different numbers of parameters, i.e. 2P versus 3P. AICc (or "AIC corrected", as in CETIS, the statistical software program the lab used) and BIC software are better choices to assess the model fit. Using AICc and BIC, one would choose the 2P linear model in this case. The IC20 for the 2P linear is 563 mg/L TDS (95% confidence intervals of 369-756 mg/L). Note: Appendix A of the CETIS manual has more details on AICc and BIC.

The shape of the dose-response curve, given the limitation of a non-monotonic situation, is best described by a straight line. One can see this by looking at the graph for the 3P Log-Gompertz (Attachment 3 in the Response to Hearing Undertakings June 10, 2014, pdf Page 76), which is nearly indistinguishable from a straight line (this curve is usually S-shaped). This presents further reason one might choose a 2P linear model and with the goal of conservatism, this lower IC20 of 563 mg/L would be a more appropriate estimate.

The best approach depends on the significance of the non-monotonic response and using a model that assumes the data is monotonic versus the prudent selection of the more conservative estimate. The re-calculated estimate of 563 mg/L would be the new lowest IC20 in the *Daphnia magna* test series and could replace 684 mg/L as the proposed SSWQO or an even more conservative approach could justify using the estimate of 310 mg/L.

- With respect to the copepod test, EC has the following concerns:
  The Technical Memorandum from Golder dated June 10, 2014 states: "The copepod test was conducted as a 20-day (d) test, with survival and growth (length) the endpoints measured. The IC20 (i.e. 20% inhibitory effect concentration) for growth effects derived from testing with this copepod species was > 1,508 mg/L. These findings of no effects at the highest tested TDS concentrations mirror those determined previously for algae, diatoms, rotifers, insect larvae, Lake Trout, and Artic Grayling (Golder 2013)." This is not entirely accurate in that there was a 20% decrease in survival from control levels observed at the 1008 and 1508 mg/L TDS test concentrations. Typically, one would be interested in a non-lethal endpoint as they are more sensitive and this may be the reason why Golder concluded "no effects" based on growth. However, with some invertebrates survival is the more sensitive endpoint because it has been noted that some species die rather than lose weight. As this test with *Cyclops vernalis* was conducted using an in-house method (i.e. not standardized), it is unclear if the growth endpoint is more sensitive than survival.

  It is important to consider that a test that evaluates survival alongside growth may have density-dependant effects (i.e. a dual effect). This is a non-toxicant effect that can occur on growth endpoints in test vessels because of resource limitations that can be inherent in lab test designs. With this design the toxicant can have an effect on mortality and consequently the surviving individuals respond to the increased space and food available (i.e. less competition for resources). As a result, growth endpoints (like length or weight) may not be very reliable because of the uncertainty as to whether the effects (or lack of them) are the result of the toxicant or reduced

competition. To evaluate this potential problem, one can plot growth as a function of surviving adults and look for a decreasing trend. EC checked the data and there is no evidence of dual effects in this data set, but it's always good practice to evaluate this before making conclusions about growth being impaired (or stimulated, or not affected at all) in the presence of partial mortalities.

Method validation for a new test is critical to determine if the organism is responding to a toxicant or to environmental conditions in the laboratory (such as temperature, lighting, food type and availability, loading densities, etc). Typical evidence of method validation would include: acclimation criteria (e.g. must be held in the lab at test conditions for 3 weeks prior to use in a test and temperature change must not exceed 3°Celsius per day during acclimation to lab conditions), culture health criteria, (e.g. minimum survival of adults 7 days before use of progeny in a test, minimum reproduction), reference toxicant data (i.e. a positive control that documents a consistent response to a known toxicant), and test performance or validity criteria (i.e. minimum control survival and length). None of this information is provided. There was also no evidence to support the use of length, as opposed to weight, as a surrogate for growth with these organisms.

The laboratory also identified the possibility of cannibalism during the last week of the test, as these are carnivorous organisms and this likely impacted the survival data, so the 20% reduction in survival due to TDS may be suspect. For these reasons, the toxicity data for *Cyclops vernalis* does not add to the weight of evidence approach in determining a SSWQO for TDS at Snap Lake.

- A clerical note - Figure 2 (Page 7 of the Nautilus Test Report) incorrectly plots the control response (length) at 0 mg/L TDS. The correct controls had 212 mg/L TDS.

In summary, while EC appreciates the additional test work undertaken by the Proponent, EC recommends a more conservative approach be considered because the conclusions drawn from the test results do not fully support the proposed water quality objective of 684 mg/L for TDS in Snap Lake.

Sincerely,

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cc: Carey Ogilvie Head Environmental Assessment North (NT & NU), EPO
EC Review Team