

From: [Marc Casas](mailto:Marc.Casas)
To: permits@mvlwb.com
Subject: FW: Proposed TDS and Strontium Plan for De Beers Snap Lake
Date: Wednesday, February 01, 2012 11:53:39 AM

From: Marc Casas [mailto:mcasas@mvlwb.com]
Sent: February-01-12 11:44 AM
To: 'Hood, Alexandra'
Cc: 'Kathy Racher - WLWB'; 'Rebecca Chouinard'
Subject: RE: Proposed TDS and Strontium Plan for De Beers Snap Lake

Thanks for keeping us updated on your progress to date. It will be posted on the registry.

We are working towards providing some guidance or framework for the development of the above mentioned plans.

Thanks,

Marc Casas
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From: Hood, Alexandra [mailto:Alexandra.Hood@debeerscanada.com]
Sent: January-30-12 4:31 PM
To: Marc Casas; permits@mvlwb.com
Cc: Tracy Covey; Zhong Liu; David White; Hanna, Bruce; Raymond, Darren; Chapman, Peter (Canada); Robinson, Michael; Raymond, Darren
Subject: Proposed TDS and Strontium Plan for De Beers Snap Lake

As above. Please let me know if you have any questions or concerns with the attached plan.

Regards, Alex



January 23, 2012

Scope of Work: Evaluation of the toxicity of TDS and strontium to aquatic organisms

To	Dr. Peter Chapman	From	James Elphick
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Nautilus Environmental is pleased to provide this study plan to conduct toxicity testing to:

- 1) evaluate the sensitivity of the early life stages of two fish species representative of fish in Snap Lake (NWT) to elevated Total Dissolved Solids (TDS);
- 2) evaluate the effect of acclimation on the sensitivity of *Ceriodaphnia dubia* to TDS; and,
- 3) evaluate the sensitivity of *Hyalella azteca* and early life stages of rainbow trout to strontium.

Evaluation of effects of TDS on early life stages of a spring spawning (Arctic grayling) and fall spawning fish species found in Snap Lake (either lake trout or round whitefish)

Toxicity tests will be conducted using early life stages of a spring spawning (Arctic grayling) and a fall spawning salmonid (either lake trout or round whitefish). The tests will be initiated using recently-fertilized eggs, and will continue until one-to-two weeks following hatch, encompassing the embryo-alevin stages described in Environment Canada (1998). The test methods will incorporate the following modifications to the Environment Canada (1998) method:

- The tests will be conducted at $7 \pm 1^\circ\text{C}$, rather than $14 \pm 1^\circ\text{C}$;
- Test solutions will be renewed three times per week, rather than daily. This modification is appropriate considering the low temperature, long test duration, and the fact that the constituent of interest (i.e., TDS) would not diminish over time in the test containers as a result of degradation, volatilization, etc; and,
- The Arctic grayling and round whitefish eggs will be treated prophylactically on a weekly basis prior to hatch using an iodophore disinfectant, so that fungal infection does not impact the eggs.

The test using Arctic grayling is expected to be initiated in May 2012. The duration of the test is anticipated to be approximately six weeks, and the test will be terminated one week after 50% of the control organisms have hatched.

Lake trout and round whitefish both spawn in the fall, and the test with one or other of these species is expected to be initiated in October or November 2012, and will be terminated two

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weeks after 50% of the control organisms have hatched. The duration of the test with the fall spawning salmonid will be approximately three months.

Ripe adult fish will be obtained from a suitable uncontaminated source, and eggs and milt will be stripped and transported to the laboratory under cool, dark conditions. If available, the gametes will be obtained from a hatchery or, alternatively, may be collected from a wild population of adult fish. If possible, the eggs will be fertilized and the tests initiated within 24 h of stripping.

Ideally, eggs from at least four females and milt from at least four males will be obtained, although fewer fish producing gametes that are of high quality may still produce acceptable results. The eggs will be dry fertilized at the laboratory (i.e., eggs and milt will be mixed together in the absence of water); milt used to fertilize the eggs will be pooled from milt batches that display good sperm motility. Following fertilization, the eggs will be introduced to the test containers, such that water hardening occurs in the test solutions. Eggs from each female will be used to initiate separate replicates (e.g., replicate A from each concentration will be initiated using eggs from the same female), so that it is possible to remove the contribution of a female that produces non-viable eggs.

Endpoints from the tests will include percent reaching eyed stage, percent hatch, percent survival and percent normally developed fry.

The testing will be conducted to establish a threshold for effect of TDS using the ratio of major ions that currently occur at the site, but elevated to 1500 mg/L TDS on a calculated basis. A 0.67 X series of dilutions (1500, 1000, 667, 444, and 296 mg/L TDS) will be tested, as well as a laboratory water control. Concentrations of major ions will be measured at test initiation and termination, as well as at the mid-point of the test, in order to confirm that target concentrations were achieved and maintained. Statistical analyses will be conducted using TDS concentrations established on the basis of gravimetrically measured concentrations of TDS (gravimetric TDS), as well as TDS calculated based on measured concentrations of major ions in the test solutions (calculated TDS).

Evaluation of the effect of acclimation on survival and reproduction of *Ceriodaphnia dubia*

Cladocerans, such as *Ceriodaphnia dubia* and *Daphnia magna*, have been shown to be among the most sensitive species to elevated concentrations of TDS. However, these species are able to adapt to changes in ionic strength; indeed, the Environment Canada (2007) method for conducting toxicity tests using *C. dubia* suggests acclimation of this species for at least two generations is appropriate to remove effects associated with transitions in ionic strength.

Earlier tests conducted to establish the sensitivity of this species to TDS did not incorporate a pre-acclimation period, because the purpose of those tests was to establish a threshold for sensitivity to TDS. Thus, since a range of TDS concentrations was being evaluated, it was not possible to pre-acclimate the organisms to the TDS level of interest.

In order to establish whether *C. dubia* is able to tolerate a higher concentration of TDS as a result of acclimation, the following testing is proposed. *C. dubia* is proposed for this testing rather than *D. magna* because of the shorter duration of the test and the lower degree of variability that

is typical in the survival and reproduction test with this species. The results of the tests described below will be used to establish whether *C. dubia* are able to acclimate to increasing concentrations of TDS over a period of four generations.

Testing will be conducted to establish a threshold for effect of TDS using the ratio of major ions that currently occur at the site, but elevated to 1500 mg/L TDS on a calculated basis. A 0.67 X series of dilutions (1500, 1000, 667, 444, and 296 mg/L TDS) will be tested, as well as a laboratory water control.

A three brood (6 to 8-day) survival and reproduction test will be conducted using *C. dubia*, in a similar manner to that previously performed. However, at the end of the test, a second test will be initiated using <24 h old organisms produced on the final day of the initial test. Thus, in this second test, second generation test organisms will be used to initiate the test, using organisms produced in each concentration to initiate exposures in that same test concentration (e.g., offspring produced on day 7 in the 1000 mg/L TDS solution will be used to initiate the 1000 mg/L TDS exposure in the next test). Similarly, organisms produced on the final day of the second test will be used to initiate a third test, again with the same test concentrations. Finally, in a similar manner, a fourth test will be conducted using organisms obtained at the end of the third test.

Concentrations of major ions will be measured at test initiation and termination of each test in order to confirm that target concentrations were achieved and maintained. Statistical analyses will be conducted using TDS concentrations established on the basis of gravimetric and calculated TDS concentrations in the test solutions. Results will be presented in terms of both measured and calculated TDS concentrations.

Evaluation of the effect of strontium on rainbow trout and *Hyaella azteca*

Toxicity tests conducted by Birge (1978) suggest that early life stages of rainbow trout are highly sensitive to strontium. However, these results have been questioned on the basis of the very large difference between the sensitivity reported by Birge (1978) and data for other species, as well as later life stages of rainbow trout, which indicate that freshwater organisms are generally orders of magnitude less sensitive to strontium than the results reported by Birge (1978).

A rainbow trout embryo-alevin test will be conducted using strontium in a similar manner to that reported by Birge (1978) in order to establish whether those initial results were erroneous, or if early life stages of rainbow trout are, indeed, unusually sensitive to strontium. The test duration will be approximately 30 days, or until 7 days after 50% of control organisms have hatched; this duration is consistent with that described by Environment Canada (1998) and is essentially equivalent to that used by Birge (1978), who terminated his tests at 4 days post-hatch. The test will be conducted at 14±1°C and the solutions will be replaced daily throughout the exposure.

As a result of the uncertainty surrounding the threshold for toxicity of strontium to rainbow trout, eight test concentrations following a 0.33-times concentration series will be employed (i.e., 10, 30, 90, 270, 810, 2430, 7290 and 21,870 µg/L strontium) rather than the more typical five concentrations using a 0.5-fold dose series, in order to cover a reasonable range of concentrations.

It is likely that the toxicity of strontium is reduced with increasing water hardness, since strontium shares a number of chemical characteristics with calcium, as a result of their proximity in the periodic table. Thus, establishing the role of water hardness in reducing the toxicity of strontium may be beneficial in gaining an understanding of the toxicological profile of this alkaline earth metal. In order to establish whether water hardness reduces toxicity, the tests described above using rainbow trout will be conducted both in low (~15 mg/L) and moderate hardness (~100 mg/L), in order to establish whether a significant difference in sensitivity is evident between the water hardnesses. These hardnesses were selected because the low hardness is the water type that is typically used as control water in our laboratory, and the higher hardness is similar to that employed by Birge (1978). Furthermore, the relatively large difference in hardnesses between these two water types (6 to 7-fold) should be sufficient to demonstrate a difference related to hardness, if one does indeed exist, without being obscured by between-test variability.

Borgmann et al. (2005) reported results for survival of *Hyalella azteca* following a 7-day exposure to strontium in which it was not clear whether actual toxicological effects had been observed in the test solutions, or if the apparent effects observed were related to natural background mortality (because the results were not corrected for control mortality). In order to reduce uncertainty associated with the sensitivity of this species to strontium, a 14-day survival and growth test will be conducted, in which strontium is introduced to the overlying water. Note that Borgmann et al. (2005) only measured survival; however, growth will also be measured to fully evaluate the potential sensitivity of this test organism to strontium. The test method is adapted from that described by Environment Canada (1997) for measuring toxicity of sediments using this species and is similar to that used by Borgmann et al. (2005), although is longer in duration. Control sediment will be used for all exposures, and strontium will be introduced at a range of concentrations to the overlying water. Test solutions will be replaced daily, and survival and growth of the test organisms will be measured at test termination. Test concentrations used for this investigation will be 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/L strontium.

In each case, strontium concentrations will be measured in all test concentrations and control at test initiation and termination and, in the case of the trout tests, at the mid-point of exposure. Test endpoints will be calculated on the basis of average measured strontium concentrations.

Please feel free to contact me should you have any questions regarding the proposed testing. We look forward to working with you on this project.

Yours truly,

James Elphick, R.P.Bio.
Environmental Toxicologist

References

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