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May 6, 2014

File: L020

Rosanna Nicol and Marc Casas
Mackenzie Valley Land and Water Board
PO Box 2130
Yellowknife, Northwest Territories
X1A 2P6

Dear: Mr. Casas and Ms. Nicol:

Re: Snap Lake Mine De Beers Canada Inc.
Chronic Toxicity Testing
Water Licence #MV2011L2-0004

As you are aware under Water License MV2011L2-0004, at SNP station 02-20, a once yearly early life stage (egg/alevin) (ELS) chronic toxicity test must be carried out using the Environment Canada Method EPS/1/RM/28. This test must be carried out with 100% lake water with no dilutions.

On October 9, 2013 De Beers wrote a letter to the MVLWB discussing the series of issues that had occurred during attempts to complete the test and detailing proposed options for alternative test methods (Attachment 1). The Board responded to this letter on November 14th, 2014 with the following advice:

"De Beers will initiate the 30 day egg/alevin ELS test under ice at edge of the mixing zone when conditions allow (De Beers proposed option 2). In order to minimize the risk of more test invalidations De Beers should implement the following precautions:

As the window approaches, De Beers should be in close contact with the lab to get feedback of when the eggs and milt will be available.

- *Ideally try and avoid use of eggs and milt that will need to be shipped long distances or overnight.*

De Beers also requested clarity on the number of attempts to complete the work that would be deemed acceptable. There is no finite number of what is acceptable it is a matter of timing. Tests should be initiated at the first opportunity to use high quality eggs and milt. They should be run until a successful test is conducted or the window for high quality eggs closes. The intent is not for De Beers to conduct a test that is unlikely to succeed."

DE BEERS CANADA INC.
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As per the Board's advice De Beers commenced discussions with Golder and laboratories capable of fulfilling the test request on January 16, 2014, once the ice was safe to traverse. Aquatox was chosen at that time to be the successful candidate over other labs (Hydroqual is no longer performing the test, No local gametes near Nautilus until April 2014) due to the proximity of local gametes. Due to unseasonably cold weather in Ontario fish were unable to spawn and produce gametes, for this reason the test was delayed for two months by the lab.

On March 16th, 2014 the first sample was collected at Snap Lake to initiate the test (Attachment 2). The test was cancelled after 7 days due to a very low viability in the control embryos (14%). Since that time the viability of test eggs has remained low and as such the test has not been reinitiated. Freshet has now commenced at Snap Lake meaning that the ice will soon no longer be safe to traverse. As such the test will not be reinitiated until full ice out and the lake is safe to access by boat.

Based on the continued difficulties experienced with the ELS test and the need to find an appropriate surrogate, De Beers requests that the Board revise the condition of an ELS Rainbow Trout (*Oncorhynchus mykiss*) to the Fathead Minnow (*Pimephales promelas*) larval test. This request is supported by the Golder and Associates Technical Memo presented as IR#2 from the Gahcho Kue Technical Session (Attachment 3).

This report states the following:

"Environment Canada (1998, 2011) states that both Fathead Minnow and Rainbow Trout can be used for early life stage toxicity tests with water samples having salinity ≤10 g/kg (i.e., 10,000 mg/L TDS). Elphick et al. (2011) investigated the chronic toxicity of chloride to Fathead Minnow and Rainbow Trout embryos, to assess effects on survival, development, and biomass. The 32-d IC25 for Fathead Minnow growth was 704 mg/L, and the 56-d IC25 for Rainbow Trout growth was 1,174 mg/L. Environment Canada (1998, 2011) has standardized protocols for conducting short-term chronic tests with early life stages of Fathead Minnow and Rainbow Trout.

*There are currently seven Canadian laboratories accredited by the Canadian Association for Laboratory Accreditation (CALA) to conduct 7-d larval Fathead Minnow tests; those laboratories are located in British Columbia (BC), Alberta, and Ontario. There are currently two Canadian laboratories, both in BC, accredited by CALA to conduct the rainbow trout (*ELS test); *other laboratories have the capability to perform this test but do not maintain accreditation. No government laboratories in Canada are currently accredited for either the Fathead Minnow or the Rainbow Trout test method; and no Canadian laboratories maintain accreditation for the Rainbow Trout EA test (although several have the capability to perform it).*

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Under the Metal Mining Effluent Regulations, Environment Canada (2002, 2012) specifies that sublethal testing of freshwater effluents for the environmental effects monitoring (EEM) program use 7-d tests with Fathead Minnow or Rainbow Trout as the early life stage fish test, except that Rainbow Trout are to be used where Fathead Minnow are not indigenous (e.g., in BC).2 The Pulp and Paper Effluent Regulations had the same testing requirement until 2008 when sublethal fish testing was removed from the regulation.

The 7-d Fathead Minnow test requires smaller sample volumes be collected and shipped, uses test organisms that are more readily available and of reliability quality, and is more widely performed by commercial Canadian testing laboratories than the 7-d Rainbow Trout E test. The sample volumes required for the Rainbow Trout EA test are four to five times larger than for the E test.

Further details on the comparability of the tests can be found in Table 2 of Attachment 3. Based on this, the two tests were found to have comparable results with the Fathead minnows having a lower tolerance level. It is therefore concluded that the Fathead minnow test would be an adequate surrogate for the ELS test.

Should you have any questions or concerns, please feel free to contact me by phone at (867) 766-7308 or by email at Alexandra.Hood@debeersgroup.com .

Sincerely,
DE BEERS CANADA INC.

A. Hood

Alexandra Hood
Environment and Permitting Superintendant
Snap Lake Mine

cc	T. Bradbury, E.Bonhomme, M. Peters	DBCI
	M. Sanderson, S. Whitaker, R. Walbourne, P. Green	AANDC
	M. Casas, R. Nicol, R. Chouinard, L. Cymbalisty	MVLWB
	K. Eggers	DFO
	S. Lacey-MacMillan	EC
	P. di Pizzo, Z. Liu	SLEMA

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October 9, 2013

File: L020

Willard Hagen, Chair
Mackenzie Valley Land and Water Board
PO Box 2130
Yellowknife, Northwest Territories
X1A 2P6

Dear: Mr. Hagen:

Re: Snap Lake Mine
De Beers Canada Inc.
Chronic Toxicity Testing
Water Licence #MV2011L2-0004

As you are aware under Water License MV2011L2-0004, at SNP station 02-20, a once yearly early life stage (egg/alevin) chronic toxicity test must be carried out using the Environment Canada Method EPS/1/RM/28. This test must be carried out with 100% lake water with no dilutions.

On September 17, 2013 De Beers contacted the MVLWB to advise that two attempts (September 3 and 10) at completing the aforementioned test by Hydroqual laboratories were invalidated due to a failure in the control (reference) test. The lab report and referenced letter are attached to this document. On September 24, De Beers initiated a third test with a second lab, Nautilus Environmental, which has had success with developing site specific benchmarks for the Mine site in support of the Water License Amendment. After 7 days, this test was invalidated, again due to a failure in the control test. The lab report for this failure is also attached.

In conversation with Nautilus, typically October and early November are prime times to collect viable gametes for this test. However, this does not necessarily coincide with safe conditions at the mine due to rough water and ice formation. Due to safety concerns the test cannot be performed a fourth time during open water, as the test is run for 4 consecutive weeks, with water being shipped to accommodate the test regularly.

De Beers strives for regulatory excellence and requests that the Board provide guidance on how to carry out this activity. There are three proposed options forward:

Option	Pros	Cons
Initiate test with water sampled at 02-17	• Easy to collect and ship • If test species can	• Should the test fail, it may be difficult to draw accurate

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	survive in pure effluent than there would be no concerns in lake	conclusions
Initiate test under ice	• Most accurate/realistic results. • Closest to design intent of test as outlined in the SNP	• Weather and ice thickness dependant
Initiate test with bulk water sample collected in lake	• Easy to collect and ship	• Water quality of stored water may degrade over time, producing invalid/inaccurate results.

Option 2, to initiate the test under ice is the preferred option. However it is heavily dependent on ambient conditions on the lake, and samples may be unable to be collected.

De Beers also requests clarity on a maximum numbers of attempts to complete this work that would be deemed acceptable. Beers would like to remind the Board that no laboratory within Canada is currently accredited for this program (EPS/1/RM/28).

Should you have any questions, comments or require further clarification, please do not hesitate to contact me at (867) 766-7308 or e-mail me at the following address: Alexandra.Hood@debeerscanada.com.

Sincerely,

DE BEERS CANADA INC.

A. Hood

Alexandra Hood
Permitting and Environmental Superintendant
Snap Lake Mine

Attachments

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Copied to:	J. Potten	MVLWB
	L. Cymbalisty	MVLWB
	D. White	SLEMA
	P. Kramers	AANDC
	D. Raymond	DBCI

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September 24th, 2013

File: L020

Willard Hagen, Chair
Mackenzie Valley Land and Water Board
PO Box 2130
Yellowknife, Northwest Territories
X1A 2P6

Dear: Mr. Hagen:

Re: Snap Lake Mine
De Beers Canada Inc.
Chronic Toxicity Testing
Water Licence #MV2011L2-0004

According to Snap Lake Mine's Water License MV2011L2-0004, a once yearly early life stage (egg/alevin) chronic toxicity test must be carried out at SNP station 02-20, using the Environment Canada Method EPS/1/RM/28. This test must be carried out with 100% effluent with no dilutions.

The test concluded on September 3rd, 2013 and resulted in an invalid result as per the information provided below:

- **7-Day Test**
  - The result of the 7-day test: Pass
  - The final report for this test was received on August 19.

- **30-Day Test**
  - The result of the 30-day test: Invalid
  - The control portion of this test did not pass and as such the test is invalid.
  - The Snap Lake 30-day embryo-alevin test was invalid because only 57% of fish survived in the control and 65% is the test acceptability criterion.

Upon receipt of the invalid results, De Beers collected a new set of water samples to be sent to Hydroqual, and a new round of the test commenced on September 10th. Hydroqual had been in discussions with hatcheries, Environment Canada method experts to review test methods for the second re-test.

De Beers was notified on September 18th, 2013 that the results are yet again invalid, based on failure of embryos to fertilize in the concurrent 7-day control toxicant test and similar results in one of the extra sacrificial control replicates that was prepared. Both invalidations were due to lab error and a failure within the control (reference) sample. This information was communicated to the MVLWB Regulatory Officer Jennifer Potten and the AANDC

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Inspector, Patrick Kramers on September 4th, 2013. A follow up call on the second invalidation was completed on September 19th, 2013.

Due to the second failure, De Beers identified a new lab for the third round of testing. Nautilus Environmental was selected, as De Beers has had success working with this lab in developing site specific benchmarks for Total Dissolved Solids and Nutrients. The laboratory will do their utmost to succeed in this test, but as noted in previous communications, no labs in Canada are currently accredited for this program (Environment Canada EPS/1/RM/28).

There is also some concern that should ice form on Snap Lake or conditions be unsafe to sample over the coming weeks, the test will again be invalidated. De Beers continues to strive to ensure compliance with the Water License as written. As such if the third test be deemed invalid a fourth test may be conducted, pending ice conditions, in November/December 2013.

Should you have any questions, comments or require further clarification, please do not hesitate to contact me at (867) 766-7308 or e-mail me at the following address: Alexandra.Hood@debeerscanada.com.

Sincerely,

DE BEERS CANADA INC.

A. Hood

Alexandra Hood
Permitting and Environmental Superintendent
Snap Lake Mine

Attachments

Copied to:	J. Potten	MVLWB
	L. Cymbalisty	MVLWB
	D. White	SLEMA
	P. Kramers	AANDC
	D. Raymond	DBCI

HydroQual Laboratories Ltd.

#4, 6125 12th Street SE Calgary, Alberta Canada T2H 2K1
Tel (403) 253-7121 Fax (403) 252-9363 www.hydroqual.ca

Transmittal

Date: 2013/09/25

From: T. McClure, B.Sc.
Quality Assurance Manager

T. Harvey, B.Sc.
Laboratory & Group Manager

To: **De Beers Canada Inc.**

Embryo-Alevin Early Life Stages (ELS) Testing

A summary of the embryo-alevin testing conducted at HydroQual Laboratories for De Beers is provided. Although there is an Environment Canada protocol for this method, this test was originally positioned as a specialized analysis as HydroQual has not previously conducted an embryo-alevin ELS test. Additionally, there are currently no other laboratories in Canada accredited for this toxicity test.

Samples for embryo-alevin early life stages were collected from the Snap Lake diffuser stations throughout July, August, and September 2013 with one test initiated on July 31, 2013 and a second test initiated on September 10, 2013.

The first test showed valid reference toxicant results after 7 days and the test was continued until day 34, following Environment Canada protocols. Upon test completion, the controls showed 58% viability, which was less than the minimum control validity requirement of 65%. The majority of the control mortality occurred in the embryo state, with few alevins dying post-hatch.

The second test showed an invalid reference toxicant test after 7 days due to inadequate fertilization (no live embryos present in any test, control, or reference toxicant treatment). The embryo-alevin test was terminated at that time due to this lack of fertilization success.

For both tests, there were no protocol deviations or technical errors found that would have contributed to these invalid results.

HydroQual Laboratories Ltd.

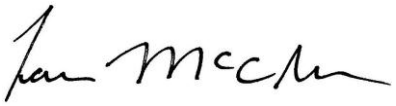
#4, 6125 12th Street SE Calgary, Alberta Canada T2H 2K1
Tel (403) 253-7121 Fax (403) 252-9363 www.hydroqual.ca

Transmittal

For Early Life Stage testing, gametes used for testing are very sensitive and the tests were initiated as soon as gametes were received to reduce the length of time they were held. Due to our geographical location, gametes must be shipped to Calgary from a supplier in Ontario and fertilization occurred shortly after receipt. Milt quality was verified microscopically; however, it is difficult to assess the quality of eggs upon receipt. Some alternate CALA accredited laboratories are able to collect gametes from nearby hatcheries. This would limit stress throughout transportation and minimize the time elapsed between gamete collection and fertilization.

We believe that fertilization success for embryo-alevin testing hinges on obtaining high quality gametes, which are transported under optimal conditions, from a reliable source. We will continue to work with our suppliers to establish optimal transportation conditions in order to obtain consistent, valid results for Early Life Stage testing.

If you have any questions or require any additional details, please don't hesitate to contact us.



T. McClure
Quality Assurance Manager

T. Harvey
Laboratory & Group Manager

Nautilus Environmental

Oct 8, 2013

Memo: Results of early life stage toxicity test conducted on sample identified as SNP

To	Alexandra Hood	From	Josh Baker
Affiliation	De Beers Canada Inc	Tel	604-420-8773
e-mail	alexandra.hood@debeerscanada.com	e-mail	josh@nautilusenvironmental.com

Nautilus Environmental initiated an early life stage development test on the sample identified as SNP. The sample was collected on Sept 24, 2013 and delivered to the Nautilus Environmental Laboratory in Burnaby, BC on Sept 25, 2013. Rainbow trout (*O. mykiss*) gametes were collected by Troutlodge Inc (Sumner, WA, US) on Sept 25, 2013 and delivered to Nautilus Environmental on Sept 26, 2013.

The 30-d embryo-alevin development test with rainbow trout was initiated using, and followed throughout the duration of the test, the Environment Canada guideline (1998, EPS 1/RM/28) for early life stage testing for salmonids. The test was initiated on Sept 26, 2013 following receival of the rainbow trout gametes. Five female gametes were deemed to be appropriate quality, mixed and used in fertilization and testing. Milt quality was deemed to be poor, as little sperm motility was observed. The fertilization was completed and 30 embryos were transferred into four replicates of the sample, respectively. In addition, a concurrent 7-d embryo development reference toxicant test using Sodium Dodecyl Sulfate (SDS) was initiated.

After 7 days the reference toxicant test was terminated and the following results (Table 1) were observed in the control (dechlorinated municipal tap water) test solution.

Table 1. *O.mykiss* survival and development test. 7-d reference toxicant results.

Sample	Survival (%) (Mean ± SD)	Development (%normal) (Mean ± SD)
Control	95.0 ± 3.3	17.5 ± 8.8

Due to the low control performance (normally developed embryos) in the reference toxicant test, which did not meet Environment Canada guideline criteria, the 30-d embryo-alevin SNP sample testing was terminated.

AquaTox Testing & Consulting Inc.
11B Nicholas Beaver Rd
Guelph ON N1H 6H9
Tel: (519) 763-4412 Fax: (519) 763-4419

April 22, 2014
File: 162705330

Alexandra Hood
Environment and Permitting Superintendent, Snap Lake Mine
De Beers – Mining Canada

The De Beers Group of Companies
De Beers Canada Inc.
Suite 300 – 5120 49th Street
Yellowknife, NT X1A 1P8
Phone: (867) 766-7308
E-mail: Alexandra.Hood@debeersgroup.com

Dear Alexandra:

Reference: Review of Failed EA (Embryo Alevin) Test Conducted on De Beer's "DBCI Sample Control Number 2014-0348" Collected March 16, 2014 (AquaTox Sample No. 39636)

AquaTox was contracted to conduct an EA test in accordance with Environment Canada's test method EPS 1/RM/28 on behalf of De Beers Canada Inc. (DBCI). A sample of the DBCI Sample Control Number 2014-0348 collected on Sunday, March 16, 2014 was received by AquaTox on Wednesday, March 19, 2014. Eggs and milt for use in conducting the test (AquaTox Batch No. EA 14-01) were obtained from Rainbow Springs Trout Farm located in Thamesford, Ontario on the previous day (Tuesday, March 18, 2014). The eggs and milt were held overnight in the dark under refrigerated conditions (temperature range 3 - 7°C). The 28-d EA test conducted on the DBCI sample (AquaTox Sample No. 39636) was initiated on Wednesday, March 19th, along with a 7-d embryo reference toxicant test using potassium chloride (AquaTox Sample No. 39637). The same batch of fish eggs were used for both tests.

The 7-d embryo reference toxicant test was terminated on day 7 (March 26, 2014). Eggs were fixed and cleared overnight using a hypersaline brine solution and examined microscopically to determine embryo viability. Results of this test are summarized in Table 1. The percentage of viable embryos determined from the control group was 14.2%. Since this test did not meet the test validity criteria (i.e., invalid if < 70% of controls are viable at the end of the test), the test initiated on the DBCI sample was terminated on the following day.

Immediately following a review of the reference toxicant test results, an internal review was initiated, including: a review of the water chemistry measurements conducted during the test, a review of the conditions during testing and follow up discussions with the fish culturist at Rainbow Springs as well as those from other local trout suppliers (e.g., Lyndon Fish Hatcheries Inc., Alma Research Station). A review of the water quality data recorded during the test showed that the levels of dissolved oxygen, pH, temperature and conductivity were within acceptable ranges for fish survival. Staff engaged in all aspects of the test, including: transport of the eggs and milt, preparation of the test solutions, testing for sperm viability, initiation of test, monitoring of the test solutions and termination of the test were not aware of any potential issues of concern which may have contributed to the poor embryo viability. However, discussions with various trout suppliers suggested that there may have been some concerns with the quality of the green (unfertilized) eggs.

Reference: Review of Failed EA (Embryo Alevin) Test Conducted on De Beer's "DBCI Sample Control Number 2014-0348" Collected March 16, 2014 (AquaTox Sample No. 39636)

DBCI was made aware of the results of the reference toxicant test and the decision by AquaTox to terminate the test on the DBCI Sample Control Number 2014-0348 (AquaTox sample 39636) by email on March 27th. A conference call involving representatives of DBCI, Golders Associates and AquaTox was conducted on April 2nd to review the test results and to prepare a plan for moving forward. It was agreed that testing of a fresh sample of the DBCI effluent should be initiated by AquaTox as soon as there was reasonable assurance from the fish suppliers that the females were once again producing viable eggs. As of the time of writing this letter report, we understand that the availability of viable eggs remains poor but is expected to improve in the near future. AquaTox will continue to monitor the situation at the various fish hatcheries and will inform DBCI of when conditions improve and this testing may proceed.

Table 1. Results of Salmonid Embryo Reference Toxicant Test (AquaTox Sample 39637) Using Sodium Chloride

[Nacl] mg/L	Replicate	Number of Embryos on Day 0	Number of Viable Embryos on Day 7	Viable Embryos (%)	Treatment Mean (%)
Control	A	40	7	17.5%	14.2
	B	40	5	12.5%	
	C	40	5	12.5%	
1441	A	40	6	15.0%	12.5
	B	40	5	12.5%	
	C	40	4	10.0%	
2058	A	40	0	0.0%	1.7
	B	40	1	2.5%	
	C	40	1	2.5%	
2940	A	40	1	2.5%	3.3
	B	40	1	2.5%	
	C	40	2	5.0%	
4200	A	40	0	0.0%	0.00
	B	40	0	0.0%	
	C	40	0	0.0%	
6000	A	40	0	0.0%	0.00
	B	40	0	0.0%	
	C	40	0	0.0%	

Sincerely,

AQUATOX TESTING & CONSULTING INC.

Keith Holtze
President, Director Laboratory Services
Tel: (519) 763-4412 Ext 308
Fax: (519) 763-4419
kholtze@aquatox.ca

TECHNICAL MEMORANDUM

DATE February 21, 2014 **PROJECT No.** 13-1365-0007/DCN-208

TO Veronica Chisholm
De Beers Canada Inc

CC Kristine Mason and Tasha Hall

FROM Cathy McPherson and Peter Chapman **EMAIL** pmchapman@golder.com; cmcpherson@golder.com

GAHCHO KUÉ PROJECT – INFORMATION REQUEST #2, TOTAL DISSOLVED SOLIDS TOXICITY TESTING WITH FATHEAD MINNOW OR RAINBOW TROUT

Information Request 2: De Beers will evaluate the viability of testing toxicity at end of pipe using the early life stage Rainbow Trout test versus the larval Fathead Minnow test, with a review of relative sensitivities for each species (specifically TDS and salinity) by February 24, 2014.

Response:

Range of Fathead Minnow and Rainbow Trout

Fathead Minnow (*Pimephales promelas*) are native to most of Canada (including the southern drainage of Great Slave Lake in the Northwest Territories) and the United States, but are not native to British Columbia or Atlantic Canada. Fathead Minnow can be found in ponds, lakes, ditches, streams, and alkaline/saline lakes; they are omnivores and will feed on invertebrates, organic matter, and detritus (Environment Canada 2011).

Rainbow Trout (*Oncorhynchus mykiss*) are native to western North America, occurring from California to Alaska; they are also cultured in hatcheries, and stocks have been introduced around the world (Environment Canada 1998). After hatching, alevins receive nutrition from their yolksac until it is absorbed; fry and juveniles feed on insect larvae and zooplankton.

Sensitivity of Early Life Stage Fathead Minnow and Rainbow Trout to Total Dissolved Solids (TDS)

Available information on the toxicity of total dissolved solids (TDS) and salinity to Fathead Minnow and Rainbow Trout is provided in Table 1 and summarized below. Information on the toxicity of TDS constituent ions, where available, is also provided.

Definitions for test endpoints are provided. The LC*x* is the concentration of test material estimated to be lethal to "*x*" percent of the test organisms, (e.g., LC50); the ECx is the concentration of test material estimated to cause a specified non-lethal effect to "*x*" percent of the test organisms (e.g., EC50). The IC*x* is the concentration of test material estimated to cause "*x*" percent inhibition in a sublethal endpoint such as growth or reproduction. The no observed effect concentration (NOEC) is the highest concentration tested where there was no statistically significant response compared to the negative control. The lowest observed effect concentration (LOEC) is the

lowest concentration tested where there was a statistically significant response relative to the negative control. The maximum acceptable toxicant concentration (MATC) is the geometric mean of the NOEC and LOEC.

Chronic Toxicity

Environment Canada (1998, 2011) states that both Fathead Minnow and Rainbow Trout can be used for early life stage toxicity tests with water samples having salinity ≤10 g/kg (i.e., 10,000 mg/L TDS).

Atton (1986) investigated the distributions of selected fish species in saline lakes in Saskatchewan, and reported that Rainbow Trout reproduction occurred at a salinity of approximately 4,500 mg/L TDS, but that populations did not survive at 20,000 mg/L TDS.

Chapman et al. (2000) conducted short-term chronic toxicity tests on Rainbow Trout embryos and swim-up fry, using synthetic effluents formulated to match the TDS composition of discharges from two Alaskan mines. One synthetic effluent was approximately 70% sulphate and 25% calcium, and the second was approximately 50% sulphate and 20% calcium. There were no adverse effects on early life stages of Rainbow Trout following 7-d exposures to either synthetic effluent, at TDS concentrations of up to 2,000 mg/L.

Elphick et al. (2011) investigated the chronic toxicity of chloride to Fathead Minnow and Rainbow Trout embryos, to assess effects on survival, development, and biomass. The 32-d IC25 for Fathead Minnow growth was 704 mg/L, and the 56-d IC25 for Rainbow Trout growth was 1,174 mg/L.

Evans and Frick (2001) reported unpublished results from early life stage toxicity tests (Beak International 1999) to assess the toxicity of sodium chloride to Fathead Minnow and Rainbow Trout. In a 7-d test with <24-h old larval Fathead Minnow, the LC50 was 5,490 mg/L, the NOEC and LOEC for growth were 2,000 and 4,000 mg/L, respectively, and the MATC for growth was 2,830 mg/L. In a 7-d embryo-larval and teratogenicity test conducted with <36-h old Fathead Minnow embryos, the NOEC and LOEC for final mortality (which combined counts for dead embryos/larvae and deformed larvae) were 1,000 and 2,000 mg/L, respectively, and the MATC was 1,410 mg/L. In a 7-d test with Rainbow Trout embryos, the EC25 for embryo viability was 1,630 mg/L, the EC50 was 2,400 mg/L, and the MATC was 2,830 mg/L. In a 27-d Rainbow Trout embryo-alevin test, the EC25 was 18,630 mg/L and the EC50 was 2,630 mg/L. Evans and Frick (2001) noted that Fathead Minnow embryos were more sensitive than larvae to sodium chloride, and that mortality of both species was similar and increased markedly between concentrations of 2,000 and 4,000 mg/L.

Birge et al. (1985) conducted a 33-d chronic test to investigate the toxicity of sodium chloride to Fathead Minnow embryos. Mean survival was only 20% at 1,650 mg/L. Point estimates were not reported. For survival, the NOEC and LOEC were 415 and 580 mg/L, respectively, and the MATC was 492 mg/L. It appears that growth was a less sensitive endpoint than survival; the NOEC and LOEC were 880 and 1,210 mg/L, respectively, and the MATC was 1,030 mg/L.

Pickering et al. (1996) conducted 7-d survival and growth tests with 1-d old larval Fathead Minnow to assess the toxicity of potassium chloride and sodium chloride. For potassium chloride, the NOEC and LOEC were 500 and 1,000 mg/L, respectively, and the MATC was 707 mg/L; these values applied for both survival and growth. For sodium chloride, the NOEC and LOEC were 4,000 and 8,000 mg/L, respectively, and the MATC was 5,700 mg/L; these values applied for both survival and growth.

Acute Toxicity

Elphick et al. (2011) investigated the acute toxicity of chloride to Fathead Minnow and Rainbow Trout. The 96-h LC50s for chloride were 4,079 mg/L for Fathead Minnow and 6,030 mg/L for Rainbow Trout.

Waller et al. (1996) conducted 24-h acute exposures of juvenile Fathead Minnow and Rainbow Trout to single concentrations of three chloride salts. Exposure to 10,000 mg/L sodium chloride or 2,500 mg/L potassium chloride for 24 h resulted in no mortality for either fish species. Exposure to 10,000 mg/L calcium chloride for 24 h resulted in 100% mortality for Fathead Minnow but only 16% mortality for Rainbow Trout.

Adelman et al. (1976) conducted acute tests with sodium chloride and reported a 96-h LC50 of 7,650 mg/L for fathead minnow. Birge et al. (1985; cited in Evans and Frick 2001) reported a 96-h LC50 for sodium chloride of 10,830 mg/L for fathead minnow.

Vosyliene et al. (2006) reported 96-h LC50s of 18,250 and 20,380 mg/L for the respective toxicities of road maintenance salt and common salt (sodium chloride) to Rainbow Trout. The authors hypothesized that the relatively small difference between the LC50s was due to the presence of additional constituents in the road salt, which increased its toxicity.

Goodfellow et al. (2000) reported a 96-h acute LC50 for Fathead Minnow of 266 mg/L for the calcium ion (Ca^{2+}), but did not report the calcium salt used. Ketola et al. (1988) reported that exposure to elevated calcium sulfate concentrations (520 mg/L Ca^{2+}) during water hardening of newly fertilized Rainbow Trout eggs resulted in only 23% survival at the eyed stage.

Moore et al. (2010) and Moore (2011) conducted acute toxicity tests with juvenile Rainbow Trout and reported the following preliminary 96-h LC50s for TDS constituents: sodium chloride (32,000 mg/L); magnesium chloride (13,000 mg/L); calcium chloride (12,000 mg/L); sulfate (9,000 mg/L); and, potassium chloride (4,000 mg/L).

Camargo and Tarazona (1991) exposed juvenile Rainbow Trout to 800 mg/L sodium chloride for eight days, as a toxicity control used in conjunction with other testing, and reported no mortality or sublethal behavioural effects.

Stoss et al. (1977) assessed the effects of different salts on the activation of Rainbow Trout milt. Spermatozoa activity was inhibited at concentrations ranging from 11,749 to 16,010 mg/L for magnesium chloride, calcium chloride, and sodium chloride; sensitivity to potassium chloride was much greater, with spermatozoa activity inhibited at 19.2 mg/L. The amount of inhibition was not reported.

Mount et al. (1997) examined the acute toxicity of over 2,900 ion solutions using Fathead Minnows in 96-h exposures. Of the chloride salts, potassium chloride alone and in combination with other compounds was the most toxic with an individual 96-h LC50 of 884 mg/L. Mount et al. (1997) reported a mean 96-h LC50 for calcium chloride of 4,632 mg/L and a 96-h LC50 for sodium chloride of 6,392 mg/L.

Summary

Limited data were available to compare the relative sensitivities of Fathead Minnow and Rainbow Trout to TDS or its constituent ions. However, it appears that Fathead Minnow have either similar or greater sensitivity than Rainbow Trout, depending on the analyte.

Comparison of Toxicity Test Methods for Early Life Stages of Fathead Minnow and Rainbow Trout

Environment Canada (1998, 2011) has standardized protocols for conducting short-term chronic tests with early life stages of Fathead Minnow and Rainbow Trout. Test conditions and sample requirements for each test method are summarized in Table 2.

The standard test with Fathead Minnow is a 7-d static-renewal test with newly-hatched larval fish; test endpoints are adverse effects on survival and biomass (as a measure of growth).[1] There is a similar standard protocol for conducting 7-d tests in the United States (USEPA 2002), and methods such as OECD (1992) can be used as guidance for conducting longer (e.g., 30-d duration) tests.

Environment Canada (1998) has multiple options for conducting early life stage rainbow trout tests, which include a 7-d embryo (E) test intended for frequent or routine monitoring, and an embryo-alevin (EA) test with an approximately 30-d duration. Both tests begin with embryo fertilization; the EA test requires sample collection and shipment weekly for the duration of the test, whereas the E test only requires sample collection once for test initiation.

There are currently seven Canadian laboratories accredited by the Canadian Association for Laboratory Accreditation (CALA) to conduct 7-d larval Fathead Minnow tests; those laboratories are located in British Columbia (BC), Alberta, and Ontario. There are currently two Canadian laboratories, both in BC, accredited by CALA to conduct the rainbow trout 7-d E test; other laboratories have the capability to perform this test but do not maintain accreditation. No government laboratories in Canada are currently accredited for either the Fathead Minnow or the Rainbow Trout test method; and no Canadian laboratories maintain accreditation for the Rainbow Trout EA test (although several have the capability to perform it).

Under the *Metal Mining Effluent Regulations*, Environment Canada (2002, 2012) specifies that sublethal testing of freshwater effluents for the environmental effects monitoring (EEM) program use 7-d tests with Fathead Minnow or Rainbow Trout as the early life stage fish test, except that Rainbow Trout are to be used where Fathead Minnow are not indigenous (e.g., in BC).[2] The *Pulp and Paper Effluent Regulations* had the same testing requirement until 2008 when sublethal fish testing was removed from the regulation.

The 7-d Fathead Minnow test requires smaller sample volumes be collected and shipped, uses test organisms that are more readily available and of reliability quality, and is more widely performed by commercial Canadian testing laboratories than the 7-d Rainbow Trout E test. The sample volumes required for the Rainbow Trout EA test are four to five times larger than for the E test.

[1] Biomass is the total dry weight of surviving fish in a replicate at the end of the test, divided by the number of fish at the start of the test, and represents a combination of sublethal effect and mortality (Environment Canada 2011).

[2] Laboratories in BC are able to import Fathead Minnow into the province provided that an approved transplant application is in place.

Table 1 Toxicity of Total Dissolved Solids (TDS) to Fathead Minnow and Rainbow Trout

Common Name	Scientific Name	Life Stage Tested	TDS Component	Test Duration (h or d)	Biological Measurement	Endpoint	Effects Concentration (mg/L)	Reference
Acute								
Fathead Minnow	*Pimephales promelas*	-	Ca^{2+}	96 h	survival	LC50	266	Goodfellow et al. (2000)
Fathead Minnow	*Pimephales promelas*	1-7 d old	$CaCl_2$	96 h	survival	LC50	4,632	Mount et al. (1997)
Fathead Minnow	*Pimephales promelas*	1-7 d old	$CaSO_4$	96 h	survival	LC50	>1,968	Mount et al. (1997)
Fathead Minnow	*Pimephales promelas*	1-7 d old	KCl	96 h	survival	LC50	884	Mount et al. (1997)
Fathead Minnow	*Pimephales promelas*	1-7 d old	$MgCl_2$	96 h	survival	LC50	2,117	Mount et al. (1997)
Fathead Minnow	*Pimephales promelas*	1-7 d old	NaCl	96 h	survival	LC50	6,392	Mount et al. (1997)
Fathead Minnow	*Pimephales promelas*	-	NaCl	96 h	survival	LC50	10,830	Birge et al. (1985)
Fathead Minnow	*Pimephales promelas*	-	NaCl	96 h	survival	LC50	7,650	Adelman et al. (1976)
Fathead Minnow	*Pimephales promelas*	juveniles	Cl^-	96 h	survival	LC50	4,079	Elphick et al. (2011)
Fathead Minnow	*Pimephales promelas*	juveniles	$CaCl_2$	24 h	survival	LC100	10,000	Waller et al. (1996)
Fathead Minnow	*Pimephales promelas*	juveniles	KCl	24 h	survival	LC0	2,500	Waller et al. (1996)
Fathead Minnow	*Pimephales promelas*	juveniles	NaCl	24 h	survival	LC0	10,000	Waller et al. (1996)
Rainbow Trout	*Oncorhynchus mykiss*	gametes	TDS (primarily $CaSO_4$)	24 h	fertilization	LOEC	750	Stekoll et al. (2003)
Rainbow Trout	*Oncorhynchus mykiss*	gametes	TDS (primarily $CaSO_4$)	24 h	fertilization	NOEC	500	Stekoll et al. (2003)
Rainbow Trout	*Oncorhynchus mykiss*	embryos	Ca^{2+} ($CaSO_4$)	fertilization to eye-up	survival	23% survival	520	Ketola et al. (1988)
Rainbow Trout	*Oncorhynchus mykiss*	juveniles	$CaCl_2$	24 h	survival	LC16	10,000	Waller et al. (1996)
Rainbow Trout	*Oncorhynchus mykiss*	juveniles	KCl	24 h	survival	LC0	2,500	Waller et al. (1996)
Rainbow Trout	*Oncorhynchus mykiss*	juveniles	NaCl	24 h	survival	LC0	10,000	Waller et al. (1996)

Table 1 Toxicity of Total Dissolved Solids (TDS) to Fathead Minnow and Rainbow Trout (continued)

Common Name	Scientific Name	Life Stage Tested	TDS Component	Test Duration (h or d)	Biological Measurement	Endpoint	Effects Concentration (mg/L)	Reference
Rainbow Trout	*Oncorhynchus mykiss*	gametes	$CaCl_2$	-	spermatozoa activity	inhibition	15,229	Stoss et al. (1977)
Rainbow Trout	*Oncorhynchus mykiss*	gametes	$MgCl_2$	-	spermatozoa activity	inhibition	11,749	Stoss et al. (1977)
Rainbow Trout	*Oncorhynchus mykiss*	gametes	NaCl	-	spermatozoa activity	inhibition	16,010	Stoss et al. (1977)
Rainbow Trout	*Oncorhynchus mykiss*	gametes	KCl	-	spermatozoa activity	inhibition	19.2	Stoss et al. (1977)
Rainbow Trout	*Oncorhynchus mykiss*	juveniles	Cl^-	96 h	survival	LC50	6,030	Elphick et al. (2011)
Rainbow Trout	*Oncorhynchus mykiss*	juveniles	NaCl (road salt)	96 h	survival	LC50	18,250	Vosyliene et al. (2006)
Rainbow Trout	*Oncorhynchus mykiss*	juveniles	NaCl	96 h	survival	LC50	20,380	Vosyliene et al. (2006)
Rainbow Trout	*Oncorhynchus mykiss*	2 month old fingerlings	NaCl	8 d	survival	NOEC	800	Camargo and Tarazona (1991)
Chronic								
Fathead Minnow	*Pimephales promelas*	larvae	KCl	7 d	survival	NOEC	500	Pickering et al. (1996)
Fathead Minnow	*Pimephales promelas*	larvae	KCl	7 d	survival	LOEC	1,000	Pickering et al. (1996)
Fathead Minnow	*Pimephales promelas*	larvae	KCl	7 d	survival	MATC	707	Pickering et al. (1996)
Fathead Minnow	*Pimephales promelas*	larvae	NaCl	7 d	survival	NOEC	4,000	Pickering et al. (1996)
Fathead Minnow	*Pimephales promelas*	larvae	NaCl	7 d	survival	LOEC	8,000	Pickering et al. (1996)
Fathead Minnow	*Pimephales promelas*	larvae	NaCl	7 d	survival	MATC	5,700	Pickering et al. (1996)
Fathead Minnow	*Pimephales promelas*	embryos	NaCl	33 d	survival	LC80	1,650	Birge et al. (1985)
Fathead Minnow	*Pimephales promelas*	embryos	NaCl	33 d	survival	NOEC	415	Birge et al. (1985)
Fathead Minnow	*Pimephales promelas*	embryos	NaCl	33 d	survival	LOEC	580	Birge et al. (1985)

Table 1 Toxicity of Total Dissolved Solids (TDS) to Fathead Minnow and Rainbow Trout (continued)

Common Name	Scientific Name	Life Stage Tested	TDS Component	Test Duration (h or d)	Biological Measurement	Endpoint	Effects Concentration (mg/L)	Reference
Fathead Minnow	*Pimephales promelas*	embryos	NaCl	33 d	survival	MATC	492	Birge et al. (1985)
Fathead Minnow	*Pimephales promelas*	embryos	Cl^-	32 d	biomass	IC25	704	Elphick et al. (2011)
Rainbow Trout	*Oncorhynchus mykiss*	embryos	TDS (70% sulphate)	7 d	viability	NOEC	1,999	Chapman et al. (2000)
Rainbow Trout	*Oncorhynchus mykiss*	fry	TDS (70% sulphate)	7 d	survival and growth	NOEC	1,999	Chapman et al. (2000)
Rainbow Trout	*Oncorhynchus mykiss*	fry	TDS (50% sulphate)	7 d	survival and growth	NOEC	2,080	Chapman et al. (2000)
Rainbow Trout	*Oncorhynchus mykiss*	embryos	Cl^-	56 d	biomass	IC25	1,174	Elphick et al. (2011)

Notes:
LCx = concentration of sample estimated to be lethal to x% of the test organisms.
ECx = concentration of sample estimated to cause a specified effect to x% of the test organisms.
ICx = the inhibiting concentration for an x% effect; it is the concentration of sample estimated to cause x% reduction in growth or fecundity of the test organisms.
NOEC = no observed effect concentration.
LOEC = lowest observed effect concentration.
$CaCO_3$ = calcium carbonate; $CaCl_2$ = calcium chloride; $CaSO_4$ = calcium sulfate; KCl = potassium chloride; $MgCl_2$ = magnesium chloride; NaCl = sodium chloride; Cl- = chloride ion; Ca^{2+} = calcium ion; TDS = total dissolved solids; h = hours; d = days; mg/L = milligrams per litre; % = percent; > = greater than; < = less than; - = no information available.

Table 2 Recommended Test Conditions for Early Life Stage Tests with Fathead Minnow and Rainbow Trout

Test Condition	Description	
Test organism	Fathead Minnow (*Pimephales promelas*)	Rainbow Trout (*Oncorhynchus mykiss*)
Test organism age	<24-h old newly hatched larval fish	Gametes (eggs and milt)
Test organism source	In-house culture or commercial supplier	Hatchery
Test organism availability and transport tolerance	Available year round and on relatively short notice; tolerate overnight shipping well	Depends on hatchery spawning cycle; may be year round or limited windows in April/May and November; gametes are best used within hours of collection and overnight shipping can reduce gamete quality
Test type	Static-renewal	Static-renewal or flow-through
Test duration	7 d	Embryo (E) test: 7 d Embryo-alevin (EA) test: 7 d after half the controls have hatched (about 28-34 d)
Sample volume required	1 concentration tested: 20 L 5 concentrations tested: 35 L	E test: (volume depends on test vessels used) - 1 concentration tested: 60 to 90 L - 5 concentrations tested: 120 to 180 L EA test: - same as E test but weekly for up to 5 weeks - 1 concentration tested: 300 to 450 L - 5 concentrations tested: 600 to 900 L
Test vessel	500-mL glass beaker	800-mL plastic beaker with slits in side suspended in plastic pail or glass aquarium
Test solution volume	≥250 mL	≥2 L depending on test vessel size
Test concentrations	7 concentrations, plus a negative (clean) control	5 concentrations, plus a negative (clean) control
Test replicates	Minimum 3 replicates per concentration; 4 replicates recommended	4 replicates per concentration
No. of organisms	10 per replicate	30 per replicate
Control/dilution water	Uncontaminated ground or surface water, reconstituted water, or dechlorinated municipal water	Uncontaminated ground or surface water, reconstituted water, or dechlorinated municipal water
Test solution renewal	Daily	Daily
Test temperature	25 ± 1°C	14 ± 1°C
Feeding	Newly hatched brine shrimp (*Artemia*) nauplii two or three times daily	E test: no feeding EA test: no feeding
Photoperiod	16:8 h light: dark	Dark until one week after hatching; 16:8 h light: dark thereafter
Aeration	Gentle aeration if needed	Gentle aeration throughout test if static-renewal, as needed if flow-through
Test endpoint	Survival and biomass	E test: embryo viability EA Test: hatchability, alevin survival, alevin deformity
Test acceptability criteria	Mean control survival ≥80%; mean dry weight of surviving control fish >250 µg/fish	E test: ≥70% mean viable controls EA test: ≥65% mean viable controls
Test protocol	Environment Canada (2011)	Environment Canada (1998)

h = hours; d = days; °C = degrees Celsius; % = percent; L = Litre; mL = millilitre; ± = plus or minus.

Closure

If you have any questions or require additional information, please do not hesitate to contact Golder Associates Ltd.

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