Final Report
Comparison of Results from Alternative Acute Toxicity Tests with Rainbow Trout for Selected Mine Effluents

To:

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EXECUTIVE SUMMARY

A general concern for timely results of toxicity tests by industrial dischargers, including members of the metal mining sector and government regulators, has resulted in the investigation of several micro/screening toxicity test procedures emerging into the market place as alternatives to methods currently in place. The purpose of this evaluation is to determine which, if any, types of micro/screening toxicity tests can be used as an alternative to the Rainbow Trout acute lethality procedures specific to the Canadian Mining sector. As more recent regulations have included the use of the *Daphnia magna* acute lethality toxicity test for compliance testing, the project evaluation also includes a comparison to this test organism.

This study evaluates the data generated from toxicity tests conducted on selected mine effluents of various mine types (ie. zinc, copper/zinc, uranium, etc.) exhibiting a range of toxicity and chemical parameter characteristic of Canadian mine effluents. The report provides a comparison of toxicity tests using Rainbow Trout, *Daphnia magna* acute lethality bioassays with various micro/screening toxicity tests, which include the *Daphnia magna* IQ toxicity test**J**, Microtox**J**, Rotoxkit F, Thamnotoxkit F and Toxichromotest.

The comparison consisted of evaluating several criteria specific to each toxicity test and comparing these results to the rainbow trout toxicity test. These criteria included costs, speed, the correlation of effluent chemistry to toxicity results, the reproducibility of toxicity results including intra and interlaboratory results, the applicability of each toxicity test and the comparability of the micro toxicity test results to the comparable rainbow trout toxicity test results.

The following points summarize the main conclusions of the Canmet study:

- No one toxicity test compared directly with the rainbow trout toxicity test for both comparability of toxicity response and correlation of endpoint results to chemistry.

- Based upon the evaluation criteria the "best" toxicity test varied depending on mine types. The "best" toxicity test varied between the Thamnotoxkit, *Daphnia magna* IQ and *Daphnia magna* acute toxicity tests depending on mine type.

- When either the *Daphnia magna* IQ or Thamnotoxkit was selected as the Abest@ test the next best test was the reciprocal procedure. When the *Daphnia magna* acute toxicity test was selected as the "best" the next selection included either the IQ or Thamnotoxkit procedure.

- From the results it has become quite obvious that the applicability of the "best" test for a specific application has to be assessed on a case by case basis. Results of the Canmet study provide direction in this assessment and would be of added value for the justification of a specific toxicity test procedure to corporate environmental mangers and/or government regulators.

- The *Daphnia magna* IQ and Rotoxkit demonstrated an increased toxic response for gold mine effluents compared to the rainbow trout toxicity test. This increased toxicity response may be a result of a specific toxicant(s) (ie. cyanide) characteristic of gold mine effluents which should be investigated further.

- The highly standardized Microtox test, availability of technical reference material, ability to provide results quickly and high concordance (presence or absence of toxicity) with the rainbow trout test does make this assay procedure attractive for use at mine sites. But such things as the initial capital costs, insensitivity to various metals and reduce ecological relevance may deter its application with mining effluents. Use of the Microtox would have to be assessed on a case by case basis with regard to its applicability to address a specific application. If use of the Microtox is considered further evaluation of exposure time and osmotic adjustment agent (ie. sucrose/NaCl or $NaClO_4$ instead of NaCl) should be considered.

- At present the micro or kit toxicity test kits evaluated in this study do not have any QA/QC requirements. Use of any one of these toxicity test procedures would require the inclusion of specific QA/QC procedures (ie. reference toxicants, duplication, reporting requirements, etc.) in order to provide credibility to results, particularly if results are to be used as a replacement for the standard acute toxicity tests.

During this study, one encompassing commercial toxicity test kit could not be identified which would fulfill the requirements for acceptance and application throughout the mining sector, the scientific community and the various government jurisdictions. However, this study does contribute to the understanding of toxicity in mining discharges, the interactions of the various toxicity tests evaluated and variations of response between the various mine types and specific mining operations.

Results in this report, and the format in which they are presented, can provide the basis by which any particular site can conduct their own independent evaluation of toxicity tests specific to their effluent type, application and priorities.

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1.0 Introduction

1.1 Background

The Metal Mining Liquid Effluent Regulation (MMLER) was introduced to establish a national baseline standard to provide protection to fish and other aquatic biota. The intent of the MMLER was to limit the discharge of deleterious materials from mining operations including base metal, uranium and iron ore facilities. Once promulgated, new, expanded or reopened mines would be subject to these regulations. The MMLER would apply as guidelines to existing mine facilities. In specific instances, more stringent effluent requirements may have been imposed by either federal or provincial regulatory bodies.

A key component of the MMLER is the guideline for conducting the 96 hour flow-through and/or 96 hour static acute fish lethality tests as a method of assessing the potential for aquatic impact from effluent discharges.

Environment Canada is presently conducting a review of the MMLER to determine its adequacy in protecting fisheries resources beyond the point of discharge. This in essence is quite similar to the intent of the Pulp and Paper industrial sector Fisheries Act regulations for environmental effects monitoring (EEM).

1.2 Current Status of Toxicity Testing in the Industry

Since the implementation of the MMLER, the science of toxicity testing and assessment has advanced quite dramatically. Environment Canada has developed a series of aquatic bioassay test protocols which incorporate a suite of organisms representative of various trophic and ecological niches. The bioassay protocols also include specific procedure endpoints that measure acute, sublethal and chronic effects.

In **most** instances the discharger contracts out testing to be completed on their behalf. As a result of the test duration, sample transport time and time to complete and report results, a discharger may not obtain results of a toxicity test until some time well after the event of the discharge.

1.3 Rationale for Project

Though the majority of the toxicity test methods presently in use are ecologically relevant, results cannot be provided immediately. A general concern for timely results by industrial dischargers, including members of the metal mining sector and government regulators, has resulted in the investigation of several micro/screening toxicity test procedures emerging into the market place as alternatives to methods currently in place. These micro tests may be used as alternative screening methods for effluent toxicity if they can be correlated to results obtained with compliance test organisms. This would be particularly relevant if monitoring frequency was increased due to effluent non-compliance and/or operational changes or upsets.

The purpose of this evaluation is to determine which, if any, types of micro/screening toxicity tests can be used as an alternative to the Rainbow Trout acute lethality procedures. As more recent regulations have included the use of the *Daphnia magna* acute lethality toxicity test for compliance testing, the project evaluation also includes a comparison to this test organism.

1.4 Benefits to be Derived

Alternative micro/screening bioassays will be evaluated utilizing effluent samples representative of the metal mining sector. The results will provide a detailed comparison of several currently available micro/screening procedures, *Daphnia magna* IQ toxicity test**J**, Microtox**J**, Rotoxkit F, Thamnotoxkit F and Toxichromotest, to the two recognized compliance test organisms, Rainbow Trout and *Daphnia magna*. The results of this study may have significant application to other industrial sectors.

As indicated in the original request for proposal, "if satisfactory alternatives can be found to provide the required information at less cost and greater speed, it would be in the best interests of both mining industry and the regulatory community to adopt and implement them".

If a micro/screening procedure is found to be comparable to a compliance testing organism is achieved it could provide:

1) an assessment tool to be used at the discharge site,

2) an assessment tool to be used for increased monitoring,

3) linkable results to ecological effects as well as specific effluent chemical parameters,

4) a method of assessment at a reduced cost per test,

5) a screening mechanism to determine if other more traditional compliance tests are required,

6) an opportunity for a discharger to evaluate different operating conditions or temporal variations at an increased frequency and at a much reduced cost,

7) a mechanism to conduct cost effective toxicity identification/toxicity reduction evaluations (TIE/TRE).

1.5 Objectives of the Project

This study is intended to evaluate practical, cost-effective test alternatives to current regulatory toxicity tests using mine effluents representing the major mine types across Canada.

A number of micro/screening toxicity test procedures were evaluated as part of the study plan. This particular study evaluates the data generated from toxicity tests completed in the first part of the Canmet study to provide a comparison of toxicity testing using Rainbow Trout, *Daphnia magna* acute lethality bioassays with various micro/screening toxicity tests which include the *Daphnia magna* IQ toxicity test J, Microtox J, Rotoxkit F, Thamnotoxkit F and Toxichromotest using selected mine effluents. Although the various end points of these tests (Mortality, Fluorescence, enzymatic inhibition) do not have the same toxicological significance the objective is to determine whether one or more of these alternative tests would consistently exhibit a response similar in magnitude to that of the standard rainbow trout test, for a variety of Canadian mine effluents.

In addition, the project will also summarize results provided by the contract laboratories regarding the toxicity test alternative in terms of cost, correlation to chemistry, speed (turn around time), reproducibility, applicability and comparability to the rainbow trout toxicity test. This information has been tabulated and included in discussions of this report.

2.0 Methodology

2.1 Approach

Testing was conducted on selected mine effluents exhibiting a range of toxicity and chemical parameters characteristic of Canadian mine effluents. Mine site effluents were collected on 1 to 4 occasions from 21 mine sites. These mine types included:

a. 5 Gold Mines
b. 1 Bitumen Mine
c. 1 Tin Mine
4. 2 Uranium Mines
5. 1 Zinc Mine
d. 4 Copper/Zinc Mines
e. 4 Nickel/Copper Mines
6. 3 Lead/Zinc Mines

The samples were collected by the mine operators and shipped to the appropriate laboratories responsible for conducting the toxicity testing and chemical analysis. The secretariat of AETE coordinated and tracked samples. The protocol for sampling can be found in Appendix E. The mine effluents were tested with Rainbow Trout, and *Daphnia magna* acute lethality bioassays, along with various micro/bio toxicity tests, which included the *Daphnia magna* IQ toxicity test **J**, Microtox **J**, Rotoxkit F, Thamnotoxkit F and Toxichromotest. The tests were completed by two laboratories; Bar Environmental Inc. and Beak Consultants Limited. The following table summarizes the tests that each lab completed and the associated endpoints of each test.

Table 2.1.1 Toxicity Tests Evaluated

LABORATORY	TOXICITY TEST	ENDPOINT
BAR Environmental Inc.	Rainbow Trout	96 hr LC50[1]
	Daphnia magna	48 hr LC50
	Daphnia magna IQ	75 Min. EC50[2]
BEAK Consultants Limited	Microtox (*Photobacterium phosphoreum)*	15 Min. IC50
	Rotoxkit (*Bachhionus calyciflorus*)	24 hr LC50
	Thamnotoxkit (*Thamnoocephalus platyurus*)	24 hr LC50
	Toxichromotest (*E. coli*)	90 Min. IC50

1 - LC50 is the estimated concentration which causes acute lethality to 50% of the test organisms.
2 - EC50 is the estimated concentration which causes immobility to 50% of the test organisms.

Chemistry testing of the mine effluents was completed by Seprotech. Split sample QA/QC (toxicity and chemistry) testing of eight samples was conducted by Environment Canada, Ontario Ministry of the Environment and Energy and Canmet laboratories. Once testing was completed, raw data results and a final report from each laboratory were forwarded to Pollutech Enviroquatics Limited and B. Zajdlik & Associates for statistical analysis and evaluation.

2.2 Toxicity Test Descriptions

The following sections provide a brief discussion on the principles of each toxicity test. Table 2.2.1 provides a synopsis of the experimental design of each toxicity test.

2.2.1 Rainbow Trout Acute Lethality Test

The LC50 toxicity test involved placing groups of fish (10 per concentration) in a range of concentrations of effluent, diluted with freshwater (to which the fish were acclimated). The tests were conducted in temperature controlled water baths held at 15 ± 1°C. Solutions were gently aerated throughout the 96 hour exposure period. Tests were conducted under static conditions with no renewal of the test solution. For all tests, temperature and photoperiod were similar to those of culture or holding conditions and kept constant between all tests. Observations for immobility or mortality were recorded after 24, 48, 72 and 96 hours. A fish was considered dead if there was no evidence of opercular or other activity, and showed no response to gentle prodding. The rainbow trout 96 hour LC50 was completed in accordance to the Federal Protocol (Environment Canada, 1990a).

2.2.2 *Daphnia magna* Acute Lethality Test

Basic test procedures are similar to those for the LC50 fish toxicity test. Each 48 hour *Daphnia magna* test involved placing groups of <24 hour old *D. magna* neonates into a range of concentrations of effluent, diluted with freshwater (to which the daphnids were acclimated). Toxicity tests with *D. magna* were conducted in 55 mL glass test tubes. For each concentration (including controls), 4 replicate test tubes were set up each containing 3 daphnids for a total of 12 daphnids per concentration. All tests were conducted in temperature controlled rooms at 20 ± 1°C. Tests were conducted under static conditions with no renewal of the test solution. For all tests, temperature and photoperiod were similar to those of culture or holding conditions and kept constant between all tests. Observations for immobility or mortality were recorded after 24 and 48 hours. A daphnid was considered to be dead if there was no visible heart beat upon microscopic examination. The Daphnia magna 48 hour LC50 was completed in accordance to the Federal protocol (Environment Canada, 1990b).

2.2.3 *Daphnia magna* IQ

The IQ test is based upon the measuring of a fluorescent substrate uptake and subsequent enzyme activity. For this test, starved *Daphnia magna* are exposed to a series dilution or concentration range of the effluent or chemical. The 2 to 10 day old Daphnids are obtained from an in-house culture similar to the acute lethality test procedure. The substrate methylumbelliferyl-β-D-galactoside (MUF-galactoside) is added to serially diluted test samples after a 1 hour incubation of juvenile *Daphnia magna*. Upon ingestion the MUF-galactoside is enzymatically hydrolyzed producing a fluorescent compound, 4-methylumbelliferone. The number of fluorescent organisms is counted after 15 minutes using a ultraviolet light. Therefore a reduction of the florescence is considered the toxic response which is expressed as and EC50 (Janssen and Persoone 1994). An ASTM standardization is underway (ASTM, 1994). The *Daphnia magna* IQ is one of several commercially available IQ test kits from Aqua Survey Inc., Flemington, N.J., USA.

2.2.4 Microtox

This is the most ubiquitous microbioassay. It was developed by Bulich (1979) and has a large volume of literature associated with it (Microbics Inc. 1994). The test is available as a kit with the apparatus and a lyophilized marine bacteria from Microbics Inc. Carlsbad, CA, USA. The luminescent marine bacterium *Photobacterium phosphoreum* (strain NRRL B-11177) which can be rehydrated in 5 minutes is exposed to serially diluted samples of the effluent, water or chemical. The measured response is the inhibition of light production as the sign of a toxic response. Since light production is a function of respiration, of which takes place in all organisms, results have to be generalized to all organisms, (Isenberg, 1993). In the special apparatus provided by Microbics the test can be completed in as little time as 5, 15 or 30 minute IC50's depending of the project objectives and response time of the toxicant(s). For the Canmet study a 15 minute endpoint was determined.

2.2.5 Rotoxkit F

The Rotoxkit F is a freshwater 24 hour LC50 bioassay performed in a multiwell test plate using neonates of the freshwater rotifer *Brachionus calusiflorus* The rotifers are provided in cyst form and can be hatched within 24 hours (Snell et al. 1991). The organisms are exposed to a dilution series of effluent or chemical along with a control. Five rotifers are placed in each test well with six repetitions for each concentration. At the end of the 24 hour period the test wells are examined and the number of dead and living rotifers is recorded to provide a 24 hour LC50. An organism is considered dead if they do not exhibit any movement in 5 seconds of observation. This toxkits is commercially available from Creasel Ltd., Deinze, Belgium.

2.2.6 Thamnotoxkit F

The Thamnotoxkit F like the Rotoxkit F is a 24 hour LC50 bioassay performed in a multiwell test plate using instar II-III larvae of the fairy shrimp *Thamnocephalus platyurus*. The test animals are provided in the form of resting eggs which can be hatched within 24 hours. The organisms are exposed to a dilution series of the effluent or chemical and a control. Ten larvae are placed in each test well with three repetitions for each concentration. At the end of the 24 hour period the test plate is checked for the number of dead and living larvae. The larvae are considered dead if they do not show any movement during 10 seconds of observation. This toxkit is also commercially available from Creasel Ltd., Deinze, Belgium.

2.2.7   Toxichromotest

A mutant strain of stressed *Eshericia coli* is exposed to a toxicant. Stressed bacteria are more sensitive to toxicants. Stressing is done by freeze drying. The test measures the inhibition of an inducible enzyme system (Reinhartz et al, 1987).   The toxicants pass through the lipopolysaccaride cell wall and inhibit the synthesis of β-galactosidase. The sample is serially diluted and mixed with the bacteria and a cocktail consisting of inducers of the enzyme system and compounds that promote successful recovery from freeze-drying. Only those bacteria that are unaffected by the toxicant will exhibit enzyme induction and hence a measurable colour change. The production of a colour is a measure of metabolic activity with inhibition considered a toxic response (Reinhartz et al. 1987) Colour change is measured using a microplate reader. This test can be completed within four hours. The usual endpoint is the EC20 or the minimal inhibitory concentration. For this study an EC50 response concentration was determined. Kits are available from EBPI (Environmental Biodetection Products Inc.), 14 Abacus Rd., Brampton, Ont. Canada. L6T 5B7.

Table 2.2.1 Summary of Toxicity Test Experimental Design

Test	Species	Endpoint	Number of Conc.	Number of Organisms/Conc.	Number of Replicates
Rainbow Trout Acute	Rainbow Trout	LC50	5-6 + control	10	1
Daphnia magna Acute	*Daphnia magna*	LC50	5-6 + control	3/rep	4
Daphnia magna IQ	*Daphnia magna*	EC50: amount of fluorescence reduction	5-6 + control	6/rep	3
Microtox	*Photobacterium phosphoreum*	IC50: inhibition of light production	≥ 4+ control	$1x10^6$/vessel	1/ conc. 2/ control
Rotoxkit F	*Brachionus calyciflorus*	LC50	5+ control	5/well	6
Thamnotoxkit F	*Thamnocephalus platyurus*	LC50	5 + control	10/well	3
Toxi-Chromotest	*Eschericia coli*	MIC20 (minimal inhibitory conc.) at 20% (colour density)	7 + control, + standard positive toxicant at 7 levels, + blank	100 µl bacterial suspension	2/test conc., 1/ std. pos. tox., 8 /blank,

2.3 Method of Evaluation

In their final reports, the primary labs (Bar and Beak) discussed several issues as they related to the toxicity tests (Rainbow trout, Daphnia magna, Daphnia magna IQ, Microtox, Rotoxkit F, Thamnotoxkit F and Toxichromotest Tests). the following criteria were evaluated utilizing information discussed in these reports and statistical analysis completed by Pollutech Enviroquatics and B. Zajdlik & Associates:

- Cost - Includes capital, material, labour and QA/QC costs
- Speed - Time required to complete tests
- Correlation to Chemistry - Environmental parameters which correlate to endpoint results
- Reproducibility - The between and within laboratory precision of the toxicity test.
- Applicability - The ability of the microtest to meet specific expectations for an application.
- Comparability to Rainbow trout toxicity test - Compares statistically results of the microtest to the rainbow trout toxicity test.

The statistical analyses completed for this project fit into the two following categories:

1) prediction of toxicity test results from effluent chemistries, and
2) comparison of toxicity test endpoints to the rainbow trout test LC50.

As a method for comparing these tests, each of these categories, listed above, for each micro/screening bioassay have been discussed and a method of scoring has been devised that allows ranking of each toxicity for each evaluation criteria. Scoring criteria for the Aapplicability@ category was not established. Since the specific application for which the toxicity tests would be utilized has not been defined a textual discussion has been provided instead that helps to provide definitions. The evaluation, where relevant, was be completed in comparison to the rainbow trout toxicity test. This approach of ranking is similar to that utilized in an environmental assessment where various options for a specific objective are being evaluated against a predefined set of evaluation criteria. Further discussion of the evaluation criteria and ranking can be found in Section 5. It should be noted that the evaluation criteria or approach of this report has been provided as a guide for users of these toxicity tests particularly if a specific application or situation has been defined.

3.0 Summary of Toxicity Test Results

The following section provides tabulated summaries of all the toxicity data. The environmental parameter data (chemistry) for the four sampling periods has been provided in Appendix A.
The sampling periods are defined as follows:

1) February 20 to March 6, 1995
2) March 20 to April 3, 1995
3) May 8 - June 2, 1995
4) May 29 - June 12, 1995

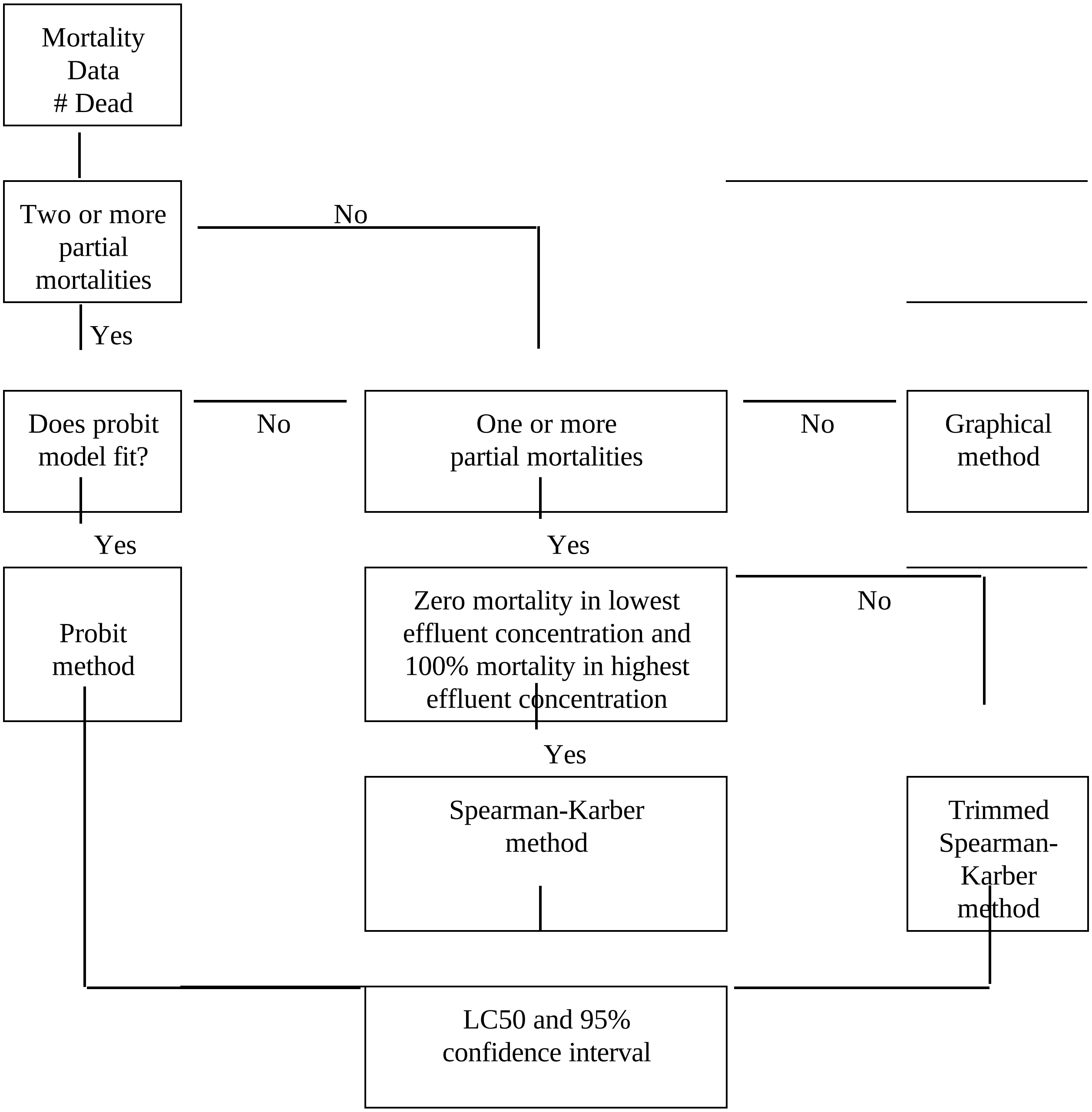
Toxicity data for the four sampling periods is summarized in Table 3.A through 3.E. Toxicity data has been segregated into tables defined by mine type. A specific site number has been assigned to each site for which samples have been received. For example, from Table 3.A Site 8, a gold mine, would be represented by Canmet sample numbers 5, 26, 45 and 67 for the first, second, third and fourth sampling periods, respectively. Laboratory Quality Control (QC) toxicity data is summarized in Table 3.F. QC analytical data has also been included in Appendix A.

The following list details the site numbers that have been assigned for Canmet sample numbers from the same mine:

Site Number	Canmet Number(s)
Site 1	9, 19, 35, 61
Site 2	8, 32, 40, 62
Site 3	2, 27, 36, 57
Site 4	6, 33
Site 5	13, 22, 37, 56
Site 6	10, 30, 41, 63
Site 7	17, 20, 46, 58
Site 8	5, 26, 45, 67
Site 9	4, 24, 43, 65
Site 10	1, 31, 38, 59
Site 11	3, 28, 42, 64
Site 12	12, 21, 47, 69
Site 13	7, 34
Site 14	11, 18
Site 15	15, 29
Site 16	16, 25, 44
Site 17	14, 23, 55
Site 18	54, 68
Site 19	50, 53
Site 20	39, 60
Site 21	48

In this report, the estimated endpoints, presented in Tables 3.A through 3.F, may have been modified from the primary reports due to adherence to the EPA paradigm for estimation of acute toxicity test endpoints, (Lewis et al., 1994, illustrated in Figure 3.0.1.). Toxicity data, presented in these tables, may have been standardized with regard to reporting format, where applicable.

Figure 3.0.1 EPA Flowchart



The following are specific comments regarding the re-analysis of the CANMET data set.

- The Microtox data was not re-analyzed. The software provided with the testing system provides the results that the user would utilize. Therefore, the results provided by the contract laboratory reflect the results that would be used in making an assessment at their respective site.

- The data from the Toxichromotest was analyzed using the graphical method recommended by the company that distributes the system. The data could be re-analyzed using a more sophisticated method but this may invalidate comparison of "real" test results. The arguments that were used for using post-treatment effluent toxicity results rather than pre-treatment effluent toxicity results hold here. For the effluents, we wanted to compare test results for representative mine effluents that may be subjected to future regulation. Here, we want to compare the results of tests using the recommended analyses which the user/site would utilize. This is the same argument for the Microtox data.

- No control mortality was assumed for the *Daphnia magna* IQ test for CANMET numbers 6,7,9,11 and 12.

Because of the lack of suitable dose responses for the compliance species, rainbow trout and *Daphnia magna* during the first and second sampling periods, additional sites were sampled and sites previously sampled were not included for the third and fourth sampling periods. For example sites 4, 13, 14, and 15 were not included during the third and fourth sampling periods, while sites 18, 19, 20, and 21 were added to the study.

At site 7, Table 3.C, for the third sampling period, the site (Canmet #46) submitted an influent sample to their treatment facility. This is different from all other samples submitted in that all other samples represent effluent discharge samples. The sample did demonstrate a relatively high toxicity to all test species. This sample was not included in the CANMET data set utilized in the final analysis.

The environmental parameter data, is tabulated by assigned site number (Appendix A). For convenience environmental parameter data is summarized in one table per site. For example, site 3 environmental parameter data from the four sampling periods, is summarized in Table A3.

Table 3.A Gold Mine Toxicity Results for each Sampling Period - Results Reported as %V/V

Site #	Sampling Period[*]	Rainbow Trout (96hr LC50)	Daphnia magna (48hr LC50)	Daphnia magna IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24hr LC50)	Thamnotoxkit (24hr LC50)	Toxichromotest[1] (90 min IC50)
8	1(5)	N.L.	N.L.	33.7	>4.9	>100	N.L.	>50
	2(26)	N.L.	N.L.	N.L.	>49.5	N.L.	>100	>50
	3(45)	>100	63.0	6.5 [4]	78.2	8.7	45.3	>50
	4(67)	77.1	44.5	2.1	>99	2.0	20.6	6.25
13	1(7)	>100	N.L.	>100	>49.5 (Q >99)	>100	>100	>50
	2(34)	N.L.	N.L.	>100	>90	>100	>100	>50
	3& 4(-)[2]							
15	1(15)	N.L.	N.L.	>100	>90	>100	>100	>50
	2(29)	N.L.	N.L.	>100	>90	N.L.	>100	>50
	3&4(-)[2]							
19	1,2&3(-)[2]							
	4a(50)[3]	43.5	13.3	0.2	54.7	6.4	17.2	6.25
	4b(53)[3]	43.5	15.0	0.5 [4]	60.5	5.5	18.3	11
21	1&2(-)[2]							
	3(48)	N.L.	>100	15.9	>99	>100	>100	>50
	4(-)[2]							

N.L. = Non Lethal
N.T. = Not Tested
N.E. = No Effect
1 - Results at 595 nm.
2 - Indicates no sample submitted for sampling period
3 - Two samples submitted for sampling period
4 - Confidence limits unavailable

Site # - Assigned by Pollutech
Q - Quality Control Sample Result
[*] The number in parentheses is the Canmet sample number for that sampling period

Shaded Cell = Data point Not Considered in the Final Analysis

Table 3.B Bitumen, Tin, Uranium and Zinc Mine Toxicity Results for each Sampling Period - Results Reported as %V/V

Mine Type	Sampling Period[*]	Rainbow Trout (96hr LC50)	Daphnia magna (48hr LC50)	Daphnia magna IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24hr LC50)	Thamnotoxkit (24hr LC50)	Toxichromotest[1] (90 min IC50)
Bit	1(9)	35.4	N.L.	34.1	45.6	>100	>100	>50
	2(19)	40.6	N.L.	19.6	41.5	>100	>100	>50
	3(35)	43.5	>100	8.4	47.4	>100	>100	21
	4(61)	35.4	N.L.	22.4	47.6	>100	>100	>50
Tin	1(10)	>100	>100	49.0	>49.5 (Q >99)	>100	>100	>50
	2(30)	>100	N.L.	36.1	>90	>100	>100	>50
	3(41)	>100	N.L.	72.2[3]	>99	> 100	>100	2.9
	4(63)	>100	N.L.	>100	>99	>100	>100	>50
U	1 (11)	N.L.	>100	>100	>49.5	>100	>100	>50
	2(18)	N.L.	>100	>100	>90	N.L.	>100	>50
	3,4(-)[2]							
U	1,2(-)[2]							
	3(39)	N.L.	N.L.	75.5	>99	>100	N.L.	>50
	4(60)	N.L.	>100	96.7	>99	>100	N.L.	>50
Zn	1(3)	N.L.	N.L.	87.8	>4.9	>100	59.3	>50
	2(28)	N.L.	N.L	>100	>90	>100	56.6	14.8
	3(42)	N.L.	N.L.	>100	>99	>100	>100	>50
	4(64)	N.L.	N.L.	>100	>99	>100	>100	>50

N.L. = Non Lethal
Site # - Assigned by Pollutech
1 - Results at 595 nm.
2 - Indicates no sample submitted for sampling period

[*] The number in parentheses is the Canmet sample number for that sampling period
Q - Quality Control Laboratory Sample Result

**3 -	Confidence limits unavailable**
Shaded Cell = Data point Not Considered in the Final Analysis

Table 3.C Copper/Zinc Mine Toxicity Results for each Sampling Period - Results Reported as %V/V

Site #	Sampling Period[*]	Rainbow Trout (96hr LC50)	Daphnia magna (48hr LC50)	Daphnia magna IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24hr LC50)	Thamnotoxkit (24hr LC50)	Toxichromotest[1] (90 min IC50)
5	1(13)	89.1	73.0	78.5	>49.5	>100	>100	>50
	2(22)	N.L.	>100	>100	>49.5	N.L.	82.0	>50
	3(37)	70.7	>100	>100	49.59	> 100	60.2	>50
	4(56)	89.1	>100	37.5	>99	N.T.	58.2	>50
7	1(17)	N.L.	>100	>100	>90	>100	>100	>50
	2(20)	N.L.	70.7	10.8	>90	>100	>100	>50
	3(46) [3]	1.1	0.4	0.1	0.44	<0.03	0.5	1.8
	4(58)	70.7	70.7	70.7	>99	N.T.	>100	>50
12	1(12)	N.L.	N.L.	89.9	>90	>100	42.8	23.71
	2(21)	N.L.	>100	>100	>90	>100	49.8	>50
	3(47)	N.L.	>100	70.7	>99	>100	35.0	9
	4(69)	>100	N.L.	>100	>99	70.7	70.7	5.8
17	1(-)[2]							
	2(14)	53.6	75.8	9.9	>49.5	>100	41.0	>50
	3(23)	43.5	>100	18.3	>99	>100	60.2	>50
	4(55)	53.6	N.L.	72.4	>99	N.T.	36.0	>50

N.L. = Non Lethal
N.T. = Not Tested
[*] The number in parentheses is the Canmet sample number for that sampling period
Site # - Assigned by Pollutech
1 - Results at 595 nm.
2 - Indicates no sample submitted for sampling period

3 - Sample represents effluent before treatment
Shaded Cell = Data point Not Considered in the Final Analysis

Table 3.D Nickel/Copper Mine Toxicity Results for each Sampling Period - Results Reported as %V/V

Site #	Sampling Period[*]	Rainbow Trout (96hr LC50)	Daphnia magna (48hr LC50)	Daphnia magna IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24hr LC50)	Thamnotoxkit (24hr LC50)	Toxichromotest[1] (90 min IC50)
3	1(2)	70.7	N.L.	72.3	>49.5	N.L	>100	>50
	2(27)	70.7	N.L.	31.3	>49.5	N.L.	>100	>50
	3(36)	70.7	70.7	8.0	>99	N.L.	>100	>50
	4(57)	70.7	N.L.	17.2	>99	N.T.	>100	>50
4	1(6)	82.0	N.L.	>100	>49.5 (Q 91.8)	>100	>100	>50
	2(33)	N.L.	N.L.	40.0	>90	>100	>100	>50
	3 & 4(-)[2]							
10	1 (1)	N.L.	N.L.	34.1	>4.9	N.L.	>100	>50
	2(31)	N.L.	N.L.	N.E.	>90	>100	>100	>50
	3(38)	N.L.	N.L.	>100	>99	N.L.	>100	>50
	4(59)	N.L.	N.L.	>100	>99	N.T.	>100	>50
18	1&2(-)[2]							
	3(54)	70.7	70.7	51.7	22.2	70.7[3]	>100	>50
	4(68)	>100	73.0	14.0	62.3	62.6	>100	>50

N.L. = Non Lethal
N.T. = Not Tested
N.E. = No Effect
[*] The number in parentheses is the Canmet sample number for that sampling period
Site # - Assigned by Pollutech
Q - Quality Control Laboratory Sample Result
1 - Results at 595 nm.
2 - Indicates no sample submitted for sampling period
3 - Confidence intervals unreliable
Shaded Cell = Data point Not Considered in the Final Analysis

Table 3.E Lead/Zinc Mine Toxicity Results for each Sampling Period - Results Reported as %V/V

Site #	Sampling Period[*]	Rainbow Trout (96hr LC50)	Daphnia magna (48hr LC50)	Daphnia magna IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24hr LC50)	Thamnotoxkit (24hr LC50)	Toxichromotest[1] (90 min IC50)
2	1(8)	70.7	>100	29.8	38.18	>100	48.0	>50
	2(32)	70.7	70.7	63.0	>90	N.L.	46.8	>50
	3(-)[2]							
	4a(40)[3]	N.L.	>100	N.E.	>99	>100	62.4	>50
	4b(62)[3]	N.L.	>100	>100	>99	>100	91.4	22
9	1(4)	N.L.	N.L.	37.2	>4.9	>100	49.3	>50
	2(24)	N.L.	>100	>100	>49.5	N.L.	8.5	>50
	3(43)	>100	70.7	29.8	>99	78.3	42.0	>50
	4(65)	73.5	73.0	>100	N.T.	N.T.	N.T.	N.T.
16	1(16)	N.L.	39.7	>100	>90	>100	9.4	>50
	2(25)	N.L.	70.7	61.0	>49.5	>100	11.2	>50
	3(-)[2]							
	4(44)	N.L.	70.7	>100	>99	N.L.	>100	>50

N.L. = Non Lethal
N.T. = Not Tested
N.E. = No Effect
[*] The number in parentheses is the Canmet sample number for that sampling period
Site # - Assigned by Pollutech
1 - Results at 595 nm.
2 - Indicates no sample submitted for sampling period
3 - Two samples submitted for sampling period
Shaded Cell = Data point Not Considered in the Final Analysis

Table 3.F Main and QA Laboratory Toxicity Results - Results Reported as %V/V

Mine Type	Sampling Period*	Rainbow Trout (96hr LC50)	Daphnia magna (48hr LC50)	Daphnia magna IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24hr LC50)	Thamnotoxkit (24hr LC50)	Toxichromotest[1] (90 min IC50)
Pb/Zn	1(8)	70.7	>100	29.8	38.18	>100	48.0	>50
	1Q(8)	80.6	73.7[2,3]	9.6	>45; 41.9	>100	61.3	>50
Ni/Cu	4(57)	70.7	N.L.	17.2	>99	N.T.	>100	>50
	4Q(57)	69.2	>100	11.2	61.8	N.L.	>100	>50
Ni/Cu	1(6)	82.0	N.L.	>100	>49.5	>100	>100	>50
	1 Q (6)	>100	>100	41.0	>45; 91.8	>100	>100	>50
Cu/Zn	4(56)	89.1	>100	37.5	>99	N.T.	58.2	>50
	4Q(56)	>100	>100	53.2	69	N.L.	69.3	>50
Tin	1(10)	>100	>100	49.0	>49.5	>100	>100	>50
	1Q(10)	N.L.	76.6[5]	63.5	>45;>99	>100	88.4	>50
Cu/Zn	4(58)	70.7	70.7	70.7	>99	N.T.	>100	>50
	4(58Q)	80.6	77.5	16.8	41.6	N.L.	>100	>50
Au	1(7)	>100	N.L.	>100	>49.5	>100	>100	>50
	1Q(7)	N.L.	N.L.[2]	>100	>45;>99	>100	>100	>50
Cu/Zn	4(55)	53.6	N.L.	72.4	>99	N.T.	36.0	>50
	4(55Q)	64.1	77.5[4]	65.8	>99	N.L.	55.8	>50

N.L. = Non Lethal
N.T. = Not Tested
Q - Quality Control Duplicate Sample, results generated by separate laboratory
1 - Results at 595 nm.
2 - Estimated at 52.11 hours due to lack of data at 48 hours
3 - Assume 0 control mortality
4 - 24 hour LC50, 48 hour data not available
5 - Confidence Limits Unavailable

Site # - Assigned by Pollutech
* The number in parentheses is the Canmet sample number for that sampling period

Shaded Cell = Data point Not Considered in the Final Analysis

4.0 Data: Trends and Concerns

This section of the report details the data trends and concerns. Comments have been categorized into general and test specific points. The following points summarize considerations and trends when assessing and reviewing the data sets:

4.1 General Points

1. It should be noted that the dose response data for each of the toxicity tests has been entered into electronic format and the test endpoint for those test results have been recalculated with the exception of the Microtox and Toxichromotest results.

2. Data for the Microtox and Toxichromotest data sets have not been recalculated. The method of analysis followed was that recommended and/or supplied by the distributor. In the instance of the Microtox test, the calculation is completed by the software provided by Microbics Inc. The Toxichromotest's recommended method is a graphical linear interpolation. The estimated endpoint reflects the method which would be utilized by the assessor at the site.

3. The toxicity data was checked for consistency in results reported. For instance in some of the acute lethality type tests where partial mortalities occurred, at any test concentration, the contract laboratory reported the results as "non-lethal", if mortality was insufficient to provide a dose response relationship. The other contract laboratory reported the results as >100% volume. For consistency the reported results were adjusted to >100% mortality if partial mortalities occurred at any test concentration. In some instances a dose response was noted in the raw data, for the highest test concentrations, but was insufficient to calculate an LC50.

4. Toxicity results for the first two sampling periods on whole were lacklustre with regard to response. For instance, with the Rainbow Trout toxicity results, only 6 out of 16 samples during the first sampling period and 4 out of 17 during the second sampling period demonstrated sufficient dose response to calculate an LC50. Results were somewhat similar for the *Daphnia magna* acute lethality toxicity results. The lack of response makes it difficult to compare the microtest results to the compliance test species, rainbow trout.

5. After the second sampling period, action was taken to select additional mine sites/samples that might provide a dose response for the rainbow trout test. In doing so, sites or sampling points previously sampled which provided no responses for the compliance species and a majority of the microtests would be deleted from further testing.

6. Microtox sample results reported as >4.9% volume and 49% volume have not been included in the Canmet data. For further discussion see section 4.5, point 1.

7. Table 4.1.1 summarizes toxicity result frequencies into three specific categories:

   Category 1 - An Endpoint Effect Can Be Calculated (ie. LC50, EC50, IC50).
   Category 2 - An Effect was Observed but was Insufficient to Calculate an Endpoint.
   Category 3 - No Effect was Observed (ie. no mortality).

8. Table 4.1.1 also compares the microtest results with the rainbow trout acute lethality bioassay. Three specific comparisons are provided and are summarized as follows:

Comparison 1 -	Rainbow trout test was positive and microtest was positive, meaning an endpoint could be estimated for both test results.
Comparison 2 -	Rainbow trout test was **not** positive and the microtest was positive
Comparison 3 -	Rainbow trout test was positive and microtest was **not** positive
Comparison 4 -	Both tests were **not** positive.

9. Table 4.1.2 provides a similar summary and comparison for the *Daphnia magna* acute and *Daphnia magna* IQ bioassays.

Table 4.1.1 Summary of Endpoint Results and Summary of Comparison with Rainbow Trout Toxicity Test

Toxicity Test	# of Samples	Summary of Endpoint Results (# Samples)			Comparison With Rainbow Trout Acute Test (# Samples) [2]			
		Endpoint Calculated (ie <100%)	Effect Noted Cannot Calculate Endpoint (ie. >100%)	No Effect	Trout Positive & Microtest Positive	Trout Not Positive & Microtest Positive	Trout Positive & Microtest Not Positive	Trout Not Positive & Microtest Not Positive
Rainbow Trout Acute	64	23	9	32	NA	NA	NA	NA
Daphnia magna Acute	64	17	17	30	10	7	13	34
Daphnia magna IQ	64	38	23	3	20	18	3	23
Microtox[1]	50	12	38	0	10	2	8	30
Rotoxkit	57	9	38	11	5	4	14	34
Thamnotoxkit	63	25	35	3	10	15	12	26
Toxichromotest	63	10	53	NA	4	6	17	36

1 - Where applicable results include the quality control laboratory result if data from the primary laboratory was not available for a particular sample.
2 - Positive result defined as a toxicity result in which an endpoint can be calculated.
NA - Not Applicable

Table 4.1.2 Comparison of the *Daphnia magna* Acute Bioassay with the *Daphnia magna* IQ Toxicity Test

Toxicity Test	Number Samples	Summary of Endpoint Results (# Samples)			IQ Toxicity Test Comparison With *Daphnia magna* Acute Test (# Samples)		
		Endpoint Calculated (ie <100%)	Effect Noted Cannot Calculate Endpoint (ie. >100%)	No Effect	Daphnid Acute Positive & Daphnid IQ Positive	Daphnid Acute Not Positive and Daphnid IQ Positive	Daphnid Acute Positive and Daphnid IQ Not Positive
Daphnia magna Acute	64	17	17	30	NA	NA	NA

Daphnia magna IQ	64	38	23	3	14	24	3

NA- Not applicable

4.2 Rainbow Trout Acute Toxicity Results

The following summarizes specific points regarding the rainbow trout acute lethality toxicity test results.

1. Of the 64 samples tested, 23 (36%) samples provided results from which an LC50 could be calculated.

2. Rainbow trout toxicity tests detected toxicity for all mine types except tin, uranium, and zinc.

3. Of the 21 mine sites evaluated, only 11 sites provided effluent samples which caused toxicity to rainbow trout.

4. There were 41 samples for which no endpoint could be calculated or no effect was noted (ie. >100% or non-lethal) for rainbow trout. Of these 41 samples, 14 samples did not demonstrate any toxicity for the micro toxicity tests and 27 of the samples did show toxicity for one or more of the microtests (this includes the *Daphnia magna* acute bioassay).

5. As indicated in Table 3.F the 8 quality control samples submitted indicate a close comparison in endpoint results between the primary and QC laboratory for the rainbow trout acute lethality bioassays.

6. Only 2 effluent samples tested provided positive responses for all the toxicity micro tests completed (Table 3.A, site 19, Canmet #50 and 53).

4.3 *Daphnia magna* Acute Toxicity Results

The following summarizes specific points regarding the *Daphnia magna* acute lethality toxicity test results.

1. Of the 64 samples tested, 17 (26.6%) samples provided results from which an LC50 could be calculated.

2. The *Daphnia magna* acute test detected toxicity in four mine types; copper/zinc, gold, lead/zinc and nickel/zinc.

3. Of the 21 mine sites evaluated, only 10 sites provided an effluent sample which caused toxicity to *Daphnia magna*.

4. Seven, or 11%, of the samples which produced an acute lethality endpoint for *Daphnia magna* did not cause acute lethality to rainbow trout. On the other hand 10, or 59%, of the positive Daphnid samples also provided an endpoint response for the rainbow trout toxicity test.

5. Nine samples which produced an acute lethality endpoint for rainbow trout did not cause an acute lethality response for *Daphnia magna*.

6. As a result of the lack of a toxicity response for the rainbow trout and *Daphnia magna* toxicity tests, it was suggested in the first interim report that it may be prudent to include in the assessment the associated immobility data from the *Daphnia magna* acute lethality tests. These results may prove to be a more sensitive indicator for response and provide an additional comparison to the micro toxicity test results. Table 4.3.1 summarizes the *Daphnia magna* acute lethality and immobility results provided by the contract laboratory for the third and fourth sampling period. Upon review of the data set it became quite obvious that the immobility data did not provide any increase in response or sensitivity. Of the 32 samples where immobility data was requested (3rd and 4th sampling periods) only four samples (Canmet # 40, 48, 60, 69) demonstrated an immobility response that appeared to be sufficiently different from the lethality response. All four of these samples provided an immobility response when no acute lethality response occurred. From this somewhat lacklustre response, further pursuit of this option would in all probability not yield any additional value to the assessment program.

Table 4.3.1 - Continued

Table 4.3.1 Summary of the *Daphnia magna* Lethality and Immobility data for the Third and Fourth Sampling Period (Modified from BAR's Bioassay Reports)

Site #	Canmet Number	*Daphnia magna*		Remarks
		48 hour LC_{50} (% v/v)	48 hour EC_{50} (% v/v)	
1	35	>100	>100[2]	No atypical signs of stress observed in surviving organisms.
	61	Non-lethal	No immobility	NP
2	40	>100	87.3	NP
	62	>100	100	NP
3	36	70.7	70.7[1]	No atypical signs of stress observed in surviving organisms
	57	Non-lethal	No immobility	NP
5	37	> 100	>100	Three immobile organisms observed at 100% effluent concentration.
	56	>100	>100	NP
6	41	Non-lethal	No Immobility	No atypical signs of stress observed in surviving organisms
	63	Non-lethal	No Immobility	NP
7	46	0.4	0.13	In addition to mortalities, nine organisms exposed to 0.36% effluent and seven organisms exposed to 0.18% effluent concentration were immobile at the end of the test.
	58	70.7	70.7	NP
8	45	63.0	63.0[1]	No atypical signs of stress observed in surviving organisms
	67	44.5	43.6	NP
9	43	70.7	70.7[1]	No atypical signs of stress observed in surviving organisms
	65	73.0	73.0	NP
10	38	Non-lethal	> 100[2]	Comment from contract lab indicates no atypical signs of stress observed in surviving organisms. This contradicts what was provided in their summary table of results.
	59	Non-lethal	No Immobility	NP

Table 4.3.1 - Continued

Site #	**Canmet Number**	***Daphnia magna***		**Remarks**
		48 hour LC_{50} (% v/v)	**48 hour EC_{50} (% v/v)**	
11	42	Non-lethal	> 100	Two immobile organisms observed at 100% effluent concentration. EC50 > 100%.
	64	Non-lethal	No Immobility	NP
12	47	> 100	> 100[1]	No atypical signs of stress observed in surviving organisms
	69	Non-lethal	70.7	NP
16	44	70.7	70.7	NP
17	23	> 100	> 100	Two immobile organisms observed at 100% effluent concentration.
	55	Non-lethal	No immobility	NP
18	54	70.7	70.7[1]	No atypical signs of stress observed in surviving organisms
	68	73.0	73.0	NP
19	50	13.3	13.3	NP
	53	15.0	8.8	NP
20	39	Non-lethal	No immobility	No atypical signs of stress observed in surviving organisms
	60	Non-lethal	70.0	NP
21	48	> 100	66.3	Ten immobile organisms observed at 100% effluent concentration.

1 - It is assumed that the EC_{50} value is the same as the LC_{50} as no additional Daphnid immobility was noted in the contract laboratory's notes.
2 - The value reported in the contract laboratory's summary table contradicts the remarks and/or raw data.
NP - Not Provided

4.4 *Daphnia magna* IQ Test

The following summarizes specific points regarding the *Daphnia magna* IQ toxicity test results.

1. Of the 64 samples tested, 38 (59.4%) samples provided results from which an EC_{50} could be calculated.

2. The *Daphnia magna* IQ toxicity test was positive for all mine types.

3. Of the 21 mine sites evaluated 18 sites provided effluent samples which caused a positive response to the *Daphnia magna* IQ toxicity test.

4. Eighteen, or 28.1%, of the samples which produced an acute lethality endpoint for *Daphnia magna* IQ test did not cause acute lethality to rainbow trout. On the other hand, 20, or 31.3%, of the positive Daphnid IQ samples provided an endpoint response for the rainbow trout toxicity test. Three samples which produced an acute lethality endpoint for rainbow trout did not cause a response for the *Daphnia magna* IQ toxicity test.

5. Comparison of the *Daphnia magna* IQ toxicity test to the *Daphnia magna* acute lethality bioassay indicates that 14 samples were positive for both tests, while 24 samples were positive to the *Daphnia magna* IQ test and not the acute lethality test. Only 3 samples showed positive results to the acute lethality test and not the IQ procedure.

6. Of the 8 samples submitted to the QC laboratory, 7 sets compare by providing positive results with somewhat similar sensitivities (Table 3.F). Only 1 sample of the 8 showed dissimilar results (>100% and 41% v/v).

4.5 Microtox Toxicity Test

The following summarizes specific points regarding the Microtox toxicity test results.

1. For the Microtox toxicity test it is difficult to test an actual 100% volume effluent sample because of the various solutions (ie. osmotic adjustment) added to the sample volume to complete the toxicity test. During the first and second sampling periods for a number of tests the contract laboratory started with the highest test concentrations of 49.5, 45 or 4.9% volume. Results were reported, for a non-toxic response, as greater than the highest concentration tested. The contract laboratory did not initiate testing of a higher dilution range in which a more accurate assessment regarding a toxicity response could be generated. Therefore, it is quite likely that the results reported as greater than for the above listed concentrations will not be useful at all. During the first sampling period the quality control laboratory did retest at a higher sample concentration once the previously tested lower concentration series were found to be non-toxic. For the third sampling period the primary

laboratory was requested to provide Microtox results for the highest test concentration possible. For samples relatively non-toxic a dilution series starting at 99% volume was to be utilized for determination of the 15 minute $IC_{50.}$ This would provide consistency in results, and fully determine if a sample tested by this bioassay procedure would elicit a response, allowing comparison with other results as well as other micro type test procedures. Therefore, for the third and fourth sampling period, samples which were determined to be non-toxic by the Microtox procedure were consistently tested with regard to dilution series and reported as >99% concentration.

2. Of the 64 samples tested, 47 of the primary laboratory sample results are useable. If we include results from the QC laboratory an additional 3 sample results can be included in the Canmet data set. This provides 50 useable Microtox sample results. Review of Tables 1A through 1E will show results preceded by a "Q" this would be an example of the QC laboratory data being substituted for the primary laboratory data (ie. Table 3.A, Site 13, 1st sampling period).

3. The Microtox test provided results for all mine types except tin, uranium, and zinc. This was similar to the Rainbow trout toxicity test.

4. Of the 21 mine sites evaluated, only 6 sites provided effluent samples which caused a positive response in the Microtox toxicity test (ie. <90 or <99% v/v).

5. Of the 23 sample results which were positive for rainbow trout, 10 samples were also positive for the Microtox test.

6. Of the 8 split samples submitted to the QC laboratory only 5 pairs of data can be used for comparison (Table 3.F). Of these 5 samples, 3 samples provide a positive response in the QC laboratory and not in the primary laboratory (ie. <99%). Two sets of samples appear to compare, one set with a positive response and one set with no response.

4.6 Rotoxkit Toxicity Test

The following summarizes specific points regarding the Rotoxkit toxicity test results.

1. For the first and second sampling periods no samples provided a response from which an LC50 could be calculated. Eight samples from the 3rd and 4th sampling period provided a sufficient response from which an LC_{50} could be calculated.

2. For the third sampling period, toxicity from the site 7 sample (which was an influent sample to the site's wastewater treatment facility), was less than the lowest test concentration tested being 0.031% volume. To provide additional value to this study it would have been prudent to retest the sample utilizing a lower dilution series in order to derive an LC_{50} value. It

should be noted that in this instance the Rotoxkit procedure did provide a toxic response somewhat comparable to all the other procedures utilized. As this was an influent sample toxicity results have not been included in the final Canmet data set.

3. During the 4th sampling period, 6 samples were not tested using the Rotoxkit. At the request of the scientific authority for the project, these 6 samples were submitted for testing by another micro type test kit. Results of this additional testing are not to be included in the final Canmet data set.

4. As a result of this diversion of samples, a total of 58 sample results are included in the Canmet data set. Of these 58 samples only 9 samples provided positive results. Of these 9 samples, 5 samples provided results when the rainbow trout test was positive and 4 samples provided results when no endpoint could be calculated for the rainbow trout test. Fourteen Rotoxkit tests provided no positive results when the rainbow trout test provided a positive response.

5. A total of 8 samples were submitted to the QC laboratory for testing. Of these 8 samples, 4 samples from the primary laboratory had been diverted for preliminary testing using another micro toxicity test procedure. This provides 4 sets of sample results for comparison purposes. Of these 4 sample sets no results provided a calculable endpoint result (ie. results were either >100% v/v or non-lethal).

6. Of the 21 mine sites tested only 4 sites provided positive results for the samples submitted.

4.7 Thamnotoxkit Toxicity Results

The following summarizes specific points regarding the Thamnotoxkit toxicity test results.

1. Of the 64 samples submitted 63 sample results are included in the Canmet data set. One sample (Table 3.E, site 9, Canmet #65) was not tested.

2. Of the 63 samples, 25 samples provided results from which an endpoint could be estimated. Of these 25 samples, 10 samples provided positive results when the trout test was positive, while 15 samples were positive when the rainbow trout was not positive. Twelve samples which tested positive for the rainbow trout test did not prove positive for the Thamnotoxkit test.

3. The Thamnotoxkit provided a toxicity response for only four mine types: copper/zinc, gold, lead/zinc and zinc.

4. Of the 8 samples submitted to the QC laboratory results indicated a good comparison (Table 3F). For example when toxicity results are positive from the primary laboratory the QC laboratory also provided positive results with the exception of Canmet #10 where the primary

laboratory results are >100% v/v and the QC laboratory provided an endpoint result of 88.4% v/v.

5. As with the rainbow trout, *Daphnia magna* and Rotoxkit for consistency in reporting, if partial mortalities occurred at any test concentration but were insufficient to calculate an LC_{50} the results were reported as >100% volume.

4.8 Toxichromotest Toxicity Results

The following summarizes specific points regarding the Toxichromotest toxicity test results.

1. The reports provided by the contract laboratory for the first two sampling periods indicate results of >100% volume or non-lethality. Due to the nature of the toxicity test, the highest concentration that can be tested is 50% volume. As such, all results which were reported as >100% volume or non-lethal were corrected to >50% volume. For the two tests in which a LC_{50} could be calculated the reported value from the contract laboratory was corrected by dividing the value by two.

2. Of the 64 samples submitted, 63 sample results are included in the Canmet data set. One sample (Table 3.E, site 9, Canmet #65) was not tested.

3. Of the 63 samples, 10 samples provided results from which an endpoint effect (ie. <50% v/v) could be calculated. Of these 10 samples, only 4 samples provided positive results when the trout test was positive, while 6 samples were positive when the rainbow trout was not positive. Seventeen samples which tested positive for the rainbow trout test did not prove positive for the Toxichromotest.

4. Of the 8 samples submitted to the QC laboratory no tests provided a sufficient dose response relationship from which an endpoint could be determined for both the primary and QC laboratory (Table 3.F).

5. The Toxichromotest appears to be quite insensitive to this application due to the general lack of response to the mining effluent and lack of comparability with the rainbow trout bioassay.

6. Toxicity results were generated for all mine types except nickel/zinc and uranium; however there was a low percentage of responses.

4.9 Sensitivity Assessment

Using a method discussed by Munkittrick et al., (1991) in a review of the Microtox compared to the rainbow trout, Daphnia magna and fathead minnow, a sensitivity value has been applied to the CANMET data set. In this case the sensitivity value is calculated by dividing the rainbow trout LC50 by the microtest endpoint value. Results less than 1 would indicate that the rainbow trout test was more sensitive and values greater than 1 indicate that the microtest results were more sensitive. Due to the data generated some assumptions had to be made in order to provide this analysis. These assumptions are summarized as follows:

- For the acute bioassay results reported as >100% volume or non-lethal, an endpoint value of 100 is used in the calculation

- For the Microtox assay results reported as >90 or 99% volume or no effect, an endpoint value of 100 is used in the calculation.

- For Toxichromotest for endpoint results reported as >50% volume an endpoint value of 100 is used in the calculation. This approach may give the perception of the assay being more sensitive. Another approach is to assign a value of 50 to the endpoint value for the calculation but this approach may give the impression of the results being either equal or less sensitive than the rainbow trout test. Some weight has to be given to these results given the fact that the Toxichromotest result provided very few positive responses and utilizes 50% volume as the highest test concentration.

- Results are provided by mine type by calculating the sensitivity value for each toxicity test, adding the sum for each mine type and then dividing by the number of samples tested by that mine type (ie. Gold 11 samples). For the overall score, each toxicity test is summed and then divided by the number of samples tested for that assay type (ie. Microtox 50 samples).

Table 4.9.1 summarizes these sensitivity values by mine type and overall. From this calculation the assays for sensitivity would be ranked, most sensitive to less sensitive, as follows:

Daphnia magna IQ (7.8)
Toxichromotest (This may be biased sensitive based upon method of calculation) (2.43)
Rotoxkit F (2.06)
Thamnotoxkit F (1.67)
Daphnia magna Acute (1.05)
Microtox (1.02)

By this approach the Daphnia magna IQ ranks as the most sensitive toxicity test compared to the rainbow trout acute toxicity test while both the *Daphnia magna* and Microtox show a similar sensitivity to each other. These sensitivity results are supported by the concordance values, presented in Table 4.10.1, of 68.7% and 80% for the *Daphnia magna* acute and Microtox assays, respectively. It should be noted that the similar sensitivity (1.02) and high concordance (80%) of the Microtox to the rainbow trout is more a result of so many effluents being non-toxic to both the rainbow trout (41/64 samples) and Microtox (38/50 samples) most of the time. From Table 4.9.1 both the *Daphnia magna* IQ (33.7) and Rotoxkit F (6.8) would appear to be quite sensitive to specific environmental parameters characteristic of gold mine effluents. If the Rotoxkit result is not included in the data set for the gold sector (Table 4.9.1) the Rotoxkit would be less sensitive than rainbow trout for all other mine sectors. The *Daphnia magna* IQ results show the greatest sensitivity compared to the rainbow trout assay as all sensitivity values, by mine type, are greater than one.

Though the discussions have focussed on an overall sensitivity value for all mine types it is more important that individual operators assess results more specifically to their particular mine type. For instance, the Microtox would appear to be less sensitive than the rainbow trout test for specific mine types. The Microtox was ineffective at detecting toxicity for the copper/zinc mine type 4 out of 5 times in which toxicity was detected by the rainbow trout toxicity test.

Table 4.9.1 Average Sensitivity Analysis by Mine Type and Overall Compared to the Rainbow Trout Acute Toxicity Test[1]

Mine Type	*Daphnia magna* (48 hr LC50)	*Daphnia magna* IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24 hr LC50)	Thamnotoxkit (24 hr LC50)	Toxichromotest (90 min IC50)
Gold	1.5	33.7	0.96	6.8	1.62	0.796
Bitumen	0.39	2.45	0.85	0.39	0.39	1.07
Tin	1	1.8	1	1	1	9.35
Uranium	1	1.08	1	1	1	1
Zinc	1	1.04	1	1	1.36	2.43
Copper/Zinc	0.92	2.13	0.91	0.91	1.4	2.9
Nickel/Copper	0.94	2.82	1.23	0.97	0.86	0.86
Lead/Zinc	1.22	1.52	1.09	0.97	4.25	1.3
Overall	1.05	7.8	1.02	2.06	1.67	2.43

1 - Results less than 1 would indicate that the rainbow trout test was more sensitive and values greater than 1 indicate that the microtest results were more sensitive.

4.10 Concordance Analysis and Predictive Value

Table 4.10.1 summarizes percentage comparison of the microtest toxicity results with the rainbow trout acute lethality test. The percentage concordance of the toxicity test is the proportion of the results, either positive or negative (ie. non-lethal, >100%, >90 or >99% for Microtox, >50% volume Toxi-chromotest), that agrees with the rainbow trout test. Also included in Table 4.10.1 is the predictive value of each toxicity test in comparison to the rainbow trout toxicity results. The predictive value is the proportion of correct results, either positive or negative, among the positive or negative results. The concordance analysis does not take into account the level of response, only that toxicity was detected or not detected.

From the results summarized in Table 4.10.1 the Microtox test has the highest concordance (80%) or the greatest number of results correctly predicted in comparison with the rainbow trout acute lethality toxicity test. As well, the Microtox also provided the lowest number of false positive toxicity results. In this analysis the term Afalse positive@ could be misleading if other microtests found toxicity but the rainbow trout did not then the microtests may be a more sensitive indicator of toxicity. Since the study has focussed on the comparison to the rainbow trout toxicity test the detection of toxicity by a microtest and not the rainbow test is considered a Afalse positive@ for purposes of the concordance analysis.

The concordance values for other tests evaluated would appear to be within the same relative range, 57.2% to 68.7%. The ability of the Microtox test to predict results correctly in comparison to the rainbow trout test is also reflected in the predictive value for positive and negative results.
The corresponding concordance analysis and predictive values for the *Daphnia magna* IQ test in comparison to the *Daphnia magna* acute toxicity test are summarized as follows:

Proportion of Positive Results Predicted	21.9%
False Negative Toxicity Results	4.7%
Proportion of Negative Results Predicted	35.9%
False Positive Toxicity Results	37.5%
Concordance (Proportion of -ve and +ve Results Correctly Predicted)	57.8%

Predictive Value (Proportion of Correct Results, Either +ve or -ve Among the +ve or -ve Results)

Predictive Value (Positive)	37.5%
Predictive Value (Negative)	35.9%

Table 4.10.1 Summary of Percentage Comparison with Rainbow Trout Toxicity Tests

Comparison Approach	***Daphnia magna*** **(48 hr LC50)**	***Daphnia magna*** **IQ (75 min EC50)**	**Microtox (15 min LC50)**	**Rotoxkit (24 hr LC50)**	**Thamnotoxkit (24 hr LC50)**	**Toxichromotest[1] (90 min IC50)**
Proportion of +ve Predicted	15.6%	31.3%	20%	8.8%	15.8%	6.3%
False Negative	20.3%	4.7%	16%	24.6%	19.0%	27%
Proportion of -ve Predicted	53.1%	35.9%	60%	59.6%	41.3%	57.1%
False Positives	10.9%	28.1%	4%	7.0%	23.8%	9.5%
Concordance (Proportion of Results Correctly Predicted)	68.7%	67.2%	80%	68.4%	57.1%	63.4%
Predictive Value (Proportion of Correct Results (either positive or negative) Among the Positive or Negative Results)						
Predictive Value (+ve)	58%	52.6%	83.3%	55.5%	40%	40%
Predictive Value (-ve)	72.3%	88.5%	79%	69.4%	68.4%	67.9%

4.11 Environmental Parameter Data

The following points apply specifically to the environmental parameter data.

1. For site 19, Canmet number 53 for the fourth sampling period, no analytical data will be available as the samples were not received by the contract laboratory in sufficient time.

2. For the QC samples the laboratory provided additional parameters which the routine contract laboratory did not provide. These additional parameters have been provided in the summary tables of this interim report.

3. For some parameters, in a small number of cases, the laboratory minimum detection limits varied from sampling period to sampling period. In some cases the laboratory provided less than values for an environmental parameter which were lower than the reported minimum detection limits on the certificate of analysis received from the contract laboratory.

4. From discussions with the Canmet advisory committee environmental parameter data for inclusion in the statistical analysis will not include total metal concentrations. This would reduce the number of parameters to be handled for this analysis.

5. The same set of analytical parameters was not always measured for every mine sample tested. This did provide some difficulty in the statistical analysis comparing toxicity results to environmental parameter data. This is discussed more in section 5.3.

6. When the detection limit was reported (ie.<10µg/L) the value (ie. 10µg/L) was utilized in the statistical analysis where it was necessary.

5.0 Evaluation of the Toxicity Tests

5.1 Cost Evaluations

To conduct the cost evaluations specific criteria must be defined. It should be noted that there is more than one approach by which a cost evaluation could be conducted. The costs are provided as a guide by which each specific site can determine costs. Upon review of the BAR and Beak reports the following criteria were developed:

5.1.1 Capital Cost

This item involves the cost of capital items necessary for the direct completion of the bioassay. This item does not include such items commonly found within a toxicity testing laboratory (ie. pH, conductivity, dissolved oxygen meters, etc.) Amortization of capital costs over a defined time period is a typical method by which private testing facilities consider capital acquisitions. For this study it was proposed to amortize capital costs for 1 or 5 years. It is suggested that capital costs that total less than $1000 only be amortized for the one year. For application of the 1 and 5 year amortization periods an annual interest rate of 10% is applied. Capital costs are detailed in Table 5.1.1 and are used in the summary provided in Table 5.1.4.

It should be noted that there are a number of ways by which capital costs can be handled by an individual mine site. Things such as readily available cash, credit standing, interest rates, depreciation rate and negotiated deal with suppliers influence the actual capital costs that would apply. For instance in the case of the Microtox system the negotiated deal may include a cash deposit and term (ie. 24 month) lease at a defined interest rate (ie. 0% to 10%). A lease buy out may then apply at the end of the lease term.

5.1.2 Disposable Costs

The disposable costs are for those materials utilized in conducting the toxicity test. In the case of the various kit tests this would also include the cost of the kit. Values provided from each of the testing laboratories will be utilized. Disposable material costs have been detailed in Table 5.1.1 and are used in the summary indicated in Table 5.1.4. It has been assumed that 10% QA/QC would apply, meaning if 100 environment samples were tested 10 reference toxicants or duplicates or a combination of both would be conducted.

5.1.3 Operator Time Including QA/QC Time Requirements

Each of the testing laboratories have provided estimates of the time spent to complete one toxicity test. The time is provided in specific categories: culturing (where required), log-in, sample preparation, pre-testing culturing, test set-up, checks, test termination, data analysis, QA/QC time and reporting. A detailed breakdown of the procedural tasks is provided in Table 5.1.2.

5.1.1. Cost Estimates for Materials and Equipment Required

BIOASSAY	ITEM	COST ESTIMATES	NUMBER REQUIRED PER TEST	TOTAL MATERIAL COST
RAINBOW TROUT	DISPOSABLE:			$ 5.10
	Plastic Liners	$ 0.45 per bag	6	
	Air Line Tubing	$ 0.15 per foot	approx. 10 ft.	
	Milk Pipettes	$ 0.15 for 1 pipette	6	
	FIXED:			$ 512.60
	Polyethylene Pails	$ 4.60 for 1 pail and 1 lid	6	
	Effluent Mixing Cylinders	$ 320.00 for 2 cylinders	1 set of 2 cylinders	
	Submersible Pump	$ 115.00 for 1 pump	1	
	Effluent Mixing Tubs	$ 50 for 1 tub	1	
DAPHNIA MAGNA	DISPOSABLE:			
	Pasteur Pipettes	$ 0.10 for 1 pipette	3	$ 00.30
	FIXED:			$ 643.60
	Test Tubes	$ 1.40 per tube	24	
	Test Tube Rack	$ 4.00 per rack	1	
	Graduated Cylinders	$ 83.00 for 3 cylinders	1 set of 3 cylinders	
	Erelenmeyer Flask	$ 23.00 for 1 flask	1	
	Microscope	$ 500.00		
DAPHNIA MAGNA IQ	DISPOSABLE:			$ 70.20
	Refill Kits	$ 70.00 for 1 refill kit	1	
	Pasteur Pipettes	$ 0.10 for 1 pipette	2	
	FIXED:			$ 862.00
	U.V. Light	$ 130.00 for 1 light	1	
	Safety Glasses	$ 20.00 for 1 pair	1	
	IQ Kit	$ 189.00 for 1 full kit	1	
	Erelenmeyer Flask	$ 23.00 for 1 flask	1	
	Microscope	$ 500.00	1	
MICROTOX	DISPOSABLE:			$ 35.00
	Reagents and Test Tubes			
	FIXED			> $ 20,000
	Luminometer	> $ 20,000	1	
	microcomputer		1	
ROTOXKIT	DISPOSABLE:	$ 45.00 per kit	1	$ 45.00
	FIXED	$500 Microscope		$500
		$1000 Incubator (optional)		(possibly $1500)
THAMNOTOXKIT	DISPOSABLE:	$ 45.00 per kit	1	$ 45.00
	FIXED	$500 Microscope		$500
		$1000 Incubator (optional)		(possibly $1500)
TOXICHROMOTEST	DISPOSABLE:	$ 38.00 per kit	1	$ 38.00
	FIXED:	$1000 Incubator		$ 1500
		$500 Multiple Pipette System		

Table 5.1.2 - Continued

Table 5.1.2. Toxicity Test Procedural Breakdown

TEST	TEST BREAKDOWN								
	Culture	Log-in	Sample Prep.	Pre-test Culturing	Test Set Up	Test Termination	Data Analysis	QA/QC	Reporting
Rainbow trout	Includes: -cleaning, maintenance -fish weights -feeding -maintenance of larvae fishes	Includes: -log-in of sample	Includes: -composite of sample -temp. adjustment if necessary	Includes: -selection of test fish	Includes: -bench tag/sheet prep. -bucket/airline prep. -dilution preparation -parameters -adding organisms	Includes: -final mortality checks -final parameters -final weights and lengths -pumping out tanks	Includes: -data analysis -benchsheet approval	Includes: -QA/QC reporting	Includes: -toxdata reports -tables and final report
Daphnia magna	Includes: -algae prep. -daily culture changeovers -new culture initiation	Includes: -log-in of sample	Includes: -initial parameters -aeration (if required) -temperature adjustments if necessary -composite of sample	Includes: -selection of daphnid neonates (<24 hours old)	Includes: -bench tag/sheet prep. -glassware prep. and labelling -dilution preparation -parameters -adding organisms	Includes: -final mortality checks -final parameters	Includes: -data analysis -benchsheet approval	Includes: -QA/QC reporting	Includes: -toxdata reports -tables and final reports
Daphnia IQ	Includes: -algae prep. -daily culture changeovers -new culture initiation	Includes: -log-in of sample	Includes: -initial parameters -aeration (if required) -temperature adjustments if necessary -composite of sample	Includes: -maintenance of >24 hour old neonates -two culture changeovers per day	Includes: -dilution preparation -addition of reagents -adding organisms	Includes: -reading of test	Includes: -data analysis -benchsheet approval		Includes: -tables and final reports
Microtox		Includes: -log-in of sample	Includes: -initial parameters	Includes: -re-hydration of bacteria	Includes: -prepare worksheet -prepare and mark test tubes -prepare dilution -incubate	Includes: -count mortality	Includes: -analyze results -worksheet approval		Includes: -table and final report

Table 5.1.2 - Continued

TEST	TEST BREAKDOWN								
	Culture	Log-in	Sample Prep.	Pre-test Culturing	Test Set Up	Test Termination	Data Analysis	QA/QC	Reporting
Rotoxkit F 8		Includes: -log-in of sample	Includes: -initial parameters	Includes: -prepare water Standard Fresh water (SFW) -hydrate cysts and transfer into petri dish -incubate at 25°C for ~19h	Includes: -prepare worksheet -prepare and mark test tubes -prepare dilution and prepare test plaque -add organisms -incubate 24 hours at 25°C	Includes: -count mortality	Includes: -analyze results -worksheet approval		Includes: -table and final report
Thamnotoxkit F8		Includes: -log-in of sample	Includes: -initial parameters	Includes: -prepare water Standard Fresh water (SFW) -hydrate and incubate cysts in a petri dish with SFW -incubate for 20-22h at 25°C -transfer from stage I to petri dish -incubate for 4h at 25°C	Includes: -prepare worksheet -prepare and mark test tubes -prepare dilution and prepare test plaque -add organisms -incubate 24 hours at 25°C	Includes: -count mortality	Includes: -analyze results -worksheet approval		Includes: -table and final report
Toxichromotest		Includes: -log-in of sample	Includes: -initial parameters	Includes: -re-hydration of bacteria	Includes: -prepare worksheet -prepare dilution and prepare test plaque -prepare dilution and test tubes -incubate	Includes: -count mortality	Includes: -analyze results -worksheet approval		Includes: -table and final report

BAR provided an estimate of the necessary QA/QC time for the acute rainbow trout and *Daphnia magna* tests. BEAK did not include a separate QA/QC time though their testing program includes reference toxicant testing. It is assumed for this situation that associated QA/QC time would be similar to the time indicated by BAR. Therefore 15 minutes has been assumed for QA/QC for all tests as well as 5 minutes for additional reporting time. For the IQ test BAR provided the total time to complete the test but did not include the necessary culture time. The culture time for the acute test is assumed to be similar for the IQ test. Utilizing the culture time per week indicated in Table 5.1.3 the additional labour has been included in the labour costs itemized in Table 5.1.4. For labour costing a technician rate of $15/hr is assumed in calculation of the personnel costs. Table 5.1.3 summarizes the time allocation for project personnel, with the associated costs provided in Table 5.1.4.

5.1.4 Considerations

From the review of the reports provided by the contract laboratories, such things as operator training time and maintenance costs were not discussed. It is therefore assumed that each of the toxicity tests would require the same time allocation for training. No allocation for training has been included. Maintenance costs are considered not to apply as most of the micro tests are self contained kits with the exception of the Microtox machine. Discussions with various Microtox operators and other technical support sources indicate very little ongoing maintenance is required. It can then be assumed that costs for training and maintenance will not have any effect on this portion of the assessment.

A general overhead cost figure has not been included in this portion of the assessment since overhead costs (rent, electricity, water, etc.) may vary between testing facilities making it difficult to assign a specific cost.

As established cost criteria have now been defined, capital purchases will take into account 1 and/or 5 year amortization time periods. On an annual basis a sample frequency of 100 samples processed per year would be a reasonable assumption for a typical monitoring program for effluent discharges from a mine site, including any necessary QA/QC testing. The 100 samples represent the minimal number of anticipated samples to be processed per year.

For this evaluation the costs of $350 per toxicity for the acute rainbow trout is utilized as typical commercial laboratory fees. For the *Daphnia magna* acute test costs are based upon information provided by the testing laboratories. It should be noted that commercial costs are approximately $250 per test. The discrepancy between the commercial laboratory cost and that provided in Table 5.1.4 probably reflects the overhead costs associated with the laboratory. Therefore to be useful, the cost comparison on a case by case basis should include a calculation for overhead costs. In this study this has not been done, since such costs would vary between facilities and mine sites. The user of the cost data contained within this report should be conscious of this exclusion but should also give consideration to their specific situation.

Table 5.1.3 Estimated Time Spent (minutes) to Complete One Toxicity Test.

Test	C*	Log-in	Sample Prep.	Pre-test Culturing	Test Set Up	Checks	Test Termination	D**	QA/QC	R***	T **!**
Rainbow Trout	350	5	10	5	41.4	11.5	13.9	5	15	22	128.8
Daphnia magna	215	5	5	5	40.3	4.4	15.8	5	15[1]	22	117.5
Daphnia IQ	215	5	5	16	16.3	-	3.0	5	15[1]	18[2]	83.3
Microtox	-	5	5	15	10	-	20	5	15[1]	15-20[2]	90-95
Rotoxkit F8	-	5	5	10	35 **!**20-30 min dilution and add in organisms **!**5 min worksheet and tube identification	-	10	5	15[1]	15-20[2]	90-105
Thamnotoxkit8	-	5	5	20-25	45 **!**20-30 min dilution and add in organisms **!**5 min worksheet and tube identification	-	10-15	5	15[1]	15-20[2]	100-125
Toxichromotest8	-	5	5	15	30 **!**20-30 min prepare plates **!**(90 min incubation 1) **!**(60 min incubation 2)	-	5	5	15[1]	15-20[2]	85-100 (+ 150 min. incubation)

* C - Culturing Time - based on culturing enough organisms to conduct an estimated 14 tests per week. Culturing time is presented as time spent on a weekly basis per test organism.
** D - Data Analysis
*** R - Reporting
! T - Total time
1 - 15 Minutes added per test for QA/QC
2 - 5 Minutes add per test for reporting QA/QC

Table 5.1.4 Breakdown Summary of Costs on a Per Test Basis and Ranking

Toxicity Test	Amortization Period	Total Capital	Annual Capital Cost	Material Costs for 100 Tests[4]	Labour Costs for 100 Tests[4]	Sample Transport Costs[2,3]	Total Cost per Test	Ranking
Rainbow Trout Acute	NA	NA	NA	NA	NA	$45	$350	4
Daphnia magna Acute	1 Year	$643.60	$707.96	$33	$6026.25	$15.50	$67.67	1
Daphnia magna IQ	1 Year	$862	$948.20	$7,722	$5,086.00	$15.50	$137.56	2
Microtox	1 Year	$20,000	$21,044.04	$3,850	$2,612.50	$15.50	$275.07	3
	5 Year	$20,000	$5,099.28	$3,850	$2,612.50	$15.50	$115.62	2
Rotoxkit	1 Year	$500	$550	$4,950	$2,887.50	$15.50	$83.88	1
Thamnotoxkit	1 Year	$500	$550	$4,950	$3,437.50	$15.50	$89.38	1
Toxichromotest	1 Year	$1,500	$1,578.24	$4,180	$2,750.00	$15.50	$85.08	1
	5 year[1]	$1,500	$378	$4,180	$2,750.00	$15.50	$73.08	1

NA - Not Applicable

1 - For the Toxichromotest amortization of the capital costs over a 5 year period does not significantly alter the cost per bioassay. Therefore, the 5 year time period will not be included in the final cost rankings.

2 - In most instances the Rainbow Trout and *Daphnia magna* tests would be conducted on the same sample. The shipment costs for the Trout are based upon the transport of 3 X 20 L pails and the *Daphnia* test the transport of 1 X 20 L pail.

3 - Transport costs/sample have been provided for information purposes, but are not included in the total cost/test

4 - Assume 10% additional QA/QC testing (ie. Reference toxicant testing)

A sample transport cost has been provided in Table 5.1.4, but is not included in the total cost per test figure. It is assumed that all micro toxicity tests have the potential to be conducted in-house and, therefore, for the micro tests a minimal sample transport cost has not been applied.

5.1.5 Cost Ranking

Once costs for each category, listed above, were tabulated a score of 1 to 5 (lowest to highest cost) was applied based on the following ranking:

Cost per Bioassay	Ranking
less than $100	1
$101 - $200	2
$201 - $300	3
$301 - $400	4
$401 - $500	5

The micro toxicity tests have been ranked according to their respective individual costs and are compared to the acute lethality bioassay commercial laboratory costs per bioassay.

It has been assumed that all other costs (ie. disposable supplies and labour) remain constant over the 5 year amortization period, for determining the 5 year ranking. Therefore, no inflationary cost factor has been provided.

Utilizing the final cost per toxicity test and ranking provided in Table 5.1.4, the associated cost rankings have been included in the overall ranking summary provided in Table 5.7.1 to 5.7.8.

5.2 Speed Evaluation

The speed evaluation was primarily based upon the discussions provided by the contract laboratories. For speed the toxicity tests have been scored on a scale of 1-5. The score will be based upon the number of days for turn around of results. For example, if a sample could be collected and results provided the same day a score of 1 would apply. If 2 days are required, then a score of 2 would apply. For instance, the rainbow trout test if conducted in-house would normally take 5 days to initiate and complete, and therefore a score of 5 would apply. The scoring method does not differentiate between minutes or hours only days for turn around. From our experience, most industrial dischargers who would utilize this data would prefer same day turn around of results. This is particularly true during upset conditions or suspicions of a contaminant release in the discharge.

Table 5.2.1 summarizes the time required to complete each toxicity test. It is assumed that each toxicity test, including the acute toxicity test, are conducted in-house. The time required is only specific to conducting the test and does not include such things as sampling time and/or transport. This table also includes the allocated ranking score, discussed above.

Table 5.2.1 Time to Conduct Toxicity Test and Associated Ranking

Toxicity Test	Time Required	Ranking Score
Rainbow Trout Acute	5 days	5
Daphnia magna Acute	2-3 days	2.5
Daphnia magna IQ	<1 day	1
Microtox	<1 day	1
Rotoxkit F	2-3 days	2.5
Thamnotoxkit F	2-3 days	2.5
Toxichromotest	<1 day	1

The time of turn around of results considers the necessary time to review and compile QA/QC data along with toxicity results into a report format for the end user.

5.3 Correlation of Chemistry to Toxicity Results

To evaluate the various microtests, the test results were statistically compared with specific chemical parameters. For determination of the correlation of environmental parameters to toxicity test results a regression method was utilized. The response of the toxicity test may vary as to the type of environmental parameters which may affect the results. Thus individual toxicity tests, tested on the same effluent, could be affected by different groupings of environmental parameters and at the same time provide a similar response or result. The types of environmental factors and/or groupings may be related to environmental contaminants found within the sample (ie. dissolved metals) or related to specific properties of the sample (DOC, turbidity, alkalinity, pH etc.) which may or may not be related to potential environmental contamination.

The physical chemical data of the Canmet study consists of a large number of ICP scans for total metals, dissolved metals and the usual water quality parameters; pH, conductivity, ammonia, alkalinity, total hardness, total suspended solids, and total dissolved solids. Note that the following discussion does not include total metals which were not considered in any regression analyses. If metals under consideration were below the analytical detection limit, this limit was used as a data point. Methods are available for correcting these censored observations but their use is not likely warranted due to the paucity of data above the detection limit.

By deleting a few problematic variables that were measured sporadically, a data set with measurements at every mine was obtained. The deleted variables are: iron, free cyanide, oil and grease, Sn, Sr, sulphate, Ti, total cyanide, total hardness and Zr. The variables considered in the regression analyses are: Ag, Al, alkalinity, ammonia, As, B, Ba, Be, Bi, Ca, Cd, Co, conductivity, Cr, Cu, Ga, K, Li, Mg, Mn, Mo, Na, Nb, Ni, pH, P, Pb, Sb, Si, total dissolved solids, total suspended solids, V, W, Y, and Zn, for a total of 35.

In a regression analysis we are trying to estimate the coefficients of variables that are linearly related to the response we are interested in. In this case the response is the endpoint (ie. LC50, EC50, IC50). The maximum number of parameters that can be fit is 2 less the total number of observations. The number of times that an EC50 was measurable in this data set was considerably less than the number of variables available for consideration. Thus some data reduction prior to model fitting was required.

The method used to reduce the number of variables is conceptually simple. The n-2 variables with the highest pairwise Pearson product-moment correlation with the EC50 of interest were retained for consideration in the regression equation. Then, a least squares estimation algorithm was used to estimate the model parameters. Mallows Cp statistic was used to determine the best subset of the initial n-2 parameters. The term "**best**" is somewhat ambiguous and is a function of the goals of the analysis. In this case we wish to find the smallest subset of parameters that is capable of describing the observed response.

Table 5.3.1 - Continued

As an example, consider the Rotoxkit data set. There were 8 times when an EC50 was measurable. Thus we can only estimate the coefficients for 6 (=8-2) out of the total of 35 available physical/chemical variables. The 6 were selected by examining the correlation between the EC50 and each of the 35 physical/chemical variables individually. The 6 variables with the highest correlations, were selected as being candidates for the model. Since some of these variables may be highly correlated with one another, further model reduction is required. This is achieved by using Mallows Cp statistic to determine the "best" subset of these 6 retained variables. This approach is Anonparametric@ in the sense that no inferences requiring distributional assumptions are made.

Note that we are attempting to determine the subset of parameters related to the response generated by a particular toxicity test and compare the subsets across tests. The focus of this section is not in creating mechanistic models of toxicity for a given test. Consequently, attention is centered upon the subsets of parameters selected by the regression approach, rather than the regression models themselves.

Table 5.3.1 summarizes results of the non-parametric regression analysis for the environmental parameter data applied against the toxicity results. The shaded cells indicate the parameters retained by the selection procedure as being considered to influence the toxicity test endpoints. Only those parameters which correlate to a toxicity test are included in the table.

Included in Table 5.3.1 are the average environmental parameter concentrations (for those parameters found significant) for the particular toxicity test in which toxicity results provided a positive result (ie. LC50 < 100 % v/v). For example, the average silver concentration is 13.1 ug/L for the 17 rainbow trout toxicity tests in which an LC50 endpoint could be estimated for rainbow trout. The same logic applies to each of the toxicity tests evaluated.

Table 5.3.1 Summary of the Non-parametric Regression Analysis for the Environmental Parameter Data

Parameter	**D. magna Acute**	**D. magna IQ**	**Microtox**	**Rainbow Trout**	**Rotoxkit**	**Thamno-toxkit**	**Toxi-chromotest**
Ag			14.7	13.1			
Al	875.3	465	346.7		1176.6		672.5
Alkalinity (mg/L)						40.0	
Ammonia (mg/L)						4.9	
B	48.3						
Bi		59.7					

Table 5.3.1 - Continued

Parameter	D. magna Acute	D. magna IQ	Microtox	Rainbow Trout	Rotoxkit	Thamno-toxkit	Toxi-chromotest
Ca						376739	
Cd			20.0				33.3
Co	58.4	32.6					43.3
Conductivity (umho/cm)	2291	1911.6			2315		
Cr	13.1			13.3		13.7	13.7
Cu			360	932.1			839.3
K		22221					
Mg				30936		32637	
Mn			387	452.6			
Mo		118.8			288.7		
Na	222048		127306	168041			
Nb	17.4						
pH (Units)		8.4		9.1		11.0	
P		160.6			148.8		
Pb				99.1			
Sb		74.7			103.9		
Si			1482.4			820.6	
TSS (mg/L)						9.8	
V		13.5		15.2			
W	186.1	117.4	91.8			148.5	
Zn						1873.4	1873.4

(Parameters retained by the selection procedure are highlighted and the average concentration for the toxicity tests providing positive results (ie. <100% v/v)) (Metals are dissolved with concentrations in ug/L unless otherwise indicated)

The *Daphnia magna* IQ and Thamnotoxkit results indicate a response to a greater number of environmental parameters, 11 and 10, respectively, than the rainbow trout, which was 9 parameters. There is no one test that has correlated parameters which are completely similar to those that

Table 5.3.1 - Continued

correlated to the rainbow trout toxicity test. The Microtox test has the greatest number of similar parameters when compared to the rainbow trout toxicity test.
The correlation of an environmental parameter does not imply causality for that parameter, particularly for a relationship to an ecological effect. For example, the presence of certain constituents like calcium, magnesium, potassium or sodium may not necessarily be a concern from an ecological viewpoint but may be correlated to toxicity results because of their relationship in certain metabolic functions that relate to the endpoint being measured.

Using a scale from 1 to 5 and the results of the regression analysis, if a toxicity test procedure is highly associated with the same environmental parameters considered contributory to the effects observed for the rainbow trout toxicity test, the toxicity test is given a score of 1. If the toxicity test is more influenced by parameters not directly related to environmental effects the toxicity test is scored a 5. The degree by which the bioassay compares with the environmental parameter data dictates the relative score. The following rank scoring will be used:

Percent Agreement with Rainbow Trout Toxicity Test	Rank Scoring
80 - 100%	1
60 - 79%	2
40 - 59%	3
20 - 39%	4
0 - 19%	5

The approach taken (Table 5.3.2) simply takes the number of parameters of the microtest found to be similar with the rainbow trout toxicity test and divides it by the number of parameters correlated to the rainbow trout toxicity test to give a percentage agreement value with the rainbow trout test. With this approach a microtest which has a lower number of correlated parameters is penalized (lower percentage agreement) while a microtest which has a greater number of correlated parameters benefits by a higher percentage agreement value.

A microtest which is more responsive (greater number of endpoint results) than the rainbow trout toxicity test to mine discharges has the opportunity for a greater number of parameters to be correlated to its toxicity results. In this instance the microtest has the potential for a higher percentage of agreement for environmental parameters with the rainbow trout test. Under the new scoring mechanism utilized the toxicity test is not penalized for being more responsive than the rainbow trout toxicity test. It has been implied by the project's technical advisory committee that a more responsive toxicity test is considered beneficial to the obtainment of the project's goals. As such, the second mechanism of determining the percentage agreement with the rainbow trout toxicity test will be utilized for determining the ranking which is summarized in Table 5.3.2. These scores are utilized in the final rank scoring.

Table 5.3.2 Comparison of Environmental Parameter Results to the Rainbow Trout Test Second Approach

Toxicity Test	Number of Environmental Parameters Correlated to Microtest Results	Number of Environmental Parameters Consistent with the Rainbow Trout Toxicity Test	Ranking Score
Rainbow Trout Acute	9	9 (100%)	1
Daphnia magna Acute	8	2 (22%)	4
Daphnia magna IQ	11	2 (22%)	4
Microtox	8	4 (44%)	3
Rotoxkit	5	0 (0%)	5
Thamnotoxkit	10	3 (33%)	4
Toxichromotest	6	2 (22%)	4

We also compared the number of environmental parameters that contributed to the results observed for the *Daphnia magna* IQ test compared to the *Daphnia magna* acute procedure. It was found that a greater number of environmental parameters contributed to the IQ results and of these, 4 of the 11 parameters were consistent with the *Daphnia magna* acute toxicity test.

5.4 Reproducibility Evaluation

For determination of the reproducibility, or precision, of the various microtests 1) intralaboratory; and 2) interlaboratory comparisons were evaluated.

5.4.1 Intralaboratory Reproducibility

Originally it was believed that an intralaboratory comparison could be conducted by evaluating the toxicity of a specific mine site effluent over several sampling periods. Due to the potential for seasonal fluctuation in contaminant concentrations and the time span that sampling and testing was conducted this was determined to be unrealistic. The second method of providing an intralaboratory comparison we looked at was to evaluate results of duplicate testing within each laboratory. Review of the laboratory reports indicates no duplicate testing of effluent samples was conducted. The third mechanism by which some sort of intralaboratory comparison could be completed was to evaluate results of reference toxicant testing. No reference toxicity testing was conducted for the *Daphnia magna* IQ test, thus making it impossible to calculate coefficients of variations (CV) for all the toxicity tests and subsequently provide some mechanism of comparison and ranking for intralaboratory reproducibility.

Reference toxicant results for the four test procedures completed by BEAK and associated comments are summarized in Table 5.4.1:

Table 5.4.1 Summary of Reference Toxicant Results From Beak's Laboratory.

Toxicity Test	Reference Toxicant	Sample Size (N)	Average Endpoint Concentration (mg/) (95% Confidence Limits)	CV (%)	Remarks Provided by Laboratory
Microtox (15 Min IC50)	Zinc Sulfate	15	0.78 (0.45-1.1)	21	Very Good
Rotoxkit (24 hr LC50)	$K_2Cr_2O_7$	17	11.8 (4.6-19)	30.5	Average
Thamnotoxkit (24 hr LC50)	$K_2Cr_2O_7$	16	0.12 (0.05-0.19)	28	Good
Toxichromotest (150 min IC50)	HgCl	22	0.22 (0.011-0.41)	45	Weak

In order to provide some mechanism of intralaboratory assessment our last alternative is to utilize published CV values for the *Daphnia magna* IQ test. In a series of toxicity tests conducted by

Janssen and Persoone (1993) the reported precision of the IQ bioassay was considered quite acceptable with CV's for replicate tests (n=3) ranging from 3% to 32%. Janssen et al. (1993) found the precision of the IQ tests (n=2) conducted on pure compounds to have CV's ranging from 10 to 24%. The precision with more complex effluents, from a pharmaceutical company, was considerably lower than the pure compound testing, with CV's ranging from 20 to 43%. Hayes et al. (1993) found in a study between two laboratories using pure compounds the intralaboratory CV's ranged from 0.0 to 39.8% depending on the compound. In a 16 laboratory study conducted by Aqua Survey Inc. (1993) using copper, the intralaboratory CV's averaged 21.8% of which 13 of the laboratories had less than 40% for a CV. By all indications from the literature, it would appear that an expected intralaboratory CV would on average range from 30-35% and could be improved given additional experience with the testing procedure.

As indicated by BAR: "Completion of the *Daphnia* IQ test involved visually comparing the fluorescence of each of the daphnids in the exposure concentrations to the controls. A decrease was related to a decrease in metabolism, indicating a toxic effect. Since the degree of fluorescence is based on visual observations only, subjectivity in endpoint measurements may result in variable test results, both within and between laboratories. This was confirmed during this study when several informal verifications were made on selected effluent samples. Although informal, these verifications reveal slight differences in results when two different technicians made final observations on identical samples." This rationale provides credence to CV's indicated in the literature and the associated intralaboratory CV value one may then expect.

The compilation of BAR's reference toxicant data for the rainbow trout (phenol) and *Daphnia magna* (sodium chloride) acute lethality bioassays allows calculation of the CV's, 10.7 and 1.93%, respectively. These values would appear to be lower than what would be expected particularly for more complex effluents. For discussion we would suggest a more traditional CV of < 20% would be expected and quite acceptable from a QA/QC viewpoint.

Based upon CV results for BEAK's reference toxicant testing, literature CV's for the *Daphnia magna* IQ test and typical values expected for the rainbow trout and *Daphnia magna* acute procedures some method of intralaboratory reproducibility rank scoring can be completed. The following rank scoring is applied to the CV values:

CV Values	Rank Scoring
0 - 10 %	1
11 - 20%	2
21 - 30%	3
31 -40%	4
> 41%	5

Based upon this scoring mechanism the intralaboratory reproducibility is tabulated and ranked in Table 5.4.2.

Table 5.4.2 Comparison of Intralaboratory Reproducibility

Toxicity Test	CV Value	Ranking Score
Rainbow Trout Acute	<20%	2
Daphnia magna Acute	<20%	2
Daphnia magna IQ	30 - 35%	4
Microtox	21%	3
Rotoxkit	30.5	3
Thamnotoxkit	28	3
Toxichromotest	45	5

5.4.2 Interlaboratory Reproducibility

The evaluation of the interlaboratory reproducibility was completed by taking into account results for the split QA/QC samples. Results of the primary and QA laboratory have already been summarized in Table 3.F. Table 5.4.3 summarizes the CV values for the split sample results. (Further pair wise testing may be completed if deemed necessary and included in the final draft report).

When we compare the split sample toxicity results and attempt to calculate a CV three different scenarios occur:

1) The data allows a CV value to be calculated
2) The data does not allow a CV value to be calculated as one laboratory produced a calculated endpoint and the other produced an endpoint which was either >100% or non-lethal.
3) No endpoint could be calculated (ie>100% and/or non-lethal).

For these different data scenarios the following ranking score is applied to each split sample comparison, depending on the scenario that applies for each data comparison that occurs.

Scenario 1

CV Values	**Split Sampling Scoring**
0 - 10 %	1
11 - 20%	2
21 - 30%	3
31 -40%	4
> 41%	5

Scenario 2 - This scoring mechanisms allows scoring for samples considered to have low and/or borderline toxicity response.

Split Sample Results	**Split Sample Scoring**
80% - >100% or non-lethal	2
60% - >100% or non-lethal	3
40% - >100% or non-lethal	4
20% - >100% or non-lethal	5

Scenario 3 - The test results are scored a 1 as no effective dose response was provided by either laboratory which would allow calculation of an endpoint (ie. both results non-lethal)

Table 5.4.3 CV 's for Primary and QA Laboratory

te #	Mine Type	Sampling Period*	Rainbow Trout	*Daphnia magna*	*Daphnia magna* IQ	Microtox	Rotoxkit	Thamnotoxkit	Toxichromotest
2	Pb/Zn	1(8)	6.54	1 WR (>100, 73.7%)	51.27	4.62	NR	12.17	NR
3	Ni/Cu	4(57)	1.07	NR	21.13	1 WR (>99, 61.8%)	NOT INCLUDED	NR	NR
4	Ni/Cu	1(6)	1 WR (82, >100%)	NR	1 WR (>100, 41.0 %)	NOT INCLUDED	NR	NR	NR
5	Cu/Zn	4(56)	1 WR (89.1, >100%)	NR	17.31	1 WR (>99, 69%)	NOT INCLUDED	8.71	NR
6	Tin	1(10)	NR	1 WR (>100, 76.6%)	12.89	NOT INCLUDED	NR	1 WR (>100, 88.4%)	NR
7	Cu/Zn	4(58)	6.54	4.59	61.6	1 WR (>99, 41.6%)	NOT INCLUDED	NR	NR
.3	Au	1(7)	NR	NR	NR	NOT INCLUDED	NR	NR	NR
.7	Cu/Zn	4(55)	8.92	1 WR (NL, 77.5)	4.78	NR	NOT INCLUDED	21.57	NR

Site # - Assigned by Pollutech

* The number in parentheses is the Canmet sample number for that sampling period

NR - No Response, comparable non-lethal and/or > 100% v/v response

1 WR - One toxicity test with calculated endpoint (actual values for primary and QA laboratory, respectively).

Table 5.4.4 Split Sample Scoring and Interlaboratory Ranking

te #	Mine Type	Sampling Period*	Rainbow Trout	*Daphnia magna*	*Daphnia magna* IQ	Microtox	Rotoxkit	Thamnotoxkit	Toxichromotest
2	Pb/Zn	1(8)	1	3	5	1	1	2	1
3	Ni/Cu	4(57)	1	1	3	3	NOT INCLUDED	1	1
4	Ni/Cu	1(6)	2	1	4	NOT INCLUDED	1	1	1
5	Cu/Zn	4(56)	2	1	2	3	NOT INCLUDED	1	1
6	Tin	1(10)	1	3	2	NOT INCLUDED	1	2	1
7	Cu/Zn	4(58)	1	1	5	4	NOT INCLUDED	1	1
.3	Au	1(7)	1	1	1	NOT INCLUDED	1	1	1
.7	Cu/Zn	4(55)	1	3	1	1	NOT INCLUDED	3	1
Total Score			10	14	23	12	4	12	8
Average or Interlaboratory Ranking			1.25	1.75	2.875	2.4	1	1.5	1

Site # - Assigned by Pollutech

* The number in parentheses is the Canmet sample number for that sampling period

It should be noted that the traditional acute toxicity tests are subject to testing conditions (ie. dilution water hardness, genetic strain) that may account for the interlaboratory variation in results. In this particular study even though the contract laboratory responsible for the rainbow trout and *Daphnia magna* acute toxicity tests had a water hardness over 300 mg/L and QC laboratory ≈ 125 mg/L (Westlake pers. comm.) there would appear to be consistent results for the rainbow trout test as indicated by the CV's found in Table 5.4.3. For the *Daphnia magna* acute toxicity test only one CV of 4.59 was generated. In three other instances where the contract laboratory generated a non-lethal or >100% volume endpoint the QC laboratory produced an endpoint (Table 5.4.3). These occurrences may be attributed to differences in the culture/dilution water noted between the two laboratories.

The split sample scoring is then totalled and a final ranking of the toxicity tests can then be completed based upon the total scoring for each toxicity test procedure which is averaged for the number of valid data points. This average is the score out of 5, as 5 is maximum value that could be set for any scoring field. Therefore, the average score provides the interlaboratory ranking which is tabulated in Table 5.4.4.

This method of ranking does create some difficulties. For instance, when we compare the split sample scoring provided in Table 5.4.4 with the actual values provided in Table 5.4.3, for the Rotoxkit and Toxichromotest no endpoint could be calculated. This provides a score of "1" for all useable split sample results (Table 5.4.4). This would suggest these toxicity tests are quite reproducible between laboratories. But one must also take into account the full picture when evaluating these toxicity test procedures, which includes the toxicity tests' sensitivity and accuracy. Key discussions of these areas can be found within this report.

5.4.3 Summary of Intralaboratory and Interlaboratory Rankings

Table 5.4.5 summarizes the intralaboratory and interlaboratory ranking scores derived from this evaluation. For the reproducibility evaluation both components will be weighted evenly. As noted both components are scored from 1 to 5 (good to poor) for the final summary.

Table 5.4.5 Intralaboratory and Interlaboratory Reproducibility Rank Scoring

Toxicity Test	Intralaboratory Reproducibility Scoring	Interlaboratory Reproducibility Scoring
Rainbow Trout Acute	2	1.25
Daphnia magna Acute	2	1.75
Daphnia magna IQ	4	2.875
Microtox	3	2.4
Rotoxkit	3	1
Thamnotoxkit	3	1.5
Toxichromotest	5	1

5.5 Applicability Evaluation

Websters dictionary defines applicability as "the state or quality of being applicable" which is "capable of being applied". Thus the applicability of a toxicity test procedure must consider the final application for which the toxicity test is to be applied. As indicated in the original "request for proposal" the objective was to determine "if satisfactory alternatives can be found to provide the required information at less cost and greater speed, it would be in the best interests of both mining industry and the regulatory community to adopt and implement them". Applicability should therefore consider the ability of each toxicity test procedure examined in this study to act as an alternative procedure to the rainbow trout toxicity test. To do this comparison to the rainbow trout, we should first briefly examine the past role of this toxicity test procedure in Canada.

In Canada the rainbow trout has become the cool-water fish for determining the toxicological impacts of contaminants and ecological impacts of complex effluent discharges (Environment Canada, 1990). As a result a considerable amount of toxicological information can be found for rainbow trout, relating toxicological effects to ecological impacts. Such studies have lead to the accepted use of the rainbow trout toxicity test as a mechanism for monitoring and as a method of compliance of effluent discharges by both Federal and Provincial jurisdictions.

The historical role of the rainbow trout toxicity test can be classified into several categories as follows:

1) **Screening** of chemicals or effluent discharges for toxicity. Allows for screening of toxicological impacts prior to implementation/use of new chemicals within a process, initiation of modified or new processes, assessment of normal and abnormal process operating conditions, periodic assessment of an effluent for toxicity and for determining chemical components associated with toxicity through toxicity identification/ toxicity reduction evaluations (TIE/TRE's).

2) **Monitoring** of ongoing effluent discharges. Provides the end user (ie. industrial manager or government regulatory) a measuring stick to gauge the operational performance of an industrial facility or process with regard to the potential of causing ecological impact.

3) **Regulation** of effluent discharges for toxicity. Provides a mechanism to implement the regulation of effluent discharges for toxicity through the use of a legal test that has relevance to the environment and has the capability of standing up to the rigours of the court room.

By examining the historical role of the rainbow trout test the applicability of an alternative test would therefore depend on the application for which the alternative test is to be applied. For screening purposes or TIE/TRE's such things as cost, sample volume requirements, turn around times and comparability with other toxicity tests becomes more critical for an alternative procedure. For monitoring, the previous discussion would apply but the endpoint results must also be able to predict the potential for real effects that may be occurring in the receiving water. For regulatory purposes relevance to the environment is essential and should take precedence over cost and turnaround time. AIf a procedure will not stand up in court, then using it, even if cheap, is a false economy@ (Westlake, Pers. Comm.). The use of an alternative test procedure for legal purposes must also address concerns regarding the use of proprietary tests, particularly for the microtests used in the Canmet study.

One of the most obvious results of the Canmet survey is that no one test stands out as an alternative test procedure that could be applied to all mine types and/or sites tested. From review of the results in Tables 3A through 3F toxicity for the microtests would appear to be more specific to mine type. When considering the use of an alternative test procedure for the rainbow trout test one would have to take into account mine type (ie. copper/zinc, bitumen, etc.). The other aspect that becomes quite obvious is that responses between mine sites of a specific mine type also varies. Therefore when considering the application of an alternative test procedure it would appear to be prudent to consider the purpose of the application, the mine type and comparative toxicity of the microtest procedure to the rainbow trout test on a site by site basis. This document should provide some guidance towards the selection of an alternative test procedure.

Table 5.5.1 summarizes which test procedure was responsive for each specific mine type. When reviewing this table it should be kept in mind the number of mines represented for each mine type and whether this number is representative for that portion of the mining sector. The other aspect to consider is that the response of a microtest may not always be comparable to the response of the rainbow trout toxicity test (See Section 5.6.5).

The other aspect that should be considered when determining the applicability of a microtest procedure is the relative level of response of the procedure in comparison to the rainbow trout toxicity test. An overly responsive toxicity test which demonstrates a higher level of toxicity or the presence of toxicity when the traditional procedure does not, may provide a false cause for concern, unless the toxicity response can be correlated to **Aecological effects@**. For example with the *Daphnia magna* IQ test the procedure was more responsive than all other toxicity test procedures including the rainbow trout acute toxicity test. The IQ procedure was responsive to all mine types while this was not the case for other toxicity test procedures. On an overall basis for all mine types the IQ test indicated toxicity at concentrations 7 times lower than the rainbow trout toxicity test. For the IQ test, the level of response compared to the rainbow trout varied between mine types, which should also be considered regarding the applicability of a toxicity test procedures (See Section 4.9) . It could be argued that such a responsive test would provide an indication of the potential for toxicity when utilized for screening and/or monitoring.

Table 5.5.1 Percent of Samples by Microtest and Mine Type in which an Endpoint can be Calculated

Mine Type (# of Sites included in study)	% Samples Providing a Calculable Endpoint						
	Rainbow Trout Acute	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox	Rotoxkit	Thamnotoxkit	Toxichromotest
Bitumen (1)	100	0	100	100	0	0	25
Copper/Zinc (4)	50	28.6	64.3	9.1	9.1	71.4	21.4
Gold (5)	27	36.4	54.5	27.2	36.4	36.4	27.3
Lead/Zinc (3)	27	54.5	45.5	4.3	10	90	10
Nickel/Zinc (4)	50	25	75	33.3	20	0	0
Tin (1)	0	0	75	0	0	0	25
Uranium (2)	0	0	50	0	0	0	0
Zinc (1)	0	0	25	0	0	50	25

5.5.1 Rainbow Trout Acute Toxicity Test

Rainbow trout have been utilized for many years as the most interpretative assessment method for determining the ecological impact of effluent discharges. Most people can relate to fish dying. If the fish dies in the effluent it can in most cases be assumed ecological impact may have occurred. The general lack of rainbow toxicity as noted by the lack of a calculable response in 41 of 64 samples submitted for testing, is a fairly good indicator of environmental performance from an industrial sector.

The applicability of the rainbow trout test has significant merits as a benchmark for measuring and monitoring environmental performance. Laboratory experience with the rainbow trout test is significant. As a result of the large volume requirements, transport time, testing time, restrictions on availability of testing organisms, culture requirements, etc., the ability of a typical mine site operation to conduct this test requires a considerable expenditure of time and funds. Therefore site operators are dependent upon private testing facilities.

5.5.2 *Daphnia magna* Acute Toxicity Test

The Daphnid acute bioassay does have its ecological value as it does represent a secondary trophic level organism. Acute toxicity of the Daphnid in an effluent discharge can also be easily interpreted as the effluent having the potential for ecological impact. Their smaller size and lack of commercial value makes them less important, from the public perspective, as compared to fish. The Daphnid acute test procedure has been utilized for many years for monitoring and compliance testing of effluent discharges. As a result a significant amount of interpretable information is available in the literature regarding contaminant impacts and Daphnid toxicity. Compared to the rainbow trout test, the Daphnid acute procedure would be much easier to apply at the site. The following advantages make the Daphnid test most attractive as a monitoring test which a mine site operation may consider for use; small sample volume, reduced transport time if conducted in-house, shortened testing time (48 hrs), the ability to culture testing organisms from an accepted genetic strain and fairly simple culturing methods. Several drawbacks to this procedure include the amount of effort and experience required to maintain a sustaining culture. In addition, specific culture and testing conditions must be maintained at the site adding to the allocation of effort and funds. Though toxicity testing results can be generated in a much shorter time compared to the rainbow trout toxicity tests, a minimum 48 hours is still required. The Daphnid acute procedure does have the ability to provide more qualitative information during the testing period, such as immobility, that could be used to interpret the potential for effluent toxicity prior to the end of the 48 hour test period.

5.5.3 *Daphnia magna* IQ Toxicity Test

The IQ procedure is the only microtest evaluated as part of this study that utilizes a testing organism that is routinely used for compliance acute toxicity tests. In this case a sublethal response is determined which would be expected to be more responsive to environmental contaminants compared to the *Daphnia magna* acute test as well as any other acute toxicity procedure. In this study the IQ test was the most responsive compared to all other test procedures (See Table 4.1.1). As in the *Daphnia magna* acute testing procedure, maintenance of a sustaining culture would be a requirement for use of this testing procedure at a mine site. Additional effort and expense would required in order to supply healthy test organisms. The question then is: if a culture is to be maintained any way why not perform the acute toxicity test procedure? The advantage of the IQ test procedure is that it is easy to conduct and little time is required to obtain results meeting what is presumed to be one of the primary objectives for a mine operation for fast turnaround of results. The test procedure would appear to be cost effective compared to traditional compliance tests. The IQ test was on average 7 times more sensitive compared to the rainbow trout procedure. It was also responsive to all mine effluent types while the rainbow trout test was not.

The testing laboratory noted several disadvantages associated with the IQ test. This includes the lack of a standardized QA/QC program, subjectivity in endpoint measurements, the potential for over sensitive estimates of toxicity (compared to the rainbow trout test), and replacement of tests that measures lethality with a test that measures a sublethal response. The subjectivity of the endpoint measurements may contribute to higher intralaboratory and interlaboratory CV's, as discussed (See section 5.4).

On an overall basis the test would address the requirements of the mine but the culture requirements, lack of literature linking results to ecological impacts and subjectivity of the sublethal endpoints should be considered. Generally the laboratory was quite satisfied with this test procedure but was of the opinion; "Based upon the results of this study it is suggested that the IQ test would be more suitable for use as an alternative sublethal bioassay or as a "screening" test, rather than a replacement or alternative to the "traditional" acute lethality tests with rainbow trout and *Daphnia magna"*.

5.5.4 Microtox Toxicity Test

A significant wealth of information is available regarding this test procedure and its comparison with more "traditional" acute lethality tests. This knowledge base augments the ability to relate toxicity that may be detected in an effluent discharge to contaminant concentrations and subsequently, the ability to detect potential ecological impacts. The procedure provides results quite quickly with results generated, manipulated and analyzed by a standardized procedure and computer software system. As such, a highly skilled labour force is not required. Testing can be easily done as part of a chemical and/or on-site QA/QC laboratory's routine sample processing. Another advantage is that the bacteria are provided in dehydrated form which are reconstituted prior to conducting the toxicity test. Therefore, maintenance of a culture is not required. Lab space requirements are considered minimal as the entire apparatus, including luminometer and computer, would take up an area 1m X 2 m. Though the apparatus is not fully portable, testing could be conducted in the field with some minor adaptations and services (ie. electricity). The testing laboratory also considered the results very reproducible. A small sample size (1 mL) is required to start the test.

The interpretability of this toxicity test is difficult as the test organism is a genetically engineered marine bacteria that does not represent a real trophic level with which individuals of the non scientific community can relate. The wealth of literature information comparing results to rainbow trout and *Daphnia magna* acute toxicity tests may off set this disadvantage. It has been our experience that concordance with traditional acute toxicity test results would be a necessity as part of a mine sites ability to adopt, interpret and apply results. The prohibitive cost of the apparatus ($20,000 Can) and the monopoly on reagents and bacterial supplies could put off potential users to more cost effective (at least initially) microtest procedures. This capital cost expenditure would have to be considered on a mine site by mine site basis. Though a highly skilled labour force may not be required, some skill in pipetting is necessary and must be done with a lot of attention and precision since the results depends on the concentrations of the bacterial biomass.

Though the Microtox test was less responsive compared to the rainbow trout toxicity test the concordance (percent of agreement with rainbow trout both - and +) was 80% (see section 4.10). The reason for this high agreement was the low number of false negative and false positive toxicity results generated (see Table 4.1.1). The Microtox test also correlated to the greatest number of environmental parameters which were in common with the rainbow trout toxicity test (See Section 5.3). The Microtox test was responsive to the same mine types as the rainbow trout toxicity test, although not to the same degree (See Table 5.5.1).

Given the development of a suitable database specific for the mine site in comparison to acute lethality tests, interpretation and application of results could easily be accomplished. The higher capital cost may deter some mine operators but in considering application of this test procedure one must look at the long term application of the test and not the short-term. The availability of standardized testing procedures by the manufacturer and Environment Canada (1992), which includes QA/QC procedures, makes the Microtox assay a suitable screening mechanism. If accepted as a screening mechanism the results could trigger a requirement for additional Atraditional@ acute lethality tests based upon site specific comparative results with the rainbow trout toxicity test. This would also be true for any of the other micro tests evaluated.

5.5.5 Rotoxkit Toxicity Test

The Rotoxkit utilizes test organisms which are in a cyst form and available commercially. The rotifers are considered ecologically relevant as they represent secondary trophic level organisms similar to the Daphnids. Hatching/culturing is only required prior to initiation of the test and organisms and apparatus for hatching/culturing is included in each test kit. The cost of the kit is relatively low and a non-specialized work force is required to complete the test. The small size of the organisms and the necessity to pipette the test organisms requires a certain level of precision and patience. The testing laboratory considered the small organisms difficult to manipulate. A small lab space and limited additional equipment is required to complete the test. This makes the procedure quite cost effective and attractive.

The Rotoxkit is relatively new (Snell et al., 1991) compared to other microtests (ie. Microtox) and therefore, only a limited amount of information regarding toxicity effects to specific compounds and complex effluent has been published. Validation specific to a mine site's effluent and the acute lethality bioassays would in all probability be required prior to interpretation and application of results that may be generated. Though the endpoint results are similar to the rainbow trout and Daphnid acute tests the time frame necessary to complete the test is 24 hours.

On the whole it is our feeling that the Rotoxkit does not adequately address detection of toxicity for mine site effluents. This is validated by the low number of positive responses, 8 out of 63 samples compared to 23 out of 64 for the rainbow trout in which endpoints could be calculated. The Rotoxkit had the least number of responses of any of the toxicity test procedures evaluated, making it ineffective for either screening or monitoring purposes by mine site operators.

5.5.6 Thamnotoxkit Toxicity Test

Like the Rotoxkit, the Thamnotoxkit toxicity procedure is a recently developed (Centeno et al., 1994), commercial kit. The organisms are provided in cyst form and are incubated just prior to their use in the toxicity test. Much of the discussion provided for the Rotoxkit would apply to the Thamnotoxkit, the difference being the tests overall responsiveness to mine effluents. The Thamnotoxkit provided the second highest number of positive responses (25 out of 63 samples) in which an endpoint could be calculated, compared to the other microtests. The testing laboratory was of the opinion that further standardization could augment the precision and degree of reproducibility of this testing procedure. Results of this study (See Section 5.4) indicate good interlaboratory and intralaboratory CV's. Further standardization through implementation of suitable QA/QC procedures may make this toxicity test particularly useful.

The Thamnotoxkit provided responses to four mine types including copper/zinc, gold, lead/zinc and zinc. With the exception of zinc this is comparable to the rainbow trout test. The rainbow trout test was also responsive to copper/zinc and nickel/zinc (See Table 5.5.1) with responses for 71.4 and 90%, respectively, of the samples tested for these mine types. Though the Thamnotoxkit results correlated to 10 environmental parameters (Table 5.3.1) there were few comparable environmental parameters with the rainbow trout toxicity results. On an overall basis for all mine types the Thamnotoxkit test was 1.67 times more sensitive than the rainbow trout toxicity test. As with the IQ test, the Thamnotoxkit=s level of response compared to the rainbow trout varied between mine types (See section 4.9), particularly for zinc mine type which was 4 times more sensitive.

5.5.7 Toxichromotest Toxicity Test

The Toxichromotest produced the second lowest number of positive results (10 of 63 samples) of the microtests evaluated. As a result, its ability to detect toxicity in mine effluents is questionable. The test method does not allow for the testing of effluent samples at a concentration >50% v/v. This is insufficient to detect effluent toxicity where the effluent toxicity could occur at a higher concentration and as such be considered out of compliance. The Toxichromotest does appear to be more useful for highly toxic samples. It does provide fast results, requires no culturing bacteria and only requires a minimum amount of additional lab equipment and space. A limited knowledge base is available. Like the Microtox test, the organisms used for the test, the *Escherichia colia*, have been transformed by genetic engineering. It was considered sensitive but not ecologically relevant.

5.6 Comparability of Toxicity Results Compared to the Rainbow Trout Test

To evaluate the comparability of the microtests, the results generated were compared statistically to the rainbow trout acute lethality bioassay. The following discusses the approach and theory involved in completion of the comparison.

5.6.1 Theory of the Sign Test

We compare the two toxicity test results for a given effluent. If both tests are equally sensitive we would expect the number of times that test A is more sensitive than test B to be approximately equal. Statistically, we count the number of times test A, (or test B) is more sensitive than the other test (we ignore ties) and assign a A+@ to this comparison. The number of pluses is binomially distributed. We compare the number of pluses we obtain from the data against the number we would expect if there were no difference between the tests. If this value is greater than the cutoff value then there is sufficient evidence to reject the null hypothesis which states that both tests are equally sensitive. Statistically we have proven that a significant difference exists. We reject the null hypothesis and accept the alternative hypotheses. Test A is significantly different than Test B.

For this analysis the alternative hypothesis was that test A is more sensitive than test B, rather than test A and B vary in sensitivity. The first test is referred to as a one-tailed test, because we reject the null hypothesis only in the case where we obtain a large number of pluses. In the second case, we have a two tailed test. We reject the null hypothesis if we have a large number of pluses or a small number of pluses.

In the case of a discrete distribution, alpha values are restricted to those probabilities corresponding to levels of the discrete random variable. Therefore, we cannot always choose the traditional alpha value of 0.05 as is done for continuous data. When we have a discrete distribution and a small sample size, the alpha value becomes unduly large when we consider a two-sided alternative. Thus the tests for comparisons between toxicity tests on a per site basis (ie. site #1) and by mine type (ie gold sector) were all one-sided. The test with the largest number of pluses, say test A, was compared to the other test, Test B to determine whether test A was more sensitive than test B.

5.6.2 Achieved Alpha Values

We are able to determine the probability of obtaining a larger value than a given random variable if we know the probability distribution function of the random variable. If the random variable arises from a continuous distribution such as the normal distribution we can find that quantile which corresponds to an alpha value of 0.05. This is the critical value which we compare our test statistic to. For example if we are doing a Z-test of equality of two means we would compare the estimated Z statistic with the critical value of 1.9645 for a two sided test at alpha = 0.05. This value is the 97.5% quantile of the standard normal distribution.

In the case of a discrete probability distribution such as the binomial, the probability distribution function jumps up in steps. Thus we cannot simply pick a critical value corresponding to a specific value of alpha, because that value of alpha may not exist for the sample size of the experiment we are working with. Instead we pick a critical value as close as possible to the value of alpha we like to assign to the test. This alpha value is labelled as the "achieved alpha". In the following summary tables, the achieved alpha value for the test is presented.

5.6.3 Power Analysis

Whenever we test a hypothesis using statistical methods we encounter the possibility of making an incorrect inference. If we reject the null hypothesis when it is true, we have made a Type I error. This is generally referred to as the "alpha" (α) value of a test. For our data set an incorrect inference would result in the statement Atoxicity test a is more sensitive than toxicity test b,@ when in fact both tests were equally sensitive. If we accept the null hypothesis when it is false we have made a Type II error. This value is usually ascribed the letter "Beta" (β). This would occur if the two tests being compared were not of equal sensitivity and we stated that both were equally sensitive.

The probability of rejecting the null hypothesis when it should be rejected is 1-Beta. This is referred to as the power of a test. The failure to reject a null hypothesis may be due to two causes. One is that there is no difference between the two toxicity tests. Another is that our experiment was insufficiently powerful to decide whether one test was more sensitive than the other. In the first case our conclusion would be that both tests were equally sensitive. In the second case the proper interpretation is to state that the test is inconclusive. A general rule of thumb is to state that a test is inconclusive if the null hypothesis is not rejected and the power is less than 0.80.

5.6.4 Categorization of Data

During the first two stages of data collection it became evident that many of the effluents were non-toxic. Other effluents were only slightly toxic; that is some response occurred but not enough to estimate an EC50. Only a small proportion of the effluents were sufficiently toxic to allow for estimation of an EC50 (see Table 4.1.1). While this is encouraging from an environmental perspective, it makes the comparison of toxicity tests with the rainbow trout bioassay more challenging. Discussions with stakeholders suggested that if possible all the data should be used as clearly the case where one toxicity test produced an estimated EC50 while another showed no mortality indicates that the former test is more sensitive to the effluent.

Consider the following possible outcomes for a bioassay; an EC50 is estimable, partial mortality occurs but insufficient to estimate an EC50 and no mortality occurs. Clearly these cases can be ordered from most sensitive to least sensitive. This data is said to be ordinal. We can analyze this data using a rank procedure which takes into account the ordinality of the data but not the relative magnitude of the values. Thus for the following hypothetical data set we would rank as follows:

Comparison of Hypothetical Data		
Endpoint: Test A	Endpoint: Test B	Comparison
EC50 = 45%	no mortality or effect	A more sensitive than B
no mortality or effect	some mortality or effect, insufficient to estimate EC50	B more sensitive than A
EC50 = 23 %	EC50 = 34 %	A more sensitive than B
some mortality or effect, insufficient to estimate EC50	some mortality or effect, insufficient to estimate EC50	A and B are equally sensitive

In the case of toxicity tests where no response was measured and an operational dilution resulted in a maximum concentration of less than 100 % effluent, the test was considered as exhibiting no mortality or no effect. This occurs for the Microtox bioassay where the maximum concentration may be 99.9, or 90 % effluent, and the Toxichromotest where the maximum possible concentration is 50 % effluent.

Appendix C contains the detailed results, by site and by mine type, of the Microtest comparisons with the rainbow trout toxicity test. Appendix C also contains similar detailed comparisons of the *Daphnia magna* acute and IQ toxicity tests.

5.6.5 Summary of Results

The comparability of the microtest was analysed on three levels; by site, by mine type and overall. The results of the site analysis, summarized in Appendix B, provides summarizes results for mines that participated in the study. As a result of the relatively small sample size for a number of the sites no conclusive comparison could be conducted in some cases. Also included in the Appendix B is a summary of the analysis comparing the *Daphnia magna* IQ test compared to the *Daphnia magna* acute toxicity test. This information is provided to address interest in the IQ test, whose test organism is also utilized in an acute toxicity procedure method endorsed by Environment Canada.

Some of the information generated out of this study is the comparison of toxicity results to the rainbow trout by mine type. This is particularly true for mine types where more than 3 sites were sampled including the gold, copper/zinc, nickel/copper and lead/zinc. By combining results of the sites into these various mine types some additional statistical strength can be achieved. Results of this analysis are summarized in Table 5.6.5.1.

During the course of this study it has been the general comment that toxicological responses to the various toxicity tests are characteristic of the general mine type by which a site can be categorized. It is expected that toxicological properties by mine site could vary from site to site for the same mine type. Variation in toxicity could probably be a result of the type of extraction process, treatment system and/or local geomorphology. Those sites which did not participate but which fall into a specific mine type would benefit from the mine type results summarized in Table 5.6.5.1.

Besides providing a comparison of rainbow trout to microtest and vice versa we have also included a comparison of the *Daphnia magna* acute test to the IQ test by mine type in Table 5.6.5.2.

Table 5.6.5.1 Summary of Results Comparing Microtest to Rainbow Trout Acute Toxicity Results by Mine Type

Mine Type	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox	Rototox	Thamnotoxkit	Toxichromotest
Gold		*Daphnia magna* IQ More sensitive		Rotoxkit more sensitive	Thamnotoxkit more sensitive	Rainbow Trout more sensitive
Bitumen	Rainbow Trout more sensitive	*Daphnia magna* IQ More sensitive	Rainbow Trout more sensitive	Rainbow Trout more sensitive	Rainbow Trout more sensitive	
Copper/Zinc		*Daphnia magna* IQ More sensitive	Rainbow Trout more sensitive		Thamnotoxkit more sensitive	
Nickel/Copper	Rainbow Trout more sensitive	*Daphnia magna* IQ More sensitive				Rainbow Trout more sensitive
Lead/Zinc	*Daphnia magna* more sensitive	*Daphnia magna* IQ More sensitive			Thamnotoxkit more sensitive	Rainbow Trout more sensitive
Tin		*Daphnia magna* IQ More sensitive	Rainbow Trout more sensitive			
Uranium	*Daphnia magna* more sensitive	*Daphnia magna* IQ More sensitive		Rotoxkit more sensitive		
Zinc		*Daphnia magna* IQ More sensitive		Rotoxkit more sensitive	Thamnotoxkit more sensitive	

Blank Field - The power analysis indicates insufficient data available to make a definitive conclusion.

Table 5.6.5.2 Summary of Results Comparing *Daphnia magna* IQ Toxicity Results to the *Daphnia magna* Acute Toxicity Results by Mine Type

Mine Type	*Daphnia magna* IQ vs *Daphnia magna* acute
Gold	*Daphnia magna* IQ more sensitive
Bitumen	*Daphnia magna* IQ more sensitive
Copper/Zinc	*Daphnia magna* IQ more sensitive
Nickel/Copper	*Daphnia magna* IQ more sensitive
Lead/Zinc	
Tin	
Uranium	
Zinc	*Daphnia magna* IQ more sensitive

Blank Field - The power analysis indicates insufficient data available to make a definitive conclusion.

By reviewing the results of Table 5.6.5.1 the comparability of the microtest varied between the various mine types. For the gold mines the *Daphnia magna* IQ, Rotoxkit and Thamnotoxkit were all more sensitive than the rainbow trout toxicity test. For Bitumen type, only the *Daphnia magna* IQ test was more sensitive out of five valid statistical comparisons.

The *Daphnia magna* acute toxicity test was considered more sensitive for lead/zinc and uranium mine types. For the 8 mine types the *Daphnia magna* IQ test was found to be more sensitive in all cases. For both the Microtox and Toxichromotest the rainbow trout test was found to be more sensitive. In the case of the Microtox this may be more a function of the categorization methods (see Section 5.6.4) used. The Rotoxkit was found to be more sensitive for the gold, uranium and zinc mine types. The Thamnotoxkit was found to be more sensitive for 4 of the 8 mine types including gold, copper/zinc, lead/zinc and zinc mine types. From Table 5.6.5.2 the *Daphnia magna* IQ test is more sensitive compared to the *Daphnia magna* acute toxicity test for all mine types except lead/zinc, tin and uranium. For the gold, copper/zinc, nickel/copper, lead/zinc and zinc mine types which have the larger number of sites which participated and the larger number of data points included in the analysis provides some credibility the results generated by mine type are representative of toxicological responses on a national scale.

5.6.6 Ranking Scoring by Mine Type

From review of the results generated it is difficult to apply a specific ranking mechanism by which we can evaluate the comparability of toxicity tests when the comparison indicates insufficient power to provide a conclusive answer. The ranking scoring used in this study utilizes a mechanism by which inconclusive results can be incorporated. It should be noted that additional testing, if completed, might suggest an amendment to the rank scoring applied in this report to the inconclusive results.

Utilizing the results for the various mine types provided in Table 5.6.5.1 the tests are ranked on a scale of 1, 3 or 5 depending on their comparability to the rainbow trout test. For instance, if the toxicity test is considered more sensitive than the rainbow trout it will be scored a 1. It is assumed that if the test is assigned a 1 it may be as sensitive, if not more sensitive, than the rainbow trout toxicity test. Therefore, the test will have the ability to detect toxicity in a mine effluent but it may or may not necessarily correlate to rainbow trout toxicity. If the rainbow trout test is found to be more sensitive it will be scored a 5. If there is insufficient power to provide a conclusive answer the test will be scored a 3. The logic for this is that the test has not been found to be less or more sensitive than rainbow trout, only that there is insufficient data available to make a definitive conclusion. Therefore the intermediate value is applied. The rank scoring for each mine type has been included in Table 5.6.5.3. This approach allows sites to evaluate results which have application specific to their mine type. In section 5.7 the mine type rank scores are applied to the overall ranking summaries by mine type for purposes of deriving information specific to a mine type.

Table 5.6.5.3 Summary of Results Rank Scoring for Each Microtest and Mine Type

Mine Type	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox	Rotoxkit	Thamnotoxkit	Toxichromotest
Gold	3	1	3	1	1	5
Bitumen	5	1	5	5	5	3
Copper/Zinc	3	1	5	3	1	3
Nickel/Copper	5	1	3	3	3	5
Lead/Zinc	1	1	3	3	1	5
Tin	3	1	5	3	3	3
Uranium	1	1	3	1	3	3
Zinc	3	1	3	1	1	3

1 - more sensitive then rainbow trout
3 - insufficient power to provide a conclusive answer
5 - rainbow trout is more sensitive

5.7 Summation of the Ranking Scores

With the exception of the applicability criteria, all other evaluation criteria were ranked on a numerical score to provide a basis for comparison between toxicity tests. It became very obvious that providing an overall ranking that included all mine types provided very little value to the study. As a result summation rankings were provided for each of the mine types. This would have a greater value for mines, particularly those which did not participate in this study, to evaluate the most appropriate microtest that would be applicable to their specific situation. Results in this report, and the format in which they are presented, can provide the basis by which any particular site which participated in the study, or did not participate, can conduct their own independent evaluation specific to their application.

Now that each of the evaluation categories have been defined, analysed and rank scoring applied, a final summation of the ranking scores can be completed. The scoring summaries have been provided by mine type. Tables 5.7.1 through 5.7.8 summarize the data matrix generated for each of the mine types. The rationale for providing results by mine type is that the toxicity results, when compared to the rainbow trout (see section 5.6), varied from site to site but also varied based on mine type. Therefore, in the final summary scores grouped by mine type, the only variation in scores will be found under the evaluation criteria titled AComparability of Toxicity Results to Rainbow Trout Acute Test@. All other evaluation criteria scores (ie. costs, speed, etc.) will be the same for all the different mine types. By providing a summary of results by mine type, users of this report, particularly sites which did not participate, will have the ability to apply the results generated specific to their type category. For those sites which did participate, and for which a statistical result is available in Appendix B, the rank scoring scheme in section 5.6 can be used to determine an overall rank scoring applied to their site. Since only one site participated for each of the bitumen, tin and zinc mine types, Tables 5.7.2, 5.7.6 and 5.7.8, respectively, these participants can easily apply results specific to their situation or application without further effort.

The total scores provided in Tables 5.7.1 through 5.7.8 are the total score out of a maximum of 30. This score has been then adjusted for a score out of 100. The lowest score is the **best** score. Though it can be interpreted that the low score is best it should be emphasized that the applicability or application (see section 5.5) for which the test is to be used must also be judged.

Table 5.7.1 Summary of the Overall Rankings for Gold Mine Sector (Based on 5 Sites)

Evaluation Criteria	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox		Rotoxkit F	Thamnotoxkit	Toxi-chromotest
			1 yr	5 yr			
Cost	1	2	3	2	1	1	1
Speed	2.5	1	1	1	2.5	2.5	1
Correlation to Chemistry	4	4	3	3	5	4	4
Reproducibility (intra/inter)	1.88	3.44	2.7	2.7	2	2.25	3
Comparability	3	1	3	3	1	1	5
Overall Score out of 30	12.38	11.44	13.7	16.7	11.5	10.75	14
Overall Score out of 100	41	38	46	56	38	36	47

Table 5.7.2 Summary of the Overall Rankings for Bitumen Mine Type (Based on 1 Site)

Evaluation Criteria	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox		Rotoxkit F	Thamnotoxkit	Toxi-chromotest
			1 yr	5 yr			
Cost	1	2	3	2	1	1	1
Speed	2.5	1	1	1	2.5	2.5	1
Correlation to Chemistry	4	4	3	3	5	4	4
Reproducibility (intra/inter)	1.88	3.44	2.7	2.7	2	2.25	3
Comparability	5	1	5	5	5	5	3
Overall Score out of 30	14.38	11.44	15.7	18.7	15.5	14.75	12

Overall Score out of 100	48	38	52	62	52	49	40

Table 5.7.3 Summary of the Overall Rankings for Copper/Zinc Mine Type (Based on 4 Sites)

Evaluation Criteria	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox		Rotoxkit F	Thamnotoxkit	Toxi-chromotest
			1 yr	5 yr			
Cost	1	2	3	2	1	1	1
Speed	2.5	1	1	1	2.5	2.5	1
Correlation to Chemistry	4	4	3	3	5	4	4
Reproducibility (intra/inter)	1.88	3.44	2.7	2.7	2	2.25	3
Comparability	3	1	5	5	3	1	3
Overall Score out of 30	12.38	11.44	15.7	18.7	13.5	10.75	12
Overall Score out of 100	41	38	52	62	45	36	40

Table 5.7.4 Summary of the Overall Rankings for Nickel/Copper Mine Type (Based on 4 Sites)

Evaluation Criteria	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox		Rotoxkit F	Thamnotoxkit	Toxi-chromotest
			1 yr	5 yr			
Cost	1	2	3	2	1	1	1
Speed	2.5	1	1	1	2.5	2.5	1
Correlation to Chemistry	4	4	3	3	5	4	4
Reproducibility (intra/inter)	1.88	3.44	2.7	2.7	2	2.25	3
Comparability	5	1	3	3	3	3	5
Overall Score out of 30	14.38	11.44	13.7	16.7	13.5	12.75	14

Overall Score out of 100	48	38	46	56	45	43	47

Table 5.7.5 Summary of the Overall Rankings for Lead/Zinc Mine Type (Based on 3 Sites)

Evaluation Criteria	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox		Rotoxkit F	Thamnotoxkit	Toxi-chromotest
			1 yr	5 yr			
Cost	1	2	3	2	1	1	1
Speed	2.5	1	1	1	2.5	2.5	1
Correlation to Chemistry	4	4	3	3	5	4	4
Reproducibility (intra/inter)	1.88	3.44	2.7	2.7	2	2.25	3
Comparability	1	1	3	3	3	1	5
Overall Score out of 30	10.38	11.44	13.7	16.7	13.5	10.75	14
Overall Score out of 100	35	38	46	56	45	36	47

Table 5.7.6 Summary of the Overall Rankings for Tin Mine Type (Based on 1 Site)

Evaluation Criteria	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox		Rotoxkit F	Thamnotoxkit	Toxi-chromotest
			1 yr	5 yr			
Cost	1	2	3	2	1	1	1
Speed	2.5	1	1	1	2.5	2.5	1
Correlation to Chemistry	4	4	3	3	5	4	4
Reproducibility (intra/inter)	1.88	3.44	2.7	2.7	2	2.25	3
Comparability	3	1	5	5	3	3	3
Overall Score out of 30	12.38	11.44	15.7	18.7	13.5	12.75	12

Overall Score out of 100	41	38	52	62	45	43	40

Table 5.7.7 Summary of the Overall Rankings for Uranium Mine Type (Based on 2 Sites)

Evaluation Criteria	***Daphnia magna*** **Acute**	***Daphnia magna*** **IQ**	**Microtox**		**Rotoxkit F**	**Thamnotoxkit**	**Toxi-chromotest**
			1 yr	**5 yr**			
Cost	1	2	3	2	1	1	1
Speed	2.5	1	1	1	2.5	2.5	1
Correlation to Chemistry	4	4	3	3	5	4	4
Reproducibility (intra/inter)	1.88	3.44	2.7	2.7	2	2.25	3
Comparability	1	1	3	3	1	3	3
Overall Score out of 30	10.38	11.44	13.7	16.7	11.5	12.75	12
Overall Score out of 100	35	38	46	56	38	43	40

Table 5.7.8 Summary of the Overall Rankings for Zinc Mine Type (Based on 1 Site)

Evaluation Criteria	***Daphnia magna*** **Acute**	***Daphnia magna*** **IQ**	**Microtox**		**Rotoxkit F**	**Thamnotoxkit**	**Toxi-chromotest**
			1 yr	**5 yr**			
Cost	1	2	3	2	1	1	1
Speed	2.5	1	1	1	2.5	2.5	1
Correlation to Chemistry	4	4	3	3	5	4	4
Reproducibility (intra/inter)	1.88	3.44	2.7	2.7	2	2.25	3
Comparability	3	1	3	3	1	1	3
Overall Score out of 30	12.38	11.44	13.7	16.7	11.5	10.75	12

Overall Score out of 100	41	38	46	56	38	36	40

Early in the project, after results of the second sampling period were made available, it was seen that the selected mine effluents were generally non-toxic. While this is positive for the receiving environment, the statistical comparison of the toxicity tests was adversely affected through a decrease in the number of data points available for analysis. The conclusion regarding comparability to rainbow trout is based on a small number of tests using a non-parametric analysis. The usual criterion for rejecting a null hypothesis is an α value of 0.05. Due to technical difficulties outlined in section 5.6, this value was sometimes as high as 0.125. Although all conclusions reached are technically sound, the weakness of the data set with regards to this section should be kept in mind.

In some situations (ie. Environmental Assessment) where a rank scoring scheme has been developed it may be desirable to modify the weights assigned to the evaluation criteria to reflect those criteria which are perceived as more or less important. The value assigned to the weighting is subjective and may vary with the disparate requirements of mine site operators and governmental regulators. To obviate lengthy debates on this subject, an analysis was completed to determine the ARobustness of Weighting@ which determines if the choice of Abest@ test is greatly affected by the choice of weights for each of the 5 evaluation criteria.

This analysis was implemented using the following paradigm, others are certainly possible. A value ranging from 1 to 100 is given to the first category. This number is subtracted from 100 and the remainder is divided up among the remaining four categories. Thus as the algorithm begins, the first category receives a weight of 1; all other categories are assigned the weight (100-1)/4. The highest value a category could receive is 100 in which case all other categories are weighted by zero. This procedure is repeated for each category. The number of times a given test was the Abest@ was counted. The analysis completed on the overall rank scoring showed that Thamnotoxkit F was the Abest@ 333 out of 500 simulations. Because of the variation shown between mine types this result has very little value for individual mine site operations. Therefore the analysis was then completed by mine type to determine if there is agreement with the unweighted results presented in Tables 5.7.1 through 5.7.8.

We can compare the test which performs the best with the results of the rank scoring (Tables 5.7.1 through 5.7.8) to determine the agreement with the unweighted scheme and the ARobustness of Weighting@ scheme. Agreement between the two schemes indicates that moderate deviation from the unweighted versus weighted would have little impact on the choice of the Abest@ test for all mine types except for uranium mines. For the uranium mine type the random weightings analysis would suggest that the Thamnotoxkit F is the Abest@ test.

6.0 Discussion

Since the use of toxicity tests has become an important regulatory requirement, mine managers are faced with the task of meeting end-of-pipe discharge limits for toxicity for the acute rainbow trout toxicity test. Government regulatory managers have the task of enforcing this toxicity compliance requirement. The difficulty is that few, if any, mine sites are capable of completing this test in-house, as well as regional government facilities. They are dependent on the assistance from either a contract or central toxicity testing facilities. As a result of this dependance, the results of toxicity tests are not generally available until some time after the discharge event. A toxicity test that provides immediate results, reflects the acute rainbow trout toxicity results, can be linked to chemical parameters in the effluent and can predict or be related to ecological effects downstream of the discharge point, would be a valuable tool for any mine site operation for assessing and monitoring environmental performance.

The development of kits or micro toxicity tests, such as those included in this study, attempt to address a growing awareness of the requirement for expedient monitoring and assessment of effluent toxicity. This ability of mining operations to conduct inhouse toxicity testing would allow industrial managers and regulators to provide a more immediate response to potential environmental concerns of an effluent discharge. To fulfill this void, these commercially available toxicity tests kits or microtests must be accepted by the scientific community and by government regulators as a valid and dependable assessment tool for the application which it is to be applied. The Canmet study attempts to compare various toxicity tests available on the market to address the environmental monitoring and assessment needs specifically for the Canadian mining sector.

The Canadian mining sector is composed of a variety of different mining operations, such as those included in the Canmet study. These differences include, but are not limited to, the specific ore being mined, geological formation in which the target ore is found, milling processes by which the ore is extracted, climate and the effluent treatment scheme utilized by a site. These inherent differences are probably reflected in the variation of response for effluent toxicity between each specific toxicity test, the different mine types and various mine sites. These various differences within the mining sector, and limitations of the study design, make it quite unlikely that one encompassing commercial toxicity test kit could be selected which would fulfill the requirements for acceptance and application throughout the mining sector, the scientific community and the various government jurisdictions. The Canmet study can contribute to the understanding of toxicity in mining discharges, the interactions of the various toxicity tests evaluated and variations of response between the various mine types and specific mining operations.

For the purpose of the Canmet study, toxicity tests were selected that have specific regulatory prominence which included the rainbow trout and *Daphnia magna* acute toxicity tests and micro toxicity tests which are commercially available and for which some scientific information is available. The commercially available kits included in the Canmet study were the *Daphnia magna* IQ, Microtox, Rotoxkit F, Thamnotoxkit F and Toxichromotest toxicity tests. From the onset of the project the focus has been on the comparison of the various toxicity tests to the acute rainbow trout toxicity test. However special effort has been made in this report to provide information comparing the *Daphnia magna* acute and IQ toxicity tests.

To address the needs of all stakeholders, efforts were directed towards evaluating several criteria specific to each toxicity test and comparing these results to the rainbow trout toxicity test. These criteria included costs, the speed or turn around time required for each toxicity test, the correlation of effluent chemistry to toxicity results, the reproducibility of toxicity results including intra and interlaboratory results, the applicability of each toxicity test and the comparability of the micro toxicity test results to the comparable rainbow trout toxicity test results.

For several of the evaluation criteria specific statistical tools were applied after implementation of the study design. It should be noted that for future evaluations of a similar nature it may be advantageous to consult with the group and/or individual responsible for such analysis prior to implementation of the study design. This may help to defer difficulties regarding the selection of appropriate statistical tools and incorporation of appropriate data parameters by all participants prior to implementation. Several difficulties encountered in the experimental design and project are summarized as follows:

- The limited number of samples for specific mine types and mine sites reduced the statistical power of the analysis and restricted the types of comparisons that could be made.

- The environmental parameters (analytical chemistry parameters) selected between mine sites and mine types were inconsistent. This was not realized by the group responsible for the statistical analysis until after the sampling was completed. It was assumed by this group that all mines would submit effluent samples for the same analytical parameters for each sampling period to allow valid comparison between all mine sites. A specific core group of parameters were maintained throughout the four sampling periods but others varied between sampling periods, sites and mine types. As a result some of the analytical data which was provided could not be included in the final analysis.

- The lack of toxicity results for the rainbow trout toxicity test required modification of the sampling design for the third and fourth sampling periods in order to target effluents which had a greater potential for toxicity. As a result, the selection of statistical tools, was restricted by availability of dose responses. This was associated with a concomitant loss of statistical power.

With the exception of the applicability criteria, all other evaluation criteria were ranked on a numerical score to provide a basis for comparison between toxicity tests. It became very obvious that providing an overall ranking that included all mine types provided very little value to the study. As a result summation rankings were provided for each of the mine types. This would have a greater value for mines, particularly those which did not participate in this study, to evaluate the most appropriate microtest that would be applicable to their specific situation. Results in this report, and the format in which they are presented, can provide the basis by which any particular site which participated in the study, or did not participate, can conduct their own independent evaluation specific to their application.

The costs for the toxicity tests evaluated in this report attempted to reflect the actual costs of conducting each specific toxicity test. However, when we look at the actual costs of the *Daphnia magna* acute bioassay of $137.56/test and compare these costs to the commercial laboratory rate of approximately $250/test there is a concern that costs presented in Table 5.1.4 do not accurately reflect the real costs. As previously noted these costs do not include either the overhead or profit margin incorporated into the commercial laboratory rates. By the time this is incorporated the actual costs may more than double what has been indicated in Table 5.1.4. Therefore the actual cost of conducting the microtests may be more in line with the rainbow trout and *Daphnia magna* acute toxicity tests of approximately $350 and $250/test respectively. Commercial laboratories have the added advantage of volume through put for samples and the establishment of baseline facilities equipped with the basic services and equipment. The actual overhead costs (rent, utilities, insurance, vehicles) would be specific to each facility. The costs provided should be sufficient to guide individual mine operations in determining associated costs for each of the toxicity tests evaluated.

Toussaint et al., (1995) provides the costs for the Microtox and Rotoxkit F as $62 and $333/test in 1994 U.S. dollars, respectively. These costs include the test kit costs, labour needed for preparation, running the test, clean-up, calculations, materials, equipment and overhead. The costs exclude the $21,000 one time cost for the Microtox. For comparison Toussaint et al., (1995) provides the cost for the *Daphnia magna* acute lethality assay as $703/test. Willemsen et al., (1995) summarized the costs, in U.S. 1995 dollars, for specific microtests included in their comparative evaluation. Costs for each of the microtests evaluated consistent with Canmet's study are summarized as follows:

Toxicity Test	**Cost/Test**	**Remarks**
Daphnia magna IQ	$ 50	Does not include culturing costs.
Microtox	$ 5-15	Does not include cost of machine
Rotoxkit F	$ 45	
Thamnotoxkit F	$ 45	
Toxichromotest	$ 50	

The costs provided by Willemsen et al., (1995) would appear to only cover direct material costs and does not attempt to provide the same detail as Toussanint et al., (1995) and this report, to determine the actual costs of conducting the toxicity test. Though this report attempts to include costs for QA/QC into the cost per test price, the level of QA/QC can vary from very preliminary QA/QC requirements to extensive requirements that includes affiliation with an accreditation agency such as CAEAL (Canadian Association of Environmental Analytical Laboratories). Such a membership could add between $5,000 and $10,000 per year to the operating costs of a testing facility.

It is one of the underlying assumptions of this study that a mine site would conduct a selected microtest at facilities already established by a mine operation or in a facility in close proximity. One of the understood objectives of this study is for the fast turn around of results (ie. < 1 day) allowing a more immediate investigation of discharges causing toxicity. If this assumption is met, the transport costs of a sample to a contract laboratory would not apply, except for the internal sample collection and transport costs which would also be required prior to shipment to a contract laboratory.

Three of the microtests evaluated were capable of providing toxicity results the same day. This included the *Daphnia magna* IQ, Microtox and Toxichromotest. But it should be noted that the IQ test requires maintenance of a culture for the supply of viable test organisms and the Toxichromotest proved to be one of the least responsive toxicity tests to mine effluents. Also for the Toxichromotest test the highest test concentration of 50% volume would deter use by those sites which would expect toxicity between 50% and 100% volume effluent. The capability for a rapid turnaround of results from the Microtox assay may be advantageous to some mine site operations which have developed (Compliance test Concordance) and applied an ongoing monitoring program for effluent discharges.

A problem which may be encountered by those facilities which attempted the use of a microtest is the correlation of results to compliance toxicity tests. As seen by the Canmet study it is quite likely that a toxicity result generated for a microtest may not necessarily coincide with a toxicity response for a compliance species. Once an appropriate microtest is selected by an individual mining operation it is quite likely the user would be required to conduct ongoing acute compliance toxicity testing until some concordance to the microtest and variation in effluent quality has been established. This would assist in the interpretation of the microtest toxicity results. If a mine is required to demonstrate correlation with a compliance test, then the whole idea of selecting an alternative test (other than perhaps Microtox with a large database) as part of the Canmet study be a moot issue.

Results from the literature for the testing of complex effluents and single compounds would support the notion of different responses by different organisms based upon the relative sensitivities of each test organism to specific contaminants. The Canmet study showed that the relative correlation of environmental parameters to the endpoint results varied somewhat when compared to the rainbow trout toxicity test. It should be noted that some of the environmental parameters which did correlate are not noted as contaminants of concern (ie. Ca, Mg, Conductivity, alkalinity) while others are (ie. Cu, Cr, Zn, etc.). For specific environmental contaminants of concern certain parameters (ie. alkalinity and metal toxicity and pH and ammonia toxicity) have the potential to influence the toxicity response of this specific contaminant of concern. This may account for the correlation of certain environmental parameters to toxicity results when individually that parameter would not be a concern.

Table 6.0.1 indicates which environmental parameters listed in the Canadian Water Quality Guidelines (CWQG) for the Protection of Aquatics Life (CCREM, 1987) are correlated to the various toxicity results.

Table 6.0.1 Summary of Parameters Correlated to Parameters Listed in the CWQG for the Protection of Aquatics Life

Parameter	D. magna Acute	D. magna IQ	Microtox	Rainbow Trout	Rotoxkit F	Thamnotoxkit F	Toxi-chromotest
Ag			+	+			
Al	+	+	+		+		+
Ammonia						+	
Cd			+				+
Cr	+			+		+	+
Cu			+	+			+
pH		+		+		+	
Pb				+			
Sb		+			+		
TSS						+	
Zn						+	+

The rainbow trout acute toxicity test, Thamnotoxkit and Toxichromotest correlated to 5 parameters for which a CWQG criteria exists for the protection of aquatic life. For the Thamnotoxkit two parameters were common with the rainbow trout toxicity test and for the Toxichromotest copper and chromium were in common. Interestingly the *Daphnia magna* IQ test only has three parameters for which CWQG criteria for the protection of aquatic life exists considering the number of toxicity responses encountered and the number of parameters correlated to the endpoint results.

Willemsem et al., (1995) compiled from the literature single compound toxicity results for 202 compounds for a variety of microtests including those included in this evaluation. Data was categorized based upon chemical class or theoretical mode-of-action. The inorganics are grouped as metals, miscellaneous inorganics and oxidizers. Organic compounds were classified as follows;

Class 1) Non-polar narcotics (ie. Aliphic alcohols, ketones and benzenes)
Class 2) Less inert narcotics, contains phenols and anilins.
Class 3) Aspecific reactive compounds (ie. Aldehydes, bromides and antibodies)

Class 4) Specific toxicants, contains many drug and pesticides ie. Pentachlorophenol, lindane and organophosphates.
Other) Non-classified (ie. nitroamines, dinitroaromatics, organic acids and detergents)

The relative sensitivities for single compounds summarized in Table 6.0.2 are supported by toxicity data presented in Appendix 1 of Willemsen et al. (1995). Willemsen et al. (1995) found toxicity results for 29 pure compounds in the literature representing all chemical groupings except osmotics, for the *Daphnia magna* IQ toxicity test. The IQ test was considered sensitive to metals, bichromate, class 1 and 4 organics and especially organophosphates. Willemsen et al. (1995) noted in their review of various microtests that the Microtox assay was not considered sensitive to metals. This is consistent with the Munkittrick et al. (1991) Microtox review which indicated that Microtox was Anot as sensitive to inorganic chemicals as *Daphnia magna* or the rainbow trout@. From Munkittrick et al. (1991) *Daphnia* were considered more sensitive to copper, chromium cadmium, arsenate, zinc, mercury and cobalt, while rainbow trout were more sensitive to cadmium, copper and zinc but less sensitive to mercury and arsenate compared to the Microtox assay. From the Canmet study the metals aluminum, cadmium, copper and silver were correlated to the endpoint results which is interesting considering the Microtox's documented insensitivity to several of the metals. This is not too surprising since only measured metals and some water quality parameters were analyzed. Something had to correlate with the result. This is one of the dangers of measuring everything and searching for correlations. Anyway the important point is that correlation does not imply sensitivity.

Table 6.0.2 Summarizes the Relative Sensitivities of the Microtests Included in the Canmet Study Modified from Willemsen et al., (1995).

Toxicity Test	Metals	Misc. Inorganics	Oxidizers	Organic Compound Classes				
				1	2	3	4	Other
D. magna IQ	+	0	+	++	0	0	+	+
Microtox	-	+	-	+	++	+	+	+
Rotoxkit F	+	+	0	+	-	-	+	+
Thamnotoxkit F	+	+	+	-	++		+	+
Toxichromotest	-	+	0	-		+	0	0

++ Very Sensitive: More than an order of magnitude more sensitive than all other tests to at least one compound
\+ Sensitive: Up to an order of magnitude more sensitive than all other tests to al least one compound
\- Not Sensitive
0 Insufficient Comparison
Note: Results should be treated as indications for potential response

For the Microtox assay it has been suggested that the exposure duration be increased (ie. 30 minutes) when metal toxicity is suspected. The slow acting toxic action of metals requires longer exposure for effects to be noted. During the first sampling period this potential was evaluated. Test endpoints were calculated for 15 and 30 minutes exposures for eleven sample submissions. For sample submissions which an endpoint could be calculated, no real difference in the results was noted. It should be mentioned though that the 100% testing protocol had not yet been initiated and as such a number of the results are reported as >4.95 (4 samples) and >49.5 % (5 samples) volume. Further testing using the 100% volume testing protocol would probably be required to confirm this observation. Smith (1991) found an increased toxicity by a factor of two or greater for a number of metals when using a longer exposure time (ie. 30 minutes) with NaCl, including cadmium, chromium (VI), lead, nickel, zinc and thallium. Metals for which toxicity results were unaffected by a longer exposure time included arsenic (V), selenium (IV), copper.

Willemsen et al. (1995) summarized 77 compound results for the Rotoxkit F and 40 compound results for the Thamnotoxkit F. The Rotoxkit F included results for all chemical classes, however the Thamnotoxkit F data was lacking for class 3. The Thamnotoxkit F was found to be generally more sensitive than the Rotoxkit F. For the Toxichromotest, Willemsen et al. (1995) summarized the available data for 30 compounds (from Reinhartz et al., 1995) which represent all classes except class 2 organics. The Toxichromotest was considered sensitive to some inorganics and class 3 organics. A comparative table of toxicity results for various metals for the toxicity tests evaluated in the Canmet study has been summarized from the literature and provided in Appendix D. The results presented in the summary table are single compound results and do not take into account the potential for synergistic, additive or antagonistic effects of the chemical matrix of a mine effluent discharge (Qureshi et al., 1983, Sellers and Ran, 1985 and Michaud et al., 1990: from Smith 1991).

Janssen and Persoone (1993) found the *Daphnia magna* IQ test to be substantially more sensitive than the Microtox assay for copper (170 x), cadmium (1216x), zinc (110x) and chromium (3457x) and similarly sensitive to mercury. Terrell et al.(SETAC Presentation) compared the Microtox and *Daphnia magna* IQ toxicity tests to the *Daphnia magna* acute toxicity test comparing results of seven common pesticides. The correlation coefficients comparing the IQ and Microtox to the *Daphnia magna* acute toxicity test were 0.87 and 0.13, respectively.

Janssen et al., (1993) compared the *Daphnia magna* IQ test to the *Daphnia magna* acute toxicity test for nine chemicals and several complex effluents. For testing of the nine compounds the R^2 values comparing the 1 hr EC50's for the IQ test and the 24 and 48 hour EC50's (immobility) are 0.98 and 0.96 respectively indicating relatively good correlation. No significant correlation was found with the testing of the complex effluents. In six of the seven cases the IQ test was considered more sensitive than the acute toxicity test. Janssen et al., (1993) found that 90% of the acute results were within a factor of four of the IQ test, by combining previous single compound testing (Janssen and Persoone, 1993). It was concluded that the *Daphnia magna* acute and IQ toxicity tests are of an approximately similar sensitivity. In another study (Aqua Survey) comparing the *Daphnia magna* acute and IQ toxicity tests, 5 single compounds, including cadmium, copper and mercury, and 4 effluents were tested. The IQ results were considered in the same order of magnitude with correlation coefficients of 0.93 for the pure compounds and 0.88 for the complex effluents.

It has been noted that sample salinity could influence toxicity of some metals in the Microtox assay (Hinwood and McCormick, 1987, Vasseur et al., 1986 and Ankely et al., 1989). For the Microtox assay either NaCl or sucrose can be used for osmotically adjusting the samples (Environment Canada, 1992). For the Canmet study during the first sampling period 6 samples were subjected to both NaCl and sucrose and both 15 and 30 minute endpoints determined. For the sucrose adjustment all results were reported as >49.5% volume for both exposure periods. For the NaCl adjustment 2 samples provided calculable endpoints which were similar for the two exposure periods. All other results using NaCl were reported as >49.5%. Ankely et al., (1990) found that single toxicant concentrations for zinc and nickel were more toxic when tested with sucrose instead of NaCl. It was also suggested that the use of sucrose may not always increase the toxicity of cationic metals as was the case with copper (Ankely et al., 1990). Of the four mine sites evaluated by Ankley et al., (1990) testing with the Microtox assay was conducted using either NaCl or sucrose for osmotically adjusting the sample for three of the sites. Two of the three EC20 results showed an increased sensitivity to effluent toxicity using NaCl while no endpoint was determined for the third mine site. In a study of metal toxicity in drinking water Smith (1991) found that use of sucrose enhanced the toxicity of certain metals including cadmium, chromium, lead, selenium IV, thallium, nickel and zinc. The use of sucrose made no real difference for either copper, mercury of selenium VI. Arsenic was more sensitive when using NaCl. Smith (1991) also noted the formation of a precipitate when using NaCl for lead and thallium. Ankley et al., (1990) indicated "that sucrose should not supplant NaCl for the osmotic adjustment for freshwater samples; if anything, it should be used only in conjunction with NaCl. Based on the effluents evaluated in this study, the use of sucrose alone would have resulted in a poorer correlation between the results of Microtox tests and fathead minnow or *Ceriodaphnia dub*ia than the use of NaCl alone."

As the presence of the chloride ion, from the NaCl osmotic adjustment agent in the Microtox test can cause decreases in sensitivity for metal toxicity Carlson-Ekvall and Morrison (1995) conducted a study to evaluate various alternative osmotic adjustment agents. Of the four alternative osmotic adjustments evaluated by Carlson-Ekvall and Morrison (1995) found that in the presence of $NaClO_4$ most metal ions were soluble not forming complexes with the chloride ion. "Neither Cu, Cd, Pb, Zn nor any other metal of environmental concern forms a complex with the ClO_4^- ion, which effectively makes $NaClO_4$ the most suitable osmotic surrogate for metal toxicity testing. Also, $NaClO_4$ showed the highest sensitivity to metals, with exception of Zn" (Carlson-Ekvall and Morrison, 1995). Comparing single compound toxicity results for NaCl and $NaClO_{4,}$ cadmium was found to be 17.4x, copper 2.3x and lead 3.9x more toxic using $NaClO_4$. No comparative toxicity evaluations have been conducted with $NaClO_4$ comparing results to other micro or kit toxicity tests similar to that included in this evaluation.

For the Microtox assay, Vasseur et al., (1986) found that sensitivity to zinc and cadmium increased at a temperature of 20°C and an exposure time of 30 minutes. Metal toxicity for zinc and cadmium were decreased through the addition of NaCl and calcium (increased hardness). Vasseur et al. (1986)

suggested that the salinity content of an environmental sample be determined prior to conducting the Microtox assay in order to adjust it to the recommended 2% NaCl level instead of systematically adding a standardized amount of sodium chloride to each sample.

A number of comparative studies have been completed for single compound and complex effluent matrices. Summaries of several comparative studies have been provided in Table 6.0.3. In a comparative study using the Microtox, fathead minnow, and *Ceriodaphnia* assays on 4 mine effluents of unknown type, the relative sensitivity was found to be approximately equal (Ankley et al., 1990) with an EC20 endpoint calculated for the Microtox assay. Munkittrick et al. (1990) summarized a number of comparative studies of complex effluents (Neiheisel et al., 1983, Bulich, 1982, Calleja et al., 1986, Qureshi et al., 1986, Dutka and Kwan, 1981, Blaise et al., 1987 Qureshi et al., 1982, Vasseur et al., 1984 and Bulich et al., 1981) with the Microtox. Results of this review have been reformatted and included in Table 6.0.3.

Table 6.0.3 - Continued

Table 6.0.3 Relative Sensitivity of Tests

Comparison of A/B	Type of Effluent	Sensitivity	Other	Reference
Microtox/(Fathead minnow, trout, *Daphnia*)	pure organic compounds, municipal wastes, highly toxic industrial waste	approximately equal		Munkittrick et al, 1991
Microtox/(Fathead minnow, trout, *Daphnia*)	inorganic toxicants, pesticides	Microtox less sensitive to metals than fathead minnow, trout, and *Daphnia*		Munkittrick et al, 1991
Microtox/(Fathead minnow, *Ceriodaphnia*)	mining effluent	approximately equal	4 mines of unknown type	Ankley et al, 1990
D. magna / Thamnotoxkit F	domestic and industrial effluent (Austria)	correlation=0.873	Of 16 samples, 6 were acutely toxic to T. platyurus but not D. magna.	Persoone et al., 1994
D. magna / Thamnotoxkit F	industrial effluent (Flanders, Belgium)	correlation=0.883		Persoone et al., 1994
Microtox/Rotoxkit F	Cd, Cu, Ni, Zn	Rotoxkit more sensitive than Microtox		Ross et al, 1991
Microtox/ *D. magna* Acute	14 Inorganic & organic compounds	Microtox <Rainbow trout< *D. magna* Acute		DeZwart and Sloof, 1983
Microtox/Rotoxkit F/ *D. Magna* Acute	Zn, Cu, Cd	Rotifer more sensitive than Microtox for Zn & Cd, not Cu	Single Compound	Toussaint et al, 1995
Microtox/Rotoxkit F/ *D. Magna* Acute	Inorganics and organics	-Rotifer similar sensitivities to the acute -Microtox sensitivity fell very close to the Standard Acute	Single Compound	Toussaint et al, 1995
Microtox/Pseudomonas fluorescence/Baker=s Yeast	metals	Microtox most sensitive for detecting toxicity of Zn, Cu & Hg but not Cd, Cr, & Ni		Codina et al, 1993
Microtox/ *D. Magna* IQ	Cu, Cd, Zn, Cr, Hg	IQ more sensitive than Microtox for Cu, Cd, Zn, & Cr, equal for Hg		Janssen & Persoone

Table 6.0.3 - Continued

Comparison of A/B	Type of Effluent	Sensitivity	Other	Reference
Microtox/ *D. magna* IQ/*D. magna* acute	Pesticides	Correlation DM acute and IQ = 0.87 Correlation Microtox and IQ = 0.13		Terrel et al. 1991
Daphnia magna Acute & IQ	20 pure compounds	r^2 = 95%, similar sensitivity		Janssen et al., 1993
Daphnia magna Acute & IQ	Complex Effluents	IQ more sensitive 6 of 7 cases		Janssen et al., 1993
Rainbow trout/ Microtox/ Fathead minnow	Wastewater + 16 unidentified organics	Range of Microtox EC50's lower than Fathead minnow and *Daphnia*		Neiheisel et al., 1983
Fish/Microtox/*Daphnia*	257 Complex Effluents	Microtox/fish 89-87% agreement Microtox/*Daphnia* 85-75% agreement[a]		Bulich, 1982
Microtox/*Daphnia*	Complex Effluents	77-91% agreement		Calleja et al., 1986
Microtox/ Rainbow trout/ *Daphnia*	Complex Wastes	Relative sensitivity varied between effluent types		Qureshi et al., 1982
Microtox/ *Daphnia* / Fathead minnow	Complex Effluents	Microtox predicted 81% of samples toxic to Fathead minnow and 62% of those toxic to *Daphnia*		Dutka and Kwan, 1981
Microtox/ Rainbow trout	Pulp and Paper Wastes	Rainbow trout positive 46/55 times, Microtox 43/51; rank agreement rainbow trout:Microtox >84%, class agreement[b] 70% within 0.5 log class; Microtox 4 times as sensitive as rainbow trout		Blaise et al., 1987
Microtox/ Rainbow trout/ *Daphnia*	Pulp and Paper Waste	Microtox 4-8 times more sensitive than trout, 10 times more than *Daphnia*	Two effluents examined	Qureshi et al., 1982
Microtox/ *Daphnia*	Industrial Effluents	*Daphnia* positive 22/39 times; Microtox 19/39 and in agreement 86% of the time		Vasseur et al., 1984b
Microtox	Complex Effluents	Microtox less sensitvie to CN, urea ethanol, NH_3		Bulich et al., 1981

Table 6.0.3 - Continued

a - Agreement based on toxic/nontoxic designation at 25% or 50% lethality. Based on a comparison of percent rank, Microtox - fish agreement was 78%, Microtox - Daphnia 63%, Fish - Daphnia 69%

b - Ranked as toxic (LC50 <25%), slightly toxic (LC50 25-100%) or on class log interval (<1%; 1-3.2%; 3.2-10%; etc.).

Bulich (1982) found in the testing of 257 complex effluents, fish and the Microtox toxicity were 87-89% in agreement and *Daphnia* and Microtox 75% -85% in agreement. In a study of pulp and paper wastes (Blaise et al., 1987), Microtox test was found to be 4 times as sensitive as rainbow trout acute toxicity test. In another study of pulp and paper waste the Microtox was 4-8 times more sensitive than rainbow trout and 10 times more sensitive than the *Daphnia* acute toxicity test.

Codina et al., (1993) in their work comparing the sensitivity of Microtox, *pseudomonas fluorescens* and baker=s yeast, found that the sensitivity of metals decreased in wastewaters, which was correlated to the presence of other organic and inorganic compounds. This has the potential to reduce the bioavailability of the metals and therefore decrease associated toxicity or sensitivity to these contaminants.

In an evaluation comparing the sensitivity of the Microtox assay to the rainbow trout and daphnid acute lethality tests the *Daphnia* was found to be more sensitive than Microtox for ammonia (1.9 to 28x more sensitive) and cyanide (2.2 to 28x) (EVS, 1989). The rainbow trout was more sensitive than Microtox for total ammonia (58x) and cyanide (89x). Both ammonia and cyanide are potential toxicants that can be found in the effluents of specific mine types (ie gold) and which would be a concern of certain mining operations. EVS (1989) found the Microtox was not as sensitive to metals (inorganics) as the *Daphnia* or the rainbow trout acute lethality toxicity tests. EVS (1989) summarized that *Daphnia* were more sensitive than Microtox to copper (60 to 370x), chromium (100x), cadmium (>60x), arsenate (65x), zinc (2.0 to 96x), mercury (1.0 to 2.7x) and cobalt (1.2x). The Rainbow trout acute lethality test was more sensitive to cadmium (400x), copper (30x) and zinc (22x) but less sensitive to mercury (0.38x) and arsenate (0.81x) (EVS, 1989).

For the rotifer toxicity test Snell et al., (1991) showed that temperatures higher and lower than 20°C resulted in greater sensitivity for the reference toxicants copper and sodium pentachlorophenol. They also found that control organisms began to die at 30 hours, thus limiting the duration of the test to 24 hours. Cysts ranging from 0 to 18 months exhibited a similar sensitivity to the reference toxicants. Ross et al., (1991) found that the Rotoxkit F was more sensitive to cadmium, copper, nickel and zinc compared to results of the Microtox assay. Toussaint et al., (1995) using zinc, copper and cadmium found the Rotoxkit F was more sensitive than the Microtox assay and less sensitive than the *Daphnia* acute toxicity test for zinc and cadmium. Toussaint et al., (1995) also found the Rotoxkit F was more sensitive to copper compared to both the *Daphnia* acute and Microtox toxicity tests.

One of the primary prerequisites for acceptance of a toxicity test is the test=s ability to reproduce results. AThe ability of any test method to provide reproducible data within and among laboratories must be known before the method is adopted for regulatory purposes@ (Grothe and Kimerle, 1995). To determine the precision of a toxicity test one can look at both the intra (within) and inter (between) laboratory results.

In a round robin evaluation of the *Daphnia magna* acute toxicity test in 1980 using single compounds the intralaboratory results showed a high degree of variability while the interlaboratory extremes differed by only a factor of 2 (Lemke, 1981). Variability in the metal compound was attributed to differences in dilution water quality such as hardness. In a second *Daphnia magna* round robin evaluation (Grothe and Kimerle, 1985) additional variables were controlled including dilution water. In this particular case, using a process and waste stream effluent, the pooled intralaboratory CV was 16% for the 48 hr endpoint. Based on the interlaboratory results Grothe and Kimerle (1985) indicated that for the A*Daphnia magna* static, acute, effluent toxicity test data can be reproduced to within a factor of 2.6 among laboratories@. In another round robin evaluation (PACE, 1989) the interlaboratory CV ranged from 9.7 to 20.2% for three laboratories testing complex wastewater and effluent samples. The range of CV=s reported for algal feed daphnids in other interlaboratory comparisons are from 16% to 21% (Grothe and Kimerle, 1985). Variations between standard toxicity tests has been attributed to nutrition, diet and health of daphnids (Cowgill, 1987). Results of the interlaboratory comparisons for the daphnid acute toxicity tests indicates the susceptibility of the toxicity test to water quality, variations in laboratory procedures and nutritional differences between laboratories as sources of variability in the daphnid acute toxicity test.

In a series of toxicity tests conducted by Janssen and Persoone (1993) the reported intralaboratory precision of the IQ bioassay compared to the daphnid standard acute toxicity test was considered quite acceptable with CV=s for replicate tests (n=3) ranging from 3% to 32% while the *Daphnia magna* acute ranged from 5% to 41%. Janssen et al., (1993) found the intralaboratory precision of the IQ tests (n=2) conducted on pure compounds to have CV=s ranging from 10 to 24% and in comparison to the *Daphnia* acute toxicity test 1% to 14% for the 48 hour endpoint. Janssen et al., (1993) found the IQ precision with effluent testing from a pharmaceutical company, was considerably lower than the pure compound testing, with CV=s ranging from 20 to 43% for the IQ test and the *Daphnia* acute CV=s ranged from 5 to 20%. Aqua Survey (1993) found the intralaboratory CV=s for single compounds ranged from 3% to 32%. Terrell et al. (Setac Presentation) found the intralaboratory CV=s using 7 pesticides for the IQ test ranged from 0.0% to 19.8%, Microtox assay from 1.2% to 24.3% and for the *Daphnia magna* 48 hour acute 2.7% to 59.6%. Intralaboratory CV=s for 5 specific metals, which may be of interest to the mining sector, have been summarized from Janssen and Personne, (1993) and Aqua Survey (1993) for the IQ toxicity test and the *Daphnia magna* acute toxicity test.

	Daphnia magna IQ CV=s		*Daphnia magna* Acute CV=s
	Janssen and Persoone, 1993	Aqua Survey, (1993)	Janssen and Persoone, 1993
Copper	18%	17%	15%
Cadmium	19%	11%	19%
Zinc	21%	11%	14%
Chromium	15%	7%	8%
Mercury	29%	21%	10%

Hayes et al., (1993) found in a comparative study between laboratories using water treatment chemicals the IQ test intralaboratory CV=s ranged from 0.0% to 39.8% and interlaboratory CV results 3.5% to 96.2%. In a 16 laboratory study (6 laboratories had prior experience with the IQ test) using the IQ toxicity test and the toxicant $CuSO_4$, the interlaboratory CV was 58% and the intralaboratory CV ranged from 4.7% to 28.1% (Aqua Survey Inc, 1993). ASTM (1994) indicates the source of the *Daphnia magna* strain used in the IQ test did not significantly effect the data for the standard toxicant $CuSO_4$ from the 16 laboratory study in which daphnids were obtained from eleven different cultures. In the 16 laboratory study water hardness ranged from 60 to 130 ppm ($CaCO_3$) and did not significantly influence results.

The reproducibility of the Microtox test has been studied extensively. Interlaboratory CV's reported by Green et al., (1985) varied from 16.5 to 133% for a variety of compounds. The coefficient of variation is considered lower compared to other toxicity tests because of the highly formalized standards, reagents (McFeters et al., 1983) apparatus and procedures. A higher variability for Microtox has been indicated by Reteuna et al., (1980) for metals compared to organic toxicants. Like most toxicity tests, the variability of the Microtox test decreases as the toxicity of a sample increases (Geen et al., 1985).

An international intercalibration exercise was conducted using several of the commercially available toxkits including the Rotoxkit F. For the Rotoxkit F, 120 laboratories participated producing a CV of 48.5% (Persoone et al., 1993) using $CuSO_4H_2O$. In this study participating Canadian labs had a CV of 27%. Results of this exercise has helped the producers of the toxkit to refine and further standardize the procedure and cyst hatchability success (Personne et al., 1993). Further experience with the toxkit by individual testing labs would probably reduce the interlaboratory CV's. Persoone (1991) indicated that intralaboratory precision of their various toxkits with various chemicals was better than 20% for a CV value. This was considered to be satisfactory. The precision of the Rotoxkit is discussed by Persoone (1992) through a comparison with the *Daphnia magna* acute bioassay for the toxicant, potassium dichromate. This evaluative testing was conducted by both student and scientists. The CV results are as follows:

	Students	Scientists
Daphnia magna	37	19
Rotoxkit F	33	14

The CV for 23 Thamnotoxkit F toxicity tests using the toxicant potassium dichromate by several different operators was 25.9% and for the same operator 15.5% (Aquasense, Holland personal communication, sited in Persoone et al., 1993). At this time there does not appear to be any comparable interlaboratory results from the literature for Thamnotoxkit. From the Canmet study interlaboratory results for the mine effluent samples ranged from 8.7 to 21.6% for the Thamnotoxkit F toxicity test.

No apparent intra or interlaboratory CV results could be found in the literature for the Toxichromotest. From the Canmet study no endpoint results could be calculated for all split samples with the QC laboratory and as such interlaboratory results are unavailable for the Toxichromotest. On the other hand intralaboratory results using the reference toxicant mercuric chloride produced a CV of 45% for 22 tests. This was considered a weak or poor CV result by the contract laboratory (BEAK, 1995).

Table 6.0.4 summarizes the intra and interlaboratory CV results for the Canmet study for comparison with CV values discussed from the literature.

Table 6.0.4 Summary of the Intra and Interlaboratory CV Results from the Canmet Study

Toxicity Test	Interlaboratory			Intralaboratory	
	No.[1]	Range CV=s	Average CV	Reference Toxicant	Average CV
Rainbow Trout Acute	4	1.1-8.9%	5.8%	Phenol	10.7%
Daphnia magna acute	1	4.6%	4.6%	NaCl	1.9%
Daphnia magna IQ	6	4.8-61.6%	28.2%	-	-
Microtox	1	4.6%	4.6%	$ZnSO_4$	21%
Rotoxkit F	0	-	-	$K_2Cr_2O_7$	30.5%
Thamnotoxkit	3	8.7-21.6%	14.1%	$K_2Cr_2O_7$	28%
Toxichromotest	0	-	-	HgCl	45%

1 - Number of split samples which produced calculable endpoint results with QC laboratory.

Overall, from the Canmet study, the CV=s for the interlaboratory and intralaboratory results are considered in the realms of biological testing with the exception of two possible concerns. The first is the interlaboratory results for the *Daphnia magna* acute toxicity test with the QC laboratory. In three instances the contract laboratory did not produce a calculable endpoint and the QC laboratory did. One possible reason for the difference is in the water hardness which has been noted to effect the toxicity of specific toxicants particularly metals. The contract laboratory has a relatively hard dilution/culture water while the QC laboratory has a medium hardness (Westlake Person. Comm.). The second potential concern is the relative large range in CV=s for the *Daphnia magna* IQ toxicity test. This may also be attributed to differences in water hardness but could be a result of the subjectivity of the determination of an effect. As indicated by BAR (1995) ASince the degree of fluorescence is based on visual observations only, subjectivity in endpoint measurements may result in variable test results, both within and between laboratories.@ BAR (1995) found that slight differences occurred in the final visual observations on identical samples between technicians which may increase variability.

Blaise et al., (1988) indicates a need for Asimple, rapid and relatively inexpensive aquatic toxicity tests (ie. screening tests) to provide data and results that can be used as an indicator of toxicity measured by prescribed Alegal@ tests such as the acute lethality rainbow trout and daphnid assays@ (Munkittrick et al. (1991). The purpose of the Canmet study was to evaluate several micro or kit toxicity tests for several evaluation criteria in comparison to the rainbow trout acute toxicity test. Based on discussions within this report and specific mine site=s needs, a decision regarding the application of the micro or kit toxicity test must be made. As mentioned earlier the potential application of the micro or kit toxicity tests are several fold including the screening of chemicals or effluent for toxicity, monitoring of effluent discharges for environmental performance particularly in remote locations and/or application for regulation.

From a regulatory view the rainbow trout toxicity test has achieved national notoriety as an accepted compliance toxicity test by Federal and Provincial jurisdictions. The daphnid acute toxicity test is also utilized in Canada for toxicity compliance in several jurisdictions. The rainbow trout and daphnid toxicity tests are both utilized for compliance toxicity requirements of Ontario=s recently promulgated Clean Water Regulation for various industrial sectors. The luminescent bacteria toxicity test or Microtox toxicity test has been specified for use by an industry in at least two Canadian provinces (Environment Canada, 1992). The other toxicity tests included in the Canmet study have not been used for regulatory compliance testing. This may be a function of their recent emergence onto the commercial market and lack of published literature and supporting technical documentation. In comparison the Microtox has a wealth of technical documentation, published Federal protocol and in specific instances has been utilized by industry for the monitoring of effluent discharges. It is quite likely that government organizations would not fully endorse commercial toxicity tests, such as those included in the Canmet study, for specific regulatory application without justification to increased scrutiny, experience, correlation with other tests, determination of ecological relevance, etc. These tests do not yet have a history. Such an endorsement may be perceived as providing exclusive marketing rights to a national market.

One of the most obvious results of the Canmet study is that no one toxicity test compares directly with the rainbow trout toxicity test for both the detection of toxicity and correlation to chemistry. This is not surprising since it is well understood that different test organisms respond differently to chemicals and/or chemical matrices either showing increased or decreased sensitivity in comparison to other test organisms. This observation was particularly evident in the Canmet survey where differences in toxicity and test response were noted between the various mine types. These differences suggest that no one toxicity test would encompass and detect toxicity in all mining effluents. Van der Wielen et al., (1993) in a multispecies toxicity (standard and micro/kit toxicity tests) assessment of effluent from three pharmaceutical plants, found that each "had an own toxicity spectrum, reflecting the different types of chemicals produced as well as the different treatment procedures of the effluents".

For the rainbow trout and *Daphnia magna* acute toxicity test, the lack of comparability and correlation to the same environmental parameters in the Canmet study supports their application as joint compliance toxicity tests such as in the Ontario Clean Water Regulation. Both toxicity tests are of ecological relevance but respond differently to the same chemical matrices allowing the detection of toxicity that normally would not be detected through the use of a singular test. Several studies indicate that no one toxicity test can always detect the presence of toxicity within an effluent discharge (Willemsen et al., 1995, Calleja et al., 1994, Van der Wielen et al., 1993, CPPI 1992, Freeman, 1986, Qureshi et al., 1982), supporting the concept of a battery of tests for detecting effluent toxicity is required.

From this discussion it becomes obvious that users of any toxicity test evaluated in this study would have to decide on the usefulness of the test procedure for the application in which the test results will be utilized. This report only provides guidance to the user in that decision process. It is quite likely that prior to application of a toxicity test the user or mine site would have to provide sufficient justification and technical data supporting use of the test procedure to corporate environmental managers and/or government regulators. This would in all probability include completion of a comparative study with the standard acute toxicity tests in order to assess the applicability of a toxicity testing procedure specific to an application. If an inexpensive alternative bioassay were available to screen wastewaters, many more samples could be processed with more extensive testing only required when a specific screening criteria has not been achieved (Arbuckle and Alleman, 1992).

From the Canmet study and based upon the evaluation criteria used, three toxicity test procedures were considered the Abest@ procedure depending on mine type (Tables 5.7.1 through 5.7.8). The Thamnotoxkit was considered the Abest@ toxicity test procedure for the gold, copper/zinc and zinc mine types while the *Daphnia magna* IQ test procedure was considered the best for the bitumen, nickel/copper, and tin mine types. Surprisingly, the *Daphnia magna* acute toxicity test was considered the Abest@ toxicity test for the lead/zinc and uranium mine types. In this instance the Thamnotoxkit was considered the next Abest@ test for the lead/zinc mine type and the *Daphnia magna* IQ and Rotoxkit were tied as the next Abest@ procedure for the uranium mine type. In any event when either the *Daphnia magna* IQ or Thamnotoxkit were selected as the Abest@ test, the next best test was the reciprocal procedure.

The IQ and Thamnotoxkit were found to be the most responsive toxicity test procedures of the Canmet study detecting toxicity in the greatest number of samples. One of the underlying assumptions of the technical advisory committee is that a more responsive toxicity test in comparison to the rainbow trout toxicity test would be a better indicator of toxicity in a mine effluent discharge. Though both of these test procedures meet this assumption the Thamnotoxkit endpoint results were found to be correlated to a greater number of environmental parameters of potential concern in comparison to the *Daphnia magna* IQ toxicity test (Table 6.0.3). When the IQ and Thamnotoxkit results are compared the concordance or proportion of results correctly predicted (positive and negative results for the detection or absence of toxicity) is 52.3%. This concordance value would suggest that results of the IQ test would only reflect the Thamnotoxkit results 52.3% of the time.

The IQ procedure has the potential to provide results the same day and does utilize a test organism which is a sentinel of a compliance standard acute toxicity test. The drawback of the IQ test is that at present a daphnid culture must be maintained to provide viable test organisms for the test procedure. Hayes (Person. Comm., 1995) has indicated that research is being conducted that would provide daphnid ephippia with the IQ test kit. If this research is successful then maintenance of a daphnid culture would not be required. Therefore, the IQ toxicity test kit provided would be similar to that of the cyst based Thamnotoxkit and Rotoxkit toxicity kits. However, the Daphnia ephippia takes 3-4 days to hatch/culture from cyst (Persoone, Person. Comm., 1996). On the other hand, though the Thamnotoxkit requires only minimal pre-culturing of the cysts, which accompany the test kits, results are not provided for 24 hours. This lag in response may not address a mine site=s requirement for a quick turnaround in results.

Based on the evaluation criteria the Microtox was not the preferred toxicity test for any of the mine types. The highly standardized Microtox test, availability of technical reference material, ability to provide results quickly and high concordance (presence or absence of toxicity) (Munkittrick et al., 1991) with the rainbow trout test does make this assay procedure attractive for use at mine sites. However such things as the initial capital costs, insensitivity to various metals in comparison to the standard acute toxicity tests as well as other parameters such as ammonia and cyanide and reduced ecological relevance may deter its application with mining effluents. If the initial capital costs are acceptable it is suggested that application of this toxicity test procedure would have to be determined on a case by case basis in comparison with standard acute toxicity tests regarding its applicability for a specific application. This is no different than other toxicity tests included in the evaluation which the ranking of the evaluation criteria have determined to be the Abest@ toxicity test for a specific mine type.

It should be noted that two new toxkits,the Daphtoxkit F and and the Algaltoxkit F, recently commercially released, were not available at the initiation of the Canmet study for inclusion in the comparison of toxicity tests. (Persoone, Person. Comm., 1996)

The following points summarize the main conclusions of the Canmet study:

- No one toxicity test compared directly with the rainbow trout toxicity test for both comparability of toxicity response and correlation of endpoint results to chemistry.

- Based upon the evaluation criteria the "best" toxicity test varied depending on mine types. The "best" toxicity test varied between the Thamnotoxkit, *Daphnia magna* IQ and *Daphnia magna* acute toxicity tests depending on mine type.

- When either the *Daphnia magna* IQ or Thamnotoxkit were selected as the Abest@ test the next best test was the reciprocal procedure. When the *Daphnia magna* acute toxicity test was selected as the "best" the next selection included either the IQ or Thamnotoxkit procedure.

♦ From the results it has become quite obvious that the applicability of the "best" test for a specific application has to be assessed on a case by case basis. Results of the Canmet study provide direction in this assessment and would be of added value for the justification of a specific toxicity test procedure to corporate environmental mangers and/or government regulators. The use of a micro and/or kit toxicity test should be given preference only if the test procedure(s) "do not imply a loss in toxicity detection power" (Van der Wielen et al., 1993).

♦ The *Daphnia magna* IQ and Rotoxkit demonstrated an increased toxic response for gold mine effluents compared to the rainbow trout toxicity test. This increased toxicity response may be a result of a specific toxicant(s) (ie. cyanide) characteristic of gold mine effluents which should be investigated further.

♦ The highly standardized Microtox test, availability of technical reference material, ability to provide results quickly does make this assay procedure attractive for use at mine sites. But such things as the initial capital costs, insensitivity to various metals and reduced ecological relevance may deter its application with mining effluents. Use of the Microtox would have to be assessed on a case by case basis with regard to its applicability to address a specific application.

♦ If the use of the Microtox is considered, further effort should be given regarding the use of a sucrose/NaCl or $NaClO_4$ instead of NaCl as an osmotic adjustment agent because of indications from the literature that sucrose and $NaClO_4$ enhances the Microtox's sensitivity to specific metals. Consideration should also be given to the use of a longer exposure time (ie 30 minutes) and/or incorporation of the highest test concentration (ie 99% volume) as most practical, particularly if the effluent does not normally demonstrate toxicity or is marginally toxic using the traditional standard acute lethality toxicity tests.

♦ At present the micro or kit toxicity test kits evaluated in this study do not have any QA/QC requirements. The "lack of a standardized QA/QC program may lead to questions about quality and reliability " (BAR, 1995) of results generated. Use of any one of these toxicity test procedures would require the inclusion of specific QA/QC procedures (ie. reference toxicants, duplication, reporting requirements, etc.) in order to provide credibility to results particularly if results are to be used as a replacement for the standard acute toxicity tests.

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APPENDIX A

ENVIRONMENTAL PARAMETER DATA BY MINE SITE

Table A1 - **Physical/Chemical Data for Bit Mine Type (Site 1) (Number in Parentheses is the Canmet Sample Number)**

Parameter	Units	Site 1			
		Period 1 (9)	Period 2 (19)	Period 3 (35)	Period 4 (61)
pH		7.95	7.92	8	7.99
conductivity	μmho/cm	1911	1808	1833	1852
ammonia	mg/L	1.85	1.66	1	2.4
alkalinity	mg/L	900	847	888	906
total hardness	mg/L	90	102	100	86
total suspended solids	mg/L	2	2	<1.0	5
total dissolved solids	mg/L	1300	1300	1304	1272
oil and grease	mg/L	5	56.7	65	49
ICP scan - Total Metals					
silver	μg/L	<20	<20	25	<20
aluminum	μg/L	311	295	457	197
arsenic	μg/L	<100	<100	<100	<100
barium	μg/L	91	98	100	77
beryllium	μg/L	<5	<5	<5	<5
bismuth	μg/L	<50	<50	<50	<50
calcium	μg/L	25900	29520	25483	21590
cadmium	μg/L	59	<10	<10	<10
cobalt	μg/L	26	<10	19	<10
chromium	μg/L	<10	11	<10	56
copper	μg/L	11	<10	17	17
iron	μg/L	2008	1970	2165	2020
gallium	μg/L	<50	142	<50	<50
potassium	μg/L	5770	6600	7020	5808
lithium	μg/L	253	46	123	100
magnesium	μg/L	8080	6860	8718	7682
manganese	μg/L	354	350	373	337
molybdenum	μg/L	<20	<20	74	21
sodium	μg/L	448000	600000	457560	401500

nickel	**μg/L**	**<20**	**<20**	**<20**	**38**
phosphorus	**μg/L**	**<100**	**876**	**<100**	**167**
lead	**μg/L**	**<100**	**<100**	**<100**	**<100**
strontium	**μg/L**	**186**	**185**	**192**	**177**
vanadium	**μg/L**	**<10**	**<10**	**<10**	**<10**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**
zinc	**μg/L**	**158**	**75**	**<10**	**<10**
ICP Scan - dissolved metals					
silver	**μg/L**	**<10**	**<10**	**<10**	**<20**
aluminum	**μg/L**	**<10**	**<10**	**48**	**<10**
arsenic	**μg/L**	**199**	**112**	**109**	**<100**
boron	**μg/L**	**1290**	**1495**	**1570**	**1514**
barium	**μg/L**	**97**	**102**	**101**	**81**
beryllium	**μg/L**	**<5**	**<5**	**<5**	**<5**
bismuth	**μg/L**	**<50**	**<50**	**<50**	**<50**
calcium	**μg/L**	**24600**	**27750**	**23580**	**22690**
cadmium	**μg/L**	**<10**	**<10**	**<10**	**<10**
cobalt	**μg/L**	**21**	**<10**	**<10**	**<10**
chromium	**μg/L**	**<10**	**<10**	**<10**	**12**
copper	**μg/L**	**<10**	**<10**	**<10**	**<10**
iron	**μg/L**	**1850**	**1841**	**1769**	**1560**
gallium	**μg/L**	**<50**	**<50**	**<50**	**<50**
potassium	**μg/L**	**5240**	**4110**	**7100**	**7210**
lithium	**μg/L**	**22**	**<5**	**132**	**105**
magnesium	**μg/L**	**7600**	**7330**	**9220**	**7966**
manganese	**μg/L**	**368**	**367**	**394**	**349**
molybdenum	**μg/L**	**29**	**<20**	**39**	**33**
sodium	**μg/L**	**460000**	**592000**	**450600**	**415900**
niobium	**μg/L**	**<20**	**<20**	**<20**	**<20**
nickel	**μg/L**	**29**	**<20**	**<20**	**<20**
phosphorus	**μg/L**	**239**	**<100**	**<100**	**159**
lead	**μg/L**	**<100**	**<100**	**<100**	**<100**

Table A1 - Continued

antimony	**μg/L**	**<50**	**<50**	**<50**	**<50**
silicon	**μg/L**	**5670**	**5370**	**5790**	**6025**
tin	**μg/L**	**253**	**265**	**255**	**314**
strontium	**μg/L**	**194**	**195**	**204**	**186**
titanium	**μg/L**	**<10**	**<10**	**<10**	**<10**
vanadium	**μg/L**	**<10**	**<10**	**<10**	**<10**
tungsten	**μg/L**	**<50**	**<50**	**<50**	**<50**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**
zinc	**μg/L**	**<10**	**<10**	**<10**	**<10**
zirconium	**μg/L**	**<10**	**<10**	**<10**	**<10**

Table A1 - Continued

Table A2 - Physical/Chemical Data for Lead/Zinc Mine Type (Site 2) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 2				
		Period 1 (8)	QA/QC (8)	Period 2 (32)	Period 4 (40)	Period 4 (62)
pH		10.9	11.2	11	6.79	7.39
conductivity	μmho/cm	1565	1760	1711	1591	1588
ammonia	mg/L	1.45	2	1	<0.1	0.3
alkalinity	mg/L	81	57.1	90	12	20
total hardness	mg/L	724	872/873	893	912	806
total suspended solids	mg/L	15	9.87	9	10	10
total dissolved solids	mg/L	1380	1492	1320	850	1435
ICP scan - Total Metals						
silver	μg/L	<20	<0.7	25	<20	<20
aluminum	μg/L	279	260	895	525	278
arsenic	μg/L	<100	<14	<100	<100	<100
barium	μg/L	67	78	72	55	62
beryllium	μg/L	<5	<0.3	<5	<5	<5
bismuth	μg/L	128	<2	<50	<50	<50
calcium	μg/L	330900	332000	358200	368600	316400
cadmium	μg/L	<10	1.2	<10	<10	<10
cobalt	μg/L	<10	9.1	36	<10	<10
chromium	μg/L	20	<11	41	28	17
copper	μg/L	<10	<31	13	13	14
iron	μg/L	132	65	110	101	157
gallium	μg/L	<50	0.4	<50	<50	<50
potassium	μg/L	3540	3800	3480	3210	3384
lithium	μg/L	207	5.5	<5	28	20
magnesium	μg/L	4895	4700	1310	3901	3478
manganese	μg/L	564	569	53	86	214
molybdenum	μg/L	68	37.5	51	68	79
sodium	μg/L	77000	79000	92770	51930	47340

Table A2 - Continued

nickel	μg/L	<20	8.1	39	<20	<20
phosphorus	μg/L	<100	<200	<100	144	<100
lead	μg/L	<100	32	197	125	<100
strontium	μg/L	289	299	253	285	267
vanadium	μg/L	<10	<7	13	<10	<10
yttrium	μg/L	<5	0.16	<5	<5	<5
zinc	μg/L	426	381	40	69	116
boron	μg/L	N/A	20	N/A	N/A	N/A
niobium	μg/L	N/A	<2	N/A	N/A	N/A
antimony	μg/L	N/A	7.8	N/A	N/A	N/A
tin	μg/L	N/A	<12	N/A	N/A	N/A
titanium	μg/L	N/A	12.5	N/A	N/A	N/A
tungsten	μg/L	N/A	<4	N/A	N/A	N/A
zirconium	μg/L	N/A	51	N/A	N/A	N/A
rubidium	μg/L	N/A	10.2	N/A	N/A	N/A
cesium	μg/L	N/A	0.36	N/A	N/A	N/A
thallium	μg/L	N/A	0.8	N/A	N/A	N/A
uranium	μg/L	N/A	0.111	N/A	N/A	N/A
mercury	μg/L	N/A	<7	N/A	N/A	N/A
ICP Scan - dissolved metals						
silver	μg/L	<10	<0.6	20	<10	<10
aluminum	μg/L	<10	26	346	217	85
arsenic	μg/L	<100	<5	<100	<100	<100
boron	μg/L	<10	9	<10	<10	<10
barium	μg/L	71	79	84	51	70
beryllium	μg/L	<5	<0.3	<5	<5	<5
bismuth	μg/L	<50	<1	<50	<50	<50
calcium	μg/L	284000	344000	302700	358800	362600
cadmium	μg/L	<10	<0.2	18	<10	<10
cobalt	μg/L	<10	0.67	<10	<10	<10

Table A2 - Continued

chromium	**μg/L**	**<10**	**2.2**	**<10**	**<10**	**<10**
copper	**μg/L**	**<10**	**<2**	**<10**	**<10**	**<10**
iron	**μg/L**	**<100**	**57**	**<100**	**<100**	**<100**
gallium	**μg/L**	**<50**	**0.5**	**<50**	**<50**	**<50**
potassium	**μg/L**	**3370**	**3900**	**3910**	**3690**	**3790**
lithium	**μg/L**	**199**	**4.5**	**<5**	**30**	**<5**
magnesium	**μg/L**	**3280**	**3200**	**975**	**3729**	**3781**
manganese	**μg/L**	**<10**	**0.36**	**<10**	**54**	**230**
molybdenum	**μg/L**	**35**	**37.3**	**67**	**60**	**81**
sodium	**μg/L**	**63100**	**80100**	**99580**	**49570**	**53050**
niobium	**μg/L**	**<20**	**<0.3**	**<20**	**<20**	**<20**
nickel	**μg/L**	**<20**	**5.9**	**<20**	**<20**	**<20**
phosphorus	**μg/L**	**<100**	**<200**	**<100**	**102**	**<100**
lead	**μg/L**	**<100**	**0.7**	**179**	**<100**	**<100**
antimony	**μg/L**	**<50**	**2.7**	**<50**	**<50**	**<50**
silicon	**μg/L**	**308**	**380**	**562**	**437**	**574**
tin	**μg/L**	**245**	**<0.3**	**255**	**<200**	**<200**
strontium	**μg/L**	**304**	**324**	**296**	**274**	**302**
titanium	**μg/L**	**<10**	**1.4**	**<10**	**<10**	**<10**
vanadium	**μg/L**	**<10**	**<7**	**<10**	**<10**	**<10**
tungsten	**μg/L**	**169**	**0.44**	**117**	**132**	**<50**
yttrium	**μg/L**	**<5**	**0.15**	**<5**	**<5**	**<5**
zinc	**μg/L**	**<10**	**5.4**	**<10**	**<10**	**101**
zirconium	**μg/L**	**10**	**<0.3**	**<10**	**<10**	**<10**
rubidium	**μg/L**	**N/A**	**11**	**N/A**	**N/A**	**N/A**
cesium	**μg/L**	**N/A**	**0.43**	**N/A**	**N/A**	**N/A**
thallium	**μg/L**	**N/A**	**0.73**	**N/A**	**N/A**	**N/A**
uranium	**μg/L**	**N/A**	**0.007**	**N/A**	**N/A**	**N/A**
mercury	**μg/L**	**N/A**	**<2**	**N/A**	**N/A**	**N/A**

N/A - not analysed

Table A2 - Continued

Table A3 -Physical/Chemical Data for Nickel/Copper Mine Type (Site 3) (Number in Parentheses is the Canmet Sample Number)

Parameter	**Units**	**Site 3**				
		Period 1 (2)	**Period 2 (27)**	**Period 3 (36)**	**Period 4 (57)**	**Period 4 (57Q)**
pH		**9.62**	**9.79**	**10**	**9.74**	**10.3**
conductivity	**umho/cm**	**2824**	**2387**	**2716**	**2334**	**2530**
ammonia	**mg/L**	**8.48**	**6.5**	**6**	**16.4**	**5.7**
alkalinity	**mg/L**	**50**	**44**	**50**	**47**	**43.3**
total hardness	**mg/L**	**1429**	**1404**	**1619**	**1286**	**N/A**
total suspended solids	**mg/L**	**16**	**22**	**14**	**19**	**12.8**
total dissolved solids	**mg/L**	**2668**	**2100**	**2592**	**2212**	**2230**
cyanide - total	**mg/L**	**0.164**	**0.246**	**0**	**<0.005**	**<0.005**
cyanide - free	**mg/L**	**0.027**	**0.062**	**0**	**<0.005**	**<0.005**
ICP scan - Total Metals						
silver	**μg/L**	**<20**	**35**	**22**	**<20**	**0.5**
aluminum	**μg/L**	**581**	**32**	**592**	**<10**	**40.9**
arsenic	**μg/L**	**<100**	**<100**	**200**	**<100**	**46.1**
barium	**μg/L**	**49**	**29**	**43**	**35**	**48.8**
beryllium	**μg/L**	**<5**	**<5**	**<5**	**<5**	**<3**
bismuth	**μg/L**	**134**	**237**	**<50**	**<50**	**1.5**
calcium	**μg/L**	**489600**	**389900**	**522494**	**451600**	**463000**
cadmium	**μg/L**	**<10**	**<10**	**58**	**19**	**<2**
cobalt	**μg/L**	**<10**	**<10**	**<10**	**<10**	**6.7**
chromium	**μg/L**	**16**	**<10**	**14**	**70**	**11.8**
copper	**μg/L**	**31**	**72**	**120**	**128**	**132**
iron	**μg/L**	**1400**	**569**	**675**	**727**	**600**
gallium	**μg/L**	**107**	**<50**	**<50**	**<50**	**<1**
potassium	**μg/L**	**45100**	**31860**	**44280**	**30480**	**25800**
lithium	**μg/L**	**59**	**<5**	**237**	**80**	**<300**
magnesium	**μg/L**	**101100**	**64320**	**75040**	**37680**	**37000**

Table A2 - Continued

manganese	μg/L	42	26	29	24	20
molybdenum	μg/L	34	48	79	109	6.4
sodium	μg/L	168800	166500	138815	95120	110800
nickel	μg/L	366	342	268	371	300
phosphorus	μg/L	577	<100	110	<100	<300
lead	μg/L	<100	<100	116	120	7.1
strontium	μg/L	730	614	738	665	600
vanadium	μg/L	<10	<10	<10	<10	800
yttrium	μg/L	<5	<5	<5	<5	0.5
zinc	μg/L	<10	11	<10	14	20
antimony	μg/L	N/A	N/A	N/A	N/A	<11
boron	μg/L	N/A	N/A	N/A	N/A	87.9
niobium	μg/L	N/A	N/A	N/A	N/A	<1
silicon	μg/L	N/A	N/A	N/A	N/A	1500
tin	μg/L	N/A	N/A	N/A	N/A	<6
titanium	μg/L	N/A	N/A	N/A	N/A	4.7
tungsten	μg/L	N/A	N/A	N/A	N/A	<3
zirconium	μg/L	N/A	N/A	N/A	N/A	<0.5
ICP Scan - Dissolved Metals						
silver	μg/L	<10	26	14	<20	<0.3
aluminum	μg/L	210	<10	<10	<10	7.9
arsenic	μg/L	<100	<100	173	<100	3.7
boron	μg/L	137	<10	<10	<5	82.4
barium	μg/L	50	32	43	34	45.6
beryllium	μg/L	<5	<5	<5	<5	<3
bismuth	μg/L	144	200	<50	<50	<1
calcium	μg/L	487900	420800	532700	451400	468000
cadmium	μg/L	<10	<10	<10	<20	<2
cobalt	μg/L	<10	14	<10	14	1.5

Table A3 - Continued

chromium	**μg/L**	**11**	**<10**	**10**	**17**	**<4**
copper	**μg/L**	**22**	**71**	**87**	**47**	**54.9**
iron	**μg/L**	**768**	**<100**	**<100**	**25**	**<20**
gallium	**μg/L**	**102**	**<50**	**<50**	**<50**	**<1**
potassium	**μg/L**	**45100**	**296000**	**35600**	**28400**	**27700**
lithium	**μg/L**	**65**	**<5**	**195**	**76**	**<300**
magnesium	**μg/L**	**97460**	**63710**	**71910**	**36730**	**38000**
manganese	**μg/L**	**<10**	**<10**	**<10**	**<10**	**<1**
molybdenum	**μg/L**	**25**	**30**	**73**	**79**	**5.4**
sodium	**μg/L**	**162600**	**167100**	**141300**	**94260**	**103900**
niobium	**μg/L**	**77**	**<20**	**<20**	**<20**	**<1**
nickel	**μg/L**	**99**	**63**	**25**	**44**	**<26**
phosphorus	**μg/L**	**659**	**<100**	**184**	**<100**	**<300**
lead	**μg/L**	**115**	**<100**	**104**	**<100**	**<3**
antimony	**μg/L**	**109**	**<50**	**<50**	**<50**	**<11**
silicon	**μg/L**	**1040**	**1330**	**1230**	**1687**	**1600**
tin	**μg/L**	**<200**	**<200**	**<200**	**<200**	**<6**
strontium	**μg/L**	**780**	**678**	**749**	**663**	**600**
titanium	**μg/L**	**<10**	**<10**	**<10**	**<10**	**<4**
vanadium	**μg/L**	**<10**	**<10**	**11**	**<10**	**62.5**
tungsten	**μg/L**	**<50**	**<50**	**65**	**<50**	**<3**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**	**<0.2**
zinc	**μg/L**	**<10**	**<10**	**<10**	**<10**	**11.4**
zirconium	**μg/L**	**11**	**<10**	**<10**	**<10**	**<0.5**

Table A4 - Physical/Chemical Data for Nickel/Copper Mine Type (Site 4) (Number in Parentheses is the Canmet Sample Number)

Parameter	**Units**	**Site 4**		
		Period 1 (6)	**QA/QC (6)**	**Period 2 (33)**
pH		**9.29**	**9.6**	**7.65**
conductivity	**umho/cm**	**1100**	**1120**	**684**

Table A3 - Continued

ammonia	mg/L	3.79	5	1.2
alkalinity	mg/L	44	38.1	30
total hardness	mg/L	405	439/440	270
total suspended solids	mg/L	<1	1.85	5
total dissolved solids	mg/L	784	83.7	492
cyanide - total	mg/L	0.036	<0.005	0.103
cyanide - free	mg/L	0.024	<0.005	0.053
ICP scan - Total Metals				
silver	μg/L	<20	<0.7/<0.7	20
aluminum	μg/L	230	<75/<75	792
arsenic	μg/L	<100	<14/<14	<100
barium	μg/L	23	25.5/26.8	16
beryllium	μg/L	<5	<0.3/<0.3	<5
bismuth	μg/L	57	<2/<2	<50
calcium	μg/L	147700	163000	95130
cadmium	μg/L	<10	<0.9/<0.9	<10
cobalt	μg/L	<10	2.25/2.44	27
chromium	μg/L	25	<11/<11	23
copper	μg/L	14	31/31	26
iron	μg/L	140	<112/<112	150
gallium	μg/L	<50	<0.3/<0.3	50
potassium	μg/L	25600	26400	11370
lithium	μg/L	191	17.1/15.0	49
magnesium	μg/L	9330	9500	5810
manganese	μg/L	25	21.3/21.1	47
molybdenum	μg/L	26	5.8/6.9	<20
sodium	μg/L	46900	46800	38140
nickel	μg/L	245	240/256	791
phosphorus	μg/L	<100	200	<100
lead	μg/L	<100	<11/<11	<100
strontium	μg/L	549	603/608	343

Table A4 - Continued

vanadium	**μg/L**	**<10**	**<7/<7**	**<10**
yttrium	**μg/L**	**<5**	**<0.1/<0.1**	**5**
zinc	**μg/L**	**<10**	**<75/<75**	**26**
boron	**μg/L**	**N/A**	**22/19**	**N/A**
niobium	**μg/L**	**N/A**	**<2/<2**	**N/A**
antimony	**μg/L**	**N/A**	**<4/<4**	**N/A**
tin	**μg/L**	**N/A**	**<12/<12**	**N/A**
titanium	**μg/L**	**N/A**	**<3.2/<3.2**	**N/A**
tungsten	**μg/L**	**N/A**	**<4/<4**	**N/A**
zirconium	**μg/L**	**N/A**	**<7/<7**	**N/A**
cesium	**μg/L**	**N/A**	**0.29/0.32**	**N/A**
thallium	**μg/L**	**N/A**	**<0.3/<0.3**	**N/A**
uranium	**μg/L**	**N/A**	**<0.08/<0.08**	**N/A**
mercury	**μg/L**	**N/A**	**<7/<7**	**N/A**
ICP Scan - Dissolved Metals				
silver	**μg/L**	**<10**	**<0.6**	**18**
aluminum	**μg/L**	**100**	**2.5**	**18**
arsenic	**μg/L**	**<100**	**<5**	**<100**
boron	**μg/L**	**<10**	**19**	**<10**
barium	**μg/L**	**21**	**27**	**19**
beryllium	**μg/L**	**<5**	**<0.3**	**<5**
bismuth	**μg/L**	**138**	**<1**	**<50**
calcium	**μg/L**	**145000**	**160000**	**81500**
cadmium	**μg/L**	**<10**	**<0.2**	**<10**
cobalt	**μg/L**	**<10**	**0.96**	**<10**
chromium	**μg/L**	**<10**	**<4/<4**	**<10**
copper	**μg/L**	**<10**	**7.5**	**20**
iron	**μg/L**	**<100**	**<21**	**<100**
gallium	**μg/L**	**<50**	**<0.3**	**<50**
potassium	**μg/L**	**25400**	**26800**	**13000**
lithium	**μg/L**	**104**	**14**	**37**

Table A4 - Continued

magnesium	**μg/L**	**8440**	**9600**	**5184**
manganese	**μg/L**	**13**	**12.4**	**29**
molybdenum	**μg/L**	**26**	**4.4**	**<20**
sodium	**μg/L**	**45600**	**47800**	**41630**
niobium	**μg/L**	**<20**	**<0.3**	**<20**
nickel	**μg/L**	**100**	**95**	**612**
phosphorus	**μg/L**	**<100**	**<200**	**<100**
lead	**μg/L**	**<100**	**<0.5**	**<10**
antimony	**μg/L**	**<50**	**0.47**	**<50**
silicon	**μg/L**	**3150**	**2800**	**2963**
tin	**μg/L**	**<200**	**<0.3**	**<200**
strontium	**μg/L**	**639**	**614**	**398**
titanium	**μg/L**	**<10**	**1.2**	**<10**
vanadium	**μg/L**	**<10**	**<7**	**<10**
tungsten	**μg/L**	**<50**	**1.2**	**<50**
yttrium	**μg/L**	**<5**	**<0.1**	**<5**
zinc	**μg/L**	**<10**	**2.3**	**<10**
zirconium	**μg/L**	**<10**	**<0.3**	**<10**
rubidium	**μg/L**	**N/A**	**50.7/51.3**	**N/A**
cesium	**μg/L**	**N/A**	**<0.3**	**N/A**
thallium	**μg/L**	**N/A**	**0.3**	**N/A**
uranium	**μg/L**	**N/A**	**<0.007**	**N/A**
mercury	**μg/L**	**N/A**	**<2**	**N/A**

N/A - not analysed

Table A4 - Continued

Table A5 - Physical/Chemical Data Copper/Zinc Mine Type (Site 5) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 5				
		Period 1 (13)	Period 2 (22)	Period 3 (37)	Period 4 (56)	Period 4 (56Q)
pH		10.23	10.25	10.46	9.88	10.4
conductivity	μmho/cm	1076	1019	1111	1170	1200
ammonia	mg/L	<0.1	0.47	1.11	1	1.7
alkalinity	mg/L	41	37	53	35	38.7
total hardness	mg/L	507	586	620	658	N/A
total suspended solids	mg/L	11	15	<1.0	9	21.4
total dissolved solids	mg/L	896	908	864	956	1017
sulfate	mg/L	533	526	549	34	N/A
ICP scan - Total Metals						
silver	μg/L	<20	<20	<10	<20	2
aluminum	μg/L	735	378	933	354	228
arsenic	μg/L	<100	<100	<100	<100	85.8
barium	μg/L	32	32	71	21	47.5
beryllium	μg/L	<5	<5	<5	<5	<3
bismuth	μg/L	<50	<50	<50	<50	<1
calcium	μg/L	237900	228500	245221	256900	249000
cadmium	μg/L	<10	<10	<10	14	<2
cobalt	μg/L	<10	33	15	<10	1.2
chromium	μg/L	<10	10	22	59	16.9
copper	μg/L	19	19	12	11	17
iron	μg/L	<100	<100	155	283	100
gallium	μg/L	<50	<50	<50	<50	<1
potassium	μg/L	6830	8280	9756	9000	7700
lithium	μg/L	<5	12	84	26	<300
magnesium	μg/L	4520	3651	1577	3887	3000

Table A4 - Continued

manganese	μ**g/L**	**103**	**68**	**12**	**127**	**100**
molybdenum	μ**g/L**	**36**	**73**	**145**	**160**	**47.2**
sodium	μ**g/L**	**23200**	**21090**	**17995**	**19980**	**21100**
nickel	μ**g/L**	**<20**	**<20**	**<20**	**60**	**<26**
phosphorus	μ**g/L**	**<100**	**1024**	**326**	**285**	**<300**
lead	μ**g/L**	**<100**	**<100**	**102**	**<100**	**3.9**
strontium	μ**g/L**	**836**	**814**	**915**	**959**	**800**
vanadium	μ**g/L**	**<10**	**<10**	**<10**	**<10**	**1422**
yttrium	μ**g/L**	**<5**	**<5**	**<5**	**<5**	**<0.2**
zinc	μ**g/L**	**222**	**119**	**66**	**310**	**300**
antimony	μ**g/L**	**N/A**	**N/A**	**N/A**	**N/A**	**<11**
boron	μ**g/L**	**N/A**	**N/A**	**N/A**	**N/A**	**58.5**
niobium	μ**g/L**	**N/A**	**N/A**	**N/A**	**N/A**	**<1**
silicon	μ**g/L**	**N/A**	**N/A**	**N/A**	**N/A**	**2.3**
tin	μ**g/L**	**N/A**	**N/A**	**N/A**	**N/A**	**<6**
titanium	μ**g/L**	**N/A**	**N/A**	**N/A**	**N/A**	**8**
tungsten	μ**g/L**	**N/A**	**N/A**	**N/A**	**N/A**	**<3**
zirconium	μ**g/L**	**N/A**	**N/A**	**N/A**	**N/A**	**1**
ICP Scan - dissolved metals						
silver	μ**g/L**	**<10**	**<20**	**<10**	**<20**	**<0.3**
aluminum	μ**g/L**	**251**	**145**	**663**	**54**	**163**
arsenic	μ**g/L**	**<100**	**183**	**<100**	**<100**	**2.9**
boron	μ**g/L**	**<10**	**24**	**<10**	**<5**	**43**
barium	μ**g/L**	**26**	**30**	**37**	**22**	**34**
beryllium	μ**g/L**	**<5**	**<5**	**<5**	**<5**	**<3**
bismuth	μ**g/L**	**<50**	**<50**	**<50**	**<50**	**<1**
calcium	μ**g/L**	**237200**	**239400**	**251100**	**249100**	**244000**
cadmium	μ**g/L**	**<10**	**<10**	**<10**	**<20**	**<2**
cobalt	μ**g/L**	**<10**	**<10**	**<10**	**<10**	**<1**

Table A5 - Continued

chromium	μg/L	<10	<10	<10	10	<4
copper	μg/L	<10	<10	<10	<10	<3
iron	μg/L	<100	<100	<100	23	<20
gallium	μg/L	<50	<50	<50	<50	<1
potassium	μg/L	5320	5520	9880	9250	8500
lithium	μg/L	<5	<5	123	<5	<300
magnesium	μg/L	4286	3544	1210	3627	3000
manganese	μg/L	<10	<10	<10	<10	3.3
molybdenum	μg/L	11	75	125	104	46.4
sodium	μg/L	18950	19900	16040	17060	19100
niobium	μg/L	<20	<20	<20	<20	<1
nickel	μg/L	<20	<20	<20	42	<26
phosphorus	μg/L	<100	234	381	305	<3000
lead	μg/L	<100	<100	<100	<100	<3
antimony	μg/L	253	<50	<50	236	<11
silicon	μg/L	1980	1950	2580	2466	2000
tin	μg/L	<200	<200	209	<200	<6
strontium	μg/L	854	860	946	913	800
titanium	μg/L	<10	<10	<10	<10	<4
vanadium	μg/L	<10	<10	<10	<10	34.1
tungsten	μg/L	<50	<50	<50	<50	<3
yttrium	μg/L	<5	<5	<5	<5	<0.2
zinc	μg/L	5	10	15	29	35.5
zirconium	μg/L	<10	<10	<10	<10	<0.5

Table A5 - Continued

Table A6 - Physical/Chemical Data For Tin Mine Type (Site 6) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 6				
		Period 1 (10)	QA/QC (10)	Period 2 (30)	Period 3 (41)	Period 4 (63)
pH		**8.47**	**8.2**	**7.21**	**7.3**	**7.36**
conductivity	**μmho/cm**	**846**	**920**	**858**	**888**	**904**
ammonia	**mg/L**	**0.4**	**0.2**	**<0.1**	**<0.1**	**<0.1**
alkalinity	**mg/L**	**44**	**13.7**	**24**	**46**	**30**
total hardness	**mg/L**	**417**	**480/485**	**502**	**487**	**465**
total suspended solids	**mg/L**	**7**	**7.44**	**3**	**7**	**2**
total dissolved solids	**mg/L**	**660**	**700**	**680**	**696**	**684**
tin	**μg/L**	**N/A**	**N/A**	**N/A**	**N/A**	**<200**
ICP scan - Total Metals						
silver	**μg/L**	**<20**	**<0.7/<0.7**	**<20**	**<20**	**<20**
aluminum	**μg/L**	**1360**	**838**	**1373**	**692**	**573**
arsenic	**μg/L**	**<100**	**<14,<14**	**<100**	**<100**	**<100**
barium	**μg/L**	**14**	**15.9/15.9**	**15**	**22**	**8**
beryllium	**μg/L**	**<5**	**<0.3/<0.3**	**<5**	**<5**	**<5**
bismuth	**μg/L**	**150**	**<2/<2**	**<50**	**147**	**<50**
calcium	**μg/L**	**177800**	**186000**	**188700**	**202500**	**181500**
cadmium	**μg/L**	**<10**	**4/4.2**	**<10**	**<10**	**<10**
cobalt	**μg/L**	**<10**	**0.79/0.81**	**<10**	**<10**	**17**
chromium	**μg/L**	**16**	**<11/<11**	**<10**	**17**	**48**
copper	**μg/L**	**12**	**<31/<31**	**<10**	**16**	**<10**
iron	**μg/L**	**148**	**48**	**169**	**<100**	**227**
gallium	**μg/L**	**<50**	**<0.3/<0.3**	**<50**	**<50**	**<50**
potassium	**μg/L**	**5580**	**5400**	**5150**	**5340**	**5304**
lithium	**μg/L**	**663**	**378/377**	**209**	**369**	**379**
magnesium	**μg/L**	**3350**	**3100**	**2700**	**3179**	**2895**
manganese	**μg/L**	**962**	**966**	**762**	**403**	**253**

Table A5 - Continued

molybdenum	µg/L	30	1.1/<1	<20	79	34
sodium	µg/L	8690	8200	13490	10190	9273
nickel	µg/L	<20	<7/<7	43	<20	38
phosphorus	µg/L	<100	<200	<100	92	<100
lead	µg/L	<100	<11/<11	<100	122	<100
strontium	µg/L	191	199/197	184	222	216
vanadium	µg/L	<10	<7/<7	11	<10	<10
yttrium	µg/L	<5	<0.1/<0.1	<5	<5	<5
zinc	µg/L	134	123	446	387	254
boron	µg/L	N/A	8.4/9.5	N/A	N/A	N/A
niobium	µg/L	N/A	<7/<7	N/A	N/A	N/A
antimony	µg/L	N/A	<4/<4	N/A	N/A	N/A
tin	µg/L	N/A	<12/<12	N/A	N/A	N/A
titanium	µg/L	N/A	<3.1/<3.1	N/A	N/A	N/A
tungsten	µg/L	N/A	<4/<4	N/A	N/A	N/A
zirconium	µg/L	N/A	<7/<7	N/A	N/A	N/A
rubidium	µg/L	N/A	79/79	N/A	N/A	N/A
cesium	µg/L	N/A	2.8/2.9	N/A	N/A	N/A
thallium	µg/L	N/A	0.36/0.36	N/A	N/A	N/A
uranium	µg/L	N/A	0.66/0.53	N/A	N/A	N/A
mercury	µg/L	N/A	7	N/A	N/A	N/A
ICP Scan - dissolved metals						
silver	µg/L	<10	<0.6/<0.6	27	<10	<20
aluminum	µg/L	642	838	<10	<100	189
arsenic	µg/L	<100	<5/<5	<100	<100	<100
boron	µg/L	<10	6.2/4.9	<10	<10	<5
barium	µg/L	19	31.9/33.6	24	28	20
beryllium	µg/L	<5	<0.3/<0.3	<5	<5	<5
bismuth	µg/L	<50	<1/<1	<50	<50	<50
calcium	µg/L	162000	187000	165600	189000	186700

Table A6 - Continued

cadmium	**μg/L**	**<10**	**2.9/2.7**	**<10**	**<10**	**<10**
cobalt	**μg/L**	**<10**	**0.76/0.82**	**<10**	**<10**	**<10**
chromium	**μg/L**	**<10**	**<4/<4**	**<10**	**<10**	**<10**
copper	**μg/L**	**<10**	**<2/<2**	**<40**	**<10**	**<10**
iron	**μg/L**	**<100**	**40**	**<100**	**<100**	**33**
gallium	**μg/L**	**<50**	**<0.3/<0.3**	**<50**	**<50**	**<50**
potassium	**μg/L**	**4980**	**5200**	**4890**	**5860**	**5920**
lithium	**μg/L**	**539**	**336/287**	**258**	**320**	**357**
magnesium	**μg/L**	**3000**	**3200**	**2767**	**2963**	**3054**
manganese	**μg/L**	**1015**	**912**	**813**	**366**	**251**
molybdenum	**μg/L**	**35**	**<0.6/<0.6**	**<20**	**63**	**76**
sodium	**μg/L**	**7090**	**7400**	**9227**	**8064**	**7903**
niobium	**μg/L**	**<20**	**0.6/<0.6**	**<20**	**<20**	**<20**
nickel	**μg/L**	**30**	**5.0/4.5**	**20**	**<20**	**<20**
phosphorus	**μg/L**	**<100**	**<200**	**<100**	**67**	**<100**
lead	**μg/L**	**<100**	**<0.5/<0.5**	**<100**	**<100**	**<100**
antimony	**μg/L**	**<50**	**<0.3/<0.3**	**<50**	**<50**	**<50**
silicon	**μg/L**	**553**	**580**	**1164**	**1124**	**1197**
tin	**μg/L**	**<200**	**<0.3/<0.3**	**<200**	**<200**	**<200**
strontium	**μg/L**	**211**	**199/205**	**207**	**206**	**223**
titanium	**μg/L**	**<10**	**<0.8/<0.8**	**<10**	**<10**	**<10**
vanadium	**μg/L**	**<10**	**<7/<7**	**<10**	**<10**	**<10**
tungsten	**μg/L**	**73**	**<0.4/<0.4**	**<50**	**89**	**<50**
yttrium	**μg/L**	**<5**	**<0.1/<0.1**	**<5**	**<5**	**<5**
zinc	**μg/L**	**11**	**25/26**	**418**	**324**	**248**
zirconium	**μg/L**	**<10**	**<0.3/<0.3**	**<10**	**<10**	**<10**
rubidium	**μg/L**	**N/A**	**81/84**	**N/A**	**N/A**	**N/A**
cesium	**μg/L**	**N/A**	**2.9/3.1**	**N/A**	**N/A**	**N/A**
thallium	**μg/L**	**N/A**	**0.38/0.28**	**N/A**	**N/A**	**N/A**
uranium	**μg/L**	**N/A**	**0.27/0.26**	**N/A**	**N/A**	**N/A**

Table A6 - Continued

mercury	μ**g/L**	**N/A**	**<2**	**N/A**	**N/A**	**N/A**

N/A - not analysed

Table A6 - Continued

Table A7 - Physical/Chemical Data Copper/Zinc Mine Type (Site 7) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 7				
		Period 1 (17)	Period 2 (20)	Period 3 (46)	Period 4 (58)	Period 4 (58Q)
pH		7.31	8.45	3.07	10.31	10.8
conductivity	μmho/cm	1038	879	1774	971	1000
ammonia	mg/L	0.1	0.64	0.8	0.4	0.9
alkalinity	mg/L	62	136	<1	45	42.9
total hardness	mg/L	469	464	477	467	N/A
total suspended solids	mg/L	3	1	10	6	5.6
total dissolved solids	mg/L	804	736	1516	756	757
ICP scan - Total Metals						
silver	μg/L	<20	<20	<20	<20	<0.3
aluminum	μg/L	402	293	16530	<10	83.9
arsenic	μg/L	<100	<100	<100	<100	65.8
barium	μg/L	15	14	17	5	14.5
beryllium	μg/L	<5	<5	<5	<5	<3
bismuth	μg/L	<50	<50	74	<50	<1
calcium	μg/L	187100	162200	97040	172700	174000
cadmium	μg/L	33	54	687	71	2.3
cobalt	μg/L	<10	<10	132	22	<1
chromium	μg/L	<10	<10	26	15	16.3
copper	μg/L	22	15	24200	15	16.8
iron	μg/L	<100	146	85570	214	40
gallium	μg/L	<50	<50	<50	<50	<1
potassium	μg/L	16200	13080	1020	12780	11000
lithium	μg/L	<5	76	19	51	<300
magnesium	μg/L	15850	14140	60460	8460	8000
manganese	μg/L	373	303	9805	17	14.6

Table A6 - Continued

molybdenum	μg/L	90	86	424	124	29.1
sodium	μg/L	20200	19130	53160	9210	9200
nickel	μg/L	43	<20	59	64	<26
phosphorus	μg/L	<100	688	785	<100	<3000
lead	μg/L	<100	<100	323	<100	12.1
strontium	μg/L	358	327	166	333	300
vanadium	μg/L	<10	<10	<10	<10	1191
yttrium	μg/L	<5	<5	9	<5	<0.2
zinc	μg/L	970	826	61460	76	117
antimony	μg/L	N/A	N/A	N/A	N/A	<11
boron	μg/L	N/A	N/A	N/A	N/A	61.3
niobium	μg/L	N/A	N/A	N/A	N/A	<1
silicon	μg/L	N/A	N/A	N/A	N/A	1.3
tin	μg/L	N/A	N/A	N/A	N/A	72.4
titanium	μg/L	N/A	N/A	N/A	N/A	4.4
tungsten	μg/L	N/A	N/A	N/A	N/A	<3
zirconium	μg/L	N/A	N/A	N/A	N/A	<0.5
ICP Scan - dissolved metals						
silver	μg/L	<10	18	14	<20	<0.3
aluminum	μg/L	188	109	15950	<10	16.8
arsenic	μg/L	<100	<100	<100	<100	<2
boron	μg/L	<10	16	<10	<5	28.4
barium	μg/L	18	15	13	8	13.7
beryllium	μg/L	<5	<5	<5	<5	<3
bismuth	μg/L	<50	<50	<50	<50	<1
calcium	μg/L	199200	171400	94280	170300	177000
cadmium	μg/L	<10	<10	703	<20	<2
cobalt	μg/L	14	<10	108	<10	<1
chromium	μg/L	<10	<10	<10	<10	<4

Table A7 - Continued

copper	µg/L	20	19	23430	<10	5.5
iron	µg/L	<100	<100	83870	29	N/C
gallium	µg/L	<50	<50	<50	<50	<1
potassium	µg/L	10700	9410	965	12500	11700
lithium	µg/L	<5	<5	59	7	<300
magnesium	µg/L	16000	15820	58650	8429	7000
manganese	µg/L	274	304	9553	<10	<1
molybdenum	µg/L	109	52	319	122	27.9
sodium	µg/L	22100	18690	15870	8507	8800
niobium	µg/L	<20	<20	<20	<20	<1
nickel	µg/L	<20	<20	46	26	<26
phosphorus	µg/L	<100	135	534	<100	<300
lead	µg/L	<100	100	281	<100	<3
antimony	µg/L	205	<50	187	<50	<11
silicon	µg/L	2200	2410	5568	1338	1100
tin	µg/L	<200	<200	<200	<200	<6
strontium	µg/L	396	342	160	336	325
titanium	µg/L	<10	<10	<10	<10	<4
vanadium	µg/L	<10	<10	<10	<10	<31
tungsten	µg/L	<50	<50	3002	<50	<3
yttrium	µg/L	<5	<5	9	<5	<0.2
zinc	µg/L	398	520	50530	18	29.7
zirconium	µg/L	<10	<10	<10	<10	<0.5

Table A8 - Physical/Chemical Data For Gold Mine Type (Site 8) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 8			
		Period 1 (5)	Period 2 (26)	Period 3 (45)	Period 4 (67)
pH	units	8.11	8.09	2.47	8.24
conductivity	µMho/cm	1424	1123	3128	810

Table A7 - Continued

ammonia	**mg/L**	**1.3**	**1**	**9.5**	**12.3**
alkalinity	**mg/L**	**329**	**239**	**<1**	**96**
total hardness	**mg/L**	**517**	**478**	**134**	**77**
total suspended solids	**mg/L**	**2**	**3**	**6**	**5**
total dissolved solids	**mg/L**	**908**	**748**	**3000**	**516**
cyanide - total	**mg/L**	**0.023**	**0.05**	**0.23**	**0.2**
cyanide - free	**mg/L**	**0.022**	**0.02**	**0.08**	**<0.005**
ICP scan - Total Metals					
silver	**μg/L**	**<20**	**<20**	**<20**	**<20**
aluminum	**μg/L**	**469**	**<10**	**416**	**294**
arsenic	**μg/L**	**<100**	**<100**	**<100**	**<100**
barium	**μg/L**	**76**	**77**	**79**	**38**
beryllium	**μg/L**	**<5**	**<5**	**<5**	**<5**
bismuth	**μg/L**	**66**	**122**	**129**	**<50**
calcium	**μg/L**	**155800**	**115500**	**56820**	**24840**
cadmium	**μg/L**	**<10**	**<10**	**<10**	**20**
cobalt	**μg/L**	**<10**	**<10**	**31**	**47**
chromium	**μg/L**	**<10**	**<10**	**15**	**<10**
copper	**μg/L**	**<10**	**<10**	**212**	**442**
iron	**μg/L**	**773**	**162**	**303**	**895**
gallium	**μg/L**	**71**	**<50**	**<50**	**<50**
potassium	**μg/L**	**9970**	**8610**	**22980**	**30480**
lithium	**μg/L**	**<10**	**9**	**<5**	**5**
magnesium	**μg/L**	**46300**	**25940**	**7952**	**3606**
manganese	**μg/L**	**64**	**168**	**128**	**45**
molybdenum	**μg/L**	**143**	**269**	**1223**	**1685**
sodium	**μg/L**	**112500**	**103200**	**88540**	**98690**
nickel	**μg/L**	**20**	**<20**	**63**	**53**
phosphorus	**μg/L**	**308**	**<100**	**149**	**<100**
lead	**μg/L**	**<100**	**<100**	**<100**	**<100**

Table A8 - Continued

strontium	**μg/L**	**13810**	**9130**	**2520**	**1744**
vanadium	**μg/L**	**<10**	**<10**	**<10**	**<10**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**
zinc	**μg/L**	**<10**	**17**	**26**	**<10**
ICP Scan - dissolved metals					
silver	**μg/L**	**<10**	**<10**	**<10**	**<10**
aluminum	**μg/L**	**89**	**29**	**84**	**195**
arsenic	**μg/L**	**<100**	**<100**	**<100**	**<100**
boron	**μg/L**	**252**	**<10**	**17**	**<10**
barium	**μg/L**	**80**	**85**	**73**	**43**
beryllium	**μg/L**	**<5**	**<5**	**<5**	**<5**
bismuth	**μg/L**	**62**	**79**	**73**	**<50**
calcium	**μg/L**	**151300**	**122300**	**41830**	**3409**
cadmium	**μg/L**	**<10**	**<10**	**<10**	**21**
cobalt	**μg/L**	**<10**	**<10**	**29**	**<10**
chromium	**μg/L**	**<10**	**<10**	**<10**	**62**
copper	**μg/L**	**<10**	**16**	**179**	**501**
iron	**μg/L**	**555**	**199**	**184**	**938**
gallium	**μg/L**	**<50**	**<50**	**<50**	**<50**
potassium	**μg/L**	**9320**	**11900**	**23100**	**31100**
lithium	**μg/L**	**14**	**42**	**<5**	**<5**
magnesium	**μg/L**	**44560**	**29360**	**7095**	**4869**
manganese	**μg/L**	**64**	**182**	**121**	**51**
molybdenum	**μg/L**	**131**	**259**	**1208**	**1872**
sodium	**μg/L**	**106900**	**106170**	**82860**	**109300**
niobium	**μg/L**	**<20**	**<20**	**<20**	**<20**
nickel	**μg/L**	**<20**	**<20**	**46**	**60**
phosphorus	**μg/L**	**348**	**<100**	**129**	**<100**
lead	**μg/L**	**<100**	**<100**	**<100**	**<100**
antimony	**μg/L**	**76**	**<50**	**96**	**<50**

Table A8 - Continued

silicon	**μg/L**	**3150**	**4740**	**1921**	**2283**
tin	**μg/L**	**<200**	**<200**	**<200**	**<200**
strontium	**μg/L**	**14830**	**10020**	**2370**	**1944**
titanium	**μg/L**	**<10**	**<10**	**<10**	**<10**
vanadium	**μg/L**	**<10**	**<10**	**<10**	**<10**
tungsten	**μg/L**	**<50**	**<50**	**<50**	**<50**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**
zinc	**μg/L**	**<10**	**12**	**<10**	**<10**
zirconium	**μg/L**	**<10**	**<10**	**<10**	**<10**

Table A8 - Continued

Table A9 - Physical/Chemical Data for Lead/Zinc Mine Type (Site 9) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 9			
		Period 1 (4)	Period 2 (24)	Period 3 (43)	Period 4 (65)
pH		8.96	7.36	5.81	5.92
conductivity	μmho/cm	1836	2772	2711	2770
ammonia	mg/L	3.06	2.9	3	3.8
alkalinity	mg/L	30	15	3	5
total hardness	mg/L	356	2455	1988	1813
total suspended solids	mg/L	11	6	22	26
total dissolved solids	mg/L	1660	3024	2872	3224
cyanide - total	mg/L	0.102	0.191	0.122	1.6
cyanide - free	mg/L	0.082	0.067	0.026	0.2
fluoride	mg/L	2	5.37	<0.1	2.6
ICP scan - Total Metals					
silver	μg/L	<20	38	106	<20
aluminum	μg/L	804	590	977	293
arsenic	μg/L	<100	<100	<100	<100
barium	μg/L	11	5	8	14
beryllium	μg/L	<5	5	<5	<5
bismuth	μg/L	130	444	<50	<50
calcium	μg/L	338700	669900	686000	577900
cadmium	μg/L	<10	<10	<10	22
cobalt	μg/L	<10	<10	<10	<10
chromium	μg/L	<10	39	34	30
copper	μg/L	<10	<10	<10	<10
iron	μg/L	3613	1620	1152	1110
gallium	μg/L	116	<50	<50	<50
potassium	μg/L	12940	21920	16310	15240
lithium	μg/L	25	<5	118	42

Table A8 - Continued

magnesium	μ**g/L**	**94820**	**95100**	**74300**	**88550**
manganese	μ**g/L**	**287**	**277**	**168**	**226**
molybdenum	μ**g/L**	**<20**	**<20**	**<20**	**<20**
sodium	μ**g/L**	**16550**	**32650**	**19800**	**20200**
nickel	μ**g/L**	**<20**	**<20**	**<20**	**<20**
phosphorus	μ**g/L**	**550**	**<100**	**82**	**<100**
lead	μ**g/L**	**167**	**<100**	**165**	**<100**
strontium	μ**g/L**	**439**	**712**	**667**	**613**
vanadium	μ**g/L**	**<10**	**<10**	**11**	**<10**
yttrium	μ**g/L**	**<5**	**<5**	**<5**	**<5**
zinc	μ**g/L**	**260**	**173**	**224**	**194**
ICP Scan - dissolved metals					
silver	μ**g/L**	**<10**	**59**	**<10**	**<10**
aluminum	μ**g/L**	**315**	**201**	**<100**	**<10**
arsenic	μ**g/L**	**<100**	**<100**	**<100**	**<100**
boron	μ**g/L**	**115**	**<10**	**<10**	**<10**
barium	μ**g/L**	**9**	**<5**	**7**	**17**
beryllium	μ**g/L**	**<5**	**<5**	**<5**	**<5**
bismuth	μ**g/L**	**142**	**445**	**<50**	**<50**
calcium	μ**g/L**	**338000**	**728100**	**680100**	**665900**
cadmium	μ**g/L**	**<10**	**<10**	**<10**	**<10**
cobalt	μ**g/L**	**<10**	**<10**	**<10**	**<10**
chromium	μ**g/L**	**12**	**<10**	**<10**	**39**
copper	μ**g/L**	**<10**	**<10**	**19**	**<10**
iron	μ**g/L**	**916**	**<100**	**<100**	**<100**
gallium	μ**g/L**	**102**	**<50**	**<50**	**<50**
potassium	μ**g/L**	**12630**	**16300**	**16600**	**15400**
lithium	μ**g/L**	**29**	**<5**	**128**	**56**
magnesium	μ**g/L**	**90200**	**107100**	**70140**	**101900**
manganese	μ**g/L**	**197**	**231**	**71**	**170**

Table A9 - Continued

molybdenum	**μg/L**	**<20**	**<20**	**<20**	**<20**
sodium	**μg/L**	**16200**	**28680**	**18350**	**22130**
niobium	**μg/L**	**61**	**<20**	**<20**	**<20**
nickel	**μg/L**	**<20**	**<20**	**<20**	**<20**
phosphorus	**μg/L**	**571**	**<100**	**54**	**<100**
lead	**μg/L**	**101**	**<100**	**158**	**<100**
antimony	**μg/L**	**96**	**<50**	**<50**	**<50**
silicon	**μg/L**	**<50**	**200**	**143**	**<50**
tin	**μg/L**	**<200**	**245**	**<200**	**<200**
strontium	**μg/L**	**470**	**759**	**634**	**696**
titanium	**μg/L**	**<10**	**<10**	**<10**	**<10**
vanadium	**μg/L**	**<10**	**<10**	**<10**	**<10**
tungsten	**μg/L**	**<50**	**<50**	**67**	**<50**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**
zinc	**μg/L**	**<10**	**109**	**<10**	**<10**
zirconium	**μg/L**	**<10**	**<10**	**<10**	**<10**

Table A9 - Continued

Table A10 - Physical/Chemical Data for Nickel/Copper Mine Type (Site 10) (Number in Parentheses is the Canment Sample Number)

Parameter	Units	Site 10			
		Period 1 (1)	Period 2 (31)	Period 3 (38)	Period 4 (59)
pH		7.46	7.7	7.54	7.76
conductivity	umho/cm	1714	1636	1176	1264
ammonia	mg/L	3.91	2	1.49	1.4
alkalinity	mg/L	56	102	51	38
total hardness	mg/L	726	756	477	496
total suspended solids	mg/L	<1	4	<1.0	<1
total dissolved solids	mg/L	1361	1252	824	948
cyanide - total	mg/L	0.142	0.088	0.155	0.01
cyanide - free	mg/L	0.021	0.083	0.069	<0.005
oil and grease	mg/L	N/A	N/A	2.2	N/A
sulfate	mg/L			407	N/A
ICP scan - Total Metals					
silver	μg/L	<20	<20	<20	<20
aluminum	μg/L	467	730	472	<10
arsenic	μg/L	<100	160	141	<100
barium	μg/L	34	30	26	17
beryllium	μg/L	<5	<5	<5	<5
bismuth	μg/L	<50	<50	<50	<50
calcium	μg/L	308200	299000	186400	192500
cadmium	μg/L	<10	<10	<10	<10
cobalt	μg/L	<10	15	<10	15
chromium	μg/L	13	34	19	66
copper	μg/L	<10	25	21	29
iron	μg/L	512	<100	156	297
gallium	μg/L	<50	<50	<50	<50
potassium	μg/L	26660	23520	17000	18720

Table A9 - Continued

lithium	**μg/L**	**10**	**<5**	**<5**	**47**
magnesium	**μg/L**	**3680**	**3010**	**2734**	**3630**
manganese	**μg/L**	**<10**	**23**	**15**	**18**
molybdenum	**μg/L**	**<20**	**37**	**70**	**98**
sodium	**μg/L**	**93170**	**106000**	**63450**	**62700**
nickel	**μg/L**	**47**	**93**	**64**	**122**
phosphorus	**μg/L**	**<100**	**<100**	**<100**	**110**
lead	**μg/L**	**<100**	**<100**	**<100**	**<100**
strontium	**μg/L**	**894**	**767**	**595**	**637**
vanadium	**μg/L**	**<10**	**<10**	**<10**	**<10**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**
zinc	**μg/L**	**<10**	**17**	**10**	**11**
ICP Scan - Dissolved Metals					
silver	**μg/L**	**<10**	**13**	**12**	**<20**
aluminum	**μg/L**	**150**	**28**	**107**	**<10**
arsenic	**μg/L**	**<100**	**<100**	**<100**	**<100**
boron	**μg/L**	**40**	**114**	**59**	**59**
barium	**μg/L**	**37**	**34**	**26**	**19**
beryllium	**μg/L**	**<5**	**<5**	**<5**	**<5**
bismuth	**μg/L**	**50**	**<50**	**<50**	**<50**
calcium	**μg/L**	**314400**	**254200**	**197300**	**207900**
cadmium	**μg/L**	**<10**	**<10**	**<10**	**<20**
cobalt	**μg/L**	**<10**	**<10**	**<10**	**<10**
chromium	**μg/L**	**<10**	**<10**	**<10**	**<10**
copper	**μg/L**	**<10**	**14**	**<10**	**<10**
iron	**μg/L**	**420**	**<100**	**<100**	**22**
gallium	**μg/L**	**58**	**<50**	**<50**	**<50**
potassium	**μg/L**	**27020**	**20700**	**16300**	**14800**
lithium	**μg/L**	**11**	**<5**	**51**	**<5**
magnesium	**μg/L**	**3688**	**3235**	**2790**	**3871**

Table A10 - Continued

manganese	**μg/L**	**<10**	**21**	**10**	**14**
molybdenum	**μg/L**	**<20**	**<20**	**81**	**77**
sodium	**μg/L**	**91440**	**123200**	**67530**	**62390**
niobium	**μg/L**	**51**	**<20**	**<20**	**<20**
nickel	**μg/L**	**25**	**46**	**<20**	**64**
phosphorus	**μg/L**	**<100**	**<100**	**<100**	**<100**
lead	**μg/L**	**<100**	**<100**	**<100**	**<100**
antimony	**μg/L**	**<50**	**62**	**134**	**<50**
silicon	**μg/L**	**1300**	**2709**	**2164**	**2542**
tin	**μg/L**	**<200**	**<200**	**<200**	**<200**
strontium	**μg/L**	**969**	**896**	**614**	**693**
titanium	**μg/L**	**<10**	**<10**	**<10**	**<10**
vanadium	**μg/L**	**<10**	**<10**	**<10**	**<10**
tungsten	**μg/L**	**<50**	**60**	**66**	**<50**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**
zinc	**μg/L**	**<10**	**<10**	**<10**	**<10**
zirconium	**μg/L**	**<10**	**<10**	**<10**	**<10**

N/A - not analysed

Table A10 - Continued

Table A11 - Physical/Chemical Data for Zinc Mine Type (Site 11) (Number in Parentheses is the Canmet Sample Number)

Parameters	Units	Site 11			
		Period 1 (3)	Period 2 (28)	Period 3 (42)	Period 4 (64)
pH		8.07	8.89	6.51	6.92
conductivity	μmho/cm	1054	933	611	687
ammonia	mg/L	0.96	1.15	0.2	<0.1
alkalinity	mg/L	83	68	36	15
total hardness	mg/L	529	574	302	373
total suspended solids	mg/L	<1	56	10	<1
total dissolved solids	mg/L	844	704	472	524
ICP scan - Total Metals					
silver	μg/L	<20	12	26	<20
aluminum	μg/L	340	<10	411	171
arsenic	μg/L	<100	<100	<100	<100
barium	μg/L	10	7	9	<5
beryllium	μg/L	<5	<5	<5	<5
bismuth	μg/L	<50	73	64	<50
calcium	μg/L	226600	217560	116000	126200
cadmium	μg/L	<10	20	<10	<10
cobalt	μg/L	<10	<10	<10	<10
chromium	μg/L	46	10	16	51
copper	μg/L	13	<10	10	<10
iron	μg/L	583	334	532	463
gallium	μg/L	<50	<50	<50	<50
potassium	μg/L	3210	3150	1720	2160
lithium	μg/L	<10	<5	<5	<5
magnesium	μg/L	10190	3498	8128	13910
manganese	μg/L	205	190	285	358
molybdenum	μg/L	26	37	76	35

Table A10 - Continued

sodium	**μg/L**	**19090**	**22680**	**7615**	**8840**
nickel	**μg/L**	**<20**	**20**	**<20**	**52**
phosphorus	**μg/L**	**869**	**<100**	**287**	**<100**
lead	**μg/L**	**<100**	**<100**	**<100**	**<100**
strontium	**μg/L**	**454**	**443**	**236**	**290**
vanadium	**μg/L**	**<10**	**<10**	**<10**	**<10**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**
zinc	**μg/L**	**<10**	**28**	**160**	**127**
ICP Scan - dissolved metals					
silver	**μg/L**	**<10**	**16**	**<10**	**<20**
aluminum	**μg/L**	**123**	**<10**	**<100**	**<10**
arsenic	**μg/L**	**<100**	**<100**	**<100**	**<100**
boron	**μg/L**	**24**	**<10**	**<10**	**<5**
barium	**μg/L**	**11**	**8**	**13**	**10**
beryllium	**μg/L**	**<5**	**<5**	**<5**	**<5**
bismuth	**μg/L**	**<50**	**62**	**<50**	**<50**
calcium	**μg/L**	**225300**	**210700**	**107900**	**129900**
cadmium	**μg/L**	**<10**	**<10**	**<10**	**15**
cobalt	**μg/L**	**<10**	**<10**	**<10**	**<10**
chromium	**μg/L**	**<10**	**<10**	**<10**	**<10**
copper	**μg/L**	**<10**	**<10**	**<10**	**<10**
iron	**μg/L**	**375**	**<100**	**<100**	**250**
gallium	**μg/L**	**52**	**<50**	**<50**	**<50**
potassium	**μg/L**	**2996**	**3850**	**1750**	**2130**
lithium	**μg/L**	**<5**	**<5**	**14**	**15**
magnesium	**μg/L**	**10330**	**3123**	**7705**	**14190**
manganese	**μg/L**	**81**	**18**	**150**	**362**
molybdenum	**μg/L**	**<20**	**55**	**73**	**41**
sodium	**μg/L**	**18450**	**23280**	**7142**	**7949**
niobium	**μg/L**	**41**	**<20**	**<20**	**<20**

Table A11 - Continued

nickel	**μg/L**	**<20**	**<20**	**<20**	**<20**
phosphorus	**μg/L**	**144**	**<100**	**240**	**<100**
lead	**μg/L**	**<100**	**<100**	**<100**	**<100**
antimony	**μg/L**	**<50**	**<50**	**<50**	**<50**
silicon	**μg/L**	**709**	**851**	**842**	**720**
tin	**μg/L**	**<200**	**<200**	**<200**	**<200**
strontium	**μg/L**	**503**	**486**	**223**	**295**
titanium	**μg/L**	**<10**	**<10**	**<10**	**<10**
vanadium	**μg/L**	**<10**	**<10**	**<10**	**<10**
tungsten	**μg/L**	**<50**	**<50**	**<50**	**<50**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**
zinc	**μg/L**	**<10**	**29**	**74**	**133**
zirconium	**μg/L**	**<10**	**<10**	**<10**	**<10**

Table A11 - Continued

Table A12 -Physical/Chemical Data Copper/Zinc Mine Type (Site 12) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 12			
		Period 1 (12)	Period 2 (21)	Period 3 (47)	Period 4 (69)
pH		6.61	7.47	6.58	6.95
conductivity	μmho/cm	2514	1615	2497	2463
ammonia	mg/L	0.1	0.74	0.4	0.3
alkalinity	mg/L	90	81	23	36
total hardness	mg/L	1293	975	1601	1440
total suspended solids	mg/L	<1	4	5	134
total dissolved solids	mg/L	2424	1548	2500	2440
ICP scan - Total Metals					
silver	μg/L	<20	<20	<20	<20
aluminum	μg/L	679	<10	521	47
arsenic	μg/L	<100	<100	<100	<100
barium	μg/L	24	19	21	35
beryllium	μg/L	<5	<5	<5	<5
bismuth	μg/L	137	<50	<50	<50
calcium	μg/L	572600	378900	610300	570600
cadmium	μg/L	34	<10	<10	<10
cobalt	μg/L	<10	<10	<10	14
chromium	μg/L	<10	14	37	15
copper	μg/L	59	<10	98	11
iron	μg/L	<100	<100	369	<100
gallium	μg/L	<50	<50	<50	<50
potassium	μg/L	15360	8508	19800	15000
lithium	μg/L	<5	<5	62	25
magnesium	μg/L	7075	6675	33340	3193
manganese	μg/L	58	72	463	<10
molybdenum	μg/L	30	53	<20	40

Table A11 - Continued

sodium	μg/L	91540	61720	94240	76420
nickel	μg/L	<20	<20	25	<20
phosphorus	μg/L	<100	850	313	<100
lead	μg/L	<100	<100	166	<100
strontium	μg/L	1154	845	2047	1653
vanadium	μg/L	<10	<10	<10	<10
yttrium	μg/L	<5	<5	934<5	<5
zinc	μg/L	320	335	934	125
ICP Scan - dissolved metals					
silver	μg/L	<10	29	<10	14
aluminum	μg/L	<10	<10	<100	<10
arsenic	μg/L	<100	<100	<100	<100
boron	μg/L	<10	20	<10	<10
barium	μg/L	24	21	7	38
beryllium	μg/L	<5	<5	<5	<5
bismuth	μg/L	<50	<50	<50	<50
calcium	μg/L	599000	395800	680100	628000
cadmium	μg/L	<10	<10	<10	<10
cobalt	μg/L	<10	<10	<10	<10
chromium	μg/L	25	16	<10	18
copper	μg/L	25	<10	19	<10
iron	μg/L	<100	<100	<100	<100
gallium	μg/L	<50	<50	<50	<50
potassium	μg/L	12300	6290	16600	14600
lithium	μg/L	<5	<5	128	25
magnesium	μg/L	6281	7253	70140	3143
manganese	μg/L	53	56	71	13
molybdenum	μg/L	42	41	<20	<20
sodium	μg/L	86320	58610	18350	80350
niobium	μg/L	<20	<20	<20	<20

Table A12 - Continued

nickel	**μg/L**	**<20**	**<20**	**<20**	**25**
phosphorus	**μg/L**	**<100**	**121**	**54**	**<100**
lead	**μg/L**	**<100**	**<100**	**158**	**<100**
antimony	**μg/L**	**187**	**<50**	**<50**	**<50**
silicon	**μg/L**	**519**	**378**	**143**	**159**
tin	**μg/L**	**<200**	**204**	**<200**	**<200**
strontium	**μg/L**	**1185**	**906**	**634**	**1798**
titanium	**μg/L**	**<10**	**<10**	**<10**	**<10**
vanadium	**μg/L**	**<10**	**<10**	**<10**	**<10**
tungsten	**μg/L**	**<50**	**<50**	**67**	**<50**
yttrium	**μg/L**	**<5**	**<5**	**<5**	**<5**
zinc	**μg/L**	**327**	**350**	**<10**	**196**
zirconium	**μg/L**	**<10**	**<10**	**<10**	**<10**

Table A12 - Continued

Table A13 - Physical/Chemical Data For Gold Mine Type (Site 13) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 13		
		Period 1 (7)	QA/QC (7)	Period 2 (34)
pH	units	8.06	8.3	6.38
conductivity	μMho/cm	3160	3290	3364
ammonia	mg/L	4.69	6	1.3
alkalinity	mg/L	47	20.2	23
total hardness	mg/L	1980	2224/2229	2749
total suspended solids	mg/L	<1	2.9	19
total dissolved solids	mg/L	2928	3465	3544
cyanide - total	mg/L	0.029	0.006	0.21
cyanide - free	mg/L	0.022	<0.005	0.081
thiocyanate	mg/L	<0.1	<0.5	<0.1
cyanate	mg/l	8.1	<0.5	1.5
ICP scan - Total Metals				
silver	μg/L	<20	<0.7	33
aluminum	μg/L	845	760	1619
arsenic	μg/L	<100	<14	239
boron	μg/L	N/A	75	N/A
barium	μg/L	17	18	11
beryllium	μg/L	<5	<0.3	<5
bismuth	μg/L	588	<2	247
calcium	μg/L	745300	738000	859000
cadmium	μg/L	<10	<0.9	<10
cobalt	μg/L	<10	5.4	<10
chromium	μg/L	<10	<11	30
copper	μg/L	14	<31	25
iron	μg/L	229	120	240

Table A12 - Continued

gallium	**μg/L**	**52**	**<0.3**	**<50**
potassium	**μg/L**	**9680**	**10600**	**6070**
lithium	**μg/L**	**191**	**77**	**<5**
magnesium	**μg/L**	**73560**	**76100**	**150400**
manganese	**μg/L**	**431**	**449**	**389**
molybdenum	**μg/L**	**<20**	**1.4**	**<20**
sodium	**μg/L**	**59200**	**56500**	**86320**
nickel	**μg/L**	**<20**	**13**	**44**
phosphorus	**μg/L**	**<100**	**<200**	**<100**
lead	**μg/L**	**<100**	**<11**	**<100**
antimony	**μg/L**	**N/A**	**<4**	**N/A**
tin	**μg/L**	**N/A**	**<12**	**N/A**
strontium	**μg/L**	**1696**	**1750**	**1553**
titanium	**μg/L**	**N/A**	**1.6**	**N/A**
vanadium	**μg/L**	**<10**	**<7**	**<10**
yttrium	**μg/L**	**<5**	**0.4**	**<5**
zinc	**μg/L**	**15**	**<75**	**36**
tungsten	**μg/L**	**N/A**	**<4**	**N/A**
zirconium	**μg/L**	**N/A**	**<7**	**N/A**
rubidium	**μg/L**	**N/A**	**23**	**N/A**
cesium	**μg/L**	**N/A**	**0.69**	**N/A**
thallium	**μg/L**	**N/A**	**<0.3**	**N/A**
uranium	**μg/L**	**N/A**	**0.39**	**N/A**
mercury	**μg/L**	**N/A**	**<7**	**N/A**
ICP Scan - dissolved metals				
silver	**μg/L**	**43**	**<0.6**	**30**
aluminum	**μg/L**	**<10**	**590**	**865**
arsenic	**μg/L**	**<100**	**<5**	**400**
boron	**μg/L**	**<10**	**62**	**<10**
barium	**μg/L**	**14**	**21**	**14**
beryllium	**μg/L**	**<5**	**<0.3**	**<5**
bismuth	**μg/L**	**610**	**<1**	**<50**

Table A13 - Continued

calcium	**μg/L**	**676000**	**763000**	**743000**
cadmium	**μg/L**	**<10**	**<0.2**	**<10**
cobalt	**μg/L**	**<10**	**4.8**	**<10**
chromium	**μg/L**	**<10**	**<4**	**<10**
copper	**μg/L**	**<10**	**6**	**10**
iron	**μg/L**	**<100**	**50**	**145**
gallium	**μg/L**	**<50**	**<0.3**	**<50**
potassium	**μg/L**	**8050**	**10800**	**7200**
lithium	**μg/L**	**270**	**60**	**<5**
magnesium	**μg/L**	**65100**	**77400**	**121600**
manganese	**μg/L**	**473**	**443**	**435**
molybdenum	**μg/L**	**<20**	**1.3**	**<20**
sodium	**μg/L**	**56300**	**58000**	**80600**
niobium	**μg/L**	**<20**	**<0.3**	**<20**
nickel	**μg/L**	**<20**	**13**	**<20**
phosphorus	**μg/L**	**203**	**<200**	**<100**
lead	**μg/L**	**<100**	**<0.5**	**<100**
antimony	**μg/L**	**163**	**<0.3**	**586**
silicon	**μg/L**	**624**	**200**	**410**
tin	**μg/L**	**204**	**<0.3**	**270**
strontium	**μg/L**	**1840**	**1870**	**1761**
titanium	**μg/L**	**<10**	**1.1**	**<10**
vanadium	**μg/L**	**13**	**<7**	**<10**
tungsten	**μg/L**	**<50**	**<0.4**	**<50**
yttrium	**μg/L**	**<5**	**0.15**	**<5**
zinc	**μg/L**	**29**	**50**	**20**
zirconium	**μg/L**	**<10**	**<0.3**	**<10**
titanium	**μg/L**	**N/A**	**1.1**	**N/A**
rubidium	**μg/L**	**N/A**	**24**	**N/A**
cesium	**μg/l**	**N/A**	**0.67**	**N/A**
thallium	**μg/L**	**N/A**	**<0.3**	**N/A**

Table A13 - Continued

uranium	**μg/L**	**N/A**	**0.38**	**N/A**
mercury	**μg/L**	**N/A**	**<2**	**N/A**

N/A - not analysed

Table A13 - Continued

Table A14 -Physical/Chemcial Data for Uranium Mine Type (Site 14) (Number in Parentheses is the Canmet Sample Number)

Parameter	**Units**	**Site 14**	
		Period 1 (11)	**Period 2 (18)**
pH		**7.51**	**7.34**
conductivity	**µmho/cm**	**2240**	**30**
ammonia	**mg/L**	**2.94**	**2250**
alkalinity	**mg/L**	**56**	**1329**
total hardness	**mg/L**	**1204**	**3.21**
total suspended solids	**mg/L**	**<1**	**2**
total dissolved solids	**mg/L**	**2160**	**2212**
uranium	**mg/L**	**62**	**39**
ICP scan - Total Metals			
silver	**µg/L**	**<20**	**62**
aluminum	**µg/L**	**505**	**536**
arsenic	**µg/L**	**<100**	**<100**
barium	**µg/L**	**45**	**54**
beryllium	**µg/L**	**<5**	**<5**
bismuth	**µg/L**	**511**	**<50**
calcium	**µg/L**	**491100**	**494500**
cadmium	**µg/L**	**<10**	**<10**
cobalt	**µg/L**	**<10**	**52**
chromium	**µg/L**	**18**	**18**
copper	**µg/L**	**11**	**<10**
iron	**µg/L**	**121**	**356**
gallium	**µg/L**	**<50**	**<50**
potassium	**µg/L**	**78900**	**57240**
lithium	**µg/L**	**366**	**44**
magnesium	**µg/L**	**27100**	**22500**
manganese	**µg/L**	**302**	**354**
molybdenum	**µg/L**	**<20**	**41**

Table A13 - Continued

sodium	**μg/L**	**31740**	**43230**
nickel	**μg/L**	**<20**	**<20**
phosphorus	**μg/L**	**<100**	**631**
lead	**μg/L**	**<100**	**146**
strontium	**μg/L**	**624**	**620**
vanadium	**μg/L**	**<10**	**<10**
yttrium	**μg/L**	**<5**	**<5**
zinc	**μg/L**	**<10**	**11**
ICP Scan - dissolved metals			
silver	**μg/L**	**10**	**21**
aluminum	**μg/L**	**<10**	**313**
arsenic	**μg/L**	**<100**	**<100**
boron	**μg/L**	**<10**	**28**
barium	**μg/L**	**28**	**31**
beryllium	**μg/L**	**<5**	**<5**
bismuth	**μg/L**	**306**	**<50**
calcium	**μg/L**	**443000**	**483640**
cadmium	**μg/L**	**<10**	**<10**
cobalt	**μg/L**	**14**	**10**
chromium	**μg/L**	**<10**	**<10**
copper	**μg/L**	**<10**	**<10**
iron	**μg/L**	**<100**	**157**
gallium	**μg/L**	**<50**	**<50**
potassium	**μg/L**	**78420**	**46480**
lithium	**μg/L**	**297**	**<5**
magnesium	**μg/L**	**23200**	**24060**
manganese	**μg/L**	**315**	**361**
molybdenum	**μg/L**	**<20**	**<20**
sodium	**μg/L**	**32100**	**39760**
niobium	**μg/L**	**<20**	**<20**

Table A14 - Continued

nickel	**μg/L**	**<20**	**29**
phosphorus	**μg/L**	**324**	**<100**
lead	**μg/L**	**<100**	**<100**
antimony	**μg/L**	**<50**	**<50**
silicon	**μg/L**	**1173**	**1040**
tin	**μg/L**	**<200**	**<200**
strontium	**μg/L**	**653**	**626**
titanium	**μg/L**	**<10**	**<10**
vanadium	**μg/L**	**<10**	**<10**
tungsten	**μg/L**	**65**	**<50**
yttrium	**μg/L**	**<5**	**<5**
zinc	**μg/L**	**<10**	**<10**
zirconium	**μg/L**	**<10**	**<10**

Table A14 - Continued

Table A15 - Physical/Chemical Data For Gold Mine Type (Site 15) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 15	
		Period 1 (15)	Period 2 (29)
pH	units	6.91	7.44
conductivity	μMho/cm	2590	2452
ammonia	mg/L	15.3	12
alkalinity	mg/L	45	38
total hardness	mg/L	1102	1285
total suspended solids	mg/L	8	2
total dissolved solids	mg/L	2484	2220
cyanide - total	mg/L	0.213	0.104
cyanide - free	mg/L	0.213	0.085
ICP scan - Total Metals			
silver	μg/L	<20	<20
aluminum	μg/L	928	679
arsenic	μg/L	225	<100
barium	μg/L	13	16
beryllium	μg/L	<5	<5
bismuth	μg/L	117	<50
calcium	μg/L	500600	456800
cadmium	μg/L	<10	<10
cobalt	μg/L	<10	30
chromium	μg/L	<10	11
copper	μg/L	16	20
iron	μg/L	144	305
gallium	μg/L	<50	<50
potassium	μg/L	87570	67920
lithium	μg/L	<5	<5
magnesium	μg/L	6290	5138

Table A14 - Continued

manganese	**μg/L**	**1239**	**1368**
molybdenum	**μg/L**	**123**	**176**
sodium	**μg/L**	**20430**	**158000**
nickel	**μg/L**	**136**	**100**
phosphorus	**μg/L**	**<100**	**<100**
lead	**μg/L**	**<100**	**102**
strontium	**μg/L**	**520**	**369**
vanadium	**μg/L**	**<10**	**13**
yttrium	**μg/L**	**<5**	**<5**
zinc	**μg/L**	**301**	**92**
ICP Scan - dissolved metals			
silver	**μg/L**	**<10**	**30**
aluminum	**μg/L**	**<10**	**64**
arsenic	**μg/L**	**<100**	**<100**
boron	**μg/L**	**30**	**58**
barium	**μg/L**	**20**	**27**
beryllium	**μg/L**	**<5**	**<5**
bismuth	**μg/L**	**<50**	**<50**
calcium	**μg/L**	**519000**	**426100**
cadmium	**μg/L**	**<10**	**17**
cobalt	**μg/L**	**16**	**<10**
chromium	**μg/L**	**11**	**<10**
copper	**μg/L**	**<10**	**<10**
iron	**μg/L**	**<100**	**<100**
gallium	**μg/L**	**<50**	**<50**
potassium	**μg/L**	**68000**	**66200**
lithium	**μg/L**	**<5**	**<5**
magnesium	**μg/L**	**5733**	**5378**
manganese	**μg/L**	**1253**	**1210**
molybdenum	**μg/L**	**81**	**146**

Table A15 - Continued

sodium	**μg/L**	**201000**	**167800**
niobium	**μg/L**	**68**	**<20**
nickel	**μg/L**	**184**	**56**
phosphorus	**μg/L**	**<100**	**<100**
lead	**μg/L**	**<100**	**<100**
antimony	**μg/L**	**385**	**220**
silicon	**μg/L**	**1010**	**931**
tin	**μg/L**	**<200**	**<200**
strontium	**μg/L**	**514**	**443**
titanium	**μg/L**	**<10**	**<10**
vanadium	**μg/L**	**<10**	**<10**
tungsten	**μg/L**	**<50**	**<50**
yttrium	**μg/L**	**<5**	**<5**
zinc	**μg/L**	**158**	**71**
zirconium	**μg/L**	**<10**	**<10**

Table A15 - Continued

Table A16 - Physical/Chemical Data for Lead/Zinc Mine Type (Site 16) (Number in Parentheses is the Canmet Sample Number)

Parameter	**Units**	**Site 16**		
		Period 1 (16)	**Period 2 (25)**	**Period 4 (44)**
pH		**6.26**	**6.16**	**6.25**
conductivity	**μmho/cm**	**3614**	**3014**	**2934**
ammonia	**mg/L**	**<0.1**	**1.38**	**<0.1**
alkalinity	**mg/L**	**15**	**8**	**3**
total hardness	**mg/L**	**1211**	**1265**	**1668**
total suspended solids	**mg/L**	**14**	**8**	**7**
total dissolved solids	**mg/L**	**3468**	**2532**	**2150**
ICP scan - Total Metals				
silver	**μg/L**	**45**	**<20**	**<20**
aluminum	**μg/L**	**578**	**410**	**413**
arsenic	**μg/L**	**<100**	**<100**	**<100**
barium	**μg/L**	**62**	**54**	**21**
beryllium	**μg/L**	**<5**	**<5**	**<5**
bismuth	**μg/L**	**<50**	**58**	**53**
calcium	**μg/L**	**414800**	**362600**	**641800**
cadmium	**μg/L**	**<10**	**<10**	**<10**
cobalt	**μg/L**	**<10**	**<10**	**<10**
chromium	**μg/L**	**<10**	**<10**	**30**
copper	**μg/L**	**12**	**<10**	**<10**
iron	**μg/L**	**<100**	**<100**	**228**
gallium	**μg/L**	**<50**	**<50**	**<50**
potassium	**μg/L**	**8030**	**5520**	**5590**
lithium	**μg/L**	**<5**	**<5**	**55**
magnesium	**μg/L**	**73800**	**41650**	**40190**
manganese	**μg/L**	**3270**	**703**	**319**
molybdenum	**μg/L**	**51**	**<20**	**<20**

Table A15 - Continued

sodium	**μg/L**	**67400**	**475700**	**176200**
nickel	**μg/L**	**<20**	**<20**	**<20**
phosphorus	**μg/L**	**<100**	**<100**	**150**
lead	**μg/L**	**100**	**<100**	**205**
strontium	**μg/L**	**637**	**525**	**593**
vanadium	**μg/L**	**<10**	**<10**	**<10**
yttrium	**μg/L**	**<5**	**<5**	**<5**
zinc	**μg/L**	**163**	**62**	**159**
ICP Scan - dissolved metals				
silver	**μg/L**	**<10**	**40**	**14**
aluminum	**μg/L**	**53**	**176**	**<10**
arsenic	**μg/L**	**<100**	**<100**	**<100**
boron	**μg/L**	**<10**	**<10**	**<10**
barium	**μg/L**	**64**	**57**	**26**
beryllium	**μg/L**	**<5**	**<5**	**<5**
bismuth	**μg/L**	**<50**	**100**	**<50**
calcium	**μg/L**	**456000**	**383000**	**608800**
cadmium	**μg/L**	**<10**	**<10**	**<10**
cobalt	**μg/L**	**<10**	**11**	**<10**
chromium	**μg/L**	**<10**	**<10**	**<10**
copper	**μg/L**	**<10**	**18**	**<10**
iron	**μg/L**	**<100**	**<100**	**<100**
gallium	**μg/L**	**<50**	**<50**	**<50**
potassium	**μg/L**	**7680**	**5540**	**5640**
lithium	**μg/L**	**<5**	**<5**	**96**
magnesium	**μg/L**	**77700**	**45980**	**32300**
manganese	**μg/L**	**3410**	**770**	**263**
molybdenum	**μg/L**	**<20**	**<20**	**<20**
sodium	**μg/L**	**668000**	**509600**	**173500**
niobium	**μg/L**	**42**	**<20**	**<20**

Table 17 - Continued

nickel	μ**g/L**	**<20**	**<20**	**<20**
phosphorus	μ**g/L**	**<100**	**<100**	**278**
lead	μ**g/L**	**<100**	**<100**	**136**
antimony	μ**g/L**	**321**	**80**	**<50**
silicon	μ**g/L**	**113**	**182**	**<50**
tin	μ**g/L**	**475**	**244**	**<200**
strontium	μ**g/L**	**669**	**557**	**545**
titanium	μ**g/L**	**<10**	**<10**	**<10**
vanadium	μ**g/L**	**<10**	**<10**	**<10**
tungsten	μ**g/L**	**<50**	**<50**	**<50**
yttrium	μ**g/L**	**<5**	**<5**	**<5**
zinc	μ**g/L**	**95**	**96**	**16**
zirconium	μ**g/L**	**<10**	**<10**	**<10**

Table 17 - Continued

Table 18- Physical/Chemical Data for Copper (Site 17) (Number in Parentheses is the Canment Sample Number)

Parameters	Units	Site 17			
		Period 2 (14)	Period 3 (23)	Period 4 (55)	Period 4 (55Q)
pH		**8.91**	**8.78**	**7.09**	**9**
conductivity	**μmho/cm**	**3091**	**2888**	**3116**	**3330**
ammonia	**mg/L**	**10.1**	**25.5**	**24.3**	**26**
alkalinity	**mg/L**	**44**	**43**	**33**	**38**
total hardness	**mg/L**	**2151**	**2083**	**1985**	**N/A**
total suspended solids	**mg/L**	**5**	**2**	**19**	**7.4**
total dissolved solids	**mg/L**	**3148**	**3112**	**3196**	**3386**
ICP scan - Total Metals					
silver	**μg/L**	**30**	**10**	**<20**	**<0.3**
aluminum	**μg/L**	**91**	**657**	**237**	**140**
arsenic	**μg/L**	**<100**	**189**	**<100**	**3.9**
barium	**μg/L**	**22**	**16**	**<5**	**15.1**
beryllium	**μg/L**	**<5**	**<5**	**<5**	**<3**
bismuth	**μg/L**	**407**	**<50**	**<50**	**<1**
calcium	**μg/L**	**663000**	**709189**	**651000**	**692000**
cadmium	**μg/L**	**<10**	**<10**	**<10**	**<2**
cobalt	**μg/L**	**<10**	**<10**	**16**	**2.1**
chromium	**μg/L**	**16**	**26**	**67**	**<4**
copper	**μg/L**	**26**	**49**	**24**	**13.1**
iron	**μg/L**	**<100**	**579**	**2189**	**1800**
gallium	**μg/L**	**<50**	**<50**	**<50**	**<1**
potassium	**μg/L**	**50010**	**54600**	**49200**	**46900**
lithium	**μg/L**	**<5**	**227**	**109**	**<300**
magnesium	**μg/L**	**67110**	**74515**	**85900**	**83000**
manganese	**μg/L**	**98**	**192**	**253**	**274000**
molybdenum	**μg/L**	**<20**	**<20**	**<20**	**<2**
sodium	**μg/L**	**95580**	**62974**	**52810**	**59600**

Table 17 - Continued

nickel	μg/L	29	<20	62	<26
phosphorus	μg/L	107	183	<100	<3000
lead	μg/L	<100	160	<100	<3
strontium	μg/L	1630	1718	1651	1400
vanadium	μg/L	<10	<10	<10	79.1
yttrium	μg/L	<5	<5	<5	0.5
zinc	μg/L	38	47	165	200
antimony	μg/L	N/A	N/A	N/A	<11
boron	μg/L	N/A	N/A	N/A	59.8
niobium	μg/L	N/A	N/A	N/A	<1
silicon	μg/L	N/A	N/A	N/A	0.2
tin	μg/L	N/A	N/A	N/A	<6
titanium	μg/L	N/A	N/A	N/A	<4
tungsten	μg/L	N/A	N/A	N/A	<3
zirconium	μg/L	N/A	N/A	N/A	<0.5
ICP Scan - dissolved metals					
silver	μg/L	38	21	<20	<0.3
aluminum	μg/L	26	184	<10	140
arsenic	μg/L	<100	206	<100	87.3
boron	μg/L	<10	<10	<5	29
barium	μg/L	34	19	<5	19.6
beryllium	μg/L	<5	<5	<5	<3
bismuth	μg/L	409	<50	<50	<1
calcium	μg/L	672300	746400	644300	673000
cadmium	μg/L	<10	<10	<20	<2
cobalt	μg/L	<10	<10	<10	1.9
chromium	μg/L	<10	<10	25	17.3
copper	μg/L	30	43	<10	41.7
iron	μg/L	108	496	2078	2000

Table 18 - Continued

gallium	μg/L	<50	<50	<50	<1
potassium	μg/L	51300	45000	46200	46200
lithium	μg/L	<5	270	91	<300
magnesium	μg/L	69160	78200	86210	86000
manganese	μg/L	103	201	256	275
molybdenum	μg/L	<20	<20	<20	<2
sodium	μg/L	87610	65300	53830	61400
niobium	μg/L	<20	<20	<20	<1
nickel	μg/L	<20	<20	43	<26
phosphorus	μg/L	<100	324	<100	<3000
lead	μg/L	<100	188	<100	<3
antimony	μg/L	135	<50	<50	<11
silicon	μg/L	168	<50	<50	100
tin	μg/L	220	<200	<200	<6
strontium	μg/L	1620	1810	1687	1600
titanium	μg/L	<10	<10	<10	4.6
vanadium	μg/L	<10	<10	<10	1304
tungsten	μg/L	<50	<50	<50	<3
yttrium	μg/L	<5	<5	<5	0.7
zinc	μg/L	<10	39	167	200
zirconium	μg/L	<10	<10	<10	1.1

Table 18 - Continued

Table 19 - Physical/Chemical Data for Nickel/Copper Mine Type (Site 18) (Number in Parenthesis is the Canmet Sample Number)

Parameter	Units	Site 18	
		Period 3 (54)	Period 4 (68)
pH		9.88	9.17
conductivity	μmho/cm	549	6620
ammonia	mg/L	<0.1	<0.1
alkalinity	mg/L	283	333
total hardness	mg/L	49	42
total suspended solids	mg/L	11	6
total dissolved solids	mg/L	500	4616
ICP scan - Total Metals			
silver	μg/L	<20	<20
aluminum	μg/L	327	179
arsenic	μg/L	345	347
barium	μg/L	73	39
beryllium	μg/L	<5	<5
bismuth	μg/L	150	<50
calcium	μg/L	13320	8944
cadmium	μg/L	<10	<10
cobalt	μg/L	110	52
chromium	μg/L	20	<10
copper	μg/L	118	112
iron	μg/L	173	108
gallium	μg/L	<50	<50
potassium	μg/L	7260	8940
lithium	μg/L	19	<5
magnesium	μg/L	6916	4891
manganese	μg/L	<10	<10
molybdenum	μg/L	86	76

Table 18 - Continued

sodium	μg/L	1162000	1183000
nickel	μg/L	183	135
phosphorus	μg/L	<100	<100
lead	μg/L	<100	<100
strontium	μg/L	169	89
vanadium	μg/L	10	<10
yttrium	μg/L	<5	<5
zinc	μg/L	20	<10
ICP Scan - dissolved metals			
silver	μg/L	10	<10
aluminum	μg/L	<10	<10
arsenic	μg/L	235	334
boron	μg/L	412	341
barium	μg/L	16	14
beryllium	μg/L	<5	<5
bismuth	μg/L	<50	<50
calcium	μg/L	8673	9797
cadmium	μg/L	14	23
cobalt	μg/L	<10	28
chromium	μg/L	<10	<10
copper	μg/L	18	47
iron	μg/L	<100	<100
gallium	μg/L	<50	<50
potassium	μg/L	7310	11900
lithium	μg/L	<5	<5
magnesium	μg/L	6754	5523
manganese	μg/L	<10	<10
molybdenum	μg/L	119	42
sodium	μg/L	1178000	1251000
niobium	μg/L	<20	<20

Table 19 - Continued

nickel	**µg/L**	**20**	**63**
phosphorus	**µg/L**	**161**	**<100**
lead	**µg/L**	**<100**	**<100**
antimony	**µg/L**	**435**	**<50**
silicon	**µg/L**	**4161**	**2260**
tin	**µg/L**	**<200**	**<200**
strontium	**µg/L**	**89**	**67**
titanium	**µg/L**	**<10**	**<10**
vanadium	**µg/L**	**<10**	**<10**
tungsten	**µg/L**	**<50**	**<50**
yttrium	**µg/L**	**<5**	**<5**
zinc	**µg/L**	**<10**	**<10**
zirconium	**µg/L**	**<10**	**<10**

Table 19 - Continued

Table 20 - Physical/Chemical Data for Gold Mine Type (Site 19) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 19 Period 4 (50)
pH		9.65
conductivity	μmho/cm	3330
ammonia	mg/L	12.8
alkalinity	mg/L	50
total hardness	mg/L	1850
total suspended solids	mg/L	14
total dissolved solids	mg/L	3280
ICP scan - Total Metals		
silver	μg/L	<20
aluminum	μg/L	503
arsenic	μg/L	<100
barium	μg/L	32
beryllium	μg/L	<5
bismuth	μg/L	<50
calcium	μg/L	592900
cadmium	μg/L	<10
cobalt	μg/L	825
chromium	μg/L	28
copper	μg/L	8374
iron	μg/L	493
gallium	μg/L	<50
potassium	μg/L	35880
lithium	μg/L	70
magnesium	μg/L	88480
manganese	μg/L	363
molybdenum	μg/L	<20

Table 19 - Continued

sodium	**μg/L**	**154700**
nickel	**μg/L**	**3221**
phosphorus	**μg/L**	**<100**
lead	**μg/L**	**<100**
strontium	**μg/L**	**883**
vanadium	**μg/L**	**<10**
yttrium	**μg/L**	**<5**
zinc	**μg/L**	**98**
ICP Scan - dissolved metals		
silver	**μg/L**	**<10**
aluminum	**μg/L**	**<10**
arsenic	**μg/L**	**<100**
boron	**μg/L**	**<10**
barium	**μg/L**	**27**
beryllium	**μg/L**	**<5**
bismuth	**μg/L**	**<50**
calcium	**μg/L**	**621000**
cadmium	**μg/L**	**<10**
cobalt	**μg/L**	**925**
chromium	**μg/L**	**19**
copper	**μg/L**	**712**
iron	**μg/L**	**<100**
gallium	**μg/L**	**<50**
potassium	**μg/L**	**35800**
lithium	**μg/L**	**77**
magnesium	**μg/L**	**94030**
manganese	**μg/L**	**<10**
molybdenum	**μg/L**	**<20**
sodium	**μg/L**	**172200**
niobium	**μg/L**	**<20**

Table 20 - Continued

nickel	μg/L	1185
phosphorus	μg/L	<100
lead	μg/L	<100
antimony	μg/L	<50
silicon	μg/L	1150
tin	μg/L	<200
strontium	μg/L	945
titanium	μg/L	<10
vanadium	μg/L	<10
tungsten	μg/L	<50
yttrium	μg/L	<5
zinc	μg/L	18
zirconium	μg/L	<10

Table 20 - Continued

Table 21- Physical/Chemical Data for Uranium Mine Type (Site 20) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 20	
		Period 3 (39)	Period 4 (60)
pH		7.25	7.45
conductivity	μmho/cm	723	705
ammonia	mg/L	0.32	0.3
alkalinity	mg/L	6	8
total hardness	mg/L	352	316
total suspended solids	mg/L	<1.0	4
total dissolved solids	mg/L	540	524
uranium	μg/L	8.1	26
ICP scan - Total Metals			
silver	μg/L	<20	<20
aluminum	μg/L	637	455
arsenic	μg/L	<100	<100
barium	μg/L	14	9
beryllium	μg/L	<5	<5
bismuth	μg/L	<50	<50
calcium	μg/L	97930	90460
cadmium	μg/L	36	17
cobalt	μg/L	16	21
chromium	μg/L	18	62
copper	μg/L	18	21
iron	μg/L	486	615
gallium	μg/L	<50	95
potassium	μg/L	7176	6228
lithium	μg/L	72	52
magnesium	μg/L	25600	21450
manganese	μg/L	158	154

Table 20 - Continued

molybdenum	μg/L	55	24
sodium	μg/L	13240	11360
nickel	μg/L	<20	37
phosphorus	μg/L	<100	<100
lead	μg/L	<100	<100
strontium	μg/L	230	215
vanadium	μg/L	<10	<10
yttrium	μg/L	<5	<5
zinc	μg/L	<10	<10
ICP Scan - dissolved metals			
silver	μg/L	<10	<20
aluminum	μg/L	235	165
arsenic	μg/L	<100	<100
boron	μg/L	<10	<5
barium	μg/L	14	10
beryllium	μg/L	<5	<5
bismuth	μg/L	<50	<50
calcium	μg/L	105700	93450
cadmium	μg/L	<10	<10
cobalt	μg/L	10	19
chromium	μg/L	<10	<10
copper	μg/L	<10	<10
iron	μg/L	<100	38
gallium	μg/L	<50	<50
potassium	μg/L	7190	7060
lithium	μg/L	35	38
magnesium	μg/L	27720	22000
manganese	μg/L	164	160
molybdenum	μg/L	66	29
sodium	μg/L	12000	11205

Table 21 - Continued

niobium	μg/L	<20	<20
nickel	μg/L	<20	25
phosphorus	μg/L	<100	<100
lead	μg/L	<100	<100
antimony	μg/L	125	<50
silicon	μg/L	994	1665
tin	μg/L	<200	<200
strontium	μg/L	244	220
titanium	μg/L	<10	<10
vanadium	μg/L	<10	<10
tungsten	μg/L	82	<50
yttrium	μg/L	<5	<5
zinc	μg/L	<10	<10
zirconium	μg/L	<10	<10

Table 21 - Continued

Table 22 - Physical/Chemical Data for Gold Mine Type (Site 21) (Number in Parentheses is the Canmet Sample Number)

Parameter	Units	Site 21
		Period 3 (48)
pH		7.33
conductivity	μmho/cm	2951
ammonia	mg/L	18.5
alkalinity	mg/L	36
total hardness	mg/L	1449
total suspended solids	mg/L	9
total dissolved solids	mg/L	2728
cyanide - total	mg/L	0.22
cyanide - free	mg/L	0.105
ICP scan - Total Metals		
silver	μg/L	47
aluminum	μg/L	417
arsenic	μg/L	<100
barium	μg/L	7
beryllium	μg/L	<5
bismuth	μg/L	<50
calcium	μg/L	577600
cadmium	μg/L	<10
cobalt	μg/L	10
chromium	μg/L	<10
copper	μg/L	38
iron	μg/L	371
gallium	μg/L	<500
potassium	μg/L	101100
lithium	μg/L	14
magnesium	μg/L	7595

Table 21 - Continued

manganese	**μg/L**	**104**
molybdenum	**μg/L**	**415**
sodium	**μg/L**	**115700**
nickel	**μg/L**	**253**
phosphorus	**μg/L**	**<100**
lead	**μg/L**	**107**
strontium	**μg/L**	**343**
vanadium	**μg/L**	**<10**
yttrium	**μg/L**	**<5**
zinc	**μg/L**	**898**
ICP Scan - dissolved metals		
silver	**μg/L**	**<20**
aluminum	**μg/L**	**<10**
arsenic	**μg/L**	**<100**
boron	**μg/L**	**<5**
barium	**μg/L**	**19**
beryllium	**μg/L**	**<5**
bismuth	**μg/L**	**<50**
calcium	**μg/L**	**568300**
cadmium	**μg/L**	**<10**
cobalt	**μg/L**	**10**
chromium	**μg/L**	**<10**
copper	**μg/L**	**29**
iron	**μg/L**	**<100**
gallium	**μg/L**	**<50**
potassium	**μg/L**	**88900**
lithium	**μg/L**	**10**
magnesium	**μg/L**	**7058**
manganese	**μg/L**	**52**
molybdenum	**μg/L**	**336**

Table 22- Continued

sodium	μg/L	120
niobium	μg/L	<20
nickel	μg/L	237
phosphorus	μg/L	<100
lead	μg/L	120
antimony	μg/L	<50
silicon	μg/L	403
tin	μg/L	<200
strontium	μg/L	348
titanium	μg/L	<10
vanadium	μg/L	<10
tungsten	μg/L	155
yttrium	μg/L	<5
zinc	μg/L	<10
zirconium	μg/L	<10

APPENDIX B

COMPARISON OF MICROTESTS TO RAINBOW TROUT ON A SITE BY SITE BASIS

Appendix B Comparison of Microtest to Rainbow Trout Results on a Site by Site Basis

(Results in Parenthesis are the Comparison of the *Daphnia magna* IQ to the *Daphnia magna* Acute)

Mine Type	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox	Rotoxkit	Thamnotoxkit	Toxichromotest
1	Rainbow Trout more sensitive	*Daphnia magna* IQ more sensitive (IQ)	Rainbow Trout more sensitive	Rainbow Trout more sensitive	Rainbow Trout more sensitive	Rainbow Trout more sensitive
2		*Daphnia magna* IQ more sensitive				
3	Rainbow Trout more sensitive	*Daphnia magna* IQ more sensitive (IQ)		Rainbow Trout more sensitive	Thamnotoxkit more sensitive	
4		(IQ)			Rainbow Trout more sensitive	
5				Rainbow Trout more sensitive		
6		*Daphnia magna* IQ more sensitive	Rainbow Trout more sensitive			
7	*Daphnia magna* more sensitive	*Daphnia magna* IQ more sensitive				
8		*Daphnia magna* IQ more sensitive (IQ)		Rotoxkit more sensitive		
9	Rainbow Trout more sensitive				Thamnotoxkit more sensitive	
10		*Daphnia magna* IQ more sensitive (IQ)			Thamnotoxkit more sensitive	
11		*Daphnia magna* IQ more sensitive (IQ)		Rotoxkit more sensitive	Thamnotoxkit more sensitive	
12		*Daphnia magna* IQ more sensitive (IQ)		Rotoxkit more sensitive	Thamnotoxkit more sensitive	Toxichromotest more sensitive
13		(IQ)				

14						
15						
16	Daphnia magna more sensitive	*Daphnia magna* IQ more sensitive			Thamnotoxkit more sensitive	
17		*Daphnia magna* IQ more sensitive (IQ)	Rainbow Trout more sensitive	Rainbow Trout more sensitive		Rainbow Trout more sensitive
18						
20						
21						

Blank Fields - The power analysis would indicate insufficient data available to make a definitive conclusion.
(IQ) - The *Daphnia magna* IQ test is more sensitive than the *Daphnia magna* acute toxicity test.

APPENDIX C

DETAILED ANALYSIS OF MICROTEST COMPARISONS

Appendix C Detailed Analysis of Microtest Comparisons

Rainbow Trout vs Daphnia magna acute Toxicity Test

Summary of Sign Test Analysis for Rainbow Trout-*Daphnia magna* Acute Toxicity Test Comparison by Individual Mine							
Site #	Number of "-"	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
1	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
2	1	4	0.9375	0.0039	4	0.0625	Insufficient data to make conclusion
3	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
4	1	1	0.5	0	.	.	Insufficient data to make conclusion
5	2	4	0.6875	0.0625	4	0.0625	Insufficient data to make conclusion
6	3	4	0.3125	0.3164	4	0.0625	Insufficient data to make conclusion
7	3	3	0.1250	1	3	0.1250	*D. magna* acute more sensitive.
8	1	2	0.75	0	.	.	Insufficient data to make conclusion
9	3	3	0.125	1	3	0.125	*D. magna* acute more sensitive.
10	0	0	.	0	.	.	Insufficient data to make conclusion
11	0	0	.	0	.	.	Insufficient data to make conclusion
12	1	2	0.75	0	.	.	Insufficient data to make conclusion
13	1	1	0.5	0	.	.	Insufficient data to make conclusion
14	0	2	.	0	.	.	Insufficient data to make conclusion
15	0	0	.	0	.	.	Insufficient data to make conclusion

16	3	3	0.125	1	3	0.125	*D. magna* acute more sensitive.
17	3	4	0.3125	0.3164	4	0.0625	Insufficient data to make conclusion
18	0	1	.	0	.	.	Insufficient data to make conclusion
19	0	2	.	0	.	.	Insufficient data to make conclusion
20	0	1	.	0	.	.	Insufficient data to make conclusion
21	0	1	.	0	.	.	Insufficient data to make conclusion

Summary of Sign Test Analysis for Rainbow Trout-*Daphnia magna* Acute Toxicity Test Comparison by Mine Type							
Mine Type	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
All Mines	21	46	0.7693	0.0124	28.578	0.05	Insufficient data to make conclusion
au	2	6	0.8906	0.0178	5	0.1094	Insufficient data to make conclusion
bit	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
cu_zn	6	13	0.7094	0.0819	9	0.0461	Insufficient data to make conclusion
ni_cu	5	6	0.1094	0.7368	5	0.1094	Rainbow Trout more sensitive.
pb_zn	9	10	0.0107	0.9872	7	0.0547	*D. magna* acute more sensitive.
sn	3	4	0.3125	0.3164	4	0.0625	Insufficient data to make conclusion
u	3	3	0.125	1	3	0.125	*D. magna* acute more sensitive.
zn	0	0	.	0	.	.	Insufficient data to make conclusion

Comparison of Rainbow Trout versus *Daphnia magna* IQ toxicity tests.

Summary of Sign Test Analysis for Rainbow Trout-*Daphnia magna* IQ Toxicity Test Comparison by Individual Mine

Canmet #	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
1	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
2	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
3	5	5	0.03125	1	5	0.0312	*D. magna* IQ more sensitive.
4	2	3	0.5	0.2963	3	0.125	Insufficient data to make conclusion
5	3	5	0.5	0.0778	5	0.0312	Insufficient data to make conclusion
6	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
7	3	3	0.125	1	3	0.125	*D. magna* IQ more sensitive.
8	3	3	0.125	1	3	0.125	*D. magna* IQ more sensitive.
9	3	4	0.3125	0.3164	4	0.0625	Insufficient data to make conclusion
10	3	3	0.125	1	3	0.125	*D. magna* IQ more sensitive.
11	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
12	3	3	0.125	1	3	0.125	*D. magna* IQ more sensitive.
13	2	2	0.25	0	.	.	Insufficient data to make conclusion
14	2	2	0.25	0	.	.	Insufficient data to make conclusion
15	2	2	0.25	0	.	.	Insufficient data to make conclusion
16	3	3	0.125	1	3	0.125	*D. magna* IQ more sensitive.
17	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
18	2	2	0.25	0	.	.	Insufficient data to make conclusion
19	2	2	0.25	0	.	.	Insufficient data to make conclusion

20	2	2	0.25	0	.	.	Insufficient data to make conclusion
21	1	1	0.5	0	.	.	Insufficient data to make conclusion

Summary of Sign Test Analysis for Rainbow Trout-*Daphnia magna* IQ Toxicity Test Comparison by Mine Type

Mine Type	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
All Mines	61	65	<0.0001	1	39.13	0.05	*D. magna* IQ more sensitive.
au	10	10	< 0.001	1	7	0.0547	*D. magna* IQ more sensitive.
bit	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
cu_zn	13	15	0.0037	0.9904	10	0.592	*D. magna* IQ more sensitive.
ni_cu	12	13	0.0017	0.9980	9	0.0461	*D. magna* IQ more sensitive.
pb_zn	10	11	0.0059	0.9866	8	0.0327	*D. magna* IQ more sensitive.
sn	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
u	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
zn	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.

Rainbow Trout vs Rotoxkit

Summary of Sign Test Analysis for Rainbow Trout-Rotoxkit Toxicity Test Comparison by Individual Mine							
Canmet #	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
1	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
2	3	5	0.5	0.07776	5	0.0312	Insufficient data to make conclusion
3	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
4	1	2	0.75	0	.	.	Insufficient data to make conclusion
5	3	3	0.125	1	3	0.125	Rainbow Trout more sensitive.
6	0	1	.	0	.	.	Insufficient data to make conclusion
7	1	3	0.875	0.037037037	3	0.125	Insufficient data to make conclusion
8	3	3	0.125	1	3	0.125	Rotoxkit more sensitive.
9	0	2	.	0	.	.	Insufficient data to make conclusion
10	0	1	.	0	.	.	Insufficient data to make conclusion
11	4	4	0.0625	1	4	0.0625	Rotoxkit more sensitive.
12	3	3	0.125	1	3	0.125	Rotoxkit more sensitive.
13	0	2	.	0	.	.	Insufficient data to make conclusion
14	0	1	.	0	.	.	Insufficient data to make conclusion
15	0	1	.	0	.	.	Insufficient data to make conclusion
16	0	2	.	0	.	.	Insufficient data to make conclusion
17	3	3	0.125	1	3	0.125	Rainbow Trout more sensitive.
18	0	1	.	0	.	.	Insufficient data to make conclusion

19	0	2	.	0	.	.	Insufficient data to make conclusion
20	0	2	.	0	.	.	Insufficient data to make conclusion
21	0	1	.	0	.	.	Insufficient data to make conclusion

Summary of Sign Test Analysis for Rainbow Trout-RotoxkitToxicity Test Comparison by Mine Type

Mine Type	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
All Mines	31	50	0.0595	0.5214	35.82	0.05	Insufficient data to make conclusion
au	9	9	0.0020	1	7	0.0195	Rotoxkit more sensitive.
bit	4	4	0.0625	0	4	0.0625	Rainbow Trout more sensitive.
cu_zn	5	12	0.8062	0.0726	8	0.073	Insufficient data to make conclusion
ni_cu	3	8	0.8555	0.0360	6	0.0352	Insufficient data to make conclusion
pb_zn	6	9	0.2539	0.3772	7	0.0195	Insufficient data to make conclusion
sn	1	1	0.5	0	.	.	Insufficient data to make conclusion
u	3	3	0.125	1	3	0.125	Rotoxkit more sensitive.
zn	4	4	0.0625	1	4	0.0625	Rotoxkit more sensitive.

Rainbow Trout vs. Thamnotoxkit F

Summary of Sign Test Analysis for Rainbow Trout-Thamnotoxkit Toxicity Test Comparison by Individual Mine							
Canmet #	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
1	4	4	0.0625	1.0	4	0.0625	Rainbow Trout more sensitive.
2	5	5	0.0312	1	5	0.0312	Thamnotoxkit more sensitive.
3	5	5	0.0312	1	5	0.0312	Rainbow Trout more sensitive.
4	1	2	0.75	0	.	.	Insufficient data to make conclusion
5	4	5	0.1875	0.3277	5	0.0312	Insufficient data to make conclusion
6	1	1	0.5	0	.	.	Insufficient data to make conclusion
7	2	4	0.6875	0.0625	4	0.0625	Insufficient data to make conclusion
8	2	3	0.5	0.2963	3	0.125	Insufficient data to make conclusion
9	3	3	0.125	1	3	0.125	Thamnotoxkit more sensitive.
10	4	4	0.0625	1	4	0.0625	Thamnotoxkit more sensitive.
11	4	4	0.0625	1	4	0.0625	Thamnotoxkit more sensitive.
12	4	4	0.0625	1	4	0.0625	Thamnotoxkit more sensitive.
13	2	2	0.25	0	.	.	Insufficient data to make conclusion
14	2	2	0.25	0	.	.	Insufficient data to make conclusion
15	2	2	0.25	0	.	.	Insufficient data to make conclusion
16	3	3	0.125	1	3	0.125	Thamnotoxkit more sensitive.
17	3	4	0.3125	0.3164	4	0.0625	Insufficient data to make conclusion
18	0	1	.	0	.	.	Insufficient data to make conclusion

19	2	2	0.25	0	.	.	Insufficient data to make conclusion
20	0	0	.	0	.	.	Insufficient data to make conclusion
21	1	1	0.5	0	.	.	Insufficient data to make conclusion

Summary of Sign Test Analysis for Rainbow Trout-ThamnotoxkitToxicity Test Comparison by Mine Type

Mine Type	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
All Mines	45	61	0.0001	0.9906	36.92	0.05	Thamnotoxkit more sensitive.
au	9	10	0.0107	0.9872	7	0.0547	Thamnotoxkit more sensitive.
bit	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
cu_zn	13	17	0.0245	0.9183	11	0.0717	Thamnotoxkit more sensitive.
ni_cu	5	12	0.8062	0.0726	8	0.073	Insufficient data to make conclusion
pb_zn	11	11	0.0005	1	8	0.0327	Thamnotoxkit more sensitive.
sn	1	1	0.5	0	.	.	Insufficient data to make conclusion
u	2	2	0.25	0	.	.	Insufficient data to make conclusion
zn	4	4	0.0625	1	4	0.0625	Thamnotoxkit more sensitive.

Rainbow Trout vs. Microtox

Summary of Sign Test Analysis for Rainbow Trout-MicrotoxToxicity Test Comparison by Individual Mine

Canmet #	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
1	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
2	1	3	0.875	0.0370	3	0.125	Insufficient data to make conclusion
3	4	5	0.1875	0.3277	5	0.0312	Insufficient data to make conclusion
4	1	2	0.75	0	.	.	Insufficient data to make conclusion
5	2	4	0.6875	0.0625	4	0.0625	Insufficient data to make conclusion
6	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
7	1	2	0.75	0	.	.	Insufficient data to make conclusion
8	1	2	0.75	0	.	.	Insufficient data to make conclusion
9	1	1	0.5	0	.	.	Insufficient data to make conclusion
10	0	0	.	0	.	.	Insufficient data to make conclusion
11	0	0	.	0	.	.	Insufficient data to make conclusion
12	2	2	0.25	0	.	.	Insufficient data to make conclusion
13	1	1	0.5	0	.	.	Insufficient data to make conclusion
14	0	0	.	0	.	.	Insufficient data to make conclusion
15	0	0	.	0	.	.	Insufficient data to make conclusion
16	0	0	.	0	.	.	Insufficient data to make conclusion

17	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
18	0	2	.	0	.	.	Insufficient data to make conclusion
19	2	2	0.25	0	.	.	Insufficient data to make conclusion
20	0	0	.	0	.	.	Insufficient data to make conclusion
21	0	0	.	0	.	.	Insufficient data to make conclusion

Summary of Sign Test Analysis for Rainbow Trout-MicrotoxToxicity Test Comparison by Mine Type

Mine Type	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
All Mines	28	38	0.0025	0.9262	24.07	0.05	Rainbow Trout more sensitive.
au	4	5	0.1875	0.32768	5	0.0312	Insufficient data to make conclusion
bit	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
cu_zn	9	12	0.0729980469	0.8423563242	8	0.073	Rainbow Trout more sensitive.
ni_cu	5	9	0.5	0.1574919932	7	0.0195	Insufficient data to make conclusion
pb_zn	2	4	0.6875	0.0625	4	0.0625	Insufficient data to make conclusion
sn	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
u	0	0	.	0	.	.	Insufficient data to make conclusion
zn	0	0	.	0	.	.	Insufficient data to make conclusion

Rainbow Trout vs. Toxichromotest

Summary of Sign Test Analysis for Rainbow Trout-Toxichromotest Toxicity Test Comparison by Individual Mine

Canmet #	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
1	3	4	0.312	0.3164	4	0.0625	Insufficient data to make conclusion
2	3	4	0.312	0.3164	4	0.0625	Insufficient data to make conclusion
3	5	5	0.0312	1	5	0.0312	Rainbow Trout more sensitive.
4	2	2	0.25	0	.	.	Insufficient data to make conclusion
5	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.
6	3	4	0.3125	0.3164	4	0.0625	Insufficient data to make conclusion
7	2	2	0.25	0	.	.	Insufficient data to make conclusion
8	1	2	0.75	0	.	.	Insufficient data to make conclusion
9	1	1	0.5	0	.	.	Insufficient data to make conclusion
10	0	0	.	0	.	.	Insufficient data to make conclusion
11	0	1	.	0	.	.	Insufficient data to make conclusion
12	3	3	0.125	1	3	0.125	Toxichromotest more sensitive.
13	1	1	0.5	0	.	.	Insufficient data to make conclusion
14	0	0	.	0	.	.	Insufficient data to make conclusion
15	0	0	.	0	.	.	Insufficient data to make conclusion
16	0	0	.	0	.	.	Insufficient data to make conclusion
17	4	4	0.0625	1	4	0.0625	Rainbow Trout more sensitive.

18	2	2	0.25	0	.	.	Insufficient data to make conclusion
19	0	2	.	0	.	.	Insufficient data to make conclusion
20	0	0	.	0	.	.	Insufficient data to make conclusion
21	0	0	.	0	.	.	Insufficient data to make conclusion

Summary of Sign Test Analysis for Rainbow Trout-ToxichromotestToxicity Test Comparison by Mine Type

Mine Type	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
All Mines	31	41	0.0007	0.9715	25.77	0.05	Rainbow Trout more sensitive.
au	2	5	0.8125	0.0102	5	0.0312	Insufficient data to make conclusion
bit	3	4	0.3125	0.3164	4	0.0625	Insufficient data to make conclusion
cu_zn	10	13	0.0461	0.8398	9	0.0461	Rainbow Trout more sensitive.
ni_cu	9	9	0.0020	1	7	0.0195	Rainbow Trout more sensitive.
pb_zn	4	5	0.1875	0.32768	5	0.0312	Insufficient data to make conclusion
sn	3	4	0.3125	0.31640625	4	0.0625	Insufficient data to make conclusion
u	0	0	.	0	.	.	Insufficient data to make conclusion
zn	0	1	.	0	.	.	Insufficient data to make conclusion

Daphnia magna acute vs *Daphnia magna* IQ

Summary of Sign Test Analysis for *Daphnia magna* Acute-*Daphnia magna* IQ Toxicity Test Comparison by Individual Mine							
Canmet #	Number of "-"	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
1	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
2	3	4	0.3125	0.3164	4	0.0625	Insufficient data to make conclusion
3	5	5	0.0312	1	5	0.0312	*D. magna* IQ more sensitive.
4	3	3	0.125	1	3	0.125	*D. magna* IQ more sensitive.
5	2	3	0.5	0.2963	3	0.125	Insufficient data to make conclusion
6	4	5	0.1875	0.3277	5	0.0312	Insufficient data to make conclusion
7	2	2	0.25	0	.	.	Insufficient data to make conclusion
8	3	3	0.125	1	3	0.125	*D. magna* IQ more sensitive.
9	2	3	0.5	0.2963	3	0.125	Insufficient data to make conclusion
10	3	3	0.125	1	3	0.125	*D. magna* IQ more sensitive.
11	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
12	3	3	0.125	1	3	0.125	*D. magna* IQ more sensitive.
13	3	3	0.125	1	3	0.125	*D. magna* IQ more sensitive.
14	0	0	.	0	.	.	Insufficient data to make conclusion
15	2	2	0.25	0	.	.	Insufficient data to make conclusion
16	1	3	0.875	0.0370	3	0.125	Insufficient data to make conclusion
17	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
18	1	2	0.75	0	.	.	Insufficient data to make conclusion

19	1	2	0.75	0	.	.	Insufficient data to make conclusion
20	2	2	0.25	0	.	.	Insufficient data to make conclusion
21	1	1	0.5	0	.	.	Insufficient data to make conclusion

Summary of Sign Test Analysis for *Daphnia magna* Acute- *Daphnia magna* IQ Test Comparison by Mine Type

Mine Type	Number of “-”	Sample size	P-value	Power	Cutoff	Nominal Alpha	Comment
All Mines	53	61	< 0.0001	1	36.92	0.05	*D. magna* IQ more sensitive.
au	10	11	0.0059	0.9866	8	0.0327	*D. magna* IQ more sensitive.
bit	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.
cu_zn	11	12	0.0032	0.9981	8	0.073	*D. magna* IQ more sensitive.
ni_cu	12	13	0.0017	0.9980	9	0.0461	*D. magna* IQ more sensitive.
pb_zn	6	10	0.3770	0.3823	7	0.0547	Insufficient data to make conclusion
sn	4	5	0.1875	0.32768	5	0.0312	Insufficient data to make conclusion
u	2	2	0.25	0	.	.	Insufficient data to make conclusion
zn	4	4	0.0625	1	4	0.0625	*D. magna* IQ more sensitive.

APPENDIX D

SPECIFIC COMPARISONS OF METALS

Appendix D Specific Comparisons of Metals: Results as mg/L

Metal	D. magna Acute (24h EC50)	D. magna Acute (48h EC50)	D. magna IQ (1h EC50)	Microtox (EC20)	Microtox 15 min. (EC50)	Rainbow Trout Acute	Rotoxkit	Thamno-toxkit	Toxichromo-test	Citiation
Ag							0.0075			6
		0.01								8
			0.021		2.39$_c$			0.008	0.052	15
Al							>3.0			6
		59.6								8
							3.02			15
As		5.4			35	43				9
Cd	1.9	0.97	0.41							1
			12							2
				35						4
								0.40		5
							1.3			6
					56.83					7
		1.88								8
		0.02-0.16			10					10
		0.046			14	0.15				11
		0.041			25					12
							1.3			13
		0.065			102		1.3			14
			0.37		23.4		3.98	0.36		15
				218						16
Co		4.7-13			16					10
					15.8				27.5	15
		0.16	0.72							17
Cr								0.11		5
		0.10-0.13			13					10
							8.3			13

Cu	0.28	0.24	0.23							1
			0.17							2
				0.19						3
				1.2						4
Cu								0.31		5
							0.026			6
					1.29					7
		0.093								8
		0.02			7.4	0.25				9
		0.01-0.06			4-20[a]					10
		0.064			0.42					12
							0.026			13
		0.065			1.3		0.026			14
			0.078		0.69		0.14	0.081		15
		1.09				0.44				19
Hg	0.03/0.005	0.01/0.001	0.02/0.006							1
				0.2						4
								0.06		5
							0.06			?
					0.03					7
		0.0052								8
		0.03			0.08	0.21				9
		0.01-0.06			0.03-0.07					10
		0.005				0.65[b]				11
			0.02		0.12		0.93	0.04	0.089	15
					0.044-0.32[a]					16
K	0.36	0.16	0.72							1
				340						4
		141.46								8
							871	407		15
Na	0.70	0.33	1.0							1

		420.6								8
							1513	1820		15
Ni				1.81						3
				55						4
							4.0			6
		7.29								8
Ni					87.5		4.57	2.19		15
Pb			0.210							2
							>4.0			6
		3.61								8
					30.2		6.31	1.62		15
Se							16			6
Zn	7.6	2.1	4.3							1
			0.340							2
				0.37						3
				5.6						4
								1.7		5
							1.3			6
					3.79					7
		0.56								8
		5.1			49.0_a	2.2				9
		1.0-1.2			$2\text{-}14_a$					10
		0.54			1.6					12
		0.56			12		1.3			14
			0.27		3.2		2.45	0.22		15
Zr					$>4.3_a$	>20				18

1 Janssen and Persoone, 1993.
2 Carlson-Ekvall and Morrison, 1995, 30 minute EC50
3 Ankley et al, 1990
4 Codina et al, 1993, EC50
5 Centeno et al, 1994
6 Snell et al, 1991
7 Greene et al, 1985
8 Khangarot and Ray, 1989
9 Qureshi et al, 1982, 5 minute EC50 for Microtox
10 Elnabarawy, 1986

11 Sloof et al, 1983, 30 minute EC50 for Microtox, 48h LC50 for Rainbow trout and Fathead Minnow bioassays
12 Miller et al, 1985
13 Snell and Moffat, 1992
14 Toussaint et al, 1995
15 Willemsen et al, 1995
16 DeZart and Sloof, 1983
17 Aqua Survey
18 Couture et al., 1989
19 Pollutech, personal communication, 1995.

a 5 minute EC50
b 48 hour LC50
c 30 minute EC50

APPENDIX E

SAMPLING PROTOCOL

PROCEDURE FOR SAMPLING AND SHIPPING OF EFFLUENT SAMPLES FOR TOXICITY TESTING AND CHEMICAL ANALYSIS

Material furnished by the Aquatic Effects Secretariat

For the first sampling period:

* Document "Procedure for sampling...analysis"
* Record of Sampling Details Form
* 1 formfit drum liner
* 1 bucket (20 L)
* 1 gallon jug with cap
* 1 siphon pump
→ * 4 jerry cans (5 gallons each)
→ * 4 or 5 bottles with preservatives, icepacks and 1 cooler

For each of the 3 other sampling periods:

* Document "Procedure for sampling...analysis"
* Record of Sampling Details Form
* 1 formfit drum liner
→ * 4 jerry cans (5 gallons each)
→ * 4 or 5 bottles with preservatives, icepacks and 1 cooler

→ **Note: If your site is choosen for external QA/QC purpose, you will receive these 2 items IN DOUBLE. The Aquatic Effects Secretariat will contact you if this is the case.**

Function of the furnished materials

* Document: Informs the mine operator about the purpose of the sample, how to take the sample and where to ship it.

* Record of Sampling Details Form: Detailed record of the sampling information including any concerns or anomalies. Has to be filled out by the mine operators and faxed to the Aquatic Effects Secretariat (613) 996-9673.

* Formfit drum liner: To put into a large sampling container (eg. 45-gallon drum) used to receive effluent.

* Bucket: Use to collect effluent and fill the large sampling container with effluent.

* Siphon pump: To sub-sample the **well mixed effluent** from the large sampling container to the smaller containers (jerry cans, bottles and gallon jug). Can also help to continue to stir the effluent during sub-sampling operation.

* Gallon jug: To bring some **well mixed effluent** from the large sampling container to your own laboratory in order to filter (0.45 µm) part of it for chemical analysis. The filtered effluent will go to bottle M(D) (see table p. 6).

* Jerry cans: Will be filled with **well mixed effluent** from the large sampling container for bioassay analyses.

* Bottles: Will be filled with **well mixed effluent** from the large sampling container for chemical analyses.

* Icepacks: To place around the bottles to keep them cool. These icepacks have to be kept frozen.

* Cooler: To contain the bottles and icepacks for shipping.

Note: The bucket, the siphon pump and the gallon jug should be rinsed well with clean water after sampling has been completed. These 3 items should be set aside for subsequent sampling events.

PROCEDURE FOR SAMPLING AND SHIPPING OF EFFLUENT SAMPLES FOR TOXICITY TESTING AND CHEMICAL ANALYSIS

1.0 **Effluent Collection**

* 1 large sampling container (eg. 45-gallon drum) should be lined with 1 drum liner (food grade polyethylene bag), rinsed twice with effluent and filled with at least 100 litres of effluent using the bucket. Ensure that the effluent sample does not contact the drum wells. Use the bag and the bucket supplied by the Aquatic Effects Secretariat. You will receive a new bag for each sampling period.

* **Stir the effluent very well** and use the siphon pump for sub-sampling for toxicity testing (step 2) and chemical analysis (step 3). **Sample transfer must be accompanied by continuous mixing of the effluent by using manual stirring (eg. with the pump) or other appropriate means. Any materials coming into contact with the sample must be inert, clean and non-toxic, and containers must be rinsed with effluent before sub-sampling.**

* Use the 1-gallon jug to bring the effluent sample to your own laboratory for filtering (chemical analysis-step 3, table p.6).

* **Fill out the record of sampling details form** (a detailed record of the sampling information including any concerns or anomalies) provided in your sampling kit, **and fax it to the Aquatic Effects Secretariat, in Ottawa (613-996-9673) after effluent sampling. The Aquatic Effects Secretariat will keep confidential all identification of the source of individual effluents, and will refer to these effluent samples only by code number and mine type**.

Please insure that samples do not freeze prior to shipment, and are keep cool.

2.0 Sub-sampling and Shipping for Toxicity Testing

2.1 Primary Sampling

For each sampling period:

* **3 jerry cans** must be rinsed 3 times with sample effluent, filled completely (no air space, **no acid**) with the siphon pump and sent by courier (air or land express) to:

B.A.R Environmental Inc.
Nicholas Beaver Park, R.R. #3
Guelph, Ontario, N1H 6H9

These samples are for trout, *Daphnia magna* and *Daphnia magna* IQ tests.

* **1 jerry can** must be rinsed 3 times with sample effluent, filled completely (no air space, **no acid**) with the siphon pump and sent by courier (air or land express) to:

Les Consultants BEAK Limitée
Carré Dorval
455 Boul. Fénélon, Suite 104
Dorval, Québec, H9S 5TB

This sample is for Microtox, Toxichromotest, Rotoxkit F and Thamnotoxkit F tests.

* The waterproof label on each container (which does not identify the individual mine to the laboratory) must be completed prior to shipment of the different effluent subsamples.

There must be no chemical preservatives added to any of the samples for toxicity testing.

Please insure that samples do not freeze prior to shipment, and are keep cool.

2.2 Quality Assurance Sampling

A small number of sites will provide duplicate samples for **QA/QC** purposes. Samples will be taken from the same effluent collection (the large sampling container, see effluent collection step, p.3) as the primary toxicity laboratories' samples . The Aquatic Effects Secretariat will notify you if your site is choosen. **If so**:

* **3 additional jerry cans** must be rinsed 3 times with sample effluent, filled completely (no air space, **no acid**) with the siphon pump and sent by courier (air or land express) to:

Gary Westlake, Manager
Aquatic Toxicology Section
Ontario Ministry of Environment & Energy
125 Resources Road
Etobicoke, Ontario, M9P 3V6

These samples are for trout, *Daphnia magna* and *Daphnia magna* IQ tests.

* **1 additional jerry can** has to be rinsed 3 times with sample effluent, filled completely (no air space, **no acid**) with the siphon pump and sent by courier (air or land express) to:

Ken Doe, Head
Toxicology Section, Environment Canada
c/o receiving stores
Bedford Institute of Oceanography
1 Challenger Drive
Dartmouth, Nova Scotia, B2Y 4A2

This sample is for Microtox, Toxichromotest, Rotoxkit F and Thamnotoxkit F tests.

* The waterproof label on each container (which does not identify the individual mine to the laboratory) must be completed prior to shipment of the different effluent subsamples.

There must be no chemical preservatives added to any of the samples for toxicity testing.

Please insure that samples do not freeze prior to shipment, and are keep cool.

3.0 Sub-sampling, Preservation and Shipping for Chemical Analysis

* The 1-gallon jug must be rinsed 3 times with sample effluent, filled with the siphon pump and transported to an on-site facility for filtration (bottle type M(D), see table following).

* The bottles must be rinsed 3 times with sample effluent, filled to the base of the bottle neck, sealed, and labelled. Samples requiring preservative, as indicated below, should be filled to the neck of the bottle prior to the addition of the preservative. Special instructions for specific bottle types are indicated below.

BOTTLE TYPE	PRESERVATIVE	CODE DOT	SPECIAL INSTRUCTION
M(T) 250mL	5mL 50% HNO_3	Blue	NIL (Plastic bottle)

M(D) 250mL	5mL 50% HNO_3	Blue	Filter with .45 µm **filter before adding acid** (Plastic bottle)
R 1L	4^oC	NIL	NIL (Plastic bottle)
G2 500mL	5mL 50% H_2SO_4	Black	NIL (Plastic bottle)
O & G 1L	5mL 50% H_2SO_4	Black	NIL (Glass bottle)
CN 500mL	2mL 6N NaOH	Red	NIL (Plastic bottle)

* Shipping: It is recommended that the samples be refrigerated after collection and during transportation. Samples should be shipped in the cooler supplied with the frozen ice packs placed around the samples. The samples should be kept between 1 and 8^oC, and preferably between 2 and 6^oC.

Ship the samples in the cooler to:

Seprotech Laboratories
5420 Canotek Road
Gloucester, Ontario, K1J 9G2

For QA/QC (the Secretariat will contact you if your site is choosen) there will be a duplicate set of bottles:

Ship the second series of the samples in the cooler to:

Henry Steger, Manager
CANMET, Chemistry Laboratory
555 Booth Street
Ottawa, Ontario, K1A 0G1

Please insure that samples do not freeze prior to shipment, and are keep cool.

AQUATIC EFFECTS SAMPLING DETAILED RECORD

INFORMATION CONFIDENTIAL TO CANMET

Company Code Number:

Mine Site:	
Location of Mine Site:	
Name of the Discharge Pipe:	
Location of the Discharge Pipe:	
Sampling Date:	
Sampling Time:	
Name of Sampler:	
Temperature:	
Method of Sampling:	
Sampling Anomalies?:	
Date of Shipping:	
Shipping Company: Waybill number:	

FAX TO:* *DANIELLE RODRIGUE* *(613) 996-9673