AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM

Laboratory Screening of Sublethal Toxicity Tests for Selected Mine Effluents

AETE Project 1.2.2

LABORATORY SCREENING OF SUBLETHAL TOXICITY TESTS FOR SELECTED MINE EFFLUENTS

Sponsored by:

Canada Center for Mineral and Energy Technology (CANMET)
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On behalf of:

Aquatic Effects Technology Evaluation (AETE) Program

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AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

Notice to Readers

Laboratory screening of sublethal toxicity tests for selected mine effluents

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of provincial governments; it is coordinated by the Canadian Centre for Mineral and Energy Technology (CANMET). The program is designed to be of direct benefit to the industry, and to government. Through technical evaluations and field evaluations, it will identify cost-effective technologies to meet environmental monitoring requirements. The program includes three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring.

The technical evaluations are conducted to document certain tools selected by AETE members, and to provide the rationale for doing a field evaluation of the tools or provide specific guidance on field application of a method. In some cases, the technical evaluations include a go/no go recommendation that AETE takes into consideration before a field evaluation of a given method is conducted.

The technical evaluations are published although they do not necessarily reflect the views of the participants in the AETE Program. The technical evaluation should be considered as working documents rather than comprehensive literature reviews.

The purpose of the technical evaluations is to document specific monitoring tools. AETE committee members would like to stress that no one single tool can provide all the information required for a full understanding of environmental effects in the aquatic environment.

For more information on the monitoring techniques, the results from their field application and the final recommendations from the program, please consult the AETE Synthesis Report to be published in September 1998.

Any comments concerning the content of this report should be directed to:

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PROGRAMME D'ÉVALUATION DES TECHNIQUES DE MESURE D'IMPACTS EN MILIEU AQUATIQUE

Avis aux lecteurs

Présélection en laboratoire des tests de détermination de la toxicité sublétale de certains effluents miniers

Le Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ÉTIMA) vise à évaluer les différentes méthodes de surveillance des effets des effluents miniers sur les écosystèmes aquatiques. Il est le fruit d'une collaboration entre l'industrie minière du Canada, plusieurs ministères fédéraux et un certain nombre de ministères provinciaux. Sa coordination relève du Centre canadien de la technologie des minéraux et de l'énergie (CANMET). Le programme est conçu pour bénéficier directement aux entreprises minières ainsi qu'aux gouvernements. Par des évaluations techniques et des études de terrain, il permettra d'évaluer et de déterminer, dans une perspective coût-efficacité, les techniques qui permettent de respecter les exigences en matière de surveillance de l'environnement. Le programme comporte les trois grands volets suivants : évaluation de la toxicité aiguë et sublétale, surveillance des effets biologiques des effluents miniers en eaux réceptrices, et surveillance de la qualité de l'eau et des sédiments.

Les évaluations techniques sont menées dans le but de documenter certains outils de surveillance sélectionnés par les membres de l'ÉTIMA et de fournir une justification pour l'évaluation sur le terrain de ces outils ou de fournir des lignes directrices quant à leur application sur le terrain. Dans certains cas, les évaluations techniques pourraient inclure des recommandations relatives à la pertinence d'effectuer une évaluation de terrain que les membres de l'ÉTIMA prennent en considération.

Les évaluations techniques sont publiées bien qu'elles ne reflètent pas nécessairement toujours l'opinion des membres de l'ÉTIMA. Les évaluations techniques devraient être considérées comme des documents de travail plutôt que des revues de littérature complètes.

Les évaluations techniques visent à documenter des outils particuliers de surveillance. Toutefois, les membres de l'ÉTIMA tiennent à souligner que tout outil devrait être utilisé conjointement avec d'autres pour permettre d'obtenir l'information requise pour la compréhension intégrale des impacts environnmentaux en milieu aquatique.

Pour des renseignements sur l'ensemble des outils de surveillance, les résultats de leur application sur le terrain et les recommandations finales du programme, veuillez consulter le Rapport de synthèse ÉTIMA qui sera publié en septembre 1998.

Les personnes intéressées à faire des commentaires concernant le contenu de ce rapport sont invitées à communiquer avec M^{me} Diane E. Campbell à l'adresse suivante :

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EXECUTIVE SUMMARY

The objective of the laboratory screening study was to evaluate nine sublethal toxicity tests through the testing of eight representative mining effluents. The evaluation considered the sensitivity, cost, and applicability of the tests.

The toxicity tests included the Microtox chronic test, the *Ceriodaphnia* survival and reproduction test, the larval fathead minnow survival and growth test, the rainbow trout embryo survival test, the nematode survival and growth/maturation test the algal growth inhibition test with *Selenastrum capricornutum*, growth inhibition of the duckweed *Lemna minor* and the multi-species microplate algal growth inhibition test, and the Mutatox test. Receiving waters were used as control and dilution water in the assays with *Ceriodaphnia*, fathead minnow, trout embryo, *Selenastrum capricornutum,* and *Lemna minor,* and in the multi-species microplate algal test.

Three assays were excluded from consideration: the nematode test, due to serious faults in the test design and protocol, the Mutatox test, since test results were of an "all or none" format, and the trout embryo test, because few of the tests were valid and the sensitivity of the test could not be evaluated.

The inhibitory concentrations for a 25% effect (IC25) were calculated and used to compare sensitivities. IC25s were compared non-parametrically (Friedman ANOVA and Kendall concordance) and by a simple ranking system. The most sensitive tests were the *Selenastrum* and phytoplankton microplate assays, followed by the *Lemna minor* and *Ceriodaphnia* tests, which are of roughly equal sensitivity, and the fathead minnow test. The Microtox chronic test is least sensitive, taking into account the stimulatory responses observed.

The relationship between effluent toxicity (IC25s) and effluent chemistry was examined by the calculation of correlation coefficients (nonparametric Spearman R). There were few significant correlations between the IC25s and the chemical parameters, possibly because of the small sample size. In addition, many analytical results were less than the limit of detection, suggesting that the sensitivities of the chemical methods used were too low for these samples.

Costs of the bioassays were estimated based on the costs of labour (number of hours allocated for testing, reporting, culturing and quality assurance/quality control) and disposable materials. The *Selenastrum* and *Lemna minor* growth inhibition tests were the least expensive assays (< $100.00 per sample, followed by the Microtox chronic and multispecies phytoplankton tests (< $200.00 per sample), and the *Ceriodaphnia* and fathead minnow tests (< $400.00 per sample). The cost of the rainbow trout embryo test is almost $700.00 per sample.

Points for applicability were awarded based on relevance and practicality of the tests. Points for relevance were awarded if the test organism was native to Canada and if the test protocol permitted the use of receiving water as a dilution water. Practicality was rated by summing the volumes of effluent and/or receiving water required to perform the tests. The most applicable tests were the *Selenastrum*, *Ceriodaphnia* and multispecies phytoplankton tests, followed by the *Lemna minor*, fathead minnow and trout embryo assays, and lastly the Microtox chronic test.

In conclusion, this report recommends the following tests for future studies involving mine effluents: growth inhibition of the freshwater alga *Selenastrum*, growth inhibition of the duckweed *Lemna minor*, survival and reproduction of *Ceriodaphnia dubia* and survival and growth the larval fathead minnow. The multi-species microplate phytoplankton growth inhibition test was the most sensitive assay, yet the *Selenastrum* test is preferred due to the availability of a standard test method. It was not possible to rank the rainbow trout embryo assays, as the sensitivity of the test could not be evaluated.

SOMMAIRE-RECOMMANDATIONS

L'objet de la présélection était d'évaluer neuf tests de détermination de la toxicité sublétale, au moyen de huit effluents miniers représentatifs. L'évaluation a porté sur la sensibilité, le coût et le domaine d'application de chaque test.

Les tests toxicologiques évalués comprenaient le test Microtox de détermination de la toxicité chronique, le test de mesure de la survie et de la reproduction de *Ceriodaphnia*, le test de mesure de la survie et de la croissance des larves de tête-de-boule, le test de mesure de la survie des embryons de truite arc-en-ciel, le test de mesure de la survie ainsi que de la croissance et de la maturation de nématodes, le test de mesure de l'inhibition de la croissance de l'algue *Selenastrum capricornutum*, le test de mesure de l'inhibition de la croissance de la lentille d'eau (*Lemna minor*), le test de mesure de l'inhibition de la croissance de nombreuses espèces d'algues sur microplaque et le test Mutatox. On s'est servi des eaux réceptrices comme témoins et milieux de dilution pour les dosages biologiques avec *Ceriodaphnia*, le tête-de-boule, les embryons de truite, *Selenastrum capricornutum*, *Lemna minor* et, sur microplaque, diverses algues.

Nous avons éliminé trois tests : le test aux nématodes, en raison de carences graves dans sa conception et son protocole ; le Mutatox, puisque les résultats étaient du type « tout ou rien » ; le test aux embryons de truite, dont peu de résultats étaient valides et dont la sensibilité n'a pas pu être évaluée.

Nous avons calculé les concentrations inhibitrices à 25 % (CI25), qui ont servi à comparer la sensibilité des divers tests au moyen de méthodes non paramétriques (analyse de la variance [ANOVA] selon Friedman et concordance de Kendall) et d'un simple système de rangement. Les tests les plus sensibles étaient ceux qui utilisaient *Selenastrum* et la croissance du phytoplancton sur microplaque. Les suivaient les tests utilisant *Lemna minor* et *Ceriodaphnia*, à peu près égaux en sensibilité, puis le test utilisant le tête-de-boule. La détermination de la toxicité chronique par le test Microtox est la moins sensible, compte tenu des réactions stimulantes observées.

Nous avons examiné la relation entre la toxicité de l'effluent (CI25) et ses caractéristiques chimiques, par calcul de coefficients de corrélation (coefficient R non paramétrique de Spearman). Nous avons décelé peu de corrélations significatives entre les CI25 et les paramètres chimiques, peut-être en raison de la petitesse des échantillons. En outre, de nombreux résultats analytiques étaient inférieurs à la limite de détection, ce qui porte à croire que la sensibilité des méthodes chimiques utilisées était trop faible pour ces échantillons.

Pour estimer les coûts des dosages biologiques nous avons tenu compte des coûts de la main-d'œuvre (nombre d'heures affectées aux épreuves, à la rédaction des rapports, à la culture, à l'assurance et à la maîtrise de la qualité) et de la consommation des matières à usage unique. Les tests les moins coûteux (par échantillon) utilisaient *Selenastrum* et *Lemna minor* (< 100 $) ; suivaient le Microtox et le test utilisant plusieurs espèces de phytoplancton (< 200 $), puis les tests utilisant *Ceriodaphnia* et le tête-de-boule (< 400 $). Le test avec embryons de truite arc-en-ciel coûte presque 700 $.

L'utilité et le caractère pratique des tests ont servi à en estimer l'applicabilité. Nous avons accordé des points à l'utilité si les organismes d'essai étaient indigènes au Canada et si le protocole expérimental autorisait l'emploi d'eaux réceptrices comme eau de dilution. Nous avons évalué le caractère pratique par le volume total d'effluent, d'eau réceptrice ou des deux exigé pour la réalisation des tests. Les plus applicables étaient ceux qui utilisaient *Selenastrum*, *Ceriodaphnia* et plusieurs espèces de phytoplancton, puis les tests avec *Lemna minor*, le tête-de-boule et les embryons de truite arc-en-ciel et, enfin, le Microtox.

Pour conclure, le rapport recommande les tests suivants pour les études à venir des effluents miniers : le test de mesure de l'inhibition de la croissance de l'algue dulcicole *Selenastrum* ; celui de la mesure de l'inhibition de la croissance de la lentille d'eau *Lemna minor* ; celui de la mesure de la survie et de la reproduction de *Ceriodaphnia dubia* ; celui de la mesure de la survie et de la croissance de la larve de tête-de-boule. Si le test de mesure de l'inhibition de la croissance de plusieurs espèces de phytoplancton sur microplaque était le plus sensible, on lui préfère néanmoins le test avec *Selenastrum*, qui est normalisé. Le test avec embryons de truite arc-en-ciel est inclassable, sa sensibilité ne pouvant pas être évaluée.

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1 INTRODUCTION

1.1 BACKGROUND

Environment Canada's Metal Mining Liquid Effluent Regulations (MMLER) are currently being reviewed. A focus of this review concerns an assessment of the adequacy of the current regulations in mitigating mining effluent impacts on receiving water ecosystems. As part of this initiative, the Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. An important focus of this program will be to evaluate and identify cost-effective technologies to meet environmental monitoring requirements.

The objective of the chronic toxicity sub-program is to evaluate sublethal toxicity tests for assessing sublethal impacts of effluents. The results of this study should identify the most cost effective and sensitive bioassays for the evaluation of sublethal effects of mining effluents. The benefit to industry is that resources allotted for environmental assessment would be most efficiently used.

1.2 OBJECTIVES

The principal objectives of the study were to compare the performance of eight (8) sublethal toxicity tests through the testing of eight representative mining effluents and to assess whether or not the sublethal toxicity test data correlate to major chemical constituents of the effluent/receiving water. These comparisons should allow a reduction in the number of required tests without sacrificing the relevance of the toxicity data. The evaluation also considers the relative cost, speed and applicability of the bioassays.

Bioassay costs were estimated by adding the costs of labour and the costs of disposable materials, as provided by the participating laboratories. The criteria for judging applicability were the relevance and the practicality of the tests. Relevance of a toxicity test was judged on how well the results could be applied to the situation in the field. Practicality was evaluated by examining the material and

technical requirements of each test.

A chemical characterization of effluent and receiving water samples was performed. Results of the toxicity tests were compared with the major chemical constituents of the effluent/receiving water.

Where the protocol permitted, toxicity tests were conducted using the local receiving water collected in the vicinity of each site as test control and dilution water, with an additional control using the laboratory's usual dilution water. Another objective was therefore to evaluate the use of local receiving water in these chronic tests.

Certain test methods recommend an acclimation procedure if local receiving waters are used as control/dilution water for toxicity tests. This procedure allows the organisms to be acclimated to the receiving waters for a certain period of time prior to conducting toxicity tests. In this study, the test organisms were not acclimated to the receiving waters before effluent testing was conducted.

1.3 PROJECT DESCRIPTION

Eight mine effluents, representing different mine types and covering a range of chemical parameters such as metal concentrations, pH, alkalinity and hardness, were tested with eight toxicity tests. Five of these tests were performed in the laboratory of B.A.R. Environmental in Guelph: the Microtox chronic test, the *Ceriodaphnia* survival and reproduction test, the larval fathead minnow survival and growth test, the rainbow trout embryo survival test and the nematode survival and growth/maturation test. A sixth test, the algal growth inhibition test with *Selenastrum capricornutum*, was performed by a sub-contractor- Les Laboratoires Eco-CNFS in Pointe Claire (Québec). Two additional bioassays, growth inhibition with *Lemna minor* and the multi-species microplate algal growth inhibition test, were performed by the Saskatchewan Research Council. A final assay, the Mutatox test was conducted by Dr. Graham Van Aggelen, of the B.C. Ministry of the Environment (Vancouver).

The mine's receiving waters were used as control and dilution water in the assays with *Ceriodaphnia*, fathead minnow, trout embryo, *Selenastrum capricornutum*, and *Lemna minor*, and in the multi-species microplate algal test. A control using the usual laboratory or test dilution water was only performed with the first four of these tests. There was some concern that the receiving waters would be of low ionic strength, reducing the growth/reproduction of some of the test animals such that some of the tests would be invalid. Prior to testing with mine effluents, *Ceriodaphnia* and fathead minnow were exposed to a range of concentrations of diluted laboratory water. A "threshold" value or TEC for low ionic strength water was determined for both animals. There were no significant effects on reproduction of *Ceriodaphnia* or growth/survival of fathead minnow at very low hardness levels (3 $mg \cdot L^{-1}$ as $CaCO_3$).

2 METHODS

2.1 SAMPLE COLLECTION AND HANDLING

2.1.1 Samples for Toxicity Testing

Mine personnel collected and prepared all samples, both effluent and receiving water, for shipment to B.A.R. Environmental's laboratory. The sample containers for both receiving waters and effluents were 20 L plastic pails fitted with a polyethylene plastic liner. The pail was filled to maximum capacity and the plastic liner was closed with a twist-tie, after expelling as much air as possible. Chain-of-Custody forms were provided by B.A.R. Environmental for use by the participating mining companies. Separate containers (200 mL polyethylene plastic bottles) were employed for samples destined for Les Laboratoires Eco-CNFS , the Saskatchewan Research Council and the B.C. Ministry of the Environment.

Seven of the eight receiving waters for Ceriodaphnid, fathead minnow, trout embryo, *Lemna minor* and phytoplankton microplate bioassays were sampled 10 - 14 days prior to sampling of the effluent, and were shipped by ground transport (Table 2-3). An exception was the receiving water for sample #960753, which was sampled and shipped at the same time as the effluent.

A sub-sample of the receiving waters was used for the *Selenastrum* test. This was shipped at the same time as the effluent sample, and was maintained in a cool environment at the mine site until then. Upon arrival at B.A.R. Environmental, receiving water samples were composited in a 2000 L polyethylene container and then returned to the original containers for storage.

Effluents were sampled during normal operations, as determined by the mine personnel, using the instantaneous grab method. Samples were shipped directly to the laboratory by express transport (ground or air). Upon arrival at the laboratory, samples were logged in and recorded according to B.A.R. Environmental standard operating procedures. Effluent samples were separated into three

batches (1, 2 and 3) for tests requiring daily renewal (rainbow trout embryo, *Ceriodaphnia* and fathead minnow bioassays). Batch # 1 was used on test days 0, 1 and 2; batch # 2 on days 3, 4 and 5 and batch # 3 on days 6 and 7. No sample numbers were assigned to the receiving waters and they were identified by adding the prefix RW to the effluent sample number (as in RW-960753). Samples were stored at 4°(± 2) C until testing, when sample temperature was brought to the appropriate test temperature before the assay was initiated. Physical-chemical parameters measured immediately prior to testing included dissolved oxygen, temperature, conductivity and pH.

With two exceptions, all of the bioassays were initiated within 72 h of the time the sample was collected. The *Lemna minor* and multispecies phytoplankton assays with samples # 960753 and # 960679 were not performed within this time period but were delayed. Effluent # 960679 was sampled on Monday April 22 and arrived at the laboratory in Saskatoon on Wednesday April 24. Sample # 960753 was collected on Monday May 6, while the receiving water for this sample arrived in Saskatoon on Wednesday May 8.

Three of the participating mines were lead/zinc mines. Samples # 960482 and # 960483 were different effluents from the same mine, and were tested with the same receiving water (RW-960482/83). The final effluents tested, # 960768 and # 960918, were also different effluents from the same mine site, a gold/silver mine. These effluents were tested with the same receiving water, which, however, was sampled at two different times. The remaining mines were copper and copper/zinc operations.

Values of dissolved oxygen, conductivity and pH of the effluent samples prior to testing are presented in Table 2-1. The conductivity of the effluent samples ranged from 61 to 3730 $\mu S \cdot cm^{-1}$ and the pH ranged from pH 6.2 to pH 9.5. A more complete chemical analysis of the effluent samples was performed by Seprotech Laboratories and these results are shown in Table 2-3. Two effluent samples, # 960768 and # 960918 were not analysed at the same time as the bioassays were performed. These effluents were re-sampled and chemically analysed several weeks following the bioassays.

Table 2-1. Physical-chemical attributes of the eight mining effluents prior to testing. The parameters were measured on arrival at B.A.R. Environmental's laboratory in Guelph, Ontario.

Sample #	Date Collected (d/m/y)	Date Rec'd Saskatoon (d/m/y)	Date Rec'd Guelph (d/m/y)	Dissolved O_2 ($mg \cdot L^{-1}$)	Conductivity ($\mu S \cdot cm^{-1}$)	pH
960482	25/03/96	26/03/96	26/03/96	11.0	1648	9.5
960483	25/03/96	26/03/96	26/03/96	10.7	61	6.2
960577	08/04/96	09/04/96	09/04/96	9.5	176	8.6
960676	22/04/96	23/04/96	23/04/96	9.7	3730	7.6
960679	22/04/96	24/04/96	24/04/96	9.7	3220	7.2
960753	06/05/96	07/05/96	07/05/96	10.3	2110	7.0
960768	06/05/96	07/05/96	08/05/96	10.2	393	8.8
960918	03/06/96	04/06/96	05/06/96	9.7	78	7.1

The pH and hardness of the receiving waters were measured upon their arrival at B.A.R. Environmental's laboratory (Table 2-2). The hardness of the receiving waters ranged from "soft" (8.0 $mg \cdot L^{-1}$ as $CaCO_3$) to moderately "hard" (180 $mg \cdot L^{-1}$ as $CaCO_3$.). The pH ranged from neutral (pH 7.0) to slightly above neutral (pH 8.0). Seprotech Laboratories also performed chemical analyses on the receiving water samples and these results are shown in Table 2-4.

In preliminary assays with diluted laboratory water, threshold values for hardness were determined for Ceriodaphnid reproduction and for fathead minnow growth/survival. The threshold value for Ceriodaphnid reproduction was 5.5 $mg \cdot L^{-1}$ as $CaCO_3$. Fathead minnow growth and survival were not affected at a hardness of 3.9 $mg \cdot L^{-1}$ as $CaCO_3$ (threshold value < 3.9). Since all samples of receiving water were above this threshold, it was not necessary to adjust the hardness of these dilution waters prior to testing.

Table 2-2. Physical-chemical attributes of the receiving waters measured on arrival at B.A.R. Environmental's laboratory in Guelph, Ontario. The receiving waters were used as dilution and control water in the *Selenastrum capricornutum*, *Ceriodaphnia dubia*, fathead minnow, embryo rainbow trout, *Lemna minor* and phytoplankton multispecies tests.

RW Sample #	Date Collected d/m/y	Date Rec'd Saskatoon d/m/y	Date Rec'd Guelph d/m/y	Hardness [a]	pH
960482/960483	18/03/96	26/03/96	22/03/96	8.0	7.0
960577	28/03/96	09/04/96	08/04/96	69	7.9
960676	15/04/96	23/04/96	24/04/96	31	7.5
960679	26/03/96	24/04/96	22/04/96	46	7.7
960753	03/05/96	07/05/96	06/05/96	180	7.4
960768	21/04/96	07/05/96	03/05/96	108	8.0
960918	17/05/96	04/06/96	03/06/96	108	8.0

[a] as $mg \cdot L^{-1}$ $CaCO_3$.

2.1.2 Samples For Chemical Analysis

Samples of receiving waters and effluents were also collected for chemical analyses. Four litres of sample were collected in a plastic container which was rinsed three times with the sample before filling. Five sub-samples were taken for measurements of total metals, dissolved metals, cyanide, ammonia and routine parameters (pH, alkalinity, etc). Approximately 250 mL of the sample was filtered (0.45 µm filter) into a plastic bottle and preserved with the addition of 5 mL of concentrated acid (50% HNO_3). This portion was reserved for measurement of dissolved metals. A second volume of approximately 250 mL was placed into a plastic bottle and preserved with 5 mL of concentrated acid (50% HNO_3), for the measurement of total metals. A 500 mL sample, destined for the analysis of cyanide, was placed in a plastic bottle and preserved with 2 mL of 6N NaOH. Another 500 mL sample, for the analysis of ammonia, was placed in a plastic bottle and preserved with 5 mL

concentrated H_2SO_4 (50%). Finally a 1 L sample was placed in a plastic bottle (without preservatives) for the analysis of routine parameters. The bottles were sealed and labelled, placed in cooler with frozen ice-packs and sent by express courier to Seprotech Laboratories in Ottawa. A list of the parameters and the results of analyses are shown on Tables 2-2 and 2-3.

Table 2-3. Chemical parameters measured in samples of mining effluents by Seprotech Laboratories (Ottawa, Ontario).

Parameter	Unit	Detection limit	Sample #:							
			960482	960483	960577	960676	960679	960753	960768	960918
TDS[a]	$mg \cdot L^{-1}$	1	1280	44	104	3910	3210	1790	296	98
TSS[b]	$mg \cdot L^{-1}$	1	7	9	11	26	21	8	10	2
total CN	$mg \cdot L^{-1}$	0.013, 0.005 [d]	<0.013	<0.013	<0.013	<0.005	<0.005	<0.005	0.035	0.006
free CN	$mg \cdot L^{-1}$	0.002, 0.005 [d]	<0.005	<0.005	<0.005	<0.002	<0.002	<0.002	<0.005	<0.005
N-NH 3	$mg \cdot L^{-1}$	0.01	0.74	<0.01	<0.01	2.20	0.8	0.53	0.88	0.04
Cd-dissolved	$\mu g \cdot L^{-1}$	10	<10	<10	20	<10	<10	<10	<10	<10
Cu-dissolved	$\mu g \cdot L^{-1}$	10	<10	67	<10	11	97	<10	<10	<10
Pb-dissolved	$\mu g \cdot L^{-1}$	100	<100	<100	<100	<100	<100	<100	<100	<100
Ni-dissolved	$\mu g \cdot L^{-1}$	20	<20	<20	<20	<20	87	<20	<20	<20
Zn-dissolved	$\mu g \cdot L^{-1}$	10	<10	600	269	<10	214	99	12	<10
As-dissolved	$mg \cdot L^{-1}$	1, 100 [d]	1	1	<100	<100	<100	<100	<100	<100
conductivity	$\mu S \cdot cm^{-1}$	2	1670	59	161	3660	3240	2070	541	119
alkalinity[c]	$mg \cdot L^{-1}$	1	29	2	63	13	179	38	57	19
pH	pH unit	0.01	9.38	6.10	8.53	8.43	7.13	7.07	10.58	8.60
hardness[c]	$mg \cdot L^{-1}$	1	799	15	83	2810	2760	1220	65	63
Cd (total)	$\mu g \cdot L^{-1}$	20	<20	<20	21	<20	<20	<20	<20	<20
Cu (total)	$\mu g \cdot L^{-1}$	10	<10	60	10	12	223	14	<10	<10
Pb (total)	$\mu g \cdot L^{-1}$	100	<100	<100	143	<100	<100	<100	<100	<100
Ni (total)	$\mu g \cdot L^{-1}$	20	<10	<20	<20	<20	99	<20	<20	<20
Zn (total)	$\mu g \cdot L^{-1}$	10	362	645	274	<10	210	96	12	<10
As (total)	$\mu g \cdot L^{-1}$	1, 100 [d]	2	1	<100	<100	<100	<100	<100	<100

[a] Total Dissolved Solids.

[b] Total Suspended Solids.

[c] as $CaCO_3$.

[d] detection limits varied, refer to text for details.

Table 2-4. Chemical parameters measured in samples of receiving waters by Seprotech Laboratories (Ottawa, Ontario). Dissolved Organic Carbon was analysed by the Water Quality Section, Saskatchewan Research Council, Saskatoon.

Parameter	Unit	Detection limit	Receiving Water Sample #					
			960482/483	960577	960676	960679	960753	960768/918
TDS[a]	$mg \cdot L^{-1}$	1	21	98	42	52	270	140
TSS [b]	$mg \cdot L^{-1}$	1	<1	<1	2	2	7	3
total CN	$mg \cdot L^{-1}$	0.005, 0.013 [d]	<0.013	<0.013	<0.013	<0.005	<0.005	<0.005
free CN	$mg \cdot L^{-1}$	0.002, 0.005 [d]	<0.005	<0.005	<0.005	<0.002	<0.002	<0.002
N-NH 3	$mg \cdot L^{-1}$	0.01	0.01	<0.01	<0.01	<0.01	0.09	<0.01
Cd-dissolved	$\mu g \cdot L^{-1}$	10	<10	<10	<10	<10	<10	<10
Cu-dissolved	$\mu g \cdot L^{-1}$	10	<10	<10	<10	<10	11	<10
Pb-dissolved	$\mu g \cdot L^{-1}$	100	<100	<100	<100	<100	<100	<100
Ni-dissolved	$\mu g \cdot L^{-1}$	20	<20	<20	<20	<20	<20	<20
Zn-dissolved	$\mu g \cdot L^{-1}$	10	<10	<10	<10	<10	<10	<10
As-dissolved	$mg \cdot L^{-1}$	1, 100 [d]	<1	<100	<100	<100	<100	<100
conductivity	$\mu S \cdot cm^{-1}$	2	29	148	70	61	452	227
alkalinity [c]	$mg \cdot L^{-1}$	1	5	61	25	23	84	69
pH	pH unit	0.01	6.33	7.71	7.48	7.11	7.08	7.84
hardness [c]	$mg \cdot L^{-1}$	1	11	65	30	40	185	108
DOC [e]	$mg\ C \cdot L^{-1}$	1	3.1	<1.0	1.1	2.4	9.5	<1.0
Cd (total)	$\mu g \cdot L^{-1}$	10, 20 [d]	<20	<10	<20	<20	<20	<20
Cu (total)	$\mu g \cdot L^{-1}$	10	<10	<10	<100	<10	17	<10
Pb (total)	$\mu g \cdot L^{-1}$	100	<100	<100	<20	<100	<100	<100
Ni (total)	$\mu g \cdot L^{-1}$	20	<20	<20	<10	<20	23	<20
Zn (total)	$\mu g \cdot L^{-1}$	10	35	<10	<10	<10	<10	<10
As (total)	$mg \cdot L^{-1}$	1, 100 [d]	1	<100	<100	<100	<100	<100

[a] Total Dissolved Solids.

[b] Total Suspended Solids.

[c] as $CaCO_3$.

[d] detection limits varied, refer to text for details.

[e] Dissolved Organic Carbon

2.2 CULTURE OF THE ORGANISMS

The cultures of *Ceriodaphnia* and fathead minnows used in testing were maintained in a natural non-chlorinated groundwater. This laboratory water was also used as a source of control water for tests requiring a second control (tests with *Ceriodaphnia*, fathead minnow and the trout embryo). The alga *Selenastrum capricornutum* was obtained from a strain cultured by the Québec Ministère de l'Environnement et de la Faune, and was maintained in AAP culture media (Environment Canada, 1992a).

Lemna minor (strain C4) cultures were originally collected from a local pond near the Saskatchewan Research Council, and thereafter maintained by weekly subculture in Hoagland's E+ medium (Saskatchewan Research Council, 1996).

Cultures of *Selenastrum capricornutum*, *Microcystis aeruginosa*, and *Nitzschia* sp. used for the multispecies phytoplankton test were cultured in modified ISO (International Standards Organization) medium. Starter cultures were maintained at room temperature, with a 12 h alternating light and dark cycle, and a light level of 10 to 30 $\mu E \cdot m^{-2} \cdot s^{-1}$.

2.3 TOXICITY BIOASSAYS

2.3.1 Growth Inhibition Test Using the Freshwater Alga *Selenastrum capricornutum*

The toxicity testing of effluent samples using this freshwater alga was performed according to Environment Canada (1992a). Microplates (sterile 96 well, with a capacity of 250 µL per well) were used for testing. Serial dilutions of the effluent sample were prepared by addition of receiving water. An inoculum (10 µL) of exponentially growing algal cells was introduced into each well with 10 µL of a nutrient solution and 200 µL of a sample dilution. The control wells received receiving water instead of sample dilution. A second control plate was prepared, using inoculum, the nutrient solution and 200 µL of reagent water. The microplates were incubated at 25 °C in constant light for

72 h. At the end of the assay, the microplate wells were individually mixed (with a micropipette) and the cells were counted with an automatic counter.

2.3.2 Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia.*

The toxicity testing of effluent samples using *Ceriodaphnia* was performed according to Environment Canada (1992b). Testing was performed at 25°C in a temperature controlled room. Ten neonates, less than 24 h old, were exposed to a minimum of five effluent concentrations and to a control consisting of laboratory well water. One individual was exposed in each test vial. A small volume of food (0.1 mL algae and 0.1 mL yeast culture) was added to each vial prior to the test. The test solutions were renewed daily by transferring the test organisms only (without their offspring) to freshly prepared solutions. Prior to solution renewal, daily measurements of pH, dissolved oxygen, conductivity and temperature were taken in control and in low, medium and high effluent exposure concentrations. Survival of organisms and number of young were recorded daily. As a measure of reproduction, at the end of the assay, the number of live neonates produced by each of the ten individuals per concentration (=10 replicates) were totalled. For measurements of survival, the ten individuals exposed at each concentration were considered as one group. The test is not completed until at least 60% of the surviving control organisms had three broods of neonates.

2.3.3 Test of Larval Growth and Survival Using Fathead Minnows *Pimephales promelas*

The toxicity testing of effluent samples using the fathead minnow was performed according to Environment Canada (1992c). Fathead minnow larvae, less than 24 h old, were exposed to a minimum of five effluent concentrations, with two controls consisting of the receiving water and laboratory well water. The exposure vessels were 1 L polystyrene beakers. Each beaker contained ten larvae, exposed to 500 mL of an effluent concentration and each effluent concentration series consisted of four replicates. Fish were randomly distributed in each beaker. Testing was performed at 25°C in a temperature controlled room. Fish were fed with 0.1 mL of a concentrated suspension of brine shrimp three times each day during testing. A double portion (0.2 mL) was given on day 6.

Fish were not fed for 12 h before the test ended. Test solutions were renewed daily. Prior to solution renewal, measurements of pH, dissolved oxygen, conductivity and temperature were taken in the control exposures and in low, medium and high effluent exposure concentrations. The number of fish surviving in each test beaker was recorded daily. At the conclusion of the test, surviving fish in each beaker were counted and oven dried at 100°C for a minimum of 2 h, but not exceeding 24 h. The pooled fish were then cooled in a desiccator and weighed to constant weight.

2.3.4 Rainbow Trout (*Oncorhynchus mykiss*) Embryo Survival Test

The principle of this test is to assess the toxicity of a sample based on survival of newly fertilized rainbow trout eggs. Embryos were exposed to a range of concentrations of an effluent for seven days under static renewal conditions. Effluent dilutions were prepared using the local receiving water. Control solutions were prepared with the receiving water and with the laboratory well water. Toxicity testing with trout embryos was done according to Environment Canada (1992d), with the following modifications: the test volumes were reduced from 6 L to 2.25 L and the test temperature was increased from 12°C to 15°C (Yee et al., 1996).

Unfertilized eggs and milt were obtained from a certified disease-free hatchery (Rainbow Springs Hatchery). Eggs were obtained from 1-3 females, and milt from at least one male. In most cases, fertilization took place immediately, but if necessary both the eggs and milt were stored for a maximum period of 24 hours. If stored, the milt was kept at a depth less than 6 mm, at 0 - 4°C, and eggs were kept no more than 3 layers thick at 0 - 3°C.

The incubation test chambers were constructed from 1 L polyethylene jars and CPVC piping. The embryos were placed on a nitex screen located at the bottom of the incubation chamber, and water was gently circulated into the chamber and over the embryos. The flow of water was monitored twice a day.

Eggs were dry-fertilized. The females were spawned into a dry, clean plastic food grade bucket, to which the milt was added to the eggs. Upon addition of the milt, the gametes were gently stirred by hand and gently mixed for 20 minutes. The test was started within 30 minutes following the 20 minute period for fertilization. Forty embryos were randomly added to each container for a total of 120 embryos per test concentration. Excess embryos, or any that appeared abnormally small/large or deformed were removed, and missing embryos were added. The test containers were kept in the dark for the duration of the test and subdued lighting was used for daily observations.

The embryos were exposed to a minimum of five concentrations of effluent. Each test concentration and control exposure consisted of three replicates. A fourth control was included to monitor embryo fertilization through out the test. The pH of the control, low, medium, and high concentrations at the start of the test and at the beginning of each renewal period was measured and recorded. Dissolved oxygen concentrations were measured at the beginning and end of each renewal interval in at least one replicate of each concentration. Temperature was measured in each of the newly made solutions prior to the first changeover and at the end of the first renewal in all replicates, and continuously thereafter in at least 1 replicate solution. Test solutions were renewed once daily for the duration of the test and a minimum of 80% of the test solution was replaced.

During the test, the number of dead embryos in each test vessel was recorded daily and any obviously dead embryos (with fungus) were removed. Embryos which were not dead, but appeared atypical were not removed until the end of the test. Any observed deformities were noted.

At the end of the test, surviving embryos were counted. Each replicate was examined under a dissecting stereo-microscope to determine if the embryos were fertilized, unfertilized and/or dead. In cases where it was difficult to determine if the dead eggs were fertilized or unfertilized, eggs were preserved in a 1:1:1 v/v solution of glacial acetic acid, methanol and water, until clear. Eggs were then examined under a dissecting stereo-microscope to check for evidence of cleavage of the germinal disc or the presence of a white streak. The test is considered valid if fertilization in the controls is $\geq$ 70% and if mortality of control embryos (not including unfertilized eggs) is $\leq$ 20%.

2.3.5 Growth Inhibition of the Duckweed *Lemna minor*

Lemna minor is a vascular aquatic plant with a floating growth habit. It forms 2 to 4 fronds 0.5 cm in length. Its maximum rate of growth is close to one doubling every 24 h. *Lemna minor* is a cosmopolitan species growing in most regions of Canada.

The *Lemna minor* growth inhibition test as developed by the Saskatchewan Research Council [SRC] Water Quality Section is a modification of the *8211 Duckweed (Proposed)* toxicity test procedure published by American Public Health Association (APHA, 1995). The major modifications include changes to the medium composition (potassium added, pH stabilized), pre-cultivation methods and the use of axenic cultures, as well as the establishment of a requirement for a greater biomass increase during the test (Saskatchewan Research Council, 1996a). The *Lemna minor* growth inhibition test was developed to provide a photosynthetic plant bioassay for the testing of metal mine wastewater. The test is limited in that effluents and receiving waters are filtered to prevent algal growth and, although simple and relatively inexpensive to perform, it is seven days in length.

Fast growing cultures of duckweed, *Lemna minor* are exposed to various concentrations of a test substance in a static system under defined conditions. Plants are cultured in Hoagland's E+ medium and acclimated to test media, (modified APHA media) for 24 h. The test is performed with an illumination of 63-72 $\mu E/m^2/s$ at a temperature of 25 ± 2°C. Eight replicates of each exposure concentration are prepared in 1 oz polystyrene cups or , polystyrene petri dish lids, with a volume of 25 mL per replicate. The biomass of the *Lemna* treated with the test substance is compared with the biomass of *Lemna* in an appropriate control over 7 days. A test substance is considered toxic when a statistically significant, dose-dependent inhibition of growth (as biomass) is observed.

2.3.6 Multispecies Phytoplankton Growth Inhibition Test

The multispecies phytoplankton growth inhibition test, developed by the Saskatchewan Research Council [SRC] Water Quality Laboratory in collaboration with the Technical University of Denmark,

is a modification of the International Standards Organization [ISO] test, "Fresh water algal growth inhibition test with *Scenedesmus subspicatus* and *Scenedesmus capricornutum*" (ISO 1989) and the Swedish National Chemicals Inspectorate "Algal microtest battery" developed by Blanck and Björnsäter (1989). The test has been designed to work at low cell densities so pH will not be affected by algal growth (Saskatchewan Research Council, 1996b). The endpoint is fluorescence, which can be measured irrespective of phytoplankton growth habits (*i.e.*, it is possible to measure filamentous, colonial, or unicellular organisms).

Phytoplankton species from three taxonomic classifications are included in the test battery since the sensitivities of phytoplankton classes may vary among different types of toxic compounds. The test should be done with multiple organisms chosen from different phytoplankton groups. Suggested organisms are the green alga *Selenastrum capricornutum* (Chlorophyta, Chlorophyceae), the blue-green algae *Microcystis aeruginosa* (Cyanophyta, Cyanophyceae), and the diatoms *Nitzschia* sp. (Bacillariophyta, Bacillariophycea).

The dilution water is either a natural receiving water spiked with nutrient stock solution or synthetic medium, aerated overnight prior to testing. Testing is performed in 96-well round bottom, sterile, non-tissue culture treated microplates. The test volumes is 240 μL and replicates of each exposure concentration are prepared. Microplates are incubated on microplate shakers at 400 rpm under 70 to 90 $\mu E \cdot m^{-2} \cdot s^{-1}$ in a temperature and humidity controlled chamber. Temperature is held at 23 to 27°C and humidity maintained at 40 - 60%. Incubation is for 45 to 52 hours, at which time the fluorescence is measured. The SRC lab uses a Fluorolite 1000 microplate fluorometer by Dynatech Corporation with an excitation filter of 440 nm (bp 20), and an emission filter of 670 nm (bp 40). Optimal conditions for fluorescence measurements is with the cells on the bottom of the plate, therefore, care must be taken not to agitate the plates.

2.3.7 Nematode Survival, Maturation and Growth Test

Nematodes or roundworms are a significant component of the benthic fauna. The nematode toxicity assay was performed according to Samoiloff (1990), which describes measurements of survival, maturation and growth of the species *Panagrellus redivivus*. The assay involves exposure of newborn animals, which are termed second stage juveniles. Over a period of four days, the juveniles pass through two additional juvenile stages and become adults. The organisms are cultured in a growth medium (M9 buffer), which also serves as dilution water.

The assay involves the exposure of second stage juveniles to a range of effluent concentrations. Assays are conducted at room temperature. The maximum effluent concentration that can be tested is 50% v/v. Ten organisms are exposed in 1.0 mL of each replicate for 96 h, at which point the survival, growth (length of the animal) and maturation in each of the replicates are recorded. The test is considered invalid if control survival is < 80% and if <40% of the control organisms reach the adult stage.

2.3.8 Microtox Chronic Test With Luminescent Marine Bacteria

The Microtox acute test has been used as a "rapid screening" test for the assessment of toxicity. The chronic test is a further development of the acute test, using the same naturally luminescent marine bacteria, *Vibrio fischeri.* These bacteria emit light through an enzymatic pathway involving luciferin, the luciferase pathway. Toxicity results in a reduction of the bacterial activity and hence their luminescence, and the reduction in light output is proportional to the effluent exposure concentration.

The Microtox chronic test was performed according to the manufacturer's specifications (Measuring Chronic Toxicity Using Luminescent Bacteria, Microbics Corp. 1994). The bioassay involves measuring changes in the bacteria luminescence after 22 hours of exposure to the toxicant. The manufacturer supplies the bacteria (lyophilized), a nutritive test media for preparing dilutions and the incubator - photometer. Tests are performed at 27 °C. The full strength effluent sample is adjusted

to 2% salinity using analytical grade NaCl and the effluent dilutions are prepared in an osmotically adjusted test media.

2.3.9 Mutatox Test With Luminescent Marine Bacteria

The Mutatox uses a mutant strain of *Vibrio fischeri*, which becomes luminescent when it undergoes a mutation to the wild type. The Mutatox test was performed according to the manufacturer's specifications (Mutatox Genotoxicity Test; Microbics Corporation, 1995). Turbid samples were centrifuged and only the supernatant was tested. A volume of sample was mixed with a vial of Mutatox Medium and ten serial dilutions (either 1:2 or 1:1.5) were prepared in the analyser. Exposures took place both with and without the presence of the enzymatic activation solution S-9. Light output readings were taken after 16, 20 and 24 hours. Samples were run with a positive control (benzo (a) pyrene or phenol) and control blanks. A positive genotoxic response is defined as a light output greater than twice the control level. The sample is considered as genotoxic if a positive response is obtained in two consecutive dilutions.

2.4 CHEMICAL ANALYSES

Concentrations of dissolved and total metals in receiving water and effluent samples were determined by Inductively Coupled Plasma Emission Spectroscopy (ICP). Total and whole acid digested cyanide, and ammonia, were determined by automated colorimetry. Total and suspended solids were determined by the gravimetric technique. Alkalinity and pH were determined by titration, conductivity by electrode, and hardness by calculation from concentrations of Ca and Mg.

Detection limits for each parameter are listed in Table 2-3 and 2-4. Several detection limits are listed for cyanide, depending on the date of analysis. Two detection limits are listed for arsenic and for total cadmium. Arsenic was determined in the initial samples by the hydride method, with a detection limit of 1 $\mu g \cdot L^{-1}$, while later samples were analysed by ICP, with a detection limit of 100 $\mu g \cdot L^{-1}$. The detection limits for total cadmium in receiving water samples also varied, depending on the sample

matrix and on the performance of the apparatus on a given day.

In some cases, the reported concentration of dissolved metals exceeds the quantity of total metal (Cu: sample # 960483, Zn: samples # 960679 and # 960753). These discrepancies are due to variation in the precision of the method.

2.5 DATA ANALYSIS

2.5.1 Toxicity Endpoints

Determination of endpoints for tests with *Selenastrum*, *Ceriodaphnia*, fathead minnow and the embryo rainbow trout followed recommendations contained in the standard test methods (Environment Canada 1992a, 1992b, 1992c, 1992d). The responses of the organisms in the laboratory water and receiving water control exposures were compared using a t-test. If the data were not normally distributed, they were transformed (arc-sine, log, power function) and re-tested. The statistics were performed using the program TOXSTAT (Gulley et al. 1989), a copy of which was provided by Environment Canada.

The LC50 values and 95% confidence limits for tests with *Ceriodaphnia*, fathead minnow and the embryo rainbow trout were calculated using either probit, moving average, or binomial methods with the program STEP (Stephan 1977). Results were adjusted for control mortality using Abbott's correction.

IC25s and IC50s with 95% confidence limits were calculated by linear interpolation (BOOTSTRP program; Norberg-King, 1988) for *Ceriodaphnia*, Microtox chronic, trout embryo, and fathead minnow assays. Endpoints for the *Selenastrum* test were determined from a linear regression of growth inhibition vs log effluent concentration. Endpoints for *Lemna minor* and multi-species algal growth inhibition tests were determined with non-linear regression models. Models were chosen which were non-symmetric around the ICp values to curve-fit the data and predict the desired ICp

values and confidence intervals. Toxicity results with effluent samples are shown as % v/v effluent. Software was provided by Environment Canada.

2.5.2 Comparisons of Toxicity Tests

The toxicity tests were compared in terms of their sensitivity and in terms of the similarity of their responses. Sensitivity was measured by ranking the toxicity tests according to the IC25s. The similarity of the responses of the tests were evaluated by correlation. Non-parametric statistics were employed due to the small sample size.

Two methods were used to compare the selected toxicity tests. A rank was assigned to a toxicity test based on the range of IC25s obtained with each sample, allowing for ties in the scoring for IC25s of similar magnitude. An average score for each bioassay was then calculated.

The test results were also compared non-parametrically using Friedman ANOVA and Kendall concordance. The Friedman ANOVA by ranks test assumes that the variables under consideration were measured on at least an ordinal (rank order) scale. The null hypothesis for the procedure is that the different variables contain samples drawn from the same population, or specifically, populations with identical medians. Thus, the interpretation of results from this procedure is similar to that of a repeated measures ANOVA. The Kendall concordance coefficient expresses the degree of association between k variables. The concordance coefficient is the average of all Spearman R's between variables and the general assumptions of this test are identical to that of the Spearman rank order correlation.

2.5.3 Correlations With Effluent Chemistry

The relationship between effluent toxicity (IC25s) and effluent chemistry was examined by the calculation of correlation coefficients. Due to the small sample size, the nonparametric equivalent to the standard correlation coefficient, the Spearman R, was used. The Spearman R assumes that the

variables under consideration were measured on at least an ordinal (rank order) scale.

A large number of the chemical analyses resulted in " less than" values, results which were less than the method's detection limit. The parameters selected were those for which measurable values were reported for at least four of the eight samples. Only ten parameters satisfied this criterion and were selected for correlation analysis. Values below the detection limit were replaced by amounts equal to one-half (0.5) this limit.

The parameters included total dissolved solids, total suspended solids, ammonia nitrogen, dissolved zinc, conductivity, alkalinity, pH, hardness, total copper, and total zinc. Two other parameters, the sum of total metals and the sum of dissolved metals, were added to this list. The total and dissolved concentrations of metals were converted into $\mu moles \cdot L^{-1}$, and then summed. This procedure accounted for metals, such as nickel and lead, which were present in only one or two samples and may have contributed to the samples' toxicity.

2.5.4 Cost Analysis Correlations

For each bioassay, the time allocated to the following tasks was recorded: testing (including sample preparation and reporting); culturing; and quality assurance/quality control (QA/QC, including: reference toxicant testing and culture health). An average time per test was then calculated and multiplied by a technician's hourly rate of $\$15.00 \cdot hr^{-1}$. Accounts of the amounts of disposable materials used for each assay were also maintained. These were added to the labour costs for a final estimated cost.

The costs of capital equipment are not considered in these totals. Most bioassays only require standard laboratory equipment, such as thermometers, pH metres, and microscopes, in order to perform the testing itself. However, certain bioassays, such as the Microtox chronic test and the multi-species algal test, require equipment that is either specialized and/or expensive. For example, the Microtox analyser, which also serves as a temperature controlled incubator for the Microtox

acute, Microtox chronic and Mutatox tests, costs approximately $26,000.00. The multi-species algal assay requires a fluorometer. While some other equipment may be optional, it's use can increase the accuracy or rapidity of the assays. For example, while the algal cells in the *Selenastrum* test can be counted by eye, the time required to perform the assays is reduced if they are counted using a particle counter.

However, major capital costs for other bioassays are required to maintain laboratory cultures of the organisms, such as *Ceriodaphnia* and fathead minnow. These may include systems for water treatment and/or dechlorination, for aquaculture (pumps, aquaria), and for control of photoperiod and temperature. These investment required to culture certain aquatic organisms would appear to be at least equivalent to the capital costs mentioned above and should be considered if these tests are to be compared to assays such as the Microtox. The only equipment costs considered in this report will be that of the disposable materials used in each assay.

3 RESULTS

3.1 TOXICITY TESTS

3.1.1 *Selenastrum capricornutum*

The cell counts of *Selenastrum capricornutum* in the control exposures were compared using t-tests. In all cases, the algae grew at least as well in the receiving water as in the laboratory dilution water. There was significant stimulation of algal (*Selenastrum*) growth in exposures to the following receiving waters: samples RW - 960482, RW - 960483, RW - 960753 and RW - 960679.

The *Selenastrum* assay was one of the most sensitive assays, as five of the eight tests resulted in IC25s which were less than 10% v/v. The toxicity of the effluents to the growth of the alga ranged from very low (IC25 >100% v/v) to fairly severe (IC25 of 0.9% v/v). A summary of the results of toxicity tests with the freshwater alga is shown in Table 3-1.

Table 3-1. Growth inhibition of the freshwater alga *Selenastrum capricornutum* after 72 h exposure to eight mining effluents. Toxicity test results are expressed as % v/v of effluent. IC25 and IC50 values are shown with 95% confidence intervals (CI).

Sample #	Test date (d/m/y)	Growth inhibition	
		IC25 (95% CI)	IC50 (95% CI)
960482	28/03/96	46.0 (40.2-51.7)	70.6 (64.8-76.3)
960483	28/03/96	0.9 (0-2.7)	2.6 (0.6-4.3)
960577	11/04/96	7.9 (0-28.7)	12.9 (0-33.8)
960676	25/04/96	3.0 (0-13.4)	9.3 (0-19.7)
960679	25/04/96	1.3 (0-5.8)	3.3 (0-7.9)
960753	09/05/96	5.7 (0-11.5)	18.9 (13.1-24.6)
960768	09/05/96	32.7 (22.0-43.4)	>100
960918	06/06/96	>100	>100

3.1.2 *Ceriodaphnia dubia*

The survivorship and number of young produced in the two control exposures were compared using t-tests. A single Ceriodaphnid test was invalid due to mortality in the receiving water control of sample # 960676. The mortality in this case was 30%, slightly more than the 20% allowed according to the test method. However, there was no significant difference in reproduction between the two controls. The responses in the receiving water controls in all of the other assays satisfied the test method criteria for acceptance. There was a significant stimulation of reproduction (at $p = 0.05$) in the control exposure to RW - 960753.

Table 3-2. Survival and reproduction of the cladoceran *Ceriodaphnia dubia* after exposure to eight mining effluents. Toxicity test results are expressed as % v/v of effluent. LC50, IC25 and IC50 values are shown with 95% confidence intervals (CI). Invalid tests are denoted by I.

Sample #	Test date (d/m/y)	Survival	Reproduction	
		LC50 (95% CI)	IC25 (95% CI)	IC50 (95% CI)
960482	28/03/96	80.4 [a]	35.8 (24.1-41.0)	>50[b]
960483	28/03/96	16.8 (13.0-25.0)	>13.0 [b]	>13.0 [b]
960577	10/04/96	18.0 (13.0-25.0)	>13.0 [b]	>13.0 [b]
960676	25/04/96	I	I	I
960679	25/04/96	> 100	37.1 (11.4-57.3)	64.8 (42.0-81.1)
960753	08/05/96	>100	33.8 (20.5-37.1)	45.5[a]
960768	08/05/96	>100	81.9[a]	>100
960918	05/06/96	>100	>100	>100

[a] Approximate value since confidence limits could not be calculated.

[b] Complete mortality at higher concentrations.

The effluents were generally of low to moderate toxicity to the invertebrate. The effluent exposures caused relatively mild effects on Ceriodaphnid reproduction, with IC25s ranging from 33.8% to > 100% v/v. However, two effluent samples, # 960483 and # 960577, caused substantial mortality during the assay, with LC50s of 16.8 and 18.0% v/v respectively. Effluent # 960676 also caused toxicity to the organisms. While this test was invalid due to mortality in the controls, none of the Ceriodaphnids survived in the full strength (100% v/v) effluent exposure. A summary of the results of toxicity tests with the invertebrate is shown in Table 3-2.

3.1.3 *Pimephales promelas*

The survivorship and growth of fish in the two control exposures were compared using t-tests. There were no significant differences in weights of the fish between the receiving water and laboratory controls at the end of the assays ($p > 0.05$).

In two cases, assays with effluent # 960577 and # 960676, mortalities in the receiving water controls were respectively 30% and 43%. Since these values were greater than the 20% mortality permitted under the test method, these tests were considered as invalid. However, growth in the receiving water controls appeared to be unaffected, since weights of the fish surviving in these controls were not different than those exposed to the laboratory dilution water. The remaining tests were all judged to be valid.

The effluents were generally of low to moderate toxicity to the larval fish. Exposures to two of the samples, # 960482 and # 960483, affected minnow survival. The IC25s for survival were 67.4 and 16.3% v/v, and the LC50s were 87.6 and 24.3% v/v, for assays with samples # 960482 and # 960483, respectively. As discussed previously, two of the assays were invalid due to mortalities in the controls. However, these effluents, # 960577 and # 960676, also affected survival of the larval fish since most (97.5% and 100%, respectively) of the fish died in the full strength (100% v/v) effluent concentrations. Growth of the minnows was also affected by exposures to samples # 960482 and # 960483, with IC25s of 87.6 and 9.2 %v/v. A modest impact on growth was observed with

exposure to sample # 960753, with an IC25 of 94.4 %v/v. The remaining samples were of low toxicity, as no IC25 values for growth or survival could be calculated. A summary of the results of toxicity tests with the minnow is shown in Table 3-3.

Table 3-3. Survival and growth of larval fathead minnow *Pimephales promelas* after 7 days of exposure to eight mining effluents. Toxicity test results are expressed as % v/v of effluent. LC50, IC25 and IC50 values are shown with 95% confidence intervals (CI). Invalid tests are denoted by I.

Sample #	Test date (d/m/y)	Survival		Growth	
		IC25 (95% CI)	LC50 (95% CI)	IC25 (95% CI)	IC50 (95% CI)
960482	27/03/96	67.4 (55.2-74.6)	87.6 (50.0-100)	87.6 [a]	>100
960483	27/03/96	16.3 (0-30.4)	24.3 (13.0-50.0)	9.2 (4.9-11.6)	24.7[a]
960577	10/04/96	I	I	I	I
960676	24/04/96	I	I	I	I
960679	25/04/96	>100	>100	>100	>100
960753	07/05/96	>100	>100	94.4[a]	>100
960768	09/05/96	>100	>100	>100	>100
960918	05/06/96	>100	>100	>100	>100

[a] Estimated value since confidence limits could not be calculated.

3.1.4 *Oncorhynchus mykiss* embryo

In general, the rainbow trout embryo test was not successful. Mortalities were severe in both laboratory dilution water and receiving water controls in five of the assays. During many of these tests, the trout eggs appeared to be of poor quality and began to fungus almost immediately.

These assays were performed in the months of March, April and May, which may have coincided with a period of poor egg or sperm viability, perhaps due to seasonal effects. The eggs and milt were supplied by Rainbow Springs Hatchery, an installation which supplies year-round spawners by

manipulating the photoperiod. This hatchery is the only aquaculture farm in Ontario that has spring spawners. During the months of March and April, the Rainbow Springs Hatchery was switching over from indoor (artificial light) to outdoor (natural light) installations and the timing of certain tests unfortunately coincided with this change-over period. A high proportion of the mature adults from the hatchery suffered from poor egg or sperm viability.

Several other hatcheries were contacted during this period (Blue Springs, Spring Valley Trout, Alma Research Station, as well as the Ontario Aquaculture Association), in attempts to obtain another source for eggs. In most cases, our contacts referred us to our original supplier. Considering that the Rainbow Springs Hatchery supplies most of the trout eggs for aquaculture in Eastern Canada, it is unlikely that trout eggs or milt could have been obtained elsewhere in this geographic area.

This lack of success with the rainbow trout embryo test may also be related to the changes to the test method introduced at the start of this project. It is possible that the increased test temperature and reduced exposure volume may also have contributed to the problem of excessive control mortality.

In summary, six of the eight assays were judged as invalid because mortalities in the controls surpassed 20%, the percentage permitted under the test method. In five of the six assays, mortalities in both the receiving water and laboratory dilution water controls were > 20%. However, in one case, the receiving water sample was toxic. In the test performed with sample # 960577, survival of fertilized eggs in the laboratory dilution water control was acceptable (ie < 20%), yet mortality in the receiving water control was severe (> 80%).

The two remaining effluents, # 960678 and # 960918, were of low toxicity to the embryos, with IC25s for survival of 51.7 and 54% v/v , and LC50s of 88.7 and 78.8% v/v. It should be noted that water from the same location was used as dilution and control water in both of these tests, since these

Table 3-4. Survival of embryos of the rainbow trout, *Oncorhynchus mykiss*, after 7 days of exposure to eight mining effluents. Toxicity test results are expressed as % v/v of effluent. IC25 and LC50 values are shown with 95% confidence intervals (CI). Invalid tests are denoted by I.

Sample #	Test date (d/m/y)	IC25 (95% CI)	LC50 (95% CI)
960482	27/03/96	I	I
960483	27/03/96	I	I
960577	10/04/96	I[a]	I[a]
960676	24/04/96	I[b]	I[b]
960679	24/04/96	I	I
960753	08/05/96	I[b]	I[b]
960768	08/05/96	51.7[c]	88.7 (50.0-100)
960918	05/06/96	54.0 (0-70.1)	78.8 (50.0-100)

[a] Test invalid due to toxicity of receiving water.

[b] Test invalid, but survival of fertilized eggs in full strength effluent was > 90%.

[c] Estimated value since confidence limits could not be calculated.

samples came from the same mine site. The receiving water body was sampled at different times to coincide with the effluent tests. Two other effluents were of low toxicity to the fish embryos. While the assays with samples # 960676 and # 960753 were invalid, survival of the embryos in the full strength (100% v/v) effluent concentrations was respectively, 100% and 94.8%. The results of these tests are shown in Table 3-4.

3.1.5 *Lemna minor*

Only receiving water controls were performed for the duckweed tests, so no comparisons with the usual test media were necessary. All of the receiving water controls satisfied the criteria for acceptance of the tests and all results and confidence limits were calculated using parametric data analysis. However, in two cases, the assays were delayed beyond the recommended period of 72 hours. Effluent sample # 960679 was tested on May 1, 1996. The receiving water, RW - 960753

arrived at the same time as the effluent sample, and this assay was therefore delayed. The test with this effluent had to be repeated, on June 5 1996, due to the presence of algae in the receiving water.

The effects of the effluent exposures on growth of the duckweed ranged from mild to relatively severe. The lowest IC25s, 0.32 and 2.82 % v/v, were obtained with samples # 960679 and # 960676, respectively. The other IC25s ranged from 8.8 to 67.0 % v/v, while growth of the plant was not affected by exposure to sample # 960753 (IC25 > 93.0%). Results of tests with the aquatic plant are shown in Table 3-5.

Table 3-5. Growth inhibition of the duckweed *Lemna minor* after 7 days of exposure to eight mining effluents. Toxicity test results are expressed as % v/v of effluent. IC25 and IC50 values are shown with 95% confidence intervals (CI).

B.A.R. Sample #	SRC Sample #	Test date (d/m/y)	Growth inhibition	
			IC25 (95% CI)	IC50 (95% CI)
960482	C28	27/03/96	67.0 (60.3 - 74.5)	81 (75.8-86.5)
960483	C27	27/03/96	24.5 (17.5 - 35.0)	49.7 (41.9-58.9)
960577	C29	10/04/96	15.7 (10.1 - 24.6)	55.1 (41.9-72.6)
960676	C30	24/04/96	2.82 (1.67 - 4.75)	18.3 (13.1-25.6)
960679	C31	01/05/96	0.32 (0.09 - 1.15)	5.6 (1.8-17.5)
960753	C33 [a]	15/05/96	> 93.0	>93.0
960768	C32	08/05/96	8.8 (2.2 - 34.5)	52.3 (19.0-100)
960918	C34	05/06/96	55.6 (41.2 - 75.1)	>93.0

[a] Receiving water arrived too late for valid test, tested June 5/96.

Note: C33 effluent required repeat testing June 5 due to algal content of receiving water.

3.1.6 Multispecies Phytoplankton Growth Inhibition Test

Only receiving water controls were performed for the multi-species algal tests, so no comparisons with the usual test media were necessary. All of the receiving water controls satisfied the criteria for

acceptance of the tests and all results and confidence limits were calculated using parametric data analysis.

Assays with two of the effluent samples were not started within the recommended 72 hour period. Effluent # 960679 was tested on May 1, 1996. Effluent # 960753 was tested once, and then re-tested on June 5,1996. Due to the presence of indigenous algae, sample RW- 960753 water was filtered with GF/C paper before being used for testing.

Effluents were shaken for 30 seconds and aliquots were drawn off to set up the test. If large particles were observed, the effluent was allowed to settle for 10 minutes before the sample was removed to allow the particulate matter to settle to bottom and not cause interference in the test. Three effluent samples (# 960577, # 960676 and # 960679) were decanted in this manner prior to being tested.

The multi-species algal test was the most sensitive bioassay evaluated. The responses to the effluent exposures ranged from none (IC25 > 90.2% v/v with samples # 960768 and # 960918) to severe (IC25 of 0.3 % v/v with sample # 960483). In four of eight tests, *Microcystis aeroginosa* was the most sensitive species while *Selenastrum capricornutum* was the most sensitive alga in the assay with sample # 960753. In the assay with effluent # 960577, all of the algae except for Nitschia sp did not meet the criteria for test validity. The last two samples # 960768 and # 960918 were of low toxicity to all of the algal species (IC25s > 90.2% v/v) and no single species was more sensitive than another during these exposures.

There is a discrepancy in the toxicity results for sample # 960753 with the algae *Selenastrum*. The results of the multispecies phytoplankton assay with this effluent suggested that *Selenastrum* was the most sensitive species, with an IC25 of 64.5% v/v. However, Eco-CNFS obtained an IC25 of 5.7 % v/v with the Environment Canada *Selenastrum* test method. These values are significantly different ($p < 0.05$, standard error of mean differences).

There are several possible reasons for this. The first is the difference in growth media. The Environment Canada (EC) method recommends a modification of the U.S. EPA Algal Assay Media while the SRC specifies an ISO media. The differences include small variations in the amounts of salts added, in the kind of chelate added (EDTA in the EC method compared with NTA) and in the addition of a vitamin solution (SRC). These media, with slight adjustments, are also used in the test concentrations.

There are differences in how the samples are treated before testing, which may be more important. First, in the EC test protocol, effluent pH is not changed (unless a second test is run without pH adjustment). In the SRC draft protocol, the effluent pH is adjusted to that of the receiving water. (According to the Seprotech data the pH of the receiving water and effluent were almost identical 7.08 and 7.07). However, the SRC protocol specifies that the effluent should be aerated for two hours before testing, and the effluent pH may have changed during this period.

Secondly, the EC protocol uses sterile alga cultures. The samples (receiving water, effluent) are also filtered (0.45 µm filter) to remove bacteria. The SRC method recommends filtering the receiving water (through a GF/C filter, which will not eliminate bacteria or some algae) if "visibly" cloudy or green. The RW # 960753 sample was filtered, since duckweed was found growing in it on arrival at the SRC. Filtration can change a sample's toxicity since the material removed by filtration (particles) may either increase or decrease the toxicity. Toxicity may substantially decrease if the sample is not filtered because other organisms (indigenous algae, bacteria) are then part of the assay. These organisms may bind to or accumaulate toxic components that otherwise might have been available to the test species. The growth of these organisms, and their effects on the test species, are also unpredictable, especially if changes in the effluent or receiving water occur.

Thirdly, the assays were started at different times. The receiving water and effluent samples for the EC assay were shipped at the same time, and the test was performed within 72 hours of collection of the effluent sample. The receiving water shipped to Saskatoon arrived too late for the assay to be performed within this 72-h period, and the test was started nine days after effluent collection. This

may have allowed some loss in toxicity during storage, due to aging of the receiving water and/or effluent. The receiving water sample may have been different too, since no evidence of duckweed was found in B.A.R. Environmental's sample.

Finally, there are differences in how the cells are counted. The laboratory that performed the EC test method uses a particle counter to determine cell numbers for the initial inoculum and for final growth, while the SRC uses fluorescence to detect cell numbers.

Results of the multi-species algal tests are shown in Table 3-6.

Table 3-6. Results of exposure to eight mining effluents, determined with the multispecies phytoplankton tests. Growth inhibition is expressed as % v/v of effluent. IC25s and IC50s are shown with 95% confidence intervals (CI). Endpoints were calculated using results of the most sensitive species.

B.A.R. Sample #	SRC Sample #	Test date (d/m/y)	Most sensitive species	Growth inhibition	
				IC25 (95% CI)	IC50 (95% CI)
960482	C28	27/03/96	*Microcystis aeruginosa*	2.1 (1.4 - 3.3)	9.3 (7.1 - 12.2)
960483	C27	27/03/96	*Microcystis aeruginosa*	0.28 (0.15 - 0.5)	0.88 (0.42 - 1.83)
960577	C29	10/04/96	Nitschia sp.[b]	5.3 (5.0 - 5.7)	8.3 (8.0 - 8.7)
960676	C30	24/04/96	*Microcystis aeruginosa*	3.62 (2.48 - 5.27)	56.0[c]
960679	C31	01/05/96	*Microcystis aeruginosa*	0.51 (0.50 - 0.53)	0.62 (0.53 - 0.73)
960753	C33 [a]	15/05/96	*Selenastrum capricornutum*	64.5 (61.9 - 67.3)	75 (73.1 - 76.6)
960768	C32	08/05/96	nd[d]	>90.2	>90.2
960918	C34	05/06/96	nd	>90.2	>90.2

[a] Receiving water arrived too late for effluent to be tested within 72 hours of collection.

[b] May not be most sensitive organism, others did not meet validity criteria.

[c] Confidence limits not available.

[d] Not determined.

3.1.7 *Panagrellus recidivus* (Survival)

Survivorship of the nematode was not affected by exposure to the effluents, as mortalities at the highest exposure concentration were always < 20% v/v. However, the effects on growth and maturation of the organisms could not be scientifically evaluated.

During this testing, a major fault in the protocol and in the design of the nematode test was discovered. This fault arises when the ages of the test organisms are determined. At the end of the assay, the number of survivors is recorded and their lengths are measured. The survivors are counted in the exposure solutions, then the replicate exposure vials are rinsed and the contents are emptied into a watch glass. The surviving animals are picked up with a micropipette and placed on a microscope slide, which is then stained and gently heated at 60 °C (or heated and stained, depending on the hydrophilic/hydrophobic properties of the staining solution) in order to kill and elongate the animals. The length of the individuals is then measured as an indication of their age/stage of development.

The major problem encountered was that there was a difference between the number of survivors counted in the vials and the number of animals measured on the microscope slide. The numbers of animals measured, after staining, were fewer than the recorded number of survivors. This difference occurred in every sample and ranged from 10 - 30% of the surviving animals. In our knowledge, there is no valid scientific method by which this data may be recuperated, unless extensive trials are run to estimate the size distribution of the missing individuals. Thus, with the exception of the survival data, the growth and maturation data must be considered as unreliable and is not reported here.

It is possible to speculate on the reasons for the discrepancy in numbers of survivors and their lengths. It is possible that not all the surviving animals are transferred from the exposure vials. However, the vials were rinsed three times, and it was rare that any animals remained in the vials after the rinsing steps. The heating/evaporation step may destroy some animals, because there is

evidence of debris. The test media is a high ionic strength solution. During the heating step, the water evaporates, leaving ridges of salt crystals. The rapid change in osmotic pressure may contribute to the "explosion" of some nematodes, but the major problem could be that some animals may be trapped and hidden within these salt ridges.

It should be noted that once this problem was realized, we communicated with the laboratory which originated the test. They informed us that they had also encountered this problem and have since altered the method of recording the growth/maturation responses in the test (Dr. Martin Samoiloff, personal communication). The technique involves classifying the surviving animals according to their life stages (J2, J3, J4, adult), at the same time that survivorship is recorded. This technique can only be performed by highly experienced personnel, since the animals are classified by visual examination - their length is not actually measured. Thus, this ranking method does not allow for the determination of endpoints such as the IC25.

3.1.8 Microtox chronic test

The toxicity of the eight effluents as measured by the Microtox chronic test ranged from low to moderate. Toxicity was noted for three sample exposures, # 960483, # 960577 and # 960679, with IC25s of 9.8, 7.6 and 31.3% v/v, respectively. Light output was not decreased by exposure to effluent samples # 960482, # 960676, # 960753, and # 960768 and # 960918, where the IC25s and IC50s were > 100% v/v.

However the overall responses of the luminescent bacteria were more complicated, since stimulation of light output was also observed, in addition to "neutral" (no effect) and inhibitory responses. IC25s could not be calculated from results of assays with samples # 960768 and # 960918, since light output was stimulated in all exposure concentrations. These results are also indicated as "IC25 > 100% v/v". Light output was also stimulated, in at least one of the exposure concentrations, in assays with samples # 960483, # 960676 and # 960679. The extent of stimulation ranged from + 25% to more

than + 100% of the control values. A summary of the results of toxicity tests with the Microtox chronic test is shown in Table 3-7.

It should be noted the expression "IC25 > 100% v/v" can indicate either "no significant effect", or stimulation, at the 100% exposure concentration. Since these stimulatory responses are difficult to compare with inhibitory responses, further evaluation of this type of responses is necessary.

Table 3-7. Inhibition of light emission from the marine bacterium *Vibrio fischeri* after 22 hours of exposure to eight mining effluents. Toxicity test results are expressed as % v/v of effluent. IC25 and IC50 values are shown with 95% confidence intervals (CI).

Sample #	Test date (d/m/y)	Inhibition of light emission	
		IC25 (95% CI)	IC50 (95% CI)
960482	27/03/96	>100	>100
960483	28/03/96	9.8 (9.0 -11.0) [a]	14.1 (11.9 - 16.3)
960577	10/04/96	7.6 (2.4 - 12.7)	12.3 (8.6 - 27.6)
960676	24/04/96	>100 [a]	>100
960679	24/04/96	31.3 (31.2 - 31.4)[a]	37.5 (37.4-37.6)
960753	08/05/96	>100	>100
960768	08/05/96	>100 [a]	>100
960918	05/06/96	>100 [a]	>100

[a] some stimulation of light output observed at one or more exposure concentrations

3.1.9 Mutatox

Samples were tested for mutagenicity with the Mutatox system, with two media - the "direct Mutatox" media and one containing the enzymatic activation media S9. Light output of the bacteria occurred after a genetic mutation. Effluents were considered to be mutagenic when the induced light output was twice that of the background rate over a minimum of two exposure concentrations. None

of the effluent samples showed any mutagenic activity in the direct media. All effluent samples showed some mutagenic activity in S9 media. Samples # 960482 and # 960676 showed mutagenic activity at a narrow concentration range, between 1.0 and 10.% v/v. Two samples, # 960483 and # 960577, showed mutagenic activity only at exposure concentrations of 50 and 100% v/v. All of the remaining samples were mutagenic over a wide range of exposure concentrations, from 2.6% to 100% v/v. A summary of the Mutatox results (for those assays performed with the S9 media) is shown in Table 3-8.

Table 3-8. Mutagenicity of mining effluent samples (for those assays performed with the Mutatox S9 media). The concentration range, in % v/v of effluent, indicated is where light output appeared consistently greater than twice the levels in the controls.

Sample #	Response	Concentration range
960482	(+)	1.0 - 10.0
960483	(+)	50.0 - 100
960577	(+)	50.0 - 100
960676	(+)	2.6 - 5.85
960679	(+)	3.9 - 100
960753	(+)	2.6 - 66.7
960768	(+)	2. 6 - 66.7
960918	(+)	8.8 -100

3.2 RELATIVE COST OF THE BIOASSAYS

Bioassay costs were estimated by adding the costs of labour and the costs of disposable materials, as provided by the participating laboratories. A technician's hourly wage of $15.00 was selected to calculate the labour costs. It is important to note that this only accounts for technician's salary and does include any allowance for overhead or administration. The estimates presented here do not represent the amount that would be charged for performing these bioassays.

Some comments regarding the bioassay time estimates are called for. The times presented by the SRC for the *Lemna minor* test are considerably less than estimates derived from B.A.R. Environmental's experience with the APHA method of this test. However, the SRC values were retained. The average time spent culturing *Ceriodaphnia* and fathead minnows was estimated from the time spent per week divided by the average number of tests performed. Ceriodaphnid culture times were based on the number of tests performed during the study period. Fathead minnow times were derived from annual estimates. Times for Quality Assurance/Quality Control (QA/QC) are estimated from reference toxicant testing. The cost estimates are shown in Table 3-9.

Table 3-9. Average costs of sublethal bioassays with mining effluents, with the time in hours for each task (testing, QA/QC and culturing) and the cost of disposables. Total costs were estimated as the sum of disposables and the cost of labour, assuming an hourly wage of $15.00.

Assay	Technician time (h)			Costs ($)		
	Testing	QA/QC	Culturing	Labour	Disposables	Total (Rank)
Selenastrum	3.8	1.0	0.2	75.00	18.75	93.75 (1)
Ceriodaphnia	11.8	2.0	10.9	370.50	1.91	372.41 (3)
fathead minnow	12.7	2.2	10.6	382.50	4.79	387.29 (3)
Microtox chronic	1.5	0.1	0	24.00	120.60	144.60 (2)
trout embryo	36	8.1	0	661.50	30.61	692.11 (5)
Lemna minor	2.5	0.5	1.0	60.00	20.21	80.21 (1)
multispecies phytoplankton	6.5	0.5	0.5	112.50	30.60	143.10 (2)

4 DISCUSSION

4.1 RESPONSES IN RECEIVING WATER CONTROLS

The responses of the organisms in the receiving water controls varied from toxicity (fathead minnow, *Ceriodaphnia*, trout embryo) to stimulation of growth (*Selenastrum*) or reproduction (*Ceriodaphnia*) Two receiving water samples caused significant mortality to two of the test species. RW-960577 was toxic to the fathead minnow and embryo trout, while RW- 960676 was toxic for the fathead minnow and *Ceriodaphnia*. In contrast, cell growth of the alga *Selenastrum* was significantly higher after incubation in four of the RW samples than in the test's usual control water. Sample RW-960753 resulted in significantly greater production of young in *Ceriodaphnia*, compared to the laboratory control. No toxicity was encountered in the *Lemna minor* or multispecies phytoplankton assays, though in some cases the receiving waters were either decanted or filtered prior to use. The responses of the organisms to the receiving waters are summarized in Table 4-1.

Table 4-1. Responses of the test organisms in the receiving water control exposures (I = invalid test, S= significant stimulation, T = toxic, NT = non-toxic).

Assay	RW Sample #					
	960482/483	960577	960676	960679	960753	960768/918
Selenastrum	S	NT	S	NT	S	S
Ceriodaphnia	NT	NT	T	NT	NT	NT
fathead minnow	NT	T	T	NT	NT	NT
trout embryo	I	T	I	I	I	NT
Lemna minor	NT	NT	NT	NT	NT	NT
multispecies phytoplankton	NT	NT	NT	NT	NT	NT

4.2 SENSITIVITY OF THE BIOASSAYS

Comparisons were performed using results from six of the nine assays. The three assays that were excluded were the nematode test, the Mutatox test and the trout embryo test. The nematode test was excluded due to the serious faults in the test design and protocol discussed previously. The Mutatox test was not considered because of the test results were of an "all or none" format - either mutagenic or non-mutagenic.

The embryo trout results were excluded due to the fact that few of these tests were successful. It is not possible to fairly evaluate the sensitivity of the trout assay in this study because there were only two valid tests where IC25 values could be calculated. However, the problem of seasonally poor gamete quality in this study can be used to judge the practicality of the test.

The six assays retained for comparisons were the *Selenastrum*, Microtox chronic, *Lemna minor*, *Ceriodaphnia*, multispecies phytoplankton and fathead minnow tests. IC25s from these assays were used in the comparisons (Table 4-2). If no effect was detected at the highest exposure concentration, the IC25 was assigned a value of 100% v/v.

To increase the sample size, results of three invalid tests - two with fathead minnow and one with *Ceriodaphnia* -were included in the ranking analysis. These tests were invalid due to mortality in the receiving water controls. However, in the assay with Ceriodaphnia and in one of the fathead minnow assays, control mortality was 30% - only 10% greater than the 20% allowed by the test method. In the third assay with fathead minnow, mortality in the receiving water control was considerably greater (43%). However, growth of the surviving fish in the exposure concentrations was not different than growth in the laboratory water control. IC25s for fathead survival were calculated by non-linear interpolation, which took into account the mortality observed in the receiving water control. An approximate IC25 for Ceriodaphnid reproduction were estimated by assuming that reproduction was zero at the exposure concentrations where mortality was 100%.

Table 4-2. Calculated and estimated IC25s from bioassays conducted with eight mining effluents. Toxicity test results are expressed as % v/v of effluent.

Sample #	*Selenastrum*	*Ceriodaphnia*	fathead minnow	Microtox chronic	*Lemna minor*	multispecies phytoplankton
960482	46.5	35.8	67.4	>100	67.0	2.1
960483	0.9	14.6[a]	9.2	9.8	24.5	0.3
960577	7.9	13.5[a]	39.4[a]	7.6	15.7	5.3
960676	3.0	19.6[a]	62.5[a]	>100	2.8	3.6
960679	1.3	37.1	>100	31.3	0.3	0.5
960753	5.7	33.8	94.4	>100	>93	64.5
960768	32.7	81.9	>100	>100	8.8	>90.2
960918	>100	>100	>100	>100	55.6	>90.2

[a] Estimated value.

It should be noted that the sensitivity of the tests and their ranking may be affected by the small sample size. In preliminary trials, a value of 50% v/v instead of 100% v/v was entered as the IC25 for a single fathead minnow assay and this error was sufficient to change the order of sensitivity determined in the analysis. In addition, one effluent, # 960918, was of low toxicity - in five of the six assays, the IC25s were > 100% v/v. The inclusion of this low toxicity effluent increased the degree of similarity of the responses, and may overly influence the rankings from such a small sample size. Thus these comparisons should be considered with proper caution.

According to the Freidman ANOVA- Kendall concordance analysis, there were significant differences in the test results (Table 4-3). The ranking of the tests, in order of sensitivity from high to low, and showing the rank in brackets, was as follows: multispecies phytoplankton (2.0), *Selenastrum* (2.6), *Lemna minor* (3.1), *Ceriodaphnia* (3.6), Microtox chronic (4.6), and fathead minnow (5.0).

Table 4-3. Results of Friedman ANOVA and Kendall Coefficient of Concordance analysis of selected toxicity tests. (ANOVA $Chi^2 = 13.67521$, $p < 0.01783$, Coefficient of Concordance = 0.39072, average rank r = 0.28917).

Test Species	Average Rank	Sum of Ranks	Mean	Std. Dev.
Selenastrum	2.642857	18.50000	27.85714	36.27855
Ceriodaphnia	3.642857	25.50000	45.21428	33.13565
fathead minnow	5.000000	35.00000	65.77143	34.90452
Microtox chronic	4.571429	32.00000	64.10000	45.40892
Lemna minor	3.142857	22.00000	37.84571	34.46630
multispecies phytoplankton	2.000000	14.00000	36.15571	43.42526

The results of the simpler comparison are shown in Table 4-4. The simpler ranking accounted for the similar magnitude of some of the IC25s (within 10% of each other) by allowing ties in the scoring, separating the assays into three groups. These, in order of decreasing sensitivity were as follows: the *Selenastrum* and multispecies phytoplankton tests (rank of 2), the *Lemna minor* and the *Ceriodaphnia* assays (rank of 3) and the fathead minnow and Microtox tests (rank of 5).

The most sensitive assays in both analyses were those involving phytoplankton. The statistical analyses revealed a ranking of 2.0 for the multispecies phytoplankton assay and 2.6 for the *Selenastrum* test. In the simpler comparison, both assays were ranked at 2. The similarity in sensitivity is not surprising since both assays involve algae, and one species is common to both tests. *Selenastrum capricornutum* is used in is the principal organism in the Environment Canada test method and is one of three organisms used in the multispecies phytoplankton test.

The next group consists of the *Lemna minor* and *Ceriodaphnia* tests (ranked at 3.1 and 3.6 in the Friedman ANOVA; ranked at 3 with the simpler comparison). These tests were of approximately equivalent sensitivity, yet it would be wise not to extrapolate from these results by generalizing this similarity in responses. The data set used in this study consisted of only eight samples and testing

with additional samples may reveal greater differences in the sensitivities of the two organisms to mining effluents.

Table 4-4. Sensitivity of eight toxicity tests to mining effluents using a simplified ranking system. Ranks were assigned based on the magnitude of IC25s obtained in each assay, allowing for ties.

Sample #	*Selenastrum*	*Ceriodaphnia*	fathead minnow	Microtox chronic	*Lemna minor*	multispecies phytoplankton
960482	3	2	4	6	4	1
960483	2	5	3	3	6	1
960577	2	4	6	2	5	1
960676	1	4	5	6	1	3
960679	3	5	6	4	1	2
960753	1	2	4	4	4	3
960768	2	3	4	4	1	4
960918	2	2	2	2	1	2
Average (rounded)	2	3	4	4	3	2

Of the six assays, the fathead minnow and Microtox chronic test were the least sensitive (ranked at 5.0 and 4.6 with the Friedman ANOVA, both ranked at 4.0 in the simpler comparison). A high proportion of the eight samples tested with these organisms resulted in IC25s >100% v/v (three tests with the fathead minnow, five with the Microtox). In the fathead minnow assays, this denoted that no significant effects on growth or survival were measured at the full strength effluent concentration, in other words a "no observable effect concentration" (NOEC) was obtained. However, in three of the Microtox chronic assays, there was significant stimulation of light output in the effluent exposures, in some cases > 1.5 times that observed in the controls.

Stimulatory effects are difficult to compare with a NOEC - such responses are not adequately expressed as an "IC25 > 100%". The toxicological implications of a stimulatory effect could be

benign, but are not necessarily advantageous. It was decided that a NOEC would be considered as a preferable response for the purposes of this study. If the stimulatory effects observed in the Microtox chronic test are taken into account, the fathead minnow test is the more sensitive test.

In summary, the six toxicity tests may be classified into four groups according to their sensitivity. The most sensitive tests, the Selenastrum and phytoplankton microplate assays may be allotted a rank of one. The next most sensitive tests are the *Lemna minor* and *Ceriodaphnia* tests, which are of roughly equal sensitivity, and may be allotted a value of 2. The fathead minnow assay occupies a third group. Finally, the Microtox chronic assay may be considered as a fourth group, once the stimulatory responses observed in this test are accounted for.

4.3 CORRELATION WITH CHEMICAL ANALYSES

The results of the correlation analyses are shown in Tables 4-5 and 4-6. There were few significant correlations between the IC25s and the chemical parameters. The toxicity of the effluents to *Selenastrum* was related to the ionic strength, since there was a negative correlation of total dissolved solids (TDS), conductivity and hardness measurements with the *Selenastrum* IC25s. There was also a negative correlation (Spearman R of -0.862) of total suspended solids and *Lemna minor* IC25s. With one exception, none of the metal parameters correlated with IC25 values. This exception was the sum of total metals and the multispecies phytoplankton IC25s, which were also negatively correlated. Similar results were obtained using other non-parametric correlation procedures (Kendall Tau and coefficient Gamma).

The general lack of correlation between observed toxicity and chemical characteristics of the samples is unfortunate, but it is not surprising. A large number of analytical results were less than the limit of detection. The detection limits for metals (≥ 10 $\mu g \cdot L^{-1}$) may have been appropriate for untreated effluents, but appears elevated, given that most metal concentrations in natural waters are below this range. The limit for another important contaminant, arsenic, was even more elevated (100 $\mu g \cdot L^{-1}$). However, it should be noted that the lack of significant correlations could also be attributed to the small sample size.

Table 4-5. Spearman Rank Order Correlations of toxicity tests and physical-chemical parameters in the effluents.

Parameters	Valid N	Spearman R	t (N-2)	p-level
TDS & *Selenastrum*	8	-.785714	-3.11127	.020815
TDS & *Ceriodaphnia*	8	-.238095	-.60048	.570156
TDS & fathead minnow	8	-.048795	-.11967	.908655
TDS & Microtox chronic	8	-.085930	-.21127	.839673
TDS & *Lemna minor*	8	-.574861	-1.72088	.136058
TDS & multispecies phytoplankton	8	-.287430	-.73508	.490015
TSS & *Selenastrum*	8	-.333333	-.86603	.419753
TSS & *Ceriodaphnia*	8	-.261905	-.66474	.530923
TSS & fathead minnow	8	-.048795	-.11967	.908655
TSS & Microtox chronic	8	-.343720	-.89656	.404486
TSS & *Lemna minor*	8	-.862291	-4.17085	.005873
TSS & multispecies phytoplankton	8	-.215573	-.54076	.608149
Ammonia-N & *Selenastrum*	8	-.431145	-1.17046	.286196
Ammonia-N & *Ceriodaphnia*	8	.191620	.47823	.649410
Ammonia-N & fathead minnow	8	.294528	.75493	.478865
Ammonia-N & Microtox chronic	8	.327260	.84833	.428791
Ammonia-N & *Lemna minor*	8	-.578313	-1.73639	.133173
Ammonia-N & multispecies phytoplankton	8	.072289	.17754	.864929
Conductivity & *Selenastrum*	8	-.785714	-3.11127	.020815
Conductivity & *Ceriodaphnia*	8	-.238095	-.60048	.570156
Conductivity & fathead minnow	8	-.048795	-.11967	.908655
Conductivity & Microtox chronic	8	-.085930	-.21127	.839673
Conductivity & *Lemna minor*	8	-.574861	-1.72088	.136058
Conductivity & multispecies phytoplankton	8	-.287430	-.73508	.490015
Alkalinity & *Selenastrum*	8	-.333333	-.86603	.419753
Alkalinity & *Ceriodaphnia*	8	-.047619	-.11677	.910849

Table 4-5. (Cont.). Spearman Rank Order Correlations of toxicity tests and physical-chemical parameters in the effluents.

Alkalinity & fathead minnow	8	.243975	.61624	.560376
Alkalinity & Microtox chronic	8	-.282341	-.72092	.498071
Alkalinity & *Lemna minor*	8	-.431145	-1.17046	.286196
Alkalinity & multispecies phytoplankton	8	.035929	.08806	.932691
pH & *Selenastrum*	8	-.023810	-.05834	.955374
pH & *Ceriodaphnia*	8	.095238	.23435	.822505
pH & fathead minnow	8	0.000000	0.00000	1.000000
pH & Microtox chronic	8	.012276	.03007	.976985
pH & *Lemna minor*	8	-.143715	-.35572	.734221
pH & multispecies phytoplankton	8	.287430	.73508	.490015
Hardness & *Selenastrum*	8	-.809524	-3.37756	.014903
Hardness & *Ceriodaphnia*	8	-.380952	-1.00924	.351813
Hardness & fathead minnow	8	-.170783	-.42457	.685955
Hardness & Microtox chronic	8	-.233239	-.58752	.578279
Hardness & *Lemna minor*	8	-.550908	-1.61694	.157018
Hardness & multispecies phytoplankton	8	-.347312	-.90721	.399264

4.4 RELATIVE COST, SPEED AND APPLICABILITY OF THE BIOASSAYS

The bioassays may be separated into four groups, according to the cost (Table 3 -9). In the first group are those assays that may be performed for < $100.00 per sample. The *Selenastrum* and the *Lemna minor* growth inhibition tests, with average costs of $93.98 and $80.21 respectively, compose this first group. The next group can be performed at < $200.00 per sample, and includes the Microtox chronic test, with an average cost of $145.60 and the multispecies phytoplankton test, with average cost of $143.10. Those bioassays costing < $400.00 per sample constitute the third group, which encompasses the *Ceriodaphnia* and fathead minnow tests. The fourth group consists of the rainbow trout embryo test, with an estimated cost of nearly $700.00 per sample.

Table 4-6. Spearman Rank Order Correlations of toxicity tests and metal concentrations in the effluents.

Parameter	Valid N	Spearman R	t (N-2)	p-level
Zn (dissolved) & *Selenastrum*	8	.152204	.37722	.718995
Zinc (dissolved) & *Ceriodaphnia*	8	-.228306	-.57440	.586569
Zinc (dissolved) & fathead minnow	8	-.103975	-.25607	.806447
Zinc (dissolved) & Microtox chronic	8	-.385827	-1.02440	.345162
Zinc (dissolved) & *Lemna minor*	8	.031900	.07818	.940229
Zinc (dissolved) & multispecies phytoplankton	8	-.280716	-.71642	.500654
Copper (total) & *Selenastrum*	8	-.195180	-.48747	.643226
Copper (total) & *Ceriodaphnia*	8	-.048795	-.11967	.908655
Copper (total) & fathead minnow	8	.125000	.30861	.768055
Copper (total) & Microtox chronic	8	-.150946	-.37403	.721245
Copper (total) & *Lemna minor*	8	-.245440	-.62017	.557947
Copper (total) & multispecies phytoplankton	8	-.417249	-1.12462	.303718
Zinc (total) & *Selenastrum*	8	-.203596	-.50938	.628675
Zinc (total) & *Ceriodaphnia*	8	-.443122	-1.21079	.271502
Zinc (total) & fathead minnow	8	-.490881	-1.38013	.216766
Zinc (total) & Microtox chronic	8	-.611297	-1.89205	.107347
Zinc (total) & *Lemna minor*	8	.078313	.19242	.853762
Zinc (total) & multispecies phytoplankton	8	-.656627	-2.13255	.076938
Dissolved Metals & *Selenastrum*	8	0.000000	0.00000	1.000000
Dissolved Metals & *Ceriodaphnia*	8	-.195180	-.48747	.643226
Dissolved Metals & fathead minnow	8	-.050000	-.12263	.906406
Dissolved Metals & Microtox chronic	8	-.352208	-.92180	.392192
Dissolved Metals & *Lemna minor*	8	-.159536	-.39585	.705906
Dissolved Metals & multispecies phytoplankton	8	-.392705	-1.04595	.335879

Table 4-6. (cont.).Spearman Rank Order Correlations of toxicity tests and metal concentrations in the effluents.

Parameter	Valid N	Spearman R	t (N-2)	p-level
Total Metals & *Selenastrum*	8	-.380952	-1.00924	.351813
Total Metals & *Ceriodaphnia*	8	-.380952	-1.00924	.351813
Total Metals & fathead minnow	8	-.365963	-.96324	.372625
Total Metals & Microtox chronic	8	-.626061	-1.96664	.096801
Total Metals & *Lemna minor*	8	-.179644	-.44731	.670344
Total Metals & multispecies phytoplankton	8	-.826362	-3.59443	.011443

A significant portion of the labour costs is associated with the rapidity of the bioassays since longer running assays usually require daily feeding of the organisms and renewal of the test media. Thus, the rapidity of the bioassays was not ranked and compared separately, since the cost of the tests accounts for this factor. The most rapid assays are those using the Microtox system (the Microtox chronic test and the Mutatox), since the results of these tests are ready within 24 hours. The algae tests, the multispecies phytoplankton and the *Selenastrum* tests, are intermediate, with a duration of 45 - 52 h (multispecies) and 72 h (*Selenastrum*). The remaining tests use higher organisms and last for a week or slightly more. These seven - eight day tests include the rainbow trout embryo, the *Lemna minor*, the fathead minnow and the *Ceriodaphnia* assays.

The applicability of these tests has been evaluated using the criteria of relevance and of practicality or usefulness. The relevance of a toxicity test was judged on how well the results could be applied to the situation in the field. The usefulness or practicality was evaluated by ranking the material requirements of each test.

The relevance of a bioassay to the Canadian mining situation would be enhanced if the test organism and test conditions are closely related to those found naturally. For example, is the test species native to aquatic habitats in the vicinity of mining activities, or does the test method permit the use of local receiving water as dilution water? Points for relevance were awarded equally for these two categories ("test organism" and "receiving water").

The scoring for test organism was based on two criteria: if the species was native to aquatic habitats in the vicinity of mining activities, and if the test organism could be used for testing throughout Canada. This scoring was broken downs as follows: 0 points - test species native to Canada and can be used in all regions; 1 point : test species native to Canada but it's use is restricted in certain regions; and 2 points: little relevance of test organism to Canadian mining environment.

Points were also allotted for test methods which permit the use of local receiving water as a dilution water. Points were awarded depending on the degree of laboratory manipulation required for use of a receiving water. Laboratory manipulation was taken to include any treatment that could alter the physical-chemical characteristics of a receiving water (ie., adjustment of pH or ionic strength, filtration, addition of nutrients). No points were awarded if the receiving water could be used with a minimum of manipulation, such as in the *Ceriodaphnia*, fathead minnow and trout embryo assays. A single point was awarded if the protocol permitted the use of a receiving water, but specified either filtration, pH adjustment or the addition of nutrients. Finally, two points were awarded if the use of a receiving water not permitted by the protocol. The points for these two categories (test organism and use of receiving water) were added for an estimate of relevance (maximum total = 4).

In these terms, most of the assays evaluated in this report are highly relevant to Canadian mining situations. The ranges of the fathead minnow (*Pimephales promelas)*, the rainbow trout (*Oncorhynchus mykiss*), the duckweed (*Lemna minor*), the cladoceran (*Ceriodaphnia dubia*) and the freshwater algae (*Selenastrum capricornutum, Microcystis aeruginosa, Nitzschia* sp) either cover all of Canada or a large portion of it (Scott and Crossman 1978; Environment Canada, 1992a; Environment Canada, 1992b; Environment Canada, 1992c; APHA, 1995). However, since testing with the fathead minnow is restricted in Canada (the species is not native to British Columbia or Newfoundland), this assay was awarded a single point.

If tests can be performed using local receiving waters as dilution water, it increases the applicability of test results to natural environments. The *Ceriodaphnia*, fathead minnow and trout embryo assays specify that receiving waters can be used as dilution/control water after a minimum of manipulation. However, the *Selenastrum*, *Lemna minor* and the multispecies phytoplankton test methods all specify filtration of receiving waters and/or the addition of a nutrient spike when these waters are used as a

dilution/control water.

In terms of relevance, the Microtox chronic test ranks extremely poorly, at least in terms of mining environments. The Microtox test organism is a marine bacteria, which requires the use of a specific dilution media during the test, so receiving waters may not be used. (None of the mines in this study discharge into a marine environment). In addition, since it is necessary to adjust salinity, the physical-chemical attributes of a sample are altered to an unknown degree.

In summary, the sum of the scores for relevance were 0 (nil) for *Ceriodaphnia* and trout embryo tests, 1 for the fathead minnow, *Selenastrum*, *Lemna minor* and the multispecies phytoplankton tests, while the Microtox chronic assay was assigned a score of 4.

The second component of applicability was practicality. The usefulness or practicality was evaluated by examining the material requirements of each test. The volumes of effluent and/or receiving water required to perform the tests may be used as a partial indication of the material requirements of a bioassay. Tests which require large volumes of liquids probably also require a large amount of laboratory space (for storage and testing), larger exposure vessels and more equipment (for mixing and transferring liquids) than smaller volume tests. The volume requirements for the selected tests are shown in Table 4-7. The requirements were calculated by assuming that tests involving *Lemna minor*, *Ceriodaphnia*, fathead minnow and embryo trout consisted of six exposure concentrations of three replicates each, in an arithmetic (0.5) dilution series ranging from 100% v/v to 3.1% v/v. Since the algae tests are conducted using microplates, the volume requirements are minimal.

Similarly to the cost comparisons, the tests can be assembled into four groups according to the volume requirements. The Microtox chronic, *Selenastrum* and multispecies phytoplankton have minimal test needs (< 1 L, including dilution water), while the *Ceriodaphnia* and *Lemna minor* requirements are moderate (< 10 L). The fathead minnow test may be considered as a third group, since it requires a considerably greater amount of effluent and receiving water, approximately 75 L. The greatest volume is required by the trout embryo test (294 L).

Table 4-7. Volumes (to the nearest 0.1 L) of effluent and receiving water samples required to perform selected bioassays. Volumes were calculated for six effluent concentrations ranging from 100% v/v to 3.1 % v/v. (na: not applicable).

Assay	Volumes in L			Rank
	Effluent	Receiving Water	Total per test	
Selenastrum	0.1	0.1	0.2	1
Ceriodaphnia	2.1	5.3	7.4	2
fathead minnow	32	43	73.5	3
Microtox chronic	0.1	na	0.1	1
trout embryo	83	211	294	4
Lemna minor	0.4	1.1	1.5	2
multispecies phytoplankton	0.1	0.1	0.2	1

Ratings for applicability were determined by averaging the scores for relevance and practicality and rounding up to a whole number. The tests which are most applicable have a ranking of 1 (the *Selenastrum*, *Ceriodaphnia* and multispecies phytoplankton tests); these are followed by the *Lemna minor*, fathead minnow and trout embryo assays with a ranking of 2. Finally, the Microtox chronic test is last with a rank of 3.

4.5 RECOMMENDATIONS

The average scores of the tests in terms of their sensitivity, cost and applicability are summarized in Table 4-10. Each category (sensitivity, cost and applicability) was assigned a value of four ranking points and an average score for each bioassay was determined. The rankings on sensitivity were derived from values shown in Table 4-4. The rankings for cost were taken from Table 3-9, while those for applicability are shown in Table 4-9.

The tests with the best "average scores" are the *Selenastrum* and multispecies phytoplankton tests, followed by the *Lemna minor* and *Ceriodaphnia dubia* assays, followed by the fathead minnow test and the Microtox chronic test.

Table 4-8. Relevance of bioassays used for testing mining effluents. Points were awarded for the species of test organism and for allowing the use of receiving water as dilution water.

Test Organism	Receiving Water	Test Species	Sum (Relevance)
Selenastrum	1	0	1
Ceriodaphnia	0	0	0
fathead minnow	0	1	1
Microtox chronic	2	2	4
trout embryo	0	0	0
Lemna minor	1	0	1
multispecies phytoplankton	1	0	1

Table 4-9. Scores for applicability determined as averages of averages of points for relevance and for test requirements (practicality).

Test Organism	Relevance	Practicality	Rounded Average (Applicability)
Selenastrum	1	1	1
Ceriodaphnia	0	2	1
fathead minnow	1	3	2
Microtox chronic	4	1	3
trout embryo	0	4	2
Lemna minor	1	2	2
multispecies phytoplankton	1	1	1

The *Selenastrum* and multispecies phytoplankton tests are both based on algae, and the same organism is used in both tests, and the two assays were equally ranked. While either of these algae tests can be recommended, the *Selenastrum* test has been used in several Canadian laboratories and a standard test method is available. At this point in time, it is more practical to recommend the *Selenastrum* test because a number of laboratories have experience with it.

The rankings of the *Lemna minor* and *Ceriodaphnia* tests were lower than the algae tests, but were comparable to each other. Since these organisms represent different trophic levels in an ecosystem, both of these tests are recommended.

The Microtox test offers several advantages. It is a rapid test since a chronic response can be obtained within 24 hours, as compared to seven days for other assays. The test media and bacterial culture are provided by the manufacturer, eliminating culture maintenance and media preparation. The methodology is straight-forward and it is an easy test to learn and to perform. However, the stimulatory response noted during this study suggest that a further evaluation of this type of response is necessary before this test can be entirely recommended. If these stimulatory responses are accounted for, the fathead minnow test becomes more sensitive than the Microtox test. The fathead minnow test also offers the advantage of representing an important component of aquatic ecosystems, and for this reason is much more relevant to the Canadian mining environment. Thus, the fathead minnow assay is preferred over the Microtox chronic test.

Table 4-10. Average scores for toxicity tests based on rankings of sensitivity, cost and applicability. Scores for sensitivity are derived from values in Table 3-1. The ranking of tests by cost appears in Table 4-4. Scores for applicability are averages of points for relevance and for test requirements (practicality) as shown in Table 4-9.

Test Organism	Sensitivity	Cost	Applicability	Average Score
Selenastrum	1	1	1	1
Ceriodaphnia	2	3	1	2
fathead minnow	3	3	2	3
Microtox chronic	4[a]	2	3	3
trout embryo	nr[b]	4	2	nr
Lemna minor	2	1	2	2
multispecies phytoplankton	1	2	1	1

[a] Accounting for stimulatory responses.

[b] Not ranked.

It was not possible to rank the rainbow trout embryo assays, as the sensitivity of the test could not be evaluated. Since only three embryo tests were valid, this screening study cannot be considered a fair evaluation of the sensitivity of the test. In terms of cost and practicality, the test scores high (ranks lower) than other tests. However, there may be reasons to retain the embryo test as an alternate to the fathead minnow test. For example, the embryo test would be useful where receiving waters are toxic to fathead minnow larvae but not to salmonid eggs. Another important aspect is the use of the embryo test in regions of the country where the fathead minnow is not native, such as British Columbia and Newfoundland. Since it is not possible to use the fathead minnow for toxicity testing in these regions, the trout embryo assay rmains as the only freshwater chronic toxicity test in a considerable portion of Canada.

In conclusion, this report recommends the following tests for future studies involving mine effluents: the phytoplankton growth inhibition test with *Selenastrum capricornutum*, growth inhibition with *Lemna minor*, the *Ceriodaphnia* survival and reproduction test and the larval fathead minnow survival and growth test. While the multi-species microplate phytoplankton growth inhibition test was the most sensitive assay, the *Selenastrum* test is preferred due to the availability of a standard test method.

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APPENDIX 1 Test Reports -Growth Inhibition Test with *Selenastrum capricornutum*

APPENDIX 2 Test Reports - Growth Inhibition Test with *Lemna minor*

APPENDIX 3 Test Reports - Phytoplankton Multi-species Growth Inhibition Test

APPENDIX 4 Instructions For Collecting and Shipping Receiving Water and Effluent Samples

Appendix 1

Test Reports: Growth Inhibition Test with *Selenastrum capricornutum*

Laboratoires ECO Laboratories

121 Boul. Hymus, Pointe-Claire, Québec H9R 1E6
Tel.: (514) 697-3400 Fax: (514) 697-2900

Certificat d'analyse • Certificate of Analysis

CONDITIONS D'ANALYSE

Description de l'échantillon:	Eff., 03/06/96, 8:30 (BAR #960918)
Date d'analyse:	06-09/06/96
Notre numéro de projet:	606092
Notre numéro de test:	14191
Organismes:	***Selenastrum capricornutum*** (4 à 7 jours)
Inoculation:	~10000cellules/mL
Milieu:	13.75X (mL, chacune des 5 sol. mères)
Eau de dilution:	eau déionisée (stérilisé)
Préparation de l'échantillon:	filtré @ 0,45µm
Protocole d'essai:	SPE 1/RM/25, Novembre 1992

concentration de l'échantillon (%v/v)	moyenne des concentrations des algues après 72 heures (cellules/mL)	inhibition (%) *	pH (non ajusté)	température (degré C)		coef. de var. (%)
				début	fin	
100	1046005	-35.0	7.3	23	23	4.6
50	1088719	-40.5	7.3	23	23	4.5
25	1066903	-37.7	7.3	23	23	3.1
12.5	991885	-28.0	7.3	23	23	1.0
6.25	1013900	-30.8	7.3	23	23	0.9
3.13	979800	-26.4	7.3	23	23	2.2
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
témoin #1	745930	n.a.	7.7	23	23	
témoin #2	813531	n.a.	7.7	23	23	⇨ 4.5
témoin #3	765193	n.a.	7.7	23	23	

REMARQUES:

L'essai avec toxique de référence: CI_{25} = 302.0 (289.8 - 314.1) mg/L(NaCl)

Limites historiques d'avertissement: Min/Max = 218.3 / 447.4

* le ' - ' indique une amplification

n.a. : non applicable

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Le 25 juillet 1996
Projet: 606092

IDENTIFICATION DE L'ECHANTILLON	NUMÉRO DE TEST	DATE DE RECEPTION	DATE D'ANALYSE	CI_{50} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)	CI_{25} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)
Eff., 03/06/96, 8:30 (BAR #960918)	14191	05/06/96	06-09/06/96	> 100 N.C.	>100 N.C.

SOMMAIRE DES RESULTATS:

Échantillon	Conclusions	
Eff., 03/06/96, 8:30 (BAR #960918)	Sans effet	Sans effet

Inf. Conf.: intervalle de confiance à 95%
N C : non calculable

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RÉSULTAT DE L'ÉVALUATION DE LA TOXICITÉ AUX ALGUES DE L'ÉCHANTILLON Eff., 03/06/96, 8:30 (BAR #960918)

PARAMETRE	unit	INHIBITION de la CROISSANCE
CSEO-72hrs (1)	%(V/V)	100.0
CMEO-72hrs (2)	%(V/V)	> 100
STC-72hrs (3)	%(V/V)	N C
CI_{50}-72hrs	%(V/V)	> 100 N.C. (5)
CI_{25}-72hrs	%(V/V)	>100 N.C. (5)

1) Concentration maximale sans effet observé
2) Concentration minimale avec effet observé
3) Seuil de toxicité chronique, = (CSEO x CMEO)^{½}
4) Intervalle de confiance à 95%
5) N C :non calculable pour des raisons de statistique
6) non applicable

Projet: 606092
Échantillon reçu le: 05/06/96
Échant. analysé le: 06-09/06/96
Protocole: SPE 1/RM/25, Novembre 1992
Statistiques: Regression linéaire.

Analyste: Elliott Picken, Tech.

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CONDITIONS D'ANALYSE

Description de l'échantillon:	28/03/96
Date d'analyse:	11-14/04/96
Notre numéro de projet:	603499
Notre numéro de test:	13517
Organismes:	***Selenastrum capricornutum*** (4 à 7 jours)
Inoculation:	~10000cellules/mL
Milieu d'enrichissement:	13.75X (mL, chacune des 5 sol. mères)
Eau de dilution:	eau déionisée (stérilisé)
Préparation de l'échantillon:	filtré @ 0,45µm
Protocole d'essai:	SPE 1/RM/25, Novembre 1992

concentration de l'échantillon (%v/v)	concentration des algues après 72 heures (cellulues/mL)	inhibition (%) *	pH (non ajusté)	température (degré C)		
				début	fin	
100	23602	75.8	7.7	24	23	
50	24245	75.1	7.7	24	23	
25	23746	75.6	7.7	24	23	
12.5	35658	63.4	7.7	24	23	
6.25	91583	6.0	7.7	24	23	
3.13	101016	-3.7	7.7	24	23	
1.56	105422	-8.2	7.7	24	23	
0.781	114324	-17.3	7.7	24	23	coef. de var. des témoins (%)
0.391	117653	-20.8	7.7	24	23	
0.195	112435	-15.4	7.7	24	23	
témoin #1	94523	n.a.	7.7	24	23	
témoin #2	95045	n.a.	7.7	24	23	3.8
témoin #3	102711	n.a.	7.7	24	23	

REMARQUES:

L'essai avec toxique de référence: Cl_{25} = 358.7 (347.9 - 369.6) mg/L(NaCl)

Limites historiques d'avertissement: Min/Max = 220.7 / 444.6

* le ' - ' indique une amplification

n.a. : non applicable

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RÉSULTAT DE L'ÉVALUATION DE LA TOXICITÉ AUX ALGUES DE L'ÉCHANTILLON
28/03/96

PARAMETRE	unit	INHIBITION de la CROISSANCE
CSEO-72hrs [1]	%(V/V)	6.3
CMEO-72hrs [2]	%(V/V)	12.5
STC-72hrs [3]	%(V/V)	8.8
CI_{50}-72hrs	%(V/V)	12.9 (<0.0 - 33.8) [4]
CI_{25}-72hrs	%(V/V)	7.9 (<0.0 - 28.7) [4]

1) Concentration maximale sans effet observé
2) Concentration minimale avec effet observé
3) Seuil de toxicité chronique, = (CSEO x CMEO)$^{1/2}$
4) Intervalle de confiance à 95%
5) non calculable pour des raisons de statistique
6) non applicable

Projet: 603499
Échantillon reçu le: 10/04/96
Échant. analysé le: 11-14/04/96
Protocole: SPE 1/RM/25, Novembre 1992
Statistiques: Regression linéaire.

Analyste: Elliott Picken, Tech.

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Le 25 juillet 1996
Projet: 603499

IDENTIFICATION DE L'ECHANTILLON	NUMÉRO DE TEST	DATE DE RECEPTION	DATE D'ANALYSE	CI_{50} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)	CI_{25} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)
28/03/96	13517	10/04/96	11-14/04/96	12.9 (<0.0 - 33.8)	7.9 (<0.0 - 28.7)

SOMMAIRE DES RESULTATS:

Échantillon	Conclusions	
	Effet	Effet
28/03/96		

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Int. Conf.: intervalle de confiance à 95%

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CONDITIONS D'ANALYSE

Description de l'échantillon:	Eff. 26/03/96
Date d'analyse:	28-31/03/96
Notre numéro de projet:	602976
Notre numéro de test:	13377
Organismes:	***Selenastrum capricornutum*** (4 à 7 jours)
Inoculation:	~10000¢/mL
Milieu:	AAM 13.75×
Eau de dilution:	Eau déionisé + nutriments
Préparation de l'échantillon:	filtré @ 0,45µm
Protocole d'essai:	SPE 1/RM/25, Novembre 1992

concentration de l'échant. (%v/v)	nombre des algues 72 heures ¢/mL	inhibition (%) *	pH	température (degré C)		
				début	fin	
100	60107	72.4	7.7	24	23	
50	161688	25.8	7.7	24	23	
25	236281	-8.5	7.7	24	23	
12.5	227273	-4.3	7.7	24	23	
6.25	212785	2.3	7.7	24	23	
3.13	214167	1.7	7.7	24	23	
1.56	191004	12.3	7.7	24	23	
0.781	183990	15.5	7.7	24	23	coef. de var.
0.391	183416	15.8	7.7	24	23	des témoins
0.195	178413	18.1	7.7	24	23	(%)
témoin #1	224490	n.a.	7.7	24	23	
témoin #2	229161	n.a.	7.7	24	23	5.9
témoin #3	199953	n.a.	7.7	24	23	

REMARQUES: L'essai avec toxique de référence: Cl_{25} = 383.3 (368.2 - 398.3) mg/L(NaCl)

Limites historiques d'avertissement: Min/Max = 217.9 / 447.6

* le ' - ' indique une amplification

n.a. : non applicable

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CONDITIONS D'ANALYSE

Description de l'échantillon:	Eff. 26/03/96
Date d'analyse:	28-31/03/96
Notre numéro de projet:	602976
Notre numéro de test:	13376

Organismes:	***Selenastrum capricornutum*** (4 à 7 jours)
Inoculation:	~10000cellules/mL
Milieu d'enrichissement:	13.75X (mL, chacune des 5 sol. mères)
Eau de dilution:	eau déionisée (stérilisé)
Préparation de l'échantillon:	filtré @ 0,45µm
Protocole d'essai:	SPE 1/RM/25, Novembre 1992

concentration de l'échantillon (%v/v)	concentration des algues après 72 heures (cellulues/mL)	inhibition (%) *	pH (non ajusté)	température (degré C)		
				début	fin	
100	6434	95.3	7.7	24	23	
50	6935	94.9	7.7	24	23	
25	7450	94.5	7.7	24	23	
12.5	14353	89.4	7.7	24	23	
6.25	36823	72.9	7.7	24	23	
3.13	58998	56.6	7.7	24	23	
1.56	86927	36.0	7.7	24	23	
0.781	103120	24.1	7.7	24	23	coef. de var.
0.391	103200	24.0	7.7	24	23	des témoins
0.195	109717	19.3	7.7	24	23	(%)
témoin #1	135821	n.a.	7.7	24	23	
témoin #2	140575	n.a.	7.7	24	23	2.8
témoin #3	131232	n.a.	7.7	24	23	

REMARQUES: L'essai avec toxique de référence: Cl_{25} = 383.3 (368.2 - 398.3) mg/L(NaCl)

Limites historiques d'avertissement: Min/Max = 217.9 / 447.6

* le ' - ' indique une amplification

n.a. : non applicable

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RÉSULTAT DE L'ÉVALUATION DE LA TOXICITÉ AUX ALGUES DE L'ÉCHANTILLON

Eff. 26/03/96

PARAMETRE	unit	INHIBITION de la CROISSANCE
CSEO-72hrs (1)	%(V/V)	< 0.2
CMEO-72hrs (2)	%(V/V)	0.2
STC-72hrs (3)	%(V/V)	N.C. (5)
Cl_{50}-72hrs	%(V/V)	2.5 (0.6 - 4.3) (4)
Cl_{25}-72hrs	%(V/V)	0.9 (<0.0 - 2.7) (4)

1) Concentration maximale sans effet observé
2) Concentration minimale avec effet observé
3) Seuil de toxicité chronique, = (CSEO x CMEO)^{½}
4) Intervalle de confiance à 95%
5) N C :non calculable pour des raisons de statistique
6) non applicable

Projet: 602976
Échantillon reçu le: 26/03/96
Échant. analysé le: 28-31/03/96
Protocole: SPE 1/RM/25, Novembre 1992
Statistiques: Regression linéaire.

Analyste: Salvador Rojas, B.Sc.

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RÉSULTAT DE L'ÉVALUATION DE LA TOXICITÉ AUX ALGUES DE L'ÉCHANTILLON
Eff. 26/03/96

PARAMETRE	unit	INHIBITION de la CROISSANCE
CSEO-72hrs [1]	%(V/V)	25.0
CMEO-72hrs [2]	%(V/V)	50.0
STC-72hrs [3]	%(V/V)	35.4
CI_{50}-72hrs	%(V/V)	70.6 (64.8 - 76.3) [4]
CI_{25}-72hrs	%(V/V)	46.0 (40.2 - 51.7) [4]

1) Concentration maximale sans effet observé
2) Concentration minimale avec effet observé
3) Seuil de toxicité chronique, = (CSEO x CMEO)$^{1/2}$
4) Intervalle de confiance à 95%
5) N C:non calculable pour des raisons de statistique
6) non applicable

Projet:	602976
Échantillon reçu le:	26/03/96
Échant. analysé le:	28-31/03/96
Protocole:	SPE 1/RM/25, Novembre 1992
Statistiques:	Regression linéaire.

Analyste: Salvador Rojas, B.Sc.

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Le 24 juillet 1996
Projet: 602976

IDENTIFICATION DE L'ECHANTILLON	NUMÉRO DE TEST	DATE DE RECEPTION	DATE D'ANALYSE	CI_{50} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)	CI_{25} - 72 HRS
Eff. 26/03/96	13376	26/03/96	28-31/03/96	2.5 (0.6 - 4.3)	0.9 (<0.0 - 2.7)
Eff. 26/03/96	13377	26/03/96	28-31/03/96	70.6 (64.8 - 76.3)	46.0 (40.2 - 51.7)

SOMMAIRE DES RESULTATS:

Échantillon	Conclusions	
Eff. 26/03/96	Effet	Effet
Eff. 26/03/96	Effet	Effet

Int. Conf.: intervalle de confiance à 95%

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CONDITIONS D'ANALYSE

Description de l'échantillon:	Eff., 06/05/96	(BAR #960768)
Date d'analyse:	09-12/05/96	
Notre numéro de projet:	604809	
Notre numéro de test:	13897	
Organismes:	***Selenastrum capricornutum*** (4 à 7 jours)	
Inoculation:	~10000cellules/mL	
Milieu:	13.75X (mL, chacune des 5 sol. mères)	
Eau de dilution:	eau déionisée (stérilisé)	
Préparation de l'échantillon:	filtré @ 0,45µm	
Protocole d'essai:	SPE 1/RM/25, Novembre 1992	

concentration de l'échantillon (%v/v)	moyenne des concentrations des algues après 72 heures (cellulues/mL)	inhibition (%) *	pH (non ajusté)	température (degré C)		coef. de var. (%)
				début	fin	
100	552261	41.7	8.4	23	23	10.0
50	563628	40.5	8.4	23	23	11.7
25	794347	16.2	8.4	23	23	8.4
12.5	963448	-1.6	8.4	23	23	4.8
6.25	932739	1.6	8.4	23	23	2.0
3.13	966240	-1.9	8.4	23	23	5.5
1.56	882527	6.9	8.4	23	23	4.6
0.781	898859	5.2	8.4	23	23	1.0
0.391	888729	6.2	8.4	23	23	8.1
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
témoin #1	979362	n.a.	8.0	23	23	
témoin #2	870842	n.a.	8.0	23	23	7.1
témoin #3	993600	n.a.	8.0	23	23	

REMARQUES:

L'essai avec toxique de référence: Cl_{25} = 300.9 (298.0 - 303.9) mg/L(NaCl)

Limites historiques d'avertissement: Min/Max = 218.3 / 448.4

* le ' - ' indique une amplification

n.a. : non applicable

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RÉSULTAT DE L'ÉVALUATION DE LA TOXICITÉ AUX ALGUES DE L'ÉCHANTILLON Eff., 06/05/96 (BAR #960768)

PARAMETRE	unit	INHIBITION de la CROISSANCE
CSEO-72hrs [1]	%(V/V)	12.5
CMEO-72hrs [2]	%(V/V)	25.0
STC-72hrs [3]	%(V/V)	17.7
CI_{50}-72hrs	%(V/V)	> 100 N.C. [5]
CI_{25}-72hrs	%(V/V)	32.7 (22.0 - 43.4) [4]

1) Concentration maximale sans effet observé
2) Concentration minimale avec effet observé
3) Seuil de toxicité chronique, = (CSEO x CMEO)^{½}
4) Intervalle de confiance à 95%
5) N C: non calculable pour des raisons de statistique
6) non applicable

Projet: 604809
Échantillon reçu le: 08/05/96
Échant. analysé le: 09-12/05/96
Protocole: SPE 1/RM/25, Novembre 1992
Statistiques: Regression linéaire.

Analyste: Elliott Picken, Tech.

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Le 25 juillet 1996
Projet: 604809

IDENTIFICATION DE L'ECHANTILLON	NUMÉRO DE TEST	DATE DE RECEPTION	DATE D'ANALYSE	CI_{50} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)	CI_{25} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)
Eff., 06/05/96 (BAR #960768)	13897	08/05/96	09-12/05/96	> 100 N.C.	32.7 (22.0 - 43.4)

SOMMAIRE DES RESULTATS:

Échantillon

Eff., 06/05/96
(BAR #960768)

Conclusions

Sans effet Effet

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Inf. Conf.: intervalle de confiance à 95%
N C : non calculable

Laboratoires ECO Laboratories

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CONDITIONS D'ANALYSE

Description de l'échantillon:	Eff., 22/04/96, 15:30 (BAR #960679)
Date d'analyse:	25-28/04/96
Notre numéro de projet:	604151
Notre numéro de test:	13691
Organismes:	***Selenastrum capricornutum*** (4 à 7 jours)
Inoculation:	~10000cellules/mL
Milieu:	13.75X (mL, chacune des 5 sol. mères)
Eau de dilution:	eau déionisée (stérilisé)
Préparation de l'échantillon:	filtré @ 0,45µm
Protocole d'essai:	SPE 1/RM/25, Novembre 1992

concentration de l'échantillon (%v/v)	moyenne des concentrations des algues après 72 heures (cellulues/mL)	inhibition (%) *	pH (non ajusté)	température (degré C)		coef. de var. (%)
				début	fin	
100	78898	94.6	7.3	25	23	5.9
50	60492	95.9	7.3	25	23	5.9
25	29783	98.0	7.3	25	23	32.0
12.5	37779	97.4	7.3	25	23	25.1
6.25	161374	89.0	7.3	25	23	12.5
3.13	738392	49.9	7.3	25	23	21.4
1.56	1080742	26.6	7.3	25	23	15.6
0.781	1278479	13.2	7.3	25	23	2.3
0.391	1335670	9.3	7.3	25	23	3.2
0.195	1430032	2.9	7.3	25	23	2.6
témoin #1	1469556	n.a.	7.3	25	23	
témoin #2	1486306	n.a.	7.3	25	23	⇨ 0.8
témoin #3	1463454	n.a.	7.3	25	23	

REMARQUES:

L'essai avec toxique de référence: CI_{25} = 256.5 (251.3 - 261.7) mg/L(NaCl)

Limites historiques d'avertissement: Min/Max = 218.3 / 449.5

* le ' - ' indique une amplification

n.a. : non applicable

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RÉSULTAT DE L'ÉVALUATION DE LA TOXICITÉ AUX ALGUES DE L'ÉCHANTILLON
Eff., 22/04/96, 15:30
(BAR #960679)

PARAMETRE	unit	INHIBITION de la CROISSANCE
CSEO-72hrs [1]	%(V/V)	0.4
CMEO-72hrs [2]	%(V/V)	0.8
STC-72hrs [3]	%(V/V)	0.6
Cl_{50}-72hrs	%(V/V)	3.3 (<0.0 - 7.9) [4]
Cl_{25}-72hrs	%(V/V)	1.3 (<0.0 - 5.8) [4]

1) Concentration maximale sans effet observé
2) Concentration minimale avec effet observé
3) Seuil de toxicité chronique, = (CSEO x CMEO)^{½}
4) Intervalle de confiance à 95%
5) non calculable pour des raisons de statistique
6) non applicable

Projet: 604151
Échantillon reçu le: 23/04/96
Échant. analysé le: 25-28/04/96
Protocole: SPE 1/RM/25, Novembre 1992
Statistiques: Regression linéaire.

Analyste: Elliott Picken, Tech.

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Le 25 juillet 1996
Projet: 604151

IDENTIFICATION DE L'ECHANTILLON	NUMÉRO DE TEST	DATE DE RECEPTION	DATE D'ANALYSE	CI_{50} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)	CI_{25} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)
Eff., 22/04/96, 15:30 (BAR #960679)	13691	23/04/96	25-28/04/96	3.3 (<0.0 - 7.9)	1.3 (<0.0 - 5.8)

SOMMAIRE DES RESULTATS:

Échantillon	Conclusions
Eff., 22/04/96, 15:30 (BAR #960679)	Effet Effet

Yves Bois, M..Sc. Arg.
Directeur
Département d' Écotoxicologie

Linda Bouffard, M..Sc.
Biologiste
Département d' Écotoxicologie

Int. Conf.: intervalle de confiance à 95%

Laboratoires ECO Laboratories

124, Boul. Hymus, Pointe-Claire, Québec H9R 1E6
Tel.: 514-697-3260 Fax: 514-697-2360

Certificat d'analyse • Certificate of Analysis

CONDITIONS D'ANALYSE

Description de l'échantillon:	Eff., 06/05/96, 13:00 (BAR #960753)
Date d'analyse:	09-12/05/96
Notre numéro de projet:	604857
Notre numéro de test:	13896
Organismes:	***Selenastrum capricornutum*** (4 à 7 jours)
Inoculation:	~10000cellules/mL
Milieu:	13.75X (mL, chacune des 5 sol. mères)
Eau de dilution:	eau déionisée (stérilisé)
Préparation de l'échantillon:	filtré @ 0,45µm
Protocole d'essai:	SPE 1/RM/25, Novembre 1992

concentration de l'échantillon (%v/v)	moyenne des concentrations des algues après 72 heures (cellulues/mL)	inhibition (%) *	pH (non ajusté)	température (degré C)		coef. de var. (%)
				début	fin	
100	102030	89.2	7.2	23	23	1.2
50	344973	63.6	7.2	23	23	2.5
25	413072	56.4	7.2	23	23	5.2
12.5	537904	43.3	7.2	23	23	5.3
6.25	809622	14.6	7.2	23	23	5.4
3.13	1043612	-10.1	7.2	23	23	5.8
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
témoin #1	954834	n.a.	7.5	23	23	
témoin #2	945142	n.a.	7.5	23	23	⇨ 0.6
témoin #3	944185	n.a.	7.5	23	23	

REMARQUES:

L'essai avec toxique de référence: Cl_{25} = 300.9 (303.9 - 298.0) mg/L(NaCl)

Limites historiques d'avertissement: Min/Max = 218.3 / 448.4

* le ' - ' indique une amplification

n.a. : non applicable

Laboratoires ECO Laboratories

121, Boul. Hymus Pointe-Claire Québec H9R 1E6
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Certificat d'analyse • Certificate of Analysis

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

Le 25 juillet 1996
Projet: 604857

IDENTIFICATION DE L'ECHANTILLON	NUMÉRO DE TEST	DATE DE RECEPTION	DATE D'ANALYSE	CI_{50} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)	CI_{25} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)
Eff., 06/05/96, 13:00 (BAR #960753)	13896	09/05/96	09-12/05/96	18.8 (13.1 - 24.6)	5.7 (0.0 - 11.5)

SOMMAIRE DES RESULTATS:

Échantillon	Conclusions
Eff., 06/05/96, 13:00 (BAR #960753)	Effet / Effet

Yves Bois, M..Sc Agr..
Directeur
Département d' Écotoxicologie

Linda Bouffard, M..Sc.
Biologiste
Département d' Écotoxicologie

Inf. Conf.: intervalle de confiance à 95%

Laboratoires ECO Laboratories

121, Boul. Hymus, Pointe-Claire, Québec H9R 1E6
Tél.: (514) 697-3400 Fax: (514) 697-2090

Certificat d'analyse • Certificate of Analysis

RÉSULTAT DE L'ÉVALUATION DE LA TOXICITÉ AUX ALGUES DE L'ÉCHANTILLON
Eff., 06/05/96, 13:00
(BAR #960753)

PARAMETRE	unit	INHIBITION de la CROISSANCE
CSEO-72hrs [1]	**%(V/V)**	3.1
CMEO-72hrs [2]	**%(V/V)**	6.3
STC-72hrs [3]	**%(V/V)**	4.4
CI_{50}-72hrs	**%(V/V)**	18.8 (13.1 - 24.6) [4]
CI_{25}-72hrs	**%(V/V)**	5.7 (0.0 - 11.5) [4]

1) Concentration maximale sans effet observé
2) Concentration minimale avec effet observé
3) Seuil de toxicité chronique, = (CSEO x CMEO)$^{1/2}$
4) Intervalle de confiance à 95%
5) non calculable pour des raisons de statistique
6) non applicable

Projet:	604857
Échantillon reçu le:	09/05/96
Échant. analysé le:	09-12/05/96
Protocole:	SPE 1/RM/25, Novembre 1992
Statistiques:	Regression linéaire.

Analyste: Elliott Picken, Tech.

Laboratoires ECO Laboratories

121 Boul. Hymus Pointe-Claire, Québec H9R 1E6
Tél.: 514 697-3200 Fax: 514 697-3909

Certificat d'analyse • Certificate of Analysis

CONDITIONS D'ANALYSE

Description de l'échantillon:	Eff., 22/04/96, 11:00 (DWTP)
Date d'analyse:	25-28/04/96
Notre numéro de projet:	603884
Notre numéro de test:	13690
Organismes:	***Selenastrum capricornutum*** (4 à 7 jours)
Inoculation:	~10000cellules/mL
Milieu:	13.75X (mL, chacune des 5 sol. mères)
Eau de dilution:	eau déionisée (stérilisé)
Préparation de l'échantillon:	filtré @ 0,45µm
Protocole d'essai:	SPE 1/RM/25, Novembre 1992

concentration de l'échantillon (%v/v)	moyenne des concentrations des algues après 72 heures (cellulues/mL)	inhibition (%) *	pH (non ajusté)	température (degré C)		coef. de var. (%)
				début	fin	
100	105120	92.2	7.8	25	23	3.4
50	255577	81.1	7.8	25	23	10.3
25	326727	75.8	7.8	25	23	4.6
12.5	448846	66.8	7.8	25	23	9.1
6.25	906058	32.9	7.8	25	23	4.8
3.13	1297463	4.0	7.8	25	23	5.7
1.56	1177696	12.8	7.8	25	23	15.7
0.781	1170398	13.4	7.8	25	23	9.7
0.391	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.195	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
témoin #1	1241030	n.a.	7.3	25	23	
témoin #2	1417988	n.a.	7.3	25	23	⇨ 7.1
témoin #3	1394896	n.a.	7.3	25	23	

REMARQUES:

L'essai avec toxique de référence: Cl_{25} = 256.5 (251.3 - 261.7) mg/L(NaCl)

Limites historiques d'avertissement: Min/Max = 218.3 / 449.5

* le ' - ' indique une amplification

n.a. : non applicable

Laboratoires ECO Laboratories

121, Boul. Hymus, Pointe-Claire, Québec H9R 1E6
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Certificat d'analyse • Certificate of Analysis

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

Le 25 juillet 1996
Projet: 603884

IDENTIFICATION DE L'ECHANTILLON	NUMÉRO DE TEST	DATE DE RECEPTION	DATE D'ANALYSE	CI_{50} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)	CI_{25} - 72 HRS % v/v (Int. Conf.) *S. capricornutum* (Algues)
Eff., 22/04/96, 11:00	13690	23/04/96	25-28/04/96	9.3 (<0.0 - 19.7)	3.0 (<0.0 - 13.4)

SOMMAIRE DES RESULTATS:

Échantillon	Conclusions
Eff., 22/04/96, 11:00	Effet / Effet

Int. Conf.: intervalle de confiance à 95%

Yves Bois, M.Sc. Agr..
Directeur
Département d' Écotoxicologie

Linda Bouffard, M.Sc.
Biologiste
Département d' Écotoxicologie

Laboratoires ECO Laboratories

121, Boul. Hymus, Pointe-Claire, Québec H9R 1E6
Tél.: (514) 697-3400 Fax: (514) 697-2090

Certificat d'analyse • Certificate of Analysis

RÉSULTAT DE L'ÉVALUATION DE LA TOXICITÉ AUX ALGUES DE L'ÉCHANTILLON
Eff., 22/04/96, 11:00
(DWTP)

PARAMETRE	unit	INHIBITION de la CROISSANCE
CSEO-72hrs [1]	%(V/V)	3.1
CMEO-72hrs [2]	%(V/V)	6.3
STC-72hrs [3]	%(V/V)	4.4
CI_{50}-72hrs	%(V/V)	9.3 (<0.0 - 19.7) [4]
CI_{25}-72hrs	%(V/V)	3.0 (<0.0 - 13.4) [4]

1) Concentration maximale sans effet observé
2) Concentration minimale avec effet observé
3) Seuil de toxicité chronique, = (CSEO x CMEO)^{½}
4) Intervalle de confiance à 95%
5) non calculable pour des raisons de statistique
6) non applicable

Projet: 603884
Échantillon reçu le: 23/04/96
Échant. analysé le: 25-28/04/96
Protocole: SPE 1/RM/25, Novembre 1992
Statistiques: Regression linéaire.

Analyste: Elliott Picken, Tech.

Appendix 2

Test Reports: Growth Inhibition Test with *Lemna minor*

technology is our business

June 27, 1996 SRC Reference #:R1640-4-C-96

B.A.R. Environmental, Inc.
Nicholas Beaver Park, R.R. 3
Guelph, ON N1H 6H9
Tel: 519-763-4410
Fax: 519-763-4419

Attn: Rob Roy

Re: Toxicity Report for CANMET Study for *Lemna minor* Growth Inhibition Test Updated - June 27, 1996

The following toxicity results for the *Lemna minor* growth inhibition test are current to June 27/96 (**note:** C31 IC_{25} result has changed). Please disregard all previous data. A brief description of the *Lemna minor* growth inhibition test, Cost and Time estimates, as well as QA/QC charts, are included as Appendix 1, Appendix 2, and Appendix 3.

Note: All results and confidence limits are calculated using parametric data analysis. All tests have been carried out in receiving water, pH not adjusted.

***Lemna minor* growth inhibition test results to June 27, 1996.**

SRC #	BAR #	IC_{25}	95% Confidence Limits	IC_{50}	95% Confidence Limits
C27	960483	24.5	17.5 - 35.0	49.7	41.9 - 58.9
C28	960482	67	60.3 - 74.5	81	75.8 - 86.5
C29	960577	15.7	10.1 - 24.6	55.1	41.9 - 72.6
C30	960676	2.82	1.67 - 4.75	18.3	13.1 - 25.6
C31*	960679	0.32	0.09 - 1.15	5.55	1.76 - 17.5
C32	960768	8.8	2.2 - 34.5	52.3	19 - 100
C33**	960753	>93%		>93%	
C34	960918	55.6	41.2 - 75.1	>93%	

* effluent received too late for valid test, tested May 1/96
** receiving water received too late for valid test, tested June 5/96
C33 problems with growth of algae required further filtration of receiving water and repeat test

.../2

	Effluent sampled	Effluent received	RW sampled	RW received	*Lemna minor* test
C27	Mar 25/96	Mar 26/96	Mar 25/96	Mar 26/96	Mar 27/96
C28	Mar 25/96	Mar 26/96	Mar 25/96	Mar 26/96	Mar 27/96
C29	Apr 8/96	Apr 9/96	Mar 28/96	Apr 01/96	Apr 10/96
C30	Apr 22/96	Apr 23/96	Apr 15/96	Apr 17/96	Apr 24/96
C31*	Apr 22/96	Apr 24/96	Apr 22/96	Apr 24/96	May 1/96
C32	May 06/96	May 07/96	***	Apr 29/96	May 8/96
C33**	May 6/96	May 7/96	May 3/96	May 8/96	May 15/96
C34	June 3/96	June 4/96	May 16/96	May 23/96	June 5/96

* effluent received too late for valid test
** receiving water received too late for valid test, tested May 15/96, results from repeat test June 5 are reported
*** chain of custody document not received
C33 problems with growth of algae required further filtration of receiving water and repeat test

Note: appended QA/QC charts are created in SigmaPlot, and imported into WP 6.1 for Windows and can only be printed correctly using a WP printer driver. If you have a problem printing the file, contact Yvonne **Tel:** 306-933-5425 or **e-mail** wilkinson@SRC.sk.ca

Originals are being sent *via* courier on June 28/96 - you should receive them by July 2/96.

Approved by:

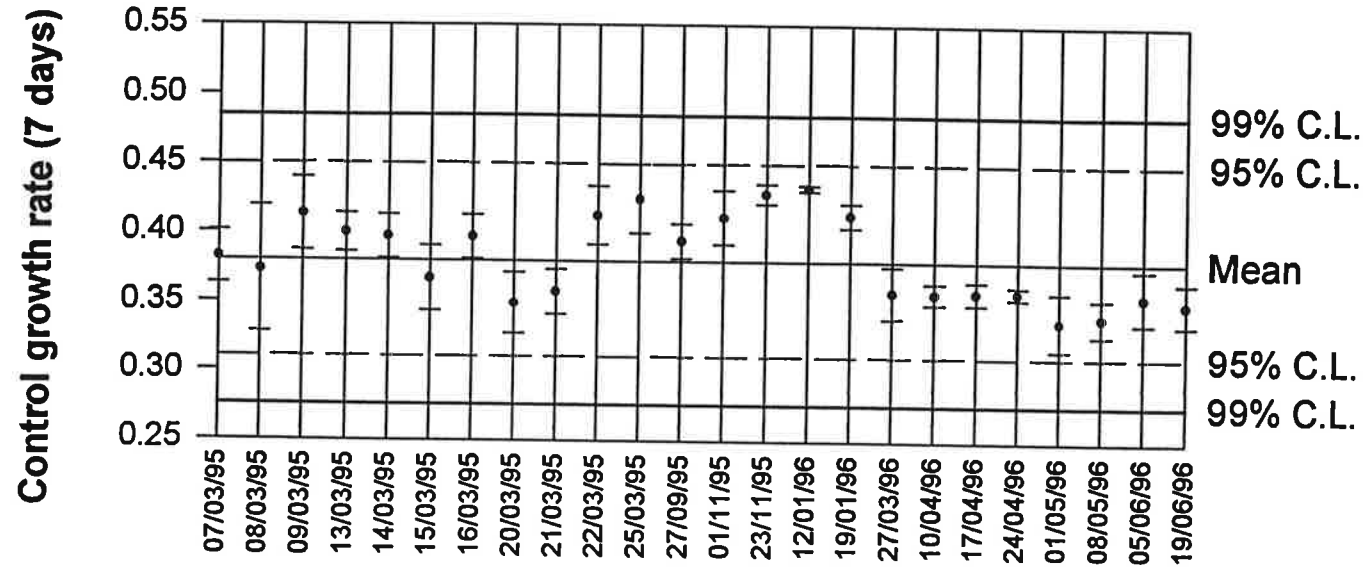
for Y. Wilkinson
H. Peterson

Hans G. Peterson, Ph.D.
Principal Research Scientist
Water Quality Section
Tel: 306-933-5445
Fax: 306-933-7446
E-mail: Hans.Peterson@sasknet.sk.ca

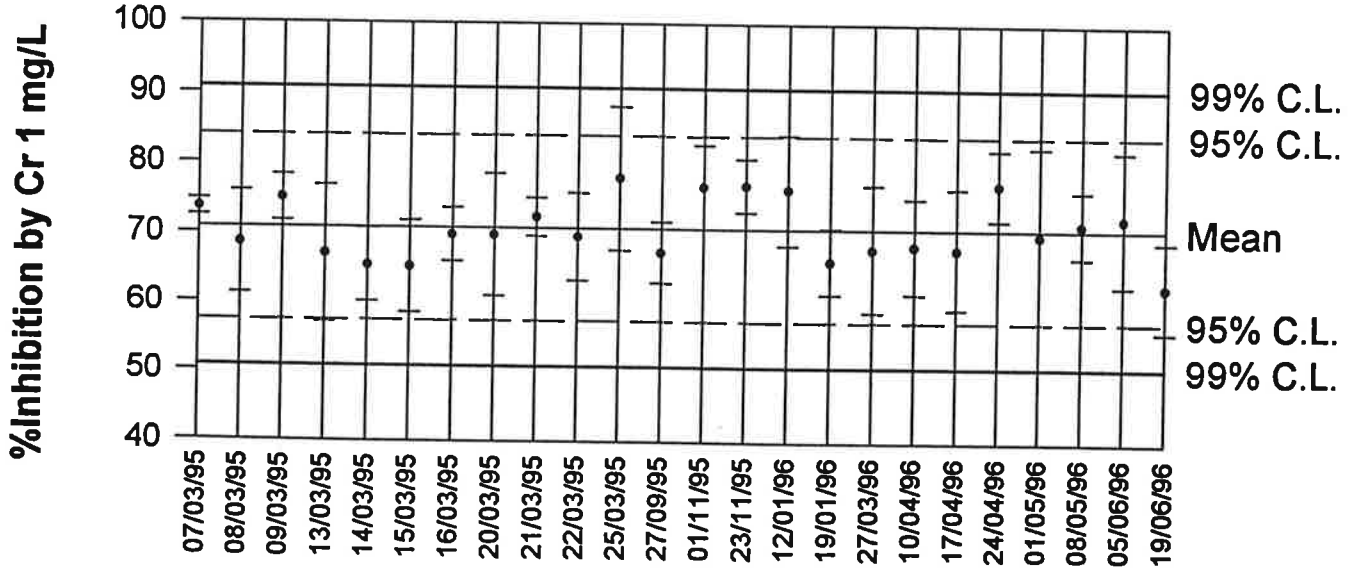
WATER QUALITY SOLUTIONS

HGP:MM:ymw

Lemna minor Control Charts 1995-1996



Mean and 95% confidence limits for each experiment



Reference Toxicant Cr 1 mg/L

Mean and 95% confidence limits for each experiment

Appendix 3

Test Reports: Phytoplankton Multi-species Growth Inhibition Test

technology is our business

June 27, 1996

SRC Reference #:R1640-5-C-96

B.A.R. Environmental, Inc.
Nicholas Beaver Park, R.R. 3
Guelph, ON N1H 6H9
Tel: 519-763-4410
Fax: 519-763-4419

Attn: Rob Roy

Re: Toxicity Report for CANMET Study for Phytoplankton Microplate Growth Inhibition Test Updated - June 27, 1996

The following toxicity results for the Phytoplankton microplate growth inhibition test are current to June 27/96. Please disregard all previous data. A brief description of the Phytoplankton Microplate Growth Inhibition Test, Cost and Time estimates, as well as QA/QC charts, are included as Appendix 1, Appendix 2, and Appendix 3.

Note: All results and confidence limits are calculated using parametric data analysis.
All testing carried out without pH adjustment of the receiving water.

Phytoplankton microplate growth inhibition test results to June 27, 1996.

SRC #	Bar #	Effluent Collected	Tested	Effluent Treatment	Receiving Water Treatment	Most Sensitive Organisms	IC_{25}	95% Confidence Limits	IC_{50}	95% Confidence Limits
C27	960483	Mar 25/96	Mar 27/96	none	none	*Microcystis aeruginosa*	0.28	0.15-0.5	0.88	0.42-1.83
C28	960482	Mar 25/96	Mar 27/96	none	none	*Microcystis aeruginosa*	2.1	1.4 - 3.3	9.3	7.1-12.2
C29	960577	Apr 8/96	Apr 10/96	settled	none	*Nitzschia*, sp.***	5.3	5.0 - 5.7	8.3	8.0-8.7
C30	960676	Apr 22/96	Apr 24/96	settled	none	*Microcystis aeruginosa*	3.62	2.48-5.27	56	n/a
C31*	960679	Apr 22/96	May 1/96	settled	none	*Microcystis aeruginosa*	0.51	0.50 - 0.53	0.62	0.53-0.73
C32	96068	May 6/96	May 8/96	none	none		>90.2		>90.2	
C33**	960753	May 6/96	May 15/96	none	GF/C filtered	*Selenastrum capricornutum*	64.5	61.9 - 67.3	75	73.1-76.6
C34	960918	Jun 3/96	June 5/96	none	none		>90.2		>90.2	

* effluent received too late to be tested within 72 hours of collection
** receiving water received too late for effluent to be tested within 72 hours of collection
*** may not be most sensitive organism, others did not meet validity criteria
n/a not available

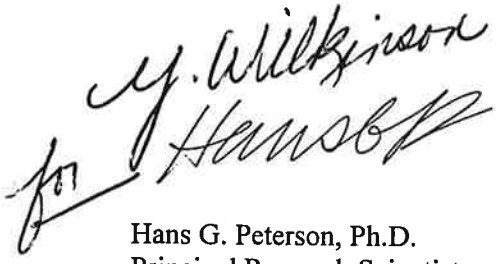
.../2

➢ Effluents were shaken for 30 seconds and aliquot drawn off to set up test. If large particles, the effluent was allowed to settle for 10 minutes before sample was removed to allow large particulate matter to settle to bottom and not cause interference in the test.
➢ Receiving waters were used without any pre-treatment, except for C33 which was highly turbid and had *Lemna* floating on top, this sample was filtered prior to experimentation.

Note: appended QA/QC charts are created in SigmaPlot, and imported into WP 6.1 for Windows and can only be printed correctly using a WP printer driver. If you have a problem printing the file, contact Yvonne **Tel:** 306-933-5425 or **e-mail** wilkinson@SRC.sk.ca

Originals are being sent *via* courier on June 28/96 - you should receive them by July 2/96.

Approved by:



Hans G. Peterson, Ph.D.
Principal Research Scientist
Water Quality Section
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E-mail: Hans.Peterson@sasknet.sk.ca

WATER QUALITY SOLUTIONS

HGP:NR:ymw

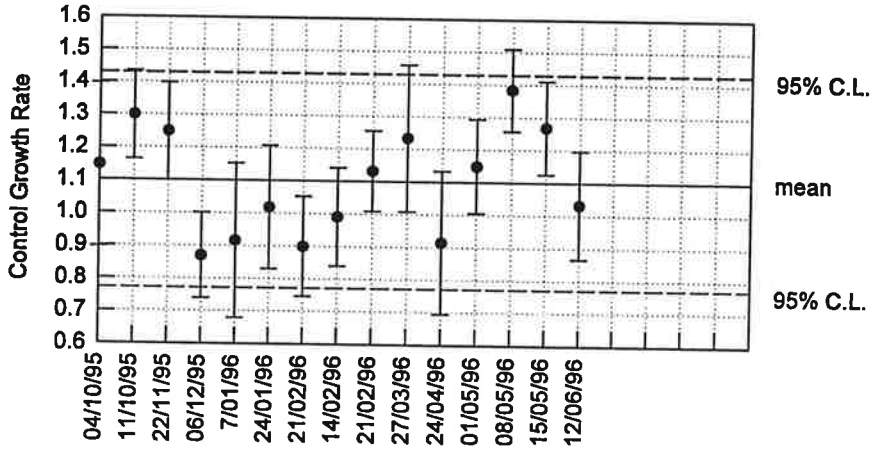
Appendix 1
Brief Description of the Phytoplankton Microplate Growth Inhibition Test

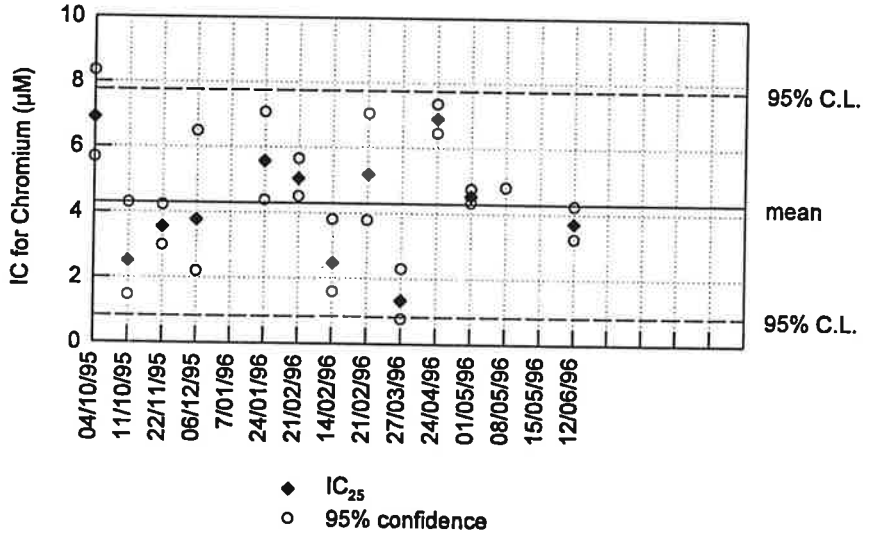
The phytoplankton microplate growth inhibition test developed by the Saskatchewan Research Council [SRC] Water Quality Laboratory in collaboration with the Technical University of Denmark is a modification of the International Standards Organization [ISO] test, "*Fresh water algal growth inhibition test with Scenedesmus subspicatus and Scenedesmus capricornutum*" (ISO 1989) and the Swedish National Chemicals Inspectorate "*Algal microtest battery*" developed by Blanck and Björnsäter (1989). SRC and TUD have set more stringent criteria for most aspects of these tests in order to reduce variability and increase sensitivity, as well as decreasing the impact phytoplankton growth has on test parameters. The development of highly sensitive microplate fluorometers has made this development possible.

The test developed by the Saskatchewan Research Council [SRC] Water Quality Laboratory in collaboration with the Technical University of Denmark [TUD] has been designed to work at sufficiently low cell densities to not affect pH, one of the primary components algal growth will modify. The endpoint is fluorescence, which can be measured irrespective of phytoplankton growth habits (*i.e.*, it is possible to measure filamentous, colonial, or unicellular organisms). It is also possible to carry out determinations cost-effectively, as reading the microplates is fully automated.

The phytoplankton microplate growth inhibition test can be used for assessing the toxicity of most water soluble compounds diluted in any aqueous environment. Phytoplankton species from three taxinomic classifications are included in the test battery. Sensitivities of phytoplankton classes may vary among different types of toxic compounds.

Appendix 3
QA/QC Charts





Quality control chart for control growth rate and IC_{25} values for *Selenastrum capricornutum*, documented at the Saskatchewan Research Council [SRC] Water Quality Laboratory in collaboration with the Technical University of Denmark [TUD].

Appendix 2
Cost and Time Estimates

Table 1 **Estimated costing of disposables used in the phytoplankton microplate growth inhibition test as developed by the Saskatchewan Research Council [SRC] Water Quality Laboratory in collaboration with the Technical University of Denmark [TUD].**

• microplates: disposable, rigid polystyrene, 96-well round bottom microplates (must be non-tissue culture treated), 5 @ $2.00	10.00
• disposable 2 to 5 mL pipette tips, 10 @ $0.08	0.80
• disposable 200 to 1000 μL pipette tips, 10 @ $0.065	0.65
• disposable 2 to 200 μL pipette tips, 75 @ $0.08	6.00
• disposable plastic reservoirs for dispensing effluent and reference toxicant dilutions, 2 @ $0.065	0.13
• disposable test tubes (16x125 mm), 20 @ $0.057	1.14
• disposable test tubes (25x150 mm), 4 @ $0.24	0.96
• nylon filtration membranes (0.2 μm pore size), 1 @ $2.10	2.10
• weighing dishes, 16 @ $0.065	1.04
• hemocytometer cover slips, 12 @ $0.16	1.92
• parafilm, 1 ft @ $0.19	0.19
• glass disposable Pasteur pipettes for aeration, 3 @ $ 0.04	0.12
• 500 mL plastic bottles (made out of highly inert plastic and used for bottling soft drinks), 2 @ 0.32	0.64
• cubitainers®: 1 qt, 2 @ $2.34	4.68
• foam plugs to plug bottles during aeration, 3 @ $0.075	0.23
Total cost of disposables	$ 30.60

Table 2 **Estimated time to conduct method used in the phytoplankton microplate growth inhibition test as developed by the Saskatchewan Research Council [SRC] Water Quality Laboratory in collaboration with the Technical University of Denmark [TUD].**

• culturing	0.5
• test preparation	0.5
• test set-up	3.0
• test completion	1.0
• data analysis and reporting	2.0
• QA/QC	0.5
Total time in hours	7.5

Appendix 4

Instructions For Collecting and Shipping

Receiving Water and Effluent Samples

1.0 PROCEDURES FOR COLLECTING AND SHIPPING SAMPLES OF DILUTION WATER AND EFFLUENT FOR TOXICITY TESTING AND CHEMICAL ANALYSES

Toxicity testing and chemical analyses will be performed on samples of mine effluents using the local receiving water as a dilution and control water.

A receiving water sample should be "collected upstream from the source of contamination, or adjacent to the source but removed from it" (Environment Canada, 1992).

You will be provided with equipment (containers, coolers, ice packs, preservatives, address labels, etc.) for shipping the dilution water and the effluent. **DILUTION WATER** will be collected in the shipping containers provided. **EFFLUENT** samples will be collected in 45 gallon drums and then shipped in the containers. All materials that come into contact with the dilution water and the effluent must be clean, non-toxic and inert. Sample transfer must be accompanied by continuous mixing using manual stirring or other appropriate means.

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

The **DILUTION WATER** should be sampled 7 days before the scheduled sampling date for the effluent. It may be shipped by ground or air, but it must arrive at the laboratories **before** the effluent samples. **EFFLUENT** must be shipped by courier (air or ground express) in order to arrive at the laboratories within **48 hours** after sampling.

The samples must not freeze during transport and should be clearly labeled. Unlabeled samples will not be tested.

Due to the availability of rainbow trout eggs, **the EFFLUENT must be sampled and shipped on a MONDAY** so it arrives at B.A.R. Environmental on or **before the Wednesday** of that sampling week.

NOTE: EFFLUENT SAMPLES THAT ARRIVE AFTER WEDNESDAY WILL NOT BE TESTED!

2.0 LIST OF SAMPLING MATERIALS PROVIDED

1. Two (**2**) formfit **drum liners** (to fit 45 gal. drum).

2. Twenty-eight (**28**) **white plastic pails** (20 L capacity, with plastic liners).

3. Five (**5**) **polyethylene bottles**, 200 mL capacity.

4. A blue or green **box** containing three (**3**) 1 gallon cubitainers.

5. One (**1**) **carboy**, 20 L capacity.

6. **Sampling directions**, icepacks, coolers, address labels, Chain of Custody Forms.

7. **Chemical analysis material**: 2 coolers with 5 bottles each, preservatives, etc.

3.0 PROCEDURE FOR COLLECTING AND SHIPPING DILUTION WATERS

PREPARATION: Ice packs should be frozen prior to sampling. A clean 1 gallon plastic container will be needed to bring a sub-sample of the dilution water back to your own laboratory for filtering. All materials that come into contact with the dilution water must be clean, non-toxic and inert.

DILUTION WATER should be sampled **7 days before** the scheduled sampling date for the effluent. Dilution water may be shipped by air or ground transport but it must arrive at the laboratories **before** the effluent samples.

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

The samples must not freeze during transport and should be clearly labeled.

1. Fill out a **CHAIN OF CUSTODY SHEET** and include with each shipment. Identify the sample, the company name and location, the type of sample (grab, composite), the date and time of sampling and the name of the person who collected the sample.

2. Insert plastic liners inside **twenty-one** (21) **20 L white plastic pails**. Rinse the pails three (3) times, fill entirely (no acid, no airspace), affix labels, and send to

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

These samples are for the rainbow trout embryo, Ceriodaphnia and fathead minnow tests.

3. Rinse **one** (1) **200 mL plastic bottle** three (3) times, fill completely (no airspace, no acid), and place into shipping box with frozen ice pack. Tape shut, affix labels, and send to:

Les Laboratoires Eco-CNFS Inc.
121 Boul. Hymus
Pointe Claire, Quebec
H9R 1E6

This sample is for the algal microplate test with *Selenastrum capricornutum*.

4. Rinse **one** (1) **20 L carboy** (3) times, fill completely (no airspace, no acid), affix labels and send to:

Saskatchewan Research Council
15 Innovation Boulevard
Saskatoon, Saskatchewan
S7N 2X8

This sample is for the multispecies algal test and the growth inhibition test with *Lemna minor*.

5. Rinse **one** (1) **200 mL plastic bottle** three (3) times, fill entirely (no acid, no airspace), and place into shipping box with frozen ice pack. Tape shut, affix labels, and send to:

BC Environment
Environmental Protection Division
Pacific Environmental Science Centre Toxicology Lab
2645 Dollarton Highway
North Vancouver, British Columbia
V7H 1V2

This sample is for the Mutatox test.

6. Use a clean **1 gallon container** to bring dilution water sample to your laboratory for filtering (chemical analysis, Section 5.0, Table p. 10).

7. When sampling is completed, fax the transporter's name and the waybill number to:

Robert Roy
B.A.R. Environmental Inc.
(519) 763-4419.

4.0 COLLECTING AND SHIPPING EFFLUENTS FOR TOXICITY TESTING

4.1 PROCEDURE FOR COLLECTING EFFLUENTS

PREPARATION: Ice packs should be frozen prior to sampling. A clean 1 gallon plastic container will be needed to bring a sub-sample of the effluent back to your own laboratory for filtering. All materials that come into contact with the effluent must be clean, non-toxic and inert. Sample transfer must be accompanied by continuous mixing of the effluent using manual stirring or other appropriate means.

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

The samples must not freeze during transport and should be clearly labeled.

Due to the availability of rainbow trout eggs, the EFFLUENT must be sampled and shipped on a MONDAY so it arrives at B.A.R. Environmental on or before the Wednesday of that sampling week.

EFFFLUENT SAMPLES ARRIVING AFTER WEDNESDAY WILL NOT BE TESTED!

4.1 PROCEDURE FOR COLLECTING EFFLUENTS (cont'd)

1. Place the plastic drumliners in two (2) **45 gal. drums** (**DRUM A** and **DRUM B**).

2. Rinse each drum twice with effluent. The effluent should not come into contact with the walls of the drum.

3. Fill the sample barrels until they are **two-thirds** full. When sampling, alternate between drums A and B. (Fill one-third of **A**, then one-third of **B**, then return to **A**, etc.).

4. Mix **A** and **B**: fill **A** from **B**, stir, then fill **B** from **A**. Repeat this transfer **6 times** until **A** and **B** are well mixed. Keep stirring the effluent while sub-samples are taken for toxicology and chemical analyses.

5. Use a clean **1 gallon container** to bring an effluent sample to your laboratory for filtering (chemical analysis, Section 5.0, Table p. 10).

4.2 SUB-SAMPLING AND SHIPPING EFFLUENTS FOR TOXICITY TESTING

1. Fill out the **CHAIN OF CUSTODY SHEETS** and include with each shipment. Identify the sample, the company name and location, the type of sample (grab, composite), the date and time of sampling and the name of the person who collected the sample.

2. Insert plastic liners inside **seven** (7) **20 L white plastic pails**. Rinse three (3) times, fill entirely (no acid, no airspace), affix labels, and send by courier (air or land express) to:

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

These samples are for the rainbow trout embryo, Ceriodaphnia and fathead minnow tests.

3. Rinse **one** (1) **200 mL plastic bottle** three (3) times, fill entirely (no acid, no airspace), and place into shipping box with frozen ice pack. Tape shut, affix labels, and send by courier (air or land express) to:

B.A.R. Environmental Inc. (address above)

This sample is for the Microtox chronic and nematode tests.

4. Rinse **one** (1) **200 mL plastic bottle** three (3) times, fill completely (no airspace, no acid), and place into shipping box with frozen ice pack. Tape shut, affix labels, and send by courier (air or land express) to:

Les Laboratoires Eco-CNFS Inc.
121 Boul. Hymus
Pointe Claire, Quebec
H9R 1E6

This sample is for the algal microplate test with *Selenastrum capricornutum*.

5. Rinse **three** (3) **4 L cubitainers** (3) times, fill completely (no airspace, no acid) and place into shipping box with frozen ice packs. Tape shut, affix labels and send by courier (air or land express) to:

Saskatchewan Research Council
15 Innovation Boulevard
Saskatoon, Saskatchewan
S7N 2X8

These samples are for the multispecies algal test and the growth inhibition test with *Lemna minor*.

6. Rinse **one** (1) **200 mL plastic bottle** three (3) times, fill entirely (no acid, no airspace), and place into shipping box with frozen ice pack. Tape shut, affix labels, and send by courier (air or land express) to:

BC Environment
Environmental Protection Division
Pacific Environmental Science Centre Toxicology Lab
2645 Dollarton Highway
North Vancouver, British Columbia
V7H 1V2

This sample is for the Mutatox test.

7. When sampling is completed, fax the transporter's name and waybill number to:

Robert Roy
B.A.R. Environmental Inc.
(519) 743-4419.

5.0 SUB-SAMPLING, PRESERVATION AND SHIPPING FOR CHEMICAL ANALYSES

1. Rinse the 1-gallon sample container 3 times with the sample (DILUTION WATER or EFFLUENT) before filling. Transport to an on-site facility for filtration (bottle type M (D), see table below.

2. Fill the sample bottles to the base of the bottle neck with DILUTION WATER or EFFLUENT. Add preservative if necessary, according to the table below:

BOTTLE TYPE		PRESERVATIVE	CODE DOT	SPECIAL INSTRUCTIONS
M (T)	250 mL	5 mL 50% HNO_3	Blue	NIL(plastic bottle)
M (D)	250 mL	5 mL 50% HNO_3	Blue	Filter with 0.45 µm filter before adding acid (plastic bottle)
R	1 L	4 ° C	NIL	NIL(plastic bottle)
G2	500 mL	5 mL 50% H_2SO_4	Black	NIL(plastic bottle)
CN	500 mL	2 mL 6N NaOH	Red	NIL (plastic bottle)

3. Seal and label the bottles, place in cooler with frozen ice-packs and send by courier (air or land express) to:

Seprotech Laboratories
2378 Holly Lane
Ottawa, Ontario
K1V 7P1

Please ensure that samples do not freeze prior to shipment, and are kept cool (between 1 and 8°C, preferably between 2 and 6 °C).