AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM

Toxicity Assessment of Mining Effluents Using Up-Stream or Reference Site Waters and Test Organism Acclimation Techniques

AETE Project 4.1.2a

TOXICITY ASSESSMENT OF MINING EFFLUENTS USING UP-STREAM OR REFERENCE SITE WATERS AND TEST ORGANISM ACCLIMATION TECHNIQUES

Sponsored by:

Canada Centre 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

Toxicity Assessment of Mining Effluents Using Up-Stream or Reference Site Waters and Test Organism Acclimation Techniques

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 Canada Centre for Mineral and Energy Technology (CANMET). The program was designed to be of direct benefit to the industry, and to government. Through technical and field evaluations, it identified cost-effective technologies to meet environmental monitoring requirements. The program included three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring.

The technical evaluations were 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 included a go/no go recommendation that AETE takes into consideration before a field evaluation of a given method is conducted.

The technical evaluations were published although they do not necessarily reflect the views of the participants in the AETE Program. The technical evaluations should be considered as working documents rather than comprehensive literature reviews. The purpose of the technical evaluations was to focus on 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.

This report collates and presents the results of toxicity tests conducted as part of the AETE 1996 Preliminary Field Studies at seven mine sites. Information regarding the selection of the sublethal toxicity tests and the results of the screening study completed previously are available in other AETE reports (AETE reports #1.2.1* and #1.2.2**). To get a broader perspective of sublethal toxicity program and the cost-effectiveness of the tests, the AETE Synthesis report should be consulted.

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 February 1999.

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

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* AETE Report #1.2.1. Review of Methods for Sublethal Aquatic Toxicity Tests Relevant to the Canadian Metal Mining Industry. April 1997.

** AETE Report #1.2.2. Laboratory Screening of Sublethal Toxicity Tests for Selected Mine Effluents. January 1997.

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

Avis aux lecteurs

Détermination de la toxicité des effluents de mines avec l'utilisation de l'eau des zones réceptrices et de la technique d'acclimatation des organismes expérimentaux

Le Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ÉTIMA) visait 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 était conçu pour bénéficier directement aux entreprises minières ainsi qu'aux gouvernements. Par des évaluations techniques et des études de terrain, il a permis 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 comportait 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 ont été menées dans le but de documenter certains outils de surveillance sélectionnés par les membres d'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 d'ÉTIMA prennent en considération.

Les évaluations techniques sont publiées bien qu'elles ne reflètent pas nécessairement toujours l'opinion des membres d'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 d'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 environnementaux en milieu aquatique.

Le présent rapport rassemble et présente les résultats des tests de toxicité menés dans le cadre des Études préliminaires sur le terrain effectuées par ÉTIMA en 1996, à sept emplacements miniers. Les renseignements concernant l'examen des essais de toxicité sublétale et les résultats des tests de préselection qui ont été menées antérieurement sont disponibles dans d'autres rapports d'ÉTIMA (Rapports numéros 1.2.1* et 1.2.2**). Pour obtenir une perspective élargie du programme de recherche sur la toxicité sublétale et sur le rapport coût-efficacité des tests, le Rapport de synthèse d'ÉTIMA devrait être consulté.

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 février 1999.

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

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* Rapport ÉTIMA #1.2.1. Examen des méthodes d'évaluation de la toxicité sublétale des effluents miniers présentant un intérêt particulier pour l'industrie canadienne des mines de métaux. Avril 1997. (disponible en anglais)

** Rapport ÉTIMA #1.2.2. Présélection en laboratoire des tests de détermination de la toxicité sublétale de certains effluents miniers. Janvier 1997. (disponible en anglais).

EXECUTIVE SUMMARY

B.A.R. Environmental Inc. conducted toxicity tests involving effluents and receiving waters from Myra Falls, (Westmin Resources, B.C.), Sullivan Mine (Cominco Ltd., B.C.), Levack Mine, (Inco Ltd., ON), Onaping site (Falconbridge Ltd., ON), Dome Mine, (Placer Dome Canada, ON), Gaspé Division, (Noranda Mining and Exploration Inc., QC) and Heath Steele Division, (Noranda Mining and Exploration Inc., N.B.). The objective is to characterize the toxicity of the seven mine effluents, using the local receiving waters as dilution and control waters. The other objectives include determining if receiving waters cause toxicity to fathead minnow and *Ceriodaphnia dubia*, and evaluating the use of acclimation procedures for receiving waters which are found to be toxic. Toxicity studies were conducted in conjunction with field studies of the receiving environments at the seven mine sites.

Toxicity testing involved growth inhibition with *Selenastrum capricornutum* and *Lemna minor*, reproduction and survival of *Ceriodaphnia dubia*, growth and survival of the fathead minnow, and viability of the rainbow trout embryo. To determine if acclimation was necessary, samples of receiving waters were collected for preliminary tests with fathead minnow and *Ceriodaphnia dubia*. Receiving water samples for effluent tests were collected at a later time.

The acclimation procedure involves gradual introduction of the organisms to the 100% v/v receiving water. Neonate ceriodaphnids and adult fathead minnows are held for 7 days in a laboratory dilution water with pH and hardness adjusted to that of the receiving water. Newly fertilized fish eggs and with third brood ceriodaphnid neonates are then introduced to a 10% concentration of receiving water. The proportion increases each day until the organisms are in 100% receiving water, when the newly hatched fish larvae and the third brood ceriodaphnid neonates are used in toxicity tests.

Toxicity tests with effluents involved several effluent exposure concentrations, using the mine's receiving waters as control and dilution water. The toxicity of the effluents was compared by averaging the results of the four successful toxicity tests in this study. The sensitivity of the toxicity tests was compared by ranking the IC25s.

In preliminary tests, the Sullivan receiving water was toxic to both *Ceriodaphnia* (30% mortality) and fathead minnows (60% mortality). The Gaspé receiving water was toxic to *Ceriodaphnia* only (30% mortality). The remaining receiving waters were not toxic in preliminary tests.

Ceriodaphnia were acclimated to Sullivan and Gaspé receiving water while fathead minnows were acclimated to Sullivan water only. Survival of both organisms improved during the gradual

acclimation. During the acclimation procedure, all ceriodaphnids survived in the Gaspé series and 80% survived in the Sullivan series. During acclimation to the Sullivan receiving water, 87.5% of fathead minnow eggs hatched into larvae.

During effluent tests, the acclimated organisms also survived in the Sullivan and Gaspé receiving water controls, leading to valid tests. The Myra Falls receiving water was toxic to *Ceriodaphnia* during the effluent test, yet did not cause toxicity during the preliminary test. During the Heath Steele assay, reproduction of *Ceriodaphnia* in the receiving water control was significantly greater than during the preliminary test. The responses of *Ceriodaphnia* in Heath Steele and Myra Falls receiving waters suggest that different samples of the same receiving waters can vary in a manner that affects reproduction and/or survival of the invertebrate.

Four trout embryo tests failed. The Gaspé receiving water was slightly toxic and tests with the Dome, Sullivan and Myra Falls effluents were invalid due to poor quality eggs and/or milt, which caused excessive control mortalities.

Most effluents exhibited little toxicity. The *Selenastrum, Lemna* and *Ceriodaphnia* tests showed a similar sensitivity to the effluents while the fathead minnow assay was less sensitive. The sensitivity of the fathead minnow and trout embryo assays is similar, when data from this study and the previous Sublethal Toxicity Screening Project are combined.

RÉSUMÉ

B.A.R. Environmental Inc. a mené des tests de toxicité sur des effluents miniers et des eaux réceptrices provenant de Myra Falls (Westmin Resources, C.-B.), Sullivan Mine (Cominco Mine Ltd., C.-B.), Levack Mine (Inco Limitée Ont.), Onaping Site (Falconbridge Limitée, Ont), Dome Mine (Placer Dome Canada, Ont.), Division Gaspé (Mines et Exploration Noranda Inc., QC) et Heath Steele Division (Mines et Exploration Noranda Inc., N.-B.). L'objectif principal des tests était de caractériser la toxicité de sept effluents miniers en utilisant les eaux réceptrices locales comme témoins et milieux de dilution. Les autres objectifs consistaient à déterminer si les eaux réceptrices étaient toxiques dans le cas du tête-de-boule et de *Ceriodaphnia dubia* ainsi qu'à évaluer l'utilisation de procédés d'acclimatation aux eaux réceptrices toxiques. Les études de toxicité ont été menées conjointement avec les évaluations sur le terrain aux sept emplacements miniers.

Les tests de toxicité portaient sur la mesure de l'inhibition de la croissance de l'algue *Selenastrum capricornutum* et de la lentille d'eau *Lemna minor*, sur la mesure de la reproduction et de la survie de *Ceriodaphnia*, sur la mesure de la survie et de la croissance du tête-de-boule et sur la viabilité de l'embryon de la truite arc-en-ciel. Afin de déterminer l'utilité des procédés d'acclimatation, des échantillons d'eaux réceptrices ont été recueillis et utilisés pour les tests de toxicité préliminaires avec le tête-de-boule et *Ceriodaphnia dubia*. Les échantillons d'eaux réceptrices utilisés comme milieux de dilution et témoins pour les tests avec les effluents ont été recueillis à une date ultérieure.

Le procédé d'acclimatation comprend l'introduction graduelle d'organismes dans de l'eau réceptrice concentrée à 100 % vol/vol. Les néonates Cériodaphnies et les têtes-de-boule adultes sont conservés pendant 7 jours dans l'eau du laboratoire dont le pH et la dureté sont ajustés à ceux de l'eau réceptrice. Les oeufs de poisson nouvellement fertilisés et les néonates Cériodaphnies de la troisième couvées sont ensuite introduits dans des eaux réceptrices concentrées à 10 %. La proportion de la concentration augmente chaque jour jusqu'à ce que les organismes se retrouvent dans de l'eau réceptrice concentrée à 100 %. Les larves de poisson nouvellement écloses et les néonates Cériodaphnies de la troisième couvées sont alors utilisés dans des tests de toxicité.

Les tests de toxicité réalisés avec des effluents comprenaient plusieurs concentrations d'effluents, les eaux réceptrices de la mine étant utilisées comme témoins et milieux de dilution. On a comparé la toxicité des effluents en établissant la moyenne des résultats des quatre tests de toxicité qui ont réussi au cours de cette étude. On a comparé la sensibilité des tests de toxicité en classant les concentrations inhibitrices à 25% (CI25).

Lors des tests préliminaires, l'eau réceptrice de la mine Sullivan s'est avérée toxique tant pour *Ceriodaphnia* (taux de mortalité de 30 %) que pour le tête-de-boule (taux de mortalité de 60 %). L'eau réceptrice de Gaspé n'a été toxique que dans le cas de *Ceriodaphnia* dont le taux de mortalité a été de 30 %. Les eaux réceptrices des autres remplacements ne se sont pas révélées toxiques au cours des tests préliminaires.

Les Cériodaphnies ont été soumis au procédé d'acclimatation avec les eaux réceptrices de Sullivan et de Gaspé et les têtes-de-boule à l'eau réceptrice de Sullivan seulement. Le taux de survie des deux organismes s'est amélioré au cours de l'acclimatation graduelle. Pendant le procédé d'acclimatation, tous les Cériodaphnies ont survécu à la série d'essais avec les eaux réceptrices de Gaspé tandis que 80 % ont survécu à la série d'essais avec les eaux réceptrices de Sullivan. Pendant la période d'acclimatation aux eaux réceptrices de Sullivan, 87,5 % des oeufs des têtes-de-boule ont éclos sous forme de larves.

Lors des tests menés sur les effluents, les organismes qui s'étaient adaptés ont également survécu dans les eaux réceptrices témoins de Sullivan et de Gaspé, ce qui a validé les tests. Les eaux réceptrices de Myra Falls se sont avérées toxiques pour *Ceriodaphnia* bien qu'elles ne se soient pas avérées toxiques au cours du test préliminaire. Lors du test effectué avec l'effluent provenant de Heath Steele, le taux de reproduction de *Ceriodaphnia* dans les eaux réceptrices témoins a été beaucoup plus élevé qu'à l'occasion du test préliminaire. Les réponses de *Ceriodaphnia* aux eaux réceptrices de Heath Steele et de Myra Falls suggèrent que divers échantillons provenant des mêmes eaux réceptrices peuvent varier de façon à affecter la reproduction et la survie de l'invertébré.

Quatre tests menés avec des embryons de truite échoués. Lors des tests, l'eau réceptrice de Gaspé était légèrement toxique et les tests menés avec des effluents provenant de Dome, de Sullivan et de Myra Falls n'ont pas été valides en raison du manque de qualité des oeufs et/ou de la laitance, ce qui a occasionné un taux de mortalité excessif dans les eaux réceptrices témoins.

La plupart des effluents se sont avérés peu toxiques. Les tests menés au moyen de *Selenastrum*, de *Lemna* et de *Ceriodaphnia* ont démontré une sensibilité vis-à-vis des effluents tandis que la sensibilité des tests menés avec le tête-de-boule était moindre. Les données compilées au cours de cette étude combinées aux données recueillies antérieurement dans le cadre du projet de préselection des tests de toxicité sublétale démontrent que la sensibilité des tests menés avec le tête-de-boule et l'embryon de truite est semblable.

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1 INTRODUCTION

1.1 BACKGROUND

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 Canada Centre for Mineral and Energy Technology (CANMET). The program is designed to be of direct benefit to industry and government. An important focus of this program is to evaluate and 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 testing.

Under the 1996 AETE Extrapolation Study, B.A.R. Environmental Inc. conducted sublethal toxicity tests of mine effluents. These tests were performed in conjunction with field studies of the receiving environments, which were carried out in the months of September, October and November 1996. Eight mine sites across Canada were involved. However, since one mine site did not have a discharge, toxicity testing was performed on seven mine effluents.

1.2 OBJECTIVE

The overall objective of the 1996 field study is to recommend five mine sites which are to be studied in the 1997 field program. The 1996 study involves characterizing the seven 1996 mine sites, including their discharges. Thus the main objective of the sublethal toxicity testing program is to characterize the seven mine effluents, using the local receiving waters as dilution and control waters. The other objectives of the study include determining if the receiving waters cause toxicity to fathead minnow and *Ceriodaphnia dubia*, and evaluating the use of acclimation procedures for receiving waters which are found to be toxic.

1.3 PROJECT DESCRIPTION

1.3.1 Toxicity Tests

The seven mine effluents were characterized with the following assays: growth inhibition with *Selenastrum capricornutum* and *Lemna minor*, reproduction and survival of *Ceriodaphnia dubia*, growth and survival of the fathead minnow, and viability of the rainbow trout embryo. The assays were chosen based on recommendations of the sublethal toxicity preliminary study and CANMET's Aquatic Toxicity subgroup. The test with *Selenastrum* was performed by Les Laboratoires Eco-CNFS in Pointe Claire (Québec). Assays involving *L. minor*, *Ceriodaphnia*, fathead minnows, and rainbow trout embryos were performed in B.A.R. Environmental's laboratory in Guelph, Ontario. Test reports for all assays, including raw data, are found in the Appendices (Mine Gaspé: Appendix 2, Heath Steele Mine: Appendix 3, Dome Mine, Appendix 4, Sullivan Mine, Appendix 5, Onaping Mine: Appendix 6, Myra Falls Mine, Appendix 7, Levack Mine, Appendix 8).

1.3.2 Preliminary Tests of Receiving Waters with *Ceriodaphnia dubia* and Fathead Minnow

Some receiving waters are toxic to laboratory test animals, reducing the organism's survival, reproduction, or growth. A toxicity test using such a receiving water may be invalid if the control animals fail the test criteria, due to excessive mortality or insufficient production of young. However, if given a period of time to adapt or acclimate, the organisms in receiving waters can often perform as well as they do in their usual laboratory culture water. To determine the necessity of acclimation, the receiving waters were screened for toxicity before the effluents were tested. The preliminary tests only involved fathead minnow and *Ceriodaphnia dubia,* since the test methods for these organisms were the only ones which described procedures for acclimation.

If a receiving water caused toxicity to either *Ceriodaphnia dubia* or fathead minnows, the organisms were gradually introduced to the receiving water. Survival (*Ceriodaphnia*, fathead

minnow) and reproduction (*Ceriodaphnia*) were monitored during this acclimation procedure.

1.3.3 Effluent Testing

Toxicity tests with effluents were conducted according to recognized test methods or according to draft protocols under evaluation by Environment Canada. Each assay consisted of several effluent exposure concentrations involving several replicates, and using the mine's receiving waters as control and dilution water. A second control exposure using the laboratory dilution water was conducted simultaneously.

1.3.4 Study Sites

The following mine sites participated in the AETE Field Study: Myra Falls, (Westmin Resources, B.C.), Sullivan Mine (Cominco Ltd., B.C.), Levack Mine, (Inco Ltd., ON), Onaping site (Falconbridge Ltd, ON), Dome Mine, (Placer Dome Canada, ON), Gaspé Division, (Noranda Mining and Exploration Inc., QC) and Heath Steele Division, (Noranda Mining and Exploration Inc., N.B.).

The collection and shipment of receiving water and effluent samples to the participating laboratories were the responsibility of the consulting firms Environmental Services For Planning (Guelph, ON), EVS Consultants (Vancouver, B.C.) and Jacques Whitford Environment Limited (Fredericton, N.B.).

2 METHODS

2.1 SAMPLE COLLECTION AND HANDLING

2.1.1 Samples for Preliminary Tests of Receiving Waters

The receiving waters in this study were sampled from the same location but on two separate occasions. The initial samples for preliminary tests were collected by mine personnel. The control/dilution waters for effluent tests were sampled by the field consultants at a later time.

Due to time constraints, it was not possible to send B.A.R. Environmental containers for these preliminary samples to every mine. Generally the samples were collected in 20 L containers, but the types of containers varied. They were either B.A.R. Environmental pails (Dome, Levack/Onaping), clean containers used for environmental sampling by the mines (Heath Steele, Sullivan, Myra Falls), or new containers purchased especially for this project (Gaspé).

Upon arrival at B.A.R. Environmental, the samples were composited and returned to the original containers for storage at 4°C. The pH, dissolved oxygen and conductivity measured in receiving water samples prior to preliminary testing are shown in Table 2-1.

The Dome mine personnel provided an initial sample of their receiving water, which was tested with both fathead minnow and *Ceriodaphnia*. However, the field consultant considered that this sample of receiving water was susceptible to contamination, since it was taken downstream of the mine effluent. A new sample of receiving water was collected and re-tested with *Ceriodaphnia* only. The preliminary test with fathead minnows was not repeated since no deleterious effects were observed after exposure to the initial sample.

If possible, tests with *Ceriodaphnia* and fathead minnow were conducted on the same date. However the initial ceriodaphnid test with the Heath Steele receiving water was discontinued due

to mortalities in the laboratory water controls. The test was re-started at a later date.

Table 2-1. Summary of physical-chemical attributes of the receiving waters measured prior to the preliminary tests.

Mine site (Receiving Water)	Date Collected (d/m/y)	Date Received (d/m/y)	Date Tested (d/m/y)	Dissolved O_2 ($mg \cdot L^{-1}$)	Conductivity ($\mu S \cdot cm^{-1}$)	pH
Gaspé (ruisseau Miller)	05/09/96	09/09/96	09/09/96	9.2	196	8.1
Heath Steele (Northwest Mirimachi R.)	29/08/96	03/09/96	04/09/96 17/09/96 [a]	10.6	47	7.5
Dome (South Porcupine R.)	03/09/96 18/09/96 [a]	04/09/96 20/09/96 [a]	04/09/96 26/09/96 [a]	10.1	277	8.1
Sullivan (St. Mary's R.)	29/08/96	30/08/96	30/08/96	9.6	62	7.6
Onaping/Levack (Onaping R.)	12/09/96	13/09/96	13/09/96 18/09/96 [a]	9.5	39	7.0
Myra Falls (Buttle Lake)	11/09/96	13/09/96	13/09/96	9.3	63	8.2

[a] ceriodaphnid test

2.1.2 Samples for Toxicity Testing of Effluents

B.A.R. Environmental supplied sampling kits for the effluent toxicity tests. Samples of effluents and receiving waters were collected and prepared by the field consultants, or by mine personnel, for shipment to the laboratories. The sample containers used for samples sent to B.A.R. Environmental 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, sent in coolers with ice packs) were employed for samples destined for Les Laboratoires Eco-CNFS.

The receiving water samples for effluent toxicity studies were collected either several days prior to sampling of the effluent or at the same time as effluent samples were collected (Table 2-2). Upon arrival at B.A.R. Environmental, receiving water samples were composited and returned to the original containers for storage. Receiving water samples can be stored for up to four weeks after collection (CANMET Aquatic Toxicity Subgroup, personal communication).

Effluents were sampled by instantaneous grab and were shipped to the laboratory, usually 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 toxicity tests). 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. All testing was performed within 72 h of sample collection.

The Levack effluent was first sampled on October 1, 1996. However, the field consultant was informed that the mine had not been releasing effluent during the sampling period, and what was collected was probably rainwater. The toxicity tests in progress were halted and the Levack effluent was sampled at a later date.

An initial sample of the Sullivan effluent was collected on September 23, 1996 and was shipped by ground transport. The sample arrived in Guelph, Ontario several days later, when the sample was more than three days old, and was not tested. The maximum delay between collection and testing permitted by the Environment Canada test methods is 72 h. A second sample was collected at a later date.

Table 2-2. Summary of physical-chemical attributes of the receiving waters measured prior to effluent testing.

Mine site (Receiving Water)	Date Collected (d/m/y)	Date Received (d/m/y)	Dissolved O_2 ($mg \cdot L^{-1}$)	Conductivity ($\mu S \cdot cm^{-1}$)	pH
Gaspé (ruisseau Miller)	16/09/96	18/09/96	9.0	206	8.2
Heath Steele (Northwest Mirimachi R.)	23/09/96	25/09/96	9.1	51	7.7
Dome (South Porcupine R.)	01/10/96	03/10/96	8.3	217	8.3
Sullivan (St. Mary's R.)	15/10/96	18/10/96	8.4	71	7.9
Onaping/Levack (Onaping R.)	01/10/96	03/10/96	8.6	39	7.5
Myra Falls (Buttle Lake)	07/10/96	23/10/96	8.7	59	7.8

The initial *Lemna minor* assay with the Gaspé sample resulted in very poor growth in the test media control. It was discovered that the distilled water used to prepare the test media was contaminated. The Gaspé mine effluent and receiving waters were re-sampled and re-tested with *Lemna minor* at a later date.

Upon arrival in the laboratory, 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 before testing included dissolved oxygen, temperature, conductivity and pH.

The pH and conductivity of the receiving waters measured prior to effluent testing are shown in Table 2-2. The conductivity of the receiving waters ranged from 39 to217 µS·cm^{-1} and the pH ranged from pH 7.5 to pH 8.3.

Values of dissolved oxygen, conductivity and pH of the effluent samples prior to testing are presented in Table 2-3. The conductivity of the effluent samples ranged from 644 to 2850 µS·cm^{-1} and the pH ranged from pH 7.2 to pH 10.2.

Table 2-3. Summary of physical-chemical data for the mining effluents measured prior to testing.

Mine site	Date Collected (d/m/y)	Date Received (d/m/y)	Dissolved O_2 (mg·L^{-1})	Conductivity (μS·cm^{-1})	pH
Gaspé	16/09/96	18/09/96	9.7	644	8.0
Heath Steele	23/09/96	25/09/96	10.2	1909	8.6
Dome	16/10/96	17/10/96	10.9	917	7.2
Sullivan	15/10/96	18/10/96	10.5	2850	8.8
Onaping	01/10/96	03/10/96	9.7	1594	7.6
Myra Falls	22/10/96	23/10/96	10.3	1207	9.8
Levack	04/11/96	05/11/96	11.2	1777	10.2

2.2 CULTURE OF THE ORGANISMS

2.2.1 *Selenastrum capricornutum*

A strain of this alga was obtained from the Québec Ministère de l'Environnement et de la Faune, and was then maintained in AAP (Algal Assay Procedure) culture media by Les Laboratoires Eco-CNFS, Pointe Claire, Québec. New cultures are started weekly and growth is regularly monitored. Maintenance of this organism in the laboratory follows recommendations in Environment Canada (1992a).

2.2.2 *Ceriodaphnia dubia*

These organisms are cultured from an original stock obtained from the Ontario Ministry of the Environment, Rexdale, Ontario, in 1988. They are maintained at 25°C with a 16 h light/ 8 h dark photoperiod in laboratory well water. New cultures are started weekly and are fed a combination of cultured alga (*Selenastrum capricornutum*) and a yeast broth mixture. Maintenance of this organism in the laboratory follows recommendations by Environment Canada (1992b).

2.2.3 Fathead minnows

An original brood stock of fathead minnows was obtained from the Aquatic Biology Unit, Ontario Ministry of the Environment, Rexdale, Ontario, with additional wild stock from Bobcaygeon, Ontario. These were used to set-up in-house laboratory cultures, which provide organisms for tests. Minnows were cultured in laboratory well water, with a photoperiod of 16 h light/ 8 h dark. Fish were fed several times a day with a brine shrimp diet. Maintenance of this organism in the laboratory follows recommendations in Environment Canada (1992c).

2.2.4 *Lemna minor*

Duckweed (strain C4) cultures were obtained from the University of Toronto and thereafter maintained by weekly subculture in Hoagland's E+ medium. The growth media was prepared by adding reagent grade salts to deionized (reverse-osmosis) water. Maintenance of this organism in the laboratory follows recommendations in the draft test method of the Saskatchewan Research Council (1996).

2.3 ACCLIMATION PROCEDURES

If a sample of receiving water caused toxicity to *Ceriodaphnia dubia* or to fathead minnows, the organisms were allowed to acclimate to the receiving water.

The step-by-step acclimation procedure employed in this study was developed by Keith Holtze of B.A.R. Environmental. The procedure consists of two steps, with each step lasting approximately one week: (1) acclimation to the pH and hardness conditions of the receiving water, using adjusted laboratory water, and (2) gradual acclimation to the full strength receiving water. The organisms are gradually introduced to the full strength solution within a reasonable amount of time, which allows tolerance to develop without selection of a resistant strain or race.

2.3.1 Acclimation of Fathead Minnows

An "adjusted" laboratory dilution water with the same pH (if pH $\geq$7.0) and hardness levels as the toxic receiving water was prepared. If the pH of the receiving water is less than pH 7.0, the pH of the dilution water was adjusted to pH 7.0. Adult fathead minnows (16-24 pairs) were transferred and held in this water for 5 days, with a water renewal rate similar to cultures in regular laboratory culture water. Acclimation of the organisms to the receiving water started with newly fertilized eggs from these fish. Newly fertilized eggs from these fish were collected and gradually acclimated to the full strength receiving water from the egg stage to hatch, over a period of six days. The proportion of receiving water to adjusted dilution water was increased at each renewal period, on a daily basis. The larvae, newly hatched (<24 hr old) in 100% receiving water, were then used in toxicity testing

2.3.2 Acclimation of *Ceriodaphnia dubia*

Neonate ceriodaphnids were transferred to "adjusted" laboratory dilution water having pH and hardness levels similar to that of the receiving water. Acclimation of the organisms to the

receiving water started with third brood neonates from this culture. The neonates were collected and placed in 10% receiving water. The amount of receiving water was increased each day until the animals were acclimated to full strength receiving water after 6 days. The proportion of receiving water to adjusted dilution water was increased every day, at each renewal period. The *Ceriodaphnia* continued to have broods of young while being cultured in the full strength receiving water. Toxicity tests were performed with the third brood of neonates from these cultures.

2.4 TOXICITY TESTS

2.4.1 Preliminary Tests of Receiving Waters

The preliminary tests of receiving waters were conducted as 7 day single concentration exposures, with four replicates. Control exposures were run in laboratory dilution water. The Environment Canada test methods recommend that controls meet certain standards if tests are to be considered valid, and these standards were used to classify a receiving water as toxic or not. Preliminary tests were only performed with *Ceriodaphnia* and fathead minnows.

A receiving water was judged as toxic to *Ceriodaphnia* if any one of these conditions were not satisfied during the test: adult survival $\geq$ 80%, mean production of young $\geq$ 15 young per female, and the production of at least three broods during the test. The numbers of young ceriodaphnids produced in receiving water and laboratory control water were also compared with a t-test.

A receiving water was judged as toxic to fathead minnows if fish survival was less than 80% during the test. Fathead minnows were not routinely weighed after the preliminary tests in order to reduce the cost of testing. However, fish development was visually monitored during the test, and if any fish had been judged as abnormally small compared to laboratory water controls, they would have been weighed. The test method criteria for individual weight gain is a minimum of 250 µg, which is approximately 50% of the normal weight gain for these fish in B.A.R.

Environmental laboratory dilution water. This difference is quite noticeable to the naked eye.

2.4.2 Toxicity Tests with Effluent Samples

Toxicity tests with effluent samples were conducted as either static (*Selenastrum capricornutum*, *Lemna minor*) or static replacement tests (trout embryo, fathead minnow, *Ceriodaphnia dubia*). Each test consisted of a minimum of five effluent concentrations and a receiving water control, with a minimum of three replicates per test concentration. A second control was conducted at the same time as the effluent test. In tests with the trout embryo, fathead minnow and *Ceriodaphnia*, this second control consisted of laboratory dilution water. The second control in the *Lemna minor* consisted of the "test media" (SRC, 1996). Since the *Selenastrum* test is performed on microplates, a second control microplate was prepared with the usual control "reagent water" specified in the test method. The test conditions of the five toxicity tests are summarized in Tables A-1.1 - A-1.5 in Appendix 1.

2.5 DATA ANALYSIS

2.5.1 Toxicity Endpoints

Determination of endpoints for tests with *Selenastrum*, *Ceriodaphnia* and fathead minnow and followed recommendations contained in the standard test methods (Environment Canada 1992a, 1992b, 1992c). Endpoints for the rainbow trout embryo test were determined according to a draft Environment Canada test method (Environment Canada, 1996). The responses of the organisms in the laboratory water and receiving water control exposures were compared using a t-test or an Analysis of Variance (ANOVA) followed by Dunnett's test or Tukey's multiple comparisons. If the data were not normally distributed, they were transformed (arcsine, log, power function) and retested. The statistics were performed using the program TOXSTAT (Gulley *et al.* 1989).

The LC50s and EC50s, including 95% confidence limits, 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 (ICpin program; Norberg-King, 1988) for *Ceriodaphnia*, fathead minnow and *Lemna minor* assays. IC25s and IC50s for the *Selenastrum* test were determined from a linear regression of growth inhibition *vs.* log effluent concentration. Calculating EC25s with the available software was not possible, so IC25s for rainbow trout embryo viability were calculated as described in Environment Canada (1992d).

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

2.5.2 Comparison of the Effluents

The toxicity of the seven effluents was compared by averaging the IC25s obtained from four of the toxicity tests. Results of the trout embryo test were not used since there were no EC50s for three of the failed tests. IC25s of " >100% v/v" were considered as 100% v/v so that an average could be taken. The effluents were rated using the average IC25, with the lowest IC25 being awarded a rank of one.

2.5.3 Comparison of the Toxicity Tests

The toxicity tests were compared in terms of their sensitivity by a simple ranking system. A rank was awarded based on the IC25s, with the lowest IC25 being assigned a rank of one. The average rank for each toxicity test, rounded to the nearest whole number, is shown in Table 4-5.

3.0 RESULTS

3.1 PRELIMINARY TESTS OF RECEIVING WATERS.

Two receiving water samples were toxic to test organisms in the preliminary tests. The Sullivan receiving water (St. Mary's River) was toxic to both *Ceriodaphnia* and fathead minnows, while the Gaspé receiving water (ruisseau Miller) was toxic to *Ceriodaphnia* only (Table 3-1).

After exposure to the St. Mary's River sample from the Sullivan mine, ceriodaphnid survival was 70%, less than the minimum of 80% according to the test method criterion. The average number of young ceriodaphnids produced was 17.6 during this exposure, more than the required minimum of 15, and not significantly different than the numbers produced in the laboratory water control ($p > 0.05$, Table 3-1).

Table 3-1. Responses of the cladoceran *Ceriodaphnia dubia* in receiving water (RW) and laboratory water (LW) exposures during preliminary tests.

Mine site (RW)	Survival (%)		Mean number of young per female (SD)	
	RW	LW	RW	LW
Gaspé (ruisseau Miller)	70	80	12.6 (9.2) [a]	16.1 (7.4)
Heath Steele (Northwest Mirimachi R.)	100	90	16.1 (5.5) [a]	23.0 (10.1)
Dome (South Porcupine R.)	100	100	29.5 (8.8)	31.2 (6.9)
Sullivan (St. Mary's R.)	70	80	17.6 (14.0)	21.4 (8.7)
Onaping/Levack (Onaping R.)	100	80	36.8 (11.1) [a]	23.9 (14.2)
Myra Falls (Buttle Lake)	100	90	27.7 (13.2)	23.7 (13.6)

[a] significant difference with laboratory dilution water exposure at $p = 0.05$.

Table 3-2. Survival of larval fathead minnows in receiving water (RW) and laboratory dilution water (LW) during preliminary tests.

Mine site (RW)	Survival (%)	
	RW	LW
Gaspé (ruisseau Miller)	100	100
Heath Steele (Northwest Mirimachi R.)	100	100
Dome (South Porcupine R.)	92.5	97.5
Sullivan (St. Mary's R.)	42.5[a]	87.5
Onaping/Levack (Onaping R.)	100	92.5
Myra Falls (Buttle Lake)	92.5	92.5

[a] significant difference with laboratory dilution water exposure at $p = 0.05$.

The Gaspé receiving water was also toxic to the invertebrate. Only 70% of the ceriodaphnids survived the exposure, and the adults only produced an average of 12.6 young per female in the preliminary test with water from the ruisseau Miller. This was less than the average produced in the laboratory water control ($p<0.05$, Table 3-1), as well as being less then the minimum of 15 young required by the protocol.

All ceriodaphnids survived during exposures to the Northwest Mirimachi River (Heath Steele), South Porcupine River (Dome), Onaping River (Onaping/Levack) and Buttle Lake (Myra Falls) water samples. Reproduction in these exposures also satisfied the criterion of an average of 15 young per female, as specified in the test method. However, ceriodaphnid reproduction in two of the receiving water exposures was significantly different than that observed in their respective laboratory controls.

During the preliminary test of the Heath Steele receiving water, only 16.1 young per female were produced. This was significantly less (t-test, $p<0.05$) than the number of young produced in the laboratory water control, which averaged 23 young per female. Despite the difference with the laboratory control, the Heath Steele receiving water was not considered toxic since the production of young was acceptable according to the protocol, and all of the organisms survived.

The Onaping River (Levack/Onaping) preliminary test also resulted in differences in ceriodaphnid reproduction between the receiving water and laboratory control exposures. The number of young produced in the Onaping River exposure averaged 36.8 young per female, significantly higher than the mean of 23.9 young per female produced in the laboratory dilution water control ($p<0.05$).

The St. Mary's River sample from the Sullivan mine was the only sample that was toxic to the fathead minnow, causing nearly 60% mortality (Table 3-2). Fathead minnow survival in the other receiving water exposures varied from 92.5 to 100%. According to visual observation, the surviving fish in these exposures gained enough weight to satisfy the minimum weight requirements specified in the tests method.

3.2 RESPONSES OF CERIODAPHNIA AND FATHEAD MINNOWS DURING THE ACCLIMATION PROCEDURE

Based on results of the preliminary tests, *Ceriodaphnia* were acclimated to the Gaspé and Sullivan receiving waters. Ceriodaphnid culture health tests were run during the final week of acclimation to the receiving water samples, when the proportion of receiving water in the acclimation exposures was gradually increased. During this period, survival of the ceriodaphnids increased. All of the invertebrates survived during acclimation to the Gaspé receiving water, while survival in the Sullivan acclimation series was 80%.

The production of young improved significantly ($p < 0.01$, t-test) during the step- by-step acclimation procedure. During acclimation to the Gaspé receiving water, the ceriodaphnids more than doubled their production of young, from 12.6 young per female during the preliminary test (Table 3-1), to 26.7 young per female (Table 3-3). During acclimation to the Sullivan receiving water, production of young increased to 31.9 young per female, compared with 17.6 young per female in the preliminary test (Tables 3-1 and 3-3).

Fathead minnows were acclimated to Sullivan receiving water (St. Mary's River) before the effluent was tested. The gradual acclimation procedure considerably reduced the toxicity of the Sullivan receiving water to the fathead minnow. Most (87.5%; Table 3-3) minnow eggs remained viable during the acclimation procedure, and hatched into larvae.

Table 3-3. Responses of *Ceriodaphnia dubia* and fathead minnows during step-by-step acclimation to receiving waters. Ceriodaphnids were acclimated to increasing concentrations of Sullivan and Gaspé receiving waters. Fathead minnows were only acclimated to the Sullivan receiving water.

Mine site (RW)	*Ceriodaphnia dubia*		fathead minnow
	Mean number of young per female (SD)	Survival (%)	% viable eggs (range)
Gaspé (ruisseau Miller)	26.7 (11.6)	100	na [a]
Sullivan (St. Mary's R.)	31.9 (10.2)	80	87.5 (50 -100)

[a] not applicable

3.3 TOXICITY TESTS WITH EFFLUENTS

3.3.1 Mine Gaspé

The Gaspé effluent exposures resulted in little toxicity to the five test species. The exposures had few effects on the survival of *Ceriodaphnia* and of fathead minnows, and the LC50s were >100% v/v. The IC25s for the other responses varied from 31.8% v/v to >100%. The most sensitive response was growth of the duckweed, with an IC25 of 31.8% v/v and an estimated IC50 of 66.9 % v/v. The exposures had negligible effects on the other organisms and the remaining IC25s and IC50 were >100% v/v.

The trout embryo test with the Gaspé sample was invalid because the receiving water was toxic, causing >70% mortality. Egg viability in the receiving water control was 56.7%, compared with 89% in the laboratory dilution water. Viability in the effluent exposures ranged from 72 to 76%, only slightly greater than egg viability in the receiving water. Since egg viability in the laboratory water controls was acceptable, this effluent was considered to have an EC50 of >100% v/v.

A summary of the results of toxicity tests with the Gaspé effluent is shown in Table 3-4.

3.3.2 Heath Steele Mine

The Heath Steele mine effluent exposures resulted in measurable toxicity to all of the test organisms. Exposure to the effluent had significant effects on survival of *Ceriodaphnia* (LC50 of 45.8% v/v), on survival of fathead minnows (LC50 of 63.1% v/v), and on trout embryo viability (EC50 of 84.6% v/v). The IC25s ranged from 19.0% v/v, for reproduction of *Ceriodaphnia*, to 47.3% v/v, for growth of the duckweed *Lemna minor*. Most of the organisms showed a similar sensitivity to the effluent, since four IC25s were in a relatively narrow range, from 19.0 to 24.0% v/v.

Table 3-4. Toxicity of the Gaspé effluent to test organisms. Test results are expressed as % v/v of effluent and are shown with 95% confidence intervals (CI). (na: not applicable).

Assay	IC25 (95% CI)	IC50 (95% CI)	LC50 (95% CI) [a]
Selenastrum capricornutum growth	>100	>100	na
Lemna minor growth	31.8 (8.5 - 49.4)	66.9 [b]	na
Ceriodaphnia dubia survival	na	na	>100
Ceriodaphnia dubia reproduction	79.4 [b]	>100	na
Fathead minnow survival	> 100	na	> 100
Fathead minnow growth	> 100	> 100	na
Rainbow trout embryo viability	> 100 [c]	na	> 100 [c]

[a] EC50 for rainbow trout embryo viability.

[b] estimated value since confidence limits could not be calculated.

[c] invalid test due to toxicity of receiving water (yet viability in 100% v/v effluent concentration was >70%).

Table 3-5. Toxicity of the Heath Steele effluent to test organisms. Test results are expressed as % v/v of effluent and are shown with 95% confidence intervals (CI). (na: not applicable).

Test Organism	IC25 (95% CI)	IC50 (95% CI)	LC50 (95% CI) [a]
Selenastrum capricornutum growth	23.3 (10.9 - 35.7)	42.1 (29.7 - 54.5)	na
Lemna minor growth	47.3 (37.8 - 55.5)	76.5 (68.1 - 83.1)	na
Ceriodaphnia dubia survival	na	na	45.8 (33.6 - 63.5)
Ceriodaphnia dubia reproduction	19.0 (16.6 - 21.7)	25.0 (21.7 - 33.0)	na
Fathead minnow survival	23.0 (12.4 - 96.1)	na	63.1 (13.0 - 100)
Fathead minnow growth	>50.0 [b]	>50.0 [b]	na
Rainbow trout embryo viability	24.0 (13.1 - 100)	na	84.6 (50. 0 - 100)

[a] EC50 for rainbow trout embryo viability.

[b] complete mortality at higher concentrations.

Effluent exposure was more detrimental to fathead minnow survival than growth. The IC25s and IC50s for fathead minnow growth were >50%, while the IC25 for survival was 23.0% v/v. Duckweed growth was less sensitive to the Heath Steele exposures than the responses of most of the other organisms. The IC25 for duckweed growth, 47.3 % v/v, was significantly higher than the IC25s for *Selenastrum* growth, for fathead minnow survival and for ceriodaphnid reproduction ($p<0.05$).

A summary of the results of toxicity tests with the Heath Steele effluent is shown in Table 3-5.

3.3.3 Dome Mine

The toxicity of the Dome mine varied considerably, with IC25s ranging from 2.8% v/v to >100% v/v. Exposure to the effluent had negligible effects on fathead minnow growth and survival, and on ceriodaphnid reproduction and survival. The IC25s and LC50s for these responses were >100% v/v. The effluent exposures inhibited growth of the alga and the duckweed, with IC25s of 2.8% v/v and 21.7% v/v respectively. Growth of *Selenastrum* was the most sensitive indicator, since the *Selenastrum* IC25 was significantly less than the duckweed IC25 ($p<0.05$). However, duckweed growth was affected over a wider range of effluent concentrations than the alga.

The trout embryo test was invalid, since mortalities were >70% in both receiving water and laboratory dilution water controls. Extensive mortalities also occurred at each effluent concentration. The eggs used for this test showed poor or very poor viability under hatchery conditions and it is probable that the failure of this test was due to the poor quality of the eggs and/or milt. This is discussed in more detail under Section 4.2.2.

A summary of the results of toxicity tests with the Dome effluent is shown in Table 3-6.

Table 3-6. Toxicity of the Dome final effluent to test organisms. Test results are expressed as % v/v of effluent and are shown with 95% confidence intervals (CI). (na: not applicable).

Test Organism	IC25 (95% CI)	IC50 (95% CI)	LC50 (95% CI) [a]
Selenastrum capricornutum growth	2.8 (0.3-5.3)	>100	na
Lemna minor growth	21.7 (13.3 - 28.2)	42.2 (38.6 - 46.0)	na
Ceriodaphnia dubia survival	na	na	>100
Ceriodaphnia dubia reproduction	>100	>100	na
Fathead minnow survival	>100	na	>100
Fathead minnow growth	>100	>100	na
Rainbow trout embryo viability	I [b]	na	I

[a] EC50 for rainbow trout embryo viability.

[b] invalid test due to poor egg viability.

Table 3-7. Toxicity of the Cominco Sullivan DWTP effluent to test organisms. Test results are expressed as % v/v of effluent and are shown with 95% confidence intervals (CI). (na: not applicable).

Test Organism	IC25 (95% CI)	IC50 (95% CI	LC50 (95% CI) [a]
Selenastrum capricornutum growth	22.2 (0 - 46.6)	30.2 (5.8 - 54.5)	na
Lemna minor growth	27.2 (17.4 - 34.7)	>93.1	na
Ceriodaphnia dubia survival	na	na	70.7 (50.0 - 100)
Ceriodaphnia dubia reproduction	12.6 (10.0 - 15.9)	18.4 (13.6 - 20.8)	na
Fathead minnow survival	>100	na	>100
Fathead minnow growth	>100	>100	na
Rainbow trout embryo viability	I [b]	na	I

[a] EC50 for rainbow trout embryo viability.

[b] invalid test due to poor egg viability.

3.3.4 Sullivan Mine

The Sullivan effluent exposures resulted in IC25s ranging from 12.6% v/v to >100% v/v. The IC25s for growth of the alga *Selenastrum* and the duckweed were similar, 22.2 and 27.2% v/v respectively. However, the corresponding IC50s were different, 30.2 and >93.1% v/v for the alga and the plant, respectively. Ceriodaphnid reproduction was the most sensitive indicator for this effluent, since this IC25, 12.6% v/v, was significantly less than either *Selenastrum* or duckweed values ($p<0.05$). The effluent exposure also affected survival of the invertebrate, with an LC50 of 70.7% v/v. The least sensitive test was the fathead minnow test, since IC25s for growth and survival were >100%.

The Sullivan sample was collected on Tuesday and arrived at the laboratory on Friday, 72 h later. The draft *Lemna minor* protocol specifies that the sample must be aerated for 12 - 16 h before concentrations can be prepared. The *Lemna minor* assay with this sample was therefore started the day after, 96 h after sample collection and 24 later than the recommended maximum delay.

Unfortunately, the trout embryo test was invalid, since mortalities were >70% in both receiving water and laboratory dilution water controls. Extensive mortalities also occurred at each effluent concentration. As discussed in Section 4.2.2, the eggs used for this test showed poor or very poor viability under hatchery conditions. It is probable that the failure of this test was due to the poor quality of the eggs and/or milt.

A summary of the results of toxicity tests with the Sullivan effluent is shown in Table 3-7.

3.3.5 Onaping Mine

The Onaping effluent was of low or negligible toxicity to three of the five test species. IC25s and IC50s for fathead minnow growth, fathead minnow survival and trout embryo viability were >100%. Most *Ceriodaphnia* survived in the full strength effluent concentration, and the LC50

was >100% v/v. The IC25 for ceriodaphnid reproduction was estimated at 80.7%, while the IC50 was >100%. Growth of *Selenastrum* was inhibited at lower effluent concentrations, with an IC25 of 30.8% v/v and an IC50 of 49.8% v/v.

The most sensitive indicator was growth of the duckweed, with an IC25 of 14.2% v/v and an IC50 of 19.8% v/v. These values are significantly less than the corresponding IC25 and IC50 for *Selenastrum* growth ($p<0.05$). Duckweed growth was the most sensitive response of all the tests, despite the possible interferences of algal growth. Algae were observed growing in the receiving water control and in the effluent concentrations during the duckweed test. This growth occurred despite the fact that the receiving water had been filtered through a glass fibre filter (GF/C grade) before use.

A summary of the results of toxicity tests with the Onaping effluent is shown in Table 3-8.

3.3.6 Myra Falls Mine

The responses of the test organisms to Myra Falls effluent exposures were variable, with IC25s ranging from 7.0 to 72.9% v/v. The exposures affected invertebrate survival, with an LC50 of 80.4% v/v. Survival of the larval fish was also affected. While no LC50 could be calculated, mortality in the 100% v/v effluent exposure was 46.7% and the IC25 for larval survival was 72.9% v/v. Growth of the fish was reduced at concentrations similar to those that affected survival. The IC25 and IC50 for minnow growth were 64.4 and 93.5% v/v respectively. Algal growth was the most sensitive indicator, with an IC25 of 7.0 % v/v and an IC50 of 13.5% v/v. The sensitivity of the duckweed was intermediate to those observed for the alga and the fish, with an IC25 of 18.3% v/v and an IC50 of 42.1% v/v.

The test with *Ceriodaphnia* was invalid, with 40% mortality in the receiving water control. This result was unexpected since water from Buttle Lake was not toxic to these organisms in the preliminary test. As was the case with all of the other receiving waters, samples for the

Table 3-8. Toxicity of the Falconbridge Onaping effluent to test organisms. Test results are expressed as % v/v of effluent and are shown with 95% confidence intervals (CI). (na: not applicable).

Test Organism	IC25 (95% CI)	IC50 (95% CI)	LC50 (95% CI) [a]
Selenastrum capricornutum growth	30.8 (24.8- 36.8)	49.8 (43.7 - 55.8)	na
Lemna minor growth	14.2 (11.9 - 15.4)	19.8 (18.2 - 21.1)	na
Ceriodaphnia dubia survival	na	na	>100
Ceriodaphnia dubia reproduction	80.7 [b]	>100	na
Fathead minnow survival	>100	na	>100
Fathead minnow growth	>100	>100	na
Rainbow trout embryo viability	>100	na	>100

[a] EC50 for rainbow trout embryo viability.

[b] approximate value since confidence limits could not be calculated.

Table 3-9. Toxicity of the Myra Ponds Outflow to test organisms. Test results are expressed as % v/v of effluent and are shown with 95% confidence intervals (CI). (na: not applicable).

Test Organism	IC25 (95% CI)	IC50 (95% CI)	LC50 (95% CI) [a]
Selenastrum capricornutum growth	7.0 (0 - 18.8)	13.5 (1.6 - 25.3)	na
Lemna minor growth	18.3 (8.9 - 30.2)	42.1 (30.2 - 48.6)	na
Ceriodaphnia dubia survival	na	na	80.4 (50.0 -100) [b]
Ceriodaphnia dubia reproduction	33.5 (5.3-37.6)	44.0 (37.8 - 55.3)	na
Fathead minnow survival	72.9 [b]	na	>100 [b]
Fathead minnow growth	64.4 (53.5 - 76.4)	93.5 [b]	na
Rainbow trout embryo viability	I [c]	na	I

[a] EC50 for rainbow trout embryo viability.

[b] estimated value.

[c] invalid test due to poor egg viability.

preliminary and effluent tests were collected at different times. It is possible that there were differences in the two Buttle Lake samples which may explain the different ceriodaphnid responses observed in the preliminary and effluent tests.

Despite the control mortality observed, it is possible to obtain estimated results using the responses in the other test concentrations. All of the adult ceriodaphnids in the laboratory water control survived, while survival in the full strength (100% v/v) effluent concentration was only 10% v/v. With the exception of the full strength effluent, ceriodaphnid survival in the other effluent exposures was $\geq$70%. In addition, there was no difference in the number of young produced in the lowest exposure concentration compared with the laboratory water control ($p<0.05$, ANOVA). Thus, the responses in the laboratory water exposure control were taken as the control values for the effluent exposures and estimated values of the endpoints (LC50, IC25 and IC50) were calculated. The estimated LC50 for adult *Ceriodaphnia* was 80.4% v/v, while the IC25 and IC50 for reproduction were 33.5 and 44.0% v/v.

The trout embryo test was unfortunately invalid, since most of the embryos died in the controls. At the conclusion of the assay, the percent embryo viability was only 22.5% in the receiving water control and 37.5% in the laboratory water control. There were no viable embryos in the 100% v/v effluent exposure. As discussed in Section 4.2.2, the eggs used for this test showed poor or very poor viability under hatchery conditions and it is probable that the failure of this test was due to the poor quality of the eggs and/or milt.

A summary of the results of toxicity tests with the Myra Falls effluent is shown in Table 3-9.

3.3.7 Levack Mine

The test organisms' responses to the Levack Mine effluent generally occurred at moderate effluent concentrations. Trout embryo viability was 60% in the 100% v/v exposure and the EC50 was >100% v/v. Fathead minnow survival was 83% in the full strength (100% v/v) effluent

exposure, with an IC25 and LC50 of >100% v/v. Growth of the minnow was reduced in the concentration range of 50-100% v/v, with an IC25 of 81.2 % v/v. Ceriodaphnid survival and reproduction were affected at the 100% v/v exposure, with 50% mortality and an average of only 8.6 young produced at this effluent concentration. Since 10% of the ceriodaphnids in the receiving water control also died, no LC50 could be calculated. The IC25 and IC50 for reproduction were 67.0 and 85.2 % v/v respectively.

Growth of the duckweed and the alga were reduced at lower effluent concentrations compared with the responses of the other test organisms. The IC25s for growth were 37.0% v/v (*Lemna minor*) and 47.6% v/v (*Selenastrum*), while the IC50s were 64.4% v/v (*Selenastrum*) and 72.1% v/v (*Lemna minor*).

A summary of the results of toxicity tests with the Levack effluent is shown in Table 3-10.

Table 3-10. Toxicity of the INCO Levack effluent to test organisms. Test results are expressed as % v/v of effluent and are shown with 95% confidence intervals (CI). (na: not applicable).

Test Organism	IC25 (95% CI)	IC50 (95% CI)	LC50 (95% CI) [a]
Selenastrum capricornutum growth	47.6 (34.4 - 60.7)	64.4 (51.2 - 77.6)	na
Lemna minor growth	37.0 (18.5 - 51.1)	72.1 (64.0 - 78.0)	na
Ceriodaphnia dubia survival	na	na	>50.0 [b]
Ceriodaphnia dubia reproduction	67.0 (44.2 - 69.7)	85.2 (73.5 - 89.4)	na
Fathead minnow survival	>100	na	>100
Fathead minnow growth	81.2 (78.2 - 89.2)	>100	na
Rainbow trout embryo viability	85.1 [c]	na	>100

[a] EC50 for of rainbow trout embryo viability.

[b] mortality in the 100% v/v effluent was 50%.

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

4.0 DISCUSSION

4.1 RESPONSES IN THE RECEIVING WATERS

4.1.1 Responses of Acclimated Organisms during Toxicity Tests with Effluents

After the acclimation procedure, survival of *Ceriodaphnia* and fathead minnow improved in the receiving water controls during the effluent tests. For example, while 57.5% of larval fathead minnows died in the Sullivan preliminary test (Table 3-2), only one individual out of thirty (3.3 %) died in the receiving water control during testing of the Sullivan effluent (raw data, Appendix 5). Similarly, while 30% of ceriodaphnids died in the Gaspé and Sullivan receiving waters during preliminary testing, survival in the receiving water controls after acclimation increased to 100% and 80%, for the Gaspé and Sullivan receiving waters, respectively. With this increase in survival, the Gaspé and Sullivan receiving water controls now satisfied the test method and these tests were valid.

Reproduction of *Ceriodaphnia* generally improved following acclimation. In the receiving water control of Gaspé effluent test, the acclimated ceriodaphnids produced 28.9 young per female, a significant improvement over the preliminary test (ANOVA, $p<0.05$). During the Sullivan effluent test, acclimated ceriodaphnids produced 19.3 young per female, not significantly different than the preliminary test. It should also be noted that reproduction increased significantly in the Heath Steele receiving water controls compared to the preliminary testing, without prior acclimation (t-test, $p<0.05$).

The major benefit of the acclimation procedure is increased survival. The acclimation procedure was clearly successful in the assays involving the Sullivan receiving water and fathead minnow survival. In addition, the improvements in ceriodaphnid reproduction in the Gaspé and Sullivan receiving waters after acclimation were partially due to an improved female survival during the exposure. Survival of ceriodaphnids was only 70% during the preliminary tests with Gaspé and

Sullivan receiving waters. This increased to 80% or more after acclimation. While there is no statistical difference in these two values, a survival rate of 80% satisfies the test method's requirements for survival in the controls. This is an example of differences that may not be statistically significant but which are most important in terms of the test method and for test validity.

4.1.2 Responses of Nonacclimated Organisms

The responses of the nonacclimated organisms to the receiving water exposures were variable, ranging from toxicity to stimulation. Two receiving water samples caused significant mortality to the test organisms. Only 56.7% of trout embryos were viable following exposure to the ruisseau Miller (Gaspé mine) control, causing the assay to fail. As previously discussed, the Myra Falls receiving water (Buttle Lake) was toxic to *Ceriodaphnia* during the effluent test, whereas it had not been toxic during the preliminary test. However, five of the seven receiving waters were beneficial to growth of the freshwater alga and of the duckweed.

Growth of *Selenastrum* was stimulated in the Gaspé, Heath Steele, Dome, Sullivan and Levack receiving waters, compared with growth in the usual test control water ("reagent water"). It should be noted that the amount of growth media added to the microplate wells is considered just sufficient for the initiation of algal growth. Algal growth in a natural receiving water is probably not nutrient limited to the same degree as it is in the usual control reagent water.

Growth of *Lemna minor* was also stimulated compared with that in the test media control, during exposures to three of the receiving waters. Stimulation occurred in exposures to the receiving waters of the Heath Steele, Dome and Onaping/Levack mines. However, it should be noted that in only one of the assays conducted during this study was growth in the test media controls satisfactory, according to the SRC draft test criteria.

Similarly, reproduction of *Ceriodaphnia* was stimulated by a sample of the Heath Steele receiving

water. During the effluent test, more young were produced in the Heath Steele receiving water control than in the accompanying laboratory water control (ANOVA, $p<0.05$). In addition, reproduction was significantly greater in this receiving water sample than in the sample used for the preliminary tests (ANOVA, $p<0.05$). These results suggest that the Heath Steele receiving water sample used for effluent testing may have differed from the sample used for preliminary testing.

The Myra Falls receiving water sample used for effluent testing may also have differed from the sample used for preliminary testing. During the preliminary test with the Myra Falls receiving water, all of the ceriodaphnids survived. However, during the effluent test with this organism, there was excessive mortality (40%) in the receiving water control, and the toxicity test was therefore invalid.

The different responses noted with the Heath Steele and Myra Falls receiving waters during testing with *Ceriodaphnia* suggest that different samples of the same receiving waters can vary in some manner that affects reproduction and/or survival of the invertebrate. This sample variability was only evident in tests with ceriodaphnids and not with fathead minnows. Survival of non-acclimated larval fish was similar in the preliminary test exposure and in the receiving water control of the effluent test.

Storage of receiving waters may influence certain characteristics that can affect algal growth. Samples from Onaping River, the receiving water for the Onaping and the Levack mines, were collected on October 1, 1996 and were received in the laboratory on October 3, 1996. After compositing, some of the receiving water was immediately used for the toxicity tests with the Onaping effluent, which commenced on October 3, 1996, and the remainder was stored at 4°C. Testing of the Levack effluent was delayed until November 1, 1996. Growth of the alga in the receiving water controls differed in the two assays. Growth of *Selenastrum* was stimulated during the Levack assay, yet no stimulation was observed during testing with the Onaping sample (t test, $p<0.05$). When compared to algal growth in the usual control reagent water, growth in receiving

waters can be stimulated by the presence of additional nutrients. It is possible that more nutrients were available in the Onaping receiving water sample, permitting more growth of the alga during the test.

The responses of both acclimated and non-acclimated organisms in the receiving water controls during effluent testing are summarized in Table 4-1.

Table 4-1. Comparisons of the responses of the test organisms in the laboratory water and receiving water controls (S: significant stimulation, NS: no significant difference, I: invalid test, T: significant reduction/toxicity).

Test Organism	Gaspé	Heath Steele	Onaping	Levack	Dome	Sullivan	Myra Falls
Selenastrum growth	S	S	NS	S	S	S	NS
Lemna growth	NS	S	S	S	S	NS	NS
Ceriodaphnia reproduction	NS[a]	S	NS	NS	NS	NS [a]	T
Fathead minnow growth/survival	NS	NS	NS	NS	NS	NS [a]	NS
Rainbow trout embryo viability	T	NS	NS	NS	I [b]	I [b]	I [b]

[a] acclimated organisms

[b] test invalid due to poor egg viability

4.2 LEMNA MINOR AND TROUT EMBRYO TOXICITY TESTS

4.2.1 *Lemna minor* Assays

Two aspects of the *Lemna minor* assay, growth in the controls and algal contamination, will be discussed.

In most cases, the plants in the control exposures did not produce enough fronds (leaves) to satisfy the criteria set out in the draft SRC protocol. The plants start out with three leaves per replicate and there must be an average of thirty at the end of the test - a 10-fold increase in 7 days. This is a considerable increase when compared to the requirements of the APHA (1995) and the ASTM (1991) test methods involving duckweeds. According to the unmodified APHA *Lemna minor* protocol, the number of leaves in the controls should increase by 2-fold over the four day test period, while the ASTM *Lemna gibba* protocol specifies a 5-fold after 7 days. While the present tests do not satisfy the draft test method, leaf production was impressive (eight-fold) and growth in the controls was fairly consistent (Table 4.2). The data were thus considered acceptable.

The growth criteria for this test are derived from the SRC experience in developing the assay, and could represent the best possible test performance. It is possible that leaf production can vary within the range of light intensities specified in the draft method, depending on other laboratory conditions (Mary Moody, SRC, personal communication). Growth may be maximized by small changes in light intensity within the range 63 - 72 $\mu E/m^2/s$.

Algal growth was observed in the test vessels of two of the *Lemna minor* asssays. Algae were evident in both the receiving water controls and effluent exposures during the Onaping assay, and in the effluent exposures with the Gaspé sample. Since algae are visibly present, these tests are not valid according to the draft protocol.

The draft protocol specifies that receiving water samples should be filtered through a GF/C filter if algae are suspected. All of the receiving waters in this study were inspected visually (microscope) before the test was started. If algal cells were detected, the sample was filtered. However, effluent samples, such as the Gaspé sample, were not filtered, and are also possible sources of algae. It would be preferable if the protocol specified that all samples be filtered, since the treatment of samples would then be standardized. It would also be advisable to use a more selective filtration for the removal of algae, such as the 0.45 µm filter specified by the *Selenastrum*

test method.

While the Levack and Onaping assays involved the same receiving receiving water, no algae were observed growing in the Levack assay receiving water controls. It is possible that the storage of the receiving water used for the Levack effluent may have influenced the presence of algae in the test.

Table 4-2. Average leaf production (± SD) by the duckweed *Lemna minor* in control exposures during toxicity tests with mining effluents.

Sample	Test date (d/m/y)	Average leaf production (SD)	
		Test Media Control	Receiving Water Control
Heath Steele	25/09/96	21.1 (3.8)	26.1 (1.7)
Onaping	04/10/96	21.5 (4.1)	26.8 (4.7)
Dome	18/10/96	22.5 (3.5)	33.6 (3.3)
Sullivan	19/10/96	19.8 (2.7)	21.1 (6.1)
Myra Falls	25/10/30	23.4 (2.5)	25.3 (4.4)
Levack	07/11/96	28.1 (3.4)	33.8 (5.3)
Gaspé	22/11/96	31.2 (4.0)	35.9 (6.5)
	Overall Mean (SD)	23.9 (5.2)	29.1 (6.9)

4.2.2 Trout Embryo Assays

There are two aspects of the trout embryo test that will be briefly discussed. The first is the number of failed tests and the second is the relative sensitivities of the two sublethal tests with fish.

4.2.2.1 Invalid tests

Three out of seven trout embryo tests assays conducted during this study resulted in valid tests. These valid tests were conducted with the Heath Steele, Onaping and Levack effluents. The four remaining tests failed the criteria for validity. The test with the Gaspé sample was invalid because the receiving water was slightly toxic, causing >70% mortality. The tests with the Dome, Sullivan and Myra Falls effluents were invalid due to excessive mortalities in both receiving water and laboratory water controls. It is probable that these test failures were due to the poor quality of eggs and/or milt used for the tests.

Eggs and milt for trout embryo assays were obtained from a provincial government fish hatchery (Ontario Ministry of Agriculture and Food, Alma Research Station, Alma, Ontario). This hatchery is primarily a research station, with exceptional facilities for temperature control and animal care. The staff at the hatchery kindly monitored the progress of eggs fertilized from the same batch of eggs and milt used for the toxicity tests. The fertilized eggs were maintained under hatchery conditions until the eyed stage. The staff provided general estimates of the success of egg development, ranging from very poor to very good (Table 4-3).

The egg batches used for the Dome, Sullivan and Myra Falls tests showed poor or very poor viability under hatchery conditions. This suggests that the failure of these tests may have been due to the poor quality of the eggs and/or milt used.

Table 4-3. Estimated viability of embryos of the rainbow trout, *Oncorhynchus mykiss*, for batches of eggs and milt used in toxicity tests. Viability was determined by the staff of the Alma Research Station fish hatchery (Ontario Ministry of Agriculture and Food). I - invalid test; V - valid test.

Sample	Test date (d/m/y)	Test Validity	Estimated viability
Gaspé	18/09/96	I	fair - poor
Heath Steele	25/09/96	V	good
Onaping	03/10/96	V	very good (≈ 14% dead)
Dome	17/10/96	I	very poor (≈50% dead)
Sullivan	18/10/96	I	very poor (≈50% dead)
Myra Falls	22/10/96	I	fair - poor (10 - 20 % dead)
Levack	05/11/96	V	good

Table 4-4. Comparison of IC25s (as % v/v effluent) for larval fathead minnow growth/survival and for trout embryo viability after exposures to mining effluents. Data are taken from the Sublethal Toxicity Screening Project and from the present study.

Sample	trout embryo	fathead minnow
# 960753	>100	94.4
# 960768	51.7	>100
# 960918	54.0	>100
Gaspé	>100	>100
Heath Steele	24.0	23.0
Onaping	>100	>100
Levack	85.1	81.2

4.2.2.2 Sensitivity

The sensitivity of the two fish assays was compared by combining data from this present study and the previous Sublethal Toxicity Screening Project. As shown in Table 4-4, the IC25s for growth/survival of the larval fathead minnow and for trout embryo viability are very similar. For five of the seven samples, the IC25s for the two species are nearly identical.

4.3 COMPARISON OF THE EFFLUENTS

A summary of the IC25 results is shown in Table 4-5, with an average IC25 for each effluent. The toxicity of the effluents is rated as follows, from most to least toxic (accompanied by the average IC25): Heath Steele (28.2), Myra Falls (30.3), Sullivan (40.5), Onaping (56.4), Levack (58.4), Gaspé (77.8) and Dome (80.4).

Table 4-5. Summary of results (IC25s) for toxicity tests conducted with mining effluents. Test results are expressed as % v/v of effluent.

Sample	*Selenastrum* growth	*Lemna minor* growth	*Ceriodaphnia* reproduction	fathead minnow growth/survival	Mean (rank)
Gaspé	>100	31.8	79.4	>100	77.8 (6)
Heath Steele	23.3	47.3	19	23	28.2 (1)
Onaping	30.8	14.2	80.7	>100	56.4 (4)
Dome	>100	21.7	>100	>100	80.4 (7)
Sullivan	22.2	27.2	12.6	>100	40.5 (3)
Myra Falls	5.1	18.3	33.5	64.4	30.3 (2)
Levack	47.6	37	67	82.1	58.4 (5)

4.4 COMPARISON OF THE TOXICITY TESTS

The average rank for each toxicity test, rounded to the nearest whole number, is shown in Table 4-6. The assays can be placed in two groups based on their sensitivity. Three assays, the *Selenastrum, Lemna* and *Ceriodaphnia* tests, showed a similar sensitivity to the effluents, with an average rank of 2. The fathead minnow assay, with an average rank of three, was slightly less sensitive and can be considered as part of a second group. The similarity in the sensitivity of the toxicity tests can be partially explained by the fact that most effluents exhibited relatively little toxicity. For example, only one IC25 was less than 10%, while twelve values were >50% v/v.

Table 4-6. Sensitivity of four toxicity tests to mining effluents using a simplified ranking system. Ranks were assigned based on the magnitude of IC25s obtained in each assay.

Sample	*Selenastrum* growth	*Lemna minor* growth	*Ceriodaphnia* reproduction	fathead minnow growth/survival
Gaspé	3	1	2	3
Heath Steele	2	4	1	2
Onaping	2	1	3	4
Dome	2	1	2	2
Sullivan	2	3	1	4
Myra Falls	1	2	3	4
Levack	2	1	3	4
Average:	2	1.9	2.1	3.3

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