AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM

Toxicity Assessment of
Highly Mineralized Waters
from Potential Mine Sites

AETE Project 1.2.4

Aquatic Effects Technology Evaluation Program

TOXICITY ASSESSMENT

OF HIGHLY MINERALIZED WATERS

FROM POTENTIAL MINE SITES

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AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

Notice to Readers

Toxicity Assessment of Highly Mineralized Waters from Potential Mine Sites

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 focused 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.

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 the spring of 1999.

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

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PROGRAMME D=
TECHNIQUES DE MESURE D=IMPACTS EN MILIEU AQUATIQUE

Avis aux lecteurs

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des eaux fortement minéralisées
d=éventuels emplacements miniers

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 -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

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.

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 au printemps 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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EXECUTIVE SUMMARY

Mines exist in geologically anomalous areas where elevated metals are a common feature of the surrounding area. Surficial mineralized zones cause elevated metal concentrations in the terrestrial and aquatic environments and the natural biota, via acclimatization, tend to reflect these highly mineralized environments. The study is to provide realistic information on the environmental effects of mining activities and the application of laboratory sublethal toxicity tests to highly mineralized waters (HMWs).

This study tested the following hypothesis: natural waters in mineralized areas which have been mined, or are likely to be mined, have no potential for chronic toxicity. The study involved submitting samples with a battery of tests, including 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. If a HMW is toxic, *Ceriodaphnia* and fathead minnow are acclimated to the sample and retested.

Criteria for selecting a HMW sample were developed following discussions with specialists in geochemistry and CANMET representatives. The criteria propose that if concentrations of metals and of sulphates in a receiving water surpass the limits listed in the British Columbia working/approved criteria for aquatic life, the receiving water would be considered as a HMW.

Only a single site, Discovery Pond, from Voisey=s Bay, Labrador (Voisey=s Bay Nickel Co. Ltd.) was tested and the sample was toxic to all of the test organisms. Acclimation of *Ceriodaphnia dubia* and fathead minnows was also not successful. Due to the toxicity of the sample, no tests could be performed with acclimated animals.

Representatives of the mining industry recommended additional sites for future HMW samples. These included B.C. sites on the Windy Craggy Deposit, Red Mountain, Bruceside Project /Sulphurets Property and Mount McIntosh/Pemberton Hills.

A method of identifying the input of HMWs in a stream or river is needed (such as conductivity) which can be simply used in the field. The water quality of HMWs can vary substantially. A large number of samples should be tested to identify the scale of problem, the degree of variation and the typical background conditions for different types of mines.

SOMMAIRE

Les mines se trouvent dans des régions d'anomalies géologiques dont la caractéristique commune est la richesse en métaux. Les zones minéralisées de surface sont à l'origine d'une forte concentration de métaux dans les milieux terrestres et aquatiques. Les organismes vivants, par l'acclimatation, témoignent aussi de l'existence de ces environnements fortement minéralisés. L'étude vise à fournir des renseignements réalistes sur les effets de l'activité minière sur l'environnement et sur l'application des essais de mesure de la toxicité sublétale en laboratoire aux eaux fortement minéralisées.

L'hypothèse à vérifier était la suivante : les eaux naturelles de régions minéralisées ayant été (ou susceptibles d'être) exploitées pour leurs mines ne posent aucun risque de toxicité chronique. On a donc soumis des échantillons à une batterie d'essais, qui mesuraient notamment : l'inhibition de la croissance de *Selenastrum capricornutum* et de *Lemna minor* ; la reproduction et la survie de *Ceriodaphnia dubia* ; la croissance et la survie du tête-de-boule ; la viabilité des embryons de la truite-arc-en-ciel. Si une eau fortement minéralisée est toxique, on y acclimate *Ceriodaphnia* et le tête-de-boule, puis on les soumet de nouveau aux essais.

On a élaboré les critères de sélection d'un échantillon d'eau fortement minéralisée après discussions avec des géochimistes et

des représentants de CANMET. Si les eaux réceptrices renferment plus que les limites de métaux et de sulfates exposées dans les critères officieux ou officiels de la Colombie-Britannique concernant la vie aquatique, on les considérerait comme fortement minéralisées.

On a soumis aux essais toxicologiques l’échantillon d’un seul lieu, l’étang Discovery, dans la région de la baie Voisey, Labrador (Voisey’s Bay Nickel Co. Ltd.), et cet échantillon s’est révélé toxique pour tous les organismes. *Ceriodaphnia dubia* et le tête-de-boule n’ont pas réussi à s’y acclimater. En raison de sa toxicité, nous n’avons pas pu effectuer d’essais avec des animaux acclimatés.

Les représentants de l’industrie minière ont recommandé des emplacements supplémentaires en Colombie-Britannique pour le prélèvement des futurs échantillons d’eau fortement minéralisée, notamment : le dépôt Windy Craggy, Red Mountain, le projet Bruceside sur la propriété Sulphurets ainsi que le secteur du mont McIntosh/collines Pemberton.

On a besoin d’une méthode qui permettra de déterminer l’apport d’eau fortement minéralisée dans un ruisseau ou une rivière (p. ex. conductimétrie), que l’on peut utiliser en toute simplicité sur le terrain. La qualité de l’eau fortement minéralisée peut varier considérablement. On devrait soumettre un nombre important d’échantillons à des essais pour cerner l’échelle du problème, sa variabilité et le contexte associé aux différents types de mines.

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1.0 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.

Mines exist in geologically anomalous areas where elevated metals are a common feature of the surrounding area. Surficial mineralized zones cause elevated metal concentrations in sediment and vegetation and these occurrences are useful exploration tools. Consequently, mining camps are surrounded by naturally elevated metal concentrations in the terrestrial and aquatic environments. Therefore, natural biota, via acclimatization, tends to reflect their highly mineralized environments.

The responses of sublethal toxicity tests where background waters are highly mineralized, such as is typical of the location of metal mines, is to be determined. This work is required to test the following hypothesis regarding HMW: ANatural waters in mineralized areas which have been mined, or are likely to be mined, have no potential for chronic toxicity.@

This work is needed to provide background data against which to assess toxicity testing for operating mines, for which pre-mining conditions could neither be determined or simulated , and to assess the utility of the proposed tools, relative to determining any impacts of mining which differ from pre-mining impacts or which cause real environmental problems compared to pre-mining conditions. This information will also provide a database which can be called upon when companies are operating, or considering operating, in areas of similar mineralized water characteristics. The importance of the work is that it will provide realistic information as to the environmental effects of mining activities and the application of specific, laboratory sublethal toxicity tests to mineralized waters.

1.2 OBJECTIVE

The principal objective of the study is to determine if highly mineralized waters (HMWs) have a potential for chronic toxicity. To meet this objective it is necessary to answer these questions:

1. Is there a potential for highly mineralized waters (HMWs) to be toxic to selected laboratory test species? Toxicity will be defined as a significant difference in test organism performance in the HMW assay, as compared to responses observed in controls with laboratory dilution water performed at the same time.

2. If HMWs are toxic, can the test organisms be acclimated to these waters? Successful acclimation occurs when the performance of the acclimated organisms satisfies the criteria in the appropriate test protocol within a defined time period.

The project is organized in two parts: (1) a screening study, testing samples with a comprehensive battery of tests, and (2) an acclimation study, where two of the organisms would be acclimated to toxic HMW samples for a finite period of time, and then retested.

1.3 PROJECT DESCRIPTION

1.3.1 Recruitment and Selection of Study Sites

At the initiation of the project, a precise definition of what constituted a highly mineralized water and an understanding of its chemical characteristics in the context of a mining environment was not available. Thus, an initial difficulty was providing a practical definition of a HMW so that samples could be collected and tested.

The first approach was to determine Atypical@ background conditions for different types of mines and define HMWs by comparing the metal levels with those contained in Water Quality Criteria (e.g., B.C., 1995 or CCME, 1996). A request for information on highly mineralized waters (HMWs) was prepared and sent to all participating CANMET mines. This letter requested background conditions from all participating CANMET mines which have collected chemical data (hardness, alkalinity, pH, metal concentrations, DOC, etc.) on their local receiving waters. The letter also enquired whether HMW should be defined by reference to Water Quality Criteria.

We received telephone responses to our request for information on HMWs, though little data was provided. The definition of a HMW was still confusing and at times contradictory, depending on different people. A conference call was then organized by CANMET, with specialists in geochemistry, CANMET representatives, and the laboratory.

Following this teleconference, the following definition of HMW was agreed upon:

AHighly mineralized waters (HMW) are the result of water coming into contact with naturally mineralized zones. These waters contain elevated levels of metals and of major ions, especially sulphate@.

Additional discussions with CANMET included the proviso that a HMW is a natural receiving water which can also support aquatic organisms such as fish and invertebrates, and is therefore not naturally toxic to the local aquatic life.

Criteria for selecting a HMW sample were developed from this definition. It originally appeared that British Columbia would be the source of most of the HMW samples for this project. Therefore, British Columbia Ministry of Environment, Lands and Parks guidelines for metals and sulphate were used to set the criteria for a HMW. A guide for the selection and sampling of HMWs was prepared for use by the mining community (Appendix 2). The guide proposes that if concentrations of metals and of sulphates are greater than certain limits, the receiving water will be considered as a HMW.

The metal and sulphate thresholds are based on the limits listed in the B.C. working/approved criteria for aquatic life.

Two sites undergoing development or exploration offered to take part in the study: Voisey's Bay, Labrador (Voisey's Bay Nickel Co. Ltd.) and Red Mountain, B.C. (Royal Oak Mines). Sampling kits were shipped to both sites in the summer and autumn of 1996 and a sample was obtained from the Voisey's Bay site. The Red Mountain site could not be sampled because the evaluation team was occupied in closing down the camp for the season.

1.3.2 Toxicity tests

The HMW sample was characterized with the following assays: growth inhibition with *Selenastrum* and *Lemna minor*, reproduction and survival of *Ceriodaphnia*, 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 screening 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.

2.0 METHODS

2.1 SAMPLE COLLECTION AND HANDLING

2.1.1 Samples for Chemical Analysis

Four litres of the HMW 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. A second 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, Ontario. A list of the parameters and the results of analyses are shown in Table 2-1.

2.1.2 Samples for Toxicity Testing

The Discovery Pond outflow sample was collected by the staff of the consulting company (Jacques Whitford Environment Limited) which was employed in evaluation of the site. B.A.R. Environmental supplied the sampling kits which were 20 L plastic pails fitted with a polyethylene plastic liner. The outflow was sampled by instantaneous grab. The pails were filled to maximum capacity and the plastic liner was closed with a twist-tie, after expelling as much air as possible. B.A.R. Environmental supplied the Chain-of-Custody forms.

Table 2-1. Chemical parameters measured in the sample of Discovery Pond Outflow HMW by Seprotech Laboratories (Ottawa, Ontario).

Parameter	Unit	Detection limit	Discovery Pond Outflow
TDS[a]	$mg·L^{-1}$	1	6
TSS [b]	$mg·L^{-1}$	1	44
total CN	$mg·L^{-1}$	0.005	<0.005
free CN	$mg·L^{-1}$	0.002	<0.002
$N\text{-}NH_3$	$mg·L^{-1}$	0.01	<0.01
conductivity	$\mu S·cm^{-1}$	1.0	79
alkalinity [c]	$mg·L^{-1}$	1.0	2
hardness [c]	$mg·L^{-1}$	1.0	21
pH	pH unit		5.90
As-dissolved	$mg·L^{-1}$	0.10	<0.1
Cd-dissolved	$mg·L^{-1}$	0.02	<0.01
Cu-dissolved	$mg·L^{-1}$	0.01	0.43
Pb-dissolved	$mg·L^{-1}$	0.10	<0.1
Ni-dissolved	$mg·L^{-1}$	0.02	1.11
Zn-dissolved	$mg·L^{-1}$	0.01	0.02
As (total)	$mg·L^{-1}$	0.10	<0.1
Cd (total)	$mg·L^{-1}$	0.02	<0.02
Cu (total)	$mg·L^{-1}$	0.01	0.43
Pb (total)	$mg·L^{-1}$	0.10	<0.1
Ni (total)	$mg·L^{-1}$	0.02	1.12
Zn (total)	$mg·L^{-1}$	0.01	0.02

[a] Total Dissolved Solids.

[b] Total Suspended Solids.

[c] as $CaCO_3$.

Upon arrival at the laboratory, the sample was logged in and recorded according to B.A.R. Environmental standard operating procedures. A sub-sample for the *Selenastrum* test was collected in a 200 mL polyethylene plastic bottle which was subsequently shipped, in a cooler with ice packs, to the laboratory in Pointe Claire, Québec.

The sample was stored at 4 (± 2) °C until testing, when its 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 (Table 2-2).

Table 2-2. Physical-chemical data measured in the Discovery Pond Outflow sample prior to toxicity testing.

Date Collected (d/m/y)	Date Received (d/m/y)	Dissolved O_2 ($mg \cdot L^{-1}$)	Conductivity ($\mu S \cdot cm^{-1}$)	pH
05/11/96	07/11/96	10.8	79	5.5

2.2 CHEMICAL ANALYSES

Concentrations of dissolved and total metals in the HMW sample were determined by Inductively Coupled Plasma Emission Spectroscopy (ICP). Cyanide (total and free) 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 2-1.

2.3 CULTURE OF THE ORGANISMS

2.3.1 *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 Algal Assay Procedure (AAP) 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).

2.3.2 *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).

2.3.3 *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).

2.3.4 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).

2.3.5 Rainbow trout embryos

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). Eggs were obtained from 1 to 3 females and milt from at least one male. Eggs and milt were transported to the laboratory on ice. During transport and storage, milt was kept at a depth less than 6 mm at 0 to 4°C, and eggs were kept no more than 3 layers thick at 0 to 3°C. The eggs were fertilized and used in toxicty testing within 24 hours of collection. Maintenance of this organism in the laboratory follows recommendations in Environment Canada (1996).

2.4 ACCLIMATION PROCEDURES

Ceriodaphnia dubia and fathead minnows were allowed to acclimate to the HMW sample. 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.

2.4.1 Acclimation of fathead minnows

An Aadjusted@ laboratory dilution water, at pH 7.0, but with the same hardness level as the HMW was prepared. The pH of the modified dilution water was not adjusted below neutrality, since reproduction of adult fathead minnows in our laboratory ceases at pH < 7.0. Adult fathead minnows (16 to 24 pairs) were transferred and held in this water for five 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. The newly fertilized eggs 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 which are newly hatched (<24 hr old) in the 100% receiving water are used in toxicity testing.

2.4.2 Acclimation of *Ceriodaphnia dubia*

Neonate ceriodaphnids were transferred to "adjusted" laboratory dilution water, with hardness and pH 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 HMW sample. Toxicity tests are performed with the third brood of neonates from these cultures.

2.5 TOXICITY TESTS

Toxicity tests were conducted as either static or static replacement tests (trout embryo, fathead minnow, *Ceriodaphnia*). The assay involved exposures to 100% v/v HMW and to control dilution water, with five replicates per exposure. In tests with the trout embryo, fathead minnow and *Ceriodaphnia,* control exposures consisted of laboratory dilution water. The control in the *Lemna minor* consisted of the Atest media@ (SRC, 1996). Since the *Selenastrum* test is performed on microplates, a second control microplate was prepared with the usual control Areagent water@ specified in the test method. The test conditions of the five toxicity tests are summarized in Tables A-1.1 to A-1.5 in Appendix 1.

Determination of endpoints for tests with *Selenastrum*, *Ceriodaphnia* and fathead minnow followed recommendations contained in the standard test methods (Environment Canada 1992a, 1992b, 1992c). Endpoints for the rainbow trout E-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. If the data were not normally distributed, they were transformed (arcsine, log, power function) and retested. The statistics were performed with software provided by Environment Canada (TOXSTAT program; Gulley *et al.* 1989).

3.0 RESULTS

3.1 CHEMICAL ANALYSIS OF DISCOVERY POND HMW

The sample of the Discovery Pond outflow contained elevated concentrations of copper and nickel, and a measurable quantity of zinc (430, 1120, and 20 $\mu g \cdot L^{-1}$, respectively). Alkalinity and hardness values were 2 $mg \cdot L^{-1}$ and 21 $mg \cdot L^{-1}$ as $CaCO_3$, respectively, which suggests that levels of the major cations, Ca^{2+} and Mg^{2+}, were not elevated. The sample was slightly acidic, with a pH of 5.9. The conductivity of the sample was 79 $\mu S \cdot cm^{-1}$, suggesting that the sulphate concentration was less than 100 $mg \cdot L^{-1}$. For comparison, a 100 $mg \cdot L^{-1}$ solution of magnesium sulphate has a calculated conductivity of about 230 $\mu S \cdot cm^{-1}$.

Table 3-1. Toxicity of the Discovery Pond Outflow to toxicity test organisms, showing endpoint measurements and significant difference with responses in laboratory control.

Assay (endpoint measurement)	Mean response		Significant difference ($p<0.05$)
	Discovery Pond	Laboratory Dilution Water (Control)	
Selenastrum capricornutum growth (cell numbers)	27992	969418	yes
Lemna minor growth (numbers of fronds; ± SD)	6.9 (1.2)	36.8 (6.3)	yes
Ceriodaphnia dubia survival (%)	0	100	yes
Ceriodaphnia dubia reproduction (no. young/female; ± SD)	0	30 (3.1)	yes
Fathead minnow survival (%)	0	96	yes
Fathead minnow growth (weight in mg; ± SD)	- [a]	0.632 (0.023)	yes
Rainbow trout embryo viability (%)	0	95	yes

[a] complete mortality

3.2 SINGLE CONCENTRATION TESTS WITH DISCOVERY POND HMW

Results of toxicity tests with the Discovery Pond outflow are shown in Table 3-1. The sample caused considerable toxicity to all of the test organisms. All of the animals involved in the testing died, though the alga and duckweed did grow during the exposures. No rainbow trout eggs were viable after 7 days of exposure to the HMW. All larval fathead minnows died within 96 hours into the test, so the growth of the exposed minnows could not be measured. *Ceriodaphnia* ceased reproduction during the exposures, and all of these animals also died. There was a significant reduction (81% inhibition) in duckweed growth in the full strength exposure (97% v/v sample). Algal growth was almost completely inhibited (97% inhibition).

3.3 ACCLIMATION OF CERIODAPHNIA AND FATHEAD MINNOWS

Acclimation of *Ceriodaphnia dubia* and fathead minnows was not successful (Table 3-2). Within days of the transfer to the 100% HMW sample, all of the organisms succumbed. The fathead acclimation procedure was repeated twice with identical results. In three culture health tests with the young ceriodaphnids exposed to the 100% v/v HMW, no young were produced prior to the animals= deaths. Due to the toxicity of the sample, no tests could be performed with acclimated animals.

Table 3-2. Responses of *Ceriodaphnia* and fathead minnows during acclimation to Discovery Pond outflow.

Ceriodaphnia dubia		fathead minnow
% Survival	Number of young per female	% viable eggs
0	0	0

4.0 DISCUSSION

4.1 TOXICITY OF DISCOVERY POND OUTFLOW

The Discovery Pond outflow was extremely toxic to all of the test organisms, and neither *Ceriodaphnia* nor fathead minnows could be acclimated to the sample. During a previous study, the pond was sampled in the winter and these samples were toxic to rainbow trout and to *Daphnia magna* (Jacques Whitford Environment Ltd., 1996). While the elevation of the pond appears to be a barrier to fish, they have been observed in the pond during the summer (Bruce Bennett, JWEL, personal communication). The toxicity of the sample is most likely due to the elevated concentrations of copper and nickel present (Table 2-1) at the low hardness conditions of the sample (since the toxicity of metals increase with decreasing water hardness). For comparison, the CCME (1996) guidelines for the protection of aquatic life list values of 25 $\mu g\ L^{-1}$ for Ni, and 2 $\mu g\ L^{-1}$ for Cu, in low hardness waters such as Discovery Pond.

4.2 SELECTION OF OTHER HMW SITES

Once an acceptable definition of HMWs was determined, Madame Danielle Rodrigue of CANMET contacted representatives of the mining industry who had expressed an interest in the toxicity of HMWs. Madame Rodrigue communicated the names of the interested parties to B.A.R. Environmental and these people were contacted. The people contacted included Mr. Marlin Murphy of Homestake, Mr. Harold Bent of Royal Oak Mines, Mr. Bruce Downing and Mr. Derek Riehm of Teck Corporation, Mr. Bill Napier and Mr. Bruce Bennett of Voisey=s Bay, Mr. Ian Sharpe of B.C. Environment, Mr. Fred Hewitt of Newhawk Gold Mines, Mr. Calvin Price of Placer Dome, and Mr. Glen Watson of INCO. One proposed site was not considered since the water was not from a natural source, but rather was drainage from a closed mine. A second site was eliminated since the input of contaminants appeared to be linked to acidic precipitation and was not due to a highly mineralized water.

Table 4.2-1 Surface water quality (in mg·L^{-1}) of Little Camp Creek Inflow, Bruceside Project, Sul

Parameters	Sampling Date		
	9/13/94	7/14/94	8/16/93
pH - on site	5.857		
pH	6.65	7.02	6.7
Conductivity	778	225	244
Total Dissolved Solids	499	156	174
Total Suspended Solids	<1	<1	20
Hardness	322	111	115
Alkalinity	41.5	30.4	33
Chloride	<0.5	0.6	<0.5
Sulphate	264	72.9	78.1
	Total Metals		
Arsenic	0.0005	0.0002	0.0027
Cadmium	0.0081	0.0015	0.0016
Calcium	120	42.2	42.8
Copper	0.026	0.003	0.012
Iron	0.212	0.217	2.23
Lead	0.005	0.001	0.006
Magnesium	5.66	1.58	2.04
Molybdenum	0.001	0.002	0.002
Silver	0.0003	<0.0001	<0.0001
Zinc	1.35	0.172	0.12
Mercury	<0.00001	<0.00001	0.00001
	Dissolved Metals		
Arsenic	0.0002	0.0002	0.0009
Cadmium	0.0079	0.0004	0.0015
Calcium	120	41.9	42.8
Copper	0.018	0.003	0.008
Iron	0.115	0.180	0.475
Lead	<0.001	<0.001	<0.001
Magnesium	5.51	1.58	2.04
Molybdenum	<0.001	0.002	0.001
Silver	0.0001	<0.0001	<0.0001
Zinc	1.34	0.169	0.097

Mr. Calvin Price, of Placer Dome, was contacted regarding the Sulphurets site. Mr. Price discussed the site with the company=s geological exploration team, who indicated that several HMWs may exist on the site. There appear to be several naturally acidic (pH 2 to pH 3) drainages containing elevated concentrations of copper and sulphates. The site is difficult to access and unfortunately, no further exploration activity is planned (Mr. C. Price, personal communication).

Mr. Bruce McLeod provided water sampling summary results from the Newhawk Gold Mines Sulphurets property (Table 4.2-1 and 4.2-2). The Newhawk Gold Mines Ltd. Sulphurets Project will be on a Care and Maintenance basis during 1997 and no environmental personnel will be available on site. Mr. Bruce McLeod states that Newhawk Gold Mines would be interested in participating in an HMW study if all costs and personnel are covered by CANMET (Mr. B. McLeod, personal communication).

The Little Creek Inflow and Outflow were sampled in August 1993, July 1994 and September 1994. The Little Creek Outflow was also sampled in July 1994. The samples contain elevated concentrations of several metals, in particular Cd, Cu, Fe and Zn, but the levels of these metals varied from sample to sample. In the Outflow, Cd ranged from <0.0002 to 0.0019 $mg{\cdot}L^{-1}$, Cu from 0.003 to 0.034 $mg{\cdot}L^{-1}$, Fe from 0.078 to 6.54 $mg{\cdot}L^{-1}$ and Zn from 0.060 to 0.183 $mg{\cdot}L^{-1}$ (Table 4.2-1). In the Inflow, Cd fluctuated from 0.0015 to 0.0081 $mg{\cdot}L^{-1}$, Cu from 0.003 to 0.026 $mg{\cdot}L^{-1}$, Fe from 0.212 to 2.23 $mg{\cdot}L^{-1}$ and Zn from 0.12 to 1.35 $mg{\cdot}L^{-1}$ (Table 4.2-2). The pH of these waters, as measured in the laboratory, ranged from pH 4.20 to pH 7.02 (Tables 4.2-1 and 4.2-2).

A single sample contained sulphate at levels greater than 100 $mg{\cdot}L^{-1}$ (the September Inflow sample with 264 $mg{\cdot}L^{-1}$; Table 4.2-1). This particular sample would qualify as a HMW since levels of metals are also elevated (Cu: 0.026 $mg{\cdot}L^{-1}$; Zn: 1.35 $mg{\cdot}L^{-1}$). The pH as measured in the laboratory was near-neutral, pH 6.65. However, it is not known if the creek is habitat for aquatic life.

Table 4.2-2 Surface water quality (in mg·L^{-1}) of Little Camp Creek Outflow, Bruceside Project, Sulphurets Property, British Columbia (data provided by Mr. Bruce McLeod).

Parameters	Sampling Date			
	9/13/94	7/14/94	9/22/93	8/16/93
pH - on site	2.727			
pH	4.20	6.39	6.73	6.2
Conductivity	207	91.3	133	83.2
Total Dissolved Solids	130	60	100	55
Total Suspended Solids	42	<1	3	11
Hardness	70.1	37.7	47.4	34.6
Alkalinity	<1.0	4.9	11.1	3.9
Chloride	0.5	0.8	1.4	<0.5
Sulphate	78.8	33.0	50.5	28.9
Total Metals				
Arsenic	0.0204	0.0009	0.0004	0.0005
Cadmium	0.0019	0.0004	<0.0002	0.0006
Calcium	27.0	14.6	17.6	12.9
Copper	0.034	0.008	0.003	0.004
Iron	6.54	0.534	0.078	0.308
Lead	0.026	0.016	0.008	0.012
Magnesium	1.20	0.558	0.849	0.569
Molybdenum	0.001	<0.001	<0.001	<0.001
Silver	0.0005	<0.0001	<0.0001	<0.0001
Zinc	0.183	0.060	0.062	0.064
Mercury	<0.00001	<0.00001	<0.00001	<0.00001
Dissolved Metals				
Arsenic	0.0009	0.0007	0.0004	0.0002
Cadmium	0.0019	<0.0002	<0.0002	0.0006
Calcium	26.3	14.2	17.6	12.9
Copper	0.031	0.008	0.002	0.002
Iron	1.50	0.448	0.078	0.171
Lead	0.018	0.013	0.004	0.005
Magnesium	1.09	0.558	0.849	0.567
Molybdenum	<0.001	<0.001	<0.001	<0.001
Silver	<0.0001	<0.0001	<0.0001	<0.0001
Zinc	0.179	0.059	0.062	0.061

Mr. Ian Sharpe of B.C. Environment provided chemical information from the Red Mountain evaluation site, which was being explored by Red Oak Mines. Seventeen stations on the site had been sampled over a period of two years. Levels of major ions, metals and general water quality parameters (e.g., suspended solids, hardness, alkalinity) were measured weekly, and later, monthly. Monthly and weekly data for the stations were compared and sampling locations with elevated levels of sulphate and metals were identified. The sulphate and metal levels (in particular Zn, Ni and Cu), at sample locations W3, W6, W11, W12 and W17 on the Red Mountain site, were elevated enough to class these waters as HMWs. Sampling kits were sent to the site in the autumn of 1996, but unfortunately the exploration/evaluation team was occupied closing their camp, and could not collect a sample. However, sampling could be conducted in the spring if the evaluation/exploration base camp is re-established.

Mr. Bruce Downing of Teck Corporation provided information on the Windy Craggy Deposit, another evaluation site in B.C. Several rivers and streams originate or have inputs from the Frobisher and Tats glaciers. Table 4.2-3 presents data provided for this site by Mr. Downing. Levels of sulphate and copper measured at station W13 suggest that Red Creek is a HMW. Levels of copper and zinc are also elevated at several other stations in the area.

Finally, Mr. Derek Riehm of Teck Corporation was contacted regarding a site on Vancouver Island near Port Hardy. The B.C. Ministry of Energy, Mines and Petroleum Resources has been collecting chemical data from this site, which is summarized in Panteleyev *et al*. (1995). Five sampling stations indicate possible HMWs (Table 4.2-4), based on the elevated levels of sulphate (> 100 mg L^{-1}) reported in Panteleyev *et al*. (1995).

Table 4.2-3 Surface water quality (in mg·L^{-1}, mean summer values) of stations in the Windy Cra

Location	Station	Sulphate	Cu	Zn
Red Creek	W12	71	1.7260	0.0307
Red Creek	W13	280	0.0012	0.0900
Turnback Canyon	W1	17	0.0308	0.0626
Frobisher Creek	W2	48	0.1800	0.2260
branch of Alsek R, not labelled	W3	2	0.0028	<0.0005
Noisy Creek	W4	4	0.0020	0.0037
Alsek R. near Noisy Creek	W16	16	0.0335	0.0653
Tats Creek near source	W11	4	<0.0005	<0.0005
branch of Tats Creek, not labelled	W5	21	0.0336	0.0193
branch of Tats Creek, not labelled	W18	11	0.0725	0.0560
Tats Creek, above Tats Lake	W6	19	0.0656	0.0510
discharge from Tats Lake	W7	6	0.0003	<0.0005
Tats Creek junction with Tatshenshini R.	W8	13	0.0350	0.0416
Tatshenshini R. between Tats Cr.& Alsek R.	W15	26	0.0610	0.1213
Tatshenshini R. between Tats & Henshi Creeks.	W9	39	0.0330	0.0800
Tatshenshini R. between Henshi Cr & O=Connor R.	W17	27	0.0598	0.1058
Shini Creek	W20	28	<0.0005	<0.0005
Shini Creek	W19	29	<0.0005	<0.0005

Discussion ensued on the selection of a sampling site for toxicity testing. This would not have been difficult if the only criteria used were elevated levels of sulphate and metals. However, if the presence of aquatic life was also considered, the extremely acidic HMW samples shown in Table 4.2-4 would not be sampled directly. It was suggested that samples be collected in the receiving environments of the HMW(s), yet near the location where the HMW(s) enter the stream/river, using the B.C. Environment data from the summer as a guide to select the sites. Mr. Riehm pointed out that the water quality measured in the summer months would most likely differ from that during the winter. Thus, a potential difficulty was using water quality data from the summer to identify sampling sites in the winter. Another difficulty involved fish habitat. The HMWs are diluted in streams which are habitat for fish. However, it would be necessary to identify locations in the receiving environment near the source of the HMWs. This would require an indicator of HMW that could be easily used in the field, such as conductivity.

Madame Lise Trudel of CANMET suggested that it would be preferable to have some knowledge of the sulphate concentration before taking samples to ensure that the sampling effort required for the toxicity tests would not be wasted (i.e. if sulphate levels were too low). The Port Hardy sites were not sampled in 1996 due to the difficulties of sampling isolated stations under winter conditions. CANMET decided that it would be preferable to wait until spring to continue the project.

Table 4.2-4 Water chemistry of possible HMW sites in the Mount McIntosh/Pemberton Hills area, Northern Vancouver Island (taken from Panteleyev *et al.*, 1995). Concentrations of sulphate and metals are in mg·L^{-1}.

Location	pH	Sulphate	Cu	Pb	Zn
Youghpan Creek, head	3.6	138	0.02	0.06	0.06
Youghpan Creek	3.3	125	0.02	0.06	0.04
H1000 Rd S. McIntosh	3.7	100	0.01	0.31	0.03
Clarklagh Cr. at CL130	3.8	110	0.02	0.21	0.03
South McIntosh	3.8	148	0.01	0.03	0.03

In conclusion, Table 4.2-5 summarizes the sites recommended for future HMW samples.

Table 4.2-5 Location of sites recommended for future HMW samples.

Location	Site
W13	Windy Craggy Deposit
Red Creek	
W3	Red Mountain
W6	
W11	
W12	
W17	
Little Camp Creek Inflow	Bruceside Project, Sulphurets Property
Youghpan Creek	Mount McIntosh/Pemberton Hills
H1000 Rd, S. McIntosh	
Clarklagh Creek at CL130	

4.3 CONSIDERATIONS IN SELECTING HMWs

The sole HMW sample tested in this study contained elevated levels of nickel and copper, relatively low levels of sulphate, and was of moderate acidity (pH 5.5). It is evident from the data in Table 4.2-4 that Port Hardy HMWs are considerably more acid, with pH ranging down to pH 3.3. There are indications from other exploration crews that similarly naturally acidic HMWs are prevalent at other sites.

The hypothesis tested in this study is Anatural waters in mineralized areas which have been mined, or are likely to be mined, have no potential for chronic toxicity.@ As stated in the Request for Proposal, testing of this hypothesis is necessary to:

1. provide background data against which to assess toxicity testing for operating mines in the case where pre-mining conditions could neither be determined or simulated; and

2. assess the utility of the tests for determining any impacts of mining. An impact of mining is defined as an impact which causes a significant (Areal@) environmental problem as compared to pre-mining conditions.

One concern regarding HMWs is that natural populations are naturally acclimated to the locally highly mineralized waters, while test organisms would find them toxic. While local organisms may not be affected by a mining effluent, the non-acclimated test organisms would be overly sensitive to the exposure. Thus, the toxicity test result would not be indicative of actual effects in the field. However, for this to be true, the HMW should not cause toxicity to the local aquatic life either.

Toxicity tests using the local receiving water as the dilution/control water should be more indicative of effects in the field. A second control of laboratory dilution water ensures that toxic or inhibitory responses in the HMW used as control/dilution water , can be quantified. If the HMW water does cause toxicity in the laboratory, the organisms can be acclimated prior to performing tests. The success of the acclimation can also be quantified by comparison to the performance of organisms in the laboratory dilution water.

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*) either cover all of Canada or a large portion of it (Scott and Crossman 1979; Environment Canada 1992a, 1992b, 1992c; APHA, 1995). The extent of this range indicates that these organisms can successfully acclimate to many different conditions, and in general, it suggests they should be able to acclimate to most HMWs which are not toxic to local biota.

Therefore, it should not be necessary to test HMWs of extreme pH (pH<4.0) to determine their toxicity. These samples are certainly toxic - few of the test organisms used in sublethal toxicity tests would survive such exposures, especially if the low pH was accompanied by elevated concentrations of metals. It is highly likely that these conditions are also too severe for successful acclimation of *Ceriodaphnia* and fathead minnow. Yet this does not imply that the test organisms are necessarily more sensitive than natural populations, since these HMWs would almost certainly be toxic to them as well.

HMWs have been shown to cause toxicity to natural populations in the field. For example, water from the Red Dog Creek in Northern Alaska was acutely toxic to native fish such as chum salmon (*Oncorhynchus keta*) eggs, juvenile and adult arctic char (*Salvelinus alpinus*) and Arctic grayling (*Thymallus arcticus*), both in the field and in the laboratory (EVS Consultants, 1995). Fish (species not identified) had been observed in Discovery Pond during the summer, and the outflow was toxic to laboratory organisms (rainbow trout and *Daphnia magna*) in the winter. However, fish had not been seen for some time prior to the collection of samples for sublethal toxicity tests (Mr. Bruce Bennett, JWEL, personal communication).

A second concern is the use of a highly mineralized receiving water as dilution/control water in toxicity tests if the HMW is known to be toxic. If the HMW control causes toxicity, the test becomes invalid. If the effluent also causes toxicity, the test is still invalid. The only occasion where an invalid test can provide useful results is when the effluent itself does not cause significant toxicity when compared to the laboratory dilution water control. There is no contradiction between field and laboratory results if a HMW causes toxicity to both field and test organisms.

A third concern is identifying the impact of HMWs on a receiving water, which is or may eventually become the receiving water for a mining effluent. Few of the HMWs identified in this study are known to support aquatic life directly. However, these HMWs generally flow into a larger body of water which is known to contain fish and other organisms. A method of identifying the input of HMWs in a stream or river is needed (such as conductivity) which can be simply used in the field.

A fourth concern is variation in the water quality of HMWs. As shown in Table 4.2-2, the water quality of the Little Camp Creek Inflow and the Outflows varied. The apparent water quality of Discovery Pond appeared to change seasonally and may have had repercussions on the fish in the pond (Mr. Bruce Bennett, personal communication). It may be preferable to test several kinds of waters, identifying the scale of problem and possible variation and identifying typical background conditions for different types of mines. This would require testing a large number of samples so as to have a representative sample size. It may not be feasible or economical to perform acclimation studies with the organisms, but a large database on several receiving waters would be gathered.

5.0 REFERENCES

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