GUIDANCE DOCUMENT FOR CONDUCTING TOXICITY REDUCTION EVALUATION (TRE) INVESTIGATIONS OF CANADIAN METAL MINING EFFLUENTS

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January 2002

EXECUTIVE SUMMARY

The draft Metal Mining Effluent Regulation (MMER) requires that all Canadian metal mines produce effluent that is non-acutely lethal to rainbow trout when tested in accordance with Environment Canada test methods. Mine operations will also be required to monitor the acute lethality of effluent to *Daphnia magna*. If a rainbow trout test produces mortality of more than 50% of the test organisms in 100% effluent, the sample is considered to "fail" the acute lethality test. In the event of a toxicity failure, the draft MMER requires that the mine implement a plan to investigate the cause of acute lethality. The Toxicity Reduction Evaluation (TRE) developed by the U.S. EPA (1989) is a commonly used step-wise approach designed to assist industrial dischargers to identify the causes of, and eliminate final effluent acute lethality.

The purpose of this Guidance Document is to provide TRE guidance specifically focused on challenges faced by the Canadian metal mining sector in order to assist mining facilities in meeting the acute lethality requirements for both rainbow trout and *Daphnia magna*. It is intended to provide mine managers with an effective tool for implementing an appropriate strategy for resolving acute lethality issues, provide laboratories with a useful guide for conducting TRE studies with metal mining effluents, and ultimately, increase the likelihood of achieving and maintaining a consistently non-acutely lethal metal-mining effluent. It is not intended to replace the existing U.S. EPA documents, but rather to provide supplementary guidance for application with Canadian regulatory test species and metal-mining effluents.

A TRE is a site-specific study designed to identify the substance(s) responsible for acute lethality, isolate the source, evaluate the effectiveness of control options, and confirm the reduction in acute lethality of the final effluent. Although the approach to any TRE may have similar components, the sequence of events or steps will be site-specific and depend on the nature of the toxicant, as well as the results and findings from each phase of work.

The initial step in the TRE process, which should begin prior to experiencing the first failure, is the development of an "Acute Lethality Response Plan". This plan will increase the speed and efficiency with which the acute lethality failures can be addressed, by facilitating the data acquisition phase (with respect to mine facilities/operations), and assist in the decision making process. The "Acute Lethality Response Plan" may include (but is not limited to):

- Description of facility processes and operations.
- Description of effluent treatment facility and process.
- Line diagrams showing the major areas of operation and the main inputs to the treatment plant (if one exists).
- Documentation of facility operations/condition during collection of samples for routine acute lethality testing.
- Characterization (for chemistry and toxicity) of process streams over time to provide baseline data to be used for comparisons to samples collected during a toxicity episode.
- Results from acute lethality tests and chemical analysis for routinely monitored parameters.
- Up-to-date list of Material Safety Data Sheets (MSDS) for chemicals used in the process and effluent treatment (with available toxicity data for rainbow trout and *Daphnia magna*).

- Selection of a response team, which may include consultants (i.e., aquatic toxicologists, engineers experienced in the TRE process, if not already available within the facility) and mine personnel (i.e., management, operations, support personnel for sampling). Good communication and exchange of complete information among all team members is critical to the success of a TRE by speeding the response to the failure and increasing the likelihood of TRE success.

Immediately after the initial acute lethality failure is experienced, a review of the acute lethality test data should be conducted, to ensure that all Environment Canada test conditions were met. Water quality parameters (e.g., dissolved oxygen, pH, conductivity) measured during the test could also provide useful clues as to the cause of acute lethality. If samples continue to demonstrate the presence of acute lethality, a review of the information gathered as part of the Acute Lethality Response Plan, and an evaluation of remedial actions to optimize facility operations (including housekeeping practices, treatment plant optimization, and chemical optimization) should be initiated. If these activities are unsuccessful in resolving toxicity, subsequent stages in the TRE could involve a variety of approaches.

Establishing the degree (i.e., magnitude) and persistency (i.e., how toxicity changes over time) of acute lethality will be important, since these can influence subsequent TRE activities. The actual number of samples required to assess these factors will be site-specific and depend largely on effluent variability. Failure to understand the variability in effluent acute lethality and individual toxicants could lead to selection of treatment options or controls that do not consistently reduce acute lethality to compliance levels (U.S. EPA, 1999).

Although common toxicants associated with metal mining effluents have been identified (including ammonia, metals, pH, dissolved salts, and cyanide), effluent characteristics and toxicants will be unique to individual mines and operations. Therefore, the choice and combination of subsequent TRE approaches will depend upon several factors including the degree and persistency of acute lethality, availability and quality of historical toxicity and chemistry data, the type of operation/process, and the nature of the toxicant(s). Furthermore, the approach to a TRE study will unfold as information about the toxic event becomes available.

Three fundamental TRE components include:

1. Toxicity Identification Evaluations (TIEs)
2. Source Investigations (SIs)
3. Toxicity Treatability Evaluations (TTEs)

An effective TRE must determine the appropriate combination of these approaches and alternative strategies to eliminate acute lethality. However, regardless of the TRE strategy selected, good communication and co-ordination between the mine operators, toxicology, chemistry and engineering groups participating in the TRE is critical to the success of a study.

The objective of the Toxicity Identification Evaluation (TIE) is to identify the specific substances responsible for acute lethality. The TIE process is divided into three phases, which usually occur sequentially, but may be conducted simultaneously when patterns of toxicity begin to emerge during Phase I.

- Phase I involves characterization of the toxicants through a variety of effluent treatments (U.S. EPA 1991a).
- Phase II involves identification of the suspected toxicant(s) (U.S. EPA, 1993a).

- Confirmation of the suspected toxicants occurs in Phase III (U.S. EPA, 1993b).

The TIE approach will be most effective if acute lethality is consistent and persistent (i.e., does not degrade over time). In this case, characterization of the toxicant(s) (Phase I TIE) should be conducted. If successful, it may be necessary to identify (Phase II) and confirm (Phase III) the specific substance responsible for acute lethality prior to conducting a TTE or SI. Alternatively, characterization of the effluent may provide sufficient information without specifically identifying the substance(s) responsible (i.e., slight adjustment of pH eliminates toxicity). The information generated during the Phase I TIE could be used to modify the existing treatment system, or implement new treatment methods (TTE approach).

The TIE approach will be less effective and more difficult to complete if acute lethality is transient or non-persistent. Random toxicity events may require the analysis of more samples and, in some cases, may even necessitate abandoning TIE work on individual toxic samples (Ausely *et al*., 1998). Alternative approaches, in combination with TTE and SI evaluations, may be more successful under these conditions.

After completion of the Phase I characterization of an effluent, the TRE can proceed to:

1. TTE to evaluate various treatment methods for removal of the toxicant,
2. SI to identify the source of the toxicant, or
3. Phase II and III TIE to identify and confirm the specific substance responsible for acute lethality prior to conducting a TTE or SI.

TTEs and SIs can be conducted with or without identification of the specific toxicant(s), but will be more effective if a specific substance can be targeted for treatment. In the case that the TTE or SI approach is selected, confirmation testing (Phase III) will still be required to ensure that the method selected consistently removes acute lethality.

SIs and TTEs may be used as strategies in combination with, or as alternatives to, a TIE. Source Investigations determine whether the toxicants may be isolated in one or more waste streams. The approach to a SI may include identification of discharge locations and inputs to the effluent treatment plant (ETP), characterization of each discharge in terms of flows, acute lethality and chemical composition, and use of a mass balance approach to identify those streams representing the largest contribution to acute lethality and chemical loading. Once a specific process stream has been identified as the source of toxicity, a TTE could be conducted to reduce or eliminate the substance(s).

A toxicity treatability evaluation (TTE) involves the systematic evaluation of various treatment technologies, combinations of technologies, or management options (i.e., process or operational changes) to assess the ability of these technologies (or operational/process changes) to reduce levels of contaminants that are causing acute lethality. Once removal of acute lethality has been demonstrated at the bench-scale level, a decision can be made apply the technique at a larger pilot-scale or directly at the existing treatment facility.

In all TRE strategies, repeated testing and evaluations must be conducted. However, the number of samples to be treated and analyzed will depend on a variety of factors, including effluent variability, number of toxicants, conclusions drawn from data, cost of remedial action, regulatory deadlines and success of each phase (U.S. EPA, 1991).

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ABBREVIATION	DEFINITION
AA	Atomic Adsorption
AETE	Aquatic Effects Technology Evaluation
AFR	Acidification, Filtration and Reneutralization
ANFO	Ammonium Nitrate - Fuel Oil
APHA	American Public Health Association
AVR	Acidification-volatization-reneutralization
BAT	Best Available Technologies
BLM	Biotic Ligand Model
CAEAL	Canadian Association for Environmental and Analytical Laboratories
CANMET	Canadian Center for Mineral and Energy Technology
CCME	Canadian Council of Ministers of Environment
COD	Chemical Oxygen Demand
CS	Case Study
D.O.	Dissolved Oxygen
DAF	Dissolved Air Floatation
DOC	Dissolved Organic Carbon
EC	Electrocoagulation
EDTA	Ethylenediaminetetracetate
EMS	Environmental Management Systems
ETP	Effluent Treatment Plant
GAC	Granular Activated Carbon
GC/MS	Gas Chromatography / Mass Spectrometry
GFAA	Graphite Furnace Atomic Absorption
GFAAS	Graphite Furnace Atomic Absorption Spectro-photometry
HPCL	High Performance Liquid Chromatography
IC	Inhibiting Concentration
ICP-AES	Inductively-coupled Plasma - Atomic Emission Spectroscopy
ICP-MS	Inductively-coupled Plasma Mass Spectroscopy
ICP-OES	Inductively-coupled Plasma Optical Emission Spectroscopy
ISO	International Standards Organization
IX	Ion Exchange
LC/MS	Liquid Chromatography / Mass Spectrometry
LC50	Medium Lethal Concentration
LC-SAX	Quaternary Amine Bonded Silica
MDL	Method Detection Limits
MISA	Municipal Industrial Strategy for Abatement (Ontario)
MMER	Metal Mining Effluent Regulation
MMLER	Metal Mining Liquid Effluent Regulations
MSDS	Material Safety Data Sheet

ABBREVIATION	DEFINITION
NALMET	Non-Acutely Lethal Mining Effluent Technologies
PAPRICAN	Pulp and Paper Research Institute of Canada
PFD	Process Flow Diagrams
pHi	Initial pH
PP	Pilot Plant
QA/QC	Quality Assurance / Quality Control
RM	Regulatory Method
RO	Reverse Osmosis
SART	Sulphidization, Acidification, Reneutralization and Thickening
SETAC	Society of Environmental Toxicology and Chemistry
SI	Source Investigation
SPE	Solid Phase Extractor
SS	Suspended Solids
STR	(Freshwater) Salinity Toxicity Relationship Model
TDS	Total Dissolved Solids
TEF	Toxicity Emission Factors
TER	Toxicity Emission Rate
TIE	Toxicity Identification Evaluation
TIME	Toxicity Investigations of Mining Effluent
TOC	Total Organic Carbon
TRC	Total Residual Chlorine
TRE	Toxicity Reduction Evaluation
TSS	Total Suspended Solids
TTE	Toxicity Treatability Evaluation
TU	Toxic Units
U.S. EPA	United States Environmental Protection Agency
UV	Ultra Violet
WAD	Weak Acid Dissociable
WET	Whole Effluent Toxicity

ACKNOWLEDGEMENTS

This document was written by Lesley Novak, Keith Holtze and Chris Wren (ESG International Inc.), and Grant Feasby, Liang Xue Liu and Richard Wagner (Lakefield Research Limited). This document was based on previous guidance documents, reports, and the published literature on Toxicity Reduction Evaluations (TREs). Richard P. Scroggins (Method Development and Application Section, Environment Canada, Gloucester, ON) was the Scientific Authority for the project, providing general guidance, detailed proofing, and technical assistance throughout the work.

Members of the Toxicological Investigation of Mining Effluents (TIME) Network Project Planning Group and Members of the TRE Project Task group are thanked for their participation in the development and review of this document: Brian Bell (The Mining Association of Canada), Christopher Doiron (Environment Canada), Alain Dubreuil (Natural Resources Canada), Jim McGeer (Natural Resources Canada), Julie Schroeder (Ontario Ministry of the Environment), Patrick Finlay (Environment Canada), Elizabeth Gardiner (The Mining Association of Canada), Yousry Hamdy (Ontario Ministry of the Environment), Charlene Hogan (TIME Secretariat), Brennain Lloyd (Northwatch), Sue Moodie (Yukon Conservation Society), Alan Penn (Cree Regional Authority), Walter Sencza (The Mining Association of Canada), Ian Sharpe (BC Environment). John Botts (Aquatic Sciences Consulting; Woodbine, MD, USA), an external reviewer, is also thanked for his technical comments on the document.

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1 INTRODUCTION

1.1 Background

The draft Metal Mining Effluent Regulation (MMER) requires that all Canadian metal mines produce effluent that is non-acutely lethal to rainbow trout when tested in accordance with Environment Canada (2000a) Reference Method EPS 1/RM/13. Mine operations will also be required to monitor the acute lethality of effluent to *Daphnia magna* in accordance with Environment Canada (2000b) Reference Method EPS 1/RM/14.

In accordance with the draft MMER, metal mines will be required to conduct monthly rainbow trout and *Daphnia magna* acute lethality tests using the full strength (100%) effluent. Once 12 consecutive passes (#50% mortality in 100% effluent) with rainbow trout are obtained, testing frequency can be reduced to quarterly assessments of acute lethality (using both species). Results from historical acute lethality tests can be used towards the 12 consecutive rainbow trout passes, provided the tests meet the required quality assurance requirements outlined in the draft MMER.

If a rainbow trout test produces mortality of more than 50% of the test organisms in 100% effluent, the sample is considered to "fail" the acute lethality test. Subsequent samples must then be assessed for acute lethality (with both species) twice per month, until 3 consecutive passes with the rainbow trout tests are achieved. Monthly tests are then conducted to obtain 12 consecutive rainbow trout passes.

In the event of a failure, the draft MMER requires that the mine implement a plan to investigate the cause of acute lethality. The Toxicity Reduction Evaluation (TRE) approach developed by the U.S. EPA (1989) is a commonly used step-wise approach designed to assist industrial dischargers in eliminating final effluent acute lethality. A TRE is a site-specific study designed to identify the substances responsible for acute lethality, isolate the source, evaluate the effectiveness of control options, and confirm the reduction in acute lethality of the final effluent. Toxicity Identification Evaluations (TIEs) are a set of procedures that identify the specific substance responsible for acute lethality, and are often applied as a subset of tools used in a TRE (Ausley *et al*., 1998).

Results outlined in two studies commissioned to evaluate TRE use by Canadian mines indicated that the TRE was a valuable tool in assisting mines achieves consistently non-lethal effluents (Beak International Inc., 2000; ESG International Inc., 1998). However, these studies identified several challenges and limitations concerning application of the U.S. EPA methods for Canadian metal mining operations, including;

- Adaptation of the generic U.S. EPA TRE methods for application with Canadian metal mines was lacking. Specifically, the U.S. EPA TIE methods were developed for use with fathead minnows (*Pimephales promelas*) and *Ceriodaphnia dubia* for a variety of effluents (i.e., pulp and paper, mining, municipal wastewater). As generic methods, they are limited in their application to Canadian metal mining operations and regulatory test species (i.e., rainbow trout).
- Advances in TIE methodologies (since the original U.S. EPA publications; 1991a,b; 1993a,b) have not been updated.
- Variable effluent quality was experienced at some mines.
- Transient and seasonal lethality was commonly observed in mining effluent.

- Acute lethality resulting from mining effluent was often caused by multiple toxicants and could be pH-dependent.
- Matrix effects (i.e., when toxicants interact with other effluent constituents in ways that change their toxicity) were often observed in mining effluent.

Based on these issues, modifications to the standard U.S. EPA TRE approach were considered necessary for use with Canadian metal mining effluents. In late 1999, the Toxicological Investigations of Mining Effluent (TIME) network was formed. The objectives of the TIME network include the sponsorship of projects aimed at broadening the collective knowledge with respect to causes of and solutions to effluent toxicity. To that end, Environment Canada and the Mining Association of Canada sponsored the development of a Guidance Document for conducting TRE investigations of metal mining effluents with a specific focus on the challenges faced by the Canadian metal mining sector.

1.2 Purpose and Scope

The purpose of this document is to provide TRE guidance specifically focused on challenges faced by the Canadian metal mining sector in order to assist mining facilities in meeting the acute lethality requirements for both rainbow trout and *Daphnia magna*. It does not address issues related to sub-lethal toxicity. This Guidance Document is intended to provide mine managers with an effective tool for implementing an appropriate strategy for resolving acute lethality issues, provide laboratories with a useful guide for conducting TRE studies with metal mining effluents, and ultimately, increase the likelihood of achieving and maintaining consistently non-acutely lethal metal-mining effluent. It is not intended to replace the existing U.S. EPA documents, but rather to provide supplementary guidance specific for application with Canadian regulatory species and metal-mining effluents. Furthermore, it is important to recognize that methods for the reduction of acute lethality are not limited to the guidance provided in this document. As new methods or approaches become available, these procedures should be included for consideration as part of the TRE. The guidance provided can be applied using both *Daphnia magna* and rainbow trout. Although limits on *Daphnia magna* acute lethality are not required in the MMER, certain provinces set limits for mines using *Daphnia magna* (Appendix A).

1.3 Literature Review

The initial phase of work involved a thorough review of all readily available information relevant to TREs in the context of the Canadian metal mining sector. A brief description of each document reviewed is provided in the following section, with a detailed summary in Appendix B. The purpose of the review was to provide the user with an understanding of the current state of knowledge for the relevant TRE documents, which form the basis for development of this metal-mining specific Guidance Document. A comprehensive understanding of the following documents will be critical to the success of a TRE for the mining sector.

A recently published TRE document, "Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants" (U.S. EPA, 1999), was not included in the literature review because of its' specificity to municipal wastewaters. However, the general principles and approaches described may be of use to some metal mining TREs. A reference is provided in the Reference List.

1. *Report on Technologies Applicable to the Management of Canadian Mining Effluents* (SENES Consultants and Lakefield Research, 1999)

Environment Canada commissioned a study to review the Best Available Technologies (BAT) for effluent treatment in the base metal, gold, uranium and iron ore mining sectors. The study included; i) a review of mines and effluent control technologies in use, ii) identification and assessment of new technologies, iii) an evaluation of passive treatment technologies, iv) identification of the effectiveness of technologies to reduce ammonia, alkalinity and other toxicants, v) selection of BAT technologies and vi) generation of cost/benefit information. The BAT technology performance was compared to the Metal Mining Liquid Effluent Regulations (MMLER's) and found that the average performance was well within MMLER objectives. SENES Consultants noted that acute lethality was not one of the MMLER requirements, but that "technologies are available to control acute lethality when the cause of toxicity is identified". It was also concluded that the application of the new TSS limit and the introduction of cyanide and non-acute lethality limits would result in increased costs to the mining industry in Canada. The application of BAT across the industry would require the use of add-on technologies that result in the use of additional chemicals (organic polymers, precipitants, and quaternary ammonium compounds). These agents could cause a toxic response and "residual levels should be controlled to assure acute lethality does not arise". The estimated cost for upgrading to BAT levels would be $646 million (Net Present Value). Additional costs were estimated for meeting non-acutely toxic effluents at $193 million to $297 million. Each facility that produced acutely toxic results would require a toxicity evaluation study at the cost of $100,000 to identify the cause of toxicity. The report cautions that costs to meet BAT limits and non-acutely lethality requirements are not necessarily directly additive.

2. *Evaluation of Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) Application to the Canadian Mining Industry* (ESG International Inc., 1998)

The Aquatic Effects Technology Evaluation (AETE) Program commissioned a study to evaluate and summarize the experience of the Canadian mining industry with Toxicity Reduction Evaluations. The objectives were: i) to complete a critical evaluation of the quality of TRE data, its benefits and limitations and, ii) to conduct a survey to evaluate the utility of the TRE strategies, including discussions on TIEs and effluent treatment, in determining and/or addressing aquatic impacts from mining operations. The report also describes the results of 5 TRE case studies. The study reported that a complete assessment of the Canadian mining sector's experience with the TRE process was not possible since less than 50% of mines responded to the survey. Of 42 mines that responded, only 25 (57%) reported having experienced acute toxicity. Of those mines reporting toxic effluents, 7 (28%) indicated that a TRE had been conducted and 17 (76%) reported having conducted at least one Phase I TIE. Very few mines reported going beyond the Phase I toxicity characterization. Ammonia was the most commonly identified contaminant of concern and effluent toxicity appeared to be highly pH dependent.

3. *Generalized methodology for conducting industrial toxicity reduction evaluations.* (U.S. Environmental Protection Agency, 1989; EPA-600/2-88/070)

The U.S. EPA document provides guidance for the implementation of TREs at industrial facilities. A generic approach for designing and conducting a TRE is described, including case studies from a variety of industries. The overall objective of a TRE is to determine those actions necessary to achieve compliance with water quality based effluent limits. The TRE may identify a remedial action as simple as improved "housekeeping" or the need to modify the wastewater treatment system. Alternatively, a TRE

may involve toxicant identification. The document provides generic methods primarily for compliance with whole effluent toxicity limits.

4. ***Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures.*** **(U.S. Environmental Protection Agency, 1991; EPA-600/6-91/003)**

The U.S. EPA document describes procedures used to assess the nature of acute effluent toxicity. It includes discussions on various topics related to the conduct of a Phase I Toxicity Identification Evaluation (TIE), including: health and safety, quality assurance, facilities and equipment, dilution water, effluent sampling and handling, toxicity test, characterization tests, additional tests and interpretation of Phase I results. The two objectives in the Phase I TIE are to: i) characterize the substance responsible for toxicity, and ii) determine if the characteristics of the substance(s) responsible for toxicity are consistently the same over time. The generic methods can be used for assessing the cause of acute lethal toxicity in wide variety of effluents, receiving waters, elutriates, pore waters and leachates.

5. ***Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity.*** **(U.S. Environmental Protection Agency, 1993; EPA-600/R-92/080).**

The original U.S. EPA Phase II document was published in 1989, and covered Phase II approaches for acute toxicity only. The revised Phase II document (1993) provides identification schemes for non-polar organics, ammonia, metals, chlorine and surfactants causing either acute or chronic toxicity. For the purposes of this study, only the acute toxicity portion of the 1993 document was summarized. In Phase II of a TIE, further effluent treatments are conducted to identify the specific substance(s) responsible for toxicity. The additional treatments and analytical methods chosen are directly related to those treatments observed to effectively eliminate or reduce toxicity during Phase I.

6. ***Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity.*** **(U.S. Environmental Protection Agency, 1993; EPA-600/R-92/081).**

The original U.S. EPA Phase III document was published in 1989, and covered Phase III approaches for acute toxicity only. The revised Phase III document (1993) provides confirmation procedures for substances causing either acute or chronic toxicity. For the purposes of this study, only the acute toxicity portion of the 1993 document was summarized. The objectives in Phase III are to: i) confirm that the substances responsible for toxicity have been correctly identified, ii) ensure that all of the toxicity has been accounted for, and iii) confirm whether the same substance(s) is responsible for the observed toxicity. A "weight-of-evidence" approach is used during Phase III to confirm that the substances responsible for toxicity have been identified. The procedures used during Phase III may include: 1) correlation analyses, 2) observations of symptoms, 3) relative species sensitivity, 4) spiking, 5) mass balance, and 6) various adjustments to water quality.

7. ***Non-Acutely Lethal Mining Effluent Technologies (NALMET) Program – 1999 Studies (Beak International Inc. 2000)***

Environment Canada commissioned a study to demonstrate the use of toxicity identification evaluation (TIE) and toxicity treatability evaluation (TTE) on representative "challenged" mine effluents, as tools that may assist in achieving compliance with the new Metal Mining Effluent Regulation. The objectives were to: i) evaluate various approaches to TIE and TTE investigations and relevant studies applicable to Canadian mine effluent acute lethality situations in support of the NALMET program of Environment

Canada, and ii) demonstrate a combined effluent TIE/TTE approach for acute toxicity challenged mines in Canada. Two mine effluents were subjected to Phase I TIEs. For Mine A, the authors indicated that during the first TIE, ammonia, copper and zinc were the most important toxicants for fathead minnows and *Daphnia magna*. During a second TIE, ammonia alone could have accounted for the observed rainbow trout mortality, but additional toxicants (cyanate, copper, nitrite) were suggested as possible contributors to *Daphnia magna* and fathead minnow mortality. Based on the TTE results for Mine A, the authors suggested a number of possible options that could be used to treat copper, ammonia and cyanate. For Mine B, the TIE results suggested that TDS was the cause of toxicity to both *Daphnia magna* and fathead minnows. Increased toxicity to *Daphnia magna* at low pH suggested the presence of a second toxicant, which may have been metal. A third toxicant (thiosalt) was suggested for rainbow trout. The authors did not recommend that a TTE study be conducted, since changes to the treatment system were in progress.

8. *Toxicity Reduction: Evaluation and Control* (Water Quality Management Library, Edited by D.L. Ford 1998)

This book provides an overview of toxicity reduction evaluations (TREs), and includes discussions on TI-REs, organic toxicant control, toxicant control using biological treatment, toxicity reduction methodologies, and case histories for a variety of industrial effluents. The case studies include TREs for the petroleum, municipal, chemical processing, and pulp and paper sector, but did not include any mining sector examples. The toxicity reduction methodologies include those for control of cationic toxicants, precipitation/co-precipitation of inorganic toxicants, inorganic anions, cyanide and ammonia.

2 THE TOXICITY REDUCTION EVALUATION (TRE) PROCESS

As defined by the U.S. EPA (1989), the objective of the TRE is to determine the actions necessary to reduce effluent toxicity to acceptable levels. The U.S. EPA approach includes six tiers;

1) Information and data acquisition
2) Evaluation of remedial actions to optimize the operation
3) Toxicity identification evaluation (TIE)
4) Identification of the source of the toxicity (Source Investigation)
5) Identification and evaluation of treatment methods to reduce toxicity in the final effluent (Toxicity Treatability Evaluation)
6) Confirmation of removal of toxicity

The six tiers of the U.S. EPA approach are briefly described in the following sections and outlined in Figure 1 (see Appendix B for a more detailed description).

Tier 1, Data Acquisition and Facility-Specific Information, involves the collection and analysis of available information and data that might be useful in designing and directing the most cost-effective study.

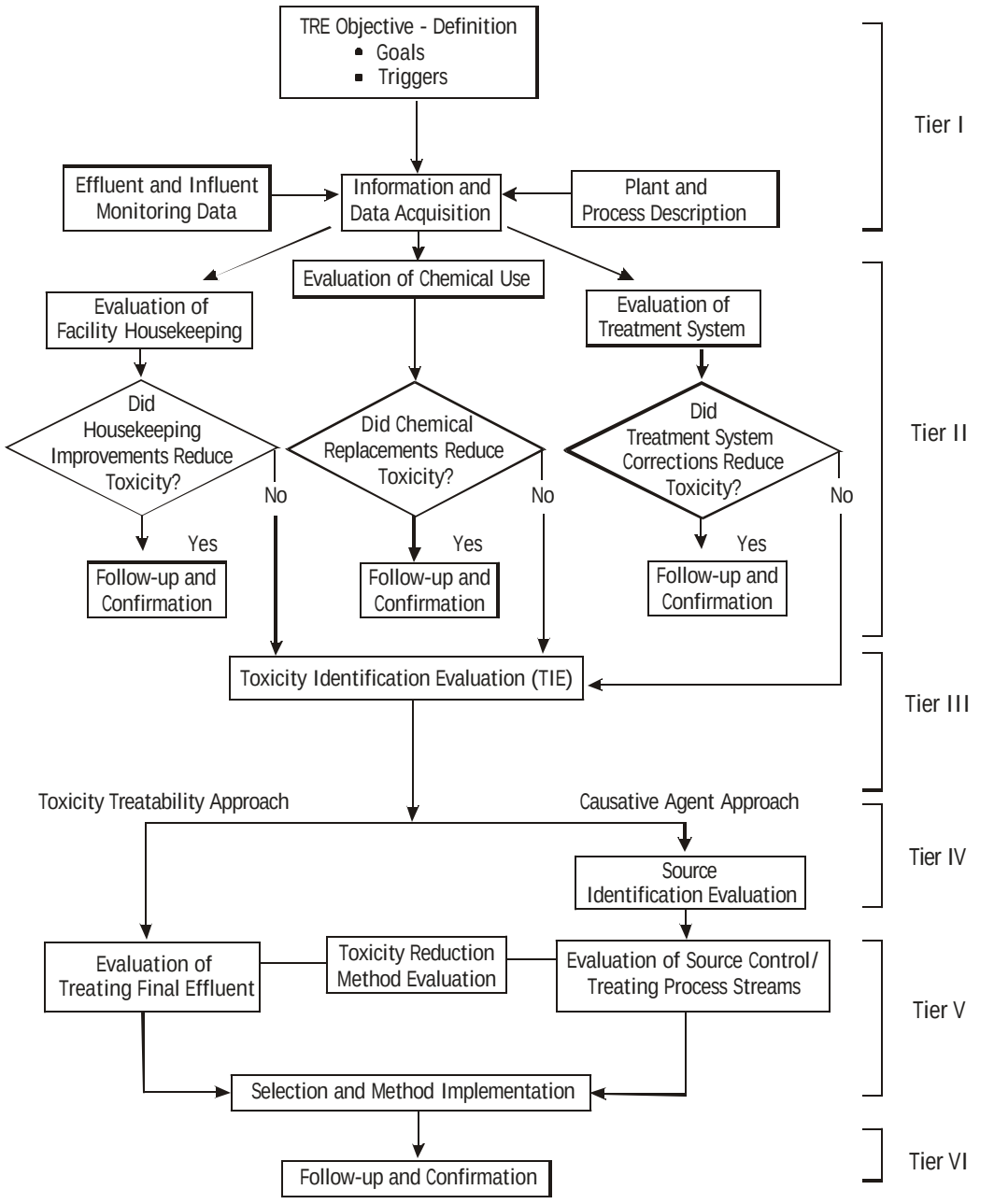
Tier 2, Evaluation of Remedial Actions to Optimize Facility Operations, includes an evaluation of: i) housekeeping practices, ii) treatment plant optimization, and iii) chemical optimization.

Tier 3 involves application of the Toxicity Identification Evaluation (TIE) procedure. The objective of the TIE is to identify the specific substances responsible for acute lethality. The TIE process is divided into three phases. Phase I involves characterization of the toxicants through a variety of effluent treatments (U.S. EPA 1991a). Phase II involves identification of the suspected toxicant(s) (U.S. EPA, 1993a). Confirmation of the suspected toxicants occurs in Phase III (U.S. EPA, 1993b). Phase I, II and III usually occur sequentially, but may be conducted simultaneously when patterns of toxicity begin to emerge during Phase I. Information from a TIE may be used to direct toxicity reduction efforts, such as chemical substitution or process/operation modifications.

Tier 4, Sources Investigations (SI), and Tier 5, Toxicity Treatability Evaluations (TTEs), may be used as alternative strategies in combination with, or as alternatives to, a TIE. Source Investigations determine whether the toxicants may be isolated in one or more waste streams. The approach to SIs may include identification of discharge locations and inputs to the effluent treatment plant (ETP), characterization of each discharge in terms of flows, acute lethality and chemical composition, and use of a mass balance approach to identify those streams representing the largest contribution to acute lethality and chemical loading.

In the case where a TTE is the appropriate course of action, the investigator will usually evaluate the performance of different effluent treatment technologies (to reduce or eliminate acute lethality) either at the bench-scale, or directly at the effluent treatment plant. Toxicity removal technologies might include pH adjustment, metals precipitation, reverse osmosis, advance oxidation processes etc. Once removal of acute lethality has been demonstrated at the bench-scale level, a decision can be made to pilot the technique on a larger scale or directly at the existing treatment facility. At this point, the acute lethality removal technique can be assessed for compatibility with the existing system. Other TTE approaches may include an evaluation of best management practices (BMP), flow sheet alteration or changes in operating practices. The selected approach will be site specific.

Tier 6, Follow-Up and Confirmation, involves implementing an appropriate monitoring program to confirm that the acute lethality control method has successfully met the required compliance limits (#50% mortality in 100% effluent).



Project Name: TRE GUIDANCE DOCUMENT	Date: January, 2002	FILE: E1157_01	Figure 1
Prepared for: ENVIRONMENT CANADA AND THE MINING ASSOCIATION OF CANADA	Revised:	U.S. EPA (1989) TOXICITY REDUCTION EVALUATION (TRE) FLOW CHART	
	Revision No.		

2.1 Application of TRE for New Mines

It is important to recognize that the TRE process does not only apply to operating mines, but can be undertaken before a mine begins operation or has an effluent. Effluent Treatment Plants (ETPs) are traditionally designed by engineers to meet specific chemical limits. Achieving compliance with acute lethality limits has only recently been a consideration in the design stages of an ETP. Assessments of acute lethality during bench, pilot and full-scale evaluations should be included in the selection of appropriate effluent treatment technologies. In cases where the proposed effluent treatment does not eliminate acute lethality, the TIE process could be used to identify the substance(s) responsible. Once the toxicant is identified, the proposed treatment system could then be modified to ensure removal of the toxicant. Toxicity results need to be tracked throughout the process since performance changes often occur when implementing any system from the bench or pilot to full-scale operation. This monitoring will increase the likelihood that the proposed treatment system will meet both chemical and acute lethality limits.

2.2 Initiation of a TRE

The draft MMER regulation requires that mines report any acute lethality failures to the appropriate government authority and initiate an investigation of the effluent toxicity event(s). A single failure (i.e., >50% mortality in 100% effluent) should not imply that all components of a TRE will be required to resolve the problem. For example, there are almost no circumstances in which a single acute lethality failure would "trigger" a TIE. In most cases, additional information should be collected to demonstrate that a TIE approach would have an opportunity for success (Ausley *et al*., 1998). The recommended actions for initiating a TRE are outlined in Figure 2, and could include (but are not limited to):

- Develop an "Acute Lethality Response Plan".
- Review acute lethality test data and facility operations during first sample failure.
- Accelerated sampling and acute lethality testing.
- Collect and analyze available information and data (Tier 1).
- Optimize facility operations, including an evaluation of: i) housekeeping practices, ii) treatment plant optimization, and iii) chemical optimization (Tier 2).

Although the initial approach to any TRE may have similar components, the sequence of events or steps taken will be site-specific and depend on the nature of the toxicant, as well as the results and findings from each phase of work.

2.2.1 Acute Lethality Response Plan

The first step in the TRE process begins prior to experiencing the first failure, and involves the development of an "Acute Lethality Response Plan". This plan will increase the speed and efficiency with which the acute lethality failures can be addressed, by facilitating the data acquisition phase (with respect to mine facilities/operations), and assist in the decision making process. The response plan should include (but is not limited to),

- Description of facility processes and operations
- Description of effluent treatment facility and process

- Line diagrams showing the major areas of operation and the main inputs to the ETP (noting that effluent retention and travel times in the system need to be considered and understood when attempting to identify cause and effect).
- Documentation of facility operations during collection of samples for routine acute lethality testing
- Characterization (for chemistry and toxicity) of process streams (including influent) over time to provide baseline data to be used for comparisons to samples collected during a toxicity episode
- Results from acute lethality tests and chemical analysis for routinely monitored parameters (summarized in an electronic format, if possible, for ease of retrieval and statistical analysis of data)
- History of compliance with other regulated chemical parameters that may influence effluent toxicity.
- Up-to-date list of Material Safety Data Sheets (MSDS) for chemicals used in the process and effluent treatment (with available toxicity data for rainbow trout and *Daphnia magna*)
- Identification of a notification protocol (who should be notified and when).

Selection of a response team may include consultants (i.e., aquatic toxicologists, engineers experienced in the TRE process, if not already available within the facility) and mine personnel (i.e., management, operations, support personnel for sampling) that are prepared to assist with TRE studies when needed.

Laboratories conducting the toxicity tests should be prepared to immediately notify mine personnel when a failure occurs, who in turn can then notify the response team. Good and immediate communication among all team members will speed the response to the failure and increases the likelihood of TRE success.

2.2.2 Review of Acute Lethality Test Data and Facility Operations

Immediately after the initial acute lethality failure is experienced, a review of the acute lethality test data should be conducted to ensure that all test conditions were met. Checklists developed by Environment Canada could be used as a tool for evaluating the test conditions (see Section 6). Water quality parameters (e.g., dissolved oxygen, pH, conductivity) measured during the test can also provide useful clues as to the cause of acute lethality. Facility processes and ETP operations at the time of sample collection should be reviewed to determine if the sample, which resulted in the failure, was collected under “normal” operating conditions (see Section 6). However, long effluent retention times at some facilities may make it more difficult to relate the toxicity failure to facility process or ETP operations. In this case, it may be necessary to determine what was happening to the effluent before treatment and discharge.

2.2.3 Accelerated Sampling and Acute Lethality Testing

Under the draft MMER, the frequency of testing in response to a rainbow trout test failure (>50% mortality in 100% effluent) is increased to twice monthly until 3 consecutive passes (#50% mortality in 100% effluent) are achieved with rainbow trout. These additional tests will provide some of the data necessary to establish the duration (i.e., length of toxicity episode) and frequency (i.e., toxicity is consistent or transient between samples) of acute lethality. However, additional testing beyond that required by the draft MMER may be necessary, particularly to establish the degree (i.e., magnitude) and persistency (i.e., how toxicity changes over time) of acute lethality, since these can influence the approach to a TRE study. It is important to recognize that TIE results can be difficult to interpret when the acute

lethality is marginal. For example, it can be difficult to discern differences in toxicity between the toxic final effluent and TIE treatments when the mortality in the full strength (100%) effluent is close to 50%.

The U.S. EPA (1991a) indicates that acute lethality should be present frequently enough and endure storage so that repeated testing can characterize, identify and confirm the substances responsible for mortality. Therefore, sufficient testing should be done to ensure consistent and persistent presence of mortality before a TIE study is initiated. The actual number of samples required to identify these factors will be site-specific and depend largely on effluent variability. Failure to understand the variability in effluent acute lethality and individual toxicants could lead to selection of treatment options or controls that do not consistently reduce acute lethality to compliance levels (U.S. EPA, 1999).

In the case where the effluent is acutely lethal to rainbow trout, potential surrogate test species to be used during a future TIE study could be included during accelerated testing (see Section 8). Because rainbow trout require large test volumes, a surrogate test species may be necessary for those TIE treatments limited by the ability to treat only small effluent volumes, or for mines located in remote areas where collection and shipment of large volumes of effluent could be difficult. The additional testing may increase the speed with which a TIE can be completed, since it will have already been determined if the species of interest (rainbow trout) and the surrogate species respond in a similar manner to the untreated effluent under a variety of conditions.

Concurrent chemical analysis for "common" metal-mining toxicants could also be conducted during the accelerated testing (see Sections 4 and 12.1). Collection of chemical data (when co-ordinated with toxicity testing) during this initial stage could provide useful information in the preliminary assessment of the causes of acute lethality. Even in cases where the effluent is non-lethal, the chemical data could prove useful for comparisons to samples where acute lethality is observed.

2.2.4 Data Acquisition and Facility Optimization

If the accelerated testing confirms the presence of acute lethality, Tier 1 (data acquisition and facility-specific information) and Tier 2 (evaluation of remedial actions to optimize facility operations) should be initiated (see Figure 1 and Section 5). Tier 1 involves the collection and analysis of available information and data that might be useful in designing an effective study. Most of the Tier 1 information should be readily available as part of the "Acute Lethality Response Plan". Tier 2, includes an evaluation of: i) housekeeping practices, ii) treatment plant optimization, and iii) chemical optimization.

If actions taken in response to Tier 1 and 2 investigations are unsuccessful in eliminating acute lethality, subsequent stages in the TRE will involve various approaches depending on the type of toxicity (see Section 6).

2.3 Rationale for Choice of Various Toxicity Reduction Efforts

An effective TRE must determine the appropriate combination of TIE, TTE, SI and alternative strategies to eliminate acute lethality. However, it is difficult to provide a generalized approach that will lead directly to specific TRE activities, since decisions must be made on a site-specific basis, by individuals experienced in the application of TRE tools (Ausley *et al*., 1998). Because effluent characteristics are unique to individual mines, the choice and combination of approaches will depend on several key factors including availability and quality of historical toxicity and chemistry data, the type of operation / process, the nature of the toxicant(s), and the type of toxicity. Furthermore, the approach to a TRE study will unfold as information about the toxic event (i.e. mine conditions at the time of sampling, nature of acute

lethality) becomes available. Therefore, the full scope of a TRE cannot be outlined at the start of the study, since the results of initial tasks often lead to directions not anticipated in the beginning. The initial scope of work can be defined (to ensure all key TRE components are addressed; see Figure 1), however, the results of each phase of study help to define the scope of subsequent tasks.

The experience of the investigator(s) and effective communication between the investigator(s) and mine personnel will also be critical in the selection of an appropriate TRE approach. In cases where the mine facility has experienced an acute lethality failure, but historically has been able to achieve a consistent non-acutely lethal effluent, information on changes in the facility, process or effluent treatment will be important in selecting the appropriate TRE approach. For example, mine personnel may be aware of changes or upsets in the mining, milling or refining process or effluent treatment, which may lead to immediate on-site controls or adjustments to eliminate acute lethality.

The following section is intended to provide some general guidance for the selection of an appropriate TRE strategy.

2.3.1 Toxicity Identification Evaluation (TIE) Approach

The TIE approach will be most effective if acute lethality is consistent, sufficient and persistent (i.e., does not degrade over time). In this case, characterization of the toxicant(s) (Phase I TIE) should be conducted. If successful, it is often desirable to identify (Phase II) and confirm (Phase III) the specific substance responsible for acute lethality prior to conducting a TTE or SI. Once the toxicants are known, specific measures can be taken to remove them. Alternatively, characterization of the effluent may provide sufficient information without specifically identifying the substance(s) responsible (i.e., slight adjustment of pH eliminates toxicity). The information generated during the Phase I TIE could be used to modify the existing treatment system, or implement new treatment methods (TTE approach).

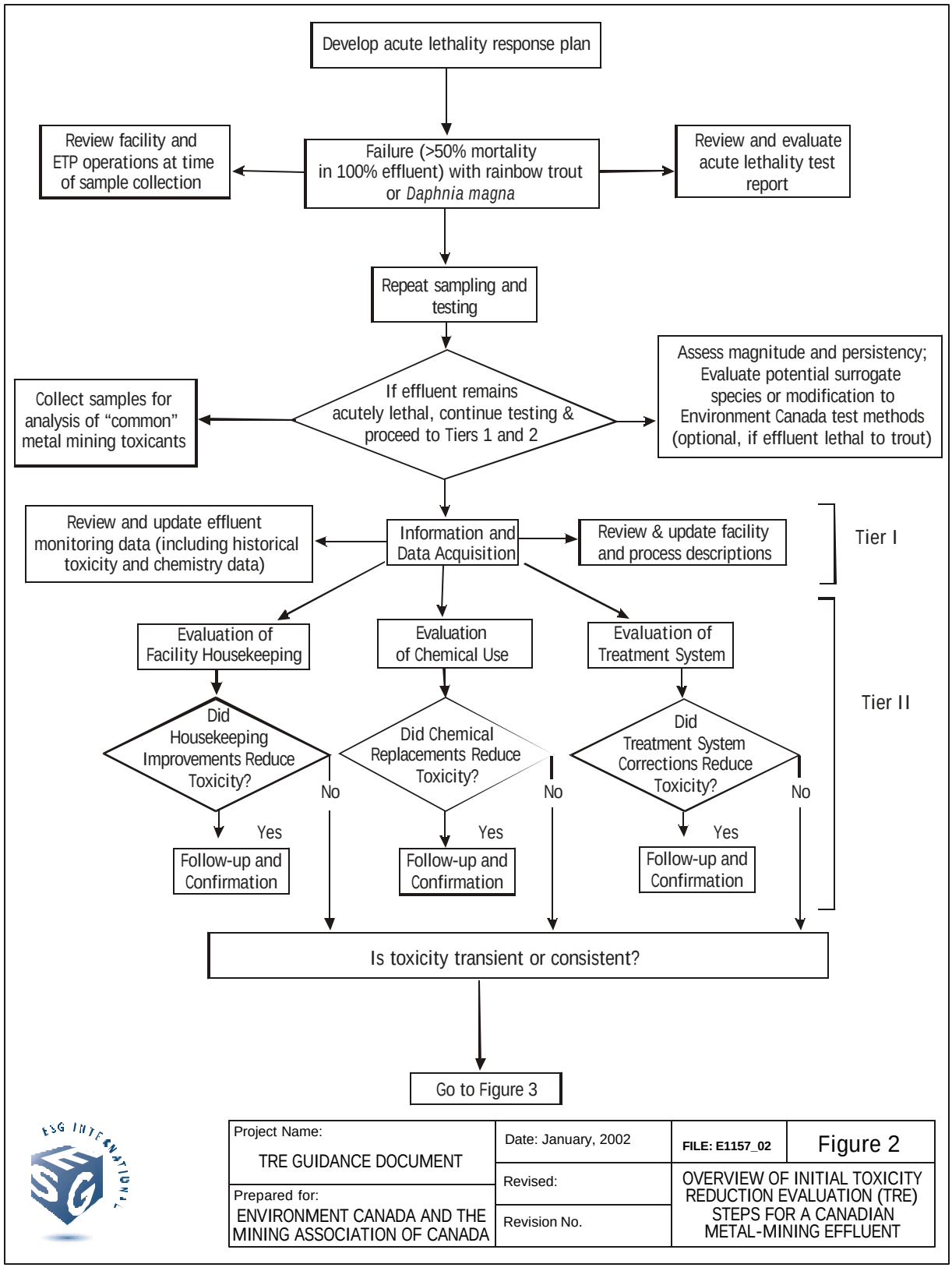
The TIE approach will be less effective and more difficult to complete if acute lethality is transient, marginal or non-persistent. Random toxicity events may require the analysis of more samples and, in some cases, may even necessitate abandoning TIE work on individual toxic samples (Ausley *et al*., 1998). Alternative approaches (see Section 7), in combination with TTE and SI evaluations, may be more successful under these conditions.

2.3.2 Toxicity Treatability Evaluation (TTE) Approach

Based on the results from the TIE, the TRE can proceed to: i) an SI to identify the source of the toxicant, or ii) a TTE to evaluate various treatment methods, chemical optimization, substitution of process chemicals, or changes in operational or management practices. The TTE is the most direct approach to elimination or reduction of a suspected toxicant and will be most effective if the toxicant(s) can be identified and treatment technologies are available to reduce or eliminate the substance(s) (see Section 12). The TTE approach can also be applied if the toxicant(s) has been characterized, but not identified. For example, the toxicant has been classified as a metal, but the specific metal(s) has not been identified. In this case, the TTE may involve evaluation of those Phase I treatments observed to eliminate acute lethality. However, there will be more uncertainty associated with TTE studies based on toxicant characteristics alone (rather than the known identity of the substance(s) responsible for acute lethality).

2.3.3 Source Investigation (SI) Approach

The SI approach to identify the source(s) of acute lethality, will be most effective when the substance(s) responsible has been identified, and discrete multiple inputs within the process (or entering the ETP) exist and can be tested. The approach would involve the use of chemical specific analysis or in-plant toxicity testing to identify and confirm which of the source streams contained the suspected toxicant (see Section 13). Once a specific process stream has been identified as the source of toxicity, a TTE could be conducted to reduce or eliminate the substance(s). The SI approach could also be a viable alternative to identify sources of toxicant types or toxicity in cases where the TIE does not conclusively identify the toxicant(s), or if acute lethality is transient or non-persistent. However, greater uncertainty will be associated with results from the SI approach if tracking of a specific substance cannot be conducted since the toxicant has only been characterized, but not identified in the final effluent (U.S. EPA, 1989).



Project Name: TRE GUIDANCE DOCUMENT	Date: January, 2002	FILE: E1157_02	Figure 2
	Revised:	OVERVIEW OF INITIAL TOXICITY REDUCTION EVALUATION (TRE) STEPS FOR A CANADIAN METAL-MINING EFFLUENT	
Prepared for: ENVIRONMENT CANADA AND THE MINING ASSOCIATION OF CANADA	Revision No.		

2.4 Summary of Key TRE Facts

In order to resolve acute lethality using a TRE, it is necessary to understand the factors that will contribute to a successful study. An overview of some of the important benefits and limitations was obtained from the U.S. EPA documents (1989, 1991a, 1993a and b, 1999), and reports produced specifically for the Canadian metal mining sector by ESG International (1998) and Beak (2000). Additional comments were also obtained from a report on the application of TIEs and TREs for effluent toxicity, produced by the Whole Effluent Toxicity (WET) Expert Advisory Panel of the Society of Environmental Toxicology and Chemistry (SETAC) (Ausley *et al*., 1998).

- Each TRE is unique. A strategy should be developed for each study that accounts for site-specific conditions and allows flexibility in the study design, including the use of alternative tools and techniques.
- The overall success of a TRE is based on a number of factors including the experience of the laboratory personnel performing the tests and the variability in effluent quality, and usually a combination of three approaches; Toxicity Identification Evaluations (TIEs), Source Investigation (SIs) and Toxicity Treatability Evaluation (TTEs).
- TREs are more likely to be successful when an effluent is consistently toxic, if the loss of toxicity is minimal over time and if the factors contributing to toxicity do not vary from one sample to the next. Conversely, the process can be rendered more difficult if toxicity is transient, if the samples quickly lose toxicity over time, if toxicity is marginal, or if the factors contributing to toxicity are variable
- A successful TRE requires teams of individuals with a variety of expertise including, aquatic toxicologists, chemists, treatment and process engineers, and mine operators
- Selection of an appropriate TRE team should be based on experience of the investigators, and not necessarily based on the lowest price.
- Good communication between the mine and TRE investigators is critical to study success
- Knowledge of the specific chemistry of the mine, its treatment system, the generic contaminants found with each class of mine, and knowledge of mine treatment technologies are important in designing a successful study
- TIEs do not "prove" the cause of toxicity, but rather use a weight of evidence approach
- The TIE process incorporates the responses of organisms into the assessment of complex effluent mixtures to determine the identity of the substance(s) responsible for acute lethality
- The TIE process allows matrix effects and bioavailability to be quantified.
- The TIE process can focus chemical analysis by providing the characteristics of the suspected toxicants; without some knowledge of the toxicant, broad-spectrum analyses (e.g., GC/MS, HPLC) are less sensitive and costly
- The majority of unsuccessful TIEs occur when toxicity is too transient to effectively apply TIE techniques, when the individuals performing the TIEs are unqualified or inexperienced in the techniques, or when the TIE treatments are ineffective due to technical limitations related to the current state of the science of TIE methodologies

- Identification of the specific substance(s) responsible for acute lethality is not always necessary in order to develop adequate control options for achieving and maintaining a consistently non-acutely lethal effluent
- The U.S. EPA TIE guidance documents are not intended to be a comprehensive list of TIE methods; other procedures have been successfully utilized
- Phase I TIEs are designed to characterize a wide spectrum of contaminants. Modification to the method (i.e., omission of the certain treatments) can result in certain contaminants being overlooked.
- The Phase I TIE can provide an essential foundation for conducting a TTE or SI
- Beyond Phase I, the TIE approach is not standardized and subsequent studies to identify the specific toxicants require experienced personnel; if Phase II (identification) and Phase III (confirmation) studies are to be successful, it is crucial that the tests are well planned and scientifically defensible.
- Through out the TRE process repeated testing is required to account for effluent variability and confirm that the cause of acute lethality is the same under all conditions
- Appropriate and relevant chemical analysis should be coordinated with toxicity testing on untreated and treated effluent samples
- The lack of statistical comparisons may not be critical at certain stages of a TIE study, where gross changes in toxicity are the primary consideration; however, large amounts of data can become unmanageable and difficult to interpret without statistical analysis
- The generation of a sufficient amount of data to provide strong evidence regarding the identification of the toxicant is critical if the mine is to consider investment in costly plant-scale remedial measures; toxicity testing must be included in all bench scale and pilot plant studies.
- The TTE is the most direct approach to elimination or reduction of a suspected toxicant and will be most effective if the toxicant(s) can be identified and treatment technologies or other approaches (i.e., operational or management practices, chemical substitution) are available to reduce or eliminate the substance(s)
- The TTE approach can be applied if the toxicant(s) has been characterized, but not identified. However, there will be more uncertainty associated with TTE studies based on toxicant characteristics alone (rather than the known identity of the substance(s) responsible for acute lethality).
- The SI approach will be most effective when the substance(s) responsible has been identified, and discrete multiple inputs within the process (or entering the ETP) exist and can be tested.
- The SI approach could be a viable alternative to eliminate final effluent acute lethality in cases where the TIE does not conclusively identify the toxicant(s), or if acute lethality is transient or non-persistent. However, there will be more uncertainty associated with SI studies based on toxicant characteristics alone (rather than the known identity of the substance(s) responsible for acute lethality).
- The SI approach may identify opportunities to recycle concentrated or heavily contaminated streams back to the process, for additional metal recovery, reagent savings, and reduced effluent acute lethality.

3 SAMPLE COLLECTION, TRANSPORT AND STORAGE

Issues related to the collection, transport and storage of effluent samples for TIEs are discussed in this section.

Guidance on sample collection, transport and storage of effluent samples for routine acute lethality tests can be found in the Environment Canada test methods for rainbow trout (EPS 1/RM/13) and *Daphnia magna* (EPS 1/RM/14). Guidance on key aspects of sample collection, storage and handling, which are important in maximizing data reliability and reducing variability among and within laboratories, can also be found in the "Guidance Document on Acute Lethality Testing for Mining Effluents" (TIME, 2002). Additional considerations related to the collection, transport and handling of effluent samples specifically for use in a TIE investigation are discussed in the following sections. A checklist is provided in Table 1.

3.1 Sample Collection

3.1.1 Mine and Lab Co-ordination

TIEs require a certain level of planning and co-ordination, since they involve a major sampling effort by mine personnel, and extensive use of laboratory staff and resources. Therefore, all activities relating to sample collection, handling, transportation and study initiation should be co-ordinated between the mine/mill site and the laboratory conducting the tests.

Good communication within a mine/mill site (i.e., between operations and environmental department, if one exists) is required to ensure samples collected are representative of "typical" or "normal" operating conditions (see Section 6). The process/production side should be aware that changes in these areas could have significant impacts on the quality/toxicity of the effluent generated.

Sample collection schedules must be co-ordinated with the test laboratory to ensure the availability of resources, including test organisms, technicians experienced in conducting TIEs, laboratory bench-space and available space for sample storage. In most instances, the laboratory will provide sampling containers, labels and chain-of-custody forms for the mine/mill site. Instructions for the proper collection of effluent samples should also be provided in order to ensure the procedures are standardized between samples.

3.1.2 Representative Samples

Samples to be used for TIE investigations should not be collected during known upsets or atypical operations. TIEs should be undertaken if correction of the upset or atypical operation has been ineffective at eliminating acute lethality (See Section 6). Therefore, documentation of facility conditions (i.e., process/production and effluent treatment system, weather conditions) at the time of collection (typically by on-site personnel) will be useful in determining if the samples for TIE investigations are representative of "typical" or "normal" operating conditions. The information documented should be included as part of the "Acute Lethality Response Plan", since it could be useful to compare conditions experienced during, and prior to, the acute lethality event.

The information documented should include:

i) the status of process/production (to assess variability in operational conditions at the time of sampling),

ii) routinely monitored parameters (i.e., TSS, metals, ammonia, pH; these may provide an indication of the operational status of either the facility or effluent treatment system),

iii) measurements of samples when collected: pH, temperature, D.O., conductivity, hardness, alkalinity, ammonia, etc. as appropriate, and

iv) weather conditions at the time of sample collection (precipitation could dilute the effluent resulting in a less toxic effluent; alternatively, potentially toxic runoff (e.g., drainage from the plant yards and waste piles) may contribute to acute lethality of the final effluent).

3.1.3 Sample Type

The selection of grab or composite samples will depend on the discharge situation, questions to be answered by the TIE and stage of the TIE. Composite samples may be easier to use during Phase I, when samples that are different from one another produce results that are difficult to interpret. If toxicity is low, not persistent or intermittent, grab samples may be preferable during all Phases (U.S. EPA, 1991a). Initially, the type of sample to be collected should be similar to those used for the tests that "triggered" the TIE investigation.

The type of sample (i.e., grab versus composite) used during Phase II should be the same as that used during Phase I. One composite or grab sample should be used for the identification and confirmation tests. However, once the substance responsible is identified, multiple samples may be analyzed for the toxicant. If multiple samples are used for one test, correlations may be difficult since the toxicant may not be present in every sample, toxicant concentrations may vary, or other toxicants may appear. Variability must be identified, but should be done after at least one of the toxicants is known (U.S. EPA, 1993b).

3.1.4 Sample Volume

Prior to collection of an effluent sample for a TIE study, the approximate volume required to complete the tests must be determined. When compared to conducting an Environment Canada acute lethality tests with rainbow trout or *Daphnia magna*, substantially larger sample volumes are required for a Phase I TIE. Estimates of the volume of effluent required for completing one full Phase I TIE (as described in Section 8) using rainbow trout and *Daphnia magna* are provided in Table 2. However, the volumes required will vary depending on the test species, test conditions, number of Phase I treatments conducted, and the amount of testing to be conducted on a single sample. Therefore, the laboratory conducting the investigations must be consulted to confirm the required effluent volumes.

Table 1. Checklist for collection, transportation and storage of effluent samples for use in TIEs

Item	Completed (Yes/No)
Initial Planning and Co-ordination	
Communicate with lab to schedule TIE, arrange for supply of sampling equipment and to discuss sample volume requirements.	
Confirm TIE test schedule with laboratory	
Confirm required sample volumes with test laboratory	
Arrange for supply of sample containers, liners, labels, chain of custody forms	
Sample Collection	
Sample type (i.e., grab, composite) identified and documented	
Sampling containers labelled with source, sample type, name of sampler, date and time of sample collection	
Document sample conditions at time of collection (i.e., temperature, D.O. pH, conductivity, total ammonia)	
Document status of operation at time of sample collection	
Document condition of treatment system at time of sample collection	
Document weather conditions at time of sample collection	
Sample Transportation	
Ensure shipping arrangements are made for immediately delivery of samples to laboratory	
Contact laboratory to confirm shipment and expected delivery date	
Ensure appropriate labels are placed on buckets and shipping forms	
Ensure chain-of-custody forms are completed and included with shipment	
Ensure samples are kept cool immediately prior to, and during shipment	
Sample Handling and Storage	
Ensure laboratory has sufficient equipment and space to store samples under appropriate conditions (i.e., at 4 EC).	
Sample thoroughly homogenized prior to test initiation and storage.	
Documented sample conditions upon arrival at laboratory (i.e., temperature, D.O. pH, conductivity, total ammonia)	
Repeated testing of untreated effluent conducted (i.e., to document changes in effluent quality during storage)	

Table 2. Estimated minimum effluent volume required for completing one full Phase I TIE (as described in Section 9)

Species	Rainbow Trout	*Daphnia magna*
Exposure Volume (assuming one replicate per test)	5 L	150 mL
Estimated Effluent Volume Required for Phase I Single Concentration Tests (using 100% effluent)	160 L	40 L
Estimated Effluent Volume Required for Phase I LC50 Tests (using 100, 50, 25, 12.5 and 6.25% effluent)	320 L	80 L

3.2 Sample Transportation

Samples should be delivered as quickly as possible to the laboratory. Environment Canada allows for a maximum 5-day storage period prior to initiation of acute lethality tests for regulatory purposes. The 5-day holding time may not be possible during TIE studies since testing can extend over several weeks depending on the nature of the toxicant (i.e., its persistence) and the amount of TIE testing (i.e., Phase I, II and III) conducted on a single sample. Alternatively, shorter holding times may be required when toxicity is non persistent. However, it is under these conditions (i.e., maximum storage of 5 days) that the sample is assessed for regulatory compliance. Consequently, the 5-day holding period should be carefully considered when sufficient data has been generated to identify the substances responsible for acute lethality, or when conducting TTE and SI studies.

Ensuring that samples are kept cool (4 EC) immediately prior to, and during transport will help to reduce changes in sample toxicity. Shipping arrangements should be made specifically for the TIE sample. Our experience has shown that shipment of large sample volumes using routine daily courier service often results in delayed delivery to the laboratory. Special attention to sample delivery will also be particularly important for those mines/mills located in remote or isolated areas where access to courier service may be limited. The samples must not be allowed to freeze during transport.

3.2.1 Evaluating Changes in Effluent Quality during Transport

Changes in effluent quality during transport from the mine/mill site to the laboratory are a problem that may be encountered by isolated facilities. Although effluent quality may change during transportation, it is the sample as received by the laboratory that is used to assess regulatory compliance. Similarly, it is under these conditions that the causes of acute lethality are investigated. However, changes in effluent quality during transport could be an important consideration during TTE studies. Often bench and pilot-scale treatment tests are conducted at a laboratory remote from the mine/mill site. If effluent quality changes during transport, the sample received by the laboratory may not be representative of the effluent as it is discharged, and the bench or pilot-scale evaluations could be misinterpreted or could misdirect pilot or full-scale treatments. This could lead to potentially costly changes without achieving a non-acutely lethal effluent.

One way to assess possible changes in effluent characteristics could involve comparisons of water quality parameters measured at the site to those measured by the laboratory upon receipt of the sample. Site personnel could collect samples for analysis of the substance responsible for acute lethality (if identified), or for "common" toxicants associated with metal mining effluents (i.e., pH, ammonia, metals). The results are then compared to the effluent as received by the testing laboratory.

An example of the need to consider changes in effluent quality during transport was provided in the AETE Case Studies (ESG International, 1998) with an integrated base metal mining, milling, smelting and refining facility (Case Study #3). Elevated pH (>10), ammonia and metals (copper) were the suspected causes of acute lethality. During the TTE studies, the investigators noted that effluent pH decreased from the time of sampling to the time of toxicity testing. Recognizing effluent pH would decline during transport, it was determined that in order to consistently pass the acute lethality tests with rainbow trout and *Daphnia magna*, the effluent pH needed to be adjusted to within the range of 8.7-8.9. For typical total ammonia concentrations measured at the site (5-16 mg/L), on-site adjustment to pH 8.8 resulted in a measured pH of 8.5 at the start of the toxicity testing, with a corresponding reduction of un-ionized ammonia values to less than lethal levels. Using this approach, the facility was able to achieve a non-acutely lethal effluent by reducing un-ionized ammonia concentrations, while avoiding potential toxicity due to metals at lower pH.

3.3 Sample Handling and Storage at the Laboratory

Upon receipt by the laboratory, the sample should be thoroughly homogenized, and care taken to prevent the entrainment of air in the sample during mixing. The minimum water quality parameters to be measured should include: pH, D.O., conductivity, temperature, ammonia and hardness. Additional measurements (i.e., metals, cyanide, thiosalts) can be taken based on historical data and knowledge of the process.

A TIE investigation can often involve storage of large volumes of samples over an extended period. Consequently, laboratories must not only have the appropriate technical knowledge to conduct the TIE, but must also have sufficient equipment and space to store the samples under the appropriate conditions (i.e., at 4EC).

Unlike testing of samples for routine regulatory purposes, TIEs or bench-scale tests can take several weeks to complete. Therefore, toxicity (and chemical composition) must be measured periodically during storage to document any changes that may occur. The extent of analysis on a single sample must be weighed against the cost of additional sampling, persistence of toxicity, representativeness of the sample, and the need to test samples that represent the range of toxicity and toxicants occurring at the site (U.S. EPA, 1991).

4 CHEMICAL ANALYSIS

The use and co-ordination of chemical analysis during a TRE is discussed in this section. Included are suggested analytical techniques for "common" metal-mining toxicants and QA/QC considerations for analytical measurements.

4.1 Co-ordination of Chemical Analyses

Reliable and accurate analysis is critical in the TRE process. Chemical analysis of the effluent can be conducted at various stages during a TRE study. Preliminary analysis of the effluent is essential, particularly if site personnel have reason to suspect a particular toxicant (e.g., ammonia, metals). For example, several studies have shown regression and correlation analysis between toxicity and corresponding chemical data (conducted during historical data reviews) can be useful in providing guidance for conducting a successful TRE study (U.S. EPA, 1989; ESG International, 1998; Beak, 2000). Evaluation of the chemical parameters measured during the toxicity test (i.e., D.O., pH, conductivity) is important in assessing potential causes of acute lethality (see Section 6). However, detailed chemical analysis will be most effective once the characteristics of the toxicant have been identified, since in the absence of TIE testing, there are no assurances that the parameters being measured are the substances responsible for toxicity.

Chemical analysis of samples collected at the mine/mill site can be useful for assessing the operational status of either the facility or effluent treatment system, in evaluating changes in effluent quality during transportation, or as a preliminary assessment of the potential causes of acute lethality. However, results will be most useful for toxicant identification if samples are collected and analyzed at the start of the toxicity test. Samples for chemical analysis collected immediately prior to addition of the test organisms will ensure that the chemistry results are representative of the concentrations to which the organisms are exposed.

4.2 Total versus Dissolved Concentrations

For metals and anions, both total and dissolved concentrations should be analyzed. Total metal, metalloids and anion concentrations provide an adequate data set for an initial assessment of the possible cause(s) of acute lethality. However, dissolved values (operationally defined as those that pass through a 0.45 micron filter) may be the most relevant in toxicant identification. While particulate bound chemicals can be a source of toxicity (e.g., through ingestion), dissolved (water soluble/hydrophilic) chemicals are more readily available to the organisms of interest and are consequently the most relevant in terms of acute lethality. However, it is also important to bear in mind that dissolved concentrations are not necessarily synonymous with biologically available metals, since methods for determining the bioavailable fraction of a metal are limited. Although there have been important advances in understanding metal bioavailability and acute lethality, the application of this information in the context of a mine effluent is rudimentary.

Samples for dissolved metals and anion analysis can be easily filtered using a 60 cc syringe and 0.45 micron syringe filters. Both the filters and syringes can be purchased through most laboratory supply companies, or provided by the analytical laboratory. All filters should be properly prepared prior to filtering of the effluent sample. Preparation methods may include rinsing of the filter with a 10% nitric acid solution (to remove trace metals), followed by rinsing with high purity rinse water.

Often the most toxic species of a substance are reasonably well known (eg. many metals, ammonia), as well as how water chemistry affects speciation. Once chemical analysis has been completed, further efforts to identify toxic species may be useful. Free ion calculations or the Biotic Ligand Model (see Section 9.2.6) may be of use, however, the latter can be more complex.

4.3 Analytical Methods

The U.S. EPA (1993a) Phase II document provides general information on analysis of metals and ammonia. Table 3 presents suggested analytical techniques for common metal mining toxicants in effluents. These techniques include recommended sample containers, preservatives, maximum holding time prior to analysis, analytical equipment, techniques and procedures for various parameters. The analytical equipment, techniques and procedures are only recommendations, and other compatible instrumentation, techniques or accredited procedures can be substituted. Not included in this summary of procedures are site-specific requirements such as organics that could range from oils and lubricants, flocculants and mineral froth flotation reagents, wood preservatives, herbicides and pesticides.

For all analyses, the method detection limits (MDLs) should be sufficiently low so as not to present impedance to data interpretation, and the detection of pertinent subtle changes in chemistry during effluent manipulations. However, However, selection of the lowest detection limits and the most sophisticated equipment will not always be necessary, unless warranted by the data (Bailey *et al.,* 1999). The selection of appropriate MDLs may depend on the effluent matrix. For example, higher detection limits for metals are likely to be obtained by ICP-OES (inductively-coupled plasma optical emission spectroscopy), atomic absorption spectroscopy or flame photometry with a multi-element and compound matrix, while lower detection limits may be obtained by ICP-MS (inductively-coupled plasma mass spectroscopy) with a cleaner matrix. Either method (ICP-OES or ICP-MS) allows for cost effective metals analysis of a large number of samples, but the selected approach and required MDLs will depend on the toxicity of each metal to the species being tested, as well as the experience of the investigator.

For metal concentrations in saline water or water containing high concentrations of dissolved salts, special procedures may be required eliminate matrix effects. For example, with tailings deposition in sea water or use of NaCN in gold flotation where sodium accumulation occurs in the circuit, the trace metals are first chelated and pre-concentrated in suspended particulate resin (which consists of immobilized iminodiacetate on a divynylbenzene polymer) and then analyzed by graphite furnace atomic absorption spectrophotometry (GFAAS) and/or ICP-MS.

Reliable analysis results will depend on a number of factors, including:

- Precise sampling techniques;
- Proper sample preservation techniques;
- Quality assurance and quality control methods in sampling and analysis; and
- Precise, error-free record-keeping and reporting.

Sampling should include field and travelling blanks for quality control assurance. Laboratory procedures should use quality control and assurance methods that conform to CAEAL standards such as ISO Guide 25 (or ISO 17025).

Handwritten field notes should describe the sampling designation, time, location and site characteristics such as processing data and flow data at the time of sampling. The relevant information, including

preservation techniques, is transferred to the laboratory system that should be in electronic format to reduce chance of error in subsequent reporting of results.

Table 3. Suggested analytical techniques for common metal-mining toxicants in effluents

Parameter	Symbol	Sample Container	Preservative	Holding Time (d=days)			Analytical Method
				U.S. EPA [1]	MISA [2] (non preserved)	EC [3]	
Acidity	-	Plastic	none-cool to 4ºC	14 d	4 d	7 days at room temperature	Titration
Alkalinity	-	Plastic	none-cool to 4ºC	14 d	4 d		Titration
Ammonia	NH_3	Plastic	1:1 H_2SO_4 to pH<2	28 d	10 d (3 d)	24 h at 4-6 EC	Colourimetry
Chemical oxygen demand	COD	Glass	1:1 H_2SO_4 to pH<2	28 d	30 d (4 d)		Colourimetry
Conductivity	-	Plastic	none-cool to 4ºC	28 d	4 d	7 days at room temperature	Conductivity Meter
Cyanate	CNO	Plastic	NaOH	14 d	7 d		Liquid Chromatography
Cyanide (free)	CN free	Plastic	NaOH to pH>12 & refrigerate in dark		7 d		Colourimetry
Cyanide (total)	CN_T	Plastic	NaOH		7 d		Colourimetry
Cyanide weak acid dissociable (WAD)	CN WAD	Plastic	NaOH		7 d		Colourimetry
Hardness [8]	-	Plastic	1:1 HNO_3 to pH<2	6 months	28 d		ICP-OES
Nitrate	NO_3	Plastic	none-cool to 4ºC	48 h		24 h	Liquid Chromatography
Nitrite	NO_2	Plastic	none-cool to 4ºC	48 h		24 h	Liquid Chromatography
Organic carbon, dissolved	DOC	Amber Glass	none-cool to 4ºC		10 d (3 d)		Carbon Analyzer
Organic carbon, total	TOC	Amber Glass	none-cool to 4ºC	28 d	10 d (3 d)	28 d	Carbon Analyzer
pH		Plastic	none-cool to 4ºC	immediate	4 d	immediate	pH Meter
Sulphate	SO_4	Plastic	none-cool to 4ºC	28 d	30 d	28 d	Liquid Chromatography
Thiocyanate	CNS	Plastic	none-cool to 4ºC		30 d		Liquid Chromatography
Thiosulphate	S_2O_3	Plastic	none-cool to 4ºC, freeze if analysis can not be done immediately				Liquid Chromatography, oxidation-titration

Table 3. Suggested analytical techniques for common metal-mining toxicants in effluents

Parameter	Symbol	Sample Container	Preservative	Holding Time (d=days)			Analytical Method
				U.S. EPA [1]	MISA [2] (non preserved)	EC [3]	
Total & dissolved metals	-	Plastic	1:1 HNO_3 to pH<2	6 months	30 d		AA [4], GFAA [5], Hydride, ICP-OES [6], ICP-MS [7]
Total dissolved solids	TDS	Plastic	none-cool to 4ºC	7 d	7 d	6 months	Gravimetric
Total suspended solids	TSS	Plastic	none-cool to 4ºC	7 d	7 d		Gravimetric

1 United States Environmental Protection Agency
2 Municipal Industrial Strategy for Abatement (Ontario)
3 Environment Canada
4 AA - Atomic adsorption
5 GFAA - Graphite furnace atomic adsorption
6 ICP-OES - Inductively-coupled plasma optical emission spectroscopy
7 ICP-MS - Inductively-coupled plasma mass spectroscopy
8 Hardness, may in some cases reduce acute lethality (e.g., decrease metals toxicity). In other cases hardness (or elevated Ca and Mg) could increase toxicity.

5 QUALITY ASSURANCE AND QUALITY CONTROL

This section discusses the importance of various aspects of quality assurance (QA) and quality control (QC) measures required for conducting TIE studies.

Recommended procedures for QA/QC implementation for conducting routine acute lethality tests can be found in the Environment Canada (2000a,b) rainbow trout (EPS 1/RM/13) and *Daphnia magna* (EPS 1/RM/14) test methods. Additional guidance on the importance of various aspects of ecotoxicity laboratory QA/QC, which are important in maximizing data reliability and reducing variability among and within laboratories, can also be found in the "Guidance Document on Acute Lethality Testing for Mining Effluents" (TIME, 2002). However, there are additional important QA/QC issues that should be considered when proceeding with a TIE investigation. QA/QC issues associated with conducting a TIE are provided in the U.S. EPA (1991a; 1993a, b) Phase I, II and III guidance documents, and are summarized in the following section.

5.1 Phase I TIE

Phase I tests do not generally follow standardized test methods, or necessarily require exacting quality control, because the data are only preliminary. Phase I, and to a lesser extent Phase II, are more tentative in nature compared to the confirmation tests conducted in Phase III (U.S. EPA 1991a). Due to the large number of effluent manipulations and the time required to conduct the treatments and tests, the level of QA/QC effort is generally reduced during Phase I. This does not imply that a QA/QC program should not exist when conducting a Phase I TIE, but rather that the level of QA effort increases as the results become more definitive.

Factors that will help ensure the generation of quality toxicity test data include, careful documentation of all observations during testing (including water quality data and organism symptoms), randomization techniques, use of similar test conditions (i.e., temperature, exposure volume), adherence to exposure times, use of organisms approximately the same age or size, and reference toxicant tests. These and other aspects of quality assurance and control are discussed in the Environment Canada (2000a, b) test methods.

In addition, all Phase I TIE tests should include system blanks or controls to detect toxic artifacts added during the effluent characterization manipulations. Common sources of toxicity artifacts include:

i) excessive ionic strength resulting from addition of acid/base during pH adjustments,
ii) contaminated acid/base solutions,
iii) formation of toxic by-products by acids/bases,
iv) contaminated air or nitrogen sources,
v) inadequate mixing of test solutions,
vi) contaminants leached from filters, pH probes, solid phase extraction columns, and
vii) contaminated reagents (U.S. EPA, 1991).

The U.S. EPA (1991a) recommends the use of two types of controls to detect artifactual toxicity: "Toxicity Controls" and "Toxicity Blanks". Toxicity Controls involve comparison of the untreated and treated effluent sample. The comparisons are used to determine if the effluent manipulation was effective a reducing acute lethality, and ensure that the manipulation does not result in an unintended increase in mortality. Toxicity Blanks involve the performance of a Phase I test on dilution water to determine if any

mortality is added by the effluent manipulation itself. Toxicity Blanks do not take into consideration if the effluent matrix affects artifactual toxicity. Dilution water controls should also be included to assess the performance of the test organisms in the water used for culturing, for dilution of the effluent (in the case of LC50 tests), and for preparation of the Toxicity Blanks.

5.2 Phase II TIE

The U.S. EPA (1993a) guidance document indicates that as Phase II proceeds, QA/QC requirements should be revisited and test methods adjusted as required. If modified test methods were used during Phase I, standardized methods should be applied during Phase II (and Phase III) to confirm that the suspected substance is responsible for the toxicity observed in the test that originally triggered the TIE. Initially, the use of modified test methods could be used during Phase II. However, once the toxicant has been identified, the toxicity tests should follow the Environment Canada (2000a, b) rainbow trout and *Daphnia magna* methods for toxicant confirmation.

If a surrogate test species was selected for use in Phase I, tests conducted in Phase II and III must confirm that the original and surrogate species are responding to the same toxicant. The U.S. EPA (1991a, 1993a) describes several methods for demonstrating that both species are responding to the same toxicant. However, significant time and resources will have been wasted if it is determined in Phase II and III that the organisms are responding to different toxicants. Therefore, it is important that the selection of a surrogate species be based on sufficient preliminary testing described in Section 8.1.

Reference toxicant tests should be used during Phase II (and Phase III) to assess the quality of the test organisms and test procedures. A standard reference toxicant (as described in the Environment Canada (2000a, b) test methods) can be used until the substance responsible for acute lethality has been identified. Once identified during Phase II, the suspected toxicant should be used as the reference toxicant for the TIE tests following the Environment Canada rainbow trout and *Daphnia magna* test methods (Environment Canada, 2000a, b; U.S. EPA, 1993a).

5.3 Phase III TIE

A more detailed QA/QC program is required for Phase II, but the maximum quality assurance and control is required for Phase III. Key QA/QC considerations for Phase III include:

- use of effluent test methods that triggered the TIE investigation to provide definitive data (and avoidance of altered toxicity test methods), with particular attention to test conditions, replication, test organism quality, representativeness of effluent sample tested, and analytical procedures,
- minimization of effluent manipulations prior to chemical analysis and toxicity testing to decrease the chance for the production of toxic artifacts
- inclusion of field replicates (to validate the precision of sampling techniques) and laboratory replicates (to validate the precision of analysis)
- use of calibration standards and spiked samples
- use of spiking experiments for assessing toxicant recovery
- calculation of confidence limits for toxicity tests and chemical measurements to determine if significant correlations exist between the toxicant concentration and effluent toxicity (U.S. EPA, 1993b).

Further detail on QA/QC measures recommended for TIE investigations is provided in the summary of the U.S. EPA guidance manuals outlined in Appendix B.

6 PRELIMINARY ASSESSMENT OF ACUTE LETHALITY

Effective tools for preliminary assessment of effluent acute lethality before initiation of TIE study are described in this section (which corresponds to Tiers I and II of the U.S. EPA TRE methods).

The first step in the TRE process includes the collection and review of available data and facility specific information, as well as an evaluation of remedial actions to optimize facility operation (U.S. EPA, 1989). The key components of this preliminary assessment are presented in Figure 2, and include an evaluation of:

- Historical toxicity and chemistry data (Section 6.1)
- Facility and process information (Section 6.2)
- Effluent treatment plant operation (Section 6.3)
- Housekeeping procedures (Section 6.4)
- Chemical usage (Section 6.5)

The rational and important steps associated with each component are described in sections 6.1 to 6.5 and summarized in Table 4. A detailed generic approach to each component is also described in the U.S. EPA (1989) TRE document.

Any actions taken as a result of the preliminary assessment phase may result in a reduction (to meet compliance limits) or elimination of final effluent acute lethality, negating the need for further investigation. In addition, at this early stage of the TRE, management attention will often lead to subtle operational changes, which in turn, may result in a reduction or elimination of acute lethality without a clearly identified cause (Ausley *et al*., 1998). However, if the preliminary assessment actions are unsuccessful in eliminating final effluent acute lethality, it will be necessary to proceed with the subsequent stages in the TRE. This may involve various approaches depending on the type of toxicity (see Section 7).

Effective communication between all team members (i.e. toxicologist, chemists, engineers, site personnel) during all stages of the TRE will increase the likelihood of success. However, it is particularly important that the TRE team have a clear understanding of the information gathered from all components of the preliminary assessments. The better understanding all TRE team members have of the mine or mill site, the greater the chance of achieving and maintaining a non-acutely lethal effluent.

6.1 Historical Toxicity and Chemistry Data

A review of the mine/mill historical toxicity and chemistry data is conducted to obtain information on the potential cause(s) of acute lethality and to assist in determining a proper course of study (U.S. EPA, 1989). A qualified aquatic toxicologist, experienced in conducting and reviewing aquatic toxicity test and chemical data, will typically conduct this component, with assistance from mine personnel. Mine personnel will be required to provide the raw data to be reviewed.

The important steps should include (but are not limited to);

1. **Evaluate the quality of the acute lethality and chemistry data with the testing lab to ensure integrity of the data.**

In the event of a failure, all test reports should be reviewed to establish that the acute lethality tests were conducted in accordance with Environment Canada (2000a, b) test methods. This review ensures that the

data that triggered the TRE is valid. Guidance on how to implement an evaluation of a test report (to ensure regulatory compliance) can be found in the "Guidance Document on Acute Lethality Testing for Mining Effluents" (TIME, 2002).

2. Evaluate water quality parameters (e.g., dissolved oxygen, pH, conductivity) measured during the acute lethality test

Water quality parameters measured during the toxicity tests should be reviewed since they can provide useful clues as to the cause(s) of acute lethality. Low dissolved oxygen and extreme pH values can be lethal to rainbow trout and *Daphnia magna* either alone or in combination with other substances. Changes in pH during a test can alter chemical forms, which can in turn alter their toxicity. This information may also be useful in defining how and why acute lethality varies. Insights about effluent variability can aid in designing the number and timing of samples to be characterized during a TIE (U.S. EPA, 1989).

Examples of the type of information that might be gained from a review of the toxicity test water quality parameters include:

- Implication of mortality due to ammonia. Aeration during the rainbow trout test can result in changes in effluent pH, which may affect effluent toxicity if the substance responsible for acute lethality is pH dependent. In the case of ammonia, as pH increases the proportion of un-ionized ammonia increases, with a corresponding increase in toxicity since the un-ionized form is substantially more toxic than the ionized form (Thurston *et al*. 1981). Depending on the initial pH of the effluent and how quickly the pH increases with aeration, concentrations of un-ionized ammonia that were not at lethal levels at test initiation could increase sufficiently during testing causing trout mortality by test completion. Additional guidance on ammonia toxicity can be found in the "Guidance Document on Acute Lethality Testing for Mining Effluents" (TIME, 2002).
- Implication of mortality due to low dissolved oxygen (D.O.). Prolonged exposure to solutions with a D.O. concentration less than 4 mg/L can contribute to rainbow trout mortality (ESG International, unpublished data).
- Implication of mortality due to major ions (i.e., Na, Ca, Cl). Elevated conductivity or total dissolved solids (TDS) may suggest mortality due to major ions. Freshwater effluents with conductivities greater than 2,000 FS/cm may contain concentrations of dissolved solids high enough to adversely affect freshwater test species (American Petroleum Institute, 1998). In our experience, conductivities greater than 6,000 FS/cm have the potential to cause *Daphnia magna* mortality. However, conductivity should only be used as a general screening tool, since toxicity may vary depending on the specific ionic composition of the effluent (Goodfellow *et al*., 2000). See section 9.2.1 for additional information on a model that can be used to predict mortality in high TDS effluents.

3. Determine if samples were acutely lethal to both regulatory species (rainbow trout and *Daphnia magna*)

Test results for rainbow trout and *Daphnia magna* should be compared, since species sensitivity comparisons can provide useful information about the possible cause(s) of acute lethality, including whether the substances responsible for mortality (or their concentrations) vary between samples (e.g., sample A is acutely lethal to rainbow trout, while sample B is acutely lethal to *Daphnia magna*), and if one or more contaminants are present in the effluent (e.g., sample A is acutely lethal to trout at high pH, and acutely lethal to *Daphnia magna* at low pH). Additional examples are provided in Section 7.3.

4. Determine if the existing acute lethality and corresponding chemistry data suggested a possible cause of mortality.

Statistical analysis of the acute lethality and corresponding chemical data (if available) may indicate those parameters that are positively correlated with acute lethality. If a single chemical is highly correlated with acute lethality, this could be considered a potential cause of mortality, and the TIE process could be used to evaluate and confirm the suspected toxicant(s) (US EPA, 1989).

5. Assess the type (transient, consistent, persistent) and degree of effluent acute lethality.

In the event of an acute lethality failure, the frequency of acute lethality testing will be accelerated as required under the draft MMER. However, additional testing may be required to assess if acute lethality is consistent or transient (see Section 6). The test data will be used to: i) characterize effluent variability, ii) evaluate the magnitude of lethality in order to determine if potential future TIEs will involve an evaluation of the full strength (100%) effluent, or require the use of LC50s, iii) assess whether or not the effluent is sufficiently toxic to conduct a TIE, and iv) determine if acute lethality changes over time within a single sample (i.e., is the toxicant(s) persistent).

6.2 Facility and Process Information

A review of facility and process information is conducted to identify what is already known, provide information on the potential cause(s) and source(s) of acute lethality, and help design a better TRE study (U.S. EPA, 1989). Mine personnel, including management representatives and operators, typically carry out this assessment. The mine personnel will be responsible for transfer of information and data to all TRE team members.

The important steps should include (but are not limited to);

1. Ensure TRE team understands type of process, facility design, configuration and operation of effluent treatment system, and contaminated water sources.

All TRE team members must have a clear understanding of how the facility/process is designed and operated. The information to be made available should include, a description of facility processes and operations, production schedules, line diagrams showing the major areas of operation and the main inputs to the ETP (including treatment steps and sequences), and contaminated water sources (i.e., mine waste rock, tailings, plant site run-off). This information should be included as part of the "Acute Lethality Response Plan" (see Section 2.21)

2. Identify if mine, mill or smelter was operating within "normal" or "typical" range of conditions when sample was collected.

Facility operations, effluent treatment plant performance and weather conditions should be documented during collection of samples for routine acute lethality testing. In the event of a sample failure, this information may lead to a quick solution (i.e., sample failure was a result of an upset, spill or atypical operation which can be identified and corrected).

3. Determine if the operation/process has changed recently

Factors to be considered include: different source/feed material, different equipment in use, different chemicals in use, and changes in process stream operation (e.g., batch versus continuous) at the time of the effluent test failure.

4. Assess if there are any relationships between a particular process, process sequence or operation and acute lethality.

The establishment of a relationship between a particular process/operation and acute lethality may point to a possible source of acute lethality, or provide supporting data to explain effluent variability.

5. Determine if any "non-routine" operations have occurred or are on-going

Non-routine operations may include construction, pest control, boiler and cooling tower blowdowns. Consistent documentation of operations and processes in the plant would facilitate tracking of non-routine operations.

6.3 Effluent Treatment Plant Operations

A review of treatment plant operations is conducted to determine if it is operating in an optimal fashion with respect to removal of its design parameters (U.S. EPA, 1989). Both mine personnel and a qualified aquatic toxicologist will be responsible for conducting this component. Interviews with treatment plant operators will be important in obtaining the necessary information.

The important steps should include (but are not limited to);

1. Assess if the effluent treatment system was operating according to design specifications

Changes in plant process over time may result in a final waste stream that contains contaminants that were not present in the original effluent at the time of ETP design. Therefore, an evaluation of the current ETP performance against design criteria is necessary (U.S. EPA, 1989). Routinely monitored effluent treatment parameters (i.e., pH, conductivity, TSS, metals) should be reviewed to determine if they were within "normal" operating ranges. These parameters may not be the cause of acute lethality, but are used to identify upset or abnormal conditions within the effluent treatment system or operation.

Variability in flow and loading of influent streams should also be considered. Flow and mass loading rates will be useful in understanding the actual capacity of the system and assessing if it has been exceeded, possibly resulting in plant upsets or pollutant pass-through (U.S. EPA, 1989).

The effluent treatment system may have been originally designed to remove or treat specific chemical constituents, with the objective of achieving compliance with regulatory chemical limits. Achieving a non-acutely lethal effluent may not have been one of the original design parameters. Comparisons of influent and effluent acute lethality may be useful in assessing the effectiveness of the treatment plant to remove/reduce acute lethality, while ensuring that toxicity is not being added during treatment (i.e., from a treatment chemical).

2. Assess if any new treatment chemicals had been used during the acute lethality event

The use of any new effluent treatment chemicals during the acute lethality event should be reviewed to determine their possible contribution to acute lethality of the final effluent (see Section 6.5).

3. Determine frequency and duration of effluent treatment plant by-passes and shock-loads

The frequency and durations of treatment plant by-passes (e.g., during or after a rainfall) and shock-loads (e.g., from spills, normal cleaning and maintenance activities) should be assessed, and a thorough effort made to correlate these events with acute lethality.

4. Determine if effluent residence times changed during acute lethality event.

Changes in effluent residence times (due to under- or over-loading of the system) may affect contaminant degradation or precipitation, with a resultant change in acute lethality. Understanding the retention time of the system should also help in selecting the frequency of testing required to detect variability in the TIE (US EPA, 1989).

6.4 Housekeeping Practices

A review of housekeeping practices is conducted in an attempt to optimize chemical usage, and reduce chemical losses that could contribute to the contaminant load and any toxicity detected in the final effluent (U.S. EPA, 1989). Mine personnel, including management representatives and operators, will typically conduct this component. The mine personnel will be responsible for transfer of information and data to all TRE team members.

The important steps should include (but are not limited to);

1. Evaluate general facility cleanliness

Mines, mills and refineries can produce acute lethality episodes as a result of routine operational events and incidental practices. Pre-scheduled cleaning or maintenance activities should be thoroughly evaluated, and an effort made to correlate these events with acute lethality.

Areas requiring special attention at mine sites include explosives handling facilities, reagent loading and unloading, chemical storage areas, fuel handling, storage and distribution, and vehicle wash-down facilities. Mine and mill facilities typically produce significant volumes of waste oil and lubricants. Although most mine facilities do an excellent job in storing waste organics, toxicity can result if spills occur.

The condition of the mining, milling and refining process operations with respect to housekeeping practices can be best evaluated by an audit of procedures in consultation with responsible personnel. A second method of assessment is by visual observation, supplemented by consultation with facility personnel.

Close co-ordination with mine personnel will assure all major areas are addressed. At all facilities it is important to note that specific department personnel (such as mill operations or environmental staff), may not always be aware of the relevant activities in other departments or areas of responsibility. Consultation with the operating department is recommended.

2. Evaluate spill prevention and control methods

Spill prevention and control is essential in preventing acute lethality episodes, since they may have a long-lasting effect if the spill was not completely contained. Spilled material may take many days or weeks to migrate though soil, waste piles and ground water before reaching the final effluent discharge.

All mine facilities include the storage of chemicals and fuels for the processes. Containment, secondary containment and active management of these facilities are important. Visual observations plus a review of records with operation personnel will assist in determining if one or more spills contributed to a toxic event.

Secondary containment of chemical and fuel storage areas (i.e., berms and dykes) is commonly in place at mine facilities. Filling of the structures by rainwater and snowmelt can result in containment failure. A review of such incidents could assist in the TRE investigation.

A procedure that commonly results in the release of potentially toxic material is the unloading of rail cars or trucks at a facility. Records and reports of spills during such procedures as well as contingency methods and the final destination of spilled material should be reviewed.

At mine sites, the use of ammonium nitrate as an ingredient in explosives may require special attention. Explosives storage, mixing and handling facilities are, by law and accepted practice, remote from the principle mine facilities and spill containment and control for these facilities should be reviewed. For bulk handling facilities, the handling of ammonium nitrate, fuel oil and emulsifiers could be reviewed for releases to the surface waters. However, frequently the most common loss location for blasting agents is at the rock face, particularly in underground mines where containment of small spills could be problematic. A review of procedures coupled with a review of mine water chemistry (ammonia data) can be used in determining the role of blasting agents in mine effluent toxicity.

3. Evaluate incidental waste handling, storage and disposal

Failure of non-process waste handling and storage facilities can have a significant effect on site effluent quality. Handling, storage and disposal methods for liquid wastes and those waste with rain-leachable components should be reviewed. A common source of leachable waste is off-specification reagents stored in small containers or drums that can deteriorate with time and release contaminants.

In the past, mine and mill facilities typically used waste rock or tailings management facilities for disposal of facility-produced wastes. Large waste rock piles can exhibit very slow release of contained water (retention time of years) and could be a source of acute lethality. In rare cases, the mine facility waste piles may have been used to dispose of domestic or other industrial wastes.

4. Record the disposal strategies for domestic (laundry and shower) wastes as well as sewage with respect to effluent from the mine site.

Disposal strategies for domestic wastes should be reviewed with operating personnel, and (if needed) a visual survey conducted. At some facilities, a dedicated wastewater treatment plant is operated to treat domestic waste, while at others the wastes are combined with mineral and process wastes in a managed facility. Typically, when domestic waste is combined with other waste streams, the effect on effluent quality (and acute lethality) is small. However, at some facilities, domestic waste can have a significant effect on effluent quality, particularly with respect to nutrients and oxygen demand.

6.5 Chemical Usage

A review of the use of process and treatment plant chemicals is conducted to identify chemicals used at the facility that have the potential to cause acute lethality. This review may provide evidence of potential toxicants that can be evaluated by follow-up TIE testing. Although no cause and effect relationship will

have been established between the chemicals and acute lethality of the final effluent, there may be some evidence these chemicals can contribute to acute lethality. The evidence may come from experience at other facilities, or from reported toxicity in the literature (U.S. EPA, 1989).

In cases where process or treatment chemicals (e.g., polymers, flocculating agents, water treatment agents, corrosion inhibitors, etc.) are suspected as a possible cause of effluent toxicity, the use of chemical specific toxicity "finger-prints" may be of use when combined with Phase I TIE results (see Section 9). For example, the Phase I TIE treatments could be conducted on the process or treatment chemical to characterize its' toxicity. Once the chemical "finger-print" is identified (i.e., C18 at pH 3, and XAD resin are effective at eliminating toxicity), these results can be compared to the "finger-print" of the wastewater. This approach may require detailed exchange of information with the chemical supplier and can often necessitate confidentiality or non-disclosure agreements, since the composition of many process and treatment chemicals are considered proprietary.

Both mine personnel and a qualified aquatic toxicologist will be responsible for conducting this component. The important steps should include (but are not limited to);

1. Review of chemical usage to ensure that only the required amounts are used.

The role of each chemical and the amounts used in the process should be examined. For each chemical identified the following should be determined: i) purpose of use, ii) the volumes used iii) whether the amount be reduced, iv) whether the chemical be re-used, and v) if it is possible to avoid discharge of the chemical (U.S. EPA, 1989a). In the case of final effluent treatment chemicals, even slight overdosing could result in potentially lethal concentrations in the final effluent, since these chemicals do not have the opportunity for degradation during normal plant operations (Bailey *et al*., 2000). Examples include polymers and chlorine.

Where possible, it may also be useful to estimate or predict the concentration of each process or treatment chemical in the final effluent. The predicted environmental concentrations could be compared to the available toxicity data on MSDS, but will not take into consideration synergistic effects, binding of the chemicals to particulates or fibres in the effluent, or by-products resulting from transformation or breakdown of the chemicals during use and in the treatment system.

2. Identify the most common toxicants in the list of process and treatment chemicals.

"Common" toxicants associated with metal mining effluent should be identified in the list of process and treatment chemicals (Section 11.1). For example, the following toxicants may be found in the process or effluent treatment at a gold mine: i) cyanide for gold extraction, ii) copper for the SO_2-air cyanide destruction process catalysis, and iii) ammonia from the cyanide destruction and from mine sources (i.e., blasting agents).

3. Review of available toxicity and biodegradability data for all chemicals used in the process

The MSDS for all process and treatment chemicals should be reviewed, with particular attention to biodegradability data and acute lethality data for rainbow trout and *Daphnia magna*. For those MSDS without acute lethality data for the regulatory species of interest, the supplier should be contacted for additional information. However, it would be beneficial for the mine procurement process to be set up to ensure that vendors submit all available toxicity data on their products used in mining, milling and refining operations (e.g., polymers, flocculating agents, water treatment agents, corrosion inhibitors, etc.) as a prerequisite to a purchasing decision.

4. Determine if less toxic/more degradable alternatives are available.

The information obtained in step #2 should be assessed to determine if; i) there are less toxic and more biodegradable products available, ii) the most serious problem chemicals can be isolated from the waste stream, or treated prior to mixing with the waste stream, and iii) the chemicals are used in quantities that result in effluent concentrations at or above lethal thresholds (U.S. EPA, 1989).

Table 4. Summary of preliminary assessment components

Item	Completed (Yes/No)
Historical Toxicity and Chemistry Data	
Evaluate the quality of the toxicity and chemistry data with the testing lab to ensure integrity of the data.	
Evaluate water quality parameters (e.g., dissolved oxygen, pH, conductivity) measured during the toxicity test	
Determine if samples were acutely lethal to both regulatory species (rainbow trout and *Daphnia magna*)	
Determine if the existing acute lethality and corresponding chemistry data suggested a possible cause of mortality	
Assess the type (transient, consistent, persistent) and degree of effluent acute lethality	
Facility and Process Information	
Ensure TRE team understands type of process, facility design, configuration and operation of effluent treatment system, and contaminated water sources	
Identify if mine was operating within "normal" or "typical" range of conditions when sample was collected	
Determine if the operation / process has changed recently	
Assess if there are any relationships between a particular process, process sequence or operation and acute lethality	
Determine if any "non-routine" operations have occurred or are on-going	
Treatment Plant Operations	
Determine if the effluent treatment system was operating according to design specifications	
Determine if any new treatment chemicals had been used during the acute lethality event	
Determine frequency and duration of effluent treatment plant by-passes and shock-loads	
Determine if effluent residence times changed during acute lethality	
Housekeeping Practices	
Evaluate general facility cleanliness	
Evaluate spill prevention and control methods	
Evaluate waste handling, storage and disposal	
Record the disposal strategies for domestic (laundry and shower) wastes as well as sewage with respect to effluent from the mine site	
Chemical Usage	
Review of chemical usage to ensure that only the required amounts are used	
Identify the most common toxicants in the list of process and treatment chemicals	
Review of available toxicity data for all chemicals used in the process	
Determine if less toxic / more degradable alternatives are available	

7 TYPES OF ACUTE LETHALITY

This section provides a discussion on TRE strategies for various types of acute lethality, including consistent versus transient, persistent versus non-persistent, and continuous versus seasonal. A discussion on the implications of single concentration tests (using 100% effluent only) versus LC50s (multiple concentration tests) in a TIE study is also provided.

The selection of an appropriate TRE strategy will be site-specific, and depends to a large degree on the type of acute lethality. The TIE approach will be easiest to apply and have the best chance of success when acute lethality is consistent, persistent and sufficient (i.e., moderate to high level of effluent toxicity). Alternatively, TIEs may be more difficult to conduct for those effluents that are sporadically acutely lethal (i.e., transient toxicity), when acute lethality dissipates over time (i.e., toxicity is non-persistent), or when toxicity is only marginal in the 100% effluent.

An overview of the approach to the various types of acute lethality is presented in Figure 3 and described in the following sections. For the purposes of this document, "consistent" is defined as the regular presence of acute lethality in a series of different samples. "Persistent" is defined as acute lethality that is constant in a single sample tested over time.

7.1 Consistent versus Transient Acute Lethality

Multiple samples collected over time will be required to determine if an effluent is consistently acutely lethal. To determine if acute lethality is persistent within an individual sample, testing should be conducted immediately upon collection (i.e., after receipt by the laboratory) and at periodic intervals during storage (e.g., day 3, 6, 9, 18).

If repeated testing demonstrates that acute lethality is consistent and persistent, the TRE can proceed to characterization of the toxicant(s) (Phase I TIE) (Figure 3). However, the decision to proceed immediately to TIE should not only be based on an assessment of the type of acute lethality, but also on the results from Tiers I and II in the TRE process (see Figure 2). The information gathered during the data acquisition and facility optimization may suggest an alternative course of action to performing a TIE (e.g., changes to ETP operation, alternative treatment or process chemicals). Therefore, it will be important that this information be evaluated (at the same time the type of toxicity is assessed) and taken into consideration when deciding to proceed with a TIE.

In the case of transient acute lethality, attempts should be made to predict effluent toxicity. This could involve establishing a relationship between acute lethality and a specific mine process or operation; or between acute lethality and available water quality data. In either case, an increase in sampling and testing frequency may be required in order to determine if toxicity pattern exists.

The transient nature of the events themselves may provide information on the cause or source of acute lethality. For example, acute lethality may be related to a change in a specific process, operation or time (i.e., seasonal toxicity – see Section 7.1.2). Thorough documentation of mill/mine activities, and effective communication within the TRE team, will be required to help identify a correlation between acute lethality and a specific process or operation (see Section 6). Water quality parameters routinely monitored at the mill/mine site (i.e., ammonia, pH, TSS, metals) or measured during the acute lethality test (i.e., D.O., pH, conductivity) may also be useful indicators or predictors of toxicity (see Section 6.1).

The use of on-site rapid screening tests (e.g., Microtox®, *Daphnia* IQ®) could be effective tools for predicting acute lethality. However, a significant correlation between the TIE test species and the

screening test must be established prior to use (ESG International Inc. 1998). The selection of an appropriate screening test must be made on a site-specific basis, and therefore may require extensive sample assessment with a battery of tests.

AETE evaluated the performance of 5 different rapid screening toxicity tests (*Daphnia* IQ ®, Microtox®, Rotoxkit F®, Thamnotoxkit F® and Toxichromotest®) as alternatives to the rainbow trout and *Daphnia magna* test methods (Pollutech, 1996). No single rapid screening test compared directly with the rainbow trout test (in terms of toxicity response or correlation of endpoint results to chemistry). The "best" toxicity test varied between the Thamnotoxkit F®, *Daphnia* IQ® and *Daphnia magna* acute toxicity tests depending on mine type. When either the *Daphnia* IQ® or Thamnotoxkit F® was selected as the "best" test, the next best test was the reciprocal procedure. When the *Daphnia magna* acute lethality test was selected as the "best", the next selection included either the IQ ® or Thamnotoxkit F ® procedure.

Once an appropriate test has been selected, mine personnel would collect sufficient sample for conducting a TIE, and immediately assess the sample using the rapid screening test. The sample would be submitted to the lab for a Phase I TIE only if the rapid screening test indicated that the sample would be acutely lethal to the TIE test species.

If acute lethality can be predicted, then the Phase I TIE characterization approach can be applied (assuming that toxicity is persistent) (Figure 3). If toxicity cannot be predicted, then Tiers 1 and 2 should be re-visited to gather more facility specific data and re-evaluate facility optimization. A Source Investigation may also be a viable alternative, but must be evaluated on a site-specific basis.

In cases where acute lethality cannot be predicted, extra sample to conduct a TIE could be shipped to the test laboratory as part of the routine test schedule. As a cost saving measure, the sample could also be stored at the mine site (assuming appropriate cold storage facilities are available). The extra sample could be stored until testing is conducted to determine whether it is acutely lethal, at which time a Phase I TIE could be initiated. However, the costs associated with this approach may be prohibitive. Continuous shipment of large volumes of effluent may be costly, particularly when conducting a TIE using rainbow trout, or in the case of isolated mill/mine sites. Extensive co-ordination with the test laboratory will also be required to ensure the availability of resources (i.e., test organisms, staff), which may be costly since staff must be on "stand-by" until an acutely lethal sample is obtained.

It should be noted that although transient acute lethality increases the difficulty of conducting a TIE, intermittent toxicity could be useful during the Phase III confirmation process by providing the necessary data to correlate toxicant concentration with mortality (Ausley *et al*., 1998)

7.1.1 Non-Persistent Acute Lethality

Non-persistent acute lethality could occur under either consistent or transient conditions. TIEs should not be used if acute lethality is not sufficiently persistent to allow a complete Phase I characterization to be completed. For a TIE study to be successful when acute lethality is not persistent, treatments and manipulations may have to be conducted as soon as the sample is received, rather than after the initial untreated test is performed (see Section 9). The decision to proceed with this approach must be based on a sufficient amount of historical data indicating how quickly toxicity is lost over time (during storage of the effluent sample at the test laboratory).

Alternatively, the TTE path may be a more useful approach since it is known that the toxicant(s) can be removed (e.g., the toxicant is non-persistent) (ESG International Inc., 1998). Longer retention time or

installation of aerators (to remove volatile substances) may be adequate for removal of final effluent acute lethality.

7.1.2 Seasonal Acute Lethality

A sufficient amount of data must be gathered to determine if acute lethality is seasonal in nature. Tracking of acute lethality test results using electronic data summaries and graphical comparisons will be useful in establishing a seasonal pattern.

The issue of seasonal acute lethality can be addressed using the TIE process, provided that toxicity is consistent during the toxic period. An accelerated approach to the TRE will be required, since the period of acute lethality may be restricted to a few months.

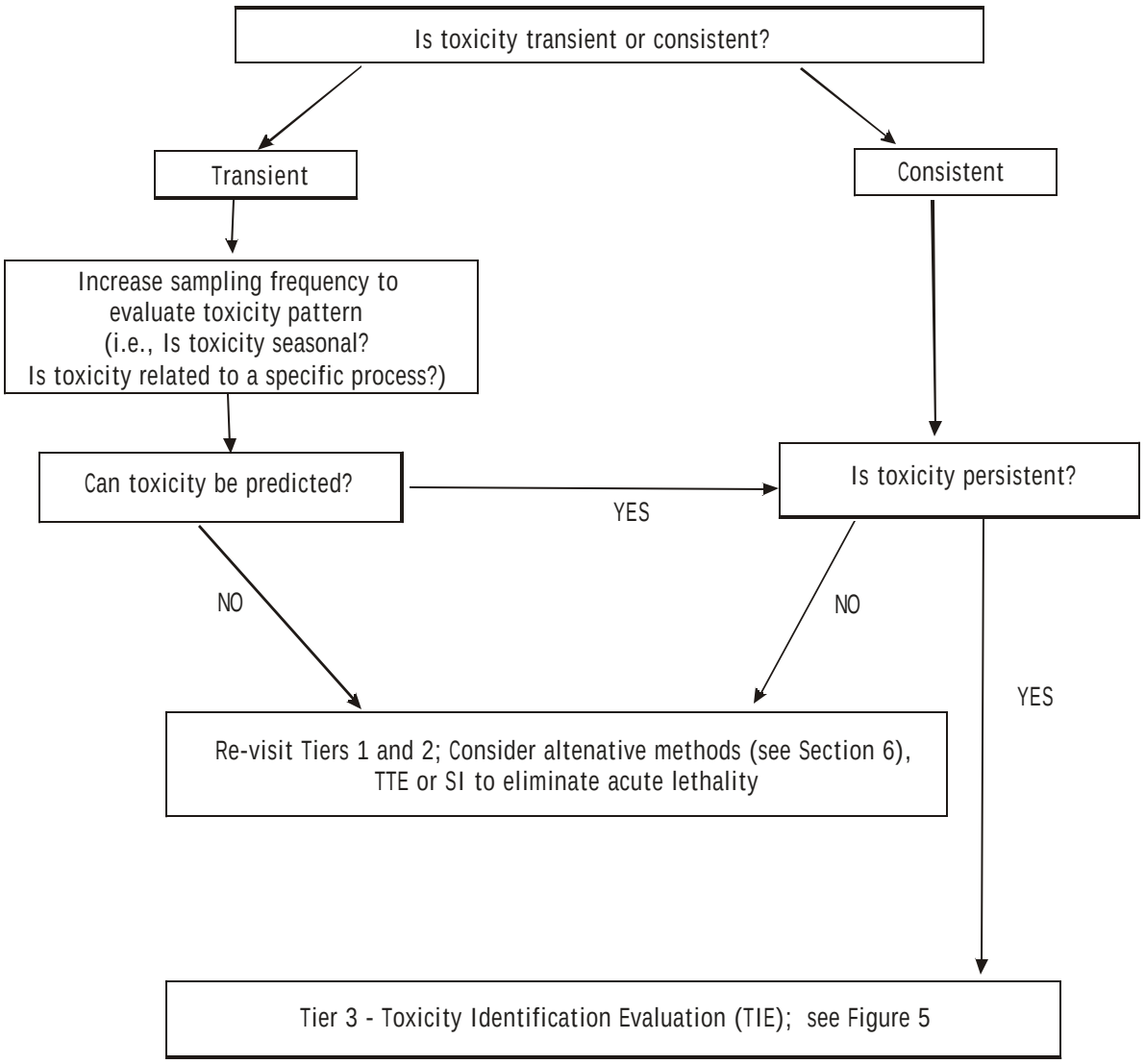
The seasonal nature of the acute lethality event may also provide an indication of the substance responsible for acute lethality. For example, higher concentrations of ammonia, thiosalts and cyanide could be expected in the winter than in the summer due to low biological activity and less penetration of ultra violet light for natural degradation under ice cover.

7.2 LC50 versus Single Concentration Tests

The magnitude of acute lethality must also be assessed in conjunction with the type of acute lethality, since it will have an effect on the level of effort required to conduct a TIE. If mortality is only observed in the full strength (100%) effluent, the TIE will involve an evaluation of the undiluted effluent (single concentration tests). Multi-concentrations (LC50) tests will be required if mortality is observed in the diluted effluent concentrations. The single concentration tests require less effluent, are easier to complete (with respect to effluent manipulations), and are therefore less costly than the LC50 tests.

There may also be circumstances in which diluted effluent concentrations (i.e., 50%, 25%) could provide useful information even if mortality was only observed in the undiluted (100%) effluent. For example, the addition of a 50% concentration in the graduated pH test may allow for detection of increased toxicity at low or high pH, which may provide insight to the toxicant's identity.

The characteristics of the dilution water used to prepare the multi-concentration LC50 test may also be an important consideration. When testing high concentrations of effluent (80%), the physical/chemical characteristics of the concentration will resemble the undiluted effluent, while the opposite is true for low concentrations (low concentrations will mimic the dilution water) (U.S. EPA, 1991a). Therefore, consideration should be given to use of dilution water with characteristics (i.e., hardness, pH) similar to that of the effluent. Use of dilution water that differs significantly from the effluent could complicate data interpretation due to the presence of a hardness or pH gradient (in addition to the toxicant gradient).



Project Name: TRE GUIDANCE DOCUMENT	Date: January, 2002	FILE: E1157_03	Figure 3
	Revised:	APPROACH TO TRE FOR CONSISTENT, TRANSIENT AND NON-PERSISTENT ACUTE LETHALITY	
Prepared for: ENVIRONMENT CANADA AND THE MINING ASSOCIATION OF CANADA	Revision No.		

8 SELECTION OF TEST SPECIES

This section of the document discusses the selection of an appropriate test species for use in TIEs, and includes guidance on the use of surrogate species, modification of the Environment Canada (2000a) Reference Method (EPS 1/RM/13) for use with TIEs, and the importance of species sensitivity comparisons.

The U.S. EPA (1991a, 1993a,b) TIE methods were developed for use with acute lethality tests using fathead minnows or *Ceriodaphnia dubia*, and have been adapted (but not standardized) by many Canadian laboratories for testing with rainbow trout and *Daphnia magna*.

The existing Phase I TIE methods can easily be adapted for use with *Daphnia magna* following the Environment Canada test methods. However, adapting the TIE methods for use with rainbow trout is generally not as straightforward, necessitating greater effort and expense since the Environment Canada method requires more sample volume.

In cases where the effluent is acutely lethal to rainbow trout, two TIE test options are presented: 1) the use of a surrogate test species or 2) modification of the Environment Canada test method. Both options may be acceptable in a Phase I TIE (and to a lesser extent Phase II). However, testing conducting in Phase III must avoid modifications to the test methods. The test methods that triggered the TIE investigation should be followed, with particular attention to test conditions, replication, test organism quality, representativeness of effluent sample tested, and analytical procedures (U.S. EPA, 1993b).

8.1 Use of Surrogate Test Species

It is generally recommended that all tests be conducted with the species that originally triggered the TIE. Even if more expensive, the benefits of using the regulatory species often outweigh the use of surrogate tests because; i) correlations are often not strong with the surrogate species, ii) the surrogate test may not be sensitive to the same toxicants affecting the regulatory species, and iii) results can led to erroneous conclusions, particularly if results are not confirmed by testing with the target species. However, the use of a surrogate test species may be necessary for those TIE treatments limited by the ability to treat only small effluent volumes (e.g., solid phase extraction with C18). A surrogate species requiring smaller test volumes may also be more practical for mines located in remote areas where collection and shipment of large volumes of effluent could be difficult.

A survey of the Canadian metal mining industry found that the fathead minnow (one of the Environment Canada test species used for sub-lethal testing) was reported to be the most common surrogate species used in a Phase I TIE (ESG International, 1998). Examples of other possible surrogate tests or species include Microtox®, *Daphnia* IQ ®, Thamnotoxkit F®, and early-life stage rainbow trout (embryo, swim-up fry) (Environment Canada, 1998; Pollutech, 1996).

Sufficient testing prior to, and during the TIE should be conducted to determine and confirm that the species of interest and the surrogate species respond in a similar manner to the untreated effluent under a variety of conditions. Considerable time and resources will be wasted if the surrogate and regulatory species are responding to different toxicants, or if the effluent is not acutely lethal to the surrogate species to begin with. The U.S. EPA (1991a) Phase I guidance document provides additional details on approaches to demonstrate that the toxicant is the same for both species, including comparison of LC50s, comparison of Phase I results, and symptom comparisons for similar organisms.

8.2 Modification of Environment Canada Rainbow Trout Test Methods For Application with TIEs

This section provides a general approach and method for modifications to the Environment Canada rainbow trout test method for use in TIEs. It is important to note that any modification to the standardized Environment Canada rainbow trout test methods are strictly intended for use in TIEs, and must not be used for compliance with regulatory test requirements.

An example of possible TIE test conditions for reduced volume tests with rainbow trout is provided in Table 5. The modifications are based on our experience conducting TIEs using modified rainbow trout tests. Modification to standardized toxicity test methods (including the Environment Canada rainbow trout test methods) for use in TIEs have been reported by other investigators (Evans *et* al., 2000; U.S. EPA, 1991a; Coombe *et al*., 1999; Chapman *et al*., 2000; Bailey *et al*., 1999). The approaches varied depending on the test method, but generally involved a reduction in the number of test organisms and exposure volume.

The key modifications presented in Table 5 include a reduction in: test duration, exposure volume, number of fish per exposure, fish weight and aeration. For each modified test condition, a description of the Environment Canada (2000a) requirement and corresponding TIE modification is summarized in the following section.

Test duration: The Environment Canada test method involves a 96-hour exposure. In cases where acute lethality is complete in less than 96-hours, a reduction in the test duration may be appropriate during a TIE using rainbow trout.

Exposure Volume: Exposure volumes in the Environment Canada method are based on a loading rate of # 0.5 g/L. Typical exposure volumes range from 10 L for fish # 0.5 g, to 50 L for fish up to 5 g in size. The exposure volumes are reduced to 1 L or 5 L during a TIE, which may result in the loading rate being exceeded (depending of fish weight and number of fish per exposure).

Number of Fish Per Exposure: The Environment Canada test methods for single concentration and LC50 tests require a minimum of 10 fish per vessel. The reduced exposure volumes used during a TIE require a reduction in the number of fish per exposure. TIE trout tests (conducted without replication) can be conducted using 5 (in 1 L volumes) or 10 fish (in 5 L volumes). Other investigators have reported using two fish in duplicate 0.5 or 1 L volumes, between 5 and 10 fish in 5 or 10 L volumes, and 3 fish in 1 L volumes (Bailey *et al*., 1999; pers. comm. Tibor Kovacs)

Fish Weight: Environment Canada method requires mean fish weight to be between 0.3 and 2.5 g. The reduced exposure volumes used for a TIE require the use of small fish, often less than or equal to an average wet weight of 0.3 g.

Aeration: EC requires all test solutions are pre-aerated for 30 minutes at a rate of 6.5 ± mL/min/L. Extended aeration (up to 90 minutes) is applied prior to addition of the test organisms if D.O. in the highest test concentration is <70% or >100% saturation. All test solutions are aerated during the test. During a TIE, the decision to aerate will be based on the initial D.O. and nature of the toxicants. Pre-aeration of the test solutions is typically not required. During TIE testing, minimal aeration (6.5 ± mL/min/L) may be applied if D.O. is sufficiently low (< 5 mg/L) as to cause mortality on its own.

Each effluent is unique, and therefore the conditions outlined in Table 5 may be adjusted depending on effluent quality and Phase I TIE tests to be conducted. Experience of the investigator and prior

knowledge of the effluent quality (i.e., pH, D.O., ammonia) will also be important in selecting the appropriate test conditions. For example, loading rate may be a concern if ammonia is a potential cause of acute lethality, since the fish may contribute additional ammonia to the effluent through excretion. In this case larger exposure volumes and small fish should be used, and ammonia measured at the start and end of each test.

Regardless of the modifications, parallel testing (with the untreated effluent) using the regulatory Environment Canada method and modified test method should be conducted prior to TIE initiation. This testing is necessary to ensure that both methods produce similar results. Furthermore, all test conditions must be similar throughout the TIE to allow for balanced comparisons of the Phase I manipulations.

The suggested modifications to the Environment Canada rainbow trout test can also be used during TTE and SI studies where effluent volume may be limited. The modifications may be particularly useful during bench-scale tests where only small volumes of treated effluent are generated. However, the standard rainbow trout test should be used when ever possible during bench-scale tests, and particularly during pilot-scale testing.

Table 5. Summary of TIE test conditions for reduced volume rainbow trout tests.

Test Species	Rainbow trout (*Oncorhynchus mykiss*)
Test Type	Static
Test Duration	96 hours, but can be completed earlier if complete mortality occurs prior to 96-hours
Temperature	15 ± 1 °C
Photoperiod	16 hrs light per 24 hrs
Exposure Volume	1 L or 5 L
Number of Organisms Per Concentration	5 (1 L exposures) or 10 (5 L exposures)
Fish size	Average wet weight ~ 0.2 to 0.3 g
Feeding During Test	None
Aeration	Optional depending on D.O.; minimal (~ 6.5 ± 1 mL/min/L) during test.

8.3 Multiple Species Testing

Rainbow trout and *Daphnia magna* are known to exhibit different tolerances to certain contaminants. As such, species sensitivity comparisons may be useful in determining the cause(s) of acute lethality. Examples for two "common" metal-mining toxicants are provided. The examples are intended only as guides and must not be used in place of a detailed evaluation of the effluent or Phase I TIE testing.

Ammonia: Rainbow trout are more sensitive to ammonia than either *Daphnia magna* or fathead minnows. At pH values > 8.0 Ninety-six hour LC50s for un-ionized ammonia ranged from 0.13 to 0.66 mg/L for rainbow trout, and from 0.2 to 1.4 mg/L for fathead minnows (Thurston *et al*., 1981). For *Daphnia magna*, a single 48-hour LC50 was found (4.2 mg/L) in the U.S. EPA Ecotox Database. Based on this information (combined with measured chemical concentrations and calculations of un-ionized values), ammonia

could almost immediately be eliminated as a possible cause of acute lethality in cases where the effluent is acutely lethal to *Daphnia magna*, but non lethal to rainbow trout.

Total Dissolved Solids (TDS): *Daphnia magna* are more sensitive than rainbow trout to total dissolved solids. For example, sodium LC50 values for rainbow trout and *Daphnia magna* have been estimated at 6.4 g/L and 2.3 g/L, respectively (unpublished data from laboratory reference toxicant tests). In our experience with effluents where acute lethality was attributed to elevated TDS, conductivities greater than 6,000 FS/cm were shown to have the potential to cause *Daphnia magna* mortality, yet were relatively harmless to rainbow trout. This information on its own cannot be used to conclude elevated TDS as a cause of acute lethality, particularly since the acute lethality of freshwater with high TDS is dependent on the specific ion composition (Mount *et al*., 1998). However, when combined with supporting TIE investigations, the differences in trout and *Daphnia magna* sensitivity can be a powerful component of the "weight of evidence" implicating TDS.

9 PHASE I TIE PROCEDURES

This section provides an overview of the U.S. EPA Phase I TIE approach, a description of new or updated TIE procedures (for "common" metal-mining toxicants), an alternative Phase I approach to address "common" metal-mining toxicants, and examples of Phase I data interpretation. Guidance on TRE options following completion of Phase I is also provided.

9.1 U.S. EPA Phase I TIE Approach

An overview of the U.S. EPA (1991a) Phase I approach is provided in Figure 4 and described briefly in the following section. A more thorough summary is provided in the literature review (Appendix B), but the original U.S. EPA document should be consulted for detailed procedures.

During a Phase I TIE, acute lethality of the untreated effluent is compared to the treated effluent following various chemical or physical manipulations. The relative degree to which the manipulations result in an improvement in acute lethality provides an indication of the types of contaminants that may be involved (e.g., volatiles, heavy metals, organics). A complete Phase I TIE consists of nine treatment categories: Initial Test, Baseline Test, pH adjustment, pH adjustment/filtration, pH adjustment/aeration, pH adjustment/C18, EDTA, sodium thiosulfate, and graduated pH. A summary of each manipulation is provided in Table 6.

The sample is initially tested for routine water quality parameters and acute lethality (Initial Test) upon arrival at the laboratory (Day 1). These results are used to assess the magnitude of acute lethality, and to identify the exposure concentrations for subsequent tests. Additional tests on the untreated effluent are conducted at the start of the TIE sample manipulations and on each day that tests on additional treatments are initiated. These "Baseline" tests are conducted in order to monitor any changes in acute lethality of the untreated effluent over time.

On Day 1, sub-samples of the effluent are also adjusted to pH 3 and 11 (pH 9 for the C18 treatment), and then filtered, aerated or passed through a C18 column. After the manipulations are complete, the samples are readjusted to the initial pH of the effluent (pH_i) and held at 4 EC overnight until testing on Day2.

Assuming the pH adjustment, aeration, filtration and C18 manipulations were conducted on Day 1, a second untreated effluent sample (Baseline Test) and the manipulations (conducted on Day 1) are tested on Day 2. The EDTA, sodium thiosulfate and graduated pH adjustments are also conducted and tested on Day 2.

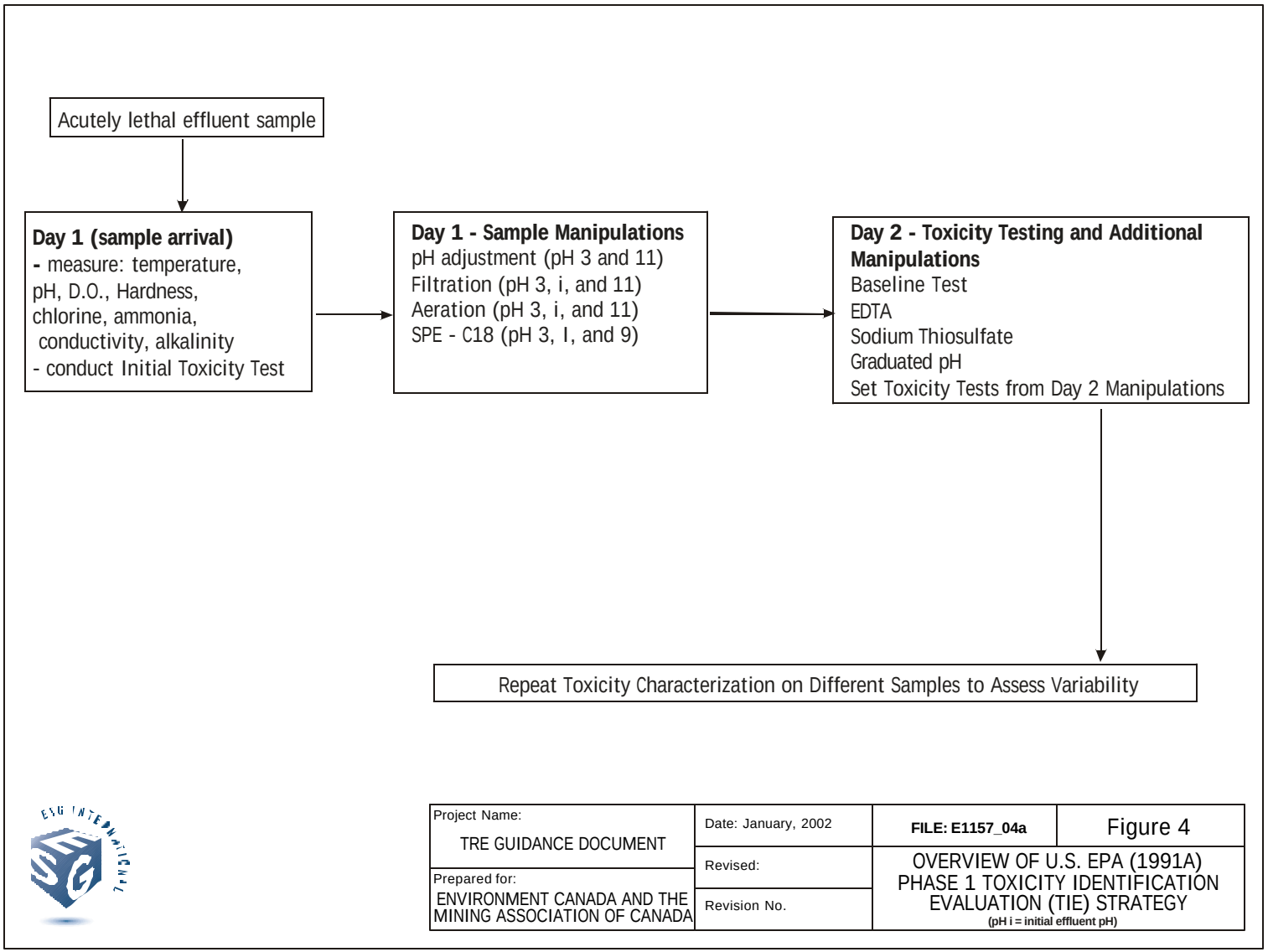
Delaying most of the toxicity testing by 24 hours, allows the test exposures to be set at concentrations bracketing the LC50 (as determined by the initial toxicity test set on Day 1), and also allows the pH-adjusted effluents to stabilize.

Table 6. Summary of U.S. EPA (1991a) Phase I TIE procedures

Test or Treatment	Description
Initial Test	Untreated effluent test started when sample is received by test laboratory. Provides an estimate of the LC50 for setting the exposure concentrations in the subsequent Phase I tests.
Baseline Test	Each day a sample manipulation is performed, an untreated effluent test (or Baseline

Table 6. Summary of U.S. EPA (1991a) Phase I TIE procedures

Test or Treatment	Description
	Test) is set. The results from each manipulation are compared to the untreated effluent to assess the effectiveness of the manipulation on reducing acute lethality.
Adjustment of pH	Adjustment of pH (to 3 and 11) provides additional information on the nature of the toxicants, and provides blanks for subsequent pH adjustment tests performed in combination with other treatments (i.e., filtration, aeration). Effluent samples are adjusted to pH 3 and 11, and then subjected to filtration, aeration, or solid phase extraction with a C18 column. The treated samples (including the pH adjusted samples without additional treatment) are re-adjusted to the initial pH of the effluent (pHi) and stored at 4 EC until testing.
pH adjustment / filtration	The pH adjustment / filtration test evaluates the effect of pH change and filtration on the toxicity of substances associated with filterable material, focusing on irreversible chemical reactions. Effluent samples, at pH 3, 11 and i are filtered (using positive pressure) through 1 micron glass fibre filters. The pH of each filtered sample is re-adjusted to pHi and stored at 4 EC until testing.
pH adjustment / aeration	The pH adjustment / aeration test evaluates the effect of pH change and aeration on the toxicity of the sample that may be due to volatile, sublatable or oxidizable substances. Effluent samples, at pH 3, 11 and i are placed in graduated cylinders and vigorously aerated for a standard time interval. The pH of each aerated sample is re-adjusted to pHi and stored at 4 EC until testing.
pH adjustment / C18 Solid Phase Extraction	The pH adjustment / C18 Solid Phase Extraction test evaluates the extent to which toxicity may be due to relatively non-polar organics and certain metals. Filtered effluent samples, at pH 3, 9 and i are passed through prepared C18 columns. The pH of each sample is re-adjusted to pHi and stored at 4 EC until testing.
Oxidant Reduction test	The Oxidant Reduction test evaluates the extent to which oxidative substances (e.g., chlorine, iodine, bromine) and some cationic metals (e.g., Cd, Cu, Ag, Hg) can be made less toxic or non-toxic by the addition of sodium thiosulfate. Sodium thiosulfate can be added as a gradient of concentrations to a single effluent concentration, or as a dilution test where effluent and thiosulfate concentrations vary.
EDTA Chelation	The EDTA Chelation test evaluates the extent to which cationic metals (e.g., Al, Ba, Cd, Co, Cu) can be made less toxic or non-toxic by the addition of EDTA (Ethylenediaminetetraacetate). A cationic metal may be suspected as the cause of toxicity if both EDTA and sodium thiosulfate reduce toxicity. EDTA can be added as a gradient of concentrations to a single effluent concentration, or as a dilution test where effluent and EDTA concentrations vary.
Graduated pH test	The graduated pH test evaluates the effect of pH on the toxicity of a variety of contaminants. Effluent samples are to three different pH values (e.g., pH 6, 7 and 8), without readjustment to pHi. The specific pH values selected will be based on the effluent characteristics.



Project Name: TRE GUIDANCE DOCUMENT	Date: January, 2002	FILE: E1157_04a	Figure 4
Prepared for: ENVIRONMENT CANADA AND THE MINING ASSOCIATION OF CANADA	Revised:	OVERVIEW OF U.S. EPA (1991A) PHASE 1 TOXICITY IDENTIFICATION EVALUATION (TIE) STRATEGY (pH i = initial effluent pH)	
	Revision No.		

9.2 Recent Advances in TIE Procedures

The TIE process outlined by the U.S. EPA was originally published in 1989, with updates published in 1991 and 1993. Since then, the investigative techniques have evolved. An overview of new and updated TIE procedures applicable to toxicants commonly associated with metal mining effluent is provided in the following sections, and include:

- methods to identify ionic imbalance as a cause of acute lethality,
- use of activated carbon, cation and anion exchange resins in Phase I TIEs,
- alternative methods for pH control during graduated pH testing with rainbow trout,
- updated EDTA and sodium thiosulfate procedures, and
- Biotic Ligand Model to predict acute lethality of various metals.

It is important to recognize that further modifications to the TIE procedures may occur as new methods are developed.

9.2.1 TIE Procedures to Identify Acute Lethality Due to Ionic Imbalance

Procedures using weight-of-evidence approaches to identify ionic imbalance as the cause of acute lethality include direct measurement, Phase I TIEs, predictive toxicity models for freshwater systems, exchange resins, mock effluents, and species sensitivity comparisons (Ausley *et al*., 1998).

A recent review paper by Goodfellow *et al*., (2000) summarized the issues of ionic strength and imbalance, provided a summary of applicable data (including 2 case studies which successfully applied TIE tools to address toxicity due to ion imbalance), and provided recommendations for regulatory options to manage these discharges. These TIE tools are summarized in the following section. The methods focus on acute lethality due to elevated ion concentrations (conditions that could be encountered at a mine, mill or refinery). However, ion imbalance may also occur as a result of ion deficiency (e.g., often observed in condensate or cooling water effluents).

Direct Measurement Approach

Preliminary analysis should include an examination of the relationship between acute lethality and conductivity, TDS or salinity. Ion imbalance may be implicated as a cause of toxicity if there is a significant correlation between mortality and TDS, conductivity or salinity. However, these parameters may not be robust predictors of acute lethality, since the toxicity of freshwater associated with high TDS is dependant on the specific ion composition. Cations and anions do not occur as individual constituents, but as combinations. The toxicity of an individual cation or anion may be masked or affected by the presence of the associated ion (Goodfellow *et al*., 2000). Therefore, it may be important to identify the specific ion combinations that are responsible for mortality using the methods described in the next sections.

Phase I TIE Approach

Useful information may also be obtained from Phase I TIEs conducted on effluent samples with relatively high conductivity. Ion imbalance may be suspected as a cause of acute lethality if none of the Phase I manipulations reduce or eliminate mortality. However, close attention must be paid to adjustment of pH

and use of ion exchange resins (during the Phase I TIE), since these treatments may cause an increase in the ionic strength of the effluent (e.g., through the addition of acid or base during pH adjustment).

Measurements of inorganic anion (i.e., HCO_3, SO_4, Cl) and cation (i.e., Ca, K,, Mg, Na) concentrations before and after various Phase I manipulations could also provide insight about the specific ions responsible for acute lethality. Concentrations of major ions should be compared to literature or laboratory derived LC50s to assess if any of the measured ions could account for all or part of the observed acute lethality.

Predictive Models

The Freshwater Salinity Toxicity Relationship (STR) model was developed by the Gas Research Institute to predict the acute lethality of seven common ions (Na, K, Ca, Mg, Cl, SO_4 and HCO_3) using three freshwater organisms, *Ceriodaphnia dubia, Daphnia magna,* and fathead minnows (Tietge *et al*., 1994). Data from over 3,000 acute lethality tests with different ion combinations was used to develop this predictive model.

The STR model can be used to predict mortality in high TDS effluents based on the concentrations of major ions. The predicted and measured acute lethality is then compared. If there is little difference between the actual and predicted acute lethality, then the observed mortality is likely due to the major ions. If measured mortality is higher than the predicted, then other toxicants may be present in the effluent.

The model has been used with varying degrees of success in a number of published studies (Mount *et al*., 1997; Sauer *et al*., 1997; Tietge *et al*., 1997). We have also successfully used the model to support the hypothesis that elevated TDS was the cause of *Daphnia magna* mortality at a metal refinery and a limestone quarry (ESG International, unpublished data). However, the model should be used cautiously, since it has also been reported to fail in its ability to consistently predict *Daphnia magna* mortality in field-collected samples (Mount *et al.,* 1997; ESG International – unpublished data). Specifically, the *Daphnia magna* model tended to over-predict mortality.

Copies of the model program and users guide can be obtained from ENSR Consulting and Engineering (4413 W. Laporte Ave., Fort Collins, CO 80521).

Exchange Resins

Anion and cation ion exchange resins (see Section 9.2.2), activated carbon (see Section 9.2.3), and zeolite have been used to separate effluent into its organic and inorganic components (Doi and Grothe, 1989). However, the use of exchange resins may not alter the TDS concentration, but rather only shift the composition of the effluent. Therefore, results from the acute lethality tests combined with chemical analysis before and after treatment, are used to determine whether a relationship exists between mortality and changes (or reductions) in the ionic composition of the sample.

A specific example of the use of an exchange resins (i.e., zeolite) to support the identification of ion imbalance as a cause of acute lethality was provided in the AETE Case Studies (ESG International, 1998) with a cobalt, nickel and precious metal refinery (Case Study #5). In that study, the historical data indicated that effluent quality was variable and that mortality often coincided with high TDS. The measurement of conductivity (which is reflective of TDS) was generally greater than 7,000 FS/cm for samples lethal to *Daphnia magna* and greater than 12,000 FS/cm for samples lethal to rainbow trout.

Zeolite was the only TIE treatment that consistently reduced or eliminated *Daphnia magna* mortality. Chemical analysis of the untreated and treated effluent samples indicated that zeolite increased the effluent concentration of calcium (Ca), potassium (K), magnesium (Mg) and strontium (Sr) and reduced the concentration of various trace metals (e.g., copper, cobalt), carbonates and bicarbonates in the treated effluent. The TDS concentration in the zeolite treated effluent remained similar to the untreated effluent, suggesting that mortality was related to the specific ionic composition of the effluent.

The addition of Ca and K to the effluent also reduced mortality. It was thought that mortality could be prevented if the Na/(Ca+K) ratio could be corrected through the addition of Ca and K, either through direct additions or indirectly by treatment with zeolite. The beneficial effect of Ca and Mg on sodium-enriched waters has also reported by Ingersoll *et al.* (1992) and Dywer *et al*. (1992). These results were used (along with regression and chemical equilibrium modeling) to support the hypotheses that elevated TDS and copper were the main causes of *Daphnia magna* mortality (see Appendix B for additional details).

Simulated Effluents

Methods using simulated or mock effluents to mimic only the TDS composition of the effluent without other toxicants are also useful in the TIE process where elevated TDS is suspected (Ausley *et al*., 1998). Two approaches for effluent simulations may be useful in the assessment of acute lethality due to elevated TDS.

In the first approach, simulated effluent testing is conducted to identify the level at which TDS may cause acute lethality for each test species and to confirm the specific components responsible for toxicity. A simulated or mock effluent can be prepared by the addition of reagent grade salts to reverse osmosis (R.O.) or de-ionized water. The selection of the specific salts added would be based on the results from correlation analysis (i.e., between major ions and mortality), while the chemical concentrations would be based data obtained from the effluent sample to be simulated. Results are compared to determine whether the toxicity of the simulated effluent matches that of the actual effluent. If they agree, then all of the mortality was accounted for (i.e., the TDS components have been identified and confirmed as the main sources of mortality).

In the second approach (described by Goodfellow *et al.,* 2000), exposure concentrations are prepared by mixing the whole effluent with various amounts of synthetic effluent (based on major ion concentrations in the effluent). For example, exposure concentrations may include: 100% effluent collected from the mine site, 75% effluent and 25% synthetic effluent; 50% effluent and 50% synthetic effluent, etc. If TDS was the cause of acute lethality, the corresponding mortality responses should be similar in each exposure concentration. However, if unidentified toxicants are present, mortality will be reduced in the lower exposure concentrations (due to dilution of the unidentified toxicants).

Species Sensitivity Comparisons

Section 8.3 provides a discussion on species sensitivity comparisons in support of TDS as a cause of acute lethality.

9.2.2 Use of Cation and Anion Exchange Resins in Phase I TIEs

Overview of Cation and Anion Exchange Resins

Ion exchange takes advantage of an exchange media's preferred affinity for the selected ion in the effluent over the ion adsorbed to the media. The ions in the effluent are exchanged for a chemically equivalent number of ions on the media, based on the charge on the ions. The exchange process is concentration-dependent and reversible; therefore, ions can also be recovered from the resin (U.S. EPA, 1993a).

Ion exchange resins can be classified as cation exchange resins, which have positively charged mobile ions available for exchange, and anion exchange resins whose ions are negatively charged. These resins can be classified into 5 categories: strong-acid cation resins, weak-acid cation resins, strong-base anion resins, weak-base anion resins, and heavy-metal chelating resins (Ford, 1998). The majority of ion exchange resins are based on a styrene or divinylbenzene backbone, which can also cause the removal of other substances, including organics (U.S. EPA, 1993a). It has also been reported that these resins can release trace organics, which may contribute to toxicity of an ion exchange treated sample (Devonic *et al*., 1999; Doi and Grothe, 1989).

Application of Cation and Anion Exchange Resin in TIEs

The use of cation or anion exchange resins in TIE studies has been reported in the published literature, but methods have not been standardized.

Doi and Grothe (1989) described a fractionation procedure to identify the causes of *Daphnia magna* mortality using cation and anion exchange resins, activated carbon and zeolite. The exchange resins used included a strongly acidic cation exchange resin (Ag50W-x8, hydrogen form, Biorad), and a strongly basic anion exchange resin (Ag 1-x8, hydroxide form, Biorad). Application of the fractionation procedures was demonstrated through case studies for chemical manufacturing facilities. In one study, treatment with either activated carbon or anion exchange eliminated *Daphnia magna* mortality in a condenser/cooling water blowdown effluent. Comparisons of metal concentrations before and after treatment revealed a correlation between mortality and the concentration of hexavalent chromium. Confirmation was achieved through removal of the source of hexavalent chromium from the process. In a second study, an effluent containing high TDS was treated using cation and anion exchange resins and activated carbon. Only treatment with the cation and anion exchange resins in sequence eliminated *Daphnia magna* mortality, while also reducing calcium and chloride concentrations. Confirmation of calcium and chloride as the cause of toxicity was carried out by simulated effluent testing.

Devonic *et al.* (1999) used ion exchange resins to identify and confirm that cationic metals (from an abandoned mine site) were the cause of toxicity to fathead minnows, *Ceriodaphnia dubia* and *Selenastrum capricornutum*. Mine leachate samples were treated using two resins: 1) Chelex 100 (Biorad, sodium form), an exchange resin used to remove cationic metals, and 2) Ag2-x8 (Biorad, chloride form), an exchange resin used to remove anionic metals and oxyanions. After ion exchange treatment, sample hardness, alkalinity and pH were adjusted to match the untreated effluent. Toxicity and copper concentrations were reduced after treatment with both resins. However, an increase in osmotic strength (due to release of sodium, chloride and sulfate ions from the exchange resins) of the treated samples was also reported.

Burgess *et al*. (1997) reported on the use of cation-exchange methodology for removal and recovery of metals in marine TIEs. However, it was suggested that the methods could also be applied using

freshwater samples. Two weakly acidic cation-exchanges resins (Supelco LC-WCX and Alltech Extra-Clean IC-Chelate – pre-packed SPE columns) were reported to have the greatest consistency in removing and eluting metals from seawater. Column operation involved four steps: 1) conditioning using deionised water or 0.01 M acid solution, 2) loading of sample, 3) eluting interfering cations (i.e., Na, Ca, K) with deionised water, and 4) eluting metals with acid (1 to 5 M HCl). Recommended flow rates for loading and eluting the resin were 2.5 mL / min and <1 mL / min, respectively. Metal (Cd, Cu, Ni, Pb, Zn) removal rates for these two columns in reconstituted seawater were 80-100%, with 85-100% recovery (65-75% for copper) in elution trials. Breakthrough studies were required to effectively characterize effluent samples. It was recommended that cation-exchange manipulation be used in Phase II (identification), if the EDTA and sodium thiosulphate treatments in Phase I suggest the presence of metal. The time required to conduct the cation-exchange methodology may be unnecessary and financially prohibitive in Phase I.

We have also had success with a strongly basic anion exchange resin (Amberlite IRA-400, hydroxide form, Rohm and Haas) with TIEs conducted using rainbow trout and *Daphnia magna* (ESG International, unpublished data). However, difficulties were encountered with the use of cation exchange resins (Amberlite IRC50 and DP-1, Rohm and Haas). Specifically, we had varying success in obtaining non-lethal cationic blanks, due to extreme changes in pH and osmotic strength following treatment of laboratory dilution water.

Guidance for Use of Cation and Anion Exchange Resin in TIEs

Guidance on the use of cation and anion exchange resins for use in TIE studies is provided in the following section. Because standardized methods are unavailable, a generic approach has been provided based on the information provided in the previously described studies, as well as guidance given by the U.S. EPA (1993a). The specific approach will vary depending on the type of resin selected, nature of the toxicant, quality and quantity of effluent to be treated, and test species selected.

1. The key to obtaining useable data from an ion exchange test is to obtain non-toxic blanks (U.S. EPA, 1993a). Therefore, preliminary trials must be conducted to ensure non-lethal blanks can be obtained using the selected resin and laboratory dilution water. These trials must be conducted prior to evaluation of the effluent, and will include steps 3 through 7.

2. Another key for successful use of cation or anion exchange will be the performance of "breakthrough studies" on new effluent samples. These tests are conducted to assess resin capacity. The approach involves passing effluent through the resin, with collection and analysis of effluent at predetermined intervals. For example, Burgess *et al.* (1997), loaded a column with 100 mL of effluent, and collected the post column effluent at 15 mL intervals. These samples were tested for toxicity and analysed chemically to determine if breakthrough occurred.

3. Select the cation or anion exchange resin to be used.

4. If non-packed resins are used, determine volume of resin and size of column. The volume of effluent needed for testing will determine the amount of resin and the size of column required. For example, we have successfully used approximately 75 mL of anion exchange resin (Amberlite IRC50) in a glass column (custom built for this application; for smaller volumes a 60 mL polypropylene syringe could be used) to treat 1 L effluent volumes. Burgess *et al.* (1997) used 2.5 g of resin per 50 mL of sample. Alternatively, the resin could be added as a slurry to the effluent (followed by mixing or stirring of the effluent+resin mixture).

5. Preparation of resin will differ according to resin type, but will generally follow the manufacturers instructions. For example, the IRC50 resin is rinsed with 2 bed-volumes of a 4% NaOH solution prior to use.
6. Rinse resin with high purity water.
7. Rinse resin with laboratory dilution water, and collect sub-samples for testing (i.e., ion exchange blanks).
8. Pass effluent through resin, and collect sub-samples for toxicity testing and chemical analysis.
9. Check pH and hardness of treated dilution water and effluent. If necessary, re-adjust to mimic untreated effluent conditions. Be aware that pH adjustment may increase the ionic strength of the effluent, which may artificially increase toxicity.
10. Conduct acute lethality tests and interpret results. The information obtained using cation and anion exchange resins should be supported by the use of other manipulations to remove metals (e.g., EDTA, sodium thiosulphate, filtration at pH 11) (U.S. EPA, 1993a). Chemical analysis before and after treatment will also be useful in determining if there is a correlation between metal/anion concentration and mortality.
11. If acute lethality is removed, it may be possible to recover cations and anions from the column by elution with a strong acid or strong base. The approach may vary depending on the resin selected and the regeneration methods outlined by the manufacturer.

9.2.3 Use of Activated Carbon in Phase I TIEs

Overview of Activated Carbon

Activated carbon is the generic term used to describe a family of carbonaceous adsorbents. It can be formed from a variety of different sources (i.e., coal wood, coconut shell, bone char), which will affect its physical, adsorptive, and regeneration characteristics. Activated carbon has the ability to remove a wide variety of organic and inorganic substances, including chlorine, metals, nitrogen and DOC (Ford, 1998; Tchobanoglous and Burton, 1991). It is typically used in either powdered or granular form.

Application of Activated Carbon in TIEs

The use of activated carbon in TIE studies has been reported in the published literature, but methods have not been standardized. The non-selectivity of activated carbon may limit its effectiveness in TIEs. Furthermore, unlike solid phase extraction with C18, toxicants cannot typically be recovered from activated carbon. However, carbon may provide useful information in cases where mortality is not removed by any of the Phase I treatments (U.S. EPA, 1991a), or when combined with chemical analysis before and after treatment.

As described in Section 9.2.2, Doi and Grothe (1989) described a fractionation procedure to identify the causes of *Daphnia magna* mortality using cation and anion exchange resins, activated carbon and zeolite.

Beak (2000) included activated carbon as part of Phase I TIE studies conducted with a gold mine effluent. Activated carbon reduced *Daphnia magna* mortality, and eliminated fathead minnow mortality (results from other treatments are summarized in Appendix B). Ammonia, nitrite and metals (i.e., copper and zinc) were suspected as the main causes of acute lethality. In subsequent TTE studies, treatment with

activated carbon (in combination with zeolite, air-stripping or hydrogen peroxide) eliminated rainbow trout and *Daphnia magna* mortality, and also reduced DOC, ammonia, nitrate, nitrite, copper and cyanate.

During a Phase I TIE for a metal refinery (which was characterized by elevated TDS and metals), activated carbon had no beneficial effect on *Daphnia magna* survival, but eliminated rainbow trout mortality (ESG International, 1998). Activated carbon reduced metal (i.e., copper, cobalt, nickel), DOC and TOC concentrations, but had little affect on TDS. Copper was the suspected cause of trout mortality. Copper and TDS were the suspected causes of *Daphnia magna* mortality.

Guidance for Use of Activated Carbon in TIEs

Guidance on the use of activated carbon in TIE studies is provided in the following section. Because standardized methods are unavailable, a generic approach has been provided based on the information provided in the previously described studies. The specific approach will vary depending on the nature of the toxicant, quality and quantity of effluent to be treated and test species selected.

1. Select activated carbon product to be used. Coal-based carbons with large pores (i.e., Calgon Filtersorb 300) may improve the likelihood of adsorbing long-chain, high molecular weight compounds.
2. Determine volume of activated carbon and size of column. The volume of effluent needed for testing will determine the amount of carbon and the size of column required. For example, we have successfully used approximately 3 L of activated carbon (Calgon Granular Activated Carbon) in a plastic column (custom built for this application) to treat 10 L effluent volumes. Contact time may also be important; therefore, the development of isotherms may be required using several effluent concentrations.
3. Rinse activated carbon with high purity water.
4. Rinse activated carbon with laboratory dilution water, and collect sub-samples for testing (i.e., carbon blanks).
5. Pass effluent through activated carbon, and collect sub-samples for testing.
6. Check pH and hardness of treated dilution water and effluent. If necessary, re-adjust to mimic untreated effluent conditions.
7. Conduct acute lethality tests and interpret results. The non-specificity of activated carbon requires that it be used in combination with other Phase I tests, as well as with chemical analysis before and after treatment (to determine if a correlation exists between mortality and removal of a specific chemical).
8. Guidance on activated carbon elution for use in TIEs was not found in the available literature. Recovery of substances adsorb to activated carbon may not be possible for the TIE application, since regeneration involves oxidation of the organic matter in a furnace (Tchobanoglous and Burton, 1991).

9.2.4 Maintenance of pH During Rainbow trout Graduated pH Tests

Maintenance of effluent pH throughout the test is the greatest difficulty encountered during the graduated pH treatment. Interpretation of the graduated pH test results could be confounded by even small shifts in pH during testing. The U.S. EPA (1993a) Phase II document provides guidance on the use of CO_2 and buffers for small volume tests with *Daphnia magna*, fathead minnows and *Ceriodaphnia dubia*.

However, maintenance of pH can be even more difficult in large volume rainbow trout tests where the solutions may require aeration (which can cause an increase in effluent pH, even after acid or base adjustment). Organic buffers and CO_2 have both been used for conducting graduated pH tests with rainbow trout.

Evans *et al*. (2000) presented a method for pH maintenance that combined CO_2 and organic buffer additions. Effluent samples were adjusted to pH 6.5, 7.5 and 8.5 using organic buffers. The buffers used were as follows:

- pH 6.5 – 5 g/L of Bis-Tris buffer and 2 mL/L 6N HCl
- pH 7.5 – 5 g/L MOPS buffer and 2 mL/L 5 N NaOH
- pH 8.5 – 2.5 g/L POPSO buffer and 0.35 mL/L 6 N HCl.

Tests were conducted in 1 L exposure vessels (3 fish per vessel) placed in 20 L sealed glass chambers with 2% CO_2 flowing into the headspace (at a rate of ~ 1.6 SCFH). Test solutions were also aerated during 96-hour exposure. Measured pH values during testing were as follows: pH 6.5 test solution – measured pH ranged from 6.5-6.66; pH 7.5 test solution – measured pH ranged from 7.46-7.65; pH 8.5 test solution – measured pH ranged from 8.36-8.45.

Use of CO_2 for pH maintenance during rainbow trout tests has also been successful (ESG International Inc., unpublished data). The effluent sample (5 L) is placed in a 10 L glass aquarium (lined with a polyethylene bag), and adjusted to the desired pH level using HCl or NaOH. Ten fish are added to each solution. A small amount of CO_2 is added to the headspace immediately prior to sealing the bag. A small hose (placed in the solution and protruding through the top of the sealed bag) is used to siphon small aliquots of effluent for measurement of pH and D.O. during testing. Aeration lines can also be added if low D.O. is a concern. However, aeration will upset the CO_2:O_2 ratio and contribute to pH shifts. The correct concentration of CO_2 could also be obtained by using a gastight box of known dimensions and a gastight syringe to measure and deliver the required amount of CO_2 (additional details are provided in the U.S. EPA (1991a) Phase I TIE methods, and in Mount and Mount, 1992).

In both examples, preliminary testing may be required to determine the exact amount of CO_2 or buffer required to maintain pH during the 96-hour rainbow trout test. It may be possible to predict the amount of CO_2 to use to achieve a specific pH through the use of chemical equilibrium modeling programs. However, these programs do require some background chemistry of the effluent, as well as experience in the application of the programs.

It is also important to include CO_2 or pH buffer treated controls, since both substances could be toxic to the organisms on their own, or cause an additional stress that may affect their response to the effluent. For example, a 1% CO_2 treatment with rainbow trout induces a severe stress, primarily due to the blood and tissue acidosis. Tris buffer alters the chemistry of the gill water interface (affecting excretion of ammonia) potentially influencing toxicant delivery to the biological surface.

The disruption of chemical equilibrium may also affect the toxicity of certain chemicals. CO_2 in the headspace has a pH effect because it equilibrates with water and changes the $H_2CO_3 <> H^+ + HCO_3^- <> H_2O + CO_2$ equilibrium. Gassing with CO_2 not only alters the H^+ concentration, but also that of CO_2 and HCO_{3-}. Consequently, beyond the effect of pH alone, the addition of CO_2 may also affect the speciation and toxicity of certain chemicals.

9.2.5 Updated EDTA and Sodium Thiosulfate Information

The amount of EDTA or sodium thiosulfate required to chelate metals or oxidants will depend on the concentrations of metals present, effluent salinity and hardness (since EDTA will bind Ca and Mg), and the toxicity of EDTA to the test species (U.S. EPA, 1991a). The U.S. EPA (1991a) provided EDTA and sodium thiosulfate LC50s in a variety of dilution waters for *Ceriodaphnia dubia* and fathead minnows. Data was not available for rainbow trout or *Daphnia magna*.

In moderately hard dilution water (~250 mg/L as CaCO3), the EDTA LC50s for rainbow trout (96-hour) and *Daphnia magna* (48-hour) were estimated at 0.74 and 0.8 g/L, respectively (ESG International, un-published data). Sodium thiosulfate LC50s for rainbow trout (96-hour) and *Daphnia magna* (48-hour) were estimated at 23 and 3.2 g/L, respectively. Because the LC50s may be higher in an effluent sample, these values are provided only as a guide for selecting the amount of EDTA or thiosulfate to be used during the Phase I tests. Furthermore, laboratories should conduct their own internal tests to determine the acute lethality of EDTA and thiosulfate in their own dilution water.

Tests conducted by Hockett and Mount (1996) characterized the effectiveness of EDTA and thiosulfate in removing toxicity of 16 different metals (i.e., Cr^{3+}, Fe^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Mn^{2+}, Hg^{2+}, Ag^{+}, Pb^{2+}, Ni^{2+}, Al^{3+} and Cr[IV]) to *Ceriodaphnia dubia*. The tests resulted in a categorization of metals toxicity to *C. dubia.* For example, both EDTA and thiosulfate removed toxicity of copper chloride, cadmium chloride and mercury chloride to *C. dubia*. However, neither treatment was effective at removing toxicity of iron chloride, chromium chloride, potassium dichromate, aluminum chloride and sodium selenite.

Van Sprang and Janssen (2001) examined the effectiveness of various fractionation techniques (EDTA, sodium thiosulfate, anion exchange (Amberlite IRA-440C, hydroxide form), cation exhange (Amberlite IR-120 Plus, sodium form), activated charcoal, graduated pH test, filtration and solid phase extraction (SPE)) in reducing toxicity of 5 metals (Cd, Cr, Cu, Ni and Zn) to *Daphnia magna*. The metal solutions were tested individually in moderately hard dilution water, with an initial pH (pH i) of 7.5.

For all 5 metals, pH adjustment (to pH 3 and 11), aeration (at pH 3, i and 11), pH 3 filtration, and pH 3 C18 SPE had no signification effect on reducing metal concentration or toxicity to *Daphnia magna*. The findings for other treatments and metals were as follows:

- Cadmium toxicity was eliminated by EDTA, and reduced by sodium thiosulfate (activated charcol, reduction to pH 6.5, filtration at pH 11, activated carbon, anion and cation exchange resins were also effective).
- Chromium toxicity was not affected by EDTA or sodium thiosulfate (only the anion exchange resin was effective at reducing toxicity).
- Copper toxicity was eliminated by both EDTA and sodium thiosulfate (activated charcol, increase to pH 8.5, filtration at pH i and 11, C18 SPE at pH i and 9, activated carbon, anion and cation exchange resins were also effective).
- Nickel toxicity was reduced by EDTA, but not by sodium thiosulfate (filtration at pH 11, C18 SPE at pH 9, activated carbon, anion and cation exchange resins were also effective).
- Zinc toxicity was reduced EDTA, but not by sodium thiosulfate (activated charcol, increase to pH 8.5, filtration at pH i and 11, C18 SPE at pH i and 9, activated carbon, anion and cation exchange resins were also effective).

Further research would be required to determine if EDTA and thiosulfate (or other fractionation techniques) were applicable to rainbow trout, or with other metals (or metal combinations).

9.2.6 Biotic Ligand Model

Chemical equilibrium modeling is a powerful tool used to predict the composition and stability of solutions. The chemical equilibrium approach assumes that a state of equilibrium exists in the environment under study. A chemical equilibrium model can be used to calculate the concentrations of different components (e.g. soluble, insoluble forms of compounds) at equilibrium, based on the initial conditions and knowledge of the chemical reactions involved. In the case of metals, the minerals determine their solubility, and will cause a metal to precipitate if conditions (metal concentration, concentrations of anions, pH, etc.) are appropriate.

Beyond chemical equilibrium modelling, the Biotic Ligand Model (BLM) is a mechanistic model that includes the influence of both biotic and abiotic ligands in the calculation of the bioavailability of metals to aquatic organisms. The model has been shown to predict the acute lethality of certain metals (e.g., copper and silver) to the TIE species of interest (specifically rainbow trout and fathead minnows, and to a lesser extent, *Daphnia magna*), across a wide range of water quality parameters. Specifically, the BLM takes into account the influence of competition of the free metal ion with other cations (e.g., Ca^{2+}, H^{+}) and complexation by inorganic and organic ligands (e.g., -DOC, -OH, $-CO^{3}$) on the binding of metals (positively charged) with negatively charged biological ligands (the site of membrane transport and route of direct uptake of dissolved metals) (DiToro *et al.,* 2000; Santore *et al*., 2001; U.S. EPA, 2000).

At this time, it is unclear how BLM may be used in the TIE process. It is a new tool, is still in development and some expertise is required to use the program and interpret the results. It is mentioned in this section because of its potential future use as a new TIE tool to predict the acute lethality of various metals.

Additional information and a copy of the BLM manual and software can be obtained from the International Copper Association (New York, NY; Tel: 212-251-7240).

9.3 Procedures to Characterize Canadian Metal Mining Effluent Toxicity

An overview of a suggested modified Phase I characterization process to focus on those toxicants commonly associated with mining effluents (Section 12.1) is provided in Figure 5 and described in the following section. The approach is based on the U.S. EPA (1991a) Phase I methods, but has been modified to include additional treatments (i.e., zeolite, activated carbon, anion/cation exchange) shown to provide useful information on the characteristics of "common" metal mining toxicants.

The timing and sequence of treatments has also been altered in order to accommodate the additional tests, and the more time-consuming rainbow trout bioassays. However, the timing and sequence will vary depending on the nature of toxicant (i.e., its persistency), experience of the investigators, availability of resources (i.e., organisms; staff to conduct the treatments), and timing of sample receipt at the laboratory.

Day 1: Upon arrival at the laboratory, water quality parameters (i.e., temperature, pH, D.O., hardness, conductivity, ammonia) are measured and an initial toxicity test (Initial Test) started. Samples for analysis of other suspected toxicants (i.e., cyanide, metals) may also be collected on Day 1.

Day 2: A second untreated effluent sample (Baseline Test #1) is tested. The effluent is subjected to the

following treatments: adjustment to pH 3 and 11, filtration at pH 3, i and 11; aeration at pH 3, i and 11. After the manipulations are complete, the samples are readjusted to the initial pH of the effluent and the acute lethality tests are initiated.

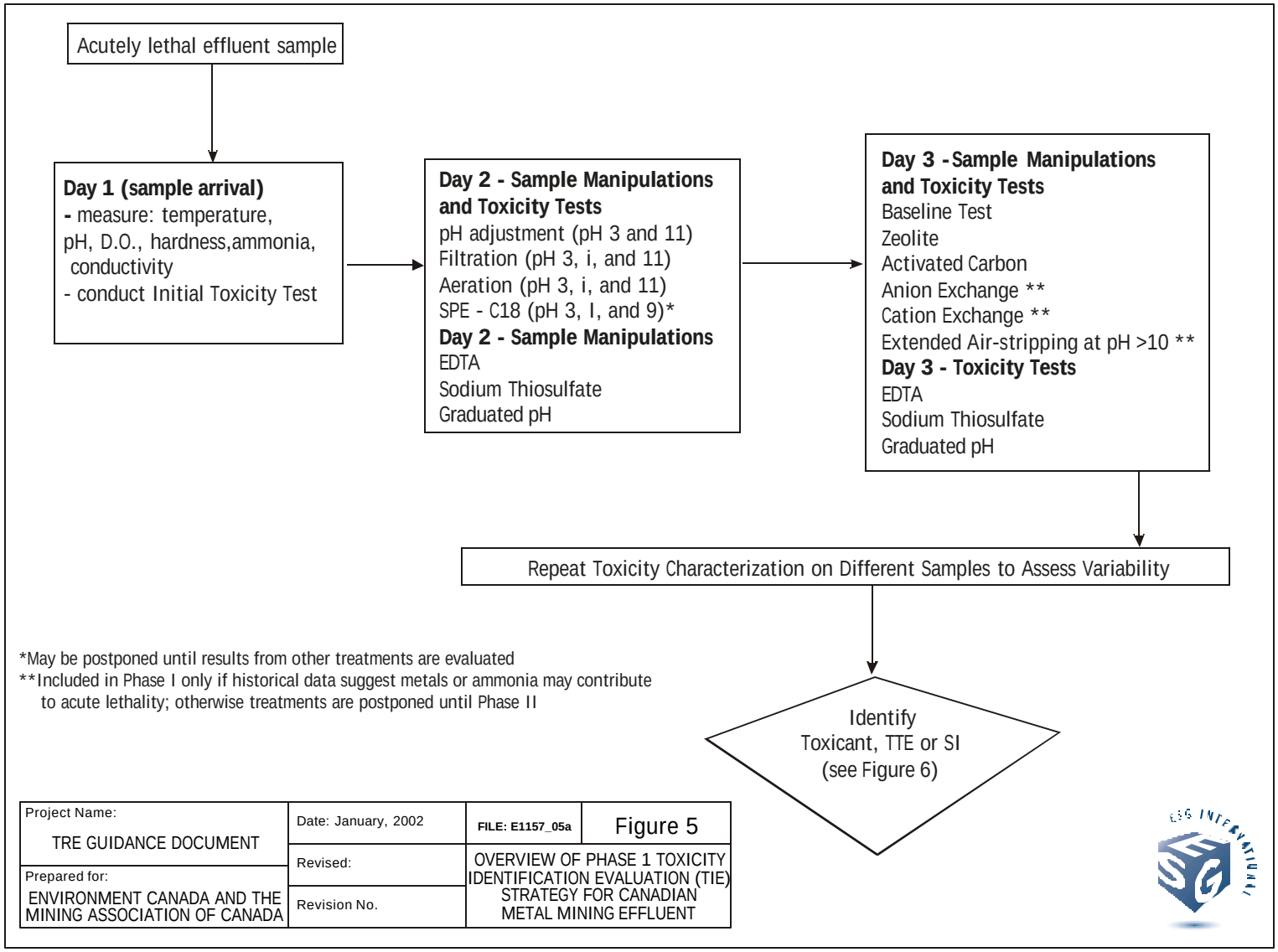
Treatment with EDTA and sodium thiosulfate, and the graduated pH adjustments are also conducted on Day 2, but testing is delayed until Day 3. The delay allows the EDTA to complex with metals, thiosulfate to react with oxidative substances and metals, and pH to stabilize in the graduated pH test. Samples may be held at test temperature in order to avoid super-saturation of D.O. resulting from warming of samples stored at cold temperatures (i.e., 4EC).

Day 3: A third untreated effluent sample (Baseline Test #2) and the manipulations conducted on Day 2 are tested. Column treatments with zeolite, activated carbon, anion and cation exchange resins, and extended aeration at elevated pH (>10) are conducted and the acute lethality tests initiated.

Because of the amount of time required to select, prepare and evaluate a cation or anion exchange resin, it is recommended that these tests be included in Phase I only if there is sufficient historical data to implicate metals or anions as the cause of acute lethality (Burgess *et al.,* 1997). Otherwise, these treatments should be conducted in Phase II, after the characterization tests implicate metals or anions as contributors to mortality. Similarly, extended air-stripping at elevated pH (see Phase II procedures in Appendix B) should only be included in Phase I if measured ammonia concentrations are at levels high enough to contribute to mortality.

In the case of a metal mining effluent, it may be possible to postpone treatment of the effluent using solid phase extraction (SPE) with C18. The C18 treatment is designed to remove relatively non-polar organics from solution; toxicants not commonly associated with metal mining effluent. However, the U.S. EPA (1991a) cautions that if the Phase I process is modified, it is possible to overlook classes of compounds that contribute to acute lethality. Abbreviated Phase I TIEs may result in the loss of valuable and necessary information about the characteristics of the substances responsible for acute lethality, which may lead to inconclusive results or erroneous conclusions (Ausley *et al.,* 1998). The decision to postpone or eliminate any Phase I treatment must be based on the experience of the investigator, and detailed knowledge of effluent chemistry and of the industrial sector.

The complete Phase I characterization should be repeated on different samples to assess effluent variability and ensure that all toxicants have been accounted for (U.S. EPA, 1991a).


Acutely lethal effluent sample
Day 1 (sample arrival)
- measure: temperature, pH, D.O., hardness,ammonia, conductivity
- conduct Initial Toxicity Test
Day 2 - Sample Manipulations and Toxicity Tests
pH adjustment (pH 3 and 11)
Filtration (pH 3, i, and 11)
Aeration (pH 3, i, and 11)
SPE - C18 (pH 3, I, and 9)*
Day 2 - Sample Manipulations
EDTA
Sodium Thiosulfate
Graduated pH
Day 3 - Sample Manipulations and Toxicity Tests
Baseline Test
Zeolite
Activated Carbon
Anion Exchange **
Cation Exchange **
Extended Air-stripping at pH >10 **
Day 3 - Toxicity Tests
EDTA
Sodium Thiosulfate
Graduated pH
Repeat Toxicity Characterization on Different Samples to Assess Variability
*May be postponed until results from other treatments are evaluated
**Included in Phase I only if historical data suggest metals or ammonia may contribute to acute lethality; otherwise treatments are postponed until Phase II
Identify Toxicant, TTE or SI (see Figure 6)
Project Name:
TRE GUIDANCE DOCUMENT
Prepared for:
ENVIRONMENT CANADA AND THE MINING ASSOCIATION OF CANADA
Date: January, 2002
Revised:
Revision No.
FILE: E1157_05a
Figure 5
OVERVIEW OF PHASE 1 TOXICITY IDENTIFICATION EVALUATION (TIE) STRATEGY FOR CANADIAN METAL MINING EFFLUENT

9.4 Interpretation of Phase I Results

This section provides examples for the interpretation of Phase I results, with a focus on two toxicants commonly associated with metal mining effluents: ammonia and metals. An overview of methods to deal with multiple toxicants is also provided.

Data interpretation is one of the most critical parts of a TIE and should be conducted by experienced investigators (Ausley *et al*., 1998). Interpretation of Phase I results can be confounded by effluent matrix effects (see Section 11), lack of good pH control (e.g., treatments may not be comparable if the pH is different among post-treated exposures), increases in conductivity during pH adjustment (e.g., caused by the addition of HCl or NaOH), the presence of multiple toxicants, variable effluent quality, and seasonal effects.

Based on the experience of the US EPA (1991a), at least one Phase I characterization test should be successful in altering acute lethality. If all mortality is not removed, other toxicants may be present in the effluent, or a single toxicant may be present at extremely elevated concentrations. Additional testing will be required to resolve these results. The approach selected (i.e., extended aeration, increased EDTA concentrations) will be dependent on the outcome of each Phase I treatment.

The U.S. EPA (1991a) also offered the following suggestions on interpreting Phase I results: i) if multiple toxicants are present, focus on identification of one toxicant (once this toxicant is identified, it should be easier to identify the other), ii) focus on those manipulations observed to have the most dramatic effect on acute lethality, and iii) concentrate on those treatments that remove the toxicant from other effluent constituents (i.e., solid phase extraction with C18, ion exchange).

The following examples represent only the simplest tools for assisting laboratories and mine operators with interpretation of the Phase I results. They are not intended as definitive diagnostic characterizations (U.S. EPA, 1991). Examples for other toxicants can be found in the U.S. EPA Phase I manual.

A cationic metal may be suspected as the cause of toxicity if:

i) toxicity is removed or reduced after EDTA addition

ii) toxicity is removed or reduced after sodium thiosulfate addition (note that some metals may not be removed by thiosulfate, but by EDTA, and visa versa; see Section 9.2.5).

iii) toxicity is removed or reduced by the C18 column

iv) toxicity is removed or reduced by filtration (under alkaline conditions)

v) erratic (non-linear) dose response is observed

Ammonia may be implicated as the substance responsible for rainbow trout mortality if:

i) the un-ionized concentration at the start or end of the test is >0.2 mg/L.

ii) rainbow trout are more sensitive than *Daphnia magna*

iii) toxicity is removed after treatment with zeolite, with a corresponding decrease in total ammonia concentrations

iv) toxicity increases as pH increases (or toxicity decreases as pH decreases)

v) toxicity is removed after extended air-stripping at high pH (i.e., pH 11)

The presence of other toxicants should be suspected if ammonia is removed by zeolite (based on measured concentrations after treatment), but the treated effluent is still acutely lethal. If the zeolite treated effluent is non-lethal it cannot be concluded that ammonia is the only toxicant, since zeolite may have removed other substances (i.e., metals) in addition to ammonia (U.S. EPA, 1993a). Results from the zeolite treated effluent must be used in combination with other manipulations (i.e., a weight-of-evidence approach) to confirm ammonia as the substance responsible for acute lethality (see Section 9.4.1). An example of a Phase I TIE implicating both ammonia and metals was reported by Beak (2000) and summarized in Appendix B.

9.4.1 Multiple Toxicants

Multiple toxicants may be suspected if: i) no single Phase I manipulation eliminates acute lethality, but several cause a reduction in mortality, or ii) different treatments reduce or eliminate toxicity to different species. If multiple toxicants are suspected, combinations of the effective characterization treatments should be conducted in sequence on a single effluent sample. The combinations are conducted after completion of the Phase I TIE, and in combination with chemical analysis of the effluent. If organism survival is increased in the combined manipulations (compared to an individual manipulation), then there is likely more than one substance responsible for acute lethality. If the results are similar, then it is likely that all of the manipulations were successful in reducing the same toxicant (U.S. EPA, 1991a).

For example, if multiple toxicants are present and aeration and EDTA both removed some mortality, the addition of EDTA to the post-aerated sample may clarify if metals are contributing to toxicity (U.S. EPA, 1991a). In a second example, ammonia and metals may be suspected toxicants if the graduated pH test exhibits greater acute lethality at low and high pH values than at intermediate test pH. In this case, the addition of EDTA to the effluent prior to conducting the graduated pH test may clarify if both ammonia and metals are contributing to mortality.

Detection of the hidden toxicants (those that do not express their toxicity because of the presence of a second toxicant) is one of the most difficult aspects of TIE testing, and will be most difficult to identify when ammonia or TDS are the primary substances responsible for acute lethality (U.S. EPA, 1993b). Because of its ability to mask the presence of other toxicants, it may be more effective to address acute lethality due to ammonia before proceeding with a full Phase I TIE. The approach would include the use of multiple species with differing sensitivity to ammonia (e.g., rainbow trout and *Daphnia magna* would be the preferred species). Effluent manipulations should include the graduated pH test, air-stripping at pH 11 and treatment with zeolite. Increased monitoring of pH change and mortality during testing (to track changes in un-ionized ammonia concentrations) should also be included, along with analysis of ammonia before and after zeolite treatment. If *all* tests (combined with measured ammonia concentrations) consistently indicated mortality due to ammonia, then it is unlikely that a hidden toxicant (i.e., metals) is present in the effluent (U.S. EPA, 1993b).

9.5 Linking Acute Lethality and Chemistry Data – The Toxic Unit Approach

Linking acute lethality and chemical data can be useful in the early stages of a TRE (see Section 6.1), but may be most valuable when the TIE treatments implicate a particular substance as the cause of mortality. One common approach in linking chemical and mortality data is through the use of Toxic Units (TUs). Lethal TUs express the degree of effluent toxicity, or the concentration of substance, as a fraction of the LC50 (Environment Canada, 1999). The TUs are dimensionless, and allow for normalization of LC50

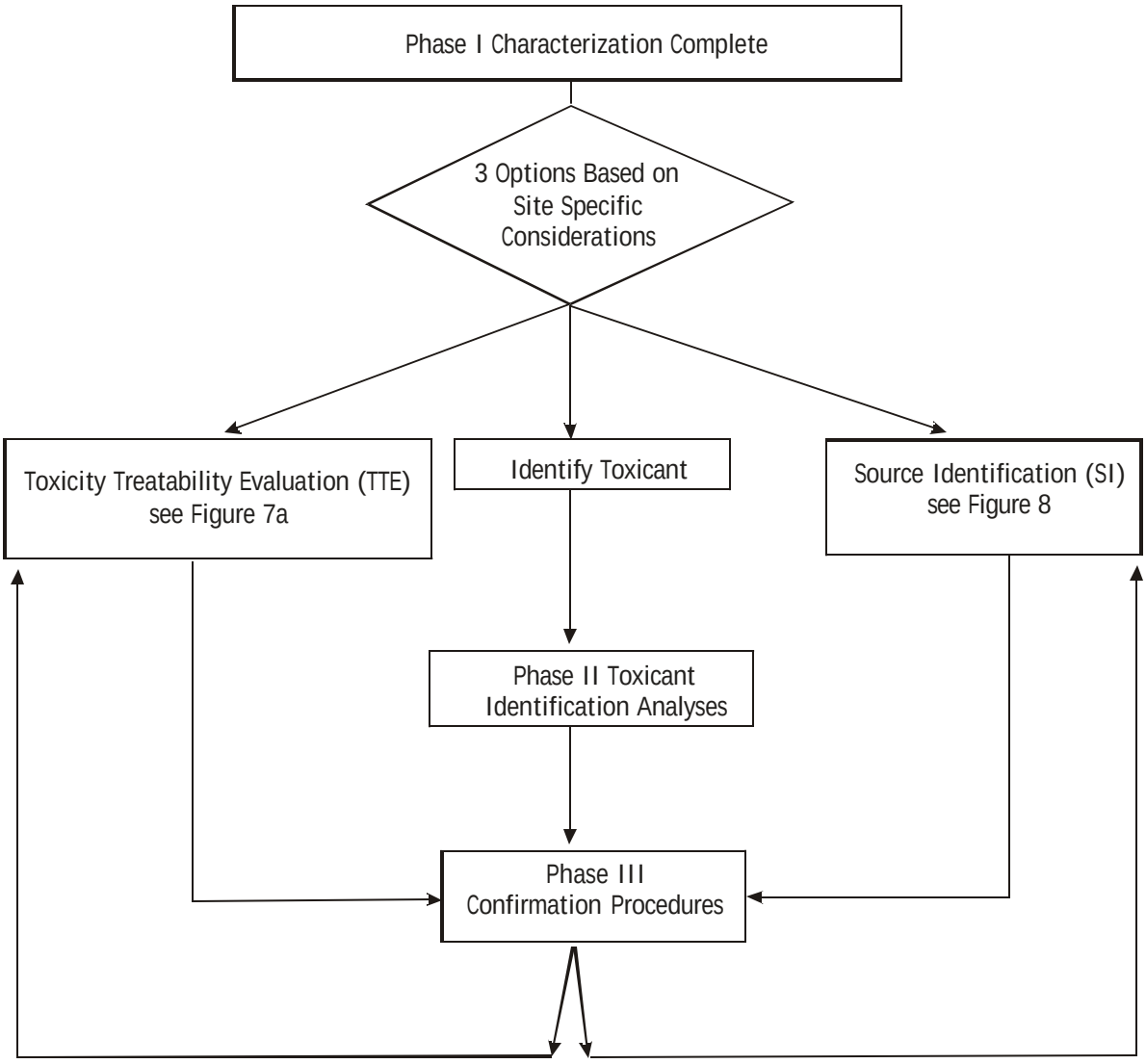
data. The TU can be used to predict the acute lethality of an effluent based on the measured toxicant concentrations.

The lethal TU for an effluent is obtained by dividing 100% by the LC50. A TU equal to 1 indicates a "marginally" lethal sample (i.e., LC50 = 100%). If the total TU > 1, then the effluent is expected to be lethal to the test organism (e.g., considered a failure in context of the MMER). The suspect toxicant concentration is converted to a TU by dividing the measured toxicant concentration by the LC50 for that toxicant. If more than one toxicant is present, the concentration of each one is divided by the respective LC50, and the TU can then be summed (Environment Canada, 1999). The total TU for the individual contaminants are compared to the actual TU for the effluent sample. If the TU are equal then it is likely that all toxicity has been accounted for.

There are limitations associated with the TU approach. First, the prediction of mixture toxicity is based on the assumption that the effects of the individual contaminants are additive (Environment Canada, 1999). The potential for synergistic or antagonistic effects is not taken into consideration. Secondly, rainbow trout and *Daphnia magna* LC50 data may not be available for those substances suspected to be responsible for mortality. The U.S. EPA Ecotox Database System (www.epa.gov/ecotox), U.S. EPA Water Quality Criteria (U.S. EPA, 1986) and Canadian Water Quality Guidelines (CCME, 1999) may be useful sources for obtaining LC50 data for a variety of substances. These databases included a range of LC50 data, as there are a number of factors that may affect acute lethality (e.g. pH, water hardness). However, it may not be possible to find LC50 data for single chemical tests conducted using water quality conditions that are similar to the effluent being investigated. In this case, attempts should be made to use relatively conservative values. Alternatively, the better choice may be to conduct additional acute lethality tests to generate LC50s for the substance of interest under water quality conditions (i.e., pH, hardness, TOC) that mimic the effluent.

9.6 TRE Options Following Completion of Phase I TIE

An overview of the TRE options available following completion of a Phase I TIE are presented in Figure 6. After completion of the Phase I characterization of an effluent, the TRE can proceed to: i) a TTE to evaluate various treatment methods for removal of the toxicant, ii) an SI to identify the source of the toxicant, or iii) a Phase II and III TIE to identify and confirm the specific substance responsible for acute lethality prior to conducting a TTE or SI. There will be more uncertainty associated with TTE or SI studies based on toxicant characteristics alone, rather than the known identity of the substance(s) responsible for acute lethality (U.S. EPA, 1989). In the case that the TTE or SI approach is selected, confirmation testing (Phase III) will still be required to ensure that the method selected consistently removes acute lethality.



Project Name: TRE GUIDANCE DOCUMENT	Date: January, 2002	FILE: E1157_06	Figure 6
	Revised:	TRE OPTIONS FOLLOWING COMPLETION OF PHASE 1 TIE (U.S. EPA, 1989, 1991A)	
Prepared for: ENVIRONMENT CANADA AND THE MINING ASSOCIATION OF CANADA	Revision No.		

10 PHASE II AND III TIE PROCEDURES

This section provides a summary of the Phase II and III TIE procedures described by the U.S. EPA (1993a,b) (see Appendix B for additional details).

The treatments, procedures and analytical methods selected for a Phase II and III TIE are directly related to those treatments observed to effectively eliminate or reduce acute lethality during Phase I. Therefore, the specific approach used can only be determined after Phase I is complete. As Phases II and III proceed, QA requirements (see Section 5) must be revisited and modified as required. If modified test methods or surrogate test species were used during Phase I, increased standardization should be used during Phase II, and particularly in Phase III, to confirm that the suspected substance is responsible for the toxicity observed in the test that originally triggered the TIE (U.S. EPA, 1993a,b).

A brief overview of possible approaches to Phase II and III (U.S. EPA, 1993a,b) is provided in the following section. A more thorough summary is provided in the literature review (Appendix B), but the original U.S. EPA document should be consulted for detailed procedures.

Phase II TIE

In Phase II of a TIE, further effluent treatments are conducted to identify the specific substance(s) responsible for toxicity. Acute lethality tests are combined with chemical analysis to obtain a quantitative measurement of the suspected toxicants. A summary of the approaches outlined in the U.S. EPA (1993a) Phase II guidance document is provided in Table 7.

Table 7. Summary of US. EPA (1993a) Phase II TIE treatments

Substance	Summary of Phase II Approach
Non-polar organics	Separation of toxic and non-toxic fractions using HPLC followed by analysis of the toxic fractions
Ammonia	Measurement of ammonia in effluent; graduated pH testing; treatment with zeolite resin to remove ammonia; air-stripping of ammonia from the effluent at high pH (i.e., pH 11).
Metals	Measurement of metals in effluent; treatment with EDTA and sodium thiosulfate; graduated pH and ion exchange tests.
Chlorine	Measurement of total residual chlorine (TRC) in effluent; treatment with sodium thiosulfate
Filterable Toxicants	Use of other filter types (i.e., nylon, Teflon); centrifugation; extraction and concentration of filtered material.

Phase III TIE

The objectives in Phase III are to: i) confirm that the substances responsible for toxicity have been correctly identified, and ii) ensure that all of the toxicity has been accounted for. A "weight-of-evidence" approach is used during Phase III to confirm that the substances responsible for acute lethality have been identified (U.S. EPA, 1993b). As with Phase II, there are many possible approaches to confirming the

substance(s) responsible for mortality. A summary of the approaches outlined in the U.S. EPA (1993b) guidance document is provided in Table 8.

If all Phases were conducted on a single sample, confirmation testing on several samples would still be required, since it could not be assumed that the substances responsible for acute lethality are the same between samples. If samples have been collected over several months, confirmation testing may need to include seasonal samples (U.S. EPA, 1993b).

The confirmation of toxicity removal must still be conducted in cases where the TTE or SI approach has been used (in place of toxicant identification). Effluent samples must be tested repeatedly over a sufficient period of time (using the species that triggered the TIE) to ensure that a full range of effluent conditions is evaluated (U.S. EPA, 1993b).

Table 8. Overview of U.S. EPA (1993b) Phase III confirmation approach

Correlation Approach	The objective is to determine if there is a consistent relationship between the concentration of the suspected toxicant(s) and effluent toxicity. A wide range of toxicity responses with several samples must be obtained in order to provide an adequate range of effect concentrations for the regression analysis.
Symptom Approach	The approach involves the use of test organism behaviour and time to death in comparing the responses of organisms to the whole effluent and then to the suspected toxicant(s).
Species Sensitivity Approach	If the suspected toxicant(s) has been correctly identified, effluent samples with different LC50s for one species should have the same ratio for a second species with different sensitivity.
Spiking Approach	In spiking tests, the concentration of the suspected toxicant(s) can be increased in the sample to determine if toxicity increases proportionally to an increase in concentration. The suspected toxicant could also be added to a non-toxic sample, to dilution water or to a sample of effluent where the suspected toxicant(s) has been removed.
Mass Balance Approach	The mass balance approach is used when the toxicant(s) can be effectively removed from the effluent and subsequently recovered.
Deletion Approach	The deletion approach involves removal of the suspected toxicant(s) from a waste stream. The suspected toxicants are removed for a short period and the effluent is tested. This approach offers the strongest evidence that the suspected toxicants identified are the correct ones.
Additional Approaches	Manipulation of pH, hardness; Measurements of body uptake, to assess bioavailability; Combined Phase I characterizations; Effluent simulations to confirm toxicity due to TDS.

11 EFFLUENT MATRIX EFFECTS

This section provides a discussion on effluent "matrix effects". A matrix effect occurs when toxicants interact with other effluent constituents in ways that change their toxicity (U.S. EPA, 1993b). As described by the U.S. EPA (1993b), matrix effects can fit into one of two categories. The first is when toxicants change form, such that they exhibit a different toxicity. For example, ammonia becomes more acutely lethal at high pH. Another example is cyanide and hydrogen sulphide, which increase in toxicity as pH decreases. The second category is when the substance undergoes a physical change (i.e., binding to particulates) making it biologically unavailable to the organism. For example, a particulate bound toxicant may be unavailable to rainbow trout, but readily available to *Daphnia magna* as the particulates are ingested via filter feeding.

Experience of the investigator as well as detailed knowledge of the chemistry of the mine effluent will be crucial in identifying potential matrix effects during a TIE. Based on the "common" toxicants associated with metal-mining effluent (e.g., ammonia, metals, cyanide) and the influence of pH on the expression of their toxicity, investigators should be prepared to expect matrix effects in metal-mining effluent samples.

In the case of metal mining effluent samples, matrix effects may be intensified during acute lethality tests with rainbow trout due to pH shifts resulting from aeration of the test solution. For example, depending on the initial pH of the effluent and how quickly the pH increases with aeration, concentrations of un-ionized ammonia that were not at lethal levels at test initiation, could increase during testing resulting in trout mortality. Therefore, increased monitoring of pH during testing (with corresponding calculations of un-ionized ammonia concentrations) may provide useful information on matrix effects and acute lethality due to ammonia.

In Phase III confirmation tests, correlation analysis will be difficult when matrix effects are present. Metals, in particular, will be difficult to implicate using correlation analysis because their toxicity is matrix dependent (i.e., pH, hardness, TOC), and less well defined when compared to ammonia. Alternatives to correlation should be used to confirm the substances responsible for mortality in cases where matrix effects are suspected (U.S. EPA, 1993b).

12 TOXICITY TREATABILITY EVALUATIONS (TTE)

This section provides generic protocol outlining the procedures to be taken during a Toxicity Treatability Evaluation (TTE). It emphasizes the systematic evaluation of chemical, biological or other treatment technologies (or combinations of technologies) to assess the abilities of these technologies to reduce the elevated concentrations of contaminants that are causing acute lethality. This section also includes a list of treatments and approaches for reduction of "common" metal mining toxicants. Detailed approaches for bench-, pilot-, and full-scale testing are described, and toxicity prevention strategies for metal mining operations are also provided.

A TTE involves the systematic evaluation of various treatment technologies, combinations of technologies, or management options (i.e., process or operational changes) to assess the ability of these technologies (or operational / process changes) to reduce elevated levels of contaminants that are causing acute lethality. TTEs can be conducted with or without identification of the specific toxicant(s), but will be more effective if a specific substance can be targeted for treatment. Treatment technology selection based on the Phase I TIE results can also help focus the TTE and increase the likelihood of success (Beak, 2000). However, it should also be noted that source reduction by waste minimization or chemical optimization is often more cost-effective than toxicity treatment.

Criteria for the selection of the preferred treatment technology or management option should be defined at the beginning of the TTE, and should include (but not limited to):

- Performance - including treatment effectiveness to consistently meet acute lethality limits, ability to handle fluctuations, and susceptibility to upsets,
- Cost - including, capital, operating and maintenance, and disposal of residuals,
- Complexity, ease of implementation and operation
- Service life and flexibility
- Application to other effluent streams

Although cost may be a primary selection criterion, the treatment technologies or management options must also offer the best potential for consistent reduction or elimination of acute lethality. The selection criteria should be used initially to screen all reasonable options. The preferred options can then undergo an evaluation using bench-, pilot- or full-scale testing. Information from these tests should be used to select the most viable option(s) based on a more thorough comparison of the criteria listed above. The final selection process may require a quantitative examination of the options using a scoring and ranking system (U.S. EPA, 1999).

Once a viable treatment or management option has been selected and implemented, a follow-up monitoring program must be established to confirm the effectiveness of the selected approach. This program may involve more frequent monitoring than is required by the regulation, but will be tailored for each facility.

As with all other aspects of the TRE process, good communication within the TRE team is critical to ensure the exchange of accurate information during all TTE or SI (see Section 13) studies. A lack of good communication or a delay in transfer of information may result in the generation of irrelevant bench- or pilot-scale data.

12.1 Treatments and Approaches for Effective Reduction of Common Metal Mining Effluent Toxicants

Common metal mining effluent toxicants include:

- Base metal mines: free acidity, depressed or high pH, dissolved metals and ammonia
- Gold mines: cyanide and cyanide related compounds, arsenic, dissolved metals, ammonia and total suspended solids (TSS)
- Uranium mines: solvent extraction organics, arsenic, uranium, TSS and dissolved metals
- Iron Ore mines: TSS

Treatments and approaches for reduction of these common metal mine effluent toxicants are summarized in Table 9. However, it is important to note that the technology selected will be site-specific and the list provided is not intended to suggest these treatments will be effective under all conditions at all sites.

Table 9. Common metal-mining toxicants and treatment techniques

Toxicants	Treatments and Approaches
Ammonia	• Reduce at source (explosives management). • Reduce chemical sources of ammonia (e.g. cyanide degradation). • Natural evolution in holding ponds. • Air sparging at elevated pH. • Biological removal of nitrogen compounds by nitrification / denitrification (efficient, but costly because of the need to warm the solution being treated). • Ion exchange (used as a pre-concentration step; zeolite has been successfully tested at the pilot plant scale). • Chemical oxidation (breakpoint chlorination has been used - expensive and produces toxic by-products). • Lower pH reduces ammonia toxicity. • Recycling elevated ammonia solutions.
Dissolved Metals (Fe, Al, Cu, Ni, Pb, Zn, Cd, and U	• Precipitation at pH with lime is the common treatment practice. • The excess alkalinity is removed by sulphuric acid or carbon dioxide addition to treated effluent. • Sulphides (NaHS, CaS) can be used to remove metals to very low levels, however, the handling of the settled solids may be problematic. Residual HS^- • Passive treatment systems have been used to remove metals in sulphate-rich systems, but the application in Canada is limited because of climate restrictions and fouling of substrates with aluminum and iron precipitates. • Metal precipitants such as polythiocarbonate and trimercapto-s- triazine trisodium salt (TMT) can be used to precipitate metals and stabilize the sludge formed. • Electrocoagulation can be used as a supplemental technology but is still in developmental stage. • Addition of metal complexing agents (e.g. EDTA)
Arsenic and Molybdenum	• Removal is commonly done by co-precipitation as ferric arsenate or ferric molybdate by addition of ferric chloride or ferric sulphate. • Process requires pH adjustment for maximum precipitation. • Oxidation of arsenic and molybdenum is frequently needed.

Table 9. Common metal-mining toxicants and treatment techniques

Toxicants	Treatments and Approaches
Mercury	• Effective technologies for Hg removal include adsorption on activated carbon and sulphide precipitation. • Metal precipitants (e.g. lime) are capable of removing Hg.
Dissolved Salts	• Calcium concentrations can be reduced by alkali metal substitution, lime/sodium carbonate softening, and gypsum or calcium carbonate precipitation.
pH	• Increases in pH are most commonly achieved with lime, sodium hydroxide, or soda ash. • Decreases in pH are most commonly achieved with sulphuric acid or carbon dioxide.
TSS	• Sedimentation (most widely practised method of removal of precipitated solids). • Coagulation and flocculation (used to promote particle aggregation to form larger flocs and promote settling; excess polymer may contribute to effluent toxicity). • Filtration (used as polishing step for removal of fine material or pre-treatment prior to ion exchange or carbon filtration).
Cyanide and Related Compounds (cyanate and thiocyanate)	• The most common technologies for cyanide destruction or oxidation include: natural degradation (which includes evolution and oxidation), INCO SO_2 /air oxidation, peroxide oxidation, ozone oxidation and alkaline chlorination. • Other site specific processes for cyanide removal/degradation include: Helmo Gold Process and Homestake's Biodegradation System. • Activated carbon adsorption can be used for very low levels, but is impractical due to high cost. • Cyanide toxicity is often supplemented by ammonia or copper toxicity that can result in acute lethality. • Cyanide recovery systems such as Cyanisorb process, acidification-volatilization-reneutralization (AVR), acidification, filtration and reneutralization (AFR), sulphidization, acidification, reneutralization and thickening (SART), etc and generation and recovery of cyanide from thiocyanate through use of ozone. • Recycle of high cyanide solutions to the leaching process is commonly practised.
Nitrate/Nitrite	• Biological denitrification (routinely applied to municipal wastes, less to mining effluents) • Ion exchange (may be costly and difficult to treat regenerate) • Electrochemical ion exchange (has not been proven on a large scale, and would not likely ever be applied for mine effluent)
Thiosalts	• Natural oxidation in holding ponds followed by pH adjustment is the most common technology. • Other technologies include: oxidation by catalysed air, ozone, chlorine, hydrogen peroxide, SO_2-air, ultraviolet radiation, electrooxidation and biological oxidation, reverse osmosis and activated carbon. These treatment technologies are high in capital and operating costs.

12.2 Approach to TTEs

A generic approach to conduct a TTE is presented in Figure 7, and includes the following important steps.

1. Review available TIE results.

The first step in the TTE process should include the review of available TIE results. Once the toxicant(s) is characterized or identified, the TTE will require the selection of technologies that will remove the specific toxicant(s) or classification of substances based on the Phase I TIE (i.e., metals). The identification of potentially effective treatment methods should also be based on best available technology information.

2. Identify potentially effective treatment methods.

The second step is the identification of effective treatment methods for removal of the suspected toxicant(s). The treatment methods identified may be commercially available, or experimental processes. The proven commercial technologies should be evaluated first. Even though experimental technologies may be attractive because they are new, exciting, or offer lower operating or capital cost, they offer the highest risk to implementation. In order to minimize risk and time delay to implementation, evaluation of experimental technologies should be conducted after the commercially available technologies have been tested. Experimental technologies should be treated as longer-term research projects to develop a treatment strategy that will minimize long term operating costs.

The treatment approaches identified (both commercial and experimental) to remove or reduce the suspected toxicant(s) should be tabulated. A list should be prepared showing all of the commercial processes that will remove the targeted contaminants. The commercial processes listed should include examples of operations that are using this treatment. Where possible the commercial process should be cross-referenced with operating flow rates and process costs. If available, treatment evaluation test conditions may begin to be explored and tabulated in another table, where operating pH, chemical dosages and retention times may be tabulated.

The facility operator may consult external experts in water treatment, or contact a commercial testing laboratory to discuss, recommend, test and evaluate the application of the commercial processes or best available technology to remove the toxicant(s). The discussions between the operator, consultants or testing laboratory will likely lead to the definition of a bench-scale program to test the commercial processes defined in the table.

3. Evaluate the effectiveness of selected technologies

The effluent treatment options should be tested and developed at the bench-scale, to assess and confirm the process constraints, efficiency, operating cost effectiveness, robustness of the technology to process upsets and to develop information for engineering scale-up and capital cost. Once this information has been evaluated, decisions will be made to pilot the technologies that have been most successful.

Samples must be tested repeatedly over a sufficient length of time to ensure a full range of effluent conditions are evaluated. Process changes, weather, seasonal changes and intermittent operations should be included during confirmation testing. It is also critical that the species that triggered the TIE be used during all testing (U.S. EPA, 1989).

4. Select the best technology

From the demonstrated effective bench treatment technologies, select one or more best options.

5. Conduct pilot testing of the best option(s)

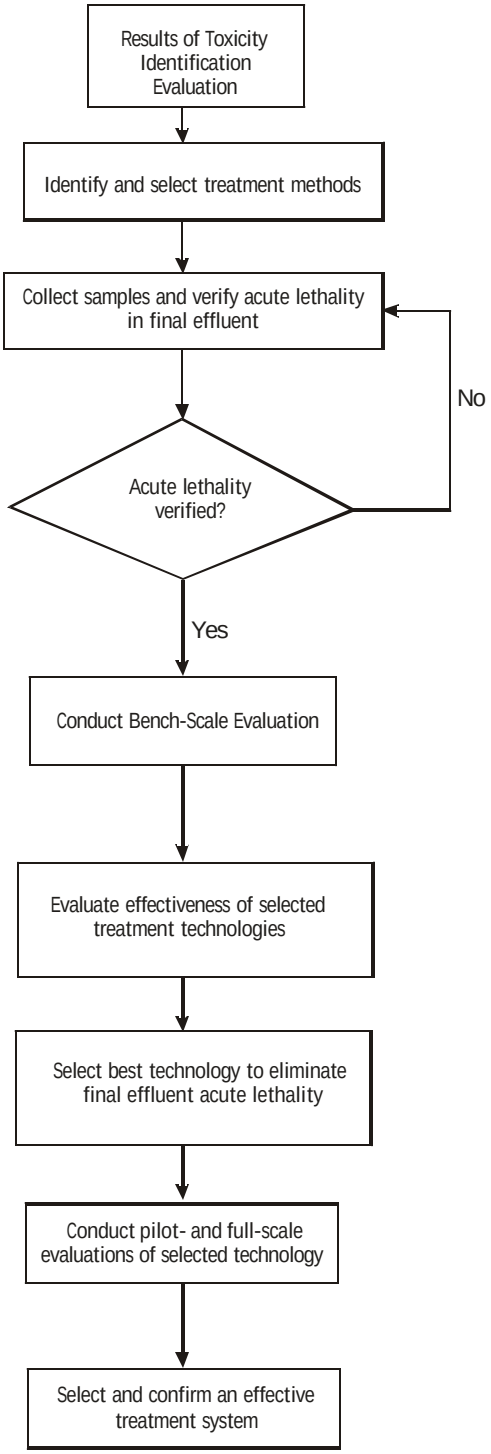
Conduct a pilot-scale test of the best option(s) for verification of treatment effectiveness, prior to feasibility of full-scale implementation. Both bench- and pilot-scale demonstrations of the effectiveness of the selected technology are required prior to actual implementation, since the implementation on-site can be potentially costly.

6. Select and confirm an effective treatment system

The final task is to confirm an effective treatment system that will consider:

- Performance - including treatment effectiveness to consistently meet acute lethality limits, ability to handle fluctuations, and susceptibility to upsets,
- Cost - including, capital, operating and maintenance, and disposal of residuals,
- Complexity, ease of implementation and operation
- Service life and flexibility
- Application to other effluent streams

Once the effective acute lethality control method has been selected and implemented, additional monitoring must be conducted to ensure the ETP is reducing acute lethality to meet regulatory requirements.



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OVERVIEW OF TOXICITY TREATABILITY EVALUATIONS (TTE) PROCESS	
Project Name: TRE GUIDANCE DOCUMENT	
Prepared For: ENVIRONMENT CANADA AND THE MINING ASSOCIATION OF CANADA	
Date: January, 2002	Revision No.

12.3 Bench-, Pilot- and Full-Scale Testing

This section describes the rationale and important steps for bench-scale, pilot-scale and full-scale testing.

12.3.1 Bench-Scale Testing

A generic protocol outlining the procedures to be taken during bench-scale testing has been provided. Bench-scale testing is undertaken as a cost-effective screening for a variety of treatment technologies used to eliminate final effluent acute lethality. Important steps in designing a bench-scale include:

1. **Assess current effluent management or treatment systems and its' operation to ensure plant optimization prior to initiation of the TTE (see Section 5.0).**

2. **Select appropriate technologies.**

The treatment methods identified to remove the suspected toxicant may be commercially available, constructed on site, or experimental processes (see Section 11.2).

3. **Design sampling methodology and acute lethality test procedures.**

A representative sample will be required for a bench-scale TTE. Samples should be characterized on-site for general water quality parameters (i.e., temperature, dissolved oxygen, conductivity, pH). It is also important that the on-site conditions be documented prior to, and during the sampling of the final effluent or source streams. This is suggested to ensure that sampling will not be conducted during unusual or atypical operating conditions. To ensure the final effluent sample is collected during normal operations, the following information is required:

i) Facility conditions at the time of sampling (i.e. status of process/production and treatment system, weather conditions). Assess if any "non-routine" operations have occurred or are ongoing (e.g. construction, pesticides for control, new mill reagents, etc.).

ii) ETP conditions at time of sampling. Determine if monitored chemical parameters, pH, conductivity, metals, cyanide, etc. are within normal operating limits. Also assess if any new treatment chemicals had been used during the toxic event.

iii) Changes in effluent characteristics during transport. At the time of sample collection, site personnel should conduct field measurements of: temperature, dissolved oxygen, pH and conductivity. If possible on-site determinations of ammonia and metals should be conducted, with a split analytical sample sent along with the larger sample for the TTE. Since the lab will test a sample that may have changed slightly due to aging, the change in effluent quality could become important during implementation of new or modified treatment methods.

Preliminary bench-scale testing studies may use modified test methods, particularly in cases where a large number of treatment technologies are to be assessed. However, subsequent tests should follow the regulatory methods that triggered the TRE investigation. LC50 tests should be conducted to determine the degree of acute lethality and to measure the effectiveness of the treatment to reduce acute lethality of a source stream (prior to combination with other untreated streams and simulation of the ETP). It is also suggested that selected samples be tested using both rainbow trout and *Daphnia magna* to ensure the proposed treatment does not increase acute lethality to a non-target species (i.e., final effluent is acute lethal only to rainbow trout, but proposed treatment increases toxicity to *Daphnia magna*).

4. Ensure existing ETP can be adequately simulated at the bench-scale.

If the mine operation has an existing ETP, then it will be critical to the success of the TTE to adequately simulate the existing treatment system at the bench-scale prior to evaluating a new or modified treatment technology. Simulation of the existing ETP is required in terms of process parameters (i.e., pH, TSS), chemical additions (i.e., polymers) and downstream conditions (i.e., settling or holding ponds) (see bullet #5). However, one of the most difficult conditions to simulate in the laboratory is temperature. Treatment plant conditions are frequently dynamic (e.g. flow) and a range of conditions should be evaluated.

For example, if a treatment technology were to be assessed on a single source stream, the treated source stream would have to be combined with the other waste streams on a flow proportional basis to simulate the influent to the ETP. The combined wastewater (after treatment of the individual source stream) must then be subjected to a simulated ETP treatment prior to acute lethality testing. However, simulation of the ETP prior to technology evaluation is necessary so that any synergistic or antagonistic effects between streams could be detected before a treatment technology was selected.

5. Develop treatment flow sheets, calculate chemical addition ratios and methods of addition, temperatures, holding/retention times and solids removal techniques to remove targeted toxicants.

Chemical addition rates in the ETP need to be determined (noting whether the rates are proportion to flow of effluent, method / strategy for control). The reliability history of the control strategy is important in determining process performance (e.g. reliability and maintenance of pH sensors for metal precipitation). Methods of addition of chemicals are also an important variable in assessing process efficiency. For example, a lime slurry added directly to a stream from a pipe is less effective than lime added into a stirred reactor.

Downstream conditions are important components of many treatment plants. The most important factors include mean retention time in ponds and short-circuiting. Mean retention time is determined by dividing the pond volume by the flow volumetric rate. Retention times are typically the lowest in spring run-off conditions at mine sites, but can also be low during and following storm events. Short-circuiting of settling ponds occurs when treated effluent does not mix well with the effluent pond volume. All settling ponds exhibit some short-circuiting, and the extent can be evaluated using dye and inorganic tracer tests.

6. Co-ordinate technology evaluation with toxicity laboratory.

If an on-site toxicity testing laboratory is unavailable, the TTE laboratory or testing facility should co-ordinate the treatment technology evaluation with the aquatic toxicity testing laboratory to ensure rapid, secure transport of samples to be tested and notification in advance of acute lethality test requirements (see Section 12.5)

7. Collect sample (usually done by mine personnel).

Proper sampling instructions (i.e., sample volumes), equipment (i.e., buckets, lids, liners) and chain-of-custody forms should be provided to mine personnel (see Section 3). The sample should be shipped immediately after collection to the facility conducting the TTE.

8. Evaluate untreated sample chemically and test for acute lethality. Results should be compared to historical data to assess if mine was operating within "normal" or "typical" range of conditions when sample was collected.

Prior to conducting TTE work, a fresh sample must be collected at the site and analysed to determine if effluent was representative of "normal" operating conditions. Routinely monitored effluent treatment parameters (i.e., pH, conductivity, TSS, metals) should be analysed to determine if they were within "normal" operating limits. Acute lethality toxicity testing will also be performed on the untreated sample to ensure the presence of toxicity prior to conducting the bench-scale tests.

On-site staff should verify the sample as truly representing the site effluent prior to testing. Verification will involve comparing the measured parameters to historical data to determine if the sample has physical, chemical and acute lethality characteristics typical of the "normal" effluent. If the sample proves to have similar characteristics to historical data, then the bench-scale TTE work on the sample may begin.

9. Conduct treatments and evaluate the treated samples chemically and test for acute lethality.

Baseline treatment tests will simulate the on-site ETP (if one exists). If no treatment plant exists on site, the sample will be subjected to test conditions that simulate the selected treatment strategy. Normally the tests are conducted in duplicate. If a treatment plant exists on the mine site, the treatment plant would be simulated at the bench scale and variations or additions to the existing technology would be tested.

Treated effluent water from the bench-scale treatment will be measured in the testing laboratory for the suspected toxicant(s), general water quality parameters (i.e., pH, conductivity, dissolved oxygen), and any important ETP operating parameters (i.e., TSS). Samples should be split for chemical and toxicity testing. Samples for chemical analyses should be preserved and sent to an analytical laboratory. The remaining sample should be shipped immediately to the aquatic toxicity test laboratory.

10. Repeat testing on different samples to consider effluent variability and provide a high degree of certainty as to the effectiveness of the treatment.

Samples should be collected to reflect the various conditions at the mine facility. The conditions could represent daily operational changes, or seasonal variations. The requirements for testing various effluent conditions are facility dependent. For example, an underground mine-only facility will likely produce constant volumes and water characteristics year round. In comparison, a complex metallurgical site with extensive surface waste management facilities can produce wide variations in effluent volumes and qualities.

11. Evaluate test results and rank technologies taking into consideration acute lethality removal efficiency and costs (including design and construction, maintenance and operation, and waste disposal).

The treatment technologies should be ranked based on acute lethality removal efficiency. The ranking of the technologies will determine the technology that will be selected for further study (pilot-scale). The removal of acute lethality, effects of temperature, ease of operation, maintenance requirements, robustness (sensitivity to upset) and operating costs should be considered during the ranking.

Example of a bench-scale test for "common" metal mining toxicants.

The following is an example of bench-scale test for common metal mining toxicants – nitrogen-based compounds.

Based on Phase I TIE results and historical data, ammonia, nitrite / nitrate and cyanate / thiocyanate were identified as suspected toxicants in a gold mine final effluent. The TTE evaluation of the effluent included the following technologies:

1. Granular Activated Carbon (GAC) plus zeolite (and in reverse order),
2. Air stripping plus granular activated carbon,
3. Biological- nutrient addition followed by aeration and denitrification treatment,
4. Multi-stage granular activated carbon,
5. Alkaline chlorination plus dechlorination, and
6. Adsorption using clay based polymers.
7. Natural degradation.

The objective of the TTE bench-scale testing was to evaluate the flow sheet options, determine operating parameters, rank the technologies, estimate costs and to develop information to allow scale-up to pilot plant size, and eventually to full scale.

The individual unit operations specifications and operating conditions of the flow sheets were developed on an individual basis. This is illustrated using the first flow sheet option with GAC and zeolite (which are adsorption and ion-exchange processes).

The quality of the GAC and zeolite were very important. GAC is manufactured from different sources, and therefore may be of different hardness and quality, with the coconut based carbons being the most robust (hard and durable) for recycle. The size of the GAC must be such that the carbon fines will be minimal otherwise GAC losses will be high leading to higher replacement costs. The GAC manufacturers are generally very willing to assist in discussing the correct carbon for the application. Similarly, all zeolites do not exhibit equal performance. Clinoptilolite is the only zeolite mineral that has unusual selectivity for ammonia ions, in preference to calcium and other ions. If this clinoptilolite mineral (zeolite) is not available, a conventional organic resin may be a substituted. The size of the zeolite (clinoptilolite) must be sufficiently coarse to allow flow through a packed-bed column. The zeolite manufacturers also provide assistance in selecting the correct grain size zeolite for the application. The loading capacity of the zeolite must also be known to ensure that a sufficient quantity of zeolite is present in the column at the start of the testing.

The column size (diameter and height) was selected to provide the desired number of bed volumes per unit time. Reasonable flow rates will minimise the testing time requirements. Since many bed volumes were to be passed through the columns during initial testing and up to 50L may be required for acute lethality testing, the column dimensions were of utmost importance.

The GAC and zeolite were tested in individual columns prior to combination. Individual testing established the loading rate of the various toxicants, onto both the GAC and zeolite, respectively. Effluent samples were collected after various bed volumes of flow, and analyses for the parameters of interest were conducted. The analyses established the kinetics of the ion exchange process and number of bed

volumes until the GAC and zeolite were spent. Changes in the number of carbon and zeolite bed volumes and residence contact times (due to under- or over-loading of the system) may effect contaminant removal, with a resultant change in acute lethality. The treated effluent was submitted for bioassays.

From the results of the bench-scale tests, conditions were selected that would produce non-acutely lethal effluents. The results indicated that pilot testing was justified, but that possible reduction in toxicants in the effluents by reduction at source (ammonia, cyanide) would be the most effective effluent management strategy.

It is important to note that the approach to each TTE is customised based on site-specific needs, combination of toxicants, and complexity of treatment applications. What works at one facility may not work at another facility. In some cases, longer term testing (i.e., biological) is much more expensive than short term chemical treatment testing.

12.3.2 Pilot-Scale

Pilot-scale testing involves experimental treatment of a portion of the facility effluent flow in actual climate conditions, or in a laboratory equipped to simulate these conditions. The following outline summarises a generic protocol for normal procedures to conduct pilot-scale testing of the chemistry and processes for treatment and removal of acute lethality in effluents at metal mining and metallurgical facilities.

For facilities with active treatment plants, pilot-scale testing is undertaken in parallel to provide an assessment of the impact of the proposed modified treatment on final effluent acute lethality. For facilities without active treatment plants, pilot-scale testing is also a reliable way to test successful bench-scale treatments. Pilot-scale testing is intended to produce information that will:

i) determine if the bench-scale treatment method is capable of producing an effluent of the desired quality on a consistent basis (over an extended long run period) and evaluate final effluent acute lethality to establish the robustness of the process to feed variability,

ii) provide equipment sizing information,

iii) provide sufficiently large volumes of treated effluent samples for acute lethality testing and sludge for chemical analysis,

iv) establish optimum reagent requirements, and,

v) provide data to help determine operating, maintenance and capital costs

Important steps in designing a pilot-scale TTE include;

1. Determine if pilot-scale testing is to be conducted at the mine site or at laboratory remote from the facility.

The advantages of on-site testing include: availability of fresh effluent samples, typical seasonal variability of effluent, typical temperature, expertise of site personnel available to evaluate conditions and recommend changes. Sample or stream access is much better for on-site pilot testing. Temperature effects (especially cold) are important for many treatment processes and on-site testing is most efficient in duplicating actual conditions. The advantages of testing off-site in a full-service laboratory include: expertise of technical staff, rapid analysis and testing of treated effluent, readily available equipment for

testing, and focused testing which is not distracted by resources being reallocated to solve operational problems. On-site pilot testing is frequently more costly if facilities have to be constructed and on-site technical and analytical expertise is not available

2. Determine if batch or continuous pilot-scale tests are to be conducted.

Batch testing can be used to optimise retention times. Large batch testing is a good approach to use when conducting work at the site or at a full-service laboratory. Batches may be sufficient for a week or more of testing. Batch testing is also more efficient in testing a range of conditions. Continuous testing may be more representative of actual full-scale application and may be best done on-site if testing is required for extensive time periods (i.e., biological treatment).

3. Obtain feed sample.

For off site testing the pilot plant test sample should be pumped and piped into clean containers (1000L carboys or tanker trucks) and delivered to the test laboratory. To feed an on-site pilot plant, the effluent is to pumped into the pilot plant feed holding tank, or if a holding tank is not used, into the pilot process on a continuous manner. Typically the pilot scale flow is a small proportion of the full effluent flow.

4. Evaluate untreated sample chemically and test for acute lethality. Results should be compared to historical data to assess if mine was operating within "normal" or "typical" range of conditions when sample was collected, or when on-site pilot testing was completed.

Prior to conducting the pilot test work, it is necessary to verify that the "batch" or continuous sample to be used for pilot testing was representative of "normal" operating conditions. Routinely monitored effluent treatment parameters (i.e., pH, conductivity, TSS, metals) should be analysed to determine if they were within "normal" operating limits. Acute lethality toxicity testing will also be performed on the untreated sample to ensure the presence of toxicity prior to conducting the pilot-scale tests.

On-site staff should verify the sample as truly representing the site effluent prior to testing. Verification will involve comparing the measured parameters to historical data to determine if the sample has physical, chemical and acute lethality characteristics typical of the "normal" effluent. If the sample proves to have similar characteristics to historical data, then the pilot-scale TTE work on the sample may begin.

5. Apply technologies selected during bench-scale to pilot-scale testing.

The bench-scale TTE work will have recommended the technology or technologies selected for further testing to optimise and fine-tune the system (including chemical dosing). The technologies selected for pilot testing program should be approved and recommended for further testing by on-site management, regulatory staff and the testing engineers, or laboratory involved. The range of pilot testing strategies is wide, and depends on site-specific factors, particularly the toxicity characteristics and chemical complexities. The guiding principles for each pilot scale tests are:

- They represent field conditions (e.g. temperature)
- Steady-state conditions are achieved for each selected variable
- Samples are taken during steady-state conditions
- Pilot-scale conditions are reproducible
- Operating and process data are obtained for full-scale tests or full-scale application.

In cases where the number of variables is high (3 or more), statistically designed pilot runs may be used to determine optimum conditions for non-acutely lethal effluents in the shortest time and at the lowest cost.

6. Evaluate treated effluent chemically and test for acute lethality at various stages during pilot trial.

The pilot study design will be more detailed than bench-scale testing (i.e., need to optimise and fine-tune system, including chemical dosing). The pilot plant will likely be operated in a combination-batch-continuous mode. The initial run-in pilot plants (PP's) will occur during a regular eight-hour shift (PP1, PP2, PP3, etc). During the last two-hours of a pilot plant run, samples should be collected for chemical analysis and acute lethality testing. The different pilot plant test conditions can be evaluated and various reagent dosages, retention times and other parameters can be tested and varied on a pilot plant batch basis. Once the optimum parameters are established, then a long and continuous pilot test run will be operated. The long continuous run will determine the stability of the process and the results will be used for the bank feasibility study.

7. Repeat testing on different samples to consider effluent variability and confirm effectiveness of the treatment.

The pilot testing can be conducted in a similar manner on feed samples taken at other times to determine the reliability of the process in removing chemical contaminants and acute lethality.

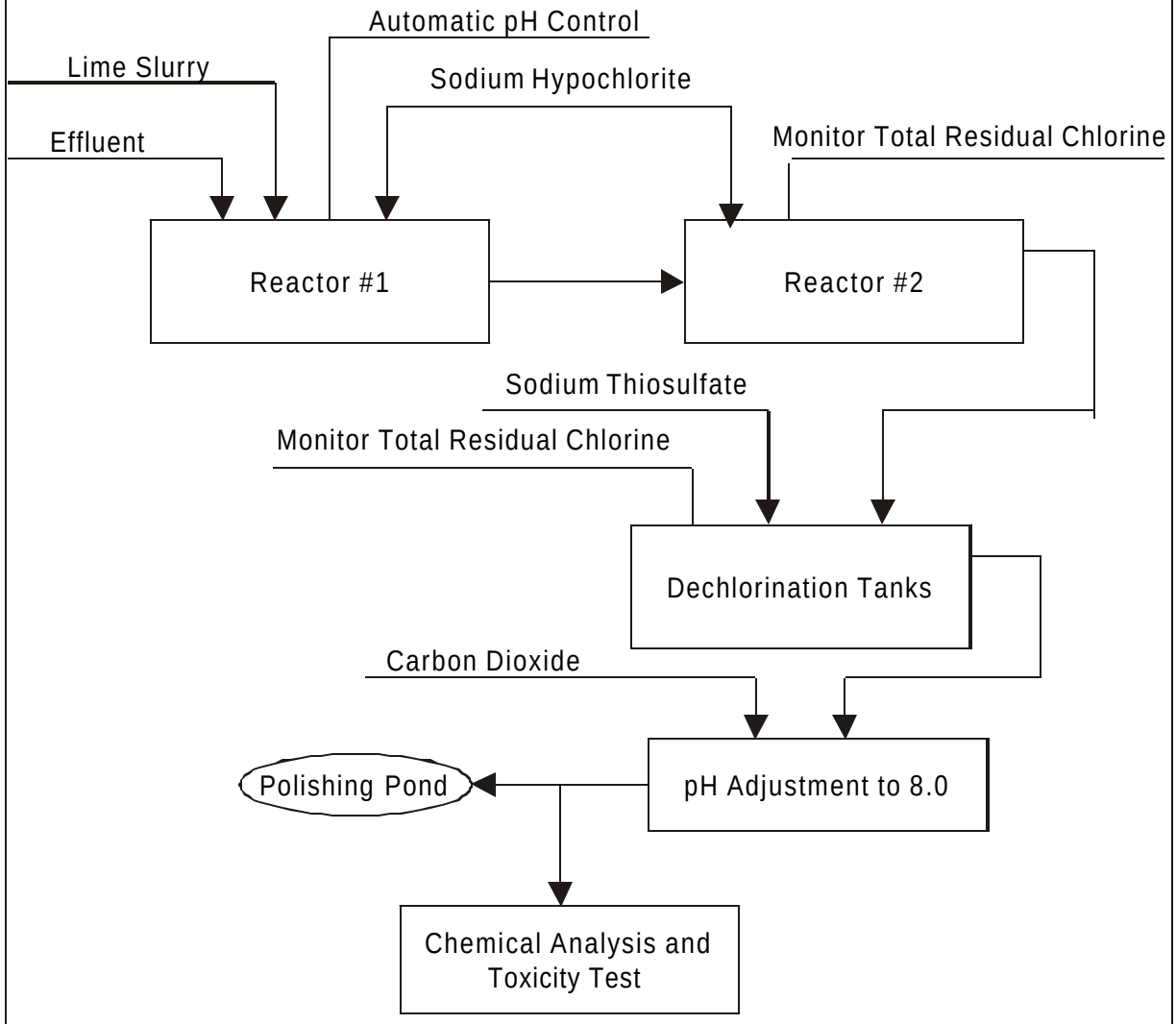
8. Use data generated to determine final design parameters.

The results obtained during the long run and the test conditions, flow rates, reagent dosages, etc., will be used to develop engineering criteria for a full-scale process. The engineering criteria are also used to determine operating and capital costs.

Example of a pilot-scale test for "common" metal mining toxicants.

An example of a pilot-scale test for cyanide and ammonia removal is provided in Figure 7b. Based on bench-scale testing, an optimum dosage of chlorine was determined and was used in the pilot-scale testing. The effluent containing cyanide and ammonia was first adjusted to pH 10.5-11 using lime slurry. Sodium hypochlorite was then added into reactor #1 in half the amount of the optimum dosage. During the testing, the pH of the solution was maintained through an automatic pH controller. After reaction for an hour, the remaining sodium hypochlorite was added into reactor #2 and mixed for another hour. The total residual chlorine was monitored in reactor #2. Depending on the total residual chlorine concentration, dechlorination was performed for 10 minutes by adding appropriate amount of sodium thiosulphate. The treated effluent was adjusted to pH 8 with carbon dioxide and discharged to a polishing pond. Samples of the treated effluent were subjected to chemical analysis and toxicity test using *Daphnia magna* and rainbow trout. In the piloting testing, 100% sodium hypochlorite was added into reactor #1. The results were compared with split distribution and the split distribution was more effective at lower reagent additions.

It is important to note that treatment options will be narrowed down as testing proceeds from bench-scale testing through pilot and full-scale testing. The selection criteria should be applied at the completion of each test series to help identify the most practical option(s).



Project Name: TRE GUIDANCE DOCUMENT	Date: January, 2002	FILE: E1157_07b	Figure 7b
	Revised:	EXAMPLE OF PILOT-SCALE TEST PROCESS FOR CYANIDE AND AMMONIA REMOVAL	
Prepared for: ENVIRONMENT CANADA AND THE MINING ASSOCIATION OF CANADA	Revision No.		

12.3.3 Full-Scale Testing

Full scale testing involves experimental treatment of all of the facility effluent in actual climate conditions. The following outline summarizes a generic approach to conducting full-scale testing of the chemistry and processes for treatment and removal of acute lethality at metal mining and metallurgical facilities.

For facilities with active treatment plants, full-scale testing is undertaken to provide a more reliable assessment of the impact on final effluent toxicity of the proposed modified treatment or additions to treatment. For facilities without active treatment plants, full-scale testing is a reliable way to test effluent treatment or modifications to effluent generation. Such testing can also be the final stage in the TTE.

Full-scale TTE for Facilities Without an Active Treatment Plant

Important steps in designing a full-scale TTE for a metal mining and metallurgical facility without an active treatment plant include:

1. **Select the most effective location for a full-scale test Effluent Treatment Plant (ETP) on site.**

Access or availability of electric power may limit the site selection. The test site should be close to the location of a continuous treatment plant should one be installed and operated. The test and permanent treatment plant will discharge treated effluent upstream of the final point of control for the facility.

2. **Using bench- and pilot-scale test data, engineer and install the temporary treatment technology.**

Because of cost and time to achieve equilibrium conditions, the number of variables for the full-scale tests should be limited. The test facility can be contained in temporary structures and containers, but the process should simulate a more robust full time treatment plant should one be installed.

3. **Commission and operate treatment plant.**

The operation could include continuous monitoring by on-site personnel as well as remotely.

4. **Confirm the removal of toxicity.**

Samples taken upstream and downstream of the test facility should be subjected to acute lethality tests to confirm removal of toxicity. Repeated testing should be conducted to evaluate effluent variability and confirm the effectiveness of the treatment.

Full-scale TTE for Facilities With an Active Treatment Plant

Options for full-scale testing for a facility with an active treatment plant may involve minor adjustments, such as pH lowering with carbon dioxide, or could involve more complex changes such as the sparging of ammonia at elevated pH in ponds or splash towers followed by pH adjustment. Important steps in designing a full-scale TTE for a site with an active treatment plant include:

1. **Assessment of current effluent management or treatment system and its' operation to ensure steady-state plant or optimisation prior to initiation of the TTE.**

This assessment should include a review of operating records, shutdown and non-performance incidents.

2. **Design of the approach for adjustment or change of existing facility.**

The design should include the provision for achieving steady-state conditions for the new treatment system and the design should include the expected range of operating conditions. One of the severe

limitations of full-scale conditions is that effluent characteristics vary substantially with the seasons. The most challenging effluent conditions (such as during spring run-off) should be tested if practical.

3. Implementation of changes or adjustments.

The changes could involve alterations to the treatment plant or to downstream holding ponds. The installation of temporary clarification capacity following chemical addition could be used as an example of a full-scale test.

4. Confirm the removal of toxicity.

Samples taken upstream and downstream of the test facility should be subjected to acute lethality tests to confirm removal of toxicity. Repeated testing should be conducted to evaluate effluent variability and confirm the effectiveness of the treatment.

Example of a full-scale test for "common" metal mining toxicants.

The following is an example of a full-scale test for a "common" metal mining toxicant - pH.

A metal mining facility has a lime treatment plant for acidity neutralisation and metals removal at the tailings pond discharge. The lime treatment plant is a simple facility with direct lime addition to the effluent stream. The limed effluent flows to a settling pond with 6 days minimum retention time. The effluent from the settling pond discharges into a small-dedicated creek (400 metres in length) flowing into a large river. The control point for monitoring purposes is the discharge of the creek into the river.

The effluent ranges from pH 9 to 10 and has been acutely lethal to rainbow trout. Laboratory tests have shown that control of effluent pH 7 to 8 using sulphuric acid or carbon dioxide will eliminate mortality. Because of safety and ease of operation, it was decided to conduct a field test with carbon dioxide rather than sulphuric acid.

The test facilities included a leased tanker trailer of carbon dioxide and a metering and control system linked to pH and flow in the final effluent stream. Three tests were planned – March, May and June. Each test was scheduled to operate 36 days (to ensure complete turnover of the limed feed pond) and would be monitored remotely by telemetry. Samples of effluent before carbon dioxide and at final discharge were collected for chemical analyses and acute lethality testing. The effluent stream was monitored weekly for visual indications of effects and calcium carbonate build-up.

The test results showed that the effluent was non-lethal in all three tests. Initial difficulties with pH sensing in cold temperatures were overcome.

Design was proceeding towards a permanent installation with secure fencing or an above ground carbon dioxide plant. The plant would be remotely operated, with operating information sent electronically to the central control facility in the mill.

12.4 Prevention Strategies

In addition to bench, pilot and full-scale testing, TTE' s could involve "prevention strategies" to eliminate acute lethality. Specific examples applicable to the metal-mining sector include:

1. Improvements in blasting agent housekeeping.

Ammonia is one of the most common toxicants and the primary source of ammonia is non-reacted ammonium nitrate in ammonium nitrate - fuel oil (ANFO) blasting agent. The application of best

management practices and training programs have been shown to be successful in reducing ammonia content in effluents.

2. Removal and recycling of cyanide.

Cyanide and cyanide chemical derivatives are also a common metal mining effluent toxicants from gold ore milling processes. There are three main processes of cyanide removal from effluents – natural evolution, SO_2-air oxidation and peroxide oxidation. Natural evolution is effective at many facilities. Chemical oxidation is also effective, but ammonia is a degradation product and can result in toxicity. Recycling of cyanide to the leaching process is cost effective at some gold mine facilities. Recycling can be achieved by several commercially available processes, where cyanide is evolved by chemical induction or by removal on cyanide specific resins. Recycling is routinely practiced by gold mining facilities by recycling dilute, untreated mill effluents to the mill feed.

3. Recycling of heavily contaminated streams to the process.

Mine water is typically one of the most toxic effluents at a metal mine as a result of ammonia concentrations. An effective treatment option for this stream is its use in process plant make-up water. In the process, the ammonia is evolved into the atmosphere as a result of aeration in grinding and other milling processes.

4. Installation of additional treatment plant feed surge pond capacity.

Acute lethality events may be common at spring run-off and following a rainstorm event when metals and other toxicants can be swept into the facility effluent. Although frequently costly, increasing surge capacity upstream of the treatment plant can reduce or eliminate this toxicity.

5. Change in chemical use.

Process chemicals such as flotation agents and surface-active agents such as cleaners can produce acutely lethal effluents. Amine type flotation agents have been discontinued in the iron ore industry because of toxicity.

12.5 Co-ordination of Toxicity Testing with TTEs

Co-ordination between the toxicology, chemistry and engineering groups participating in the TTE will be critical to the success of a study. During the TTE, acute lethality testing is used to assess the effectiveness of the treatment option in reducing toxicity. In most cases, an engineering group will be performing the bench- or pilot-scale treatments, and will provide sub-samples of the treated effluent to an analytical and toxicology laboratory for testing. It will be important to ensure sufficient sample volumes are treated (particularly at the bench-scale) to allow for both acute lethality testing and chemical analysis of the treated effluent. Treatment of a sufficient volume of effluent will be particularly important in the case of rainbow trout, since these tests require large test volumes.

Modified acute lethality test methods may be used during an initial screening assessment of a large number of treatment options. However, as the number of treatment options is narrowed, all tests should follow the Environment Canada test methods to verify that the treatment selected will be able to meet the compliance limit.

Repeated evaluation of the proposed treatment options must be conducted. However, the number of samples to be treated and analyzed will depend on a variety of factors, including effluent variability,

number of toxicants, conclusions drawn from data, cost of remedial action, regulatory deadlines and success of each phase (U.S. EPA, 1991).

13 SOURCE INVESTIGATION

This section provides a generic protocol outlining the procedures to be taken during a Source Investigation (SI). Detailed descriptions for process stream characterization and use of the mass balance approach are also provided.

The purpose of a SI is to identify the upstream source of the toxicant, followed by implementation of controls (i.e., treatment technologies or alteration of upstream management systems) that will translate into elimination of final effluent acute lethality. A key advantage is that treatment of smaller, more concentrated streams can often be performed more efficiently and economically than treatment of larger, more dilute streams (the final effluent) (U.S. EPA, 1989). Source investigations can also be a viable alternative to eliminate final effluent acute lethality in cases where the TIE does not conclusively identify the toxicant(s), or if acute lethality is transient or non-persistent. The selection of a SI versus a TTE investigative approach will be site-specific. However, performance of SIs before TTEs may be beneficial and more cost-effective, since the source of toxicity can be segregated and then treated.

13.1 Generic Approach to a Source Investigation

The following describes the U.S. EPA (1989) generic protocol outlining the procedures to be taken during a SI (Figure 8). The detailed steps associated with each generic component are described in sections 13.2 (process stream characterization) and 13.3 (mass balance of acute lethality and toxicants). Additional details are provided in Appendix B. The steps involved in a SI may include;

1. Review historical data, including TIE results.
2. Identify sewers, discharge locations and inputs to the final effluent or ETP (if one exists).
3. Select sampling locations, protocols, methods for flow monitoring and implement sampling program.
4. If the toxicant has been identified, i) use chemical specific analysis for tracking the sources, and ii) evaluate the effects of the treatment plant on altering the toxicant (where possible).
5. If toxicant has not been identified, i) use bench-scale model to simulate treatment plant and track toxicity, and ii) characterize the bench-scale treated samples using Phase I treatments (where necessary).
6. Analyze data from 3, 4 and 5.
7. Select the number of streams to be treated and the treatment technology (or management option) to be used for each stream.
8. Proceed to TTE (Section 12)

13.2 Process Stream Characterization

The specific objectives of the process stream characterization phase of a SI are to identify discharge locations and inputs to the final effluent or ETP (if one exists), including a description and available information for each location and input, and characterize each discharge in terms of flows, acute lethality and chemical composition. This information is used to conduct a mass balance (see Section 12.3) to identify those streams representing the largest contribution of toxicity and chemical loads to the final effluent. This approach also allows non-acutely lethal streams to be ruled out as sources of toxicity.

The key steps in process stream characterization are outlined in the following section.

1. Review historical data, including TIE results.

During the early stages of the TRE (and prior to conducting the SI) a review of historical data (including TIE results) and operational or process changes may assist in identifying subtle modifications that may have contributed to acute lethality. The results from the TIE will assist in determining if chemical specific analysis or toxicity tracking will be used to identify the source of acute lethality. The source(s) of acute lethality may be identified as a result of the historical changes in process or addition of new streams. Involving all team members (i.e. toxicologist, engineers, site personnel) during all stages of the SI will increase the likelihood of identifying these sources. To investigate this, the site personnel need to clearly document the process in place prior to, and after the appearance of acute lethality in the final effluent (see Section 5).

2. Identify discharge locations and inputs to the final effluent or ETP (if one exists).

A site inspection should be conducted to map out the effluent sources and review potential sources of effluent variability. The main streams entering the ETP (if present) should be identified, along with each sub-component stream. Inputs should include process, site-runoff, storm water and groundwater sources. A description of the process/operation at each discharge location should also be provided (including frequency of operation - i.e., continuous or intermittent). Locations with existing monitoring equipment and flow control devices should also be identified.

By the completion of the site inspection, a line drawing schematic illustrating how the effluent sources are combined should be produced. Proposed sampling locations, methods for sample collection and flow monitoring options should also be identified.

3. Select sampling locations and protocols, methods for flow monitoring and implement sampling program.

The sampling locations should be readily accessible and the sample should be well mixed and representative of the stream being sampled. The choice of grab or composite samples will depend on the variability of the individual stream.

Flow data should be obtained from each sampling location in order to determine relative contributions of acute lethality and toxicant concentration. For each sampling location, a method for measuring flow rate must be established. In many cases, simple methods (i.e., using a bucket and stopwatch) may be sufficient, while other locations may require more complicated approaches (i.e., installation area-velocity meters). For certain locations, where it is not possible to measure flow directly, the sum of individual upstream flows could be used as an estimate.

For each stream, flow measurements are taken and samples for chemical analysis and acute lethality testing are collected. Preliminary source stream studies may use modified test methods, particularly in cases where a large number of influent streams are to be assessed. However, subsequent tests should follow the regulatory tests that triggered the TRE investigation. Samples should also be assessed using LC50 tests, to determine the degree of acute lethality. We also suggest that samples be tested using both rainbow trout and *Daphnia magna*, since species sensitivity comparisons may eliminate some suspect streams (i.e., final effluent is acute lethal only to rainbow trout, but source stream is only acutely lethal to *Daphnia magna*).

The sampling program should be conducted several times to consider effluent variability. For example, production schedules and natural precipitation (dry and wet seasons) can vary the source stream toxicant flow and concentration. Knowledge of scheduled changes or events in process stream operation (i.e., batch, continuous or intermittent) in combination with acute lethality and chemical data, could provide strong evidence as to the possible sources of final effluent acute lethality, or provide reasons for effluent variability. Therefore, the sampling program should take into consideration variability due to weather conditions, production schedules and processes.

4. If the toxicant has been identified, i) use chemical specific analysis for tracking the sources, and ii) evaluate the effects of the treatment plant on altering the toxicant (where possible).

If the TIE identified and a confirmed a specific substance, a chemical-specific approach can be used to locate the source of the toxicant within the influent streams. This approach involves testing the source streams for the toxicant using chemical-specific analysis techniques.

Chemical specific analysis should also be conducted on the combined influent and effluent streams to assess the effects of the ETP on the toxicant. Effluent residence times within the system should be considered in order to ensure the same "slug" of wastewater is being tested in the influent and effluent (U.S. EPA, 1989).

Once the source of the toxicant(s) has been identified, the SI could go further into the process to identify sub-component streams, or the TRE could proceed to a TTE for the source stream(s) (Section 12).

5. If toxicant has not been identified, i) use bench-scale model to simulate treatment plant and track toxicity, and ii) characterize the bench-scale treated samples using Phase I treatments (where necessary).

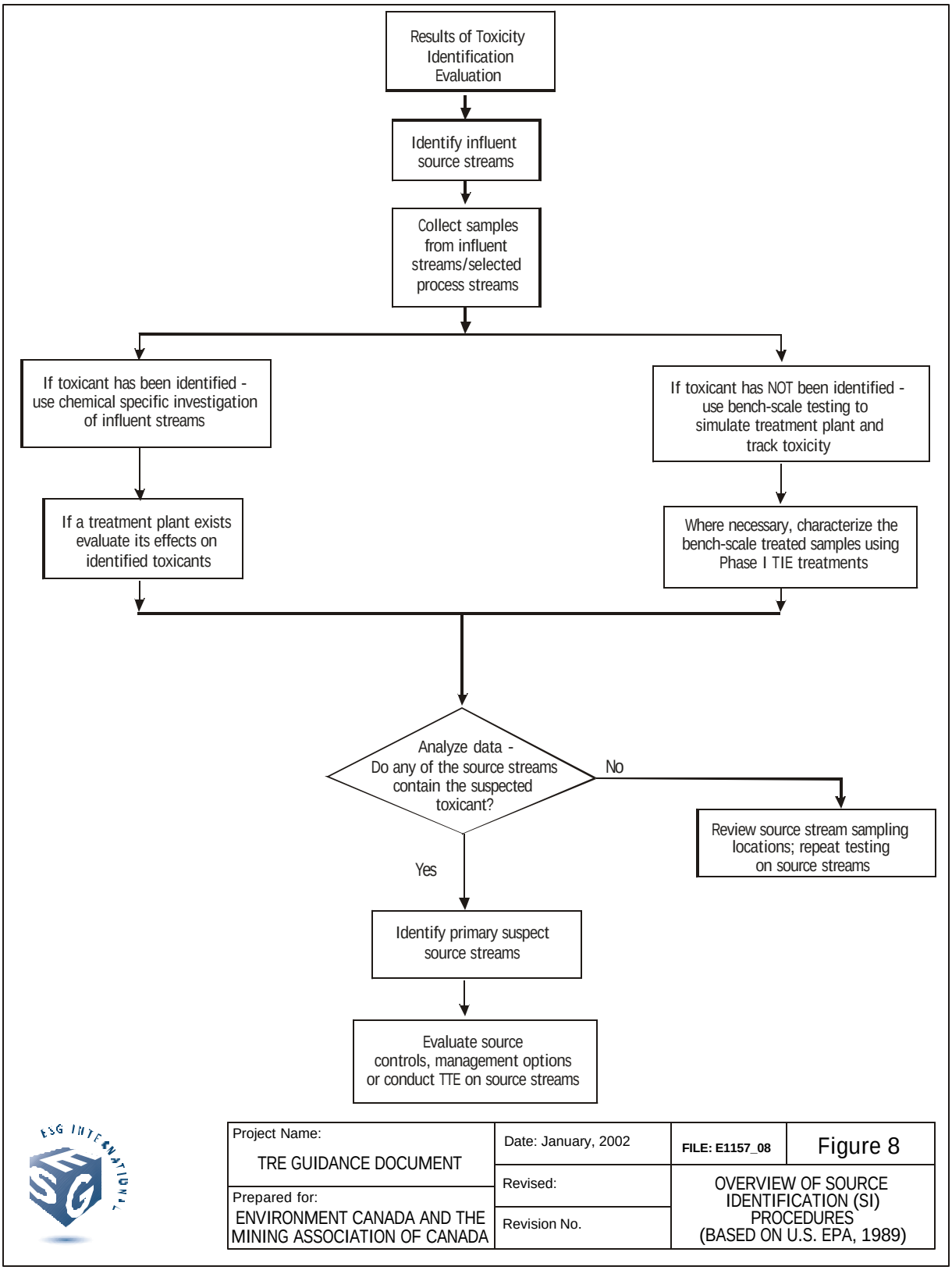
It will be more difficult to select "suspect" streams, if the toxicant has been sufficiently characterized, but not identified. In this case, the most effective approach would involve systematic sampling which utilizes the process of elimination to track toxicity to the source (U.S. EPA, 1989). Toxicity tracking can be used in cases where acute lethality is transient, non-persistent, or the TIE only characterized the substance(s) responsible for acute lethality. This process involves collection of samples from each source, followed by simulation of the ETP and testing for acute lethality.

A key component of every SI (and TTE) is the ability to simulate the ETP (if one exists). The amount of acute lethality that could potentially pass through the treatment system must be estimated by treating each source stream in a simulation of the ETP prior to acute lethality testing (U.S. EPA, 1999). Using this approach, the primary sources of acute lethality should be identified because they are sufficiently lethal and their lethality is not degraded by the ETP (U.S. EPA, 1989).

The suspect source streams could also be characterized using the Phase I TIE methods used for the final effluent. The Phase I results for the final effluent and source streams are compared to provide additional certainty that the source streams contain similar classes of toxicants (U.S. EPA, 1989).

6. Analyze data

If multiple toxicant sources have been identified, or if the toxicity tracking approach was used, the data generated during the process stream characterization (flow, acute lethality and chemical data) should be analyzed to identify those streams representing the largest contributors (in terms of acute lethality and chemical load) to the ETP (Section 13.3).



Project Name: TRE GUIDANCE DOCUMENT	Date: January, 2002	FILE: E1157_08	Figure 8
	Revised:	OVERVIEW OF SOURCE IDENTIFICATION (SI) PROCEDURES (BASED ON U.S. EPA, 1989)	
Prepared for: ENVIRONMENT CANADA AND THE MINING ASSOCIATION OF CANADA	Revision No.		

13.3 Mass Balance Approach to Acute Lethality and Chemical Load

The specific objective of the mass approach is to identify those streams that represent the largest contributors (in terms of acute lethality and chemical load) to the final effluent or ETP (if one exists). This approach could be used if the identified toxicant is found in multiple streams, or if the substance(s) responsible for toxicity is only suspected, but has not been conclusively identified. In the latter case, the risk associated with source stream misidentification is increased.

The key steps in mass balance approach are outlined in the following section.

1. Calculate the mass loading of acute lethality

Using the data gathered during the process stream characterization, TUs are calculated for each stream, and then multiplied by the proportion of the total flow for that stream to arrive at a value representing "relative" contribution to final effluent acute lethality.

Effluent streams of different volumes and toxicity can also be compared in terms of a Toxicity Emission Rate (TER). The TER allows effluent flow to be normalized according to the toxicity each effluent stream contains (Environment Canada, 1999). The TER is calculated for each stream by multiplying the TU for that stream by the volume of flow in m^3 per day.

Effluent quality performance among different operations within an organization can also be compared using Toxicity Emission Factors (TEF). The TEF expresses the amount of toxicity in terms of production, taking plant size and water use efficiency into consideration (Environment Canada, 1999). The TEF is calculated for each process by multiplying the TU by the volume of flow in m^3 per tonne of industrial goods produced.

2. Compare TUs, TERs and TEFs to determine which stream contributed the greatest acute lethality to the final effluent

TUs, TEFs or TERs are ranked to determine which streams contributed the greatest to acute lethality.

3. Calculate the mass loading for measured chemical parameters.

For each stream, the parameter mass loading must be calculated. To do this, individual chemical concentrations for each stream are multiplied by the proportion of total flow for that stream, to arrive at a total loading for each parameter. For example, in the case of copper, determine the kilograms of copper contributed by each stream. Tabulate the streams from the highest kilogram loading of copper to least toxic. Total the copper kilograms and then divide the total kilograms into each of the individual stream copper kilograms, and multiply by 100. This result is the % distribution of copper metal originating from each stream.

4. Compare loadings to determine which stream contributed the greatest chemical load for each water quality parameter.

Rank the streams that contribute the highest chemical loading for each water quality parameter.

5. Select the number of streams to be treated and the treatment technology or management option to be used for each stream.

If the toxicant has been identified, proceed to a TTE on those stream(s) representing the greatest contributors to acute lethality and chemical load for the toxicant. The majority of a specific-chemical loading may originate from one stream source. In such a case it is suggested that this stream should be a

candidate to proceed to a TTE designed to remove this specific chemical. It is also possible that the SI may suggest multiple sources of acute lethality or the suspect toxicant(s). This may occur if the sources of toxicants are widely dispersed throughout the process. The inability to locate the toxicant(s) may also indicate that the discharge sampling points did not include all possible sources of the toxicants. In this case, it may be necessary to evaluate additional input lines in the collection system (U.S. EPA, 1999).

6. If the toxicant has not been conclusively identified in the final effluent, approach to TTE must be determined cautiously.

In this case, a reduction in the acute lethality of the source streams by removal of specific contaminants does not guarantee an elimination of final effluent acute lethality. Streams selected for TTE should be based on some knowledge of the type of process stream, effect of the effluent treatment plant on source stream acute lethality, or Phase I TIE results. For example, a Phase I TIE conducted on the suspect source stream(s) may be used to ensure that the suspect source stream and final effluent contain the same class of toxicant(s). The Phase I TIE may also suggest a possible treatment option for the TTE (i.e., addition of EDTA remove acute lethality).

13.3.1 Advantages and Disadvantages of the Mass Balance Approach

The advantages of a mass balance approach to a source stream investigation include (but are not limited to);

1. Simplicity of concepts and approach.
2. Calculates specific chemical loading and acute lethality and allows ranking of each stream from highest to lowest loading.
3. Rapid focus on the source streams that are problematic and contributing the highest chemical loading and acute lethality.
4. Potential to eliminate the acute lethality at the source without need for effluent treatment. Source investigation is an opportunity for the mine operator to optimize water management, decrease acute lethality and lower water treatment costs. Source investigation and treatment could enhance final treatment plant removal of acute lethality, eliminate the need for a large plant, or reduce treatment pressure to allow natural attenuation and degradation systems to handle the remaining load.
5. The TTE may be immediately conducted to evaluate the opportunity to remove the identified toxicant from the source stream.
6. Source investigation may identify an opportunity to recycle a heavily contaminated stream back to the process, for additional metal recovery, reagent savings, and reduced effluent acute lethality. If individual concentrated sources may be treated prior to discharge it will prevent the contamination of larger volumes which are more dilute and more expensive to treat.

The disadvantages of a mass balance approach to a source stream investigation include (but are not limited to);

1. Need for precision - In stream flow measurement, inaccuracies in the flow rate measurements within the source streams will impact the loading calculation.
2. Need for Accuracy - Need for correlation of analytical concentrations to flow rate. The retention time of the effluent will vary within the discharge network as a result of wet and dry seasons, or as

production levels are adjusted, as chemical loadings are reduced to reactions, evolution or precipitation and will cause variability in the parameter concentrations and chemical loading.

3. Difficulty in identifying transient sources of toxicity - The activity of solar (low UV in winter) and biological processes (nitrification is more active in warmer temperature) will vary by season. As with other approaches to toxicant identification, the cost will be higher when acute lethality is transient.

13.3.2 Examples of the Mass Balance Approach

Both the TER and TEF approach have been successfully used in the pulp and paper sector to identify key source streams and processes contributing to combined effluent acute lethality (Scroggins, 1986). The objectives of this study were to identify the main in-plant sources of effluent toxicity, and identify in-plant controls to reduce toxicity. The first step involved in-plant surveys to collect samples for toxicity testing and flow measurements at 33 different locations. The data was used to identify and prioritize the major streams contributing to final effluent acute lethality. By comparing TERs, it was determined that the pulp mill sewer contributed 55% of the total effluent toxic load, while two other sewers (combined condensate and acid sewers) contributed 25% and 20%, respectively. Calculation of TEFs allowed the author to compare the toxicity of two different kraft mills (e.g., hardwood versus softwood pulping) within the mill complex. A 40% decrease in TEF, and a 55% decrease in TER, was observed when the mill switched from softwood to hardwood pulping. This approach led to in-plant controls and system modifications to reduce final effluent acute lethality and chemical load (Sikes and Almost, 1986).

A similar approach was used in the AETE Case Study #5 with a metal refinery effluent (ESG International, 1998). In this study, the SI first involved identification and mapping of the main and secondary inputs to the ETP. Samples (for acute lethality testing and chemical analysis) were collected and flow measurements taken at 22 locations on four different occasions. Determination of the mass balance of acute lethality involved calculation of TUs for each stream. Each TU was then multiplied by the proportion of total flow for that stream to arrive at a value representing the "relative contribution" to the acute lethality of the combined final effluent. These values were ranked according to toxic contribution, and grouped according to stream component. For chemical loading, the chemical concentrations were multiplied by the observed flows for each main and subcomponent stream to arrive at a total loading for each parameter. The loadings were compared to determine which stream contributed the greatest chemical load for each water quality parameter. Using this approach, three process streams were identified as the main contributors to final effluent toxicity. The remaining streams were either non lethal or did not contribute significantly to mortality. Effluent from one stream contributed the greatest portion of toxic levels of Cu, Co and Ni, while the remaining two streams contributed the greatest proportion of TDS contaminants (Na, SO_4, S, HCO_3, Cl). These results allowed the subsequent TTE studies to focus on specific streams, which led to operational changes to eliminate acute lethality.

14 RECOMMENDATIONS

The following recommendations are based on data and information gaps that were uncovered during preparation of this metal-mining specific TRE Guidance Document:

1. A toxicity threshold database for "common" toxicants (i.e., metals, ammonia, cyanide) associated with metal-mining effluents should be developed. Often, one of the first steps in the TRE process will be a comparison of the concentration of selected effluent constituents to toxicity data reported in the literature for *Daphnia magna* and rainbow trout. This is done as a very preliminary assessment to determine if any of the measured parameters could account for all or part of the observed acute lethality. Although there are a number of sources where LC50s can be obtained, data is not always readily available for Canadian test species or under water quality conditions commonly encountered in metal mining effluents. Therefore, it would be beneficial to all TRE participants to have a readily available database with LC50s for common metal mining toxicants conducted using Environment Canada methods. Although some of the information could be obtained from the published literature, in certain cases it may be necessary to generate the LC50 data using Canadian methods and test species.

2. A toxicity database for common process and treatment chemicals used by the metal mining industry should be developed in co-operation with chemical suppliers. This list may include acute lethality data for Canadian regulatory species, as well as identification of substance specific toxicity "finger-prints". Close consultation with the manufacturer or supplier will be required to ensure that even minor changes or alterations in chemical formulations (which can impact toxicity) are documented and users are informed.

3. Additional research should be conducted to characterize the effectiveness of EDTA and thiosulfate in removing toxicity of different metals (and metal combinations) to both rainbow trout and *Daphnia magna*. Although there is some available published information on the effectiveness of EDTA and thiosulfate to remove or reduce toxicity of various metals to *Daphnia magna*, we found no such information for rainbow trout.

4. Standardized methods for use of effective cation and anion exchange resins and Canadian metal-mining effluents should be developed. Currently, guidance on their application with metal-mining TIEs is limited. Studies should be conducted to develop and validate the use of various cation and anion exchange resins with metal mining effluents. Standardized methods should include a description of resin selection (with particular attention to the evaluation of ion exchange resins in which non-toxic blanks can be obtained), methods for establishing resin capacity, effluent treatment approaches (i.e., slurry versus column), and elution or toxic recovery techniques.

5. The TIME network should be used as a forum to exchange information on both successful and unsuccessful Canadian metal mining TREs. Most TRE information remains unpublished, however, this does not imply that there are few evaluations being done. The lack of readily available metal-mining specific TRE data can be unfortunate, since this limits the exchange of information, which is vital if experience with metal mining TREs is to advance. Regularly scheduled TIME meetings would facilitate the open exchange of study approaches and results among all parties participating in the TRE process (i.e., aquatic toxicologists, mine representatives, chemists and engineers).

REFERENCES

American Petroleum Institute. 1998. The toxicity of common ions to freshwater and marine organisms. Document 0300-029. Washington, DC.

Andrews, S. 1999. Analysis of National Acute Lethality Database on Canadian Mining Effluents. Draft Report to Environment Canada. 32 p.

Ankley, G. T. and M.K. Schubauer-Berigan. 1995. Background and overview of current sediment toxicity identification evaluation procedures. J. Aquat. Ecosystem Health 4: 133-149.

Ausley, L.W., R.W. Arnold, D.L. Denton, W.L. Goodfellow, M. Heber, R. Hockett, S. Klaine, D. Mount, T. Norberg-King, R. Ruffler, W.T. Waller. 1998. Application of TIEs/TREs to whole effluent toxicity: principles and guidance. A report by the Whole Effluent Toxicity TIE/TRE Expert Advisory Panel. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Bailey, H., R. Krassoi, J.R. Elphick, A. Mulhall, P. Hunt, L. Tedmanson and A. Lovell. 2000. Whole effluent toxicity of sewage treatment plants in the Hawksbury-Nepean watershed, New South Wales, Australia, to *Ceriodaphnia dubia* and *Selenastrum capricorunutum*. Environmental Toxicology and Chemistry. 19:72-81.

Bailey, H., J.R. Elphick, A. Potter, E. Chao, D. Konasewich and J.B. Zak. 1999. Causes of toxicity in stormwater runoff from sawmills. Environmental Toxicology and Chemistry. 18:1485-1491.

Beak International Inc. 2000. Non-Acutely Lethal Mining Effluent Technologies (NALMET) Program – 1999 Studies.

Burgess, R. M., J.D. Charles, A. Kuhn, K.T. Ho, L.E. Patton, and D.G. McGovern. 1997. Development of a cation-exchange methodology for marine toxicity identification evaluation applications. Environmental Toxicology and Chemistry. 16:1203-1211.

CCME (Canadian Council of Ministers of the Environment). 1999. Canadian environmental quality guidelines. Canadian Council of Ministers of the Environment, Winnipeg.

Chapman, P.M., H. Bailey and E. Canaria. 2000. Toxicity of total dissolved solids associated with two mine effluents to chironomid larvae and early life stages of rainbow trout. Environmental Toxicology and Chemistry. 19:210-214.

Coombe, V.T., K.W. Moore and M. J. Hutchings. 1999. TIE and TRE: an abbreviated guide to dealing with toxicity. Water Science and Technology. 39:91-97.

Deanovic, L., V.M. Connor, A.W. Knight, and K.J. Maier. 1999. The use of bioassays and toxicity identification evaluation (TIE) procedures to assess recovery and effectiveness of remedial activities in a mine drainage-impacted stream system. Archives of Environmental Contamination and Toxicology. 36:21-27.

DiToro, D.M., H.E. Allen, H.L. Bergman, J.S. Meyer, P.R. Paquin and R.C. Santore. 2000. A biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environmental Toxicology and Chemistry 20:2383-2396

Doi. J. and D.R. Grothe. 1989. Use of fractionation/chemical analysis schemes for plant effluent toxicity evaluation. In: G.W. Suter II (Editor), Aquatic Toxicology and Environmental Fate. 11:123 ASTM STP 1007, American Society for Testing and Materials, Philadelphia, PA.

Duff, Sheldon. Ph.D UBC Pulp and Paper Centre and Associate Professor Dept of Chemical Engineering, personal communication.

Eckenfelder, W.W. and P.W. Lankford. 1992. Protocol for source toxicity evaluation. Water Science and Technology. 25:45-54.

Environment Canada. 2000a. Biological test method: reference method for determining acute lethality of effluents to rainbow trout. Environmental Protection, Conservation and Protection, Environment Canada. Ottawa, Ontario, Reference Method EPS 1/RM/13, Second Edition, December 2000.

Environment Canada. 2000b. Biological test method: reference method for determining acute lethality of effluents to *Daphnia magna*. Environmental Protection, Conservation and Protection, Environment Canada. Ottawa, Ontario, Reference Method EPS 1/RM/14, Second Edition, December 2000.

Environment Canada. 1999. Guidance document on application and interpretation of single-species tests in environmental toxicology. Environmental Protection, Conservation and Protection, Environment Canada. Ottawa, Ontario, Reference Method EPS 1/RM/34.

Environment Canada. 1998. Biological test method: toxicity tests using early life stages of salmonid fish (rainbow trout). Environmental Protection, Conservation and Protection, Environment Canada. Ottawa, Ontario, Reference Method EPS 1/RM/28, July 1998, Second Edition.

ESG International Inc. 1998. Evaluation of Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) Application to the Canadian Mining Industry. AETE Report No. 1.2.5.

Evans, K., M.J. Burke, PR. Krause. 2000. Effluent TIE techniques to control pH in large volume chambers using rainbow trout (*Oncorhynchus mykiss*). Presented at the 21st Annual SETAC meeting. Nashville, TN. November, 2000.

Ford, D.L. (editor). 1998. Toxicity Reduction: Evaluation and Control. Volume3, Second Edition. Water Quality Management Library. 356 pp.

Goodfellow, W.L., L.W. Ausley, D.T. Burton, D.L. Denton, P.B. Dorn, D.R. Grothe, M.A. Heber, T.J. Norberg-King, and J. H. Rodgers. 2000. Major ion toxicity in effluents: a review with permitting recommendations. Environmental Toxicology and Chemistry. 19: 175-182.

Hockett, J.R. and D.R. Mount. 1996. Use of metal chelating agents to differentiate among sources of acute aquatic toxicity. Environmental Toxicology and Chemistry. 15: 1687-1693.

Ince, N. H., and G. Erdogdu. 1998. Toxicity screening, assessment, and reduction in an industrial wastewater treatment plant. Water Environment Research. 70: 1170-1177.

Jop, K.M., R.B. Foster, and A.M. Askew. 1991. Factors affecting toxicity identification evaluation: the role of source water used in industrial processes. Aquatic Toxicology and Risk Assessment: Fourteenth Volume, ASTM STP 1124, M. A. Mayes and M. G. Barron, Eds., American Society for Testing and Materials, Philadelphia, 1991, pp. 84-93.

Kovacs, T. and B. O'Connor. 1996. Insights for toxicity-free pulp and paper mill effluents. Produced by the Pulp and Paper Research Institute of Canada (PAPRICAN). 23 pp.

Mount, D.R. 1997. Evaluating TIE data and conclusions. Paper presented at 18th Annual Meeting of the Society of Environmental Toxicology and Chemistry.

Mount, D.R. and D.I. Mount. 1992. A simple method of pH control for static and static-renewal aquatic toxicity tests. Environmental Toxicology and Chemistry. 11:609-614.

Mount, D.R., D.D. Gulley, J. Russell Hockett, T.D. Garrison and J. M. Evans. 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimphales promelas* (fathead minnows). Environmental Toxicology and Chemistry. 16:2009-2019.

Pollutech. 1996. Environmental Comparison of Results from Alternative Acute Toxicity Tests with Rainbow Trout for Selected Mine Effluents. AETE Report No. 1.1.4.

Rand, G. M. 1995. Fundamentals of aquatic toxicology. Effects, environmental fate and risk assessment. Second Edition. Taylor and Francis, Washington, D.C. 1125pp.

Santore, R. D.M. DiToro, P.R. Paquin, H.E. Allen and J.S. Meyer. 2000. A biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and Daphnia. Environmental Toxicology and Chemistry 20:2397-2402.

Sauer, T.C., H.J. Costa, J.S. Brown, and T.J. Ward. 1997. Toxicity identification evaluation of produced-water effluents. Environmental Toxicology and Chemistry. 16::2020-2028.

Schubauer-Berigan, M.K., J.R. Dierkes, P.D. Monson and G.T. Ankley. 1993. The pH-dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia, Pimephales promelas, Hyalella azetca* and *Lumbriculus variegatus*. Environmental Toxicology and Chemistry. 12:1261-1266.

Scroggins, R.P. 1986. In-plant toxicity balances for a bleached kraft pulp mill. Pulp Paper Can 87:T344-348 (September 1986).

SENES Consultants Limited and Lakefield Research. 1999. Report on Technologies Applicable to the Management of Canadian Mining Effluents.

Sherman, J.D. 1978. Ion exchange separations with molecular sieve zeolites. American Institute of Chemical Engineers (Symposium Series) 44:98-116.

Sikes, J.E. G. and S. Almost. 1986. Black liquor spill control at Terrace Bay. Pulp and Paper Canada 87:496-500.

Stumm, W. and J.J. Morgan. 1981. Aquatic chemistry - an introduction emphasizing chemical equilibria in natural waters. John Wiley and Sons, New York, NY. 583 p.

Tan, K.G., R.E. Mlnar and MSL Staff. 1995. Ammonia Control Consortium – Pilot Campaign Results and Final Report. CANMET Mineral Sciences Laboratories Division Report MSC 95-17(CR): 88p (confidential report not available for this study).

Tietge, J.E., D.R. Mount and D.D. Gulley. 1994. The Gas Research Institute freshwater salinity toxicity relationship model and computer program: overview, validation and application. Topical Report. Chicago, IL, USA.

Tietge, J.E., J. Russel and J. M. Evans. 1997. Major ion toxicity of six produced waters to three freshwater species: application of ion toxicity models and TIE procedures. Environmental Toxicology and Chemistry. 16:2002-2008.

Tchobanoglous, G. and F. L. Burton. 1991. Wastewater engineering. Treatment, disposal and reuse. Tata McGraw-Hill Publishing Company Limited, New Delhi. 1334 pp.

Thurston, R.V., R.C. Russo and G.A. Vinogradov. 1981. Ammonia toxicity to fishes. Effect of pH on the toxicity of the un-ionized ammonia species. Environmental Science and Technology 5: 837-840.

Toxicological Investigations of Mining Effluents Network (TIME). 2002. Guidance document for acute lethality testing of metal mining effluents. Prepared by ESG International Inc.

U.S. Environmental Protection Agency (U.S. EPA). 1985. Ambient water quality criteria for ammonia. EPA-440/5-85/001.

U.S. Environmental Protection Agency (U.S. EPA). 1986. Quality Criteria for Water. EPA-440/5-86/001.

U.S. Environmental Protection Agency (U.S. EPA). 1989. Generalized methodology for conducting industrial toxicity reduction evaluations. EPA-600/2-88/070.

U.S. Environmental Protection Agency (U.S. EPA). 1991a. Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures. EPA-600/6-91/003.

U.S. Environmental Protection Agency (U.S. EPA). 1991b. Toxicity identification evaluation: characterization of chronically toxic effluents, Phase I. EPA-600/6-91/005.

U.S. Environmental Protection Agency (U.S. EPA). 1993a. Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity. EPA-600/R-92/080.

U.S. Environmental Protection Agency (U.S. EPA). 1993b. Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. EPA-600/R-92/081.

U.S. Environmental Protection Agency (U.S. EPA). 1999. Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants. EPA/833B-99/002.

U.S. Environmental Protection Agency (U.S. EPA). 2000. An SAB report: review of the biotic ligand model of the acute toxicity of metals. EPA-SAB-EPEC-00-006.

Van Sprang, P.A. and C.R. Janssen. 2001. Toxicity Identification of metals: development of toxicity identification fingerprints. Environmental Toxicology and Chemistry. 20:2604-2610.

APPENDIX A

SUMMARY OF PROVINCIAL AND TERRITORIAL ACUTE LETHALITY REQUIREMENTS FOR MINES

Appendix A Summary of Provincial and Territorial Acute Lethality Requirements for Mines (In: Environment Canada, 1999. Toxicology Subgroup Final Report: Recommendations on the Use of Acute Lethality in the Amended MMLER. Prepared for the MMLER Amendment Working Group

Provincial Acute Lethality Requirements for Mines							
Province	**Acute Lethality Compliance Testing Requirement**		**Acute Lethality Monitoring Testing Requirement**		**Frequency of Testing**		**Comments**
	Method / Species	**Compliance Limit**	**Method / Species**	**Action Triggers**	**Compliance Test**	**Monitoring Test**	
Ontario	RM 13 rainbow trout	#50% mortality at 96h	No requirement	---	Once a month until 12 consecutive passes then quarterly; trigger back to monthly when there is a failure	---	
	RM 14 *Daphnia magna*	#50% mortality at 48h	No requirement	---	Same as above	---	
British Columbia	RM 13 rainbow trout	#50% mortality at 96h	RM 14 *Daphnia magna*	#50% mortality at 48h	Once a quarter	Once a month	*Daphnia magna* monitoring required taken from new permits
Québec	MMLEG 1977 rainbow trout	#50% mortality at 96h	No requirement	---	Once a year	---	Acute lethality monitoring compulsory for new mines (ie: after 1972); voluntary for old mines; Microtox once a year as required monitoring
	APHA 1985 *Daphnia magna*	#50% mortality at 48h	No requirement	---	Once a year	---	
Newfoundland	No requirement	---	RM 13 rainbow trout	#50% mortality at 96h	---	Once a month	Acute lethality monitoring only in new Certificates of Approval
New Brunswick	No requirement	---	RM 13 rainbow trout	#50% mortality at 96h	---	Site specific (twice a year or quarterly)	Acute lethality monitoring in most permits
Manitoba	No requirement	---	No requirement	---	---	---	Some voluntary acute lethality testing by industry

Provincial Acute Lethality Requirements for Mines							
Province	**Acute Lethality Compliance Testing Requirement**		**Acute Lethality Monitoring Testing Requirement**		**Frequency of Testing**		**Comments**
	Method / Species	**Compliance Limit**	**Method / Species**	**Action Triggers**	**Compliance Test**	**Monitoring Test**	
Saskatchewan	No requirement	---	RM 13 rainbow trout	#50% mortality at 96h	---	Dependent on permit	
Nova Scotia	No requirement	---	RM 13 rainbow trout	#50% mortality at 96h	---	Site specific (twice a year or quarterly)	Acute lethality monitoring in most permits

Territory/AECB Acute Lethality Requirements for Mines							
Territory / AECB Province	**Acute Lethality Compliance Testing Requirement**		**Acute Lethality Monitoring Testing Requirement**		**Frequency of Testing**		**Comments**
	Method / Species	**Compliance Limit**	**Method / Species**	**Action Triggers**	**Compliance Test**	**Monitoring Test**	
Yukon	RM 13 rainbow trout	#50% mortality at 96h	No requirement	---	Quarterly	---	Applies to all Water Board Licences
Northwest Territories	No requirement	---	RM 13 rainbow trout	#50% mortality at 96h	---	Mine specific	Currently reviewing whether or not all Water Licences should require no acute lethality
AECB Ontario	RM 13 rainbow trout	#50% mortality at 96h	No requirement	---	Quarterly	---	
	RM 14 *Daphnia magna*	#50% mortality at 48h	No requirement	---	Quarterly	---	
AECB Saskatchewan	RM 13 rainbow trout	#50% mortality at 96h	No requirement	---	Annually or semi-annually (1 permit was quarterly)	---	AECB Licences require non-acutely lethal effluent; 1 permit requires also Microtox for monitoring

--- = No requirement

APPENDIX B

LITERATURE REVIEW

Appendix B Literature Review

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TABLES

1.0 INTRODUCTION

This initial phase of work for the Guidance Document involved a thorough review of all readily-available information relevant to Toxicity Identification Reduction Evaluations (TI-REs) in the context of the Canadian metal mining sector. The documents included for review were:

- Report on Technologies Applicable to the Management of Canadian Mining Effluents (SENES Consultants Limited, 1999);
- Evaluation of Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) Application to the Canadian Mining Industry (ESG International Inc., 1998);
- Generalized methodology for conducting industrial toxicity reduction evaluations. (U.S. Environmental Protection Agency, 1989; EPA-600/2-88/070)
- Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures. (U.S. Environmental Protection Agency, 1991; EPA-600/6-91/003)
- Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity. (U.S. Environmental Protection Agency, 1993; EPA-600/R-92/080).
- Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. (U.S. Environmental Protection Agency, 1993; EPA-600/R-92/081).
- Non-Acutely Lethal Mining Effluent Technologies (NALMET) Program – 1999 Studies (Beak International Inc. 2000)
- Toxicity Reduction: Evaluation and Control (Water Quality Management Library, Edited by D.L. Ford 1998)

Each document is summarized in the following sections (but will be part of the Appendices in the final Guidance Document). The purpose of this review is to provide the user with an understanding of the current state of knowledge for the relevant TI-RE documents, which form the basis for development of the metal-mining specific Guidance Document. The Guidance Document was not intended to replace the existing U.S. EPA documents, but rather to provide supplementary guidance specific for metal-mining effluents. Consequently, an understanding of the above documents will be critical to the success of a TI-RE for the mining sector.

Based on this review and the information provided in the original RFP, a number of specific challenges and limitations were identified with respect to the performance of a TI-RE for the Canadian mining sector. These challenges and limitations included:

- U.S. EPA methods provide a generic approach for application with any industrial effluent. As such, they lack any specific focus on the issues relevant to the mining industry. For example,
  - The methods were not developed for use with Canadian regulatory species. Specifically, modifications to the rainbow trout test protocol for use in TIEs are lacking.

- The most “common” toxicants associated with mining effluents are not addressed at the start of a Phase I TIE.
- Co-ordination of chemical analyses specific to metal mining effluents is not addressed.

- Recent advances in TIE methodologies are lacking.
- Guidance related to Source Investigations (SIs) and Toxicity Treatability Evaluations (TTEs) specific for mill/mine sites are not provided.
- Transient and seasonal lethality is commonly observed in mining effluents.
- Acute lethality resulting from mining effluents is often caused by multiple toxicants and can be pH-dependent.
- High variability in effluent quality has been encountered at some mines.
- Matrix effects are often observed in mining effluents.
- Based on these limitations and challenges, modifications to the standard U.S. EPA TIE and TTE approaches are required for use with metal mining effluents in Canada. The development of a TIE and TTE Guidance Document specifically for the Canadian mining industry will increase the likelihood of achieving and maintaining a consistently non-acutely lethal effluent.

2.0 LITERATURE REVIEW

The following information is intended as an overview of each of the relevant TI-RE documents. The information was taken directly from each document, however the user should consult the original text to obtain detailed information and specific methodologies.

2.1 Report on Technologies Applicable to the Management of Canadian Mining Effluents (SENES Consultants Limited and Lakefield Research. 1999. Report on Technologies Applicable to the Management of Canadian Mining Effluents. 124pp.)

Environment Canada commissioned a study to review the Best Available Technologies (BAT) for effluent treatment in the base metal, gold, uranium and iron ore mining sectors. The study included; i) a review of mines and effluent control technologies in use, ii) identification and assessment of new technologies, iii) an evaluation of passive treatment technologies, iv) identification of the effectiveness of technologies to reduce ammonia/alkalinity, v) selection of BAT technologies and vi) generation of cost/benefit information.

2.1.1 Description of Metal Mines

The authors provide an overview of the commonly used milling and processing methods. The producers, mining and processing methods, chemical usage, tailings disposal and effluent treatment were discussed for base metal, gold, uranium and iron ore mines in Canada

The most common elements and chemical species that must be reduced to meet effluent guidelines are provided for each sector. Free acidity, depressed or high pH, dissolved metals and ammonia are identified as the primary contaminants of concern for base metal mines. In comparison, cyanide, arsenic, dissolved metals, ammonia and TSS are reported as the contaminants of concern for gold mines. In the uranium sector, the primary contaminants of concern were radium-229, arsenic, uranium, TSS and dissolved metals. TSS was identified as the primary contaminant of concern for the iron ore sector.

2.1.2 Pollution Prevention and Control Techniques

The authors provide a description of common and emerging technologies for pollution prevention and control, with emphasis on technologies that include the implementation of environmental management systems (EMS). Recommended strategies for pollution prevention include:

- Collection and management of all potentially contaminated site water,
- Separation of contaminated flows from uncontaminated flows (thereby reducing the need to large volumes of dilute water),
- Re-routing of surface waters away from the site,
- Recycling and re-using process water (thereby reducing fresh water input), and
- Implementation of EMS to establish protocols and BAT for handling of regents and wastes.

Effluent treatment technologies (Table 1) are reviewed for the following parameters.

- Metals (Fe, Al, Cu, Ni, Pb, Zn, U and Cd)
- Total cyanide and Weak acid dissociable (WAD) cyanide
- Arsenic and Molybdenum
- Radium-226
- Thiosalts
- Ammonia
- Nitrate / Nitrite
- Total suspended solids (TSS)
- pH
- Dissolved Salts
- Mercury
- Acute lethality

Table 1. Overview of mine effluent treatment technologies summarized in the SENES report.

Dissolved Metals (Fe, Al, Cu, Ni, Pb, Zn, U and Cd)	• The removal of dissolved metals is routinely practiced by the use of lime (CaO) which is almost always slaked to $Ca(OH)_2$. • Frequently, the excess alkalinity is removed by the addition of sulphuric acid or carbon dioxide to ameliorate lethal toxicity. • H_2S or Na_2S can be used to remove metals to very low levels, however, the residual toxicity is problematic. • Passive treatment systems have been used to remove metals in sulphate-rich systems, but the application to Canada is limited because of climate restrictions; low temperatures inhibiting bacterial activities and rapid run-off conditions.
Cyanide	• The technologies for the removal of cyanide species are extensively discussed in the report. Included are the most common technologies; natural degradation, INCO SO_2 –air oxidation, and peroxide oxidation. • Other site specific processes for cyanide removal / degradation include: 1) Helmo Gold Process and 2) Homestake's Biodegradation System • The applications and limitations of the processes are outlined including the residual oxidation products (ammonia or copper) that can result in acute lethality. • Emerging technologies include biodegradation and cyanide recovery systems (i.e., Acidification-volatilization-reneutralization (AVR)).

Table 1. Overview of mine effluent treatment technologies summarized in the SENES report.	
Arsenic and Molybdenum	• Both are common contaminants in several metal ores. Removal is commonly done by precipitation as ferric arsenate or ferric molybdate.
Radium-226	• Commonly removed by co-precipitation of barium-radium sulphate. • Ion exchange was used at one facility, with removal efficiencies of 90-95% (system is now by-passed)
Thiosalts	• Thiosalts are commonly produced by alkaline oxidation of sulphide minerals in base metal plants. • Natural oxidation in holding ponds followed by pH adjustment was listed as the most common technology. • Investigations at developing engineered solutions for thiosalt control were reported to be unsuccessful to date.
Ammonia	• Can result from incomplete oxidation of cyanide or from the use of nitrogen-based explosives. • Treatment approaches discussed included; - Natural degradation (most widely used method) - Air-stripping (was not reported to be used in mining industry) - Biological removal of nitrogen compounds by nitrification / denitrification (efficient, but costly because of the need to warm the solution being treated) - Ion exchange (IX) (used as a pre-concentration step; zeolite has been used at the pilot plant scale) - Chemical oxidation (breakpoint chlorination has been used, but could be expensive and generate toxic by-products) • Since one of the primary sources of ammonia is from blasting agents, the report also emphasized the benefits of ammonia-nitrate-fuel oil (ANFO) management.
Nitrate/Nitrite	• Treatment approaches discussed included; - Biological denitrification (has been applied for municipal wastes, but not mining effluents) - IX (may be costly and difficult to treat regenerant) - Electrochemical IX (has not been proven on a large scale, and would not likely ever be applied for mine effluent)
TSS	• The control of suspended solids is common to all mines. • Common technologies discussed included; - Sedimentation (most widely practiced method of removal of precipitated solids) - Coagulant and flocculent additions (used to promote particle aggregation to form larger flocs and promote settling; excess polymer may contribute to effluent toxicity) - Filtration (used as polishing step for removal of fine material or pre-treatment prior to IX or carbon filtration).
pH	• Increases in pH are most commonly achieved with lime, sodium hydroxide, or soda ash. • Decreases in pH are most commonly achieved with sulphuric acid, hydrochloric acid, or carbon dioxide.
Dissolved Salts	• Calcium concentrations can be reduced by product substitution, lime / sodium carbonate softening or gypsum precipitation.

Table 1. Overview of mine effluent treatment technologies summarized in the SENES report.	
Mercury	• Effective technologies for Hg removal include, activated carbon and sulphide precipitation
Acute Lethality	• Common causes of acute lethality summarized in the report included: extreme pH, elevated metals levels and ammonia. Process reagents were reported as another potential cause of toxicity. • The interrelation of these toxicants was briefly described. Some of the common methods to over come the causes of toxicity, included; - Installation of a pH control system to control toxicity due to extreme pH (or to control pH-dependent toxicants) - Lime neutralization to reduce metal concentrations (the most commonly used technology). Alternative technologies include the addition of metal precipitants, chelating agents, IX or reverse osmosis. - Source control, best management practices, pH adjustment and those technologies listed in the ammonia section above were reported as possible methods to control toxicity due to ammonia.

The authors also provide a review of new technologies that have been tested or applied around the world to treat mine effluent. The technologies described include; biological sulphate reduction, addition of chelating agents (EDTA) to reduce metal concentrations and toxicity, ion exchange, advance membrane processes, and the Cyanisorb™ Process for cyanide recovery

2.1.3 Effluent Standards

This section of the report summarizes effluent standards for Canadian and international mines. Chemical limits varied by province and country. In terms of toxicity limits, British Columbia, Ontario, Quebec, Nova Scotia and the Yukon require that effluents are non-acutely lethal. No toxicity limits were listed in the requirements for other provinces.

Compliance data between 1982 and 1997 for Canadian mines was also reviewed. Full (100%) compliance ranged from 59% to 85% for regulated parameters. Acutely lethal toxicity was not included in the analyses. The most commonly reported reasons for non-compliance include:

- start-up problems,
- high run events (storms and spring),
- system upsets, and
- unexpected conditions.

2.1.4 Best Available Technology

The authors provide a review of the Best Available Technology (BAT) for the metal mining sector in Canada. The report focuses on technology that was capable of achieving discharge limits and producing a non-acutely lethal effluent (in the majority of cases).

To assess current industry performance, the authors used data from the Ontario MISA program and the Quebec monitoring program, since they provided the best source of data describing monthly mine effluent quality. Data on the acute lethality of metal-mining effluent were also obtained from two other reports; Andrews (1999) for Environment Canada and Westlake and Hamdy (1998) for the Ontario Ministry of the Environment.

The Ontario and Quebec data indicated that; i) effluent quality for the gold and base metal mining sector was similar in both provinces, ii) effluent contaminant concentrations for iron ore mines were higher in the Environment Canada database (likely due to the large representation of iron ore mines in Newfoundland), and iii) effluent contaminant concentrations for uranium mines were higher in the Environment Canada database (likely due to the large representation of uranium mines in Saskatchewan).

The Environment Canada (Andrews 1999) report indicated that out of the 103 effluent discharges from active and closed mines, 37% reported one or more acute lethality failures with rainbow trout over a 5-year period. Of these, 16 reported a single failure, and 22 reported one or more failures. These results were compared to the 1997 and 1998 data from the Ontario Ministry of Environment, which showed 3 out of 13 base metal mines (23%) failed both rainbow trout and *Daphnia magna* lethality tests, while 18% of gold mines failed the rainbow trout and *Daphnia magna* tests.

The authors also analyzed the performance of operating plants, by parameter (i.e., TSS, metals, cyanide, radium-226 and pH), using BAT technologies. No specific BAT for producing non-acutely lethal effluents was described, but it was reported that plant performance was improving. For example, Ontario data showed that the percentage of mines producing a non-acutely lethal effluent improved from 39% in 1995 to 81% in 1998.

The BAT technologies were described and evaluated by sector (e.g., base metal, gold). In summary, these are shown as:

- Base metal - high density sludge lime treatment with effluent polishing
- Gold and Silver - cyanide destruction plus ferric iron precipitation of arsenic; or
  - cyanide recovery and recycle; or
  - biological degradation of cyanide
- Uranium - barium chloride for radium removal plus ferric iron for arsenic removal
- Iron ore - flocculation and/or coagulation plus settling for suspended solids.

When the BAT was compared to the Metal Mining Liquid Effluent Regulations (MMLER's), it was found that the average performance was well within MMLER objectives (i.e., 95% of the effluent samples were compliant with the MMLER). The most common parameter exceeded was TSS. However, the report indicates that acute lethality was not one of the original MMLER requirements, but that "technologies are available to control acute lethality when the cause of toxicity is identified".

2.1.5 Benefits/Effects of Regulatory Change

The authors used the Ontario and Quebec databases to provide a comparison of plant performance with (then current) MMLER objectives. The report also predicts the level of compliance to be achieved should the MMLER limits be reduced by 25%, 50% and to BAT achievable levels.

It was concluded that small improvements in loading to the environment would result from the widespread application of BAT. For example, the reduction in zinc and copper loading would be 22% and 5%, respectively. The reduction in TSS and total cyanide would be 16% and 23%, respectively. Based on the 1994 Environment Canada data, the report also concluded that the changes proposed by Environment Canada would result in the following:

Parameter	Number of Mines out of Compliance
TSS reduced from 25 to 15	39 out of 117 (33%)

Total Cyanide at 1 mg/l monthly average	3 out of 28 (11%)
Non-acute lethality	22 out of 103 (21%)

The application of the new limit and the introduction of cyanide and non-acute lethality limits would also result in increased costs to the mining industry in Canada. The application of BAT across the industry may require the use of add-on technologies that result in the use of additional chemicals (organic polymers, precipitants, and quaternary ammonium compounds). These agents could cause a toxic response and "residual levels should be controlled to assure acute lethality does not arise".

2.1.6 Generic Capital/Operating Costs for BAT

Typical costs were provided for the Canadian mining industry to meet: i) MMLER limits, ii) BAT, iii) proposed Environment Canada changes to the MMLER, and iv) non-acutely lethal effluents on an industry wide basis. All costs were based on 15-30 year Net Present Value, and included treatment plant capital, water management, and annual operating costs. The estimated costs for upgrading to MMLER limits, BAT levels and revised MMLER limits were $244-374 million, $841-1,300 million and $637-980 million, respectively.

Additional costs for meeting non-acutely toxic effluents were estimated to range from $193 to $297 million. It was noted that the BAT technology described in the report might not produce a non-acutely lethal effluent. However, the technologies described could be applied once the cause of toxicity was identified. The costs estimates to achieve a non-acutely effluent were based on the following assumptions:

- 20% of mines produce acutely lethal effluents and require add-on technology
- 20% of these are ammonia related
- 30% are pH related
- 50% were from other causes that have not been specifically identified (assumed 50% due to metals and 50% due to organics).

For these causes of effluent toxicity, the report identifies technologies that would eliminate acute lethality, including; i) pH control, ii) ammonia removal, iii) metal reduction with lime treatment or coagulation, iv) advanced technology for organic contaminant removal, and v) polishing ponds for natural degradation.

Each facility that produced an acutely lethal effluent would also require a toxicity evaluation study at the cost of $100,000 to identify the cause of toxicity.

In the final section of the report, the authors caution the reader on the validity of the assumptions in producing cost data for producing acutely non-lethal effluents.

2.2 Evaluation of Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) Application to the Canadian Mining Industry

(ESG International Inc. 1998. Evaluation of Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) Application to the Canadian Mining Industry)

The Aquatic Effects Technology Evaluation (AETE) Program commissioned a study to evaluate and summarize the experience of the Canadian mining industry with Toxicity Identification-Reduction Evaluations (TI-REs). The objectives were: i) to complete a critical evaluation of the quality of TI-RE data, its benefits and limitations and, ii) to conduct a survey to evaluate the utility of the TI-RE strategies, including discussions on TIEs and effluent treatment, in determining and/or addressing aquatic impacts from mining operations.

2.2.1 Literature Review

This study found little pertinent published information directly related to Canadian mining and TI-RE studies and therefore the application of TI-REs to Canadian mines could not be assessed on this basis. The limited number of articles reviewed did not imply that few evaluations were being conducted, since the overwhelming majority of studies are never published in the primary scientific literature.

2.2.2 Survey

The authors could not conduct a complete assessment of the Canadian mining sector's experience with the TI-RE process, since less than 50% of mines responded to the survey. Of 42 mines that responded, only 25 (57%) reported having experienced acute toxicity. Of those mines reporting toxic effluents, 7 (28%) indicated that a TRE had been conducted and 17 (76%) reported having conducted at least one Phase I TIE. Very few mines reported going beyond the Phase I toxicity characterization. Of the 25 mines that reported their effluents as being toxic, 9 (36%) reported that toxicity was consistent, compared with 16 (64%) that experienced transient toxicity.

Ammonia was the most commonly identified contaminant of concern and effluent toxicity appeared to be highly pH dependent. Several mines reported difficulties with the identification of secondary causes of toxicity. Five mines reported making changes to their treatment system or process based on the results of the TI-RE. Two mines reported that toxicity was eliminated following the change, one mine reported that toxicity was reduced and two mines reported no change in toxicity. The treatment system or process changes varied from product substitution to implementation of a full-scale effluent treatment facility.

2.2.3 Case Studies

The report also summarized 5 TI-RE case studies (CS). The main objective was to provide examples of TI-REs conducted by Canadian mines and the rationale behind their relative success or lack thereof in implementation in terms of toxicant identification, effluent treatment changes and toxicity reduction or elimination. The case studies were reviewed and summarized in 1998. However, ESG was aware that two of the mines (CS #4 and #5) had made significant progress in their TI-RE investigations since this report was published. With the approval of each mine, the additional information was included as part of this literature review.

In CS #1, a copper/zinc mine, the primary toxicants were identified as copper and ammonia, but secondary toxicants (silver, aluminium and total dissolved solids (TDS)) were also suspected. The TIE lead to the identification of a strategy for reduction of ammonia toxicity to rainbow trout, the main concern of the client. The mine closed, but effluent continued to be discharged and was occasionally toxic.

In CS #2, a uranium mine, the primary toxicant was identified as an aliphatic alcohol (isodecanol). The results lead to process modifications and modifications to the treatment system to resolve the toxicity problem. The effluent is currently non-lethal to trout.

In CS #3, a copper/nickel mine, the primary toxicant was identified as ammonia, but secondary toxicants (metals) were suspected. The treatment system was augmented through pH control, and toxicity was subsequently reduced. Mine personnel felt that the results of the TIE were not cost-effective since the conclusions were based mostly on speculation, rather than on statistically relevant results. Treatability studies provided more relevant and applicable information, particularly related to establishing appropriate limits for pH. Historically, the mine's effluent had been acutely lethal to rainbow trout and

Daphnia magna in most toxicity tests. Most recently, the effluent has been consistently non-lethal to both species on all occasions of testing.

In CS #4, a gold mine, the general characteristics of the suspected toxicant(s) were identified (e.g., metals - most likely copper), but were not confirmed using the TIE process. Treatability investigations included bench-scale evaluations, water reclamation and pilot plant studies. A full effluent treatment plant was installed based on the results of the treatability studies. However, the target level for copper used during these studies was at the *Daphnia magna* LC50. Effluent tested during the pilot plant trials indicated that all treated samples were non-lethal to rainbow trout. However, partial *Daphnia magna* mortalities (20%) were observed in those samples exceeding the total copper target levels. During the most recent discharge period, the effluent was toxic to both species (~ 80% mortality was observed). Ammonia, produced during the destruction of cyanide, was the suspected cause of trout mortality, while metals were possible causes of toxicity to *Daphnia magna*.

Due to the continued toxicity observed in the final effluent, the mine decided to conduct additional testing to evaluate the performance of EDTA addition to treated effluent for removing toxicity due to metals (e.g., copper). The initial concentration of EDTA to be added to the final effluent was 5 mg/L, a level not expected to have any adverse effect on the receiving environment (based on historical testing and literature data). Bioassays conducted on the effluent before and after treatment indicated that the addition of EDTA had a beneficial effect on *Daphnia magna* survival; 0% survival in 100% effluent prior to the addition of EDTA, and 100% survival after EDTA additions.

The authors noted that during the initial EDTA additions to the effluent, dissolved metal concentrations increased slightly downstream of the discharge point when compared to discharged metal levels. These increases were not observed in the absence of EDTA in 1998. Therefore, a laboratory study was undertaken to assess the potential impact of EDTA containing effluent on remobilization of metals from the effluent treatment plant (ETP) iron hydroxide (FeOH) sludge and sediments. The results indicated that metal solubilization from sediments occured at a specific "free" EDTA concentration; 625 – 1,000 µg/L for both FeOH and receiving water sediments. The report noted that applying these results directly to the downstream sediment system required caution, as the majority of the EDTA found in the ETP discharge is complexed after exposure to trace metals remaining in the effluent. The actual concentration of uncomplexed EDTA remaining available to solubilize metals from any sediment would be low.

No adverse environmental effects were noted during the 1999 discharge season with EDTA added to the effluent. All samples collected downstream of the mine were non-lethal to rainbow trout and *Daphnia magna*. No significant reproductive inhibition was observed to *Ceriodaphnia dubia* exposed to receiving water samples. *C. dubia* reproduction tests with the final effluent yielded an inhibition of reproduction (IC25 = 45.5%). However, this result was an improvement from historical data (1997 IC25 values ranged from 6 to 8%). A similar response was observed for fathead minnow growth.

Following selection of the preferred chelating product, laboratory tests were conducted to determine lowest EDTA concentration necessary to eliminate toxicity to *Daphnia magna*. The addition ratio was approximately 13.5:1 (EDTA:copper), which was very close to the theoretical chelating agent equivalent of 15.4:1 (chelating product:copper). The addition ratio allowed ETP operators to adjust the EDTA dosage rate based on copper concentrations, eliminating overdosing while providing a "buffer" to pass the acute lethality toxicity tests.

EDTA addition to the final effluent commenced on August 11, 1999, and was continuous for the rest of the discharge season (July 19th to November 29, 1999). All toxicity samples collected after August 11th were non-lethal to rainbow trout and *Daphnia magna*. Rising water levels in the tailings pond forced the mine to commence effluent discharge before approval for treatment with EDTA was received. Therefore, the effluent discharged in late July and early August was not treated with EDTA, and resulted in failure of the *Daphnia magna* toxicity tests. All samples collected in 2000 were non-lethal to both species.

The maximum concentration of EDTA used during the 1998 discharge season was 5 mg/L. Based on an 1999 average discharge copper of 0.150 mg/L, it was expected that an average EDTA dosage of 2 mg/L would be realized in 2000. This would be a reduction to the actual average EDTA addition of 3.6 mg/L in 1999.

During 1999, a total of 3,023,591 m^3 of effluent was treated and discharged at a cost of $367,300 or $0.12/$m^3$. Of this total discharge, 2,502,402 m^3 (83%) was treated with EDTA. Operating costs attributed solely to EDTA product was $27,000.

In CS #5, a cobalt/nickel and precious metals refinery, several possible causes of toxicity were suspected, but not conclusively identified. Standard TIE tests indicated that zeolite was the only treatment that eliminated toxicity to *Daphnia magna*. It was hypothesized that sodium levels were sufficient to account for at least 50% of the *Daphnia magna* mortality. Copper, potassium and carbonates were identified as potentially important factors in explaining *Daphnia magna* mortality. Atypical ion balance was also a suspected cause of daphnid mortality. Based on the limited available data, it was suspected that periodic peaks in sodium and/or copper concentrations contributed to the sporadic trout toxicity.

The TIE approach to toxicant identification (used in 1996/97 and 1998) proved ineffective with the final effluent. Therefore, an alternative approach was required to resolve the problem of effluent toxicity (in 1999 and 2000). This included; 1) characterization of the upstream sources of toxicity within the refinery (Source Identification), and 2) determination of viable treatment technologies and possible management options for the removal of the toxicant(s) (Toxicity Treatability Evaluation).

A mass balance approach was used to identify 3 (out of 22) streams that represented the largest contributors, in terms of toxicity and chemical load, to the ETP. Based on the results from the mass balance, the specific objectives of the next study were to investigate possible treatment options for these streams.

Removal of the most toxic stream (Stream #1) from the process (full-scale adjustment) resulted in a decrease in Ni, Cu and Co concentrations, and was beneficial to rainbow trout survival. In the case of *Daphnia magna*, it was suspected that high levels of salts from the two remaining streams (Streams #2 and #3) contributed to toxicity even in the absence of elevated copper concentrations from Stream #1 (suggesting that metals alone were not the only contributing factor to *Daphnia magna* mortality).

The bench scale testing of potential treatment technologies focused on the TDS components in Streams #2 and #3. The following technologies were included for bench-scale testing: i) Ion Exchange (IX) with K and Ca, ii) Zeolite, iii) Evaporation, and iv) Selective Precipitation. Results from the bench-scale tests indicated that only evaporation of Stream #3 eliminated toxicity to *Daphnia magna*. Elimination of toxicity following removal of both Stream #1 and Stream #3 during the bench-scale testing, provided strong evidence to support the hypothesis that both copper (from Stream #1) and elevated TDS (from Stream #3) were the main causes of *Daphnia magna* mortality.

A feasibility study for the evaporation treatment of Stream #3 was also conducted. The technology selected was mechanical vapor compression evaporation, a technology that adds energy for evaporation by compressing and recycling the vapor produced by the evaporation process. The total construction cost of the equipment was estimated at $11.2 million, with operating costs estimated at about $1.5 million per year.

Although elevated TDS (or conductivity used as a surrogate measurement) could not account for all of the observed toxicity (due to the presence of copper), the data generated to date indicated a significant relationship between *Daphnia magna* mortality and TDS. Furthermore, the hypothesis that both copper (from Stream #1) and elevated TDS (from Stream #3) were the main causes of *Daphnia magna* mortality was supported by results generated during the bench-scale testing (i.e., removal of both streams eliminated toxicity). Based on this information, it was concluded that conductivity could be used as a tool to manage the quality of the final effluent following the removal of Stream #1.

Stream #1 was successfully removed from the process (approximate cost was $400,000), with the added benefit of recovery of the metals back into the refining process. Further testing is currently underway to determine a conductivity threshold in the absence of toxicity due to copper. The effluent has been consistently non lethal to both rainbow trout and *Daphnia magna*.

2.2.4 AETE Study Conclusions and Recommendations

The authors provided the following conclusions and recommendations based on the survey responses and case studies regarding TI-REs as applied in the Canadian mining industry:

- TIEs do not "prove" the cause of toxicity, but rather use a "weight-of-evidence" approach.
- Full transfer of information and communication between the mine and the testing laboratory is critical to the success of a TI-RE study.
- The experience of the laboratory personnel and variability in effluent quality will influence the overall success of a TI-RE.
- Beyond Phase I, the TIE approach is not standardized and subsequent studies to identify the specific toxicants require experienced personnel.
- If identification and confirmation studies are to be successful, it is critical that the tests are scientifically defendable.
- TI-REs are generally more likely to be successful when an effluent is consistently toxic, if the loss of toxicity is minimal over time and if the factors contributing to toxicity do not vary between samples.
- Repeated testing is required in order to account for effluent variability and confirm that the cause of toxicity is the same under all conditions.
- Appropriate and relevant chemical analyses should be coordinated with toxicity testing on untreated and treated effluent samples.
- Statistical comparisons may not be critical at certain stages of a TIE study, where gross changes in toxicity are the only consideration. However, large amounts of data can become unmanageable and difficult to interpret without statistical analysis.

- The generation of a sufficient volume of data to provide strong evidence regarding the identification of the toxicant is critical if the mine is to consider investment in costly plant-scale remedial measures.
- All bench-scale and pilot-plant studies must include toxicity testing.
- Modifications to the standard U.S. EPA Phase I TIE approach should be investigated and specific treatment methods or approaches developed for the Canadian mining industry.
- The use of rainbow trout in a Phase I TIE study often requires greater effort and expense since trout require large test volumes. However, surrogate test species (e.g., fathead minnows) may not be appropriate in a Canadian context. Modifications to the standard Environment Canada rainbow trout protocol should be developed and standardized for use in TIEs.

2.3 Generalized methodology for conducting industrial toxicity reduction evaluations (U.S. Environmental Protection Agency (U.S. EPA). 1989. Generalized methodology for conducting industrial toxicity reduction evaluations. EPA-600/2-88/070)

This document provides guidance for the implementation of Toxicity Reduction Evaluations (TREs) at industrial facilities. The overall objective of a TRE is to determine those actions necessary to achieve compliance with water quality based effluent limits. A generic approach for designing and conducting a TRE is described, including case studies from a variety of industries. An overview of the TRE approach is provided in Table 2.

Flexibility in the design and conduct of a TRE is essential and the approaches used must be facility-specific. Communication between the industrial facility, permitting authority, and contractors involved are also critical in identifying and understanding the objectives for the TRE, and establishing reasonable schedules.

Table 2. Summary of TRE approach.

Tier 1 **Data acquisition and facility-specific information**	• Define regulatory objectives and targets for successful completion of study. • Review of historical toxicity and chemistry data. • Review of facility and process information, describing the configuration and operation of the facility. • The information generated is used to define study objectives, identify what is already known, and possibly provide information on the causes and sources of toxicity.
Tier 2 **Evaluation of remedial actions to optimize facility operations**	• Three areas of facility operation are considered: Housekeeping, Treatment Plant Operation and Selection and Use of Process and Treatment Chemicals • The information generated is used to identify obvious problem areas, plan and perform remedial actions, and determine if the actions reduce final effluent toxicity. • If the effluent continues to be toxic, then the study will likely proceed to a TIE.

Table 2. Summary of TRE approach.	
Tier 3 **Toxicity Identification Evaluation (TIE)**	• The objective of a TIE is to characterize, identify and confirm the substance(s) responsible for effluent toxicity • Once the TIE is completed, Tiers 4 or 5 can be conducted (note - they are not necessarily mutually exclusive)
Tier 4 **Source Identification**	• The objective is to identify those streams that are significant sources of final effluent toxicity.
Tier 5 **Toxicity Reduction Methods**	• The objective is to identify methods for reducing toxicity in the final effluent or source streams.
Tier 6 **Follow-up and Confirmation**	• Continued effluent testing is conducted to confirm that the selected toxicity reduction method has been effective and that the toxicity reduction target has been achieved and maintained.

2.3.1 Tier 1

Tier 1, data acquisition and facility-specific information, involves the collection and analysis of available information and data that might be useful in designing and directing the most cost-effective study. The information generated is used to define study objectives, identify what is already known, and possibly provide information on the potential causes and sources of toxicity. The information gathered generally falls into three categories: i) regulatory information, ii) effluent monitoring data, and iii) plant and process information.

2.3.2 Tier 2

Tier 2, evaluation of remedial actions to optimize facility operations, includes an evaluation of: i) housekeeping practices, ii) treatment plant optimization, and iii) chemical optimization.

Good housekeeping practices will reduce the chemical contributions to the toxic loading in the final effluent. It includes plant practices and operations that may affect effluent quality, such as: i) general facility cleanliness, ii) spill prevention and control, iii) waste and materials storage areas, iv) materials handling (including loading stations, on-site transport, piping and valve assemblies), v) waste handling and disposal, and vi) run-on/off control.

The objective of treatment plant optimization is to ensure that the treatment plant is operating in optimal fashion with respect to removal of its' design parameters. Plant optimization can be conducted simultaneously with housekeeping improvements and chemical optimization, and includes: i) identification of available information, ii) identification and evaluation of influent waste streams, iii) description and analysis of treatment systems, and iv) implementation of corrective actions

The objective of the chemical optimization process is to identify simple solutions to toxicity by removing possible causative agents. Although no cause-and-effect relationship will have been established between the chemicals and final effluent toxicity, there may be some evidence these chemicals may cause toxicity. The evidence could come from experience at other facilities, or from reported toxicity in the literature. The initial steps will include: i) review of chemical product usage to ensure that only the required amounts are used, ii) review of available toxicity data for all chemicals used in the process (with attention to data for those species used to test the effluent), iii) review of biodegradability information, and iv) determination if less toxic/more degradable alternatives are available.

2.3.3 Tier 3

Tier 3 involves application of the Toxicity Identification Evaluation (TIE) procedure. The objective of the TIE is to identify the specific substances responsible for effluent toxicity. In certain cases, only the characteristics of the suspected toxicant may be determined. In either case, the information obtained will be useful in evaluating treatment methodologies, or investigating the sources of final effluent toxicity. In TREs, where Tier 2 suggested causes or sources of toxicity, TIEs will usually still be required to provide additional "weight-of-evidence" and confirmation of the suspected toxicants. Phase I TIE procedures can be conducted concurrently with the facility information gathering and operations assessment steps.

The TIE process consists of 3 Phases. Phase I involves toxicity characterization, which is designed to determine the class or group of the compound(s) causing toxicity. The frequency of performing Phase I will be based on the variability of effluent toxicity. In this regard, it is unlikely that a single toxic sample will be sufficient.

Phase II of the TIE is designed to identify the specific substance. The number and type of analyses will depend on the results from Phase I. Phase III is the confirmation step. In cases where Phase II identification was not successful, confirmation should still be conducted. Confirmation of Phase I results will be particularly important where treatability studies are to be conducted, with possible construction of, or modifications to, treatment facilities.

It should be noted that the Phase I, II and III methods in this particular document were described prior to publication of the final TIE methods document. Therefore, detailed procedures were not summarized from this document.

2.3.4 Tier 4

Tier 4 involves application of the Source Identification Evaluations (SI). After completion of Tier 3 (TIE), two options are available. One option is to evaluate various treatment methods for removal of the toxicant. A second option is to determine the source of the toxicant. Source controls, such as chemical substitution, spill control, and treatment of the source stream may be more efficient and economical than treating the final effluent.

Although treatability studies are the most direct approach, they may also be expensive. Identification of the source of the toxicant could result in a more cost-effective solution, but could be difficult in facilities with numerous process streams. However, treatment of smaller concentrated streams could be conducted more efficiently and economically than treatment of larger, more dilute streams (e.g., the final effluent).

Selection of the treatability study versus SI must be made on a site-specific basis. In cases where the SI approach is selected, the document suggests five steps that might be useful:

1. Set a search image.

2. Select sampling locations.

3a. If the toxicant has been identified, use chemical-specific analysis for tracking the sources.

3b. Evaluate the degradation effects of the treatment plant on altering the toxicant (where possible).

4a. If the toxicant has not been identified, use bench-scale model to simulate treatment plant and track toxicity.

4b. Characterize the bench-scale treated samples using Phase I treatments (where necessary).

5. If specific process streams have been identified as sources, go further into the process to identify toxic side-streams.

Source streams will only be identified if they are sufficiently toxic and are not de-toxified by the treatment system, or if they contain the suspected toxicant.

2.3.5 Tier 5

Tier 5 describes approaches for Toxicity Reduction Methodologies. If the effluent still exhibits toxicity after completing Tiers 1 through 3 (Housekeeping, Treatment Plant Operation and Selection and Use of Process and Treatment Chemicals), other approaches are required, such as: i) source reduction technologies, and ii) improvement of waste treatment operations.

Source reduction involves using the most practical, cost-effective and permanent practices and procedures to reduce or eliminate toxic loads. It assumes that a source was identified and may involve: i) material substitution, ii) process modifications, iii) waste stream mixing, iv) pre-treatment, materials recovery, or v) waste recycling. Source reductions are highly case specific and dependent on factors such as waste stream composition, physical constraints and flow variability. Lab, bench and pilot scale demonstrations of the effectiveness of the technology would be required prior to actual implementation. If toxicity still appears in the effluent, then end-of-pipe treatments must be examined.

The most direct means of improving waste treatment operations is through plant optimization. If plant operations are at an optimal level and the effluent remains toxic, then other treatment modifications are required based on the results from the TIE. Possible areas to be examined include: hydraulic and mass loading, chemical feed rates, biological enhancement, source batching or segregation, effluent polishing and additional treatment steps.

Factors to be considered prior to implementation of a new treatment technology include: cost, performance, complexity of solution, ease of implementation, expected life of modification, flexibility of modification, and application to various waste streams. The relative importance of each factor must be established on site-specific basis. After the methodology is selected, confirmation must be conducted using bench-scale or pilot-scale approaches. The document emphasized that this confirmation step is essential, since the implementation on-site can be potentially costly.

2.3.6 Tier 6

Tier 6, follow-up and confirmation, involves implementing an appropriate monitoring program to confirm that the toxicity control method has successfully met the required compliance toxicity limits for the final effluent.

The document also provided 10 case studies as Appendices. These are summarized in Table 3. It should be noted that all case studies were conducted prior to 1989, before publication of the U.S. EPA TIE methods.

Table 3. Summary of TRE case studies.					
Case Study	**Facility Description**	**TRE Steps Used**	**Toxicant Identified**	**Toxicity Reduction Approaches**	**Conclusions**
1	Specialty Chemical Plant	Data & Information Acquisition, TIE, SI	Pesticide (dichlorvos)	Source of pesticide was removed, but also considered using carbon / resin adsorption, hydrolytic destruction, biological removal, in-plant controls and process modifications.	Toxicity was eliminated. TRE process was useful, but toxicity was variable due to an improperly operating treatment plant. Additional toxicity testing would have been useful.
2	Petroleum Refinery	Data & Information Acquisition, TIE, SI	Neutral organic chemicals	Treatability studies were in progress at time of document preparation. Options under consideration included: activated carbon, and increased residence time in surface impoundments.	Final toxicity reduction solution not selected at time of document preparation. Unplanned upsets and planned process changes increased variability and made planning of evaluations difficult.
3	Oil Refinery	Data & Information Acquisition, TIE	Oil and grease, ammonia, amines, flocculation polymers and suspended solids (SS)	Oil and grease – installation of an oil removal unit and powdered activated carbon; Ammonia – sustained nitrification in activated sludge basin; Amines (inorganic nitrogen) – process change; Flocculation Polymers – discontinued use of problem polymer; SS – no action taken (contribution to toxicity thought to be minor).	Toxicity was eliminated. Studies took 8 years to complete (in part, because of variable toxicity). Used correlation approach to relate effluent toxicity to manufacturing processes. Tested various process streams to focus on those processes that were correlated with effluent toxicity.
4	Textile Mill	Data & Information Acquisition, TIE	Non-biodegraded non-ionic surfactants (linear alcohol ethoxylates)	Extended biological treatment and chemical substitution.	Toxicity was eliminated. Based on a comparison to literature values, metals were suspected as a possible cause of toxicity. However,

Table 3. Summary of TRE case studies.					
Case Study	**Facility Description**	**TRE Steps Used**	**Toxicant Identified**	**Toxicity Reduction Approaches**	**Conclusions**
					additional testing with cationic exchange resins suggested that metals were not a major contributor.
5	Metal Product Manufacturer	Data & Information Acquisition, TIE	Metals (zinc and copper) and Chlorine	Treatability studies were in progress at time of document preparation. Options under consideration included: lime addition and reduction of residual chlorine levels by aeration, cascading or chemical treatment.	Treatability studies were in progress at time of document preparation.
6	Texas Instruments Facility	Data & Information Acquisition, TIE	Metals	Insoluble sulfide precipitation.	Toxicity was eliminated. Pilot plant testing indicated selected methodology would successfully meet toxicity requirements at full-scale.
7	Chemical Plant (organic dyes, epoxy resins and fine chemicals for textile, paper and plastic)	Data & Information Acquisition, TIE, SI	Non-biodegradable organics	Source reduction and treatment (8 of 27 streams treated at source; 5 precipitated to remove Cu and Cr; 14 sulfide-containing streams were air-oxidized), elimination of 6 waste streams and treatment system optimization (conversion of existing biological treatment system to Powdered Activated Carbon Treatment).	TOC loadings reduced by 23%. Follow-up studies to confirm toxicity removal were underway at the time of document preparation.

Table 3. Summary of TRE case studies.					
Case Study	**Facility Description**	**TRE Steps Used**	**Toxicant Identified**	**Toxicity Reduction Approaches**	**Conclusions**
8	Chemical Plant (surfactants)	Data & Information Acquisition, TIE, SI	NPEO (nonlyphenol ethoxylate)	Powdered Activated Carbon Treatment.	Toxicity was eliminated.
9	Pulp Facility	Data & Information Acquisition, TIE	Ammonia	Air Stripping.	Document did not indicate if reduction methodology was implemented or if toxicity was eliminated.
10	Monsanto Chemical Manufacturing Facility (Three Sites)	Data & Information Acquisition, TIE	Site 1 - ammonia; Site 2 – hexavalent Cr; Site 3 – Ca and Cl	Several reduction options were considered for each site. Site 1 – activated carbon, cation and anion exchange resins and zeolite; Site 2 – activated carbon and anion exchange; Site 3 – anion and cation exchange resins.	Sites 1 and 3 – the document provided no follow-up information; Site 2 – permanent removal of hexavalent Cr eliminated toxicity.

2.4 Methods for aquatic toxicity identification evaluations (TIE): Phase I toxicity characterization procedures (U.S. EPA. 1991a. Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures. EPA-600/6-91/003)

This document provides regulated industries with procedures used to assess the nature of acute effluent toxicity. It includes discussions on various topics related to the conduct of a Phase I Toxicity Identification Evaluation (TIE), including: health and safety, quality assurance, facilities and equipment, dilution water, effluent sampling and handling, toxicity test, characterization tests, additional tests and interpretation of Phase I results.

The two objectives in the Phase I TIE are to: i) characterize the substance responsible for toxicity, and ii) determine if toxicity is consistently caused by the same substance. The generic methods can be used for assessing the cause of acute lethal toxicity in wide variety of effluents, receiving waters, elutriates, pore waters and leachates.

Phase I toxicity tests are designed to alter or render biologically unavailable a group of toxicants, such as oxidants, cationic metals or volatiles. Toxicity tests are performed before and after the effluent manipulation to provide an indication of the effectiveness of a treatment. Characterization tests are conducted over time on different samples to confirm that the substances responsible for toxicity are consistent.

At the completion of Phase I, toxicants can be classified as metals, non-polar organics, oxidants, and others. The next steps after the completion of Phase I can include: 1) toxicant identification (Phase II) and confirmation (Phase III), 2) toxicity treatability, or 3) source investigations.

An overview of different elements of a Phase I TIE is provided in Table 4 and is described in further detail in the following sections.

Table 4. Overview of Phase I TIE approach.

Objective	• Characterize the substance responsible for toxicity. • Determine if toxicity is consistently caused by the same substance.
Preliminary Testing	• Sufficient testing must be conducted prior to the TIE to ensure consistent presence of toxicity.
Quality Assurance and Quality Control	• Toxicity tests performed in the early phases of the TIE generally do not require exacting quality control because the data generated are only preliminary. • Two types of controls are used to detect artifactual toxicity: 1) Toxicity controls involve comparison of the untreated and treated effluent sample, and 2) Toxicity blanks involve the performance of a Phase I test on dilution water to determine if any toxicity is added by the test procedure.
Test Species	• When selecting a test species, consideration should be given to: size, age, availability, adaptability to test conditions, differences in species sensitivity, and the regulated species. • If a test organism other than the regulatory species is selected for the TIE, testing must be conducted to demonstrate that the toxicant is the same for both species.
Toxicity Test Procedures	• Standard acute test methods do not have to be used during Phase I. • Tests conducted for Phase I should be as inexpensive as possible because of the large number of tests that are needed.

Table 4. Overview of Phase I TIE approach.	
Effluent Sampling and Handling	• Sampling must be sufficiently extensive to ensure that the effluent samples are representative of the discharge over time. • Sample analysis should begin immediately after sample collection. • Testing on a single sample can continue indefinitely, provided that the effluent remains toxic. However, fresh samples should be used during the early stage of a TIE since the cause of toxicity may change with age (even though the degree of toxicity may not change).
Treatments	• For one complete Phase I TIE, there are nine treatment categories: Initial Test, Baseline Test, pH adjustment, pH adjustment / filtration, pH adjustment / aeration, pH adjustment / C18, EDTA, sodium thiosulfate, and graduated pH.
TIE Test Procedures	• When the sample arrives at the laboratory (Day 1), routine water quality parameters (i.e., temperature, pH, D.O., hardness, conductivity, ammonia, chlorine) are measured and an initial toxicity test (Initial Test) is started. • Sub-samples of the effluent are adjusted to pH 3 and 11, and then filtered, aerated or passed through a C18 column. After the manipulation is complete, the sample is readjusted to the initial pH of the effluent (pH_i) and held at 4 EC overnight. • A second untreated effluent sample (Baseline Test) is tested on Day 2. The EDTA, sodium thiosulfate and graduated pH adjustments are also conducted on Day 2. • The amount of testing required beyond Phase I will depend upon the stability of effluent toxicity, the nature of the toxicity, and previous Phase I results.
Additional Tests	• Recommended approaches include the use of multiple Phase I manipulations (aeration / filtraton / pH adjustment / C18 in various combinations), activated carbon, other specific ion columns and other ligands.
Interpretation of Test Results	• If multiple toxicants are present, focus on identification of one toxicant (once this primary toxicant is identified, it should be easier to identify secondary toxicants). • Focus on those manipulations observed to have the most dramatic impact on toxicity. • Concentrate on those treatments that removed the toxicant from other effluent constituents. • Examples on interpreting various Phase I results were provided for non-polar organics, TDS, surfactants, cationic metals, ammonia and oxidants. The examples are only guides and not definitive diagnostic tools.

2.4.1 Preliminary Testing

The Phase I document clearly states that toxicity must be present frequently enough and endure storage (i.e., toxicity is persistent) so that repeated testing can be conducted to characterize, identify and confirm the substance responsible for toxicity. Sufficient testing must be conducted prior to the TIE to ensure consistent presence of toxicity. This testing is conducted in order to establish the sufficient and frequent presence of toxicity.

2.4.2 Quality Assurance (QA) and Quality Control (QC)

The document indicated that a standard QA/QC program for TIEs could not be described since each study will be unique. However, a general QA program may increase the probability of success. Toxicity tests performed in the early phases of the TIE generally do not require exacting quality control because

the data generated are only preliminary. Phase I, and to a lesser extent Phase II, are more tentative in nature compared to the confirmation tests conducted in Phase III.

System blanks are used throughout the TIE to detect toxic artifacts (e.g., excessive ionic strength resulting from addition of acid/base during pH adjustments, contaminated air or nitrogen sources) added during the effluent manipulations. Two types of controls are used to detect artifactual toxicity: 1) Toxicity controls that involve comparison of the untreated and treated effluent sample; and 2) Toxicity blanks that involve the performance of a Phase I test on dilution water to determine if any toxicity is added by the test procedure.

2.4.3 Test Species

Important considerations when selecting a test species include: appropriate size, age, availability, adaptability to test conditions, differences in species sensitivity, and regulated species. If a test organism other than the regulatory species is selected for the TIE, testing must be conducted to demonstrate that the toxicant causing the response is the same for both species.

2.4.4 Toxicity Test Procedures

Toxicity test procedures for Phase I TIEs are different from those used for standard regulatory tests. Removal of stress (low D.O.) and loading rates may not be important for a Phase I TIE, since relative sensitivity is used. However, it is important to ensure test that conditions are similar for all tests. Tests conducted for Phase I should be as inexpensive as possible because of the large number of tests that are needed. Therefore, standard acute lethality tests (conducted as per the required regulatory methods) do not have to be used during Phase I.

2.4.5 Sample Collection and Handling

With respect to sample collection and handling, the authors indicated that sampling must be sufficiently extensive to ensure that the samples are representative of the discharge over time. Site information obtained at the time of collection may also be useful in determining if the sample is representative.

Analysis should begin immediately after sample collection. Samples should be kept cool (< 4 EC) during transport and storage at the lab. Testing on a single sample can continue indefinitely, provided the effluent remains toxic. However, fresh samples should be used during the early stage of a TIE since the cause of toxicity may change with age (even though the degree of toxicity may not change).

2.4.6 Treatments

For one complete Phase I TIE, there are nine treatment categories: Initial Test, Baseline Test, pH adjustment, pH adjustment/filtration, pH adjustment/aeration, pH adjustment/C18, EDTA, sodium thiosulfate, and graduated pH. A brief summary of each manipulation is provided.

- The **Initial Test** provides an estimate of the untreated effluent LC50 for setting the exposure concentrations in the subsequent Phase I tests. The Initial Test should be set as soon as the sample arrives at the laboratory.
- Each day a sample manipulation is performed, an untreated effluent test (or **Baseline Test**) is set. The results from each manipulation are compared to the Baseline Test to assess the effectiveness of the manipulation on reducing toxicity. By comparing these tests, the physical/chemical characteristics of the toxicant can be obtained.

- **Adjustment of pH** (to pH 3 and 11) is used throughout the Phase I tests to: i) provide additional information on the nature of the toxicants, and ii) provide blanks for subsequent pH adjustment tests performed in combination with other treatments (i.e.., filtration, aeration). Changes in pH can have profound effects on a number of chemical and physical properties of toxicants including: solubility, polarity, speciation and stability. pH can also change the ratio of ionized to un-ionized forms of toxicants with a corresponding change in toxicity. Changes in pH can also affect the equilibrium between metal ion complexes, which can in turn affect solubility, bioavailability and toxicity. The general test procedure involves adjustment of dilution water and effluent to pH 3 and 11, followed by filtration, aeration or solid phase extraction with a C18 column. The treated samples (including the pH adjusted samples without additional treatment) are then readjusted to the initial effluent pH (pHi) and stored at 4 EC until testing.

- **T**he **pH adjustment/filtration** test evaluates the effect of pH change and filtration on the toxicity of substances associated with filterable material, focusing on irreversible chemical reactions. Filtration of the effluent can result in an immediate removal of toxicants. Substances typically in solution in the unadjusted effluent, but insoluble or associated with particulates at extreme pH's, are also removed by filtering pH-adjusted samples. The samples are filtered prior to readjustment to pH_i, thereby preventing dissolution of the toxicants in the effluent. Chemicals associated with particulates may be less biologically available, but could also cause toxicity through ingestion (e.g., filter-feeding cladocerans may ingest toxic particles). The general test procedure involves adjustment of high purity water, dilution water and effluent to pH 3 and 11 using solutions of NaOH or HCl as required. Individual glass fibre (1 micron) filters are first prepared by rinsing with high purity water adjusted to pHi (the initial pH of the effluent), 3 or 11. Each dilution water sample is filtered and collected (for the toxicity blanks). The same filters used for each dilution water sample are then used to filter each effluent sample (i.e., pH 3 dilution water and effluent are passed through the pH 3 prepared filter). Positive pressure filtration is used, since a vacuum may result in the loss of volatiles. The pH of each dilution water and effluent sample is then re-adjusted to pHi and stored at 4 EC until testing.

- The **pH adjustment/aeration** test evaluates the effect of pH change and aeration on the toxicity of the sample that may be due to volatile, sublatable or oxidizable compounds. If toxicity is reduced or eliminated in any aerated sample, the mechanism of removal (i.e., sparging, oxidation, sublation) must be established. If tests with air and nitrogen produce the same results, the removal process is likely due to sparging. Oxidation is the likely mechanism, if only air removes toxicity. Toxicant recovery from the sides of the test vessel, using dilution water or solvents (e.g., methanol) suggests that toxicity is due to subltable substances. If nitrogen sparging removed toxicity, additional tests using "volatile toxicant transfer" may allow the volatile components to be isolated. The general test procedure involves adjustment of dilution water and effluent to pH 3 and 11 using solutions of NaOH or HCl as required. Solutions are placed in graduated cylinders and aerated at a moderate rate for a standard time interval. The pH of the solutions are checked and readjusted at regular intervals. Aerated samples are siphoned from each vessel such that the treated sample does not contact the sides of the container. This particular step is critical since the sides of the cylinder may contain surface-active material that accumulated during the aeration process. The pH of each dilution water and effluent sample is then re-adjusted to pH_i and stored at 4 EC until testing.

- The **pH adjustment/C18 Solid Phase Extraction** test evaluates the extent to which toxicity may be due to relatively non-polar organics and certain metals. By adjusting the pH of the effluent, certain organic acids/bases can be made less polar by shifting the equilibrium to the un-ionized form,

thereby allowing these substances to sorb to the C18 column. The general test procedure involves adjustment of high purity water, dilution water and effluent to pH 3 and 9 using solutions of NaOH or HCl as required. The pH is adjusted to 9 (instead of 11) for the C18 test, since this is the tolerable limit for many columns (specific column tolerance information should be obtained from the manufacturer's data). Dilution water and effluent are pre-filtered as described in the pH adjustment/filtration methods. Individual C18 columns are first pre-conditioned by rinsing with methanol followed by high purity water adjusted to pHi, 3 or 9. Each filtered dilution water sample is passed through the column and collected (for the toxicity blanks). The same columns used for each dilution water sample are then used for each effluent sample (i.e., pH 3 dilution water and effluent are passed through the pH 3 prepared C18 column). The column must not be allowed to run dry during the entire process. The pH of each dilution water and effluent sample is then re-adjusted to pHi and stored at 4 EC until testing.

- The **Oxidant Reduction** test evaluates the extent to which oxidative substances (e.g., chlorine, iodine, bromine) and some cationic metals (e.g., Cd, Cu, Ag, Hg) can be made less toxic or non-toxic by the addition of sodium thiosulfate. Sodium thiosulfate can be added as a gradient of concentrations to a single effluent concentration, or as a dilution test where effluent and thiosulfate concentrations vary. For the gradient approach, concentrations of sodium thiosulfate (equal to and lower than the LC50 for the test species) are added to the 100% (or 4x-LC50) effluent. For the dilution approach, a matrix of three effluent concentrations and three thiosulfate concentrations are tested. LC50s can be calculated using the dilution approach (to determine how much toxicity has been removed).
- The **EDTA Chelation** test evaluates the extent to which cationic metals (e.g., Al, Ba, Cd, Co, Cu) can be made less toxic or non-toxic by the addition of EDTA (Ethylenediaminetetraacetate). A cationic metal may be suspected as the cause of toxicity if both EDTA and sodium thiosulfate reduce toxicity. EDTA can be added as a gradient of concentrations to a single effluent concentration, or as a dilution test where effluent and EDTA concentrations vary. For the gradient approach, concentrations of EDTA (equal to and lower than the LC50 for the test species) are added to the 100% (or 4x-LC50) effluent. For the dilution approach, a matrix of three effluent concentrations and three EDTA concentrations are tested. LC50s can be calculated using the dilution approach (to determine how much toxicity has been removed). The EDTA spiked effluent should be allowed to stand for at least two hours prior to test initiation, since the complexation of metals with EDTA will not be immediate. Because EDTA is an acid, test solution pH should be checked and readjusted prior to test initiation.
- The **graduated pH** test evaluates the effect of pH on the toxicity of a variety of contaminants. The substances of concern addressed in the graduated pH test are those with a pK_a that allow dissociation to occur within a tolerable pH range (i.e., pH 6 to 9). Ammonia, cyanide and hydrogen sulfide are common ionizable substances found in wastewater. Metal toxicity can also be affected by changes in solubility and speciation at different pH values. The general test procedure involves adjustment of effluent to three different pH values, without readjustment to pHi. The pH values selected will be based on the effluent characteristics. For example, the pH many effluent samples will increase with exposure to air. If the pH at air equilibrium is 8.0, it may be appropriate to use pH's of 6, 7 and 8 for the graduated pH test. The greatest difficulty encountered during the graduated pH test is maintenance of effluent pH through out the test. The US EPA recommended the use of CO2, HCl or NaOH for pH adjustment and control. The use of pH buffers was also discussed, but their experience was limited.

2.4.7 TIE Test Procedures

This section provides further details on the Phase I TIE process described in Table 4. When the sample arrives at the laboratory (Day 1), routine water quality parameters (i.e., temperature, pH, D.O., hardness, conductivity, ammonia, chlorine) should be measured and an initial toxicity test (Initial Test) started. The Initial LC50 test results are used to determine the degree of toxicity and identify the exposure concentrations for subsequent tests.

On Day 1, sub-samples of the effluent are also adjusted to pH 3 and 11 (pH 9 for the C18 treatment), and then filtered, aerated or passed through a C18 column. After the manipulations are complete, the samples are readjusted to pHi and held at 4 EC overnight until testing on Day2.

Assuming the pH adjustment, aeration, filtration and C18 manipulations were conducted on Day 1, a second untreated effluent sample (Baseline Test) and the manipulations (conducted on Day 1) are tested on Day 2. The EDTA, sodium thiosulfate and graduated pH adjustments are also conducted and tested on Day 2.

Delaying most of the toxicity testing by 24 hours (until Day 2), allows the test exposures to be set at concentrations bracketing the LC50 (as determined by the initial toxicity test set on Day 1). The delay also allows the pH adjusted effluents to stabilize. However, sample manipulations can proceed on Day 2 when the toxicity of the effluent is unknown, thereby preventing treatments from being conducted on a non-toxic sample.

The amount of testing required beyond Phase I will depend on the stability of effluent toxicity, the nature of the toxicity, and previous Phase I results. Usually, a change in the LC50 equal to one concentration interval can be considered a “significant” change. However, the decision as to what a significant reduction in toxicity is, must be based on the experience of the investigator.

Based on the experience of the US EPA, at least one Phase I characterization test should be successful in altering toxicity. If not all toxicity is removed, other toxicants may be present in the effluent, or a single toxicant may be present at extremely elevated concentrations. Additional testing is required to resolve these results (i.e., extended aeration, increased reagent concentrations).

If several Phase I tests remove or reduce toxicity, multiple toxicants may be present in the effluent, or a single toxicant may be removed by more than one manipulation. These results can be resolved by conducting all of the effective manipulations on a single sample of effluent. If toxicity removal is increased in the combined manipulations (compared to an individual manipulation), there is likely more than one substance responsible for toxicity. If the results are similar, then it is likely that all of the manipulations are successful in reducing the same toxicant.

The US EPA also emphasized that assumptions not be made about the synergistic or additive nature of the toxicants during Phase I. Furthermore, based on the experience of the US EPA, selected tests should not be used to confirm a suspicion that a particular substance is responsible for toxicity. Nor should any Phase I test be eliminated on the basis that it is not suspected to be present in the effluent. The Phase I tests should be conducted without any preconceived notions as to the cause of effluent toxicity.

2.4.8 Additional Tests

Alternative approaches must used for those cases where the Phase I manipulations yield little or no information as to the characteristics of the toxicants. Recommended approaches included the use of multiple Phase I manipulations (aeration/filtraton/pH adjustment/C18 in various combinations),

activated carbon, other specific ion columns and other ligands.

2.4.9 Interpretation of Test Results

The US EPA provide suggestions on interpretation of Phase I results, including:

i) if multiple toxicants are present, focus on the identification of one toxicant (once this primary toxicant is identified, it should be easier to identify the secondary toxicant(s)),

ii) focus on those manipulations observed to have the most dramatic impact on toxicity, and

iii) concentrate on those treatments that remove the toxicant from other effluent constituents.

Examples on interpreting various Phase I results were provided for non-polar organics, TDS, surfactants, cationic metals, ammonia and oxidants. The examples are only guides and not definitive diagnostic tools.

Non-polar organics are suspected as the cause of effluent toxicity if:

1. toxicity is removed in the post C18 column effluent
2. toxicity is recovered in the methanol elution of the C18 column

TDS is the suspected cause of effluent toxicity if:

1. pH adjustment has no effect on toxicity (unless a precipitate is observed in the pH adjustment, pH adjustment / filtration and pH adjustment / aeration tests)
2. toxicity is not reduced in the post C18 column effluent (or a partial loss in toxicity is observed with no change in conductivity)
3. toxicity is not reduced in the graduated pH test, or after addition of EDTA or sodium thiosulfate

Additional tests that can be used to assist in determining if TDS is the cause of toxicity include:

1. ion exchange resins (TDS could be the cause if toxicity is reduced after treatment)
2. activated carbon (TDS could be the cause if toxicity is not reduced after treatment)

It was also noted that artifactual toxicity could be produced if the addition of acid / base during the pH adjustment test causes an increase in TDS above lethal thresholds. Conductivity should be measured before and after adjustment to avoid this situation.

Surfactants are a suspected cause of effluent toxicity if:

1. toxicity is removed or reduced after filtration
2. toxicity is removed or reduced by aeration
3. toxicity can be recovered from the walls of the test vessel
4. toxicity is removed or reduced by the C18 column
5. toxicity degrades over time (in an effluent kept in cold storage) and is slower in glass than in plastic

Cationic metals are a suspected cause of effluent toxicity if:

1. toxicity is removed or reduced after EDTA or sodium thiosulfate addition
2. toxicity is removed or reduced by the C18 column

3. toxicity is removed or reduced by filtration (particularly when combined with pH adjustments)
4. an erratic dose response is observed

Ammonia is a suspected cause of effluent toxicity if:

1. the concentration of total ammonia is $ 5 mg / L
2. toxicity increases as pH increases
3. effluent is more toxic to fathead minnows than to *Ceriodaphnia* or *Daphnia magna*

Oxidants are a suspected cause of effluent toxicity if:

1. toxicity is removed or reduced after sodium thiosulfate
2. toxicity is removed or reduced after aeration (without pH adjustment)
3. toxicity degrades over time (in an effluent kept in cold storage)
4. effluent is more toxic to *Ceriodaphnia* than fathead minnows

2.5 Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity

(U.S. EPA. 1993a. Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity. EPA-600/R-92/080)

The original U.S. EPA Phase II document was published in 1989, and covered Phase II approaches for acute toxicity only. The revised Phase II document (1993) provides identification schemes for non-polar organics, ammonia, metals, chlorine and surfactants causing either acute or chronic toxicity. For the purposes of this study, only the acute toxicity portion of the 1993 document was summarized.

In Phase II of a TIE, further effluent treatments are conducted to identify the specific substance(s) responsible for toxicity. The additional treatments and analytical methods chosen are directly related to those treatments observed to effectively eliminate or reduce toxicity during Phase I. Phase III (confirmation) should be initiated as soon as the Phase II studies support one or more specific substances as the cause of effluent toxicity.

An overview of the different elements of a Phase II TIE is provided in Table 5 and is described in further detail in the following sections.

Table 5. Overview of Phase II TIE approach.

Objective	• Identify the specific substance(s) responsible for toxicity.
Preliminary Testing	• Phase II methods are directly related to those treatments that eliminated or reduced toxicity during Phase I characterization.
Quality Assurance and Quality Control	• Increased QA / QC required as Phases II and III proceed. • Standardized test methods should be applied to confirm that the suspected substance is responsible for the toxicity observed in the test that originally triggered the TIE. • Information from all tests should be maximized during Phases II and III. • Reference toxicant tests should also be used during Phases II and III. Once identified, the suspected toxicant should be used as the reference toxicant for the TIE tests.
Test Species	• Recommend that all tests be conducted with the species that originally triggered the TIE.

Table 5. Overview of Phase II TIE approach.	
	• If an alternative species is selected, tests conducted in Phases II and III must demonstrate that the original and alternative species are responding to the same toxicant.
Toxicity Test Procedures	• Sample toxicity must be tracked during the TIE to assess if the substance responsible has degraded over time. • Tighter concentration intervals may also be required for Phase II and III toxicity tests in order to detect smaller incremental changes in toxicity.
Effluent Sampling and Handling	• Type of sample (i.e., grab versus composite) used during Phase II should be the same as that used during Phase I. • One composite or one grab sample should be subjected to both Phases II and III. • Once the substance responsible is identified, multiple samples may be analyzed for the presence of the toxicant.
Treatments	• Treatments used are specific for each suspected toxicant • Non-polar organics – separation of toxic and non-toxic fractions; subsequent analyses using HPLC and GC / MS. • Ammonia – measurement of ammonia in effluent; graduated pH testing; testing using zeolite resin to remove ammonia; air-stripping of ammonia from the effluent at high pH (i.e., pH 11). • Metals – measurement of metals in effluent; treatment with EDTA and sodium thiosulfate; graduated pH and ion exchange tests. • Chlorine – measurement of total residual chlorine (TRC) in effluent; treatment with sodium thiosulfate • Filterable Toxicants – use of other filter types (i.e., nylon, Teflon); centrifugation; extraction and concentration of filtered material.
TIE Test Procedures	• Specific for each suspected toxicant (see discussion below)
Interpretation of Test Results	• Specific for each suspected toxicant • Interpretation of TIE results is often different from the "standard" acceptance or rejection of a hypothesis. The TIE test will usually allow the hypothesis to be accepted, but not rejected. • The presence of multiple toxicants complicates interpretation of TIE results. • Recommended focusing on the toxicant that appears easiest to identify. • Effect of effluent toxicants are not always additive. At least one toxicant must be identified before additivity can be established.

2.5.1 Quality Assurance (QA) and Quality Control (QC)

As Phases II and III proceed, QA requirements should be revisited and modified as required. If modified or altered test methods were used during Phase I, standardized test methods should be applied during Phases II and III to confirm that the suspected substance is responsible for the toxicity observed in the test that originally triggered the TIE.

Information from all tests should be maximized during Phases II and III. Observations, such as time to mortality (e.g., LT50), may provide subtle indications of small or incremental changes in toxicity. Use of randomization, accurate exposure time readings, similarly-aged organisms, and increased replication might assist in reducing differences in test results. The investigator should also not rely solely on

statistical analysis of the data to determine if a treatment had a significant effect on toxicity. Experience of the investigator conducting the test will help to guide the interpretations.

Reference toxicant tests should also be used during Phases II and III to assess the quality of the test organisms and test procedures. A standard reference toxicant can be used until the substance responsible for toxicity has been identified. Once identified (and if it is feasible), the suspected toxicant should be used as the reference toxicant for the TIE tests.

2.5.2 Test Species

The US EPA recommend that all tests be conducted with the species that originally triggered the TIE. However, if an alternative species was selected, tests conducted in Phases II and III must demonstrate that the original and alternative species are responding to the same toxicant. Several methods can be used to demonstrate that both species are responding to the same toxicant. First, several samples of effluent can be tested over time using both species. If the LC50s change proportionally, then it can be assumed the organisms are responding to the same toxicants. Second, if the toxicant is the same for both species, the same characterization manipulations should alter toxicity to both species. Phase III approaches will confirm if the two species are sensitive to the same toxicant. However, it was noted that significant time and resources will have been wasted if the organisms are responding to different toxicants.

2.5.3 Sampling Collection and Handling

The type of sample (i.e., grab versus composite) used during Phase II should be the same as that used during Phase I. One composite or one grab sample should be used for both Phases II and III. Once the substance responsible is identified, multiple samples may be analzyed for the toxicant. Sample toxicity must also be tracked during the Phase II TIE to assess if the substance responsible has degraded over time. Tighter concentration intervals may be required for Phases II and III toxicity tests in order to detect smaller incremental changes in toxicity.

In some cases, the use of a single sample for Phase I treatments may reduce the volume of effluent available for subsequent tests. If the available effluent is limited, Phase II treatments may only involve assessment of the presence or absence of toxicity (i.e., the degree of toxicity is not measured). Effluent dilutions are used once the toxicant is identified.

2.5.4 Treatments and Test Procedures

The identification schemes for non-polar organics, ammonia, metals, chlorine and filterable substances are summarized in the following sections.

Non-polar Organic Compounds

This section of the document describes procedures for identification of non-polar organics based on the separation of toxic and non-toxic fractions of a whole effluent. It is assumed that during Phase I, the C18 SPE reduced or eliminated toxicity and elution of the column with methanol recovered toxicity.

The general procedure involves removal of the non-polar organics by a C18 column. The substances are then selectively removed by eluting the column with varying solvent/water mixtures that are increasingly less polar. The fractions are tested to determine toxicity. The toxic fractions are further concentrated, followed by a secondary fractionation using a reverse phase HPLC (high performance liquid chromatography) column, and tested again to determine toxicity. The toxic HPLC fractions are concentrated on a C18 column, tested for toxicity and analyzed using GC/MS (gas

chromatography / mass spectrometry). Estimated concentrations of the suspected toxicants are then compared to literature toxicity data.

If this process points to specific toxicants, the testing can proceed to Phase III (i.e., mass balance testing). If a toxicant is not suspected, a longer analysis time on the HPLC may help by increasing the separation time between toxic and non-toxic fractions. It may also be useful to increase the concentration factor, by increasing the volume of effluent passed through the C18 column. Other methods of analysis (i.e., LC / MS, direct probe mass spectrometry) may be required if no toxicants are identified using GC / MS.

Ammonia

This section of the document describes procedures for the identification of ammonia as the substance responsible for toxicity. It is assumed that the Phase I manipulations and measured concentrations implicated ammonia as the main toxicant. It was noted that other pH-dependent substances might be implicated and lead to confounding results. However, the Phase II tests are designed to help distinguish toxicity due to ammonia from toxicity resulting from other pH-dependent substances.

During Phase II, three procedures (in addition to measurement of ammonia in the effluent) are used to identify ammonia as the cause of effluent toxicity: 1) graduated pH testing, 2) testing using zeolite resin to remove ammonia, and 3) air-stripping of ammonia from the effluent at high pH (i.e., pH 11).

Graduated pH Test

The objective of the graduated pH test is to provide additional definitive evidence that ammonia is the substance responsible for effluent toxicity. The document provided three recommended approaches for conducting the graduated pH test; 1) acid / base adjustments, 2) CO_2 adjustments and 3) Buffer pH adjustments. Results using two different methods of pH control should be similar and used as the basis for identifying ammonia as the substance responsible for toxicity.

Zeolite Resin Method

Phase II testing can involve the use of zeolite to selectively remove ammonia from the effluent. Zeolites are crystalline aluminosilicates, which exhibit high selectivity for ammonia, but can also remove some heavy metals (Sherman, 1978). When placed in water, the positively charged group of the zeolites are mobile and can undergo exchange with other cations in water.

The general approach involves rinsing the zeolite resin with high purity water followed by dilution water. A portion of dilution water (blank) should be collected for toxicity testing. The 100% effluent is passed through next and tested to determine toxicity. Samples of effluent should be analyzed for ammonia before and after treatment. Temperature and pH should be documented at the start of testing in order to calculate un-ionized ammonia concentrations. Ammonia removal efficiency will be affected by column packing, effluent pH, ammonia concentrations, and flow rate. Confirmation tests involve spiking the post-zeolite sample with ammonia at concentrations similar to those observed in the untreated effluent (Phase III).

Air-Stripping of Ammonia

Air-stripping of ammonia from an effluent sample takes advantage of the fact that relatively volatile un-ionized ammonia is present in solutions with pH greater than 9.3. This method requires stirring of the sample at high pH for an extended period (i.e., >1 hour) in a container with a large surface area to volume ratio. Ammonia will be strongly suspected as contributing to toxicity, if ammonia and toxicity are both

reduced after air-stripping. These results should be compared with other Phase I tests and graduated pH tests, since other substances could precipitate (and not re-dissolve) after air-stripping.

Metals

Initial evidence that metals are the cause of toxicity comes from the EDTA tests conducted during Phase I. Other Phase I tests that may suggest toxicity due to metals include the sodium thiosulfate test, pH adjustment/filtration test, and pH adjustment/C18 test. Atypical dose-responses may also be observed when metals are present in the effluent. Additional Phase I tests with anion or cation exchange resins may also suggest metals are the causative toxicant.

This section of the Phase II document should be used if the EDTA test demonstrated a removal or reduction in effluent toxicity. Additional test information may be required if other Phase I tests suggested toxicity due to metals, but EDTA did not. Professional judgement and experience is required to determine if sufficient evidence has been gathered to justify proceeding to metals analysis.

Prioritization

After conducting tests with one sample, a list of suspected metals should be developed. Prioritizing metals for analysis will be of particular use when AA (atomic absorption) is used, since each metal is analyzed individually. Prioritization is less important with ICP (inductively coupled plasma), since numerous metals can be measured simultaneously. A list of metals and method detection limits (MDLs) are required for both methods.

The document also provided a prioritization scheme based on acute toxicity data for *Ceriodaphnia dubia*. However, at the time of publication, its applicability to other test species had not been determined.

Metal Analysis Methods

Three types of instrumentation were described for metals analysis: 1) AA (atomic absorption), 2) ICP-AES (inductively-coupled plasma-atomic emission spectroscopy, and 3) ICP-MS (inductively-coupled plasma-mass spectrometry). The MDLs selected for analysis will depend on the toxicity of the metals for the TIE species (if available). The document provides estimated MDLs for selected metals using AA, ICP-AES and ICP-MS.

Metal Speciation

Determining metal speciation may be important in the TIE, since the toxicities are different for various forms of a metal. For example, hexavalent chromium (Cr^{6+}) is more toxic than trivalent chromium (Cr^{3+}). Methods are available for the measurement of Cr^{6+}, while Cr^{3+} is determined by the difference between total and Cr^{6+}. Similar examples were also provided for arsenic and selenium.

Identification of Suspected Metal Toxicants

The initial step in the identification of metals as the suspected toxicant includes comparison of total (and if necessary, dissolved and suspended) metal concentrations to effluent toxicity. The suspect metal concentrations are also compared to literature data. Toxicity tests conducted under water quality conditions similar to that observed in the effluent (i.e., pH, hardness, TOC, TSS) may be required if toxicity data is not readily available for the metal of concern.

If a sample is to be filtered, a membrane filter (0.45 micron) should be properly prepared (i.e., rinsed with high purity water, followed by dilution water for a blank) prior to filtering of the effluent sample. The filtered sample (and dilution water) is then analyzed for metals and tested to determine toxicity. A

sample of the filtered high purity rinse water should also be analyzed for possible metals contamination from the filter or filter apparatus. If toxicity is removed, analysis of the filter for metals may be helpful in data interpretation.

Dissolved metals have been defined as those that pass through a 0.45 micron filter, but should not be considered synonymous with biologically-available metals. The document also indicates that other than the use of an aquatic organism, methods for determining the bioavailable fraction of a metal are limited. Furthermore, only rudimentary methods are available for identifying the individual species of a metal, organic complexes and chelates, metal species bound to high molecular weight organic material or metal species in the form of dispersed colloids. Stumm and Morgan (1981) were referenced by the authors as a source for general methods for identifying individual metal species.

The effect of variable water quality characteristics on metals toxicity must also be evaluated on effluent samples collected over time. Several samples could be collected and tested over a short time period (i.e., 24 hours) to generate data that is less influenced by TOC, hardness and TSS (assuming that water quality characteristics will vary less during a short period). However, metal concentrations must vary substantially enough to provide a sufficient range to make it appropriate for correlation analysis.

Additional Toxicity Testing Methods

The additional manipulations used during Phase II to identify metals as the cause of toxicity include: EDTA, sodium thiosulfate, graduated pH, and ion exchange tests.

EDTA Addition Test

During Phase I, varying amounts of EDTA and sodium thiosulfate are added to a single concentration of effluent. The Phase II confirmation tests are conducted with effluent dilutions to provide further evidence supporting metals as the cause of toxicity. The US EPA provided an example of how the data generated might be interpreted for *Ceriodaphnia dubia*. Toxicity of both copper and zinc would be removed by the addition of EDTA, while only copper toxicity would be removed by the addition of sodium thiosulfate. Furthermore, copper is more toxic at high pH, while zinc is not. Depending on the outcome of these treatments, one of these metals could be eliminated as the potential cause of toxicity.

Further evidence supporting a suspect metal as the toxicant can be generated by conducting simultaneous EDTA addition tests on effluent and the suspected metal(s) in dilution water. If the results are similar, the suspect metal is likely the substance responsible for toxicity. If the dilution water is non-toxic, but the effluent is toxic, either the metal is not responsible for toxicity, or there are strong matrix effects from the effluent. It is also noted that EDTA could reduce toxicity due to some cationic surfactants.

Sodium Thiosulfate Addition Test

If Phase I tests suggested metals may be the cause of toxicity, but the sodium thiosulfate test alone did not reduce toxicity, the use of SO_2 followed by sodium thiosulfate addition is recommended as an additional test. The presence of non-toxic oxidants in the effluent may cause a reduction in the thiosulfate available to complex with toxic metals. The addition of SO_2 may reduce non-toxic oxidants, allowing the thiosulfate to complex with the toxic metal.

As with EDTA, additional manipulations with sodium thiosulfate should also include tests with effluent and the suspected metal(s) in dilution water. These results, in combination with analytical measurements, the EDTA, graduated pH and ion exchange tests can provide sufficient evidence to proceed to Phase III confirmation testing.

Graduated pH Tests

The graduated pH test is performed during Phase I to evaluate the presence of pH-dependent toxicants. The US EPA recommend that for samples where toxicity is enhanced at elevated pH, the Phase II tests focus on ammonia. However, several TIEs conducted by other researchers (Schubauer-Berigan *et al.* 1993) suggested that toxicity caused by metals could be affected by pH within the range of 6 to 9. This research showed that Zn, Ni and Cd exhibited greater toxicity to a variety of species at elevated pH, while Cu and Pb exhibited greater toxicity at pH 6.2. These types of pH-dependent responses could be used to identify and confirm toxicity caused by these metals.

Ion-Exchange Test

Both cation and anion exchange resins have been used as part of Phase II TIEs (Doi and Grothe, 1989; Phase II zeolite test). For cation exchange resins, removal of toxic cations (e.g., Cd^{2+}, Pb^{2+}) occurs with release of cations such as H^+ and Na^+ into solution. For anion exchange resins, removal of toxic anions (e.g., $Cr_2O_7^{2-}$, AsO_4^{2-}) occurs with release of anions such as OH^- and Cl^-. The exchange process is concentration-dependent and reversible; therefore, ions can also be recovered from the column.

The US EPA had little specific experience with ion exchange columns. However, general guidance and information for conducting tests with ion exchange resins was provided in the document and is summarized below:

- Ion exchange resins remove a wide range of cations and anions; they are not chemical specific.
- Wide changes in the pH of the treated effluent can occur.
- Many ion exchange resins are based on styrene or acrylic divinylbenzene backbone, which can cause the removal of other toxicants (i.e., organics).
- Preparation methods differ according to resin type.
- The key to obtaining useable data from an ion exchange test is to obtain non-toxic blanks.
- The volume of effluent that can be treated will depend on effluent hardness, bed volume of the column, strength and type of ion exchange resin, effluent toxicity, and test species.
- General effluent treatment procedures involve column preparation, followed by a rinse with high purity water. Dilution water is passed through the column, with sub-samples collected for testing. The effluent is then passed through the column and sub-samples collected for testing. Effluent pH must be checked and readjusted if necessary.
- It should be possible to recover cations and anions from the column by elution with a strong acid or strong base.
- If toxicity is not removed, and the blanks are non-toxic, it is likely that the toxicant is not a cation or anion.
- If toxicity is removed, and the blanks are non-toxic, it cannot be concluded that the toxicant is a cation or anion, since other substances may have been removed by the resin. This information should be supported by the use of other manipulations to remove metals (e.g., EDTA)

Chlorine

Measurement of total residual chlorine (TRC) should be conducted as soon as the sample is received. The measurements of TRC combined with the sodium thiosulfate treatment will provide data to support the need for additional investigations to identify chlorine as the cause of toxicity. However, the US EPA indicate that the Phase I results do not confirm that chlorine is responsible for toxicity, since sodium thiosulfate can remove other oxidants and some metals.

If TRC is not detected in the sample, chlorine is not a suspected toxicant. If TRC is detected, attempts should be made to collect samples prior to, and immediately subsequent to chlorination. The TRC in most effluents degrades in 2 to 5 days in samples stored at 4 EC. Therefore, baseline tests should be set at pre-determined intervals to evaluate if toxicity is degrading. TRC measurements should also be conducted on each baseline test. If chlorine is a toxicant, then toxicity should decrease in the post-chlorinated sample. After the decay of TRC, the post- and pre-chlorinated sample toxicities should be similar.

The document indicates that the various forms of chlorine (i.e., free aqueous chlorine, hypochlorous acid, hypochlorite ion, chloramines) can have different toxic impacts on different species, and the effect levels are not well defined. Furthermore, analytical methods are not chlorine specific (other oxidizing substances can be quantified as chlorine). Therefore, it might also be necessary to generate additional toxicity data using dilution water and the TIE test species.

Identifying Toxicants Removed by Filtration

If the Phase I results indicated that the toxicant can be removed by filtration, a careful evaluation of all Phase I treatments should be undertaken before trying to recover and identify the material retained by the filter.

If filtration was the only treatment successful at reducing or eliminating toxicity, other filter types (i.e., nylon, Teflon) should be used to help determine if the filter type influenced removal of toxicity. Centrifugation of the sample is another recommended option for toxicants removed by filtration.

Attempts should also be made to extract, concentrate and identify the substance(s) retained on the filter. The first step involves passing a known volume of effluent through a filter or series of filters (the volume of sample passed should be recorded in order to calculate concentration values). The second step is to extract the substances retained on the filter using a solvent (i.e., methanol, methylene chloride, pH 3 high purity water). A blank filter should also be prepared and treated identically to the sample filters. The filter extracts are then concentrated and tested at a concentration similar to that of the original sample. Additional Phase I tests can be used to characterize the toxicant in the filter extract, followed by analysis using Phase II methods.

2.5.5 Interpretation of Test Results

The document states that interpretation of TIE results is often different from the "standard" acceptance or rejection of a hypothesis. The TIE will usually allow the hypothesis to be accepted, but not rejected. The US EPA provide an example with ammonia as the suspected cause of toxicity. If ammonia is removed by zeolite and the treated effluent is still toxic, it can be concluded that other toxicants are also present. However, if the treated effluent is non-toxic, it cannot be concluded that ammonia is the only toxicant, since zeolite may have removed other substances in addition to ammonia.

The presence of multiple toxicants complicates the interpretation of TIE results. The US EPA recommend focusing on the toxicant that appears easiest to identify. This toxicant will usually involve one that can be

separated from the whole effluent, and which can be measured using readily available analytical techniques.

Based on the US EPA's experience in conducting Phase II TIEs, effluent toxicants were found to not always be additive, which can also complicate interpretation. Minor or secondary toxicants might not be revealed until the primary toxicant has been removed from the effluent. Furthermore, at least one toxicant must be identified before additivity can be established.

2.6 Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity

(U.S. EPA. 1993b. Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. EPA-600/R-92/081)

The original U.S. EPA Phase III document was published in 1989, and covered Phase III approaches for acute toxicity only. The revised Phase III document (1993) provides confirmation procedures for substances causing either acute or chronic toxicity. For the purposes of this study, only the acute toxicity portion of the 1993 document is summarized.

The objectives in Phase III are to: i) confirm that the substances responsible for toxicity have been correctly identified, and ii) ensure that all of the toxicity has been accounted for. A "weight-of-evidence" approach is used during Phase III to confirm that the substances responsible for toxicity have been identified.

The procedures used during Phase III may include: 1) correlation analyses, 2) observations of symptoms, 3) relative species sensitivity, 4) spiking, 5) mass balance, and 6) various adjustments to water quality. An overview of the approaches described in the Phase III document is provided in Table 6 and is described in further detail in the following section.

Table 6. Overview of Phase III approach

Correlation Approach	• The objective is to determine if there is a consistent relationship between the concentration of the suspected toxicant(s) and effluent toxicity. • A wide range of toxicity responses with several samples must be obtained in order to provide an adequate range of effect concentrations for the regression analysis. • Two key problems associated with the correlation approach are; 1) lack of additivity requires careful data analysis, and 2) correlation analysis is difficult when matrix effects are present.
Symptom Approach	• The approach involves the use of test organism behaviour and time to death in comparing the responses of organisms to the whole effluent and then to the suspected toxicant(s). • Different toxicants could produce similar or different symptoms in a test species. If symptoms are different, the toxicants are unquestionably different, but similar symptoms could indicate the toxicants are the same or different. • If organisms exposed to the effluent and the suspected toxicant display different symptoms, the substance thought to be responsible for toxicity is either not the actual toxicant, or is not the only one present.
Species Sensitivity Approach	• If the suspected toxicant(s) has been correctly identified, effluent samples with different LC50s for one species should have the same ratio for a second species

Table 6. Overview of Phase III approach	
	with different sensitivity. • When two or more species exhibit different sensitivities to the suspected toxicant during single chemical testing, and the same pattern is observed in the whole effluent, this provides supporting evidence that the chemical tested is the cause of effluent toxicity.
Spiking Approach	• In spiking tests, the concentration of the suspected toxicant(s) can be increased in the sample to determine if toxicity increases proportionally to an increase in concentration. • The suspected toxicant could also be added to a non-toxic sample, to dilution water or to a sample of effluent where the suspected toxicant(s) has been removed. • Matrix effects, toxicant solubility, and equilibrium time could all impact upon the outcome of spiking experiments.
Mass Balance Approach	• The mass balance approach is used when the toxicant(s) can be effectively removed from the effluent and subsequently recovered. The document provided a detailed example of the mass balance approach for C18 SPE fractions. The authors indicate that the approach would be similar for other toxicants that could be removed from the effluent. However, they had little experience with other applications of the mass balance approach.
Deletion Approach	• The deletion approach involves removal of the suspected toxicant(s) from a waste stream. The suspected toxicants are removed for a short period and the effluent is tested. • This approach offers the strongest evidence that the suspected toxicants identified are the correct ones.
Additional Approaches	• Manipulation of pH, hardness. • Measurements of body uptake, to assess bioavailability. • Combined Phase I characterizations. • Effluent simulations to confirm toxicity due to TDS.
Hidden Toxicants	• Additional guidance on the identification of hidden toxicants was provided, since this is one the most difficult aspects of confirmation testing. • The best approach to identify hidden toxicants involve methods that alter the effluent the least, can remove and recover the toxicant, and are specific for a few toxicants. • Hidden toxicants are most difficult to identify when ammonia is the primary toxicant. • To ensure that all toxicants have been accounted for, thorough confirmation testing must be conducted, and include consideration of non-additive toxicity and seasonal changes.
When Treatability Approach Is Used	• Various treatment methods are used and assessed to determine which would remove toxicity without identification of the specific toxicant. • Samples must be tested repeatedly over a sufficient period of time (using the species that triggered the TIE) to ensure that a full range of effluent conditions are evaluated.

The US EPA reported that not all of the approaches described in the Phase III document need to be used with every effluent. Additional approaches may need to be developed and applied. The choice, sequence and number of methods used will depend on the specific effluent, the quality and reliability of results generated from each approach, and the experience of the investigator. However, the completeness of Phase III testing will determine the reliability of the conclusions. The amount of confidence in the confirmation tests that is required will depend on the importance of the decision that will be based on the results. For example, a higher degree of certainty is required if potentially costly treatment modifications are to be implemented.

Phases I, II and III usually occur sequentially, but could be conducted concurrently depending on the test results. If definitive results were generated during the Phase I TIE, time and resources could be conserved if all Phases could be performed on one sample. Earlier documents suggest that multiple Phase I characterizations should be performed on several samples before proceeding to Phase II and III. However, the revised Phase III document (1993) states that initiation of Phases II and III earlier in the process could be useful, particularly when regulatory agencies require immediate action on the first exceedance of toxicity.

If all Phases of the TIE were conducted on a single sample, confirmation testing on several samples would still be required, since it could not be assumed that the substances responsible for toxicity were the same among samples. If samples have been collected over several months, confirmation testing may need to include seasonal samples. Furthermore, if potentially costly treatment changes are to be implemented, there would need to be some certainty that toxicity from a specific substance is consistently present and that all toxicants have been identified. Treatment modifications may differ when toxicity is caused by a variety of toxicants at different concentrations, versus one single consistent toxicant.

Differences in results among samples could make data interpretation more difficult and less meaningful in Phase III. For example, if one sample is significantly different from the others, correlation analysis could be less meaningful. However, if it could be demonstrated (using Phase I and II techniques) that a different toxicant was responsible for toxicity, that particular data point could be eliminated from the analysis.

In the case of multiple toxicants, the US EPA indicate that (from their experience) toxicants are not typically additive or the toxicant present at lower concentrations may not be easily detected. Additive toxicity can be defined as the toxicity of a mixture of chemicals that is approximately equal to that expected from a summation of the known toxicities of the individual chemicals present in the mixture (Rand, 1995). Detection of hidden toxicants (i.e., those that do not express their toxicity because of the presence of a second toxicant) is one of the most difficult aspects of confirmation testing. Comparisons of chemical concentrations to toxicity values obtained from the literature cannot be used on their own to confirm causes of effluent toxicity, since either the hidden toxicant or primary toxicant could be missed due to matrix effects in the effluent sample.

All TIE results should be explainable at the completion of Phase III. If some results do not make sense, then it is likely that a toxicant has been missed or identified incorrectly, and the confirmation stage is incomplete. The likelihood of this happening can be reduced by participation of investigators with a broad knowledge of chemistry and toxicology, as well as the ability to logically synthesize the information generated.

Quality Assurance (QA) and Quality Control (QC) measured used during Phase III must be suitable to provide reproducible and defensible results. Modifications to the toxicity test methods must be avoided in order to provide definitive data. The effluent test protocols that triggered the TIE investigation should be followed, with particular attention to test conditions, replication, test organism quality, representativeness of effluent sample tested, and analytical procedures.

2.6.1 Correlation Approach

The objective of the correlation approach is to determine whether there is a consistent relationship between the concentration of the suspected toxicant(s) and effluent toxicity. For this approach to be effective, a wide range of toxicity responses with several samples must be obtained in order to provide an adequate range of effect concentrations for the regression analysis.

The first step in the correlation approach requires that the toxicity data (i.e., LC50s) be transformed into toxic units (TUs) in order to evaluate whether a linear relationship exists. Effluent TUs are obtained by dividing 100% by the LC50. The suspect toxicant concentration is converted to TUs by dividing the measured toxicant concentration by the LC50 for that toxicant. If more than one toxicant is present, the concentration of each one is divided by the respective LC50 and the TUs can then be summed (under direct additivity assumption). The higher the TU, the greater the acute toxicity.

The correlations conducted by the US EPA typically involved data from toxicity tests without manipulations, in combination with measured chemical concentrations of the suspected toxicant(s). In cases where toxicity is marginal (i.e., LC50>100%) or sample toxicity does not vary substantially, effluent manipulations could be used to change toxicity so that the data could be used with the correlation approach. Examples of when effluent manipulations could be used in Phase III include altering of pH (with an acceptable physiological range), to change the toxicity of ammonia. Similarly, toxicity could be altered for some metals, by changing the pH and hardness.

Two examples of correlation analysis were provided where the suspected toxicant was a pesticide. Some of the key issues from these examples are summarized below.

- Independent variables (chemical TUs) are plotted on the x-axis.
- Dependent variables (effluent TUs) are plotted on the y-axis.
- r^2 values should be calculated to provide a measure of how much the observed effluent toxicity is correlated to the measured toxicant.
- Less confidence can be placed on the slope and intercept of the regression line at lower r^2 values.
- Scatter about the regression line should be evaluated by setting lower limits on the r^2 value.
- The minimum r^2 value to be chosen should be based on the consequences of the decision. An r^2 of 0.8 was recommended where minimal chance of an incorrect decision was required.
- A lower r^2 value (e.g., 0.6) could be used when an increased risk of an incorrect decision is acceptable.

There are two key problems associated with the correlation approach. First, the lack of additivity requires careful data analysis. Second, correlation analysis is difficult when matrix effects are present. Matrix effects can vary among samples and there are no standard analyses for measuring the biologically available form of the toxicant.

Interpretation of the data must take into consideration whether or not the toxicants are additive, or if hidden toxicants are present. In either case, the data must be interpreted as if the toxicants are non-additive. Summing toxicants that are non-additive could increase the error associated with the regression analysis. Secondary toxicants should not be included in the data set, when they are present in concentrations that will not contribute to the effect concentration. The error could be large or even negate the correlation significance, if the relative amounts of toxicants vary among samples and minor TUs are included in the analysis. It is recommended that the data be evaluated in regression plots to assess the significance of the contribution of the secondary toxicant, particularly if the toxicants appear to be additive.

Problems with correlation analyses may also be encountered when matrix effects occur. Matrix effects occur when toxicants interact with other effluent constituents in ways that alter their toxicity. Matrix effects fit into one of two categories. The first is when toxicants change form, such that they exhibit a different toxicity. One example is ammonia, which increases in toxicity as pH increases. A second example is HCN, which increases in toxicity as pH decreases. The second category is when the toxicant undergoes a physical change (i.e., sorption or binding to particulates) making the toxicant unavailable to the organism. The biological characteristics of the organism may also change the availability of the toxicant. For example, a particulate-bound toxicant may be unavailable to fish, but readily available to daphnids as the particulates are ingested via filter feeding. The key problem in dealing with both types of matrix effects is the difficulty associated with measuring the biologically available portion of the toxic substance.

The authors indicate that removal of the toxicant from the effluent, and subsequent use the effluent as dilution water may appear to be a useful way to evaluate matrix effects. However, there is virtually no way to remove a specific substance from the effluent and ensure that other characteristics have remained unchanged. Furthermore, even small changes in toxicant form or availability could affect the comparisons of effluent toxicity to measured concentrations of the toxicant. Therefore, manipulations must be minimized when correlation analyses are conducted.

For effluent samples that lose their toxicity over time, both the non-toxic effluent and dilution water could be spiked with the suspected toxicant(s) to clarify matrix effects. Substantially different test results would reflect matrix effects. If toxicity is persistent, separate correlations could be developed by spiking the toxicant into two different effluent samples with different toxicant concentrations.

The US EPA note that the correlation approach could be difficult to apply with metals, since the toxicity of metals is very matrix dependent. In dealing with toxicity due to metals, the first step must involve the identification of matrix effects on a specific metal. The specific metal(s) should be tested in dilution water and subjected to the Phase I and II procedures. Once the matrix effects are understood, the next step involves determining if the effluent toxicant behaviour is consistent with the matrix effects for the specific metal.

The effect concentration will also influence the impact of matrix effects on the toxicant. For highly toxic effluents (e.g., LC50<10%), the exposure solutions will closely resemble the dilution water matrix. In comparison, matrix effects will closely resemble the effluent in samples with LC50s in the range of 50 to 100%. Therefore, the importance of effluent matrix effects decreases as the sample becomes more toxic.

2.6.2 Symptom Approach

The symptom approach involves the use of test organism behaviour and time to death in comparing the responses of organisms to the whole effluent and then to the suspected toxicant(s).

Different toxicants could produce similar or different symptoms in a test species. If symptoms are different, the toxicants are unquestionably different. However, similar symptoms could indicate that the toxicants are the same or different. If organisms exposed to the effluent and the suspected toxicant display different symptoms, the substance thought to be responsible for toxicity is either not the actual toxicant, or is not the only one present.

Behaviour monitoring of fish or cladocerans should be documented throughout the Phase I and II tests, since the information gathered could be used during the confirmation stage. The observations made should cover the entire exposure period, since distinct symptoms may be displayed at different stages of the test.

Symptoms may also change with exposure concentration. Therefore, comparisons should be made at exposure concentrations that have similar TUs, so that both the effluent and pure substance can be compared at the same toxicity level.

The use of multiple test species may also provide additional symptom information, since organisms respond to toxicants differently. For this approach to be useful, all comparisons must be made at equitoxic concentrations.

2.6.3 Species Sensitivity Approach

Differences in species sensitivity can be used to provide further evidence as to the cause of effluent toxicity. If the suspected toxicant(s) has been correctly identified, effluent samples with different LC50s for one species should have the same ratio for a second species with different sensitivity. The same ratio of LC50s for two species implies the same toxicant in both effluent samples. Similarly, when two or more species exhibit different sensitivities to the suspected toxicant during single chemical testing, and the same pattern is observed in the whole effluent, this provides supporting evidence that the chemical tested is the cause of effluent toxicity (Ankley and Schubauer-Berigan, 1995).

Comparisons of species sensitivity may also provide a warning that the suspected toxicant may not be the cause of toxicity. If the species that triggered the TIE and the test species are responding to different toxicants, the investigator should review the Phase I and II results. The characterization may have to be repeated to identify the additional toxicants.

2.6.4 Spiking Approach

In spiking tests, the concentration of the suspected toxicant(s) can be increased in the sample to determine if toxicity increases proportionally to an increase in concentration. If this occurs, supporting evidence that the suspected toxicant is in fact the cause of effluent toxicity is gained. It was also indicated that this conclusion might not be true, if an unidentified toxicant has a toxicity similar to the suspected toxicant, and if both substances were strictly additive. However, the probability of this occurrence was thought to be small.

Alternatively, the suspected toxicant (in pure form) could be added to a non-toxic sample, to dilution water or to a sample of effluent where the suspected toxicant(s) has been removed. In the case of dilution water, it will not be possible to simulate all of the effluent characteristics. Although certain

characteristics, such as hardness, can be easily simulated, others (e.g., TOC, SS) are more difficult to simulate.

Spiking tests that involve the removal of the suspected toxicant must be interpreted cautiously, since other toxicants could also be removed from the effluent at the same time. The document provided an example using zeolite and the removal of ammonia. Zeolite can be used to remove ammonia from an effluent sample. If toxicity is restored in the post-zeolite treated effluent by spiking with ammonia, then it can be concluded that there is sufficient ammonia in the sample to cause toxicity. However, it cannot be concluded that ammonia alone is the cause of toxicity, since zeolite can remove other toxicants (including metals).

Matrix effects, toxicant solubility, and equilibrium time can all impact upon the outcome of the spiking experiments. The authors indicate that for most chemicals (in a relatively simple system, such as reconstituted soft water), doubling of the chemical concentration should double the toxicity. However, the toxic outcome will be more difficult to predict or explain, if there are effects from effluent characteristics (i.e., SS) or the toxicant is near its solubility. Furthermore, after addition of the suspected toxicant, equilibrium might not be established prior to addition of the test organisms or even during the test period.

As with the symptom and species sensitivity approaches, these tests are not conclusive, but rather provide supporting evidence that the suspected toxicant is in fact the cause of effluent toxicity.

2.6.5 Mass Balance Approach

The mass balance approach is used when the toxicant(s) can be effectively removed from the effluent and subsequently recovered. The document provides a detailed example of the mass balance approach for C18 SPE fractions. The authors indicate that the approach would be similar for other toxicants that could be removed from the effluent. However, they had little experience with other applications of the mass balance approach. The C18 example is summarized in the following section.

The first step involves passing the effluent through a C18 column to remove toxicity, followed by elution of the column with varying concentrations of methanol and testing of the fractions. "Add-back" tests are then conducted to determine whether all of the toxicity in the original sample was accounted for in the SPE fractions. The "add-back" tests consist of three separate trials: 1) all-fraction test, 2) toxic-fraction test and 3) non-toxic fraction test. Each fraction is added to dilution water or the post-C18 treated effluent (non-toxic effluent). The fractions are added back such that each is present at the original effluent concentration. This mass balance approach should identify whether or not the toxicity in the toxic-fraction test equals the effluent toxicity. If all toxicity is observed in the toxic-fraction test, the all-fraction test and the toxic-fraction test results should be the same as in the untreated effluent. Toxicity should not be observed in the non-toxic fraction test.

Several problems or difficulties with this approach were discussed by the US EPA. First, toxicity may be observed in the post-C18 treated effluent, that will not allow the mass balance approach to be used on its own. In this case, the add-back tests should be conducted using dilution water and compared to tests conducted with the post-C18 treated effluent. If a residual toxicant is present in the post-C18 treated effluent, the toxic-fraction and all-fraction test should show more toxicity in effluent than when added to dilution water. Second, bacterial or fungal growth may be observed in the add-back tests. The US EPA found that the conditioning of the column with acetonitrile helped to alleviate biological growth. Lastly, the results need to be interpreted cautiously when toxicity is found in more than one SPE fraction. In this

instance, toxicity of the individual fractions should not be expected to equal the toxicity of the untreated effluent, because the toxicants might not be additive or some toxicity that cannot be detected in the individual fractions might add to the whole effluent toxicity.

2.6.6 Deletion Approach

The deletion approach involves removal of the suspected toxicant(s) from a waste stream. The suspected toxicant(s) is removed for a short period and the effluent is then tested for toxicity. This approach offers the strongest evidence that the suspected toxicants are the correct ones. However, the US EPA indicate that care must be taken to ensure that other substances are not removed, or that another effluent characteristic (i.e., pH) does not change.

2.6.7 Additional Approaches

This section of the document includes an overview of other steps that can be used to confirm the cause of toxicity. The additional approaches were:

- Manipulation of pH, hardness
- Measurements of body uptake, to assess bioavailability
- Combined Phase I characterizations
- Effluent simulations to confirm toxicity due to total dissolved solids (TDS)

2.6.8 Hidden Toxicants

The document also provides additional guidance on the identification of hidden toxicants, since this is one the most difficult aspects of confirmation testing. Two situations may produce the problem of hidden toxicants. In the first case, hidden toxicants may occur when unequal ratios of TUs for two toxicants are present in the effluent. In other words, because the effect concentration is measured by diluting the effluent, the substance of lesser toxicity (i.e., the toxicant present in fewer TUs in the 100% effluent) is so low at the effect concentration that its contribution cannot be detected (its contribution to toxicity may only be detected in the undiluted effluent). This problem could exist if the toxicants are additive or non-additive, but would not likely occur in only marginally toxic effluents (i.e., LC50 between 75 and 100%). In the second scenario, hidden toxicants may occur if the toxicants are non-additive or partially additive.

The US EPA suggest that the best approach to identifying hidden toxicants involved methods that alter the effluent the least, can remove and recover the toxicant, and are specific for a few toxicants. The US EPA also indicated that hidden toxicants would be most difficult to identify when ammonia was the primary toxicant. The following tests were recommended as a "weight-of-evidence" that ammonia was the only toxicant; zeolite, graduated pH, and air-stripping at pH 11. If these tests consistently implicated ammonia as the substance responsible for toxicity, then it was likely that hidden toxicants were not present in the effluent.

If the hidden toxicant was additive, but occurred in unequal ratios, then the confirmation process should focus on confirming the primary cause of toxicity. If the remedial action for the primary toxicant was simple (e.g., product substitution), the search for the hidden toxicants could also take place after removal of the primary toxicant.

To ensure that all toxicants have been fully accounted for, thorough confirmation testing must be conducted, and should include consideration of non-additive toxicity and seasonal changes.

2.6.9 Treatability Approach

After completion of the Phase I effluent characterization, three approaches could be used to remove toxicity: 1) toxicant identification, 2) source control, and 3) effluent treatability. In the case of effluent treatability, various treatment methods are used and assessed to determine which would remove toxicity without identification of the specific toxicant. However, confirmation is still required to ensure that the method selected consistently removes toxicity.

Samples must be tested repeatedly over a sufficient length of time to ensure a full range of effluent conditions are evaluated. Process changes, weather, seasonal changes and intermittent operations should be included during confirmation testing. It is also critical that the species that triggered the TIE be used during all testing.

2.7 Non-acutely lethal mining effluent technologies (NALMET) program (Beak International Inc. 2000. Non-Acutely Lethal Mining Effluent Technologies (NALMET) Program – 1999 Studies)

Environment Canada recently commissioned a study to demonstrate the use of toxicity identification evaluation (TIE) and toxicity treatment evaluation (TTE) on representative "challenged" mine effluents, as tools that may assist in achieving compliance with the new Metal Mining Effluent Regulation (MMER). The objectives were to: i) evaluate various approaches to TIE and TTE investigations and relevant studies applicable to Canadian mine effluent acute lethality situations in support of the NALMET program of Environment Canada, and ii) demonstrate a combined effluent TIE/TTE approach for acute toxicity challenged mines in Canada.

The authors provide a review of mine effluent treatment technologies based on the SENES (1999) report on technologies applicable to the management of mining effluents and a report on pilot studies to eliminate ammonia (Tan *et al*., 1995), as well as their own professional experience. The mine treatment technologies reviewed for the NALMET report are summarized in Table 7 (a detailed review of the SENES report is provided in a separate section of this literature review).

Table 7. Overview of mine effluent treatment technologies summarized in NALMET report.

Removal of Metals from Solution	Precipitation – common treatment practice which can be achieved by increasing pH. Solids can also be precipitated as sulphides using chemical or microbiologically generated sulphide. Oxidation state of metals will influence conditions for removal.
	Co-precipitation – common treatment practice for anions (i.e., arsenate, molybdate). Co-precipitation with hydroxides or carbonates is commonly achieved by addition of ferric chloride, ferric (or ferrous) sulphate, or alum. Process may require pH adjustment and can generate large quantities of solids. New technologies include electrocoagulation (EC).
	Adsorption – activated carbon, zeolite, synthetic absorbents or wetlands have application in treating some mine effluents. Requires small contaminant loading rates, high specific adsorption capacity, low adsorbent cost and practical long-term management of spent adsorbent.
	Ion Floatation – some applications are operating in the mining sector. Process uses floatation reagents that react with the surface solid particles to render minerals with hydrophobic surfaces.

Table 7. Overview of mine effluent treatment technologies summarized in NALMET report.	
	Coagulation – no mining application currently exists for this technology. Process applies to separation of fine particles from the effluent stream. Solids that are formed must be separated from bulk volume of treated water for discharge. Difficulty in achieving separation is related to size and density of particles.
Removal of Nitrogen-based Compounds	Cyanide – destroyed or removed by oxidation. Oxidation by sulphur dioxide / air, hydrogen peroxide, ozone or alkali chlorination is the most widely used destruction method. Cyanide toxicity problem is often replaced by ammonia or copper toxicity problem (but both can be resolved by additional treatment and careful control in cyanide destruction). Acidification-volatilization-reneutralization (AVR) is the only widespread method of cyanide recovery. Other methods for removal involve the reaction of cyanide on insoluble iron compounds, biological treatment, and natural degradation.
	Ammonia – can result from incomplete oxidation of cyanide or from the use of nitrogen-based explosives. Common treatment approaches include pH control and biological removal of nitrogen compounds by nitrification and denitrification. Wetlands have also been used.
Adjustment of Effluent to Detoxify	Adjustment of pH – increases in pH is achieved most economically with lime. Decreasing pH is often achieved by addition of sulphuric acid or carbon dioxide.
	Complexing of Ionic Metal Forms – one mine in Canada reported the addition of EDTA to its treated effluent as a cost-effective method to complex copper.
Separation of Precipitated Solids from Treated Liquids	Sedimentation – most widely practiced method of removal of precipitated solids. Problems associated with this method include accumulation of solids resulting in reduced effectiveness for storage and settling. Polymer coagulant and flocculent additions can be used when settling is poor or the sedimentation pond is undersized. Stored solids may result in re-introduction of metals into solution, or increased solids loading to the discharge.
	Filtration – few applications for mining wastewater (with the exception of passive infiltration) due to high cost. Limited to polishing filters on treatment clarifier overflows.
	Froth Floatation – widely used in mineral processing operations, and occasionally used in treating mine effluent. Methods include dissolved air floatation (DAF), induced air floatation and column floatation. Residence time is significantly shorter (minutes) compared to sedimentation (days).
Operational and Engineering Control of Treatment Systems	Flow – varies according to operation and season. Effective control systems for chemical addition in the effluent treatment plant (ETP) incorporate flow rates into the control package. Flow control by the addition of surge capacity to limit the range of flows to be treated is usually beneficial.
	Wastewater pH – generally adjusted by the addition of lime to precipitate metals (as their metal hydroxides), and by sulphuric acid or carbon dioxide to re-adjust to within regulatory requirements.
	Contaminant Load – may impact some wastewater treatment approaches (i.e., solids settling or sulphide precipitation).

Table 7. Overview of mine effluent treatment technologies summarized in NALMET report.	
	Management of Residuals – effective dewatering of metal precipitates determines the quantities of materials for long-term management.
Pollution Prevention Techniques	Techniques are mine-specific, but may include: - separation of contaminated waste streams into component streams containing specific contaminants that are more amenable to specific treatment technologies; - separation of relatively clean runoff entering tailings ponds from contaminated mine water or mill effluent sources; and, - alternation of the mining or milling technology, chemical reagents used, mass of blasting charges used, or mill process flow sheets to improve mining or milling efficiencies and simultaneously achieve environmental objectives.

2.7.1 Mine Selection

The authors base their selection on a database of over 60 mines (Andrews, 1999). Out of these 60 mines, 20 were identified as potential candidates that had experienced recent acute lethality problems. After direct contact with these mines, the authors found that most had resolved their acute lethality problem. However, two "challenged" mines were identified and used to develop a combined TIE/TTE approach. Both mines experienced acute lethality problems with rainbow trout and *Daphnia magna*.

Mine A was a gold mine, but also produced silver, copper and zinc. The mill operation consisted of cyanide leach for gold (with recovery for copper and zinc). The effluent treatment plant (ETP) consisted of natural degradation, followed by peroxide treatment to remove cyanide. Plans were in place to install an SO_2-air process for cyanide destruction.

Mine B was a nickel-cobalt mine, which produced a single concentrate that contained nickel, cobalt, copper and other precious metals. The major process chemical was soda ash (Na_2CO_3) that maintained an alkaline pH in the mill circuit. The final effluent was characterized by a pH of 9, and a total dissolved solids (TDS) level of approximately 6,000 mg/L.

2.7.2 Toxicity Identification Evaluation (TIE)

The authors indicate that the TIE treatments focussed mainly on Phase I characterization procedures, which acted as a guide for selection of candidate treatments (TTE). The report indicated that although rainbow trout was one of the species that triggered the TIE, fathead minnows were used as a surrogate test species. The authors report that fathead minnows were similar in sensitivity to most toxicants, and were more cost-effective than trout (based on effluent volume requirements for testing). TIEs for both mines started with a review of historical data, followed by one or more Phase I TIE investigations.

<u>Mine A</u>

Based on the historical data review, the authors suspected that ammonia, cyanate, DOC and copper might be causes of effluent toxicity. The effluent was also observed to be more toxic to *Daphnia magna* than to rainbow trout.

TIEs were conducted in December 1999 and February 2000. The Phase I treatments were based on the standard U.S. EPA procedures, but also included the use of zeolite (typically used during Phase II), an LC-SAX column (a quaternary amine bonded silica), and carbon column extraction. During both TIEs,

graduated pH testing indicated that the toxicant(s) were pH-sensitive. Increased acute lethality was observed at low and high pH, suggesting that more than one toxicant was potentially involved.

During the first TIE, no single treatment was successful in eliminating acute lethality to *Daphnia magna*, although a few treatments (aeration at pH 11, filtration at the initial effluent pH, post-filtration zeolite, and additions of EDTA and sodium thiosulphate) reduced toxicity. For fathead minnows, acute lethality was eliminated by adjustment to pH 11 and by post-filtration zeolite. Acute lethality to fathead minnows was reduced by adjustment to pH 3 and completely removed by aeration and filtration at pH 3.

During the second Phase I TIE, no treatment was successful in eliminating acute lethality to *Daphnia magna*. Filtration at pH_i (the initial pH of the effluent) and granulated activated carbon (GAC) were the only treatments observed to reduce toxicity. For fathead minnows, mortality was reduced after aeration of the sample at pH 3, filtration at pH 3 and 11, addition of EDTA, and after passage through a C18 column at pH 3. Passing the sample through GAC eliminated acute lethality to fathead minnows.

Chemical analyses were performed on both samples subjected to the Phase I TIE procedures. The data were used to compare the predicted toxicity of the effluent samples, based on the concentration of individual substances and their known toxicity, to the actual acute lethality test results of the initial untreated sample. The authors indicate that in the first sample, ammonia, copper and zinc were the most important toxicants for fathead minnows and *Daphnia magna*, with an additional toxicant (nitrite) suspected in explaining trout mortality. In the second sample, ammonia alone could have accounted for the observed rainbow trout mortality, but additional toxicants (cyanate, copper, nitrite) were suggested as possible contributors to *Daphnia magna* and fathead minnow mortality.

Based on the results of the TIE, the authors recommend that a TTE study be undertaken to investigate options for effluent treatment.

<u>Mine B</u>

Toxicity and corresponding chemistry data were limited for Mine B, and therefore preliminary correlations could not be conducted. Based on the limited data, the authors indicate that most metals concentrations (with the exception of occasional copper spikes) were below levels expected to cause acute lethality. However, thiosulphate concentrations were consistently above levels expected to cause toxicity to fathead minnows and *Daphnia magna*.

One TIE was conducted in January 2000. The Phase I treatments were based on the standard U.S. EPA procedures, but also included the use of zeolite. Graduated pH testing indicated that the toxicant(s) were pH-sensitive. Increased toxicity to *Daphnia magna* was observed at low pH, which was consistent with metal toxicity. No differences in toxicity to fathead minnows were observed at any pH tested. During the TIE, only zeolite was effective in removing acute lethality to both species.

As with Mine A, results from chemical analyses were compared to toxicity threshold data. A computer salinity model was also used to predict toxicity due to major ion concentrations. The results suggested that TDS (or the specific ions that make up the TDS) were the cause of toxicity to both species. Increased toxicity to *Daphnia magna* at low pH suggested the presence of a second toxicant, which may have been a metal. A third chemical (thiosulfate) was suggested as a causative toxicant for rainbow trout.

The authors did not recommend that a TTE study be initiated at this point, since changes to the treatment system were in progress. It was suggested that further TIE or TTE studies be considered after the changes were implemented.

Cost of TIE Investigations

The authors reported that the estimated cost for the TIE work conducted during this project was $20,000. This includes pre-TIE full chemical characterization of the effluent and definitive acute lethality testing and three Phase I TIEs. The Phase I TIEs includes toxicity characterization, project management, sample/test co-ordination, historical data review, report preparation and disbursements (e.g., shipping, communications, etc.).

2.7.3 Toxicity Treatment Evaluations (TTE)

The authors define a TTE as an investigation that involves the use of various effluent treatment technologies or a combination of technologies to assess the ability to reduce or eliminate elevated contaminants or acute lethality. Testing is typically conducted at the bench scale until a promising technology is identified.

For Mine A, two TTEs were conducted. Based on the results from Phase I, ammonia and metals were the targeted substances. Activated carbon (for metals removal) and zeolite (for ammonia removal) were selected as the principal treatments in the study. Air-stripping was also assessed, since it is known to have the ability to remove ammonia from solution. Hydrogen peroxide was assessed for its ability to oxidize nitrite to nitrate.

During the first TTE, activated carbon reduced toxicity to *Daphnia magna*, but had no effect on fathead minnow survival. Activated carbon combined with zeolite eliminated toxicity to both species, and removed most of the DOC, ammonia, nitrate, nitrite, copper and cyanate. Activated carbon combined with air-stripping also eliminated toxicity to both species, but was less effective in removing ammonia and nitrite. Similarly, activated carbon combined with hydrogen peroxide eliminated toxicity to both species, but caused some control mortality.

The results obtained during a second TTE indicated that activated carbon plus zeolite effectively eliminated acute lethality to rainbow trout. Activated carbon combined with either air-stripping or hydrogen peroxide only reduced acute lethality.

Cost of TTE investigations

The authors reported that the estimated cost for the TTE investigation conducted for Mine A was $12,500. This includes pre-TTE full chemical characterization of the effluent, definitive acute lethality testing and three TTEs. The TTEs include testing to confirm the treatments ability to reduce or eliminate elevated contaminants and acute lethality, project management, sample/test co-ordination, historical data review, report preparation and disbursements (e.g., shipping, communications etc.). It was cautioned that the approach to each TTE is customized based on the study objectives, combination of toxicants, and complexity of treatment applications.

2.7.4 Conceptual Effluent Treatment Options

Based on the TTE results for Mine A, the authors of the NALMET report suggested a number of possible options that could be used to treat copper, ammonia and cyanate, including:

- Adsorption using activated carbon or zeolite to treat copper, ammonia and cyanate;
- Wetlands to treat copper, ammonia and cyanate;
- Hydrogen peroxide or sulphide precipitation to remove copper; and,

- Oxidation, nitrification / denitrification, air-stripping and algal ponds to remove cyanate.

Process flow diagrams (PFDs) were provided in order to depict how the various treatment options could be configured. PFD 1 illustrated an activated carbon treatment set-up, and PFD 2 included an activated carbon plus zeolite configuration. PFD 3 provided a configuration for an air-stripping column or tower followed by an activated carbon bed. PFD 4 involved the addition of hydrogen peroxide through a chemical metering pump. PFD 5 illustrated a combination of activated carbon and hydrogen peroxide additions.

Based on the effectiveness of the flow sheets for consistently achieving a non-acutely lethal effluent, the authors felt that only PFD 2 and 3 were worth further consideration for Mine A. It was noted that additional information from larger bench-scale or pilot studies would be required to size the treatment units and complete the treatment circuits.

2.7.5 NALMET Study Conclusions and Recommendations

The authors provide a summary of the study, as well as some important perspectives on techniques for conducting TTEs. These perspectives are provided in the following section.

1. Several methods used during the study provided guidance for conducting an effective TTE, including:

- Regression analysis between toxicity data and corresponding chemical data in the historical data review;
- TIE evaluations (EDTA, pH adjustment, air-stripping);
- Acute lethality modelling (e.g., salinity model) with threshold effects data for specific toxicants;
- Laboratory method of chemical additions to evaluate specific toxicants; and,
- Non-specific treatment technologies (e.g., activated carbon).

2. The following knowledge can assist in designing a cost-effective TTE study: the specific chemistry of the mine; the mine's treatment system; general contaminants observed with each mine type (i.e., gold, base metal); mine treatment technologies.
3. TIEs should not be eliminated from the process, as they provide an essential foundation for conducting the TTE.
4. Activated carbon should be included earlier in the TIE / TTE study process.
5. The use of activated carbon as a study tool and treatment technology should be better documented in the TIE / TTE literature. The authors suspect that the lack of specificity of carbon for specific toxicants was the reason there was little available documentation on its use.
6. Pollution prevention concepts were demonstrated for Mine B, which was considering mill modification for recycling of process water. The authors suggest this approach could result in a non-acutely lethal effluent with minimal treatment technologies.

2.8 Toxicity Reduction: Evaluation and Control (Ford, D.L. (editor). 1998. Toxicity Reduction: Evaluation and Control. Volume3, Second Edition. Water Quality Management Library. 356 pp.)

This book provides an overview of toxicity reduction evaluations (TREs), and includes discussions on TIREs, organic toxicant control, toxicant control using biological treatment, toxicity reduction methodologies, and case histories for a variety of industrial effluents. The case studies include TREs for the petroleum, municipal, chemical processing, and pulp and paper sector, but did not include any mining sector examples. The toxicity reduction methodologies include those for control of cationic toxicants, precipitation/co-precipitation of inorganic toxicants, inorganic anions, cyanide and ammonia.

The goal of a TRE is to reduce or eliminate effluent toxicity or a specific chemical toxicant. In certain cases, a Toxicity Identification Evaluation (TIE) may be an important part of a TRE and could direct the focus of the TRE. The authors indicate that there is no definitive TRE protocol that is universally applicable. However, in order to avoid significant waste of time and money, toxicity reduction in all cases should be approached in three sequential phases. The first phase is data validation, in which the effluent toxicity problem is verified through additional testing. The second phase, initiated after completion of the first, involves existing treatment system optimization. The third phase of a toxicity reduction project, not to be started until completion of the second phase, consists of the following four parallel, concurrent efforts: toxicity identification, best management practices (BMP) implementation, source treatment, and treatment system upgrading.

The authors also provide a brief review of the standard U.S. EPA effluent toxicity testing protocols available and an overview of TIE and TRE methodologies based on the U.S. EPA guidance documents (1989, 1991a,b and c). A detailed review of these U.S. EPA documents is provided in a previous section of this literature review.

2.8.1 Organic Toxicant Control

This section of the document focuses on organic toxicant control. The methods reviewed include the use of activated carbon and chemical oxidation. However, organic toxicant control is not a common issue for metal mines except uranium mines, and occasionally organics used in floatation and heavy use of organic polymers in flocculation. The theory and concepts of granular activated carbon are still applicable in the removal of heavy metals such as mercury (Hg).

2.8.2 Toxicant Control Using Biological Treatment

This section of the document focuses on removal of toxic organics using biological treatment. The removal of toxic organics may occur through one or more mechanisms: sorption, stripping, or biodegradation. As mentioned above, organic toxicant control is not a common issue for metal mines.

2.8.3 Toxicity Reduction Methodologies

This section of the document provides toxicity reduction methodologies that include:

1) alternative technologies for the control of cationic toxicants,

2) precipitation/co-precipitation technologies for the control of inorganic toxicants,

3) various treatment technologies for the control of inorganic anions, and

4) control of cyanide and ammonia.

This particular chapter was most relevant to the metal mining industry with respect to the removal of metals, cyanide and ammonia in the effluent.

Alternative Technologies for Control of Cationic Toxicants

Common cationic toxicants include: cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), Lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn). In some instances, the oxidation state of the metals determines how a particular treatment technology performs, or if a process control requires multi-staging. The authors note that although metals are present in wastewaters from an extremely broad spectrum of industries, it is common to observe these concentrations at certain typical levels.

Various treatment technologies have been used for control of cationic toxicants. The authors provide a list of the more common technologies, including:

- Precipitation – hydroxide / oxide, carbonate, sulfide
- Co-precipitation
- Ion exchange
- Membrane process
- Electrolytic recovery
- Evaporative recovery
- Adsorption processes

Among these technologies, precipitation with co-precipitation accounted for 60-80% of the treatment systems described in electroplating facilities. The alternative technologies discussed in this section of the document include:

- Ion exchange;
- Membrane technologies; and,
- Electrolytic methods.

Ion Exchange

Ion exchange is an established technology, particularly for water softening. Although the potential of ion exchange for metals pollution control is well recognized, its applications are quite limited. Ion exchange is applied for pollution control either as a tertiary treatment of effluent before discharge or for direct recovery (chromate recovery in plating rinse waters). The advantages and disadvantages of the ion exchange process include:

Advantages

- Operates on demand;
- Is relatively insensitive;
- There is essentially zero level of effluent contaminant possible;
- A large variety of specific resins are available; and,
- Beneficial selectivity reversal commonly occurs upon regeneration.

Disadvantages

- Potential for chromatographic effluent peaking;
- Spent regenerant must be disposed of;
- Variable effluent quality with respect to background ions; and,
- Usually not feasible at high levels of total dissolved solids.

Three major areas of application of ion exchange in the metal finishing industry have been demonstrated:

- Wastewater purification and recycling;
- End-of-pipe pollution control; and,
- Chemical recovery.

In the second application (which was relevant for mining effluent), toxic heavy metals are removed selectively from waste streams prior to discharge. Ion exchange can be used in two different forms for end-of-pipe pollution control: i) as a means of polishing the effluent from conventional hydroxide precipitation to lower the metal concentration in the discharge, or ii) as a means of directly treating wastewater to remove heavy metals. In each of these approaches, wastewater pre-treatment involves pH adjustment, to ensure that pH is within the operating range of the resin, and filtration, to remove suspended solids that would foul the resin bed.

Membrane Technologies

Membrane processes may involve physical separation (e.g., ultrafiltration) and physical-chemical phenomena (e.g., reverse osmosis, electrodialysis). The latter are of most direct interest with respect to control of inorganic cationic toxicants.

Several membrane processes have been applied for water and wastewater mercury treatment. These include ultrafiltration, charged filtration, crossflow microfiltration, magnetic filtration and reverse osmosis.

Electrolytic Methods

Electrolytic processes are applicable for the removal and reclamation of heavy metals, including toxic metals from concentrated wastewater, and for polishing streams containing dilute metals. It is not generally useful in treating dissolved organics, organic waste streams, or viscous and tarry liquids. Research has focused on the destruction of cyanide and the treatment of chrome wastes.

Electrolytic metal recovery has been used for many years in the mining industry for electrowinning and electrorefining of ores, mostly in copper removal and recovery. No details were given on the destruction of cyanide by electrolytic process.

Precipitation/Co-precipitation Technology for Inorganic Toxicants Control

Precipitation treatment is the most widespread technology applied for inorganic toxicants control. Its application is particularly prevalent for the control of metals in the mining effluent. Some factors influencing precipitation treatment efficiency include:

- Treatment pH
- Salt formed
- Formation kinetics
- Complexing agents
- Co-precipitants formed
- Suspended solids removal efficiency

The following is a summary of precipitation/co-precipitation technology performed in full-scale for the control of various inorganic toxicants. It must be remembered that many full-scale precipitation treatment systems operate at compromised conditions that represent overall optimum treatment for the regulated parameters of concern. These conditions may not be optimal to treat specific parameters. For

instance, the difference in treatment pH for the removal of metals may require the design of a multi-staging treatment system.

Cadmium (Cd)

Hydroxide and sulfide precipitation are frequently applied for Cd control. Co-precipitation using iron and aluminum salts has also been reported. Hydroxide precipitation performs better at higher pH (i.e., >10). Incorporation of filtration after precipitation can achieve Cd levels well below 0.1 mg/L. Sulfide addition following hydroxide precipitation appears to enhance the effluent quality for Cd. Carbonate has also been reported to enhance Cd precipitation treatment.

Chromium (Cr)

Hydroxide precipitation is the predominant trivalent chromium (Cr^{3+}) treatment technology. Sulfide precipitation has also been reported. There is some uncertainty in the literature regarding the appropriate treatment pH range. Therefore, field treatability tests or system fine-tuning may be required.

Copper (Cu)

The Cu oxidation state, the type of salt precipitated, and the protocol whereby the target treatment pH is achieved, all influence the treatment results. Sulfide addition after hydroxide precipitation does not appear to improve Cu removal.

Lead (Pb)

Hydroxide precipitation of Pb is inefficient. However, a small amount of carbonate in the wastewater promotes the formation of lead carbonate and lead hydroxycarbonate salts. Sulfide precipitation is reported to be very inefficient for Pb. Co-precipitation can produce results similar to or greater than carbonate precipitation.

Mercury (Hg)

Sulfide precipitation is one of the most commonly used precipitation methods for inorganic Hg. This process is usually combined with pH adjustment and flocculation, followed by solids separation. Most effective precipitation, with regard to minimizing sulfide dosage, occurs at near-neutral pH range. Precipitation efficiency decreases significantly at pH above 9. Drawbacks of sulfide precipitation processes include:

- Lack of capability to reduce Hg below 10-100 µg/L;
- The potential problem of toxic residual sulfide in the treated effluent;
- Difficulty of clarification and sludge processing; and,
- Disposal of the sulfide sludge.

Removal of both inorganic and organic Hg by coagulation/co-precipitation has been reported for a variety of wastewaters. Coagulants used include aluminum sulfate (alum), iron salts and lime. At comparable initial Hg levels, both alum and iron provide equivalent treatment results, with effluent Hg concentrations in the range 0.5-5.0 µg/L.

Nickel (Ni)

Hydroxide precipitation is the most commonly used treatment technology for Ni, with effluent concentrations being routinely reported below 0.5 mg/L. Hydroxide precipitation is most effective in

solution where Ni is not strongly complexed. Sulfide precipitation may improve efficiency in strong complexing wastewaters.

Zinc (Zn)

The most frequently used precipitation process for Zn includes pH adjustment and precipitation of zinc hydroxide. Sulfide addition does not produce consistent results. Clarification and filtration after precipitation can achieve effluent Zn levels below 0.5 mg/L.

Adsorption Processes

Adsorption process can offer significant potential to achieve high removal efficiencies of certain metals such as mercury (Hg). Granular activated carbon (GAC) is the most commonly used adsorbent system used in industrial waste treatment applications. GAC can achieve effluent Hg concentrations of <0.001 mg/L. The cost for this process would be high, primarily due to the cost of replacing GAC. In addition, loaded GAC would be considered a hazardous waste and would require special disposal.

Control of Inorganic Anions

Inorganic anions include arsenic (as arsenite or arsenate), hexavalent chromium (as chromate), selenium (as selenide, selenite, or selenate), cyanide and fluoride. These anionic pollutants may occur from direct industrial use in their anionic form (e.g., chromate use in metal plating), as a natural, but anthropogenic processes (e.g., acid mine drainage), or as a by-product of manufacturing activity (e.g., metal ore smeltering). A broad spectrum of physical-chemical treatment and toxicity control technologies are applied for these pollutants, including:

- Chemical reduction
- Precipitation/co-precipitation
- Adsorption
- Anion exchange
- Evaporation

Chemical Reduction

Chemical reduction is mainly applied to the control of hexavalent chromium (Cr^{6+}) in the plating and tanning industries and to the removal of mercury from caustic/chlorine electrolysis effluents. Hexavalent chromium control includes reduction of Cr^{6+} to Cr^{3+} at an acidic pH range (2-3) and subsequent hydroxide precipitation of trivalent chromium. The most common reducing agents are: sulfur dioxide, sulfite salts, ferrous sulfate, powdered waste iron, powdered waste aluminum and powdered metallic zinc. Hexavalent chromium levels of 0.01 mg/L have been achieved by reduction at pH 2.5-4.0. Use of H_2SO_4 and SO_2 reduced Cr^{6+} from 8.75 mg/L to 0.01-0.03 mg/L in an automotive wastewater containing plating wastes and cooling water blowdown.

One disadvantage of chemical reduction for waste treatment is that it may introduce new ions into the effluent, resulting in additional treatment requirements.

Precipitation

Precipitation is applied primarily for fluoride (F) among the anionic toxicants. This technology has also been reported for arsenic and selenium. The technology involves the addition of a calcium reagent to induce the precipitation of a calcium salt.

Fluoride treatment involves two-chemical precipitation processes, first with lime to treatment pH 9 and then with supplemental calcium added as calcium chloride. As the reaction pH increases above 9, atmospheric carbon dioxide enters the reaction solution and, as carbonate, scavenges out the calcium ion, resulting in higher fluoride solubility at pH above 9. At the pH boundary of 9, an effluent fluoride concentration of 10-20 mg/L is achievable.

Arsenic precipitation as calcium arsenate is reported to occur at pH 12 with lime addition. At normal high treatment pH, arsenite is rapidly converted to arsenate, which then reacts to precipitate.

Adsorption and Co-precipitation

a) Arsenic removal

The removal of arsenic from water or wastewater depends on the concentrations of arsenic in solution, the mass of adsorbate present, the redox potential, and pH. Arsenic from industrial wastewaters containing heavy metals is removed through co-precipitation upon precipitation of other heavy metals. Co-precipitation involves both adsorption of the soluble ions by the forming oxide, and entrapment within the bulk precipitate. Chemical oxidation of arsenite to arsenate prior to co-precipitation has been found to enhance arsenic removal.

The amount of arsenite and arsenate adsorbed onto iron oxides and hydroxides depends on the concentrations of the adsorbing species and the solution pH. The removal of arsenite and arsenate by iron hydroxide was found to be a function of the solution pH. A pH of 4 was optimum for the removal of arsenate, and a pH of 7 was optimum for the removal of arsenite.

Ferrous hydroxide co-precipitation to remove arsenic from water supplies involves the addition of ferrous sulfate for precipitation and subsequent filtration. This results in an effluent arsenic concentration below 0.05 mg/L. Ferric iron was used to remove arsenic from a continuous flow system with greater than 90% removal at iron dosage of 14-28 mg/L.

Arsenic can also be removed by adsorption onto aluminum oxides and hydroxides. The optimum pH of removal was between 6 and 8.5.

b) Selenium Removal

Removal of soluble selenium is difficult from many industrial wastewaters. Lime precipitation was reported to be ineffective. Co-precipitation/adsorption by aluminum and ferric oxides, however, has been shown to be effective.

c) Phosphorus Removal

The removal of phosphorus (P) from waters is accomplished by adsorption through the addition of excess concentrations of coagulants: aluminum, iron or lime. In addition, co-precipitation of phosphate during calcium carbonate precipitation is also effective for P control. Both alum and ferric chloride are used to precipitate orthophosphate, and optimum removal occurs between pH 5.5 and 6.5. Domestic wastewater requires a dosage of 100-200 mg/L as calcium hydroxide to remove 80% of the phosphate.

Ion Exchange

Ion exchange is used for hexavalent chromium recovery, and for polishing effluent treatment for arsenic, chromium and selenium.

Control of Cyanide and Ammonia

The technologies applied to control of cyanide and ammonia include:

- Stripping
- Chlorination (oxidation)
- Ozonation (oxidation)
- Electrolytic decomposition
- Ion exchange
- Evaporative recovery

Air and Steam Stripping

Ammonia removal from industrial wastewaters can be achieved by various methods including physical, chemical, and/or biological treatment. Optimal ammonia stripping is influenced by pH, temperature, relative ammonia concentration, and gas-liquid ratio. Air stripping has been reported to be 60-95% effective, yielding effluent ammonia concentrations of 1-30 mg/L. One method of increasing the efficiency is by steam stripping. The cost of steam stripping is high and may be offset by the recovery and reuse of ammonia.

Oxidation

The primary use of chemical oxidation for wastewater treatment is in the conversion and destruction of cyanide from the effluent. There are many oxidizing agents available. The more commonly used agents are: ozone, air, chlorine gas, chlorine dioxide, sodium hypochlorite, calcium hypochlorite, potassium permanganate and hydrogen peroxide.

Chlorination

Many plating and metal finishing plants use chemical oxidation methods to treat their wastes. Oxidation of cyanide (CN^-) to cyanate (CNO^-) with Cl_2 or NaOCl is the most common method of treatment.

There are problems associated with alkaline chlorination of cyanide if iron or certain other transition metal ions are present. Complete oxidation of cyanide in ferri- or ferrocyanide wastes has been reported by alkaline chlorination at pH>10 and temperature 90°C and by ultraviolet irradiation with excess sodium hypochlorite.

Breakpoint chlorination has been successfully applied for ammonia treatment. The actual requirement for chlorine in practicing breakpoint chlorination of wastewater depends on the temperature, pH, and the concentrations of reacting species, and reaction may require as high as 9-10 mg chlorine for each mg NH_4-N oxidized. The problem is that the residual chlorine may be high enough to exceed the regulatory limit. If this occurs, hydrogen peroxide is applied to destroy excess chlorine.

Ozonation

In ozone treatment, cyanide is rapidly oxidized to cyanate at pH 9-12 and in the presence of trace amounts of copper. It has had limited full-scale use.

Electrolytic Decomposition

Wastes containing high concentrations of cyanide are most successfully treated by electrolytic decomposition. The advantages of this method are that cyanide is decomposed at the anode while heavy

metals are collected at the cathode, without generating a sludge problem. The full-scale application is still in the research and development stage.

Ion Exchange

Ion exchange is applied for the control of ammonia, and to a much lesser extent for cyanide treatment.

a) Removal of Ammonium by Ion Exchange

It is difficult to remove ammonium ions selectively by ion exchange due to its low position in the cation exchange selectivity sequence and its low concentrations relative to other cations. Natural inorganic zeolite (clinoptilolite) has unusual selectivity for ammonium ions in preference to calcium, magnesium and sodium ions. The unusual selectivity of zeolite, which makes it attractive for ammonia removal, is caused by structurally related ion sieve properties. The extent of ion sieving exhibited by a zeolite depends primarily on the size of the openings into the ion cages contained in the three-dimensional lattice structure and on the energy with which the structural water is bound to the zeolite framework. The selectivity of zeolite for various inorganic cations has the following order: $Cs^+ > Rb^+ > K^+ > NH_4^+ > Ba^{2+} > Sr^{2+} > Na^+ > Ca^{2+} > Fe^{3+} > Al^{3+} > Mg^{2+} > Li^+$. The total exchange capacity of zeolite is slightly less than that of the conventional organic resin.

Packed beds of zeolite have been used to remove ammonium from municipal sewage treatment plant effluent. Typically 100-300 bed volumes of wastewater containing 10-20 mg/L NH_4^+-N can be treated before regeneration.

b) Removal of Cyanide by Ion Exchange

The application of ion exchange for the removal of cyanide involves the conversion of cyanide to ferrocyanide, followed by ion exchange. Because extremely low effluent levels of cyanide are required, a typical two-column roughing-and-polishing sequence is used in which the polishing column is a freshly regenerated bed.

c) Other Physical-Chemical Technologies

Other technologies reported for cyanide and ammonia control include evaporative recovery, membrane processes, catalytic decomposition, and thermal oxidation. These technologies are not widely used.