Synopsis of Environment Canada Guidance Documents on Acute Lethality and Toxicity Reduction Evaluation (TRE)

The Metal Mining Effluent Regulations (MMER) requires that all Canadian metal mines produce effluent that is non-acutely lethal to rainbow trout when tested in accordance with Environment Canada reference 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.

Recognizing that some mines may be challenged in meeting the acute lethality requirements of the MMER, a multi-stakeholder network (Toxicological Investigations of Mine Effluent (TIME) Network) was established in 1999, with representation from governments, industry, environmental non-governmental organizations, the consulting community, and academia. 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 two Guidance Documents:

1. **Acute Guidance Document for Acute Lethality Testing of Metal Mining Effluents**

2. **Guidance Document for Conducting Toxicity Reduction Evaluation (TRE) Investigations of Canadian Metal Mining Effluents**

The purpose of the first document is to aid in the understanding of key aspects of testing mine effluent for acute lethality and to provide guidance aimed at maximizing data reliability. It improves upon, and provides greater detail on the specific guidance already provided in the Environment Canada rainbow trout and *Daphnia magna* test methods. It is intended to assist mine personnel in the collection and submission of samples and the evaluation of the resulting toxicity test reports, and enhance the efforts of laboratories to produce highly reliable data.

The second document provides mine operators with an effective tool for implementing an appropriate strategy for resolving effluent acute lethality issues, provides laboratories with a useful guide for conducting toxicity investigations with metal mining effluents, and ultimately, increases the likelihood of achieving and maintaining a consistently non-acutely lethal metal-mining effluent.

Neither document is intended to replace or supersede existing methods, but rather to provide additional detail, clarification and supplementary guidance specific to metal-mining effluents.

The key components of each document are highlighted in the following section.

Acute Guidance Document for Acute Lethality Testing of Metal Mining Effluents

Personnel from the mine and toxicity test laboratory both have critical roles to play in acute lethality testing. Several of the key elements necessary to maximize data reliability and minimize toxicity test variability are under the direct control of mine personnel, and include:

- selection of a toxicity test laboratory;
- development of a sampling plan; and
- collection of samples.

Selection of an Ecotoxicity Test Laboratory

The first critical element in the assurance of data reliability is the evaluation and selection of a capable and experienced ecotoxicity laboratory. A laboratory site assessment is one of the key ways of ensure the laboratory has the appropriate facilities, personnel, documentation (e.g., standard operating procedures), and a complete quality assurance and control program. The site visit also provides an opportunity for mine personnel to gain a better understanding of the Environment Canada test methods themselves. Laboratories considered for providing acute lethality testing services should also be accredited for both the rainbow trout and *Daphnia magna* reference methods. Accreditation (e.g., Canadian Association of Environmental and Analytical Laboratories (CAEAL)) is the process by which a laboratory quality assurance and control system is evaluated through regular site assessments by the accrediting body.

Development of a Sampling Plan

Prior to collection of any samples, a sampling plan should be developed to help ensure that the sampling program is conducted properly, reliably, and in a consistent fashion. The sampling plan should include: the sampling schedule; sample type and volume; a description of the sampling locations and sampling equipment; and, standard operating procedures for sample collection, labeling, handling and shipping. Joint involvement of key staff from the toxicity test laboratory, those involved in sample collection (generally mine environmental or technical staff), and data users (i.e., mine operators) is an important part of the overall planning process.

Sample Collection

Sample collection is another area of the effluent testing process where mine personnel play a key role, since in most instances mine personnel collect the samples for toxicity testing. Persons responsible for collection should ensure that the samples are representative, and that adequate measures are taken to preserve sample integrity during transit to the testing laboratory. Special attention to sample transportation will be particularly important for those facilities located in remote or isolated areas where access to courier services may be limited.

Split Samples

- On occasion, mine personnel may also collect "split samples" to assess the inter-laboratory variability associated with the testing of the mine's effluent. A split sample is one sample from a given source that has been subdivided and tested at two or more laboratories.
- The process involves the collection, homogenization, transport, handling, and reception of the sample, and implementation of the test.
- Approaches to minimize variation include: thorough homogenization of sample to be split for toxicity testing; co-ordination of sample delivery and test initiation; use of laboratories with similar dilution water quality characteristics (e.g., hardness); and, similar test practices (e.g., fish size, method of sample aeration).

Factors Influencing Test Method Variability

Once the sample arrives at the test laboratory, potential factors that may influence test method variability include (but are not limited to): inherent sample variability (e.g., toxicant type and concentration); abiotic conditions (e.g., changes in pH during testing); dilution water characteristics (e.g., hardness); test organisms (e.g., size, health); test conditions (e.g., aeration of test solutions); and, statistical analysis of test data.

Although the aforementioned factors can be critical in relation to the variability of test results, the magnitude of variability observed in the Environment Canada test methods is similar to that reported of analytical chemistry methods.

Specifically, a review of CAEAL round-robin test results revealed that the median Coefficients of Variations (CVs) for rainbow trout and *Daphnia magna* acute lethality tests were 15.7% and 12.9%, respectively. In comparison, CVs for regulated MMER metals (e.g., As, Cu, Ni, Pb and Zn) ranged from 2 to 9%.

Further reductions in test method variability and maximization of data reliability can be achieved by following the guidance provided in the acute lethality document.

Inherent Sample Variability

- Changes in chemical concentrations between samples from a given site can contribute to test variability. Common toxicants associated with metal mining effluents include ammonia, pH, metals, thiosalts, cyanide, suspended solids and process chemicals. Even small changes in the concentration of these substances can result in differences between samples, particularly for toxicants that interact with other effluent constituents in ways that modify their toxicity (e.g., pH and ammonia).
- Methods to reduce variability between samples exist. Optimization of effluent treatment operations and thorough documentation of available effluent chemistry (when co-ordinated with toxicity testing) may help explain differences between samples.

Dilution Water Quality

- Dilution water quality parameters such as hardness, pH, alkalinity and organic content are known to influence toxicity by modifying the bioavailability of contaminants. Thus, different dilution waters can produce different effluent test results.
- Differences in dilution water quality among laboratories may contribute to variability in multiple-concentration (LC50/EC50) tests, although these differences will have no impact on the results of single-concentration (100% effluent) tests, where dilution of the effluent is not required. Using the same water source should minimize variability within a laboratory.
- Alternate sources of dilution water may be more appropriate in tests that are intended to eliminate gradient effects due to differences in pH or hardness of the effluent relative to the dilution water.

Fish Size

- Size of test fish used can influence the outcome of acute lethality test results. Recent amendments to the Environment Canada test method have narrowed the size range allowed.
- Holding fish at lower water temperatures to reduce the rate of growth and extend the period of time that small fish are available can further reduce variability related to fish size.
- Planning and scheduling of samples will also be important to ensure that fish of the appropriate size and range are available as needed for testing.

Sample Aeration

- Aeration of the test solution can affect sample pH and the dissolved oxygen concentration. Furthermore, the method and rate of aeration can alter the rate of

change of these parameters. In the Environment Canada test methods, aeration rates are defined and pH adjustment is not allowed. However, there are two options for the method of aeration: silica-glass air diffuser, or disposable glass pipette.

- Laboratory experience has shown that the efficiency of these two aeration methods is not necessarily equivalent, which can result in differences in the dissolved oxygen concentration and pH of the test solution. This can be of concern, in the case of metal mining effluents, where the toxicity of certain contaminants (i.e., metals, ammonia and thiosalts) may be affected by the pH and oxygen concentration of the test solution.
- Standardizing the method of aeration can reduce variability. However, the potential for inter-laboratory variability remains, since laboratories currently have two choices available for aerating test solutions.

Statistical Analysis

- Methods for statistical analysis are common to both rainbow trout and *Daphnia magna*. The LC50 is based on dead test organisms, whereas the EC50 is based on impaired animals (e.g., *Daphnia magna* test EC50 accounts for dead plus immobilized organisms).
- It is generally accepted that acute lethality effects for rainbow trout are generally complete within the standard 96-h exposure period. However, this is not always the case in the 48-h tests involving *Daphnia magna*, where immobility of the test organisms is common. The presence of immobile organisms can be a source of variability, if the laboratory is not careful in distinguishing between dead or immobilized animals, or if the results are reported only in terms of an LC50 (i.e., mortality only).
- If immobile *Daphnia magna* are observed, a second statistical estimate can be made to calculate an EC50 (i.e., mortality and immobility).

Guidance Document for Conducting Toxicity Reduction Evaluation (TRE) Investigations of Canadian Metal Mining Effluents

The Toxicity Reduction Evaluation (TRE) process, developed by the U.S. EPA, is a commonly used step-wise approach designed to assist industrial dischargers to identify the causes of, and eliminate final effluent acute lethality.

What is a TRE?

A TRE is a site-specific study designed to identify the substance(s) responsible for acute lethality in effluents, 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.

Acute Lethality Response Plan

The initial step in the TRE process should begin prior to experiencing the first failure, in the form 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):

- A description of facility processes, operations, and effluent treatment facilities.
- Line diagrams showing the major operations areas and inputs to the treatment facilities.
- Documentation of facility operations/conditions 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.
- An 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 and mine personnel. Good communication and exchange of complete information among all team members will be critical to the success of a TRE by speeding the response to the failure and increasing the likelihood of TRE success.

Reviewing Toxicity 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 of the Environment Canada reference method 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.

Magnitude and Persistency of Toxicity

Establishing the degree (i.e., magnitude) and persistency (i.e., how toxicity changes over time) of acute lethality will be important, since these factors 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).

TRE Components

The 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.

Although common toxicants associated with metal mining effluents have been identified, 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.

Toxicity Identification Evaluation (TIE)

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 (e.g., 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 (Ausley *et al*., 1998). Alternative approaches, in combination with TTE and SI evaluations, may be more successful under these conditions.

What to do after a Phase I TIE?

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 Investigation (SI)

Source Investigations determine whether the toxicants may be isolated in one or more wastewater 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).

Toxicity Treatability Evaluation (TTE)

A toxicity treatability evaluation (TTE) involves the systematic evaluation of various treatment technologies, combinations of technologies, or management options (e.g., 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.

Repeated Testing

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).