GUIDANCE DOCUMENT FOR ACUTE LETHALITY TESTING OF METAL MINING EFFLUENTS

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June 2002

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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 reliability of data generated by these tests is, therefore, an important issue in the context of maintaining confidence in the use of these tests as a basis for assessing regulatory compliance.

The Toxicological Investigations of Mining Effluents (TIME) Network was established with representation from governments, industry, environmental non-governmental organizations, the consulting community, and academia, to address toxicological issues related to the amended Metal Mining Effluent Regulation (MMER). During the first TIME workshop, held in November 1999, several potential projects were prioritized, including the development of a guidance document for acute lethality testing of mine effluents. Concerns have been expressed by industry in the past regarding the variability and repeatability of effluent toxicity test results. Therefore, this guidance document has been prepared for industry personnel, aquatic toxicity testing laboratories, and regulatory authorities to aid in the understanding of key aspects of acute lethality testing and to provide guidance aimed at maximizing data reliability.

An overview of the current state of knowledge pertaining to this topic is provided in the document, including: a historical background of aquatic toxicology in Canada, the current regulatory framework in which toxicity testing is conducted, common metal mining contaminants and their potential impact on effluent toxicity, a literature review of test method variability, and a summary of test system deviations observed in a review of metal mining effluent toxicity data.

A literature review based primarily on toxicity test methods used in the United States (i.e., U.S. EPA methods) provided some insight into the potential sources of variability associated with biological test methods in general. Analyst proficiency and judgment, as well as test organism condition and health were considered to be the largest sources of variability. Additionally, a strong QA/QC program was considered essential in helping to control test method deviations, which can lead to test variability.

Variability associated with test results specifically conducted using the Environment Canada test methods was evaluated using data sets obtained from the Canadian Association for Environmental Analytical Laboratories (CAEAL) (proficiency testing (PT) program) and from nine volunteer laboratories that provided reference toxicant test results to the Ontario Ministry of the Environment (OMOE). Coefficients of variation (CVs) were estimated using variance components analysis for all intra-laboratory (within lab) reference toxicant data. The within-laboratory CVs for rainbow trout reference toxicity tests were as follows: 13.3% using phenol as a reference toxicant, and 38.5% using dissolved zinc as a reference toxicant. The within-laboratory CVs for *D. magna* reference toxicity tests were: 8.7% using sodium chloride as a reference toxicant, and 33.3% using dissolved zinc as reference toxicant. Inter-laboratory (among-lab) CVs were estimated from the CAEAL PT data set also using variance components analyses. These CVs were estimated using the

date and results from the testing of the CAEAL PT sample. This analysis yielded 52 CVs for the rainbow trout PT data set. CVs ranged from 8.0 to 60.4% with a median CV = 15.7%. Similarly, 28 CVs were estimated from the *D. magna* CAEAL PT data set. The CVs ranged from 7.5 to 53.1% with a median CV = 12.9%. Overall, the magnitude of variability observed in these biological test methods is within the range of (and in some cases, lower than) the variability observed in analytical chemistry methods.

The main portion of the document provides guidance on aspects of the Environment Canada General and Reference Methods relating to: sample collection and handling (including collection of split-samples), test organism culture and holding, test method requirements, statistical analyses, and reporting requirements, all for the purpose of maximizing data reliability. All parties involved with the testing program have critical roles to play, whether collecting the sample, performing the tests, or reviewing the test for compliance with the MMER.

Information on laboratory accreditation programs in Canada, laboratory assessments, and their importance in reducing test method variability is also provided for background in the understanding of toxicity laboratory quality assurance. In addition, guidance is provided to mine personnel for the selection of a competent ecotoxicity laboratory, as well as the implementation of test report evaluations of acute lethality data, and second-party laboratory assessments.

The guidance document improves upon, and provides greater detail on the specific guidance already provided in the rainbow trout and *D. magna* Reference Method documents (Environment Canada, 2000a,b). It will assist mine personnel in the collection and submission of samples and the evaluation of the resulting toxicity test reports, and it will enhance the efforts of laboratories to produce highly reliable data. Furthermore, the guidance document will also be of assistance to a broad range of stakeholders with an interest in acute lethality testing. This document does not supersede current government guidance, policy, or regulation including Environment Canada's Reference Methods EPS 1/RM/13 and EPS 1/RM/14 (Environment Canada 2000a,b).

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LIST OF ACRONYMS

AECB	Atomic Energy Control Board
AETE	Aquatic Effects Technology Evaluation
ANFO	Ammonium Nitrate Fuel Oil
AQUAMIN	Assessment of the Aquatic Effects of Mining in Canada
AWWA	American Water Works Association
BLM	Biotic Ligand Model
BOD	Biochemical Oxygen Demand
CAEAL	Canadian Association for Environmental Analytical Laboratories
CANMET	Canadian Centre for Mineral and Energy Technology
CCME	Canadian Council of Ministers of the Environment
CV	Coefficient of Variation
CWQG	Canadian Water Quality Guidelines
EC	Environment Canada
EC50	Median effective concentration (i.e., the concentration estimated to cause a lethal or sublethal effect (e.g., immobilization) on 50% of the test organisms). [Note: An EC50 is determined on the basis of dead as well as stressed organisms, while an LC50 is based on dead organisms alone].
EEM	Environmental Effects Monitoring
EPA	Environmental Protection Agency
ISO	International Organization for Standardization
LC50	Median lethal concentration (i.e., the concentration of material in water that is estimated to be lethal to 50% of the test organisms).
MENVQ	Ministère de l'Environnement du Québec
MISA	Municipal-Industrial Strategy for Abatement
MMER	Metal Mining Effluent Regulation
OMA	Ontario Mining Association
OMOE	Ontario Ministry of the Environment
PE	Performance Evaluation
PPER	Pulp and Paper Effluent Regulations
PT	Proficiency Testing
QA	Quality Assurance

QC	Quality Control
QM	Quality Manual
SCC	Standards Council of Canada
SETAC	Society of Environmental Toxicology and Chemistry
SOP	Standard Operating Procedure
SRM	Standard Reference Material
TIME	Toxicological Investigations of Mining Effluent
TSS	Total Suspended Solids
U.S. EPA	United States Environmental Protection Agency
WET	Whole Effluent Toxicity

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Many of the terms provided below may have meanings other than those provided in this document. The terminology and corresponding definitions outlined in this section are provided specifically in the context of acute lethality testing of industrial effluents.

Accuracy – bias of an analytical method, which reflects the closeness of a measured value to the true value of a sample.

Analyst – person trained to conduct and/or report on specific techniques or procedures for calibration and testing, according to accepted and current standard operating procedures/work instructions.

CAEAL Proficiency Testing Program – national inter-laboratory testing program aimed at assuring the quality of environmental analyses of various chemical, toxicological and microbiological parameters. On a twice-yearly basis, participating laboratories are sent "blind" reference toxicant samples for testing; laboratories then provide CAEAL with their results from the analysis in question. CAEAL then conducts statistical analyses of the inter-laboratory data, in order to score laboratories on their performance in the testing round.

Chain of custody – the documented and traceable transfer of a sample from the point of collection to reception at the testing laboratory.

Coefficient of variation (CV) – calculated as the standard deviation divided by the mean. The CV is a measure of the variability in a group of measurements. The variability is indexed by the mean so that the resultant CV is unitless. Since the CV is unitless, it can be used to compare CVs from different "experiments". For example, one can directly compare the CV's from rainbow trout and *Daphnia magna* tests. The CV can be multiplied by 100%, so that it is expressed as a percentage.

Confidence interval – range of values estimated by a sample within which the true population value is expected to fall. For example, if an LC50 and 95% confidence interval are estimated from a toxicity test, the true population LC50 is expected to fall within the interval, 95% of the time.

Confidence limits – the upper and lower boundaries of the confidence interval.

Control chart – graphical plot of test results with respect to time or sequence of measurement upon which control and warning limits are set to guide in detecting whether the test system is in a state of control.

Control limits – limits or combination of limits which, when exceeded, trigger analyst intervention. These limits may be defined statistically or based on test method requirements. Control limits may be assigned to method blanks, check standards, spike recoveries, duplicates and reference samples. Most control limits for toxicity tests are based on 3X the standard deviation of the mean (i.e., one in every 100 tests would be expected to exceed the control limits, due to chance alone).

Duplicate – a quality control sample, often chosen randomly, from a batch of samples and undergoing separate, but identical sample preparation and analysis whose purpose is to monitor method precision and sample homogeneity. Duplicate testing also aids in the evaluation of analyst proficiency.

Effluent – waste water discharged from an industry; for the purposes of this document, effluent, as defined by the MMER, includes: mine water effluent, mill process effluent, tailings impoundment area effluent, treatment pond or treatment facility effluent, seepage and surface drainage.

Holding time – the time elapsed between the end of sample collection or sample preparation and the initiation of testing.

Inter-laboratory – a term that refers to "among-laboratory activities"; for example, inter-laboratory variability evaluates the reproducibility of similar analyses by different laboratories. Estimation of inter-laboratory variability addresses a measure of quality assurance of laboratories (see section 2.6, Environment Canada 1999).

Intra-laboratory – a term that refers to "within-laboratory activities"; for example, intra-laboratory variability evaluates repeatability of an analysis within the same laboratory system. Estimation of intra-laboratory variability of data is a principal quality control measure of a laboratory (see section 2.6, Environment Canada 1999).

Laboratory – a body or part of an organization that is involved in calibration and/or testing.

Laboratory accreditation – formal recognition, by a registered accrediting body, of the competence of a laboratory to conduct specific functions. The process by which a laboratory quality system (i.e., laboratory management system) is evaluated through regular site assessments by the accrediting body, and may also include a proficiency testing program.

Laboratory certification – formal recognition, by the certifying body, of the proficiency of a laboratory to conduct specific tests.

Mean – the arithmetic mean or average; the sum of n data points, divided by n, the sample size.

Multiple-concentration test – a test that determines the degree of toxicity of an effluent. The test is generally based on a minimum of five concentrations including full strength (100%) effluent, plus a clean, negative control (based on Environment Canada, 2000a).

Parameter – a limit, state, constant or defined physical and/or chemical characteristic that describes a variable or group of variables.

Precision – the degree of agreement among replicate analyses of a sample, usually expressed as the standard deviation. Precision is affected by random errors and is a measurable and controllable parameter. Precision can be separated into two further concepts (i.e., repeatability and reproducibility). Repeatability is the closeness of agreement between successive measurements of the same effluent conducted under the same conditions (within runs). Reproducibility is the closeness of agreement between the results of measurement of the same effluent conducted under different conditions of measurement (between runs). Between-run precision includes variability due to calibration on different days, and many other factors.

Quality assurance – an integrated system of internal and external activities involving quality planning, quality control, quality assessment, quality reporting and quality improvement to ensure that data meet the laboratory's own quality objectives and the needs of its users.

Quality control – a component of quality assurance through which regular internal checks and reviews of laboratory operations and systems are conducted.

Quality manager – person who has responsibility and authority to implement and maintain the laboratory's quality system.

Quality system – the collection of documented policies, processes and procedures for ensuring the production of high quality and traceable data according to defined quality objectives.

Reference material – a material consisting of one or more substances whose properties are sufficiently well established to be used for the calibration of a test system.

Reference toxicant testing – a toxicity test procedure in which a chemical is used to provide results that can be compared within or among laboratories. Test- and substance-specific reference toxicant testing (also referred to as 'positive controls') is conducted by a laboratory on a regular basis to demonstrate consistency in toxicity test method performance (i.e., within a defined and limited range of variability). The test system can be affected by such influences as: changes in test organism sensitivity over time as a result of size, reproductive status; genetic differences between stocks of organisms obtained from different sources; and, performance of technical staff. Reference toxicant test results falling outside the normal range may indicate test organism or technician insufficiencies. Warning charts (also known as control charts) are established with the results from reference toxicant tests, and are regularly updated to demonstrate that test reproducibility is within established limits.

Sample – a portion of a lot or population consisting of one or more single units.

Sample preparation – all procedures applied to a sample prior to testing; may include pre-treatment (e.g., filtration, homogenization).

Sample pre-treatment – all procedures applied to a collected sample prior to testing, including removal of unwanted material, removal of moisture, sub-sampling and/or homogenization.

Single-concentration test – a test that determines the presence or absence of toxicity. The test is based on exposure of the test organisms to a single concentration of effluent (full strength unless otherwise specified) plus a clean, negative control (based on Environment Canada, 2000a).

Standard deviation – the square root of the sample variance.

Standard Operating Procedure (SOP) – a written, authorized and controlled quality document that details instructions for the conduct of laboratory activities; laboratories develop SOPs when adopting a standard method or when developing laboratory-specific procedures (e.g. glassware cleaning).

Traceability – the property of an item such as a record, method, measurement, or qualification that completely demonstrates the origin or validity of the item.

Variance – a measure of dispersion of data in a dataset, calculated as the sum of squares of the differences between each data point and the mean, divided by the number of data points.

Variance components analysis – a class of statistical analyses that partitions variability due to different sources; for example, LC50's observed from different laboratories at different times are variable, partially as a function of the laboratory and partially as a function of time.

Warning limit(s) – a boundary or combination of limits which, when exceeded, may trigger analyst intervention; most toxicity laboratories use 2X the standard deviation of the mean to create warning limits (i.e., one in every 20 tests would be expected to exceed the warning limits, due to chance alone).

ACKNOWLEDGEMENTS

This guidance document was prepared and co-authored by Guy Gilron (Project Manager), Keith Holtze (Project Director), Shawna Kirkpatrick, and Lesley Novak, all of ESG International (Guelph, ON, Canada), and Barry Zajdlik of B. Zajdlik & Associates (Rockwood, ON, Canada). Teresa Carter provided production assistance for all drafts and the final copy of the Guidance Document. Julie Schroeder (Standards Development Branch, Ontario Ministry of the Environment, Toronto, ON) acted as the Scientific Authority for the project, providing general direction and guidance, detailed review, and technical and logistical assistance during the project.

The project team gratefully acknowledges the cooperation, assistance and contributions provided by the members of the Project Steering Committee, in particular, Richard Scroggins (Method Development and Applications Section, Environment Canada, Gloucester, ON), Jim McGeer (Natural Resources Canada, Ottawa, ON), and Wally Senzca (Newmont Canada Ltd., Marathon, ON). Special thanks are also extended to Debbie Macey and Andrew Morris (Canadian Association for Environmental Analytical Laboratories (Ottawa, ON)), who provided assistance in the data review aspects of the project. Moreover, the project team acknowledges the following Canadian aquatic toxicity testing laboratories, who provided valuable reference toxicant data in support of the data review: Environment Canada (Pacific Environmental Sciences Centre, North Vancouver, BC; Toxicology Laboratory, Environmental Science Centre, Moncton, NB; EP Laboratories, Prairie and Northern Region, Edmonton, AB; Centre St. Laurent, Montreal, PQ), Ontario Ministry of Environment (Etobicoke, ON), BEAK International (Brampton, ON), ESG International (Guelph, ON), Lakefield Research (Lakefield, ON), and Pollutech Enviroquatics (Sarnia, ON).

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1.0 INTRODUCTION

1.1 Background

Once amended, the Metal Mining Effluent Regulation (MMER) will require that all Canadian metal mines produce effluent that is non-acutely lethal to rainbow trout when tested in accordance with Reference Method EPS 1/RM/13 (second edition; Environment Canada, 2000a). Mine operations will also be required to monitor the acute lethality of effluent to *Daphnia magna* in accordance with Reference Method EPS 1/RM/14 (second edition; Environment Canada, 2000b). The December 2000 second edition versions of the rainbow trout and *D. magna* Reference Methods cited above incorporate the amendments (to the original 1990 edition) of May 1996 and December 2000.

In accordance with the amended MMER, metal mines will be required to conduct monthly rainbow trout and *D. magna* acute lethality tests using full strength (100%) effluent. Once 12 consecutive "passes" (i.e., 12 monthly tests with $\leq$50% mortality in 100% effluent) with rainbow trout are obtained, the acute lethality testing frequency can be reduced from a monthly to a quarterly basis (for both species). At the time when the amended MMER comes into force, 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 Environment Canada's Reference Methods. A monthly testing frequency is maintained until 12 consecutive rainbow trout "passes" are achieved, at which time the frequency of testing may be reduced to a quarterly schedule.

If a rainbow trout test produces more than 50% mortality of the test organisms in 100% effluent, the sample is considered to have "failed" the acute lethality test. Subsequent samples must then be assessed for acute lethality with both species on a twice per month basis, until 3 consecutive rainbow trout "passes" are achieved. Additionally, it is recommended that the mine implement a plan to investigate the cause(s) of acute lethality. To this end, the reader is directed to information provided in the Guidance Document for Conducting Toxicity Identification/Reduction Evaluations (ESG 2002).

Recognizing that some mines may be challenged in meeting the toxicity requirements of the amended MMER, a multi-stakeholder network was established in 1999, with representation from governments, industry, environmental non-governmental organizations, the consulting community, and academia. This network, called the Toxicological Investigations of Mine Effluent (TIME) Network, focused on toxicological issues related to the amended Metal Mining Effluent Regulation (MMER). Specifically, the TIME Network was committed to the following major objectives:

- To undertake projects that will broaden the knowledge base with respect to the causes of, and solutions to, effluent toxicity;
- To investigate and develop methodologies to identify causes of, and solutions to, reduce or eliminate toxicants;
- To look for cost-effective and environmentally sound pollution prevention and control treatment technologies to consistently achieve non-acutely lethal effluents; and,

- To provide a mechanism for information dissemination.

During the first TIME workshop, held in November 1999, a number of potential projects were identified, and in May 2000, four projects were selected that focused on issues of concern to all stakeholders. One of the projects was to develop a guidance document for acute lethality testing of metal mining effluents.

1.2 Scope and Structure of this Guidance Document

This guidance document was prepared for use by industry personnel, aquatic toxicity testing laboratories, and regulatory authorities to aid in the understanding of key aspects of acute lethality testing and to provide guidance aimed at maximizing data reliability. The guidance document improves upon, and provides greater detail on, the specific guidance already provided in the rainbow trout and *D. magna* Reference Method documents (Environment Canada, 2000a,b). It will assist mine personnel in the collection and submission of samples and the evaluation of the resulting toxicity test reports, and it will enhance the efforts of laboratories to produce highly reliable data. Furthermore, the guidance document will also be of assistance to a broad range of stakeholders with an interest in acute lethality testing. This document does not supersede current government guidance, policy, or regulation including Environment Canada's Reference Methods EPS 1/RM/13 and EPS 1/RM/14 (Environment Canada 2000a,b).

The guidance document is organized as follows:

- Section 2 presents an overview of the current state of knowledge pertaining to this topic, including: a historical background relating to aquatic toxicology in Canada, the current regulatory framework in which toxicity testing is conducted, common metal mining contaminants and their potential impact on test variability, a literature review of toxicity test variability, and a summary of test system deviations observed in a review of metal mining effluent toxicity data;
- Section 3 presents a summary of the acute toxicity data review, conducted to determine the magnitude and extent of variability using Environment Canada acute lethality test methods with rainbow trout and *D. magna*;
- Section 4 provides supplementary guidance aimed at maximizing data reliability in all aspects of the Reference Methods;
- Section 5 provides information on laboratory accreditation programs in Canada, laboratory assessments, and their importance in reducing test method variability; and,
- Section 6 provides guidance to mine personnel regarding the selection of a competent ecotoxicity laboratory, as well as the implementation of test report evaluations of acute lethality data, and second-party laboratory assessments.

2.0 CURRENT STATE OF KNOWLEDGE

2.1 Development of Aquatic Toxicology in Canada

The field of aquatic toxicology in Canada was born in the early 1950's, and developed rapidly into the 1960's, with research on water pollution biology by a number of workers, most notably, Donald Alderdice, John Neil, John Sprague, Gerard Leduc, Terry Howard, James Servizi, and Thomas Beak (see historical overview provided by Sprague, 1995). Much of the early research in this field focused on the impact of various chemicals (such as pesticides and metals) on a variety of domestic fish and aquatic invertebrate species. One of the major advantages of toxicity testing (vs. chemical analysis of environmental media) is that it is an integrative indicator of biological impact. In other words, test organisms respond to all toxicants present in a sample, thereby measuring the bioavailability and the true toxicity potential of its constituents. Environment Canada (1999; section 1.4) provides a detailed account of the benefits and limitations of toxicity testing in this regard. Due to growing societal awareness of the potential impacts of water pollution on aquatic biota, this pioneering work was applied to industrial effluent discharges, mainly, pulp and paper and metal mining effluents.

In the mid- to late-1980's, the Ontario Ministry of Environment (OMOE) developed test methods for evaluating acute toxicity to rainbow trout (Craig *et al*., 1983) and *D. magna* (Poirier *et al*., 1988) and implemented acute toxicity limits in the mid-1990s under the Municipal-Industrial Strategy for Abatement (MISA) program. The development of effluent discharge regulations for the protection of aquatic life, most recently, the federal Pulp and Paper Effluent Regulations (PPER), catalyzed the development and establishment of standard methods for the evaluation of effluent toxicity across Canada. These test methods have been developed, reviewed, and published (and amended as needed) by the Method Development and Applications Section of Environment Canada to provide test-specific guidance on how to conduct the toxicity tests, with full descriptions of culture and test conditions (for different types of test media), quality assurance and quality control measures, and reporting requirements.

2.2 Current Regulatory Framework for Acute Lethality Testing in the Metal Mining Sector

In Canada, provincial and federal effluent discharge regulations for a variety of industrial sectors (e.g., pulp and paper, metal mining, petrochemical, iron and steel, electric power, industrial minerals, inorganic chemicals, metal casting, organic chemicals, manufacturing) often include, among other chemical and biological parameters (e.g., pH, biochemical oxygen demand (BOD_5), total suspended solids (TSS), ammonia) indicators of aquatic toxicity, such as acute lethality to rainbow trout and/or *D. magna*. The provincial, territorial and Atomic Energy Control Board (AECB) acute lethality requirements for mines in Canada are provided in Appendix A.

Extensive research and consultation has been undertaken to assist in the development of the amended MMER. For example, the Aquatic Effects Technology Evaluation (AETE) program, coordinated under the auspices of Natural Resources Canada, and conducted between 1994 and 1998, was a cooperative effort involving the Canadian mining industry, and federal and provincial government departments. The program was established to review, apply and recommend

methods appropriate for assessing the impacts of mining effluents on the aquatic environment. With respect to toxicity testing the results of this program were detailed in three separate reports. The first study evaluated standard acute toxicity tests with selected mine effluents and data from three tests (rainbow trout, *D. magna*, and *D. magna* IQ) were generated (AETE, 1995a). In the second study, four alternative acute toxicity tests (Microtox™, Rotoxkit F, Thamnotoxkit F and Toxichromotest™) were conducted with the same mine effluents (AETE, 1995b), and the final integrative study evaluated the data generated from the previous two studies. Selected Canadian mine effluents of various types, exhibiting a range of toxicity and chemical characteristics, were tested. The final report from this integrative study focused specifically on a comparison of the two regulatory acute toxicity tests (i.e., Rainbow trout and *D. magna*) with various micro/screening tests, including: *D. magna* IQ toxicity test™, Microtox™, Rotoxkit F, Thamnotoxkit F and Toxichromotest™ (AETE, 1996).

2.3 Common Metal Mining Effluent Contaminants and Their Potential Effects on Toxicity

2.3.1 Chemical Characteristics of Mining Effluents

Mining effluents are complex waste waters that may be comprised of many different constituents (Table 1). These constituents may vary in terms of their concentration and form in response to factors such as: process changes, quality of the ore bodies, waste treatment practices, or environmental conditions (e.g., temperature) which can affect their relative toxicity. The following text provides a brief discussion of the potential effects of some of the listed constituents on toxicity. It is important to remember that toxicity associated with individual substances is often different from tests of the same substance in an effluent due to matrix effects. See section 2.3.2 for details regarding matrix effects on toxicity.

Table 1. Examples of Constituents Present in Mining Effluents[1]

Process Chemicals[1]	Acids (H_2SO_4, HCl, HNO_3)
	Alkalis (CaO, $Ca(OH)_2$, $CaCO_3$, Na_2CO_3)
	Frothers (e.g., pentyl alcohol, propylene glycol) and collectors (xanthates)
	Modifiers (surface active organics and inorganics such as NaCN, $CuSO_4$, $AlCl_3$, $Pb(NO_3)_2$, silicates and chromates
	Sodium cyanide (for precious-metal cyanidation and as depressant for copper minerals in flotation process)
	Al and Fe salts, and organic polymers (used as coagulants)
Trace and Other Elements[1]	Process effluents can contain (among others): Al, Ar, Cd, Cu, Fe, Pb, Mn, Ni, Zn and radium
Thiosalts[1]	Partially oxidized sulphur oxyanions which originate from grinding and flotation of sulphide ores (e.g., thiosulphate, trithionate, and tetrathionate)
Suspended Solids[1]	Range from colloidal (non-settleable) to settleable materials
Ammonia	Primary source is from the use of explosives, ammonia nitrate-fuel oil (ANFO)

[1] Environment Canada, 1987

Xanthates

Xanthates (e.g., sodium ethyl xanthate) are used in the mining industry as collectors during the processing of sulphide ore by flotation (Rao and Dekker, 1971 in NICNAS). Sodium ethyl xanthate is not readily biodegradable. Hydrolysis is the principal factor in determining its fate in the environment, but this process is pH- and temperature- dependent. The half-life of sodium ethyl xanthate at 25°C decreases from over 500 hours at alkaline pH (8 – 11) to about 260 hours at neutral pH. Under acidic conditions, it is hydrolytically unstable and rapidly hydrolyzes to ethanol, carbon disulphide and caustic soda (Rao and Dekker, 1971 in NICNAS).

The results of two studies involving an assessment of the acute lethality of different xanthates to *D. magna* (Hawley, 1977) and rainbow trout (Webb *et al*., 1976) suggest that toxicity varies with the specific chemical and species tested. In tests conducted with *D. magna* (Hawley, 1977), the toxicity range (i.e., range of concentrations in which the LC50 is expected to fall) reported for sodium ethyl and potassium ethyl xanthate was reported to be between 0.1 and 1.0 ppm, while the toxicity range for potassium hexyl and sodium isobutyl xanthate was reported to be between 0.6 and 32 ppm. Corresponding toxicity ranges for rainbow trout were generally 1 to 2 orders of magnitude higher (Webb *et al.,* 1976). For example, the toxicity range for sodium ethyl xanthate was reported to be between 0.1 and 1.0 ppm for *D. magna* (Hawley, 1977) while the corresponding range for trout was reported to be 10 to 50 ppm (Webb *et al.,* 1976). Although the results from these two studies are not directly comparable (since the tests were conducted under different water quality conditions, and used different suppliers for the test material), they suggest that xanthates are more toxic to daphnids than to trout. A number of studies summarized in a review by Campbell (1995) show that xanthates have the potential to enhance metal uptake, forming lipophilic complexes with certain metals including: cadmium, nickel, mercury, and lead although the bioavailability and toxicity of these metal complexes is not well understood. Therefore, the presence of xanthates in mine effluents may affect toxicity in one of two ways, either by exerting a direct toxic effect, or indirectly, by increasing metal uptake.

Thiosalts

Thiosalts are partially oxidized sulphur oxyanions containing sulphur-sulphur bonds that are meta-stable intermediates in the oxidation of sulphides or elemental sulphur to sulfite. They originate mostly in the grinding and flotation of sulphide ores (e.g., pyrite, pyrrhotite), under alkaline conditions. The main chemical species of concern include: thiosulphate ($S_2O_3^{2-}$), trithionate ($S_3O_6^{2-}$) and tetrathionate ($S_4O_6^{2-}$) (Environment Canada, 1987). Although thiosalts have relatively low toxicity, they are of concern because they generate sulfuric acid according to the following reactions (Wasserlauf and Dutrizac, 1982):

$$S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2H^+ + 2\ SO_4^{2-}$$

$$S_3O_6^{2-} + 2O_2 + 2H_2O \rightarrow 4H^+ + 3\ SO_4^{2-}$$

$$S_4O_6^{2-} + 7/2\ O_2 + 3H_2O \rightarrow 6H^+ + 4\ SO_4^{2-}$$

Thiobacillus bacteria present in water can catalyze the aerobic oxidation of thiosalts to produce sulphuric acid, resulting in an increase in acidity and a decrease in the effluent pH. In addition to pH causing direct or indirect effects (through the alteration of metal toxicity, for example) thiosalts themselves may have the potential to cause toxicity, although little is known about the relative toxic effects of the various thiosalts. At some mines, the problem of thiosalts is seasonal. Natural

oxidation is slower in winter and faster in summer. Thiosalts are difficult to measure, unstable and must be frozen immediately until the time of analysis. With aeration, thiosalt concentration will decrease, yielding a decrease in pH, and an increase in the concentration of sulphate (Bechard, 1997). Thiosalts may also influence toxicity by binding metals, for example, thiosulphate has been shown to bind metals and reduce metal ion uptake and toxicity (Janes and Playle 1995). The neutral metal-thiosulphate complex has also been shown to increase overall metal bioavailability (Fortin and Campbell 2001).

Ammonia

The presence of ammonia in mining effluents is primarily related to unspent ammonium nitrate fuel oil (ANFO), a blasting agent. Other sources include: its use as a pH regulator (e.g., uranium precipitation), as a reagent (e.g., copper and nickel processes), as a flotation reagent (also amines), and as a decomposition product from cyanide wastes.

Ammonia toxicity is attributable to the free or un-ionized (NH_3) form as opposed to the ionized (NH_4^+) species (Thurston *et al.,* 1981). The relative concentration of un-ionized ammonia increases proportionately with pH and water temperature. Table 2 provides the percentage of un-ionized ammonia in aqueous total ammonia solutions as a function of pH and temperature. [To calculate the concentration of un-ionized ammonia using the values presented in Table 2, the measured total ammonia concentration is multiplied by the corresponding value for the appropriate pH and temperature of the solution. For example, for a total ammonia concentration of 10 ppm, the corresponding concentration of un-ionized ammonia at pH 8.5 and a temperature of 15 ºC, is 0.800 ppm (i.e., 10 x 8/100)].

Thurston *et al*. (1981) showed that the toxicity of un-ionized ammonia to rainbow trout varied with pH and alkalinity. Over the range of pH (6.5 to 9.0) and alkalinity (75 to 196 mg/L as $CaCO_3$) tested, un-ionized ammonia toxicity was inversely proportional to both of these parameters. That is, while more un-ionized ammonia is formed at higher pH, the same concentration of un-ionized ammonia is more toxic at lower pH and alkalinity. For example, concentrations of un-ionized ammonia as low as 0.13 mg/L (Thurston *et al.,* 1981) have caused acute toxicity to rainbow trout in waters with low pH (i.e., 6.4 to 6.7) and alkalinity (i.e., 62 – 86 mg/L as $CaCO_3$). However, this value is higher with increasing pH and alkalinity (e.g., 0.66 mg/L in water with pH 8.2 – 8.8 and alkalinity ~ 190 mg/L as $CaCO_3$). Consequently, effluent toxicity can be variable even among samples having the same total ammonia concentration, which can be interpreted as test data variability.

Table 2. Percent NH_3 in aqueous total ammonia solutions for 10 – 20 °C and pH 6 – 9.5*

Temp. °C	pH							
	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5
10	.019	.059	.19	.59	1.8	5.6	16.	37.
11	.020	.064	.20	.63	2.0	6.0	17.	39.
12	.022	.069	.22	.68	2.1	6.4	18.	41.
13	.024	.074	.24	.74	2.3	6.9	19.	43.
14	.025	.080	.25	.80	2.5	7.4	20.	45.
15	.027	.087	.27	.86	2.7	8.0	22.	46.
16	.030	.093	.29	.93	2.9	8.5	23.	48.
17	.032	.10	.32	1.0	3.1	9.1	24.	50.
18	.034	.11	.34	1.1	3.3	9.8	26.	52.
19	.037	.11	.37	1.2	3.6	11.	27.	54.
20	.040	.13	.40	1.2	3.8	11.	28.	56.
21	.043	.14	.43	1.3	4.1	12.	30.	58.
22	.046	.15	.46	1.4	4.4	13.	32.	59.
23	.049	.16	.49	1.5	4.7	14.	33.	61.
24	.053	.17	.53	1.7	5.0	14.	35.	63.
25	.057	.18	.57	1.8	5.4	15.	36.	64.

*from Emerson *et al*., 1975

Dissolved Metals

All metals to be regulated in the amended Metal Mining Effluent Regulation (including arsenic, copper, lead, nickel and zinc) can be toxic to aquatic biota at relatively low levels (i.e., part per billion (ppb) range). The mode of action of acute metal toxicity in fish has generally been associated with the disruption of ion regulation mechanisms (Playle *et al*., 1993), particularly sodium (Na^+), chloride (Cl^-) and calcium (Ca^{2+}) at the surface of the gills. For example, accumulation of copper on the gills has been shown to reduce Na^+-K^+ ATPase activity thus inhibit Na^+ uptake and leading to a loss of internal Na^+ and death (Playle *et al*. 1993, McDonald and Wood, 1993). Brief summaries of acute toxicity for two selected metals (copper and nickel) and the factors that modify their toxicity are discussed below. For an in-depth discussion of the acute toxicity of other relevant metals, the reader is directed to information provided in the document, "Literature Review of Environmental Toxicity of Mercury, Cadmium, Selenium and Antimony in Metal Mining Effluents" (BEAK, 2002), another TIME Network-sponsored document.

The toxicity of dissolved copper (Cu) to aquatic organisms has been studied extensively. Copper toxicity is influenced by various water quality parameters including: pH, water hardness and concentration of major ions (in particular Na) and total organic carbon (TOC). In alkaline waters, copper toxicity decreases with increasing pH in response to the formation of inorganic copper species (Cu-carbonates and Cu-hydroxide complexes). Hardness cations, calcium and to a lesser extent, magnesium (Welsh *et al*., 2000), as well as sodium (Erickson *et al.,* 1996) can mitigate copper toxicity by competing with Cu for binding sites at the gill surface. Copper toxicity also decreases with increasing TOC (Alabaster and Lloyd, 1982) and alkalinity (Spear and Pierce, 1979). The lowest acute lethality value of 0.003 mg/L was obtained for rainbow trout in very soft water (Cusimano *et al*., 1986). However, the majority of acute lethality values for copper are above 0.025 mg/L. The lowest acute lethality copper value for *D. magna* in hard water was 0.0065 mg/L (U.S. EPA, 1985).

Dissolved nickel toxicity is affected by various water quality parameters including: water hardness, pH, dissolved oxygen and suspended solids (U.S. EPA, 1980). Toxicity of nickel is also increased in the presence of copper (Anderson and Weber, 1976). The lowest acute value for rainbow trout was 8.1 mg/L (Nebeker *et al*., 1985). The lowest acute value for *D. magna* was 0.095 mg/L (Biesenger and Christensen, 1972).

2.3.2 Modifying Factors of Toxicity

Abiotic Factors

Physico-chemical factors including: temperature, pH, dissolved oxygen, light intensity and photoperiod, hardness can influence toxicity (Sprague, 1985). Therefore, controlling the factors that influence toxicity is also important in controlling variability.

Water temperature can be a modifying factor of toxicity for many contaminants, either by influencing the metabolism of the test organisms (and therefore altering the rate of uptake) or by altering the form of the contaminant, thereby affecting its bioavailability. For example, as mentioned above, ammonia speciation is directly related not only to pH, but also to temperature (Thurston *et al.,* 1981). Additionally, oxygen solubility in water is reduced at higher temperatures. Therefore, effects on organisms due to low dissolved oxygen concentrations are more likely at higher temperature. Water temperature requirements in the EC methods were selected to be within the optimum range for trout (15 ± 1° C) and *D. magna* (20 ± 1° C) and standardized over a narrow range. This minimizes the effects of temperature both as a stress factor and as a modifying factor of toxicity.

The life cycle of fish and daphnids is influenced by the number of hours of light and dark (photoperiod), as well as light intensity (Greene *et al*. 1988, Peltier and Weber 1985, Pennak 1978). The lighting regime specified in the EC test methods, with respect to the photoperiod (i.e., 16h light: 8h dark) and light intensity (i.e., 400 and 800 lux at the water surface) is optimal for the production of actively-reproducing cultures of *D. magna* and for maintenance and holding of rainbow trout. Changes in conditions outside this range, could stimulate natural physiological changes in the organisms. For example, increasing the hours of darkness can trigger the production of males in *D. magna* cultures, and in turn, the production of ephippial eggs; cultures with ephippia are unsuitable for testing. Therefore, regular monitoring of culture conditions is

important for identifying when conditions fall outside the optimum and allow for correction of the problem.

Dissolved oxygen concentration can also be a modifier of toxicity. For example, low dissolved oxygen concentrations can cause stress, which may be lethal to aquatic organisms. In addition, low dissolved oxygen can increase the toxicity of certain dissolved metals (e.g., zinc, lead, and copper), cyanide and ammonia (CCME, 1999). The Canadian Water Quality Guideline (CWQG) for dissolved oxygen is 6.5 mg/L (CCME, 1999) for cold-water fish species (including rainbow trout). *Daphnia spp.* are able to tolerate dissolved oxygen concentrations as low as 3 mg/L (Greene *et al.*, 1988). Aeration is not permitted in Environment Canada's test method with daphnids, because they are sensitive to turbulence in the test vessel but tolerant of low oxygen conditions. However, aeration is a requirement of Environment Canada's test method for rainbow trout. The required rate of 6.5 ± 1 mL/min·L is usually sufficient to maintain the dissolved oxygen concentration in the control solution within the range 70% to 100% of the oxygen saturation value, but is kept to a minimum in recognition of the fact that excessive aeration can increase the rate of pH change and the removal of volatile compounds.

It is well known that water hardness can influence the toxicity of certain dissolved metals (e.g., beryllium, cadmium, copper, lead and nickel), as discussed in section 2.3.1. In recognition of this fact, federal and provincial water quality guidelines have set limits for some of these metals based on different hardness levels. The federal water quality guidelines for cadmium, copper, lead and nickel (CCME, 1999) are based on four general categories of water hardness including: soft (0-60 mg/L as $CaCO_3$), medium (60-120 mg/L as $CaCO_3$), hard (120-180 mg/L as $CaCO_3$) and very hard (>180 mg/L as $CaCO_3$). Elsewhere, in the U.S. for example, numerical limits are determined using formulae which require a value for water hardness (CCME, 1999).

The toxicity of many common mine effluent contaminants is heavily influenced by pH. Examples include: ammonia, metals, sulfide and cyanide (Mount and Mount, 1992). Numerous studies have shown that ammonia toxicity to fish is higher with increasing pH (i.e., above 7.0) as more of it is transformed from the lesser toxic ionized form (NH_4^+) to the more toxic un-ionized form (NH_3). The solubility of certain chemicals (e.g., metals, sulfide, cyanide) can also be influenced by pH, thereby rendering them more or less toxic. For example, copper toxicity decreases with increasing pH over the range 7.2 to 8.6, due to greater Cu-carbonate and Cu-hydroxide formation and adsorption to dissolved organic material (Santore *et al.,* 2001). In contrast, copper toxicity increases at lower pH due to increased free copper formation. Solubility curves for different metals (including aluminum, copper, lead, nickel and zinc) as a function of pH are available in the literature (e.g., Environment Canada 1987, Stumm and Morgan, 1996). Speciation profiles for different dissolved metals as a function of pH can be generated by using available software packages (see section 2.3.3). Of note is that, at lower pH, increased hydrogen ions (H^+) may successfully compete with some metal ions, thereby reducing their toxicity (e.g., nickel). However, this mitigative effect is eventually lost when the concentration of H^+ ions increases to toxic levels. In static acute lethality tests, a small increase in pH (i.e., less than 1 pH unit) is typically observed as a result of the release of carbon dioxide until the solution reaches equilibrium with air. However, changes in pH may also occur in response to physical and chemical reactions within solution (Stumm and Morgan, 1996). For example, thiosalts (as noted above) can cause a reduction in pH.

Due to the pH sensitivity of many toxicants, even small changes in pH can have a marked effect on toxicity. The use of static-renewal or flow-through test systems can reduce pH drift but they do not necessarily reduce toxicity, since many toxicants are less available at higher pH. Although such test designs are not permitted in the Environment Canada Reference Methods, the procedures may be applied as tools for further understanding and investigating the possible causes(s) of toxicity. It should be noted that these tests are more costly than the static acute test due to increased sample volume, complexity, and level of effort required.

Biotic Factors

Biotic factors are those related to the test organism and include: species sensitivity, life history stage, health and fitness, and acclimation. Species differ in their relative tolerances to environmental stress factors, as well as chemical contaminants. For example, rainbow trout, a euryhaline organism (Scott and Crossman, 1973), are able to tolerate a wider range of salinity relative to *D. magna*, a non-euryhaline organism. Rainbow trout are also known to tolerate a wider range of water hardness than do *D. magna* (Greene *et al*., 1988). In terms of chemical stressors, rainbow trout show greater sensitivity to ammonia than do *Daphnia*, while the reverse is generally true for most metals. A review by McKim (1977) indicated that early life history stages of fish were generally more sensitive to chemical contaminants than older developmental stages. The Environment Canada test methods specify both the test species and life stage tested, so that these factors are reduced, as a source of variability.

Other factors including organism fitness (i.e., the ability of aquatic organisms to tolerate physical and chemical stress factors), health, and acclimation are also limited, as much as possible, as potential sources of variability in the Environment Canada test methods through the standardization of procedures. These relate to culture, maintenance and holding of test organisms, by the setting of performance criteria pertaining to test organism health and reproductive fitness, and by including QA/QC practices including the testing of negative (clean) controls, and positive (reference toxicant) controls, and the use of control charts for plotting performance of the test system over time. However, the methodologies are generally designed to establish the minimum acceptable performance requirements and biotic factors can still represent a potential source of variation that operators should be aware of, and develop an understanding for (see also sections 2.4, 4.5 and 4.6).

Matrix Effects

Interactive or "matrix" effects among co-contaminants in an effluent, or between contaminant and other constituents of the dilution water may also influence toxicity. Matrix effects occur when toxicants interact with other effluent constituents in ways that modify their toxicity (U.S. EPA, 1993). Due to matrix effects, the toxicity of a substance may be quite different when tested as part of an effluent than when tested individually in laboratory dilution water (e.g., tests using metal-rich effluent versus tests using metal salts). Matrix effects can fit into one of two categories: complexation/speciation changes and competition. The first category includes complexation of toxicants by particulate or dissolved species to the extent that their bioavailability is increased or reduced. For example, metal bioavailability may be reduced by complexation with dissolved or particulate organic material. Of note is that a particle-bound toxicant may be unavailable to rainbow trout, but readily available to *D. magna*, since these particulates may be more easily ingested via filter feeding.

As mentioned above, effluent pH can play an important role in toxicant speciation. The degree to which the effluent resists pH changes during testing is in part a function of the matrix and is an important factor in the expression of toxicity. Examples of pH-sensitive toxicants include: copper, cyanide and hydrogen sulphide, which increase in toxicity as pH decreases, and ammonia, which increases in toxicity as pH increases. Increased monitoring of pH during testing may provide useful information on matrix effects and acute lethality due to pH-sensitive toxicants. The second type of matrix effect is competition of ions with the toxicant for uptake at the biological receptor (e.g., gill membrane). As mentioned above, metal toxicity is lower in high hardness solutions. This is mostly due to competition of calcium ions with the metal ion for uptake by receptor cells. Therefore, toxicity of a metal in an effluent containing sufficiently high concentrations of competing ions may be much lower than in other samples containing the same concentration of the metal. See section 2.3.3 for further information on competition.

Based on the "common" toxicants associated with metal mining effluent (e.g., ammonia, metals, cyanide), and the influence of organic material, particulates, pH and competing ions on the expression of their toxicity, testing laboratories should be prepared to anticipate matrix effects in the testing of metal mining effluent samples.

2.3.3 Emerging Tools

The Biotic Ligand Model (BLM) is a mechanistic model comprising 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 rainbow trout and fathead minnows, and to a lesser extent, *D. 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 positively-charged metals 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 2000a). Mortality is predicted in aquatic organisms when the modeled concentration of metal bound to the biotic ligand (e.g., fish gill) exceeds a certain threshold concentration. The water chemistry-specific toxicity predictions are based on modeling the biotic and abiotic influences on metal uptake and linking them to tissue burdens known to cause acute toxicity. More detailed information regarding the BLM is provided in a U.S. EPA document entitled "Integrated Approach to Assessing the Bioavailability and Toxicity of Metals in Surface Waters and Sediments" (U.S. EPA, 1999).

At this time, the model is a new tool, is still in development (for example, models for dissolved zinc, cadmium and lead are being developed) and some expertise is required to use the program and interpret the results. 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).

2.4 Literature Review Summary

One of the objectives of the development of this guidance document was to conduct a literature review of variability associated specifically with Environment Canada acute lethality test methods. However, due to the lack of published Canadian information, the literature review was restricted to recent (post-1990), readily-available studies based on U.S. EPA test methods. Detailed summaries of each document are provided in Appendix B and key highlights are included below.

The recent U.S. EPA study on Method Variability in Whole Effluent Toxicity (U.S. EPA, 2000b) concluded that the U.S. EPA test methods are currently sound, although some modifications could be made to improve the interpretation of results. Furthermore, the authors indicated that the precision of currently promulgated U.S. EPA whole effluent toxicity (WET) methods are within the range of precision of other frequently-required chemical analyses. For example, a book chapter by Ausley (1996) cited CVs for various chemical analytes ranging from 11.8% to 291.7%; however, CVs for acute and chronic toxicity parameters were much lower, ranging from 14.8% to 67.6%. This supports the findings of the U.S. EPA (1991), which suggested that test method variability for both acute and chronic tests was similar to accepted analytical procedures for individual chemicals. Similarly, Rue *et al*. (1988) compared the distributions of CVs for the EPA's priority pollutants with effluent toxicity data from their study, and found that the CVs were generally in the same range. Additionally, Denton and Norberg-King (1996) cite a number of studies that show a good comparison between analytical and toxicity test methods.

As a comparison to the information in the U.S. literature, three major analytical laboratories in Ontario were surveyed by ESG International regarding intra-laboratory variability data for five standard reference materials (SRMs). For five metals to be regulated under the amended MMER, namely: arsenic, copper, nickel, lead and zinc (analyzed by Inductively Coupled Plasma), the intra-laboratory CVs ranged from 2.8 - 4%, 2 - 5.6%, and 3.5 - 9%, for each of three laboratories, respectively (n=18-100), with a mean CV for all metals of 4.0%. This intra-laboratory variability is considered very low, and represents excellent repeatability within a laboratory. These low values are likely due in part to: (a) the manipulation of the analytical sample prior to testing (i.e., acidification of the sample to pH 2); and (b) the CV's for the chemical analyses are based on total (not dissolved) metals, and once preserved, measurement becomes more simple. Moreover, it should be noted that the levels of metals in the SRMs represent concentrations far above the MMER limits (i.e., two orders of magnitude higher).

Variability in toxicity test data may be divided into three major categories: intra-test, intra-laboratory and inter-laboratory. Intra-test variability is the variability within a test such as differences in organism response or in physical and/or chemical conditions among test vessels. High variability within a test reduces sensitivity and may confound the interpretation of test results. Intra-laboratory variability is the variability of repeated or replicate tests conducted by one laboratory. Sources of intra-laboratory variability include: differences in test conditions, test organism health, and/or analyst performance. High variability within a laboratory reduces test precision. Inter-laboratory variability is the variability associated with testing of the same sample by two or more laboratories. Inter-laboratory variability includes aspects such as differences in dilution water sources, but also includes the sum of intra-test and intra-laboratory variability (Arnold *et al.*, 1996).

Factors identified in the literature that are generally considered to be critical in relation to the variability of test results are as follows:

- Analyst experience and judgment;
- Test organism health;
- Test conditions and abiotic parameters such as: water quality, temperature, pH, and light intensity and photoperiod;

- Experimental design factors: test vessel volume, organism size and reproductive health, numbers of test organisms exposed, number of exposure concentration, and numbers of test replicates; and,
- A strong QA / QC program.

Standardization of test methods has generally been an effective means of controlling many of these sources of variability and modifications and improvements to the existing methods can be made as more experience with the methods is gained over time. A summary of potential factors influencing test method variability is provided below in Table 3.

Table 3. Summary of Potential Factors Influencing Test Method Variability

Key Aspects	Potential Factors Influencing Variability	Measures of Variability	
		Intra-laboratory	Inter-laboratory
Sample Collection, Storage & Handling	Sample representativeness (issues relating to sampling location, frequency and type, sample volume, container, preservation methods and holding time)	X	X
	Sampling procedures	X	X
	Sample storage and handling	X	X
	Sample manipulation	X	X
Sample Variability	Chemical composition (nature of contaminants present)	X	X
	Seasonal variability	X	X
Abiotic Conditions	Test temperatures	X	X
	Changes in pH	X	X
Exposure and Variability	Static vs. flow through	X	X
	No. of concentrations and dilution series	X	X
	Test duration	X	X
Sample Toxicity and Variability	Test endpoints to be less variable for effluents having steep concentration-response curves and vice versa	X	X
Food	Quantity and quality (diet)	X	X
Dilution Water Characteristics	Source	X	X
	Potential modifying effects on toxicity due to characteristics (i.e. pH, water hardness, alkalinity etc)	X	X
	Potential modifying effects on organism sensitivity, fitness and health	X	X
	Artificially prepared or adjusted dilution waters (age of solutions)	X	X
Species Sensitivity	Most commonly used test species have acceptable ranges of variability	N/A	N/A
Organism History & Handling	Source of test organisms	X	X
	Culture conditions	X	X
	Acclimation	X	X
	Handling during testing	X	X
	Randomization (to evenly distribute the variability within the testing environment and the organisms)	X	X

Table 3. Summary of Potential Factors Influencing Test Method Variability

Key Aspects	Potential Factors Influencing Variability	Measures of Variability	
		Intra-laboratory	Inter-laboratory
Organism Numbers	Loading rates	N/A	N/A
	Ability to detect effects increases with number of organisms tested	X	X
Organism Quality	Effect of age on organism sensitivity to contaminants	X	X
Adherence to Test Methods	Deviation from methods may increase level of variability (issues relating to procedures, experimental design, quality control and test acceptability criteria)	X	X
	Sample manipulation	X	X
	Organism sensitivity	X	X
	No. of treatment reps	X	X
	Experimental design (issues relating to randomizing of treatments, organisms, replicates, specifying the number of organisms, replicates and treatments)	X	X
	Pre-aeration (type of aeration)	X	X
	Age/size of test organism	X	X
	Test acceptability criteria	X	X
Analyst Expertise	Conducting the statistical analysis to determine the effect concentration	X	X
Statistical Analysis of the Data		X	X
Selection of Testing Laboratory		X	X

The Environment Canada biological test methods (Environment Canada 2000a,b), and the Standards Council of Canada (SCC)/Canadian Association for Environmental Analytical Laboratories (CAEAL) and Ministère de l'Environnement du Québec (MENVQ) laboratory accreditation programs already address many of these factors, through test method standardization and laboratory facility and data assessment, respectively. However, there are still a number of areas in which additional guidance may assist both mine personnel and private sector aquatic toxicology laboratories in generating highly reliable acute lethality data.

2.5 Test System Deviations Observed in Ontario Mining Effluent Acute Lethality Testing

Recently, a third party review of acute lethality data generated by the Ontario metal-mining sector was conducted to determine compliance with the Province of Ontario's Municipal-Industrial Strategy for Abatement (MISA) Ontario Regulation 560/94 (OMOE, 1994), which applies to the metal mining sector (ESG, 2000). The study reviewed data and test reports submitted to the MISA program. A total of 391 data sets were reviewed, most of which involved acute lethality to rainbow trout and *D. magna*. All testing was conducted according to Environment Canada's Reference Methods for acute lethality tests with rainbow trout (EPS 1/RM/13) and *D. magna* (EPS

1/RM/14) (Environment Canada, 2000a,b). In total, 768 toxicity test reports were reviewed to determine compliance with the testing and reporting requirements.

The study identified deviations relating to sample collection and handling, test method procedures, reporting, and QA/QC. A partial listing of the noted deviations is provided in Table 4. Examples of deviations relating to sample collection and handling included: missing samples for toxicity testing or chemical analysis, missing sample identification information (e.g., sample date, sample temperature on arrival), late delivery of samples and exceedance of the maximum sample storage time of 5 days, and non-compliance with the minimum requirement of 15 days between sample collection for a given sampling location (NOTE: this is a MISA requirement). Test method deviations included testing of samples that were partially frozen upon receipt, exceedance of fish loading rates or size range (0.3 to 5.0 g), testing of samples that exceeded the maximum sample storage time, and the use of dilution water which exceeded the acceptable range of water hardness for *D. magna* (80 to 250 mg/L as $CaCO_3$). Only a few deviations were noted relating to QA/QC. These included occasional exceedance of reference toxicant warning limits (all but one event was reported on the test report), and the use of neonate daphnids from brood stock that failed the minimum requirements for culture health.

In addition to test method and QA/QC deviations, some of the most common reporting deviations included: failing to report sample temperature upon arrival, source of dilution water and loading rates in tests involving both rainbow trout and *D. magna*. The most serious reporting deviations related to discrepancies between observed and reported percent (%) mortality of test organisms. In a number of tests, the mortality documented on the laboratory bench sheet (i.e., raw data) was different than that noted in the test report. In several cases, the actual observed mortality was > 50% (fail), but the report indicated a pass (≤ 50% mortality).

It was noted that SCC-accredited laboratories had fewer method deviations than non-accredited laboratories. Recommendations to reduce test methodology variations included periodic laboratory visits/inspections by clients (see section 6.0 for further guidance) and the use of a laboratory accredited by the SCC/CAEAL program (see section 5.0).

Table 4. Summary of Sample Test Method Deviations from Review of Ontario MISA Toxicity Test Reports

Category	Description of Deviation
Sample Collection, Storage & Handling	Sample partially frozen upon arrival
	Sample date not provided
	Missing samples for toxicity testing or chemical analysis
Test Conditions	No indication as to pre-aeration of Dm test solution
	No pre-aeration (30 min minimum) of Rbt test solution
	Maximum loading rate for Rbt exceeded
	Size range limit for Rbt (0.3 to 5 g) exceeded
	Unequal numbers of replicates for Rbt
	Min # of test organisms/replicates in Dm test not met
	Sample storage time exceeded
	Dm dilution water hardness > max (250 mg/L as $CaCO_3$)
	Dm dilution water hardness < min (80 mg/L as $CaCO_3$)
	Dm dilution water D.O. < min (90% saturation)
	Rbt dilution water D.O. > max (100% saturation)
	Rbt dilution water D.O. < min (90% saturation)
	Test temperature outside 14 - 16 ^{o}C range
QA/QC	Exceedance of reference toxicant warning limit (Dm/Rbt)
	Dm mean # of neonate/brood < min (15)
	Time to first brood exceeds culture health limit

Rbt – rainbow trout

Dm- *D. magna*

3.0 EVALUATION OF VARIABILITY ASSOCIATED WITH THE ENVIRONMENT CANADA ACUTE LETHALITY TEST METHODS

Another objective of the guidance document development was to conduct an evaluation of variability associated with the results of tests conducted specifically using the Environment Canada acute lethality test methods. Data sets were obtained from the Canadian Association for Environmental Analytical Laboratories (CAEAL) (proficiency testing program) and from nine volunteer laboratories that provided reference toxicant test results to the Ontario Ministry of the Environment (OMOE). A full report detailing the data review phase is provided in Appendix C. This section of the guidance document presents the highlights and conclusions of the detailed data review.

3.1 Evaluation of Intra-Laboratory Variability

An evaluation of intra-laboratory variability was conducted using reference toxicant test data volunteered by nine laboratories from both the private and public sectors. The data sets were submitted to the project Scientific Authority (OMOE), who then consolidated the information for subsequent review and analysis by ESG International and B. Zajdlik & Associates. The data sets comprised the 20 most recent reference toxicant tests conducted using rainbow trout and *D. magna*. Eight laboratories submitted data for rainbow trout tests. Of these, 4 tested phenol as a reference toxicant, while 3 tested zinc chloride as a reference toxicant; one laboratory tested both reference toxicants. Eight laboratories submitted data for *D. magna* tests. Of these, 5 tested sodium chloride as a reference toxicant while 3 tested zinc chloride.

Coefficients of variation (CVs) were estimated using variance components analysis for all intra-laboratory reference toxicant data. The within-laboratory CVs for rainbow trout reference toxicant tests were as follows: 13.3% using phenol as a reference toxicant, and 38.5% using dissolved zinc as a reference toxicant. The within-laboratory CVs for *D. magna* reference toxicant tests were: 8.7% using sodium chloride as a reference toxicant, and 33.3% using zinc chloride as areference toxicant.

These results indicate that the choice of toxicant may significantly influence the magnitude of intra-laboratory variability in test results. Greater variability observed when dissolved zinc was used as a reference toxicant may be a consequence of the mode of toxic action of dissolved zinc relative to phenol for rainbow trout, and sodium chloride for *D. magna.* Additionally, variability may be introduced by the formation of precipitates when zinc stocks are prepared or stored due to the use of concentrations at or above the solubility limit or due to changes in dilution water selection.

Overall, these results also indicate that test system variability can, in some cases, be very low (i.e., 8.7-13.3%), but even in the case of higher CVs (i.e., 33.3-38.5%), variability is generally within the guidelines recommended in the Environment Canada guidance document on reference toxicants (Environment Canada, 1990c); this document suggests CVs in the range of 20-30% as acceptable quality control using reference toxicants.

3.2 Evaluation of Inter-Laboratory Variability

Two data sets were examined for the purpose of estimating inter-laboratory variability in rainbow trout and *D. magna* toxicity test results. These data sets were obtained from two sources: (i)

CAEAL proficiency testing evaluations, and (ii) the reference toxicant test data volunteered by nine toxicity testing laboratories.

3.2.1 CAEAL Proficiency Testing (PT) Data

The CAEAL data set consisted of proficiency testing (PT) results collected as part of the accreditation program since 1994. Four coded samples were submitted for testing to CAEAL-accredited laboratories semi-annually and a total of 33 laboratories produced results. Some laboratories have been participating in the CAEAL program since 1994, and have participated in a total of 13 performance evaluations. Other laboratories have participated in as few as one performance evaluation. Participating laboratories estimated LC50s using one or both of the acute lethality test methods (Environment Canada, 2000a,b).

Among-laboratory CVs were estimated from the CAEAL PT data set by variance components analysis using the date and results from the testing of the CAEAL PT sample. This analysis yielded 52 CVs for the rainbow trout PT data set, which are summarized in Table 5. The CVs ranged from 8.0 to 60.4% with a median CV = 15.7%. Similarly, twenty-eight CVs were estimated from the *D. magna* CAEAL PT data set (Table 6). The among-laboratory CVs ranged from 7.5 to 53.1% with a median CV = 12.9%.

Table 5. Summary of Among-Laboratory CVs from Rainbow Trout CAEAL PT Data

Date	Coefficients of Variation (%)			
	Sample 1	Sample 2	Sample 3	Sample 4
10/31/1994	17.6	13.8	20.7	20.6
3/31/1995	16.4	16.2	16.9	19.1
10/31/1995	16.0	14.2	15.7	16.9
3/31/1996	14.6	17.0	15.9	14.4
10/31/1996	16.2	15.6	11.1	9.8
3/31/1997	16.7	16.1	15.8	11.5
10/31/1997	14.9	18.9	13.5	12.3
3/31/1998	15.6	16.7	16.7	13.0
10/31/1998	15.5	18.1	17.0	18.9
3/31/1999	12.6	8.0	13.9	60.4
10/31/1999	15.4	14.3	14.3	12.9
3/31/2000	12.9	13.5	14.6	31.1
10/31/2000	16.4	15.1	17.8	10.5

Table 6. Summary of Among-Laboratory CVs from *D. magna* CAEAL PT Data

Date	Sample 1	Sample 2	Sample 3	Sample 4
10/31/1997	53.1	30.0	30.8	21.4
3/31/1998	8.7	12.7	9.6	8.7
10/31/1998	15.9	16.6	16.6	16.3
3/31/1999	10.6	9.1	10.5	11.7
10/31/1999	15.9	12.5	15.0	12.8
3/31/2000	13.0	13.7	7.5	8.0
10/31/2000	14.7	11.1	17.1	9.6

3.2.2 Reference Toxicant Data

Among-laboratory CVs were also estimated from the reference toxicant data set. The among-laboratory CVs for rainbow trout reference toxicant tests were: 3.5% using phenol as a reference toxicant, and 34.6% using zinc chloride as a reference toxicant. The among-laboratory CVs for *D. magna* reference toxicant tests were: 4.6% using sodium chloride as a reference toxicant, and 27.3% using dissolved zinc as a reference toxicant.

Overall, these results also indicate that test system variability can, in some cases, be very low (i.e., 3.5-4.6%), but even in the case of higher CVs (i.e., 27.3-34.6%), variability is generally within the guidelines recommended in the Environment Canada guidance document on reference toxicants (Environment Canada, 1990c); this document suggests CVs in the range of 20-30% as acceptable quality control using reference toxicants.

These analyses (in comparison with those results provided above) show that the variability within a laboratory (or day-to-day variability) is greater than the variability among laboratories for both tests. This result may be in part, a consequence of the extra within-laboratory variability induced by using reference toxicant data sets rather than a "true" round-robin data set where a stock solution is used to distribute identical samples.

3.3 Conclusions of the Data Review

The following are the major conclusions of the data review:

- Intra-laboratory test results indicate that the choice of toxicant may significantly influence the magnitude of intra-laboratory variability; and,
- Intra-laboratory variability is generally within the guidelines recommended in the Environment Canada guidance document on reference toxicants (Environment Canada, 1990c);

- For inter-laboratory variability, the CVs calculated for the CAEAL PT and volunteer laboratory data, indicate that Environment Canada acute lethality test results are very reproducible across laboratories. These results are especially favourable, based on a comparison to results in inter-laboratory reviews of other acute lethality test methods (see section 2.4 and Appendix B). Moreover, the toxicity test variability is within the range of (and in some cases, lower than) the variability observed in analytical chemistry methods. Of note is that the CVs for phenol (for rainbow trout) and sodium chloride (for *D. magna*) calculated from the volunteer laboratories

met the exceptional CVs reported for metals analyses at chemical analytical laboratories in Ontario (see section 2.4). It is likely that the toxicity CVs for these substances could be further improved if they were prepared as standard reference materials in the same way that metals are prepared for chemical analysis.

4.0 SUPPLEMENTARY GUIDANCE ON KEY APSECTS OF ACUTE LETHALITY TESTING AND MAXIMIZING DATA RELIABILITY

In 1990, Environment Canada published a series of biological test method documents for conducting acute lethality tests. The generic methods, "Acute Lethality Test Using *Daphnia* spp." (EPS 1/RM/11) and "Acute Lethality Test Using Rainbow Trout" (EPS 1/RM/9) (Environment Canada 1990d, 1990e), provide general or universal conditions and procedures for conducting acute lethality tests on a variety of test materials including: chemicals, elutriates, leachates, effluents and receiving waters. As such, the general methods provide detailed guidance that supports the shorter, and more specific, Reference Methods.

The Reference Methods, "Reference Method for Determining Acute Lethality of Effluents to *D. magna*" (EPS 1/RM/14) and "Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout" (EPS 1/RM/13) (Environment Canada 2000a, 2000b), were developed specifically for determining acute lethality of effluents, and have been used across Canada by the federal, provincial and territorial levels of government in the monitoring and control of industrial effluents. The methods provide instructions for: holding and/or culturing of the test animals, facilities and water supply, handling and storage of samples, preparation of test solutions, test conditions, observations to be made, endpoints with methods of calculation, test reporting, and the use of reference toxicants. Instructions provided in the Reference Methods generally take the form of either required (i.e., "must" statements) or recommended ("should" statements) tasks.

4.1 Impact of Improvements to Acute Lethality Reference Methods on Test Data Variability

The Environment Canada Acute Lethality Reference Methods have been used for industrial effluent monitoring and control since 1990. Based on feedback received from aquatic toxicologists who work for government and private sector laboratories, inspections conducted under laboratory accreditation programs, and different *Fisheries Act* court cases, Environment Canada has amended the rainbow trout and *D. magna* acute lethality Reference Methods on two occasions, in May 1996 and in December 2000. In both cases, improvements were made to reduce the potential sources of variability (i.e., to maximize data reliability) in acute lethality tests. For example, in the May 1996 amendments to the rainbow trout acute lethality method (i.e., EPS 1/RM/13), new "must" requirements for maximum fish loading density in test tanks, minimum fish size range and preparation of reference toxicant control/warning charts were introduced to reduce method variability. Also, to reduce the risk of using weak or diseased fish, the maximum fish mortality in holding tanks (i.e., rate of fish mortality in the holding tank) was changed from "should" to "must" to be less than 2% during seven days prior to the use of these fish in acute lethality testing. In the December 2000 amendments to EPS 1/RM/13, the size range of fish for use in tests was reduced from an average wet weight of between 0.3 and 5.0 grams to between 0.3 and 2.5 grams. This restriction was made based on research demonstrating that fish in the 2.5 to 5.0 gram range were less sensitive to industrial effluents than smaller fish in the 0.3 to 2.5 gram size range (Riebel and Gilron, *unpublished data*).

Similarly, there have been significant amendments to EPS 1/RM/14 (*D. magna* acute lethality method), such as new requirements for the preparation of reference toxicant control charts (May 1996 amendment) as well as clarification of culture health criteria, and their linkage to the brood stock producing neonates for testing (December 2000 amendment).

The intent of the following sections is to provide further guidance to industry, ecotoxicity laboratories and regulators, as appropriate, on the key aspects of acute lethality testing and on maximizing data reliability relating to Environment Canada's Reference Methods.

4.2 Sample Collection, Labeling, Transport, Storage and Handling

In addition to the guidance presented here, Environment Canada is currently in the process of completing a guidance document to aid in the sampling and analysis of metal mining effluents (Environment Canada Draft 6, April 2000), to which the reader may refer for additional information.

4.2.1 Sample Collection

Key Guidance: Collection of a representative sample (i.e., one which is representative of the entity being sampled) is one of the key components of any sampling program. Sample collection is one area of the effluent testing process where mine personnel play a key role, since, in most cases, sample collection is carried out by company personnel. It is in the best interest of mine personnel to ensure that their sampling programs meet their regulatory monitoring requirements, that the samples they collect are representative, that adequate measures are taken to preserve sample integrity during transit to the testing laboratory and that their testing is conducted by a competent and qualified laboratory.

Key Guidance: Development of a sampling plan based on the guidance provided in the Reference Methods will help to ensure that the sampling program is conducted properly, reliably, and in a consistent fashion. The sampling plan should include: sampling schedule, sample type and volume, a description of the sampling locations, sampling equipment and standard operating procedures for sample collection, labeling, handling and shipping. An important part of the overall planning process is the joint involvement of the key staff involved, from the data users (mine managers) to those involved in sample collection (mine environmental staff).

Sampling Plan. The Reference Methods outline specific "must" required steps to be followed, but also provide some general recommendations on sample collection, labeling, transport and storage. Table 7 provides a summary of this information. However, since conditions can vary on a site-specific basis, some latitude is given in the areas of sample collection, handling and volume requirements. Therefore, procedures should be developed on a site-specific basis, that are appropriate for the mine effluent collection site, time of year, etc. The key components of a sampling plan are discussed below.

Sampling Schedule. A schedule should be prepared to ensure that effluent sampling events meet the required legal obligations under federal or provincial regulatory requirements. Appropriate sampling equipment and materials must be available at the time of sample collection, and procedures should be in place to ensure timely delivery of the sample to the testing laboratory. Communicating information about the sampling schedule to the testing laboratory will ensure that the sample is processed in a timely fashion once it arrives. An advance call to the testing laboratory ensures that time and effort spent collecting a sample is not wasted, in the event that a

laboratory cannot carry out the intended testing at the time, and allows for re-scheduling of the sampling and testing events.

Table 7. Summary of Environment Canada's general procedures for sample labeling, transport and storage.

Reference Method Guidance	*D. magna*	Rainbow Trout
Sample Volume	2 L recommended for single or multiple concentration (LC50/EC50) tests	25 to 50 L recommended for single concentration tests 50 – 100 L recommended for multiple concentration (LC50) tests
Sample Containers	Made of non-toxic material (e.g., glass, polyethylene or polypropylene)	
Filling of Sample Containers	Prior to filling, container should be rinsed with clean water then with the sample being collected. Sample container should be filled with the sample to exclude air, then sealed.	
Preservation	No preservatives added.	

Sample Type. The type of sample can contribute to variability in effluent toxicity test results, particularly if the mine's effluent quality is variable over time. Environment Canada's Reference Methods describe various sample types including: "grab", "batch", and "24-h composite with sub-samples at 1-h intervals". A grab sample consists of a single sampling event (i.e., one point in time). Typically a grab sample is collected within a relatively short period of time (i.e., usually within seconds for small volumes). Batch or composite samples are collected over time (e.g., 24 hours) and may be collected either manually or by using an automatic sampling device. Table 8 lists the major advantages and disadvantages of the various sample types that should be considered when determining the type of sample to be collected.

Generally, grab samples are appropriate in cases where variability in effluent quality is expected to be low. Batch or 24-h composite samples may be more appropriate in situations where effluent quality is likely to be highly variable. Standardization of the sample type is necessary to reduce variability and likely to be dictated in any regulated effluent monitoring programs.

Water quality parameters, such as pH and conductivity, can be relatively easy to measure (even in the field) and can provide some measure of effluent quality in terms of its variability. As pH can modify the toxicity of contaminants present in mining effluents (e.g., metals, ammonia), variability in pH from one sample to the next can result in variability in toxicity test results. Conductivity provides an indication of the concentration of total dissolved solids and although there is no direct relationship between conductivity and toxicity, measurable changes in conductivity often reflects a change in effluent quality.

Table 8. Summary of Advantages and Disadvantages of the Various Sample Types

Sample Type	Advantages	Disadvantages
Grab Sample	• Easiest to collect • Provide a measure of instantaneous toxicity • Lowest cost in terms of equipment, manpower, and time	• Highest probability of missing contaminant spikes
Batch	• Increases the probability of capturing contaminant spikes • Low cost in terms of sampling equipment required	• Requires longer time commitment than simple grab sample
24-h Composite Sample	• Further improves the probability of capturing contaminant spikes relative to the other two methods	• Highest cost in terms of equipment and operating costs • Effects of a toxicity spike may be masked by dilution

Sample Volume. Sample volume can influence the results of acute lethality tests. Sample volumes that are too small may increase the potential of collecting a sample that is not representative or may affect the rate of change in sample quality over time. The Reference Methods specify the volume of sample required for conducting both the single-concentration (i.e., 25 to 50 L) and multiple-concentration, LC50 (i.e., 50 to 100 L) tests (Table 7). The amount of sample required for testing is related to the type of test (single- or multiple-concentration) and the specified loading rate (i.e., volume of sample per unit number/size of the test organisms). The loading rates specified in the Environment Canada methods are intended to be sufficient to compensate for the potential loss of toxicant over time (i.e., due to volatilization, adsorption to the test container, or uptake by the test organism) is negligible. In the case of rainbow trout, the volume of sample required is based on a loading rate of 0.5 gram of fish per litre of effluent. Table 9 illustrates the relationship of fish size to sample volume based on this requirement.

Table 9. Minimum sample volumes required for single- and multiple-concentration tests with rainbow trout.

Fish Size (g)	Minimum Sample Volume (L)*	
	Single-concentration Trout Test	Multiple-concentration (LC50) Test
0.3 (minimum size)	11	22
1.0	20	40
1.5	30	60
2.0	40	80
2.5 (maximum size)	50	100

* Sample volumes are based on the Reference Method requirement that there is a 15 cm minimum height of the test solution which must be considered when determining appropriate sample volumes, in addition to the loading rate requirement of 0.5 g/L. Estimates are also based on the use of a standard 23 L cylindrical plastic pail (Diameter x Height (cm): 30.5 x 38.1).

The volume of sample collected can also affect the sample shipping costs, particularly in the case of the rainbow trout test, where the volume of effluent can be substantial (11 to 100 L per sample). As

the size of fish available for testing can vary over time for any given laboratory, effective communication between the sampling personnel and the testing laboratory will help to ensure that sample volumes are adequate for testing purposes, and kept to a minimum to avoid shipping excessive amounts of sample.

Sampling Location. The sampling location(s) should be clearly identified in the sampling plan so that there is little or no uncertainty about the designated sampling point (e.g., may include a physical description of the site, schematic drawing or photograph). Where possible, the sampling site should be clearly marked by some visible means (e.g., tag, flag, or sign). The sampling location may change in response to site conditions (e.g., process change, seasonal conditions which may affect accessibility to the sampling location, etc.). It is important that the sampling location be documented and reported, since effluent characteristics may be quite different at another location and may impact upon the results of a test. Knowledge regarding sampling location variation may also be useful in understanding variations in test results.

Sampling Equipment. Sampling materials that come into contact with the test solution (e.g., sampling equipment, pumps, hoses, sample containers) need to be clean and thoroughly rinsed with a small amount of the sample being collected. The Reference Methods provide a list of recommended materials that are considered to be relatively inert and therefore appropriate for the purpose of sample collection and storage (e.g., sampling buckets made of stainless steel, sample containers made of glass, polyethylene, or polypropylene). Toxicity testing laboratories will normally provide sampling kits consisting of sample containers (e.g., plastic carboys or pails with plastic liners, glass or plastic sample jars, shipping labels and chain-of-custody or sample submission forms).

It is important to ensure that these materials are available and ready for use at the time of the sampling event. Therefore, it is recommended that the sampling equipment and materials are stored in a safe, clean area with limited access. Use of other "non-standard" equipment or materials may result in contamination of the sample and must be avoided.

Food-grade plastic liners are available and recommended for use with the standard 23 L (5 gallon) plastic pails when the containers are being recycled. The liner is used to prevent contact of the sample with the walls of the container, thus reducing the potential for contamination of the sample. These liners may leak if not handled properly, thereby allowing direct contact of the sample with the wall of the container, and the potential for contamination. This can be avoided by taking extra care when filling the sample container or by using a second liner (i.e., double bagging).

It should be noted that plastic liners have the potential to leach materials, under certain conditions, which may be toxic to aquatic biota. This was reported by at least one laboratory, wherein samples of very warm effluent where collected in containers fitted with a plastic liner (Moran *et al*., 2000). It was discovered that a particular batch of liners leached with hot water, released a plasticizer compound that caused toxicity in tests. It may be advisable for laboratories to check each batch of bags received from a supplier with a hot water test, particularly if these are being supplied to the mine. Environment Canada's test methods recommend that samples be cooled prior to shipping. It may be advisable for mine staff to cool samples prior to transferring the sample into pails fitted with the food-grade liners.

Standard Operating Procedures. Standard operating procedures (SOPs) should be prepared for use by mine technical staff for sample collection, labeling, handling and shipping of effluent samples. This standardization is important and will increase the likelihood of testing a representative sample. Failure to follow sampling SOPs can result in inconsistencies in the manner in which samples are collected over time, increase the risk of sampling errors, and introduce uncertainty relating to the source and integrity of the sample. The SOP should identify all sampling locations and equipment required for sampling, contain information relating to sample type and volume, sample identification and labeling, use of chain-of–custody or sample submission forms, preservation details, mode of shipping, and address and contact name at the testing laboratory. Environment Canada is developing a template to assist mine personnel in the preparation of a sampling SOP (Environment Canada, Draft 2000/04/06).

Chain-of-Custody (COC). A Chain-of-Custody (COC) or sample submission form should be included with each sample to document the details of collection and handling of the sample during transport. Environment Canada requires labeling of samples to include the sample type, source, date and time of sample collection and name of sampler(s). Failure to provide a COC or sample submission form with the sample can result in compositing errors by the testing laboratory, exceedance of sample holding time (i.e., maximum time allowed between sample collection and initiation of testing), or failure to conduct the appropriate test.

Mode of Sample Transportation. Sampling personnel should establish a means of sample transportation that is reliable, and can provide prompt delivery of the sample to the testing laboratory. Lack of an established procedure can result in shipping errors that may cause samples to arrive 'late' (i.e., exceed the sample holding time).

Sample Labeling. Most laboratories supply labels that include prompts for obtaining all of the required sample identification information. The labels should be completed at the time of sampling. The Reference Methods require samples to be labeled with the sample type, source, date and time of collection, and name of sampler(s). This ensures that the sample integrity is traceable. Missing information or errors in sample identification may result in rejection of the test data by the regulatory authorities. Labeling the sample container is critical for ensuring proper identification of the sample when it arrives at the testing laboratory and ensuring that errors are not made when compositing samples collected from the same source. When using the 23 L plastic pails with the snap-on lids, it is important to label both the lid and the pail, since the lids can become separated from the container during shipping.

4.2.2 Sample Transport

Key Guidance: The time spent in transit should be minimized by the selection of an appropriate method of shipping. In general, ground transport is the most commonly used and cost-effective mode of transportation where delivery is expected to occur within 48 hours of sample collection. Air transport is recommended in cases where ground transport cannot ensure delivery of the sample within the maximum allowable holding time (i.e., 5 days). Air transport may also be used in cases where the source of toxicity is known to be related to the presence of contaminants with low persistence or possibly in support of Toxicity Identification Evaluations (TIEs) where holding time may be critical.

Sample integrity can be affected during transport by temperature and total time spent in transit. Sample integrity is most likely affected during either winter or summer months when the potential

for temperature extremes is the greatest. While many of the common mining effluent contaminants are relatively stable at moderate temperatures (i.e., 4 to 20 ºC), others, (e.g., thiosalts, xanthates) may be affected by either high or low temperatures. Heating of samples during transit is of concern during the summer months, while freezing of samples is a concern during winter months. Environment Canada's Reference Methods specify that "samples must be kept from freezing" during transport as this may have an impact on sample integrity. Furthermore, it is recommended in the test methods that samples should be kept dark, at a temperature from between 1 to 8ºC (preferably 4 ± 2ºC) if they spend more than two days in transit.

Samples collected for conducting tests with *D. magna* (i.e., 2 – 4 L) can be shipped in coolers packed with ice or ice packs. In practice, keeping the larger 20 L volume samples cool during transport is more difficult. While ice packs can be inserted between the sample liner and the wall of the container as a means of keeping the sample cool, this practice is likely to be of limited value if the samples are warm to begin with (i.e., > 8ºC). Cooling these samples prior to shipping is recommended. If ice packs are required, use of a second plastic liner is recommended as a precautionary measure to prevent possible contamination of the sample in the event of a leak in the liner. Alternatively, 12 L plastic collapsible containers can be utilized in place of the 23 L plastic pails or carboys. Two of these containers will fit inside a cooler (≈ 0.06 m^3), packed with ice or ice packs.

Collecting samples late in the week should be avoided if sample delivery within the regular work week (i.e., Monday to Friday) cannot be assured. While most toxicity testing laboratories operate on a 7-day work week, delivery of samples over weekends may not be possible. Arrangements for weekend deliveries, if required, should be discussed with the testing laboratory. Where a sample is collected on a Friday and weekend delivery is not possible, the sample should be refrigerated and shipped the following Monday.

Information regarding the sampling schedule and shipping arrangements should be made available to the testing laboratory. Providing advance notice to the testing laboratory of the sampling schedule will help to ensure that adequate resources (i.e., staff, space, and test organisms) are available to process the sample in a timely fashion. Providing additional shipping information (i.e., mode of transport, name of carrier, waybill number, date sample was shipped) will assist the laboratory in the early detection of a sample that is missing or lost in transit. Notice of late or missing samples should prompt the laboratory to follow up with a call to the mine to help resolve the issue.

4.2.3 Sample Storage

Key Guidance: The general assumption is that the toxicity of a sample is most likely to decrease with holding time, due to factors such as contaminant biodegradation, hydrolysis and absorption. However, this may not be the case, for example, for samples having a high biological or chemical oxygen demand, where prolonged storage could lower dissolved oxygen levels and lead to increased toxicity. These factors can be minimized by ensuring that air is excluded when filling sample containers and by using refrigerated storage or ice during shipment, where appropriate (as recommended in the Reference Methods).

Environment Canada's test methods describe various options relating to the storage of samples upon arrival at the laboratory. In relation to the requirement that acute lethality testing should begin within three days and must commence no later than five days after termination of sampling,

the Reference Methods provide three options for sample storage. These include: (1) adjusting the sample immediately to the test temperature and commencing the test; (2) adjusting the sample overnight (i.e., gradually) to the test temperature and commencing the test; and (3) cooling the sample to 8ºC or less, preferably 4 ± 2ºC, if longer storage time is required (up to the maximum of five days from the date of sample collection).

Depending upon the type(s) of contaminant(s) present in the effluent, the choice of storage method can potentially influence the outcome of the test. For example, rapid warming of a sample to achieve the desired test temperature may result in a supersaturated solution (in terms of dissolved gases). If this condition arises, there is a requirement to pre-aerate the test solution (to a maximum of 30 minutes for tests with *D. magna* and to a maximum of 120 minutes for tests with rainbow trout (30 minutes initial aeration plus 90 minutes pre-aeration)). This may have implications for toxicity, particularly if volatile toxicants are present. Storing the sample overnight at the appropriate test temperature can eliminate the potential for supersaturation of dissolved gases to occur (provided that the containers are exposed to the air), but this adds additional storage time. In this case, test results may be affected if toxicity associated with the sample is related to the presence of non-persistent contaminants. Longer storage of samples at cold temperature (4 ± 2ºC) can also affect test results if sample toxicity is associated with volatile or non-persistent contaminants.

In the case of mining effluents, contaminants including ammonia and certain heavy metals (e.g., copper, nickel, iron, zinc, silver), should not be greatly influenced by storage conditions over the 5-day holding period. However, other contaminants (such as thiosalts), are only stable at extremely low temperatures (i.e., below 4ºC) and will degrade over time as temperature increases (some thiosalt species more readily than others). [Note: The presence and subsequent degradation of thiosalts in an effluent may be recognizable by the occurrence of a downward pH shift of the sample over time; this also depends upon the buffering capacity of the water.]

4.2.4 Sample Handling

Key Guidance: The sample should be homogeneous and representative of the effluent to be tested. Agitation, mixing, and compositing of samples should be done with care to avoid entrainment of air which may alter sample integrity.

Water quality parameters, such as hardness and pH, are known to influence toxicity. For example, metals such as copper and zinc, are more bioavailable and hence more toxic in low hardness waters than in high hardness waters. Their toxicity is also affected by pH. Thus adjusting either of these water quality parameters may influence the toxicity of the sample or the response of the organisms to other effluent constituents. To minimize this influence, Environment Canada test methods generally limit the treatment of samples before and during testing. For example, the Reference Methods do not allow any adjustment in sample pH during regulatory testing. However, if the sample pH is outside the recommended range, a second test with pH adjustment could be conducted in parallel. In two situations, the sample can be treated to eliminate a known stress to the organism. One is a hardness adjustment of the effluent when testing low ionic strength effluents with *D. magna*. The other example is the practice of aerating the test solution in rainbow trout tests. These noted exceptions are permitted in order to reduce potential stress to the test organisms. As the low ion content and dissolved oxygen conditions in the effluent prior to adjustment may be lethal to those *Daphnia* and trout (respectively), adjustments are permitted to

attempt to ensure that the test response is related to the presence of effluent contaminants and not to poor water quality conditions.

4.3 Guidance for the Collection of Effluent Samples for Split-Sample Testing

Details related to split-sample testing are not included in the Environment Canada Reference Methods but are discussed here since it is a practice that has been used by government, private industry and non-government organizations. By simple definition, a split-sample is one sample from a given source that has been subdivided into two or more sub-samples. Split-sample testing is used in laboratory proficiency testing programs for measuring the performance of individual laboratories and in round- robin testing exercises designed to develop and validate new test methods. From time to time, mine personnel are interested in determining the inter-laboratory variability associated with the testing of the mine's effluent. To this end, they will conduct a "round-robin" with an effluent tested at two or more testing laboratories. The "round-robin" (or inter-laboratory) exercise comprises the collection, homogenization, transport, handling, reception of the sample, and the implementation of the test. The goal of properly implementing split-sample testing is to ensure that the test data from the participating laboratories are comparable, since, if the samples are collected and homogenized properly, the laboratories are, in fact, testing the same sample.

The critical steps in collecting samples for split-sample testing are as follows:

1. ensure the integrity and cleanliness of all sampling equipment and materials prior to sample collection;
2. ensure that a representative sample is collected;
3. ensure that mixing is adequate to ensure the homogeneity of the sample (if possible, on-site measurement of sample conductivity, temperature, or pH);
4. collect equal volume sub-samples in appropriate storage containers;
5. ensure that an appropriate mode of transportation is secured in order to achieve timely delivery of samples to all participating laboratories;
6. ensure that the testing laboratories are provided with a study schedule and study design including adequate instructions for sample handling (including compositing of samples, if required), storage (if appropriate), testing, data collection, and reporting; and,
7. ensure that the sample temperature is recorded upon arrival.

4.3.1 Sample Collection

The most critical aspect of sample collection is obtaining a homogeneous sample. The method used to achieve this will depend upon the quality of the sample at the sampling point, specifically in terms of its variability over time. If the sample is homogeneous and identical samples can be withdrawn repeatedly, then sub-samples can be drawn directly from the sampling location. However, if this is not the case, then successive samples will need to be composited in a single container, large enough to contain the total volume of sample required. Once this has been achieved, mixing is required to achieve homogeneity of the sample prior to the collection of sub-

samples. Homogeneity of the sample can be confirmed by measurement of relevant water quality parameters (e.g., conductivity, pH).

4.3.2 Sample Delivery

A well-planned sampling program (with particular attention paid to all of the critical steps identified above) is essential to successfully meeting the goal of split-sample testing. The sampling program and sample transport schedule should ensure that samples are delivered to all of the participating laboratories within a reasonable time frame (e.g., within 24-h of sample collection). It is also advisable to use the services of a transport company that can adequately track samples while they are in transit. This can assist in the decision-making process of whether to proceed or to delay the testing based on missing samples. Finally, shipping addresses and contact names for each of the participating laboratories should be verified in advance.

4.3.3 Sample Reception and Test Initiation

The potential for changes to the sample integrity of the various sub-samples due to sample transport times, storage conditions etc., makes the testing of split-samples particularly sensitive to timing. Therefore, a great deal of effort and attention must be paid to scheduling both the sample collection and the testing. Vital to both aspects, is the transportation of the samples from the field to the participating laboratories, maintaining adequate temperature control, and sample container sealing. It is also important that testing by all of the participating laboratories be initiated within a similar time-frame (i.e., within the same working day).

4.3.4 Additional Considerations

All data produced must be generated in accordance with the same test method and related quality assurance/quality control (QA/QC) procedures. Furthermore, all test results from controls must meet the required test acceptability criteria (i.e., control survival), and all data and calculations produced should be made available to the organizing body and be capable of being verified.

4.4 Culture/Dilution Water Characteristics

4.4.1 Source

Key Guidance: The water supply should be of uniform quality, adequate quantity, should not contain contaminants that could produce toxicity and should be able to sustain the survival, health, and/or reproductive fitness of test organisms on a year-round basis.

Environment Canada's methods allow for the use of different sources of water for the purposes of culture, holding and/or testing. These include "an uncontaminated supply of groundwater", "surface water", "dechlorinated municipal drinking water", "reconstituted water adjusted to the desired hardness" or "upstream receiving water taken from a water body to be tested".

The chlorine concentration in municipal tap water has the potential to be lethal to both test species. It is important to note that municipalities periodically increase the chlorine content of their water supplies; therefore, testing laboratories that rely on a municipal water supply must have a rigorous system in place to monitor and remove chlorine and chlorinated compounds. Environment Canada's requirements pertaining to the use of municipal water is that "it must be free of harmful concentrations of chlorine or chlorinated compounds (e.g., chloramines)". The target value for total residual chlorine is ≤ 0.002 mg/L (Environment Canada, 1990a,b).

Measurements of water hardness, pH, conductivity, dissolved oxygen, and total dissolved gases, should be made frequently and as necessary to document variation. More detailed analyses (i.e., for metals, PCBs, organochlorine and organophosphorus pesticides) may be conducted periodically, including total residual chlorine (if municipal water is used).

4.4.2 Water Quality

Culture/Holding

Key Guidance: Optimally, the water used for culture/holding should consistently support good survival, health and growth of test organisms and should be the same or similar to the water used for dilution of the effluent (i.e., in the case of a multiple concentration LC50/EC50 test) and for use as the negative control.

Environment Canada's Reference Method for conducting tests with *D. magna* allows organisms to be cultured in a range of water hardness levels, depending on the source of water used. If natural water is used, the test methods recommend a water hardness of 80 to 250 mg/L, while cultures maintained in reconstituted water can have a hardness of 80 to 100 mg/L. There are no similar requirements pertaining to the holding of rainbow trout (i.e., no limits for water hardness).

The normal geographic distribution of *D. magna* is generally limited to waters in northern and western North America which have hardness values > 150 mg/L (Pennak 1978; Greene *et al.* 1988, Environment Canada 1990d; EPS 1/RM/11), but healthy laboratory cultures can be maintained over a range the 80 to 250 mg/L (Environment Canada, 1990d). In Canada, *D. magna* is used to test effluents, receiving waters and cooling waters with low water hardness levels (i.e., < 25 mg/L as $CaCO_3$). In recognition of their preference for hard water, the Reference Method (Environment Canada, 2000a) allows for an adjustment of low hardness effluents to 25 ± 1 mg/L (as $CaCO_3$) when *D. magna* is the test organism. While the lower level of 25 mg/L is considerably lower than the recommended hardness level of 80 mg/L for culturing this organism, it has not been shown to cause adverse effects on survival in short-term (i.e., ≤ 48-hr) exposures.

There are no similar hardness tolerance issues concerning the holding of rainbow trout, since rainbow trout are tolerant of a wide range of salinity and water hardness levels (Scott and Crossman, 1973)

In recognition of the fact that *D. magna* prefer hard water, Environment Canada's generic method EPS 1/RM/11 recommends the use of *D. pulex* when testing soft water samples. This species can be found in low hardness waters. It occurs over most of North America with the exception of the tropics and high Arctic (Weber, 1993).

Water quality of the culture/holding water can be a contributing factor to the variability of test results. For example, studies have shown that animals cultured in high hardness, high alkalinity waters were generally more tolerant to certain contaminants (e.g., metals) than animals cultured under conditions of low hardness and low alkalinity.

Dilution Water

Key Guidance: 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. Variability within a laboratory should be minimized by using the

same water source, provided that the quality of the water is closely monitored and controlled. Variability among laboratories may be expected when laboratories use different dilution waters.

The objective of acute lethality testing for regulatory purposes is to determine the inherent toxicity of the effluent. This objective can be met by the use of natural or synthetic (artificially prepared) dilution waters as described in Environment Canada's methods. However, as noted above, dilution water quality conditions can modify the outcome of the test if the contaminants causing toxicity are influenced by factors such as pH and water hardness (e.g., ammonia and metals). In certain cases, 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. Also, dilution water quality is less likely to be a source of variability within a given laboratory where differences in water quality should be less variable.

There are certain instances where alternate sources of dilution water may be more appropriate. For example, tests intended to eliminate gradient effects due to differences in pH or hardness of the effluent relative to the dilution water, may require adjustment of the dilution water quality to match that of the effluent.

4.5 Organism History and Handling

Key Guidance: Environmental stress and associated disease problems are minimized by high water quality standards, optimum rearing densities, and adequate nutrition. Variability of test results related to organism history and handling can be reduced by adherence to health criteria and the standardization of methods.

Stress can play a major role in the susceptibility of organisms to disease. "The difference between organism health and sickness depends on a delicate balance resulting from the interactions of the disease agent, the animal and the environment" (Piper *et al*., 1982). Behavioral changes, increased mortality or reduced tolerance to standard reference toxicants can be used to monitor the level of stress in test organism stocks and are included as part of QA/QC practices described in EC's test methods.

Disease can generally be defined as any deviation of the body from its normal or healthy state causing discomfort, sickness, or death. Disease-causing organisms to rainbow trout or *D. magna* include: viruses, bacteria, fungi, protozoans, and a range of invertebrate organisms. Disease may be recognizable by changes in behaviour, or other obvious signs. In fish, behavioural changes in response to disease, parasite or other physical affliction can include: loss of appetite, abnormal respiration, coughing, abnormal distribution in the tank (e.g., swimming at the surface, along the tank sides or in slack water), abnormal swimming patterns (e.g., flashing, darting, whirling or loss of equilibrium), and loss of vitality or reduced tolerance to handling. Physical signs of disease or parasitic infection may include: discolouration; eroded areas or sores on the body, head or fins; swelling; popeye; haemorrhages; and cysts or lesions.

Prevention of disease can be best achieved through minimizing key causative factors. Nutritional deficiencies resulting from improper balance of the major components of the diet (including proteins, amino acids, fats, carbohydrates, and fibre) are often the major cause of secondary bacterial, fungal, and parasitic disease. The Reference Method for the rainbow trout acute lethality test states that "chemical treatment of diseased fish should be avoided". However, if the use of

chemically-treated fish cannot be avoided, they must not be used in a test for at least two weeks subsequent to their treatment. If it is necessary to resort to the treatment of fish, the proper treatment method should be implemented based on the pathology of the diseased fish. Table 10 provides some examples of treatment alternatives for various types. Regardless of the type of treatment, it is important to follow prescribed instructions to ensure that the fish are not stressed or impaired by the treatment itself. Care should be taken in order to ensure that disease does not spread to other batches of fish in the testing facility.

Table 10. Examples of Treatment Alternatives for Various Types of Infections Common to Fish

Disease/Disorder	Symptoms	Treatment
Bacterial Gill Disease[1,2]	Fish gasping at surface ("sharking") or congregating at water inlet. Listless, little movement. Flared gills, reduced appetite. Sharp increase in mortality over a three-four day period.	Chloramine T – Used as prolonged dip (1 g in 200 L of water for 60 min.). First add 1 g to 20 L, then apply this to holding tank with 180 L of water, under static conditions. Apply heavy aeration. After 1-h, drain tank to minimum level and refill. Repeat 1-h dip for 3 consecutive days
		Formaldehyde (formalin) - Used as a prolonged dip (1:4,000 to 1:8,000 for 30 to 60 minutes). Apply heavy aeration. After 1-h, drain tank to minimum level and refill. Repeat 1-h dip for 3 consecutive days
		Combinations of the above treatments can be used as well (e.g., Day 1- chloramines T, Day 2- Formalin, Day 3- chormamine T or alternate starting with formalin)
Fin Rot[1]	Dark coloured skin. Fins and tail eroded (ragged edge). Lethargic.	Same as for gill disease.
Furunculosis[1] (caused by *Aeromonas hyophilla*)	Short period of reduced appetite before mortalities visible. Darkening or swelling of shoulders and back.	Sacrifice stock. Furunculosis can reoccur after treatment.
Fungus[3]	Fungus on fish and eggs	Malachite green - Used as a dip for fish (1:15,000 solution of malachite green for 10 to 60 seconds, repeated over 2 to 3 times); For eggs (1:200,000 concentration for 1 hr)
		Wescodyne, Argentyne (1:300 concentration for 10 minutes). Wescodyne is harmful to fish at concentrations ≥ 1:20,000

[1] J. Schroeder, Ontario Ministry of the Environment, *pers. comm.*

[2] J. Reid, ESG International Inc., *pers. comm.*

[3] Piper *et al.,* 1982

The assessment and treatment of disease in fish is a complex process requiring considerable expertise; a full discussion of this topic is beyond the scope of this document. Many of the substances used to treat disease in fish are themselves toxic, and therefore, proper care and procedures must be applied in handling and disposal.

In *D. magna* cultures, ephippial eggs are a direct indication of stress or change in environmental conditions in the culture. Changes or stressors include, but are not limited to: temperature, light intensity, photoperiod, etc. The production of an ephippial egg is the daphnid's natural survival response to adverse conditions. Under favorable environmental conditions, daphnids reproduce

parthenogenetically (i.e., females produce females and no males are required for reproduction). Males and females capable of sexual reproduction are produced in the event of unfavorable conditions. Fertilization results in the production of an ephippial, or "resting" egg. The egg will not hatch until it undergoes harsh stress, such as freezing or drying.

Requirements specified by Environment Canada, and listed in Table 11, provide a means to ensure that the practices used within a laboratory and by different laboratories for the culture and maintenance of test organisms are consistent and meet a standard level of performance in terms of organism health and fitness. The health and fitness of the animals used in tests is key to minimizing test variability and maximizing the applicability of test results for environmental protection. However, several of the requirements listed above allow for a range of conditions and may have the potential to contribute to variability in test results. These requirements are discussed below.

4.5.1 Nutrition/Food

Key Guidance: Nutritional deficiencies resulting from improper balance of the major components of the diet can be a major cause of stress, secondary bacterial, fungal, and parasitic disease, which can lead to reduced health, reproductive impairment or death of trout or daphnids.

It is important to establish the proper feeding conditions to ensure good health of the test organisms. Overfeeding may have a detrimental effect on water quality, whereas underfeeding may cause malnutrition. Either of these conditions may lead to undue stress on the test organisms. For rainbow trout, the Reference Method recommends that feeding should be once or more per day with a standard commercial pelleted fish food (i.e., at a rate of 1 to 5% of wet body weight) as recommended by manufacturer. Feeding at the recommended rate will help to ensure a healthy culture.

To determine the appropriate feeding rate, accurate records of fish size and density (number of fish per tank) are required. Weekly estimates of fish mass may be made by determining wet weights of a representative sub-sample of fish from the holding tank. This may be done either by sacrificing a representative number of fish from the tank or by carefully netting, blotting, and transferring a sub-sample of live fish to a tared beaker of water. Individual fish weight estimates may then be multiplied by the number of fish in the holding tank and these data may be used to determine appropriate feeding rates. It is necessary to maintain accurate records of the number of fish contained in the holding tank in order to keep feeding rates current. This may be done simply by recording numbers of fish initially moved to individual holding tanks and amending this number daily by recording mortalities and the number removed for testing.

Table 11. Summary of Environment Canada's requirements relating to Organism History and Handling.

Requirement	Test Method	
	D. magna	**Rainbow trout**
Source	Laboratory stock culture	Commercial fish hatchery (fish free of known diseases)
Culture system	All equipment and materials in contact with test organism must be non-toxic	
Acclimation	Cultures must be maintained for 7 d (min) in water with a hardness equal or similar to the control/dilution water to be used in tests	Fish must be acclimated to test conditions for at least two weeks immediately preceding the test and must continue until mortality in the stock tank is < 2% in the 7-d period immediately preceding the test. If mortality exceeds 10% per week and is related to disease or contaminants in the water, that batch of fish must not be used. Fish must not be used for at least two weeks following treatment for disease.
Feeding	Algae (min. of one species), mixture of two or more algal species is preferred in addition to a supplement of yeast, Cerophyll™ and trout chow	Single or multiple feedings, daily with a standard commercial pelleted fish food, at a rate of 1 to 5% of wet body weight
Maintenance	Replace culture water on a weekly basis	Fresh water supplied at a rate of ≥ 1.0 L/min per kg of fish held. Daily removal of dead or moribund fish. Tanks to be kept clean and free from excess food and faeces.
Density	≤ 20 animals/L	Tank volume must be ≥ 1.0 L water per 10 g of fish.
Handling	Transfer animals by pouring, pipetting or siphoning	Transfer animals by netting, and as quickly as possible to reduce stress
Health Criteria	Daily monitoring to establish cultures are free of ephippia	< 2 % mortality in the stock tank, in the 7-d period immediately prior to testing
	Adults must have 1st brood within 12 days of age	Mortality of test fish must be monitored and recorded at least 5 days per week in the stock tank.
	2 – 5 week old adults must deliver an average of ≥ 15 neonates/brood	
	≤ 25% mortality in brood stock during 7 d prior to a test in a culture of mixed ages	

Feeding activity is a sign of fish health and should be monitored frequently. Trout should be actively and vigorously taking food from the surface as soon as it is introduced. As a general rule, each ration of food should be completely consumed within five minutes of introduction, and the food should not be allowed to accumulate on the bottom of the tank. Food build up at the bottom of a tank can be indicative of overfeeding or the loss of fish appetite (which can be caused by disease, infection, or a number of other stressful conditions).

The health of a daphnid culture depends largely on the feeding regime and quality of food. The diet should be sufficient to maximize metabolic and reproductive activity. The use of unhealthy or stressed daphnids in a test could bias the results. Although at least one algal species must be used for feeding daphnids, a mixture of at least two algal species is recommended for feeding, as well as a supplement such as yeast, Cerophyll™ and trout chow.

Research suggests that maintaining cultures on an artificial diet alone can result in neonates that are more susceptible to toxicants and have a shorter life span (Cowgill, 1989). To reduce variability with regards to organism sensitivity in toxicity tests, it is important to maintain a consistently healthy and thriving culture of daphnids. As food is prepared on a batch basis, procedures relating to preparation, storage, and general feeding regime should be well documented and followed. Environment Canada's test methods provide guidance on food preparation and feeding; however, the choice of diet, ration and feeding regime is left to the discretion of the laboratory. Culture health data is a good indicator of the food quality, assuming that other factors relating to culture water quality and/or handling are not at issue.

Excess food and waste may tend to build up in the culture vessels, causing a change/degradation in water quality (e.g., fluctuations in pH and concentrations of dissolved oxygen, gradual increase in water hardness, conductivity, and ammonia). It is important to maintain constant conditions within the culture to avoid any possible variability in the quality of test organisms. Minimal fluctuations in culture water pH may not adversely affect the daphnids, however, if algal production within the culture is in excess of the amount being consumed, pH will tend to rise. In some waters, pH may increase to levels above the range recommended in the Reference Methods. Thus, frequent water replacement will help to control water quality within the cultures. The Reference Method recommends that water in cultures be almost completely replaced at least once a week. More frequent water replacement may be required if there are noticeable changes in either water quality (e.g., pH, hardness) or condition of the test organisms (e.g., presence of males and/or ephippia in cultures).

4.5.2 Acclimation

Key Guidance: Use of test organisms that have not been properly acclimated to the test conditions can result in additional stress to the organisms, which can result in variability of effluent acute lethality test results. Physico-chemical factors contributing to stress on fish and daphnids held under laboratory conditions include: handling, crowding, water quality, and physical disturbances. Environmental stress and associated disease problems are minimized by high water quality standards, optimum rearing densities, and adequate nutrition.

Acclimation in relation to the use of the Environment Canada test methods refers to "the time period prior to the initiation of a toxicity test in which aquatic organisms are maintained in untreated, toxicant-free dilution water with physical and chemical characteristics (e.g., temperature, pH, hardness) similar to those to be used during the toxicity test." For compliance with the Environment Canada's regulatory test methods for rainbow trout and *D. magna*, most laboratories use the same water (i.e., one of the water sources recommended by Environment Canada) for culturing, holding and testing purposes. As noted in Section 4.4.2 (Dilution Water), other sources of dilution water may be more appropriate than the standard laboratory dilution water. For either of the stated examples, additional acclimation of the test organisms could be required where the hardness level of the culture/holding water used for rearing the test organisms differs from that of the dilution water by more than 20% (Environment Canada, 2000a,b).

4.6 Test Method Requirements

Key Guidance: Deviations from the requirements provided in the Environment Canada test methods can potentially contribute to variability of effluent acute lethality test results, lead to unnecessary additional testing and costs to comply with the monitoring requirements or cause false positive or negative test results.

One of the key factors relating to minimizing variability of acute lethality test results relates to adherence to the test method (Grothe *et al*., 1996). Environment Canada's test methods provide a set of standardized procedures and conditions for conducting the tests. Standardization of the test method is critical to controlling, as much as possible, conditions of the test that might otherwise contribute to variability. However, to ensure that these requirements are being adhered to and the testing is being carried out in a consistent fashion, laboratories should develop an internal structure based on standardized laboratory practices. This includes a quality manual, standard operating procedures (SOPs) for all activities relating to organism culture, holding, maintenance, testing and related QA/QC practices, appropriate tools for workload management, and a well-documented mechanism for staff training and performance. A description of each of these items is provided in detail in Subsection 6.1.2 (Quality System Documentation). Infrastructure designed to promote and support these processes will help to ensure that reliable, valid, and consistent test results are delivered, and that test failures due to reduced fitness of the test organisms, or inconsistencies and errors in the documentation and/or performance relating to the conduct of a test are minimized.

Table 12 summarizes the basic requirements of the test as identified in the Reference Methods for *D. magna* and rainbow trout. Most of these requirements are sufficiently restrictive to minimize much of the potential variability associated with the performance of the test. However, several of these requirements allow for a range of conditions, which has the potential to contribute to test method variability, particularly when applied to mining effluents. These are discussed in detail below.

Table 12. Summary of Reference Method Test Requirements for *D. magna* and rainbow trout (including May 1996 and December 2000 amendments).

Requirement	Test Species	
	D. magna	**Rainbow trout**
Sample Storage Time:	5 days from completion of sample collection	
Storage Conditions upon receipt at testing laboratory	Adjust to test temperature and initiate test on the day of receipt; store overnight at test temperature and commence testing; and refrigerated storage at 1 – 8°C if longer storage is required	
Test Method (for regulatory purposes):	EPS 1/RM/14	EPS 1/RM/13
Dilution Water Hardness:	80 to 250 mg/L as $CaCO_3$ if groundwater surface water, or municipal tap water is used; 80 to 100 mg/L as $CaCO_3$ if re-constituted water is used	No range specified
Dilution Water pH:	6.0 to 8.5	
Test Type:	Acute, static	
Test Duration:	48-hr	96-hr
Test Temperature:	20 ± 2°C	15 ± 1°C
Light Intensity:	400 – 800 lux at water surface	100 – 500 lux at water surface
Photoperiod:	16 ± 1-h light: 8 ± 1-h dark	
Feeding:	Terminated prior to testing	Terminated 16-h prior to testing
Pre-aeration (prior to test):	None unless D.O. is < 40% or > 100% air saturation value. Up to 30 min (max) if required.	30 min (mandatory), and up to an additional 90 min (max) if D.O. is < 70% or > 100% air saturation value
Aeration of Test Solutions:	None	6.5 ± 1 mL/min·L (continuous)
Loading rate:	1 animal per 15 mL of test solution	≤ 0.5 g of fish per litre (based on 96-h exposure)
Age/Size of Test Organism	Neonate (≤ 24-h old)	0.3 to 2.5 g
Test Vessel:	Glass or plastic	
Test Volume:	≥ 150 mL, identical volumes in all test vessels	≥ 15 cm solution depth, identical volumes in all test vessels
No. of animals per test concentration	30 (min) for a SC test 10 (min) for an LC50 test	10 (min) for a single- concentration test 10 (min) for an LC50 test
Observations for Mortality	0 and 48-h	0, 24, 48, 72 and 96-h
Water Chemistry:	Temperature, pH, D.O. at start and end of test. Conductivity and hardness at start of test	Temperature, pH, D.O. at start and end of test. Conductivity at start of test
Test Endpoint(s):	Mortality/Immobility	Mortality
Validity Criteria	≤ 10% mortality or abnormal behaviour in controls, or if ≥ 2 organisms in one test vessel exhibit atypical or stressed behaviour	≤ 10% mortality or abnormal behaviour in controls

4.6.1 Aeration of Test Solutions

Key Guidance: 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. Since both of these factors can contribute to variability of test results, the Environment Canada Reference Methods restrict dissolved oxygen levels in the test solution to a specific range and do not allow for adjustment of sample pH. Potential intra-laboratory variability relating to these factors can be further reduced by standardizing the method of aeration. However, the potential for inter-laboratory variability remains, since laboratories currently have two choices available to them for aerating test solutions.

Aeration of the test solutions during the test is provided in tests with rainbow trout, but not in tests with *D. magna*. The requirement for aeration of test solutions, in the case of rainbow trout, is provided to ensure that conditions of low dissolved oxygen do not contribute to the test outcome.

The Reference Methods for aeration of test solutions permit a choice of two options. The options include "bubbling compressed air through a clean, silica-glass air diffuser or disposable glass pipette." In either case, aeration of the test solution is provided at a fixed rate of 6.5 ± 1 mL / min·L. Laboratory experience has shown that the efficiency of these two methods of aeration are not necessarily equivalent, which in turn 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.

This issue influences inter-laboratory variability, in that different laboratories may have a preference for one method over the other. During investigations involving split-sample testing, the participating laboratories should be encouraged to use the same aeration method. Intra-laboratory variability can be minimized by ensuring that the method of aeration is standardized.

4.6.2 Size range of fish

Key Guidance: The size of fish used in tests can influence the outcome of effluent acute lethality test results. Intra-laboratory variability related to fish size can be reduced if laboratories employ measures to narrow the size range. Proper planning and scheduling of test loads is important to ensure that fish of the appropriate size and range are available as needed for testing. Inter-laboratory variability can also be reduced by further narrowing the range of fish sizes used for testing.

Based on feedback received by Environment Canada and an upcoming publication reporting research that supports this (Riebel and Gilron, *unpublished data*), regarding the potential for variability due to the size of fish used in tests, the requirement has recently been narrowed. The amended requirement has reduced the upper size limit of 5.0 g to 2.5 g. This change is reflected in the second edition of the test method (Environment Canada, 2000a).

Reducing the size range is an important step towards minimizing both inter-, and intra-laboratory, variability. Additional steps that can be utilized by testing laboratories to minimize intra-laboratory variability due to fish size is to implement procedures to further narrow the size range of fish used in testing. This can be achieved by holding fish (eggs and fry) at lower water temperatures to reduce the rate of growth and extend the period of time that small fish are available (Note: the Environment Canada test methods permit holding fish at temperatures ranging from 4 to 18ºC). In addition, laboratories can obtain fish from hatcheries that artificially induce spawning of trout outside the normal spawning period.

4.6.3 Range of Dilution Water Hardness

Key Guidance: Dilution water quality can contribute to the variability of test results. This will be most pronounced when the contaminants present in the effluent interact with characteristics (e.g., pH, hardness) in the dilution water in ways that modify their toxicity.

These conditions will be minimized in instances where the dilution water is similar in its water quality characteristics (e.g., pH, alkalinity, hardness) to that of the effluent. Where this is not the case, high concentrations of effluent will more closely resemble the physical/chemical characteristics of the effluent. In contrast, low concentrations of the effluent will resemble those of the dilution water. See section 4.4.2 (Dilution Water).

4.7 Statistical Analyses for LC50 Calculations

Key Guidance: The Probit Method is preferred when calculating a median lethal concentration of an effluent to estimate the concentration where 50% of the test organisms would die within a defined period of exposure (LC50), provided the assumptions of the method are met. The calculated result should be a reasonable estimate that reflects the raw data values. Confidence limits should bracket the LC50 within the concentrations tested. Environment Canada's Reference Methods for rainbow trout and D. magna require that an LC50 for mortality be calculated. However, in tests with D. magna, if an immobility response is observed, a second statistical estimate can be made to calculate an EC50 (effective concentration where 50% of the test organisms would be affected (i.e. dead or immobilized) by exposure to the effluent).

In Section 6 of each of the Reference Methods, guidance on statistics for calculating LC50 and confidence limits are specified (Environment Canada 2000a,b). The methods of statistical analysis are common to both species. The LC50 is based on dead test organisms, whereas the EC50 is based on impaired animals (e.g., *D. magna* test EC50 accounts for dead plus immobilized organisms).

It is generally accepted that acute lethality effects on fish are generally complete within the standard 96-h exposure period. However, this is not always the case in the 48-h tests involving *D. magna*, where immobility of the test organisms is more common. The presence of immobile organisms at the end of a test is suggestive that acute lethality is not complete. This can be a source of variability, in particular, 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., results reported for mortality only).

Various methods for calculating LC50s, EC50s and their confidence limits are provided in Environment Canada's generic methods (1990a, 1990b) including the recommended Basic computer program (Stephan 1977). The methods also provide information on how to obtain a copy of this program. Results calculated using the Probit method are preferred and should be reported. The Probit method functions well if there are at least two partial mortalities in the data set. The calculated result should be a reasonable estimate that reflects the raw data values. Confidence limits should bracket the LC50 within the concentrations tested.

The binomial method can be used to provide an LC50 estimate with conservative confidence limits, if the results do not include at least two partial mortalities. The data trimming technique of the Spearman-Karber method is not recommended for the calculation of the LC50, as users of the program are often not be familiar with the implications of trimming off the ends of the dose-response data.

It is often desirable to compare the results of two compliance (i.e., single-concentration) tests. For example, one may wish to determine if a change in a certain proportion of mortality over two sample periods, are significantly different. This can be done using a two-sample comparison of proportions. For example, the proportion mortality from one 100% effluent exposure can be compared to the proportion mortality for the second exposure. Tables used to make this comparison (i.e., Fisher's Exact Tables) are provided in Appendix D, and can be used to make these types of comparisons for the most commonly-encountered sample sizes used in compliance testing (i.e., when test sample sizes are 10, 30, or 36).

For a comprehensive discussion on the use of statistics in calculating and reporting test endpoints, users of the Reference Methods should refer to the upcoming Environment Canada guidance manual on statistical analysis (Environment Canada, *in preparation*).

4.8 Reporting Requirements

Key Guidance: Provision of accurate and complete documentation relating to the effluent sample, test facilities, conditions and results is ultimately the responsibility of the mine as incomplete test reports could result in rejection of the test data by regulatory authorities. While this information does not have any direct effect on variability in effluent toxicity test results, it will help to reduce uncertainty due to sampling errors, deviations in test methods, verification of test endpoints, and test organism health and performance.

Environment Canada requirements for minimum test reporting are provided in Section 8.1 of the Reference Methods and summarized in Table 13 below. Section 8.2 of the Reference Methods lists additional information that must be kept on file by the testing laboratory for a minimum of five years.

Table 13. Summary of mandatory reporting requirements from Section 8.1 of the Reference Methods for rainbow trout and *D. magna*.

Reporting Requirement	Test Organism	
	D. magna	**Rainbow Trout**
Sample Data	Name and location of effluent source	
	Date and time of sample collection	
	Sample method	
	Description of sampling point	
	Type of sample	
	Name of person(s) collecting the sample	
Test Organism Information	Species	
	Most recent time to first brood	% Mortality of fish in stock tank for 7-d period prior to test commencement
	Mean # neonates/brood % Mortality of the brood stock adults in the week before testing	
Test Facilities	Name and location of testing laboratory	
	Name of technicians performing the test and verifying the results	
Test Method	Type of Test	
	Method Used	
	List of test deviations, if any	
Test Conditions	Date and time test started	
	pH, temperature, dissolved oxygen, and conductivity prior to preparation of test solutions	
	Confirmation of no pH adjustment	
	Conditions of pre-aeration of effluent	
	Test concentrations, volume and # of replicates	
	pH, temperature, dissolved oxygen, and conductivity for each test solution at start and end of test	
	# of animals per test level	
	Loading rate	
	Hardness of sample	Rate of aeration of test solutions
		Mean fork length and wet wt. of control fish (± 2SD), range of weights and sample size
Test Results	# of mortalities in each test solution and controls at the end of the test	
	# of animals controls showing atypical or stressed behaviour	
	48-h LC50/EC50	96-h LC50
	Most recent reference toxicant data including LC50, 95% confidence limits, chemical used and date tested	
	Historical geometric mean LC50 and warning limits (± 2 SD)	

5.0 THE ROLE OF ACCREDITATION IN LABORATORY QUALITY ASSURANCE

5.1 Introduction

In light of the importance of quality assurance measures for producing highly reliable data, this section provides an overview of the role of laboratory accreditation in aquatic toxicology laboratories in Canada. In this section, accreditation is defined and described, an overview of the current regulatory programs requiring or recommending the use of accredited laboratories is provided, summary descriptions of the current major accreditation programs in Canada are presented, and the role of inter-laboratory and proficiency testing in laboratory accreditation is described. Finally, the explanation of primary, secondary and third party laboratory assessment is provided.

Laboratory accreditation is defined as formal recognition, by a registered accrediting body, of the competence of a laboratory to conduct specific functions. It is the process by which a laboratory quality system (i.e., laboratory management system) is evaluated through regular site assessments by the accrediting body, and twice yearly proficiency testing rounds. Laboratory certification is formal recognition, by the certifying body, of the proficiency of a laboratory to conduct specific tests.

5.2 Canadian Environmental Laboratory Accreditation Programs for Aquatic Toxicology Laboratories

5.2.1 Introduction

In Canada, there are currently two major organizations offering accreditation to aquatic toxicology laboratories conducting the rainbow trout and *D. magna* acute lethality tests. These organizations are: 1) the Canadian Association for Environmental Analytical Laboratories (CAEAL) which operates the technical program on behalf of the Standards Council of Canada (SCC); and, 2) the Ministère de l'environnement du Québec (MENVQ). An overview of accreditation programs offered through the above organizations is provided below, and detailed program descriptions are also provided in Appendix E.

5.2.2 Canadian Association for Environmental Analytical Laboratories (CAEAL)

The Canadian Association for Environmental Analytical Laboratories (CAEAL) is a not-for-profit association established in 1989 to address the quality management interests of public and private sector environmental laboratories in Canada. The association's principal objective is to promote and maintain a high level of assurance in analytical test data. To this end, SCC/CAEAL offers a full accreditation program suited to meet the specific needs of environmental laboratories in Canada. This program includes:

- biannual site assessments; and,
- a proficiency testing program.

These are discussed briefly below.

Site assessments, in which the laboratory's quality system is assessed against the International Organization for Standardization (ISO) guide for environmental analytical laboratories (ISO/IEC Guide 17025), are conducted every 2 years, for member laboratories. The assigned scope of testing is based on application information provided by the laboratory. Qualified and trained assessors conduct the assessments on site, by interviewing staff, examining laboratory records, reviewing technical documentation, and inspecting facilities, equipment and the conduct of laboratory testing. In all cases, the assessment is made relative to specific requirements and as a part of the assessment. Any significant non-conformances are noted and corrective actions identified. The prescribed corrective actions may be either non-test-specific (i.e. based on a Rating Guide checklist) or test-specific (i.e., based on a Rating Guide Appendix checklist).

The Proficiency Testing (PT) program targets high-volume testing in the major disciplines of inorganic chemistry, organic chemistry, toxicology, occupational health and microbiology. This program currently includes testing with the following environmental matrices: water, waste oil, soil/sediment, air collection media, and asbestos.

5.2.3 Standards Council of Canada (SCC)

The Standards Council of Canada (SCC) was established in 1970 by order of Parliament under the *Standards Council of Canada Act* (SCCA; amended in 1996) to promote voluntary standardization in Canada, facilitate domestic and international trade, and further international co-operation in relation to standards. In addition the SCC represents Canada in international standards organizations such as the ISO, and the International Laboratory Accreditation Cooperation (ILAC), and is the official accrediting body for ISO in Canada. The SSC also accredits standards development organizations, certification organizations, quality system registrars, auditor course providers, auditor certifiers, and calibration and testing laboratories. One such organization is CAEAL, and the SCC/CAEAL partnership is outlined below in section 5.2.4.

As part of the SCC accreditation program, the Program for Accreditation of Laboratories – Canada (PALCAN), provides formal recognition of the competence of laboratories to manage and conduct specific tests or types of tests listed in the scope of accreditation approved by the SCC. Accreditation is available for all types of tests, measurements and observations and is currently offered in a variety of testing fields. Environmental testing is assigned, as appropriate, to the biological, chemical and physical fields of testing.

5.2.4 The Linkage between SCC/PALCAN and CAEAL

In 1994, a partnership agreement merged the environmental component of the SCC laboratory accreditation program (PALCAN) with the CAEAL site assessment program, to provide a single national program that through its affiliation with the SCC allows for both national and international recognition. International recognition in this field is becoming increasingly crucial as the provisions of international trade agreements are implemented. These provisions will require that the suppliers of environmental analytical laboratory services meet the requirements of the ISO/IEC Guide 17025 standard. Similarly, at the national level, there is an increasing trend for both government and private sector contracting policies to require laboratory accreditation. Under the terms of the SCC/CAEAL Accreditation Partnership Agreement, CAEAL conducts the site assessments and operates the proficiency testing program (as described above). The granting and maintenance of accreditation is operated under the authority of the SCC, upon the

recommendation of CAEAL. The accreditation is based on satisfactory participation in the proficiency testing program, where such testing is offered as part of the accreditation.

5.2.5 Ministère de l'Environnement du Québec (MENVQ)

The Centre d'Expertise en Analyse Environnementale du Québec, an agency operating on behalf of the Ministère de l'Environnement du Québec (MENVQ) accredits private, municipal, and institutional laboratories for the purpose of achieving environmental regulations. The accreditation program is based upon the minister's rights in the Environmental Quality law of Québec. The accreditation program comprises an array of standards and requirements that facilitate quality assurance for laboratory processes. The program was initiated in 1984 with the goal of ensuring a high level of quality of analyses conducted by accredited laboratories for the monitoring of drinking water, ground water, municipal/industrial effluents, clays from purification industries, contaminated soils, dangerous wastes, used oils, and atmospheric discharges. The objective of the program is to ensure that analytical data quality is maintained at a high standard so that clients relying on these laboratories can use the analytical information produced with confidence. This program applies to all private, public, and semi-public laboratories producing environmental data in the province of Québec. Participants in the program can be commercial, industrial, municipal, governmental, or institutional laboratories. The accredited laboratories are recognized by MENVQ, according to the law for environmental quality, and conforms to the standards and requirements of environmental analytical laboratory accreditation (ISO Guide 25). The accreditation program involves analytical expertise related to chemistry, microbiology and toxicology. It applies to all analytical parameters targeted by environmental management programs in Québec.

5.3 Overview of Current Regulatory Requirements

Canadian laboratory accreditation programs have grown and developed throughout the 1990's. Moreover, there has been a significant increase in the government regulation of water quality for various uses (e.g., aquatic life, drinking, agriculture, wildlife, recreation) by regulatory bodies, such as provincial environment ministries and federal natural resource departments (e.g., Fisheries and Oceans Canada, Natural Resources Canada, Environment Canada, etc.). As a result, these regulatory bodies have begun to develop specific requirements related to the use of accredited laboratories for the generation of quality data for regulatory purposes (e.g., compliance testing).

Current provincial regulatory requirements for the use of accredited laboratories are provided on the CAEAL web site (http://www.caeal.ca/provregs.html), and are summarized in Table 14 below. Currently (as of 2001), only two provinces (i.e., Alberta and Newfoundland) require laboratories to be accredited for aquatic toxicity testing for regulatory purposes.

Table 14. Regulatory Requirements for Laboratory Accreditation in Canada

Province	Parameter Category/ies	Requirement
Alberta	All	Accredited by the Standards Council of Canada through CAEAL
British Columbia	Microbiology (drinking water) and environmental monitoring data	Accredited by the Standards Council of Canada through CAEAL
Newfoundland	All	Recognized form of accreditation (e.g., the accreditation offered jointly by the Standards Council of Canada and CAEAL
Nova Scotia	Analytical tests Microbiology	Accredited by the Standards Council of Canada or another agency recognized by the Nova Scotia Department of Environment and Labour or maintaining an acceptable standard in a proficiency testing program conducted by CAEAL for all parameters being reported
Ontario	Microbiology (drinking water)	Accredited by the Standards Council of Canada, which works in tandem with CAEAL

In addition, there are also regulatory programs that currently do not require but recommend the use of accredited laboratories for the generation of aquatic toxicity data. For example, the Environmental Effects Monitoring (EEM) program recommended as part of the Canadian Pulp and Paper Effluent Regulations (PPERs) that private sector laboratories conducting testing on behalf of the pulp and paper mills be accredited by CAEAL. The Ecological Monitoring & Assessment Network (EMAN) of Environment Canada recognizes the importance of quality laboratory data. This network states that a key mechanism to achieve quality products is by using the guidelines established by the SCC and CAEAL.

CAEAL has strengthened the international markets for Canadian laboratory expertise. For example, the National Cooperation for Laboratory Accreditation (NACLA) website (http://www.nacla.net/Links/links.html) lists CAEAL as a related organization along with other organizations, such as the American Association for Laboratory Accreditation (A2LA). Moreover, the National Association of Testing Authorities in Australia highlights the success of the CAEAL accreditation program. The division on inspection and testing of the Inventory of National Practices on Standards, Technical Regulations and Conformity Assessment in the Western Hemisphere has an arrangement with accrediting organizations, which includes CAEAL. The SCC has mutual recognition agreements with the US National Institute of Standards and Technology (NIST), the National Voluntary Accreditation Program (NVLAP) and with A2LA. This agreement provides SCC-accredited testing laboratories with the reciprocal status of laboratories accredited by these organizations.

5.4 The Role of Inter-Laboratory and Proficiency Testing in Improving Acute Lethality Test Reliability

One of the most important ways to test the success of quality assurance is participation in inter-laboratory testing, sometimes referred to as "round-robin" testing. In this type of program, ecotoxicity laboratories are required to test specific reference toxicant samples usually sent by the certification or accreditation program and submit the test results for evaluation. Proficiency testing

results help the laboratory demonstrate that their testing capability is in agreement with, or similar to the results of other laboratories using a standard test method with standard test organisms (in this case, rainbow trout and *D. magna*).

Proficiency testing is conducted on a regular basis to assist in the evaluation of laboratory competence. For Canadian laboratories participating in the joint SCC/CAEAL or MENVQ accreditation programs, all tests appearing in the laboratory's scope of testing must be supported by PT, in those cases where PT samples are offered by the program. Laboratories may also choose to be recognized by CAEAL for proficiency testing by participating only in the PT program. In these cases, they cannot claim full accreditation, but still receive recognition for test-specific proficiency (also called certification). All laboratories participating only in the Proficiency Testing program must comply with the Proficiency Testing Related Policies (see CAEAL web site at http://www.caeal.ca). Laboratories applying for SCC/CAEAL accreditation must pass at least one PT study before accreditation can be granted.

Almost all Canadian toxicology laboratories participate in proficiency testing rounds as part of maintaining their accreditation for acute lethality testing with rainbow trout and *D. magna*. Under the SCC/CAEAL or the MENVQ accreditation programs, laboratories must perform proficiency testing on a twice-yearly basis and must obtain an acceptable score to maintain their accreditation.

5.5 The Role of Primary, Secondary and Third-Person Laboratory Assessment

There are three functional tiers of laboratory assessment conducted by personnel from different organizations. These three tiers are often referred to as primary, secondary or third person assessments. Each of these types of assessments is conducted by a particular person (or team), but the purpose differs. These three types of assessments are outlined in greater detail below.

A primary person laboratory assessment is an internal laboratory assessment, often conducted by the lab Quality Assurance (QA) Officer assigned by the laboratory's management. This QA Officer is generally a technically-trained staff member who is not involved in testing, reports directly to management, and usually has training in laboratory QA issues. This type of assessment, conducted on a regular or as-needed basis using lab-specific or generic (i.e., test-specific) checklists, is the laboratory's way of assuring its own quality system and serves as a routine check on the quality assurance/quality control (QA/QC) measures used in the laboratory. The results of this type of assessment are reported to the laboratory's management and serves as information in the annual laboratory quality management review.

A secondary person laboratory assessment is a laboratory assessment conducted by an external body, mainly staff assigned by the client/sponsor/user of the testing. This client representative can either be a trained QA professional or an environmental manager and is familiar with aquatic toxicity testing. This type of assessment is conducted on an as needed basis (i.e., to evaluate the laboratory initially, to determine ongoing compliance, or to investigate complaints or non-conformities), uses either client-specific or generic checklists and is the client's assessment of the laboratory's quality system. This type of assessment is often conducted for due diligence purposes, that is, the client/sponsor takes responsibility for assuring that the laboratory producing data on its behalf conforms to the quality system required for accreditation and/or for regulatory compliance.

A third person laboratory assessment is a formal assessment conducted by an external body, specifically, an accrediting or certifying organization (such as CAEAL, SCC or MENVQ). The assessor or team of assessors is/are always trained QA professionals with technical experience/expertise in the area being assessed. This type of assessment is conducted on a regular (and sometimes unannounced) basis using standard checklists. It is the formal objective assurance of the laboratory's quality system, and serves as a routine check on the QA/QC measures used in the laboratory. The final report for this type of assessment serves as findings that must be reviewed and dealt with through a series of corrective actions in order to maintain corrective actions, to the laboratory's accreditation.

In support of the SSC/CAEAL accreditation program for toxicology laboratories, Environment Canada has prepared detailed checklists for 18 toxicity test methods for which accreditation can be sought. These detailed checklists highlight the "must" and "should" requirements of each methodology. Checklists for the rainbow trout and *D. magna* acute lethality tests are available for use by laboratory assessors. As well, Environment Canada and CAEAL conduct 2- or 3-day training sessions involving CAEAL assessors with a toxicology background on a biannual basis. Training of the CAEAL assessors leads to more thorough laboratory inspections and helps reduce the variability of acute lethality and sublethal toxicity test results.

6.0 GUIDANCE FOR THE SELECTION AND EVALUATION OF ECOTOXICITY LABORATORIES

6.1 Guidance for Selection of an Ecotoxicity Laboratory

The selection of a capable and experienced ecotoxicity laboratory for the conduct of testing is a critical element in the assurance of data reliability. A careful evaluation of the laboratory, its operating capability, and the qualifications of its staff are paramount for ensuring high quality ecotoxicity data.

This section provides mining industry personnel with an overview and discussion of the key issues related to laboratory qualifications including: accreditation status, quality system documentation, staff qualifications, experience and training, facilities, reference toxicant testing, and how to conduct a second-party assessment of an aquatic toxicity laboratory.

6.1.1 Accreditation Status

The definition and explanation of accreditation, accreditation programs (which include site assessments and proficiency testing programs), and summary descriptions of Canadian laboratory accreditation programs are provided in Section 5.2 and Appendix E.

The laboratory considered for providing acute lethality testing services should be accredited and have a current accreditation certificate, however, it should be noted that in the Environment Canada methods, accreditation is recommended but not essential. In the case of the SCC/CAEAL program, the accredited laboratory would have been through at least one site assessment and have met all of the required actions outlined in the final assessment report. During the SCC/CAEAL site assessment, the laboratory would have also indicated the *Scope of Testing* for its accreditation and thus, would have been evaluated during the site assessment. This Scope of Testing is extremely important since it contains the list of tests whose quality has been evaluated by the assessors. CAEAL also requires all accredited laboratories to have completed PT testing rounds for most tests for which the lab requests accreditation.

Just as important is a laboratory's maintenance of accreditation. There are numerous reasons why a laboratory may not be able to maintain its accreditation (e.g., failure of two consecutive PT rounds and failure to submit responses to corrective actions from site assessments). It is important for the mining industry client to ensure that the laboratory's accreditation is current, and that the laboratory has been able to maintain this status consistently. Industry personnel can determine this status by requesting a copy of the laboratory's accreditation certificate and Scope of Testing, upon initiating an evaluation of the laboratory. This information is also available through the SCC website (http://www.scc.ca).

6.1.2 Quality System Documentation

This section provides an overview of the key elements of quality system documentation used by an accredited laboratory and the rationale for each of the different types of documents. Non-accredited laboratories may also have all of the required elements of a quality system in place.

Quality Manual

The laboratory's Quality Manual (QM) is the principal document that outlines how the laboratory meets required policies and procedures of an ISO quality system, and the goals of the laboratory for service and quality and describes how they are maintained, evaluated and remediated. It also contains all of the central information pertaining to the laboratory's staff, day-to-day operations, facilities, equipment, and quality assurance/quality control program. The QM provides an overview and details pertaining to all other quality system documentation (e.g., list of Standard Operating Procedures, SOP revision history, equipment inventory, etc.).

The QM serves two major functions as part of the laboratory's quality system. First and foremost, it is an educational and reference resource for laboratory staff. New staff should be required to read and become familiar with all elements in the document. Existing staff should be using it to refresh their knowledge of the quality system (including changes to the system), particularly in those areas that they are least familiar. Secondly, the QM serves as a primary reference document for all secondary and tertiary assessors visiting the laboratory.

The typical content of a Quality Manual is as follows:

- Organization
- Management
- Quality Policy
- Facilities
- Personnel
- Services
- Equipment
- Supplies
- Methodology
- Sample management
- Data management
- Workload management
- Traceability of measurement
- Quality control

The Quality Manual is a document which the mine's assessment team should request and review prior to visiting the laboratory.

Standard Operating Procedures (SOPs)

A Standard Operating Procedure is a written document that details specific activities carried out by laboratory or field staff relating to a specific procedure or collection of procedures.

Standardized routine procedures exist for all aspects of laboratory operations, and therefore, a full range of SOPs are established, and are continually updated by the laboratory. These SOPs are controlled, dated, and an ongoing schedule of review (e.g., semi-annually) is implemented with these SOPs. SOPs should be written for all laboratory-related procedures including (among others): test methods, equipment calibration and maintenance, test organism care and culturing and procedures for handling, treatment, storage of samples and reagents, and cleaning procedures for test chambers. All SOPs should be updated whenever a deviation from conventional practice

has been implemented to improve the performance or efficiency of the methods. These are documents that must be reviewed by the client's assessment team.

6.1.3 Staff Qualifications, Experience and Training

As indicated above, critical elements of a laboratory's data quality and reliability are the qualifications, experience and training of its staff. All laboratory personnel should have education, experience, and training commensurate with their assigned functions in the laboratory. *Curricula vitae* / Résumés, job descriptions, diplomas, special training certifications and analyst proficiency records of all individuals working for the laboratory should be maintained in a personnel file and updated regularly.

The laboratory should also have a regular, documented training program that the client's assessors should review. CAEAL-accredited laboratories are required to document staff training and analyst proficiency. Staff training should include familiarity with the relevant regulatory framework, reference test methods and in-house SOPs relating to the culture, holding and testing of laboratory organisms. Staff proficiency can be verified by conducting tests on PT samples (or by participation in other round-robin testing exercises) and in-house reference toxicant tests.

6.1.4 Facilities

Laboratories should be equipped with the basic and specialized equipment required for the culture, holding, and testing of aquatic organisms (e.g., temperature control, light intensity and photoperiod control, emergency back-up power, water treatment facilities, etc.). The key aspects include: an organizational chart, suitable facility size, and staff complement that reflect the volume of testing conducted, general housekeeping procedures (i.e., laboratory is clean, tidy and well-organized), adequate facilities for sample storage, culture, holding and testing of laboratory test organisms. The client's assessment team should make a note of the above features of a good laboratory in their tour of the facility.

6.1.5 Reference Toxicant Testing

A reference toxicant is a chemical used in toxicity testing to provide results that can be compared within or among laboratories (Environment Canada, 1990c). Test-specific reference toxicant testing (also referred to as 'positive controls') should be conducted by an ecotoxicity laboratory on a regular basis to demonstrate consistency in test method performance (i.e., within a defined and limited range of variability) that might be affected by such influences as: changes in test organism sensitivity over time as a result of size, reproductive status, genetic differences in sensitivity between stocks of organisms obtained from different sources, and performance of technical staff. Control charts should be established and regularly updated to demonstrate that test reproducibility is within established limits. Test-specific standard reference toxicants should be used and reference tests should be conducted at regular intervals as required by the test method, and as outlined in the in-house SOP. Stocks of test organisms not cultured in-house should be tested shortly after organism acclimation to laboratory conditions, and towards the end of stock utilization, as well as monthly, as long as the organism supply lasts.

For all Environment Canada aquatic toxicity test methods, including the rainbow trout and *D. magna* acute lethality tests, reference is made to the Environment Canada guidance document on Control of Toxicity Test Precision Using Reference Toxicants (Environment Canada, 1990c). This

document describes the use of reference toxicants within a laboratory for control of toxicity test precision over time. Moreover, guidance on the establishment and interpretation of control charts is provided. In addition to this general guidance document, each test method provides specific guidance relating to the conduct of reference toxicant tests to be used for that method.

6.1.6 Laboratory Turnaround Time

Turnaround time of toxicity test results is also an important aspect of the selection process for several reasons. Test results must be reported within a specified period of time to meet client- and government-specific reporting requirements. Companies that fail to do so, can be found to be in non-compliance with their regulatory requirements and may be charged accordingly. Similarly, clients need to be notified immediately in the event of a toxicity failure (i.e., in the event that a test on an effluent sample results in death of more than 50% of the test organisms), since this may cause a change in the nature or frequency of future effluent toxicity tests. Failure to respond to this requirement in a timely fashion may also result in non-compliance.

6.2 Guidance for Conducting a Test Report Evaluation

Section 8 of each of the Environment Canada test method documents (Environment Canada, 2000a,b) includes requirements for the provision of reporting. In particular, information on sample collection, culture, and test condition information, raw data and test results (i.e., statistical endpoints) for the acute lethality tests is included in test reporting. The purpose of a test report evaluation is to determine whether a given report meets these requirements. Although the test report itself is not critical in the variability associated with the test itself, this exercise is critical in ensuring regulatory compliance. Test report evaluation checklists (developed by Environment Canada) for the two acute lethality methods should be used in this type of evaluation and is provided in Appendix H. This checklist contains all of the 'must' and 'should' requirements for test reporting (i.e., Section 8.1 of Environment Canada's acute lethality test methods).

6.3 Guidance on How to Conduct a Second Party Assessment of an Aquatic Toxicity Laboratory

Mining company personnel can conduct a second party laboratory assessment in order to evaluate the laboratory's ability to consistently provide high quality data in support of the mine's regulatory compliance requirements vis-à-vis acute lethality tests. While this latter aspect is the main focus of the assessment, the laboratory's general competence should also be of interest. The following section provides recommended guidance for assessing a laboratory already conducting tests for the mine.

Preparation for the Assessment. In preparation for a laboratory site assessment, it is recommended that one or several test report evaluations (as outlined above in section 6.2, and using the appropriate checklists; Appendix H) be conducted prior to the visit. Moreover, the following preparatory tasks are recommended:

1. Develop a tentative agenda for the site assessment, in consultation with the laboratory. An example of a tentative agenda is provided below:
    a. Introductions
    b. Laboratory Tour

c. Examination of Key Documentation
d. Review of Mine's Test Data
e. Review of QA / QC Data

2. Confirm the date, time and logistics of the assessment with the laboratory; and,

3. Confirm the availability of a lab staff member for accompanying the assessor.

For general laboratory competence, the Checklist for Laboratory Qualifications (provided in Appendix E) will be useful. For the acute lethality tests of interest (i.e., rainbow trout and *D. magna*) the test-specific and test report checklists (Appendices G and H) can be used as a guide in determining what types of documentation and questions to be asking staff during the site assessment.

Introductions. Arrange to have a brief meeting for introductions and to state the purpose and nature of the assessment. It is helpful for the second-party assessor to have a staff member available for answering any questions during the visit (preferably the lab's QA officer).

Laboratory Tour. Initially, a tour of the laboratory's facilities should be conducted in order to evaluate the physical facility and good housekeeping practices. The tour should include an assessment of the laboratory's facilities for water treatment, fish holding and culturing, data processing and record retention, water treatment and sample storage as well as environmental control systems for temperature, lighting, photoperiod.

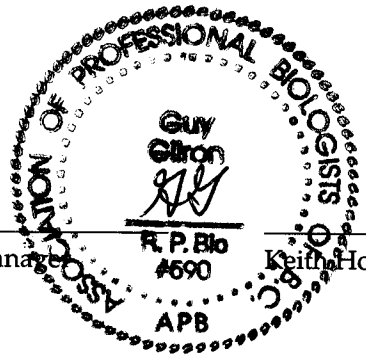
Examination of Key Documentation. The following outlines, in greater detail, the key elements provided in the Checklist for Laboratory Qualifications (provided in Appendix D) for assessors to review and evaluate:

- Quality Manual - A review of the Quality Manual should provide a second-party assessor with an impression of how the laboratory conducts its business and how it complies with its own quality policies and procedures, in addition to external guidelines (e.g., health and safety, due legal process, animal care). A comprehensive discussion of the critical elements of the Quality Manual is outlined above in section 6.1.2.1. During the assessment, some key areas that should be evaluated are as follows: data management issues (e.g., how are data recorded, checked, and stored securely), workload management issues (e.g., volume of workload vs. available resources), and quality policy evaluation (e.g., internal audit / evaluation, response to customer complaints, management review).
- SOPs - The lab's Standard Operating Procedures (SOPs) are the most critical quality documents, since they detail the procedures that are adhered to by the laboratory in their implementation of test methods. The types of issues that need to be assessed are as follows: Are SOPs available to lab staff at all times? How are interim revisions to SOPs handled, and how often are the SOPs updated? How are deviations to SOPs handled?
- Laboratory personnel - The expertise, experience, roles and responsibilities of laboratory personnel are also key critical evaluation elements. Some key areas that require assessment relate specifically to: the establishment of a comprehensive (and documented) training program for new staff, criteria for new staff initiating testing for client samples, maintenance of staff confidentiality agreements, and chain-of-command according to the lab's organizational chart.

- Facilities – The establishment and maintenance of environmental systems within the laboratory is also key to the generation of high-quality data. To evaluate this, the assessor should check the lab's monitoring records relating to environmental parameters, such as: temperature, water quality, and lab equipment (e.g., meters, environmental chambers, water baths, etc.). These records should be based on frequent checks and should be up to date. Preventative maintenance and frequent external calibration of laboratory equipment should also be conducted.
- Reference Toxicant Testing – The frequency with which reference toxicant testing is conducted in a crucial element to quality control of the laboratory. For rainbow trout, reference toxicant testing with a frequency of at least once per month (or using every new batch of fish received) should be demonstrated. Similarly, for *D. magna*, the frequency should be biweekly. The procedure for dealing with exceedances of warning and control limits on reference toxicant control charts should be detailed in an SOP, and possibly in the Quality Manual. The procedure for notification of clients when these exceedances occur should also be evident.

Review of Mine's Test Data. A focus of the laboratory assessment should be to confirm that acute lethality data previously submitted to the mine for compliance purposes in test reports, is precise and accurate. As such, it is critical that the information held "on-file" (i.e., not necessarily contained in the test report) be evaluated; see the checklist in Appendix G under the appropriate section for details. This information relates to reported data for quality control (e.g., reference toxicant data), and adherence to the reference methods (e.g., compliance with validity criteria).

QA/QC Review. Evaluation of the QA/QC system of the laboratory is a crucial part of the assessment. The QA Officer should be available and interviewed. Documentation of all accreditations/certifications, and Scope of Testing held by the lab should be provided. Similarly, information on the laboratory's participation in proficiency test programs should be covered. Reference toxicant control charts, records of control limits and documentation of non-conformities, assist in verifying that the testing is being done according to the test methods. The storage and retention of documentation (all bench sheets and test reports, QA/QC data, and other documentations) should be archived by a documented system.



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APPENDIX A

SUMMARY OF PROVINCIAL, TERRITORIAL AND ATOMIC ENERGY CONTROL BOARD (AECB) 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

GUIDANCE DOCUMENT FOR ACUTE LETHALITY TESTING OF METAL MINING EFFLUENTS: LITERATURE REVIEW

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1.0 INTRODUCTION

This initial phase of the Acute Lethality Guidance Document involved a thorough review of recent (post-1990) readily available information (obtained through a comprehensive literature search and consultation with the Scientific Authority) relevant to inter-and intra-variability in acute lethality testing, in the context of the Canadian metal mining sector. The documents included for review were as follows:

- Arnold, W.R. *et al.* 1996. Effluent Toxicity Test Variability (Chapter 5). In: D. Grothe, K. Dickson and D. Reed-Judkins (eds.) *Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving Water Impacts.* SETAC Press . pp. 131-156.
- Warren-Hicks, W. *et al.* 2000. Assessment of Whole Effluent Toxicity Test Variability: Partitioning Sources of Variability. *Environ. Toxicol. Chem.* 19(1): 94-104.
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Each of the documents is summarized in the following sections. The purpose of this review is to provide the user with an understanding of the current state of knowledge for relevant documents pertaining to the subject of test method variability, which will help to form the basis for development of the Acute Lethality Guidance Document. The Guidance Document is not intended to replace the existing Environment Canada test method documents, but rather to provide supplementary guidance specific for acute lethality testing with metal-mining effluents.

2.0 LITERATURE REVIEW

The following information is intended as an overview of each of the relevant acute lethality test method variability documents (listed in Section 1.0 above). The information presented is based on information from each document, however the user should consult the original text to obtain further detailed information.

It should be noted that most of these documents contain information on both acute lethality and chronic sublethal tests. Due to the nature of the Guidance Document, emphasis in the review has been placed on those aspects of these documents that relate to acute lethality testing, and any aspects of the chronic sublethal tests that are relevant to all test methods. Moreover, the documents reviewed discuss methods other than Environment Canada test methods; most relate specifically to U.S. EPA test methods. Therefore, they are not directly relevant to these (i.e., Environment Canada) methods, but can provide important information on what aspects of test methods can be attributable to test variability.

2.1 Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving System Impacts. (Chapter 5, Effluent Toxicity Test Variability). (Arnold, W.R. *et al.* 1996)

This book presents the proceedings of the 25th "Pellston Workshop" on Whole Effluent Toxicity. Chapter 5, Effluent Toxicity Test Variability, is of particular interest, in context of the guidance document, and was therefore selected for this review. The conclusions are based specifically on U.S. EPA methods for all commonly applied effluent toxicity compliance tests (both acute and chronic) used in the United States.

Chapter 5 summarized the discussion relating to the potential sources of variability and how they are measured and presented approaches for addressing and reducing that variability. Factors identified as being key to variability in toxicity test results were:

- Characteristics of the conditions established for the test; and
- The associated experimental design factors.

Standardization of test methods has generally been an effective means of controlling these sources of variability but modifications and improvements to the existing methods can be made as more experience with the methods is gained over time. Two general categories of variability of greatest concern were:

- Analyst experience, as it relates to both conduct of the tests and interpretation of results; and
- Condition / health of the test organisms (which may also be related to analyst experience).

Experience of the regulator, although not a contributing factor to variability of test results, was discussed in terms of the broader issues of development and implementation of WET limits. Specific concerns such as how to assess single failures and false positives / false negatives so that rationale and resource–effective decisions are made were considered to be of equal or greater importance to concerns about the existing methods. A technical support document (US EPA 1991) was cited as a source for additional guidance for these types of issues.

Sources of variability were categorized as follows:

- Intra-test (within-test) variability;
- Intra-laboratory (within-laboratory) variability; and

- Inter-laboratory (between-laboratory) variability.

Aspects of the test methods considered to be key factors that can influence the variability of test results are provided in Table 1.

Table 1. Factors influencing variability of test results.

Aspects	Key Factors Influencing Variability
Sample Collection, Storage and Handling – Issues related to collection of a representative sample	• Sample volume (issue of representativeness related to small volume and sample-container interactions) • Sampling method - grab vs. composite (issues related to effluent variability and use of appropriate method) • Sample storage and handling (issues relating to sample stability, if unknown) • Sample manipulation (e.g., salinity adjustment)
Abiotic Conditions	• Test temperature (variation in temperature may alter sample integrity by altering chemical form or concentration and/or influence organism response) • Changes in pH may alter nature or form of contaminants in solution
Exposure and Variability	• Static versus flow through conditions • Number of concentrations and dilution series • Test duration
Sample Toxicity and Variability	• Test endpoints tend to be less variable for effluents having steep concentration-response curves and vice versa.
Food	• Potential variability due to food quantity and quality • Presence of food can alter exposure, affect chemical activity of toxic constituents
Dilution Water	• Dilution water can affect effluent dilutions by modifying availability of contaminants • Dilution water characteristics can affect test organism sensitivity • With respect to synthetically prepared dilution waters, age of solutions can affect organism sensitivity
Species Sensitivity	• Most commonly used test species have acceptable ranges of variability in sensitivity.
Organism History and Handling	• Collection • Culture conditions • Acclimation • Handling during test • Randomization (to evenly distribute the variability within the testing environment and the organisms)
Organism Numbers	• Loading rates can affect test results • Ability to detect effects increases with number of organisms tested
Organism Age and Quality	• Age of test organism can affect sensitivity to contaminants

Methods of quantifying and controlling intra-test variability and intra- and inter-laboratory variability were discussed. The factors involved in quantifying and controlling WET variability are presented in Table 2.

Table 2. Key Factors in Quantifying and Controlling Intra-test and Intra- and Inter-laboratory Variability in WET Testing

Aspects	Key Factors in Quantifying and Controlling Variability
Intra-test Variability	• Deviation from methods may increase level of variability to a point that may adversely affect the test results and could lead to unnecessary additional testing, or erroneous data (false positives / false negatives)
Intra- and Inter-laboratory Variability	• Repeatability defined as variability between independent test results obtained from the same laboratory – Intra-laboratory variability (ASTM 192) • Reproducibility defined as the variability between test results obtained from different laboratories – Inter-laboratory Variability (ASTM 192) • CV considered to be simplest measure of repeatability and reproducibility • CV defined as the standard deviation of repeated tests (s), divided by the mean of the repeated tests (m), multiplied by 100 (CV = s / m x 100)
Quality Management Considerations	• Reference toxicity tests are used to monitor a laboratory's performance, in terms of analyst technique and health and condition of the test organisms. • Use of control charts help to determine when potential problems occur • Control charts provide an indication of a laboratory's capability to reproduce the desired endpoints of a reference toxicant test (very wide control limits and / or many control points outside the limits, can be cause for concern and suggest that the test results may be suspect.

A number of factors were considered important in reducing variability of test results. These are summarized in Table 3.

Table 3. Key factors involved in reducing variability.

Aspects	Factors in Reducing Variability
Following Testing Guidelines	• Deviation from methods may increase level of variability to a point that may adversely affect the test results and could lead to unnecessary additional testing, or erroneous data (false positives / false negatives)
Increasing Analyst Expertise	• Experienced staff (in all aspects relating to culture, testing and interpretation of results) reduces deviations from method
Selecting Contract Laboratories	• Quality of the laboratory along with organism health were considered to be one of the most important factors affecting test variability • Educational qualifications and experience of the technical and supervisory staff should be reviewed. • Laboratory capability should extend beyond routine effluent toxicity testing in order to meet all potential needs (i.e. be able to assist with regulatory interaction and toxicity reduction evaluations)

Although this information was primarily based on U.S. EPA test methods for various acute and chronic tests, the following conclusions of this chapter are relevant to acute toxicity test methods in general:

- To some degree, there is variability in all inter-test, intra-laboratory, and inter-laboratory toxicity test results;
- The variability of each of the test methods has not yet been accurately determined;
- Analyst experience and judgment, and test organism condition health are considered to be the largest sources of variability;
- A good QA / QC program can help to control deviations from test methods, that can lead to test variability; and
- Strengthening analyst training and experience can also reduce deviations from test methods, therefore reducing test variability.

Finally, a number of recommendations were made describing regulatory initiatives to aid the process of reducing variability and assist in interpretation of variability including:

- Establishment of WET test specific variability limits for inter-test, intra- and inter-laboratory variability;
- Development of a quality assurance and audit program;
- Providing procurement guidance for selection of high quality laboratories;

- Establish a multidisciplinary Technical Advisory Group to resolve problems associated with 1) determining test acceptability and appropriate levels of variability, 2) determining meaningful exceedences, 3) dealing with atypical effluents and 4) analyzing and interpreting unique data sets; and
- Develop guidance on data interpretation of toxicity test variability, test result interpretation and incorporation into the regulatory decision making process.

2.2 Assessment of Whole Effluent Toxicity Test Variability: Partitioning Sources of Variability. (Warren-Hicks, W. *et al.* 2000)

This Environmental Toxicology and Chemistry (ET&C) journal article discusses quantifiable sources of variation in whole effluent toxicity testing, and the relative magnitudes of these variance components. A national data set was developed consisting of raw reference toxicant data from marine and freshwater tests conducted using commonly used species, test methods and laboratories. The test methods discussed and the results evaluated, pertained primarily to U.S. EPA acute and chronic test protocols. Variances were calculated for aspects such as: the choice of laboratory (i.e. inter-laboratory variance), variance associated with the concentration series used (i.e. between test concentration variance), variability of toxicity tests conducted over time (between test variance), and random error (i.e. variance not explained by any of the previously mentioned sources of variability). Factors such as organism suppliers, dilution water quality, and laboratory conditions were included in the random error component, since the database did not include information to identify and qualify these additional sources of uncertainty. The following results were provided:

- The concentration series variance component accounted for the majority of the total variance (CV=31.7 to 92.8% of the total variance across all protocols and test species) for most test species and reference toxicant combinations;
- The second largest variance component in this study was the random error component (4.1 to 33% of total variance); and
- The test date variance component resulted in 0 to 22% of the total variance.

The above findings indicated that concentration series variance was the dominant source of variability (suggesting that variance is a function of toxicity), followed by the random error component (indicating that the laboratory and the test are less dominant than might be expected).

The article indicated that toxicity is a relative, rather than an absolute quantity, as it depends on the sensitivity of the test species, life stages, chemicals in the effluent, test method used, test conditions, and the reliability of the benchmark used to gauge toxicity. Some of the recommendations that may apply to acute, as well as chronic testing (upon which this article was based), include:

- All test methods should be evaluated for comparability of results, related to both intra-laboratory and inter-laboratory factors;
- Test methods with poor comparability and reproducibility should be revised as necessary; and
- Consideration should be given to requiring multiple test results. Intra-laboratory results would be useful if reproducibility is an issue. Inter-laboratory testing would be useful for overall comparability of results.

2.3 Intra- and Inter-treatment Variability in Reference Toxicant Tests: Implications for Whole Effluent Toxicity Testing Programs. (Moore, D.R.J. *et al.* 2000).

The article presents the results of a study conducted to determine if the results of whole effluent toxicity tests are strongly influenced by intra-laboratory variability (e.g. as a result of changes in test conditions, organism health and condition, or analyst performance from test to test) and inter-laboratory variability (e.g. as a result of differences in sources of test animals and dilution water, technical expertise, or sample and organism shipping effects). The specific objectives of the study were to quantify the intra-laboratory variability for several species-data type combinations; to determine whether the amount of intra-laboratory variability is consistent among laboratories, species and data-types; to quantify inter-treatment variability between laboratories for the same species-data type combination used to quantify intra-treatment variability; and to compare the relative magnitudes of inter-laboratory and intra-laboratory variability for different species-data combinations. The results are based on chronic test methods conducted according to the U.S. EPA protocols. A brief summary of the results is provided in Table 4.

Table 4. Evaluation of intra- and inter-laboratory sources of variability.

Variability Type	Results of Analyses
Intra-laboratory Variability	• Overall, there is considerable variability in toxicity estimates for some laboratory- and species-data type combinations. • The coefficient of variation (CV) for one species-data type can range from 15.7% to 53.5% among laboratories. • There was no apparent consistent relationship between intra-laboratory variability and data-type or species.
Inter-laboratory Variability	• Mean intra-laboratory EC50 values varied among laboratories for two of the endpoints, however this variability was smaller than the inter-laboratory variability. CVs for inter-laboratory variability did not reflect total data set variability. • Inter-laboratory CVs for *Menidia beryllina* (inland silverside) mortality and growth were 65.8 and 117%, respectively.

Several useful points are made in the discussion section of this article. Intra-laboratory variability can be quite high in some laboratories, despite the use of standardized protocols, test species, and test substance. The article indicates that although Environment Canada suggests a maximum CV of 20% for LC or EC50's, only 3 of 16 laboratories in this study were below the 20% mark for EC25's. Six out of 16 possible intra-laboratory CV's were below 30%, and eight had CV's of 40% or less. Overall, measures of intra-laboratory variability for EC25s had CVs of 30% or less.

The authors suggested rejecting results from laboratories that have unacceptably high variability in reference toxicant test results over time as a means to ensure regulatory decisions are not unduly influenced by intra-laboratory variability. While consensus on what constitutes "unacceptably high" variability does not presently exist, Environment Canada (1990) suggested an objective CV in the range of 20% to 30% for LC50s or EC50s.

There was significant inter-laboratory variability for one of the two species in this study. The CV for *M. beryllina* ranged from 65.8 to 117% for mortality and biomass, respectively. The CV's for *Ceriodaphnia dubia* mortality and number of young were 17.3% and 13.4%, respectively. Based on the analyses conducted, the following conclusions were presented:

- Further investigation of some of the factors contributing to both intra- and inter-laboratory variability is suggested. Suggested areas were: analyst technique and experience, dilution water characteristics, and organism health and condition;
- Results of the study indicated that intra- and inter-laboratory variability from reference toxicant testing is often above desirable limits (CV's > 30–40%);
- Combining the effects of both intra- and inter-laboratory variability worsens (i.e., increases) the variation problem;
- The authors recommend that a study including additional species and data-types be conducted to determine which test methods have low intra- and inter-laboratory variability among results. Laboratories should be compared to standardized acceptance criteria for the methods that perform well. It is recommended that laboratories conform, and receive accreditation for performing compliance testing; and
- Additional testing should be conducted when point estimates of effluent toxicity are close to allowable limits. This approach would reduce some of the uncertainty that revolves around intra-laboratory variability. Inter-laboratory testing should be conducted for methods with moderate inter-laboratory variability (e.g., CV=30 – 50%) to ensure that the test results are not biased.

2.4 Performance Characteristics of Effluent Toxicity Tests: Summarization and Evaluation of Data. (Parkhurst, B.R. *et al.* 1992).

In this ET&C journal article, the precision of effluent toxicity tests was evaluated using published and unpublished data from 23 intra- and inter-laboratory reports relating to variability for both acute and chronic tests, based on U.S. EPA, or similar test methods. Both effluent and single chemical test data were included in the evaluation.

Standard practices for determining precision and bias as related to methods for analyzing chemicals in water have been established by the American Society for Testing and Materials (ASTM), Committee D-19 on water. The authors adopted the ASTM standard for this study, since there are no methods or guidelines for evaluating the adequacy of data on variability and precision of aquatic toxicity test methods. For inter-laboratory studies, the standard practice requires a minimum of six laboratories producing six usable data sets. For intra-laboratory studies that evaluate the precision of single technicians, there must be a minimum of six technicians providing usable data. The study results are summarized in Table 5.

Table 5. Intra- and Inter-laboratory variability for single chemical and effluent toxicity tests.

Variability Type, Test Substance	Results of Analyses
Intra-laboratory Variability, Single Chemical	• CVs ranged from 3 to 72% for *Daphnia magna* acute lethality testing (*Daphnia* spp. were the most extensive database). • Data for other species was limited. • Only 15 of 22 studies conducted a minimum of 6 or more tests. • There was an extensive database available for chronic studies; CVs for chronic studies ranged from 2 to 83% (mean of 32%).
Intra-laboratory Variability, Effluent	• CV's ranged from 0 to 49% for *D. magna* acute lethality tests (which represented the most extensive data base). • Data for other species was limited, and did not meet the minimum of 6 or more tests. • CV's for chronic testing with *Pimephales promelas* and *Ceriodaphnia dubia* ranged from 0 to 20% (mean of 7%). • Intra-laboratory variability are lacking for chronic testing with effluents.
Inter-laboratory Variability, Single Chemical	• CV's for *Daphnia magna* acute tests ranged from 30 to 143%. • Acute lethality test data were also available for *Daphnia* spp., *P. promelas*, and *Cyprinodon variegatus*. CV's ranged from 22 to 143% (mean of 47%) for these species. • CV's for chronic studies using *P. promelas* and *C. dubia* ranged from 7 to 71% (mean 39%).
Inter-laboratory Variability, Effluent	• CVs for *D. magna* acute lethality testing ranged from 0 to 110%. • Acute studies included *Daphnia* spp., *P. promelas*, and *Mysidopsis bahia*, with CV's for all studies ranging from 0 to 166% (mean 34%). • Only *P. promelas* and *C. dubia* chronic studies were done with at least six laboratories; CV's ranged from 0 to 83% (mean 34%).

Most of the inter-laboratory studies reviewed/evaluated in this article were not considered true round-robin studies. Round-robin studies provide estimates of the inherent variability in tests, such as test species sensitivity, whereas non-round-robin inter-laboratory studies provide estimates of realistic variability that can be expected with routine testing. This "realistic" variability includes all potential sources of variability that would be reflected in the test method including: toxicological, chemical, biological, physical, and technical variations.

Little data were available on toxicity test variability using the most current U.S. EPA methods at the time this article was written (i.e., 1991). The most extensive data sets were available for acute lethality tests with *Daphnia spp*. (daphnid), *P. promelas* (fathead minnow) and *M. bahia* (mysid shrimp). The largest data sets for chronic testing included: *P. promelas*, *C. dubia*, and *C. variegates* (sheepshead minnow). The following conclusions were presented:

- Additional non-round-robin studies are needed to quantify variability associated with routine compliance testing;
- CV's for intra-laboratory precision were smaller than those for inter-laboratory studies;
- CV's for chronic tests were less than or equal to CV's for acute lethality tests; and
- CV's for effluent tests were lower than CV's for single chemical tests.

2.5 Performance Characteristics of Effluent Toxicity Tests: Variability and its Implications for Regulatory Policy. (Warren-Hicks, W. and B.R. Parkhurst. 1992).

The main objective of the study was to evaluate the amount of variability associated with the toxicity test measurements. The authors examined round-robin test data for *Daphnia magna*, fathead minnows, and *Ceriodaphnia dubia* for intra- and inter-laboratory variability using U.S. EPA test methods. Measurements of toxicity exhibit some uncertainty associated with variability. Sources of variability include: intra- and inter-laboratory variability with regard to test organism sensitivity, culture methods, diet, implementation of all aspects of test methods, recording of data, etc. Variability in terms of % survival at each test concentration, as well as a point-estimate data (LC50) were evaluated. The results of the evaluation were as follows:

- The variability in percent survival among laboratories for some test concentrations was as large as 100%;
- The variability in percent survival is much greater near the average LC50 value;
- Large inter-laboratory variation was observed in percent survival at the same effluent concentration, for all three test organisms;
- Variations in percent survival were lowest in the concentrations with the highest and the lowest toxicity (i.e., two extremes of test concentrations); and
- In a chronic study, the variability in survival was much larger than that based on the LC50. Comparisons of LC50s and CV's were based on fathead minnow 7-day survival data.

The relationship between variation in percent survival and test concentration is significant in cases where NPDES permit limitations are expressed in terms of a specific survival limit (percent). Intermediate levels of effects (between 0 and 100% survival) exhibited the greatest variability. In routine effluent testing, the uncertainty associated with predicting a response as a function of test concentration would

increase over the variability reported in the round-robin studies analyzed. The article suggests that calculating the uncertainty surrounding an LC50 could be misleading with regards to the precision of survival data from toxicity tests using single effluent concentrations.

2.6 A Review of Inter- and Intra-laboratory Effluent Toxicity Test Method Variability. (Rue, W.J. *et al.* 1988)

This study, which was reported in the annual ASTM book, evaluated both intra- and inter-laboratory precision of common acute aquatic toxicity test methods by combining both published and unpublished effluent toxicity test data. The specific methods referenced were published prior to the publication of the U.S. EPA test methods. The document asserts that, before a test method is used for regulatory purposes, the ability of that test to provide reproducible data within and between laboratories should be determined. Results of the evaluation are summarized in Table 6.

Table 6. Inter- and intra-laboratory variability for effluent toxicity test studies.

Comparison	Results of Analyses
Inter-laboratory Variability	• 81.6% of the tests for which inter-laboratory data was available had CV's of ≤ 40%. • 74.5% had CV's of ≤ 30%. • 80.3, 78.6 and 81.4% of the tests with rainbow trout, *Daphnia* spp., and *Photobacterium phosphoreum* (Microtox™) respectively, yielded CV's of ≤ 40%. • 72.4, 78.6 and 72.1% of the tests with rainbow trout, *Daphnia* spp., and *P. phosphoreum* (Microtox) respectively yielded CVs of ≤ 30%. • In inter-laboratory testing with accepted (i.e., standard) analytical chemistry methods, 76 to 83% of the test data had CVs of ≤ 50%.
Intra-laboratory Variability	• 89.2% of the lab studies had CVs of ≤ 40%. • 78.3% had CVs of ≤ 30%. • Almost 95% of the tests using *Daphnia* spp. had CVs of ≤ 30%. • 90% of tests using several species yielded CVs of ≤ 30%. • 73% of the *Daphnia* spp. tests had CVs ≤ 10%. None of these tests had CVs above 50%. • In intra-laboratory testing with accepted analytical chemistry methods, 90 to 94% of the test data had CVs of ≤ 50%.

The authors concluded that generally, the intra- and inter-laboratory variability comparisons among chemical methods and standardized acute toxicity test methods for effluents are within the same range.

It is noted that the levels of precision presented may not be representative of all effluent test methods (i.e., acute and chronic), and complex chemical mixtures. Further work is required in order to determine the levels of precision that can be expected from effluent toxicity test methods, and how test method precision can be incorporated into effluent safety assessments.

2.7 Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program. (U.S. EPA, 2000)

This recent document was developed by the U.S. EPA's Office of Water's Headquarters, Office of Enforcement and Compliance Assurance, Office of Research and Development, and Regional Staff. An external peer review of the document was also conducted following EPA's peer review guidelines. This document provides guidance to laboratories, NPDES regulatory authorities and permittees involved in whole effluent toxicity (WET) testing. The potential sources of variability, how to minimize it, and how to address variability specifically within the NPDES program, are discussed. Although only U.S. EPA published acute and chronic test methods were included in the study, the key points relating to acute toxicity test variability and some general conclusions are relevant.

The goals of the document, defined to address issues of WET variability, included:

- Quantify the variability of promulgated test methods and report a coefficient of variation (CV) as a measure of test method variability (Chapter 3 and Appendix A);
- Evaluate the statistical methods for determining the need for and deriving WET permit conditions (Chapter 6 and Appendix G); and
- Suggest guidance for regulatory authorities on approaches to address and minimize test method variability (Chapter 6).
- To provide guidance to regulatory authorities, permittees, and testing laboratories on conducting the biological and statistical methods and evaluating test effect concentrations (Chapter 5).

Chapter 2 provided a definition of terms used and ways in which variability can be quantified.

Within-test (intra-test) variability refers to the variability in test organism response within a concentration averaged across all concentrations of the test material in a single test. Sources of variability include the number of treatment replicates, the number of test organisms exposed per replicate, and the performance of the control.

Within-laboratory (intra-laboratory variability is the variability that is measured when tests are conducted using specific methods under reasonably constant conditions in the same laboratory. Sources of variability include those described for intra-test variability as well as differences in test conditions, organism health and analyst performance. ASTM uses the term repeatability to describe within-laboratory variability.

Between-laboratory (inter-laboratory) variability is the variability between laboratories and reflects the degree of precision that is measured when the same sample or standard is analyzed by multiple laboratories using the same methods, but subject to their individual conditions. ASTM uses the term reproducibility to describe between-laboratory variability.

Whole Effluent Toxicity (WET): Whole effluent toxicity is the aggregate toxic effect of an aqueous sample (e.g. effluent, receiving water) measured directly by an aquatic toxicity test.

Three measures of variability were applied to WET tests including:

- Determine the variability of the biological end point response (e.g. growth, survival);
- Quantify the uncertainty of each test point estimate (e.g. LC50, Ec25, or LC50) using confidence intervals, which reflect within-test variability; and
- Use the standard deviation to quantify the uncertainty in the mean of the replicate response at each concentration within a particular test.

Chapter 3 discussed the variability of the effect concentration estimates (EC25, LC50, NOEC) and the variability of endpoint measurements (survival, growth, and reproduction). Two of the relevant acute test species included in this study were *Daphnia magna* and rainbow trout. Forty-eight *Daphnia magna* tests were conducted in five different laboratories. The median intra-laboratory CV for *D. magna* LC50 was 23%. Considerably less data were available for rainbow trout, with only one lab participating, with 10 tests. The median intra-laboratory CV for the one laboratory producing data for rainbow trout was 23%. In general, depending upon the method, 75 percent of the laboratories had CV's in the range of 19 to 27%.

The data sets analyzed in this study did not include information that may have been useful in determining the causes of inter-laboratory variability. Suggestions of possible causes include: differences in concentration series used, incorrect calculation or reporting of concentration (e.g., concentration of metal ion versus salt), differences in laboratory dilution water characteristics (specifically pH and hardness), differences in laboratory cultures and culture diet. Differences in mean endpoints between laboratories, is partly random, reflecting the intra-laboratory variance. Other differences among laboratories can only be evaluated reliably if laboratories use the same test method, same reference toxicant, test concentrations, similar dilution waters, and conduct a sufficient number of tests.

Chapter 4 included a discussion of WET variability in the context of chemical-specific method variability. Results of independent studies have generally concluded that currently promulgated WET methods are technically sound and that the observed precision is within the range of precision of other chemical specific analyses. The general conclusions and recommendations were as follows:

- US EPA methods (1985, 1988, 1989) are technically sound, but certain modifications could be implemented to improve endpoint interpretation including improvements to current statistical procedures, establishing acceptable limits for MSD values, and adding confidence limits to WET test endpoints.
- Problems of WET tests relate to misapplication of tests, misinterpretation of the data, lack of competence of the laboratories conducting the test, poor condition / health of the test organisms, lack of training of laboratory personnel, regulators, and permittees and lack of an effective QA / QC program.

The suggested practices to control within-test variability included:

- Controlling within-test sensitivity;
- Following well-defined test methods;
- Using well trained and experienced laboratory personnel; and
- Using rigorous QA / QC practices; and,

- Maintaining communication within the regulatory community.

Chapter 5 provided guidance to permittees, testing laboratories and regulatory authorities to minimize test method variability. A summary of recommended steps for minimizing test method variability included:

- Obtaining a representative sample;
- Conducting the tests properly according to well-standardized test methods; and
- Conducting the appropriate statistical analysis to obtain defensible effect concentrations.

Some of the key factors that affect variability were discussed including:

- Sampling procedures;
- Sample representativeness;
- Deviations from standardized test conditions (e.g. temperature, test duration, feeding);
- Test organisms;
- Source of dilution water; and
- Analyst experience and technique in conducting the toxicity tests properly.

The conclusion of groups of scientists and researchers is that the observed precision of currently promulgated WET methods, are within the range of precision of other frequently required analyses, and are technically sound. The document also suggests considerations for minimizing variability, as described in several papers. A number of conclusions were presented, as follows:

- A laboratory's experience and success in conducting aquatic toxicity tests is the most important consideration in producing precise data. Experienced laboratories are able to produce the most reliable information, and interpret anomalous conditions in tests or results;
- Laboratories should follow test methods appropriately. Tests should not be used in the regulatory process if they do not meet specific protocol requirements, or if the associated QC (e.g., reference toxicant) tests are beyond control limits;
- Tests conducted with effluents that have not met the required holding times or temperatures should not be used in the regulatory process; and
- Regulatory authorities and permittees should ensure that rigorous laboratory QA practices, or good laboratory practices, are in place, whether by national laboratory accreditation, State regulatory certification, direct client oversight, or contractual agreement with the laboratory.

Specific guidance to regulators, permittees and laboratories involved in WET testing was provided in areas relating to:

- Collecting representative samples (e.g. issues relating to sampling location, frequency and type, sample volume, container, preservation methods and holding time);
- Conducting the biological test methods (e.g. procedures, experimental design, quality control, test acceptability criteria);

- Quality Control charts and laboratory audits (e.g., Use of control charts to ensure QC procedures are properly maintained, use of checklists by authorities to assist in evaluating and interpreting test results);
- Experimental design (e.g., randomizing the treatments, organisms, replicates, specifying the numbers of organisms, replicates, treatments);
- Test acceptability criteria (e.g., Minimum requirements for control survival, growth or reproduction); and
- Conducting the statistical analysis to determine the effect concentration.

Chapter 6 provided guidance to regulatory authorities on how to determine reasonable potential (RP) and derive permit limits or monitoring triggers and evaluate self-monitoring data. Finally, a summary of EPA's principal conclusions and guidance to laboratories, permittees and regulatory authorities were summarized in Chapter 7. These included:

- Design a sampling program that collects representative samples to fully characterize effluent variability for a specific facility over time;
- Ensure proper application of WET statistical procedures and test methods;
- Incorporate both upper and lower bounds using the percent minimum significant difference (PMSD) to control and to minimize within-test method variability and increase test sensitivity; and
- Participate in an Environmental Laboratory Accreditation Program and routine performance audit inspections to evaluate laboratory performance.

Encourage WET testing laboratories to maintain control charts for PMSD and the control means and report the PMSD with all WET test results.

2.8 Effects of Several Variables on Whole Effluent Toxicity Test Performance and Interpretation. (Markle, P.J. *et al.* 2000)

This article addresses selected procedural or method-related protocol changes contained within U.S. EPA whole effluent toxicity tests that have the potential to affect toxicity test performance and interpretation of results. Procedural changes evaluated in the study included: changes in the *P. promelas* chronic growth endpoint definition from final mass to biomass, differences between haemocytometer and fluorometer measurements in the *Selenastrum capricornutum* growth test, and options for statistical interpretation of species sensitivity in multiple test/species screening bioassays. Method changes evaluated in the study included: age-specific acute responses between fish ranging in ages 1 to 14 day old and 14 to 90-day old fathead minnows (*Pimephales promelas*). Procedural changes evaluated in this study were not considered relevant to the specifics of the Environment Canada acute lethality test method, and so are not discussed here. The following discussion summarizes the results of the study finding relating to the effects of age on variability of organism response based on the U.S. EPA acute lethality test (1985, 1995) using *P. promelas*.

Methods for measuring acute toxicity of effluents to fathead minnows based on the U.S. EPA (1985) permit testing of fish up to 90 days old. A more recent version of the method (U.S. EPA 1993) further limited this range to include fish between 1 and 14 days old. Based on the 1985 method, acute toxicity

tests were initiated using 1, 4, 7 and 14 day old fish. Each batch of test organisms was exposed to various toxicants including hexavalent chromium, (Cr^{+6}), sodium dodecyl sulfate (SDS), sodium pentachlorophenate (NaPCP) and ammonia, in moderately hard water. Similar testing, based on the 1993 method, was initiated using 14, 30 and 90 day old fish. In these tests, fish were exposed to copper (Cu) and ammonia (NH_3).

Within the 1 to 14 day age group, 1-day-old fish had significantly higher LC50's (greater tolerance) for NaPCP and SDS than older stages (4 to 14 days) and were able to survive higher concentrations of (Cr^{+6}). No age-related differences in sensitivity to ammonia were observed.

Furthermore, inter-test precision estimates using CV indicated that LC50 data generated using 1 d old larvae were generally more variable than data using older fish. For the 14 to 90 day old test group, 14-day-old fish had lower LC50s (lower tolerance) for Cu and NH_3 than older fish (90 d).

The data demonstrated that age of the organism can be a modifying toxicity factor for certain contaminants, not only during the early stages of development, but in older fish. Age of the test organism used for testing needs to be selected and/or specified by the testing laboratory in order to ensure uniform sensitivity and maximize precision.

2.9 Reducing Variability in *Daphnia* Toxicity Tests – A Case for Further Standardization. (Bradley *et al.* 1993)

The various challenges inherent in reproducibility of *Daphnia* toxicity tests (including the 21-day life cycle and 48-hr EC50 tests) are discussed in this book chapter, published in 1993. The issues of reproducibility are dealt with by considering the test system as a 'multi-component' system. The major components of this system, specifically, the test organism, the culture system and the test system, are considered separately with respect to issues of standardization. These issues are discussed in light of numerous studies dealing with sources of variability in *Daphnia* toxicity tests conducted by the authors and others reported in the primary literature.

Although this information was based on European test methods for various acute (48-hr) and chronic (21-day) daphnia tests, the following conclusions of this chapter are relevant to all toxicity test methods:

- interclonal variation may be important for certain contaminants; these were found to be more important in chronic versus acute tests; and
- the culture system specifically related to organism density, food quality, and culture medium, may also play a role in test variability and need to be addressed on a test method-specific basis.

Project Manager

Project Director

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APPENDIX C

DATA REVIEW

GUIDANCE DOCUMENT FOR ACUTE LETHALITY TESTING OF METAL MINING EFFLUENTS: DATA REVIEW

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EXECUTIVE SUMMARY

Two data sets were examined with the purpose of estimating coefficients of variation in rainbow trout EPS 1/RM/13 and *Daphnia magna* EPS 1/RM/14 toxicity test results. Data sets were obtained from CAEAL performance evaluations and from reference toxicant tests submitted by volunteer laboratories.

Among-laboratory coefficients of variation (CVs) were estimated from the CAEAL PE data set using variance components analyses, and within- and among-laboratory CVs were estimated from the reference toxicant data set. Among-laboratory CVs were estimated stratifying on date and CAEAL PE sample to produce 52 CVs for the rainbow trout PE data set. These CVs ranged from 8.0 to 60.4% with a median CV = 15.7%. Twenty-eight CVs were estimated from the *Daphnia magna* CAEAL PE data set. The among-laboratory CVs ranged from 7.5 to 53.1% with a median CV = 12.9%. The CVs were much larger in 10/31/1997, possibly reflecting the change in CAEAL reference toxicant from phenol to NaCl on this date.

The within and among-laboratory CVs for rainbow trout reference toxicity tests using phenol as a reference toxicant are 3.5, 13.3%. The within and among-laboratory CVs for rainbow trout reference toxicity tests using Zn as a reference toxicant are 34.6 and 38.5% The within and among-laboratory CVs for *Daphnia magna* reference toxicity tests using NaCl as a reference toxicant are 4.6 and 8.7%. The within and among-laboratory CVs for *Daphnia magna* reference toxicity tests using Zn as reference toxicant are 27.3 and 33.3%.

The analyses show that that the variability within a laboratory or day-to-day variability is greater than the variability among laboratories for both tests. This result may be in part, a consequence of the extra within-laboratory variability induced by using reference toxicant data sets rather than a round-robin data set where a stock solution is used to distribute identical samples.

Also, LC50 estimates are less variable within laboratories, when Zn is used. This may be a consequence of the mode of toxic action of Zn relative to phenol for rainbow trout, and NaCl for *Daphnia magna*. A highly toxic substance will produce a steeper dose response than a less toxic substance inducing a reduction in variability in the sample of LC50 estimates.

Overall, the magnitude of variability observed in the two acute lethality test methods presented in this data review are comparable to, or lower than, the variability associated with those reported for the U.S. EPA test methods. Moreover, the toxicity test variability is within the range of (and in some cases, lower than) the variability observed in analytical chemistry methods.

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1.0 INTRODUCTION

1.1 Background

The study objective of this project is to prepare a guidance document for toxicity testing laboratories, industry, and regulatory authorities that addresses the key aspects of acute lethality testing for mining effluents, and will provide guidance aimed at maximizing data reliability. The content of the document will be sufficiently detailed to enhance the efforts of laboratories to produce reliable data, and will include summaries to support the review of data and test results by the metal-mining sector.

To provide background to this issue, and in order to gain a better understanding of what is known about the reliability of data produced with the conduct of acute lethality testing following Environment Canada's Reference Method EPS 1/RM/13 (rainbow trout) and 1/RM/14 (*Daphnia magna*) (Environment Canada, 1990a,b), coefficients of variation were estimated.

1.2 Purpose of Data Review

Two distinct data sets were examined with the purpose of estimating coefficients of variation associated with rainbow trout EPS 1/RM/13 (Environment Canada, 1990a) and *Daphnia magna* EPS 1/RM/14 (Environment Canada 1990b) toxicity test results. Data sets were obtained from the Canadian Association of Environmental Analytical Laboratories (CAEAL) and from nine volunteer laboratories.

The CAEAL data set consists of PE results collected since 1994. Four coded samples were submitted to CAEAL-accredited laboratories biannually. A total of 33 laboratories produced results. Some laboratories have been participating in the CAEAL program since 1994 and have participated in 13 performance evaluations. Other laboratories have participated in as few as 1 performance evaluation. Participating laboratories estimated LC50s using one or both of the toxicity test methods.

The data sets collected from volunteer laboratories comprised the last 20 reference toxicant tests conducted for either the rainbow trout or *Daphnia magna* tests. Eight laboratories submitted data for rainbow trout tests. Of these, 4 used phenol as a reference toxicant, while 3 used Zn and one laboratory used both reference toxicants. Eight laboratories submitted data for *Daphnia magna* tests. Of these, 5 used NaCl as a reference toxicant while 3 used Zn.

1.3 Document Overview

The following describes the rationale for the investigation of the data sets. This section and the conclusion section should provide sufficient detail for casual readers to understand the intent and conclusions of this document. Detailed descriptions of methods and results are provided in sections 2 and 3, respectively.

Both within- and among-laboratory coefficients of variation (CVs) were estimated using the reference toxicant data sets. Exploratory data analysis tools described in section 2.2 were used to ensure validity of data entry/transcription, explore the distribution and variability of results, and check for aberrant results. Variance components analyses described in section 2.3, and performed in section 3.2.4 were used to estimate the CVs.

2.0 METHODS

This section describes the statistical tools used in the various evaluations at two levels. An introduction is provided that is intended for the non-statistician. The introduction provides the purpose of the tool and provides some rationale for why the tool was chosen.

2.1 Frequency Histograms

A frequency histogram divides a data set into "bins" or "classes", and counts the number of observations that fall within each bin. This number is divided by the total number of observations in the data set, to produce a frequency. The frequency within a bin may be plotted using a bar chart.

Observations arising from the commonly encountered normal distribution produce a bell-shaped frequency histogram. Thus, the shape of a frequency histogram can be used to determine what distribution the observations might arise from.

The width of the histogram provides a visual assessment of the variability of the observations. A variable data set will produce a wider histogram than a less variable data set.

2.2 Exploratory Data Analysis

Exploratory data analysis is a quasi-subjective exploration of a data set. The focus of the exploration depends upon the analyst's interest. In this document, exploratory data analyses were used to check for data entry and transcription errors, explore relative variability among and within laboratories, across dates and due to different contaminants, to visually assess the distribution of observations and to identify outliers.

2.3 Variance Components Analysis

Reference toxicant data sets were obtained from volunteer laboratories for rainbow trout and *Daphnia magna* tests. These laboratories represent a random sample from the population of laboratories of interest. Of interest is determining how the variability within a laboratory compares with variability among laboratories. This is similar to a round-robin study. However, in this case, there are not within-laboratory split-sample results. We have a group of 20 tests conducted by a laboratory using the same test method. The within-laboratory variance estimate includes the operator effects, differences in test organisms, etc. that would be measured by the within-laboratory variance component in a round-robin study but also includes variability due to changes in water quality and culture health over time, errors in sample preparation/dilution, etc. Therefore, the within-laboratory variance component estimated here is a more realistic estimate of the range of variability encountered within a laboratory than that obtained by the usual round-robin estimate.

We fit models of the form:

$LC50 = Laboratory_i + Toxicant_j + \theta_{ij}$, or

$LC50 = Laboratory_i + \theta_{ij}$, or

Where:

Laboratory is the random effect due to laboratory after adjusting for toxicant in the first model, or the toxicant-specific laboratory effect in the second model,

Toxicant is the random effect due to toxicant, and;

ε is the within-laboratory error.

We assume that $Laboratory_i \sim N(0, \sigma_1^2)$, $Toxicant_j \sim N(0, \sigma_2^2)$, and $\varepsilon_{ijk} \sim N(0, \sigma^2)$. We also assume that observations are independent of one another. Model assumptions are evaluated using normal quantile-quantile plots, but model diagnostics are not presented herein.

The software implementation was SPlus 2000 Professional, using restricted maximum likelihood.

3.0 RESULTS

3.1 CAEAL PE Data

3.1.1 Exploratory Data Analysis of Rainbow Trout Data Set

The data sets were initially explored by plotting the rainbow trout LC50's versus date. Two laboratories produced 8 aberrant observations that were deleted from the data set. Frequency histograms[1] were then plotted by date and CAEAL PE sample number (C11-1 to C11-4). These are presented in Appendix A and summarized below.

In the histograms, a relatively large degree of variability is observed from October 1994 to March 1996. There is a general reduction in variability over time. Given the number and nature of variables that can potentially affect an LC50 estimate, the variations about a mean are expected to be normally distributed. The distribution of results approaches the expected normal distribution during 1996, although in the early stages of the program, the distribution of rainbow trout LC50's was not normally distributed.

Date and sample-specific comments are:

- The observations in March of 1999 are remarkably homogeneous.
- For sample C11-3, it appears that mean LC50 values increase from October of 1994 to October of 1996.
- For sample C11-4, observations in March of 1999 show an aberrant result attributable to one laboratory. Moreover, values prior to 1995 are much higher than subsequent values. In March 2000, one laboratory produces a result that differs from the group of results.

3.1.2 Exploratory Data Analysis of *Daphnia magna* Data Set

A plot of the *Daphnia magna* CAEAL PE data sets against date revealed a marked difference in results before and after 10/31/1997. Prior to this date phenol was used as the CAEAL reference toxicant. Due to problems with this toxicant, NaCl was substituted in 10/31/1997. Data analyses treated only the NaCl PE data (i.e., subsequent to October 1997).

While examining quantiles, maxima and minima, it was noted that the maxima for 3 of the 4 CAEAL PE samples were identical. These 3 values were produced by a laboratory on 10/31/1997.It is extremely unlikely that a laboratory could produce identical LC50 estimates for 3 different samples on the same day. The same laboratory also produced the maximum estimated LC50 in the entire data set on this same date. These 4 observations were treated as erroneous entries and were omitted from the data set. Frequency histograms[2] were then plotted by date and CAEAL PE sample number (C12-1 to C12-4). These are presented in Appendix B.

Date and sample-specific comments are:

- For sample C12-1, quite peaked distributions were observed on 03/31/1998, 10/31/1998 and 03/31/2000. More than 40% of the laboratories produced LC50 estimates slightly less than 20 mg/l.
- For sample C12-2, a larger than expected proportion of laboratories produced results in the central portion of the distribution.

[1] Number of bins chosen using Freedman-Diaconis (1981) method.

[2] Number of bins chosen using Freedman-Diaconis (1981) method.

- For sample C12-3, there is a relatively large degree of variability in 10/31/1998. Moreover, two sampling dates exhibited kurtotic frequency histograms.
- For sample C12-4, results on 10/31/1998 were also variable. Upon re-examination of frequency histograms for samples C12-1 and C12-2 on the same date, it is apparent that the LC50 estimates are also slightly more variable than results on other dates. The relatively large variability on this sampling date implies variability in the physical distribution of PE samples.

3.1.3 Estimating Among-Laboratory Coefficients of Variation

The original purpose of the analyses was to estimate the within- and among-laboratory coefficients of variation (CVs) for the rainbow trout and *Daphnia magna* tests using the CAEAL PE data set. However, the lack of replication precludes a simple estimate of the within-laboratory variance component. It may be possible to use the coefficients of the expected mean squares to derive a suitable variance component for this data set, but to the best of our knowledge this has not been done for an unbalanced, unreplicated data set.

It is possible, however, to ignore date effects and estimate among-laboratory variances for each CAEAL sample accounting for the date effect. However, given homogenous variance estimates across the concentration range used, differences among coefficients of variation will merely reflect differences in means. A judicious choice of exposure concentration would allow an experimenter to generate any desired coefficient of variation. The CVs are estimated in a suboptimal manner by stratifying on both date and CAEAL sample.

Table 1: Summary of Among-Laboratory CVs from Rainbow Trout CAEAL PE Data

Date	Coefficients of Variation (%)			
	C11-1	C11-2	C11-3	C11-4
10/31/1994	17.6	13.8	20.7	20.6
3/31/1995	16.4	16.2	16.9	19.1
10/31/1995	16.0	14.2	15.7	16.9
3/31/1996	14.6	17.0	15.9	14.4
10/31/1996	16.2	15.6	11.1	9.8
3/31/1997	16.7	16.1	15.8	11.5
10/31/1997	14.9	18.9	13.5	12.3
3/31/1998	15.6	16.7	16.7	13.0
10/31/1998	15.5	18.1	17.0	18.9
3/31/1999	12.6	8.0	13.9	60.4
10/31/1999	15.4	14.3	14.3	12.9
3/31/2000	12.9	13.5	14.6	31.1
10/31/2000	16.4	15.1	17.8	10.5

Among-laboratory CVs for the rainbow trout test, range from 8.0 to 60.4% with a median = 15.7%.

Table 2: Summary of Among-Laboratory CVs from *Daphnia magna* CAEAL PE Data

Date	C12-1	C12-2	C12-3	C12-4
10/31/1997	53.1	30.0	30.8	21.4
3/31/1998	8.7	12.7	9.6	8.7
10/31/1998	15.9	16.6	16.6	16.3
3/31/1999	10.6	9.1	10.5	11.7
10/31/1999	15.9	12.5	15.0	12.8
3/31/2000	13.0	13.7	7.5	8.0
10/31/2000	14.7	11.1	17.1	9.6

Among-laboratory CVs for the *Daphnia magna* test range from 7.5 to 53.1% with a median CV of 12.9%. Note that coefficients of variation are much larger in 10/31/1997. This sampling date likely reflects the change in CAEAL reference toxicant from phenol to NaCl.

3.2 Reference Toxicant Data Analyses

3.2.1 Exploratory[3] Data Analysis of Rainbow Trout Data Set

The data set was initially explored by plotting frequency histograms of the rainbow trout LC50s by laboratory. These frequency histograms are presented in Appendix C. The frequency histograms showed that:

- Laboratory C, shows an unexpected distribution of LC50 estimates. These values appear more uniformly distributed than randomly (hence, normally) distributed about some mean. Laboratory E exhibits a skewed distribution.
- Laboratories E and F exhibit a skewed distribution. Interestingly, Laboratory E also produced a skewed distribution of LC50 results when the reference toxicant was phenol.

3.2.2 Exploratory Data Analysis of *Daphnia magna* Data Set

The data set is initially explored by plotting frequency histograms of the *Daphnia magna* LC50s by laboratory. These frequency histograms are presented in Appendix D. The frequency histograms showed that:

- Laboratories A and B produce skewed distributions of LC50s. The distribution of results from laboratory C is unusually precise
- Laboratories F and I produce skewed distributions of LC50s. The distribution of results from laboratory E is less precise than that of other laboratories.

3.2.3 Sources of Variability in Reference Toxicant Data Sets

Variance components analyses were used to estimate the within- and among-laboratory variability. The two variance components of interest are presented below. A more general variance component analysis incorporating reference toxicant as a variable was used to estimate variance components.

[3] Freedman-Diaconis (1981) binning is used to create histograms. Results are compared (but not presented) with Sturge's and Scott's binning criteria to ensure that interpretations are not artifactual.

Table 3: Within and Among-Laboratory Coefficients of Variation

Model	Variance Components		Coefficients of Variation	
	Among Laboratory	Within Laboratory	Among[1] Laboratory	Within[1] Laboratory
RT[2], phenol-specific	0.128	1.833	3.505 %	13.280 %
RT, Zn-specific	0.032	0.040	34.588 %	38.497 %
Dm[3], NaCl-specific	70034.1	247219.5	4.618 %	8.676 %
Dm, Zn-specific	0.078	0.116	27.267 %	33.284 %

[1]Uses global mean.

[2]RT = rainbow trout.

[3]Dm = *Daphnia magna*.

4.0 SUMMARY AND CONCLUSIONS

4.1 Summary

4.1.1 CAEAL PE Data Sets

Among-laboratory CVs were estimated stratifying on date and CAEAL PE sample to produce 52 CVs for the rainbow trout data set. These CVs range from 8.0 to 60.4%.

Twenty-eight CVs were estimated from the *Daphnia magna* CAEAL PE data set. The among-laboratory CVs ranged from 7.5 to 53.1%. The CVs are much larger in 10/31/1997, possibly reflecting the change in CAEAL reference toxicant from phenol to NaCl on this date.

4.1.2 Reference Toxicant Data Analyses

The rainbow trout phenol LC50 within-laboratory variation is much larger (by a factor of more than 14) than the among-laboratory variation due to the effects of one laboratory. The rainbow trout Zn LC50 within-laboratory variation is approximately the same as the among-laboratory variation.

The *Daphnia magna* within-laboratory variation is larger (by a factor of approximately 3.5 times using NaCl and 1.5 times using Zn) than the among-laboratory variation.

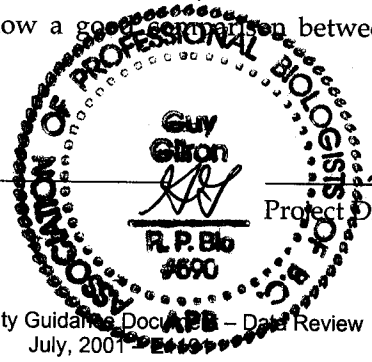
The analyses show that that the day-to-day variability within a laboratory is greater than the variability among laboratories for toxicity tests. Also, results are less variable within laboratories when Zn is used. This may be a consequence of the mode of toxic action of Zn relative to phenol for rainbow trout and NaCl for daphnids.

4.2 Conclusions

Overall, the magnitude of variability observed in the two acute lethality test methods presented in the data review (Appendix C) are comparable to, or lower than, the variability associated with those reported for the U.S. EPA test methods (see literature review; Appendix B). Moreover, the toxicity test variability is within the range of (and in some cases, lower than) the variability observed in analytical chemistry methods.

The U.S. EPA (1991) suggests that test method variability for both acute and chronic tests were similar to accepted analytical procedures for individual chemicals. Similarly, Rue *et al.* (1988) compared the distributions of CVs for the EPA's priority pollutants with effluent toxicity data from their study, and found that the CVs were generally in the same range. The U.S. EPA document on Method Variability (U.S. EPA, 2000) cites several studies with similar findings. For example, Ausley (1996) found that CVs for various chemical analytes ranged from 11.8% to 291.7%, however CVs for acute and chronic toxicity parameters were much lower, ranging from 14.8% to 67.6%. According to a U.S. EPA document, Denton and Norberg-King (1996) cite a number of additional studies that show a good comparison between chemical analytical and toxicity test methods.

Project Manager



Project Director

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Freedman, D. and P. Diaconis. 1981. On the histogram as a density estimator: L_2 theory. *Zeitschrift für Wahrscheinlichkeitstheorie und verwandte Gebiete*. 57:453-476.

APPENDIX A

FREQUENCY HISTOGRAMS FOR RAINBOW TROUT CAEAL PE DATA

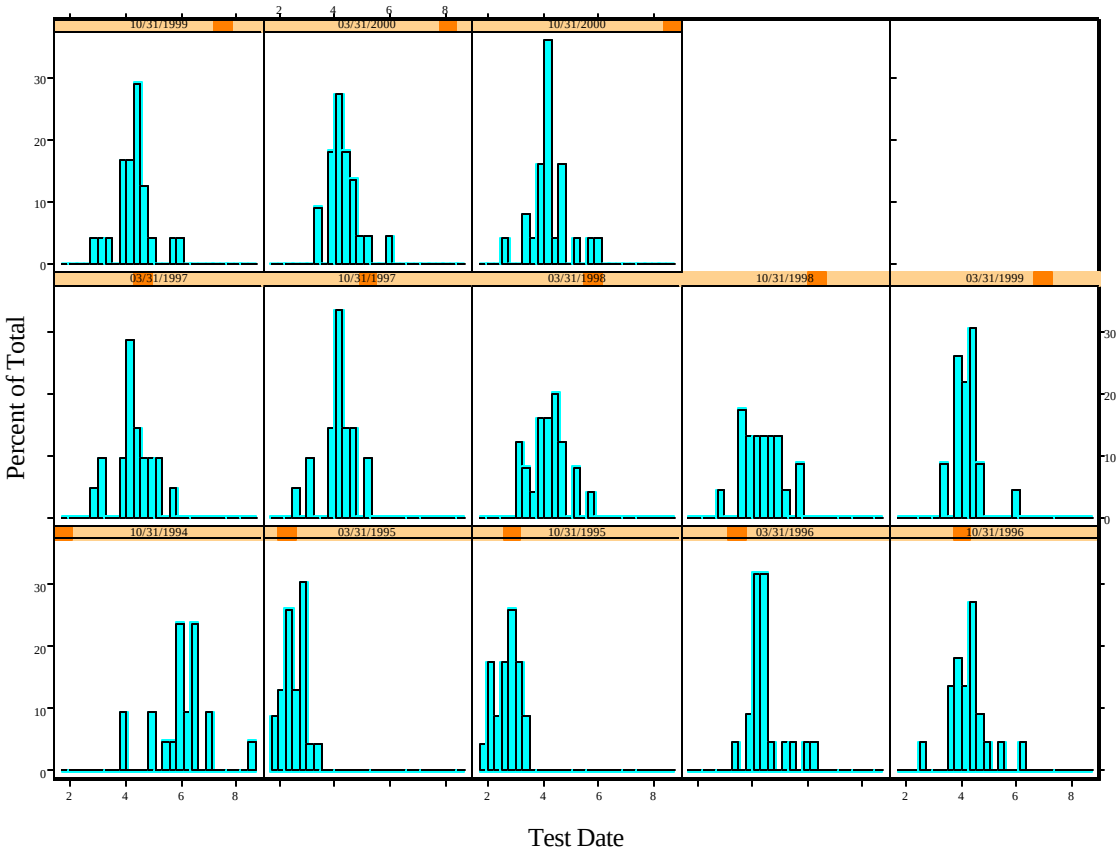


Figure A1: Frequency Histogram for Rainbow Trout LC50 Estimates using Sample C11-1

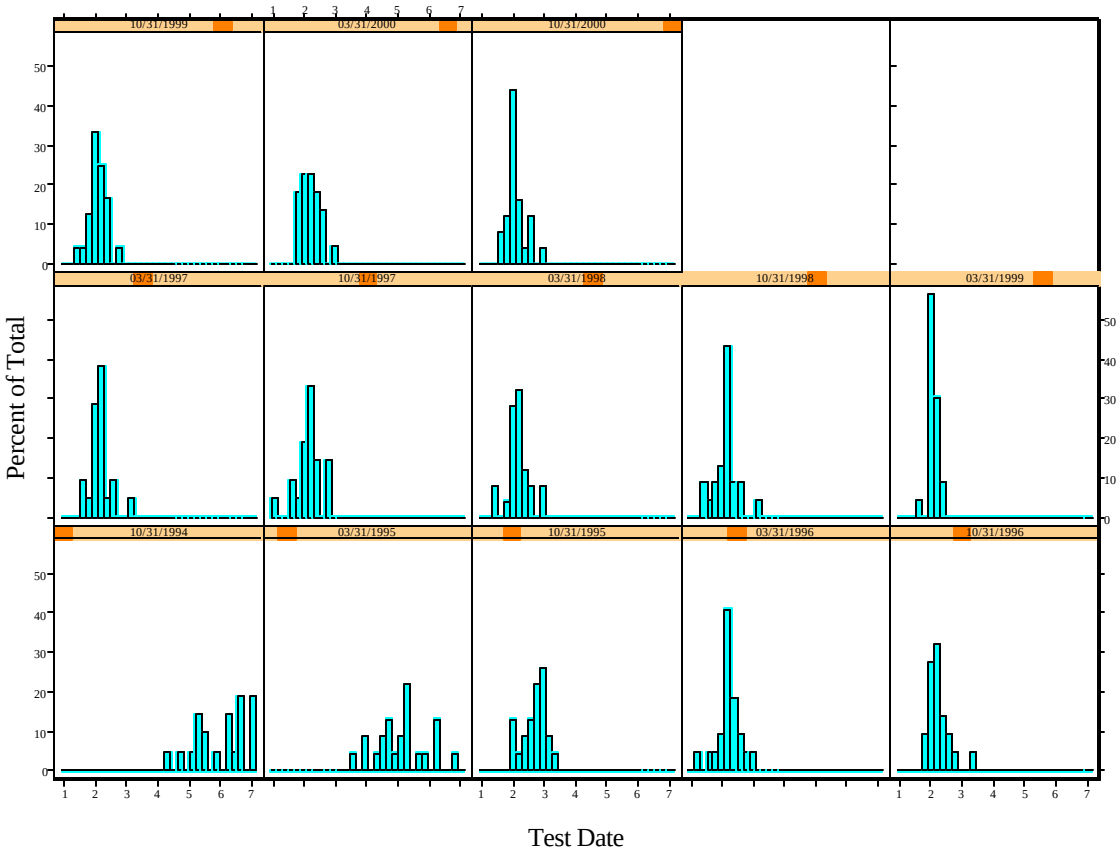


Figure A2: Frequency Histogram for Rainbow Trout LC50 Estimates using Sample C11-2

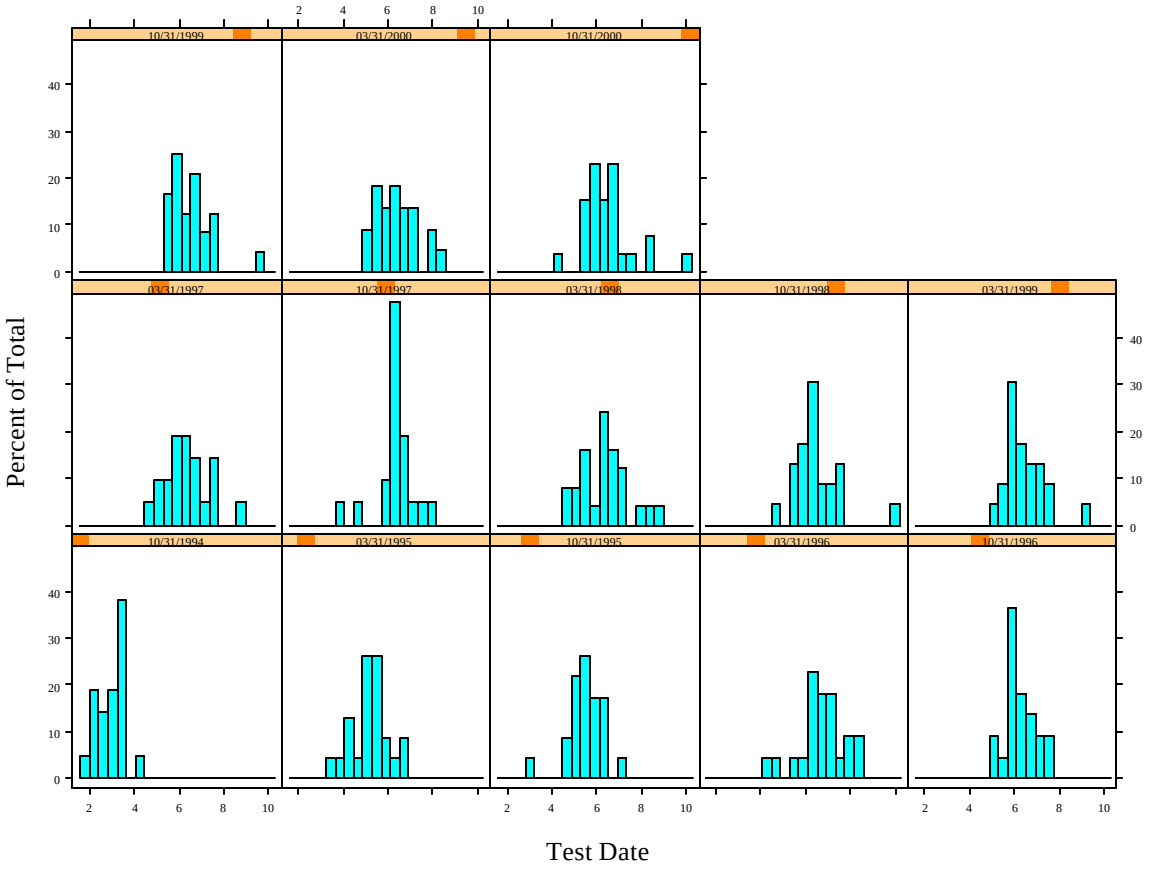
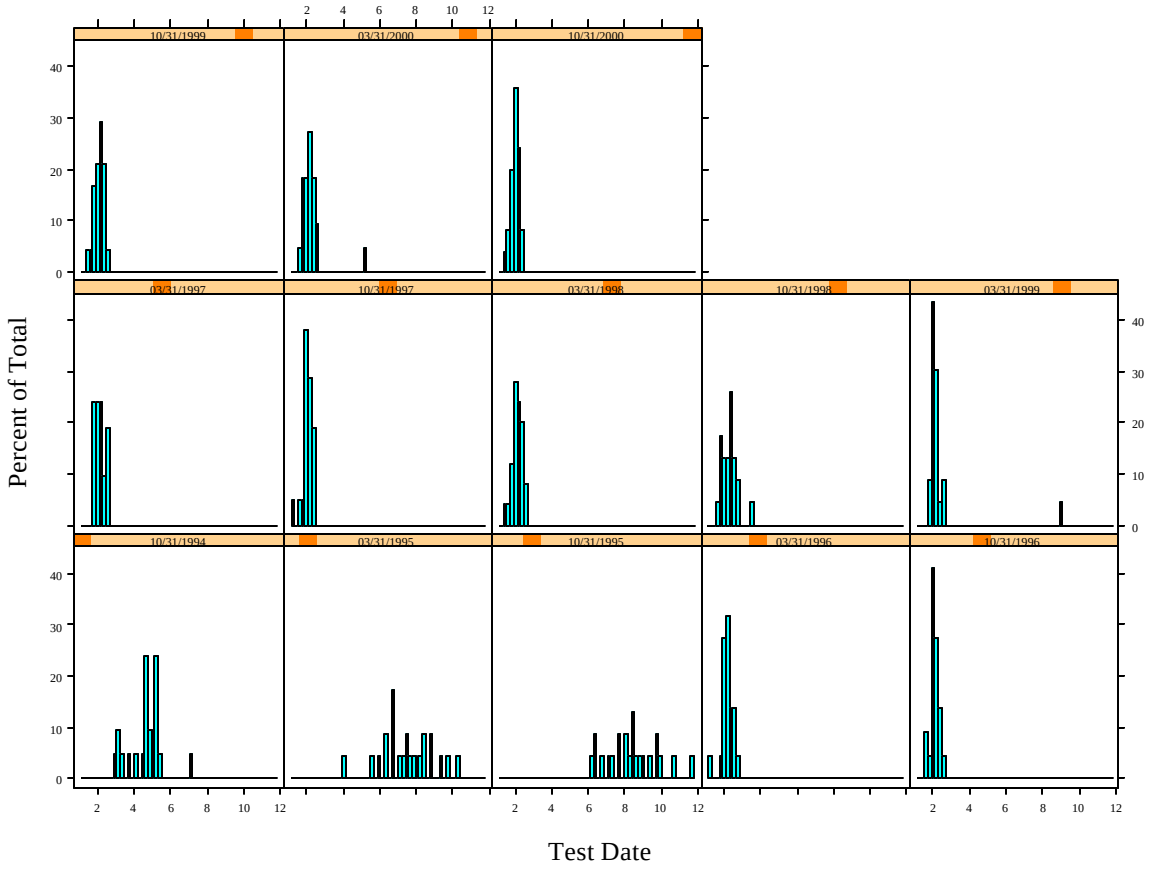


Figure A3: Frequency Histogram for Rainbow Trout LC50 Estimates using Sample C11-3



FigureA4: Frequency Histogram for Rainbow Trout LC50 Estimates for Sample C11-4

APPENDIX B

FREQUENCY HISTOGRAMS FOR *DAPHNIA MAGNA* CAEAL PE DATA

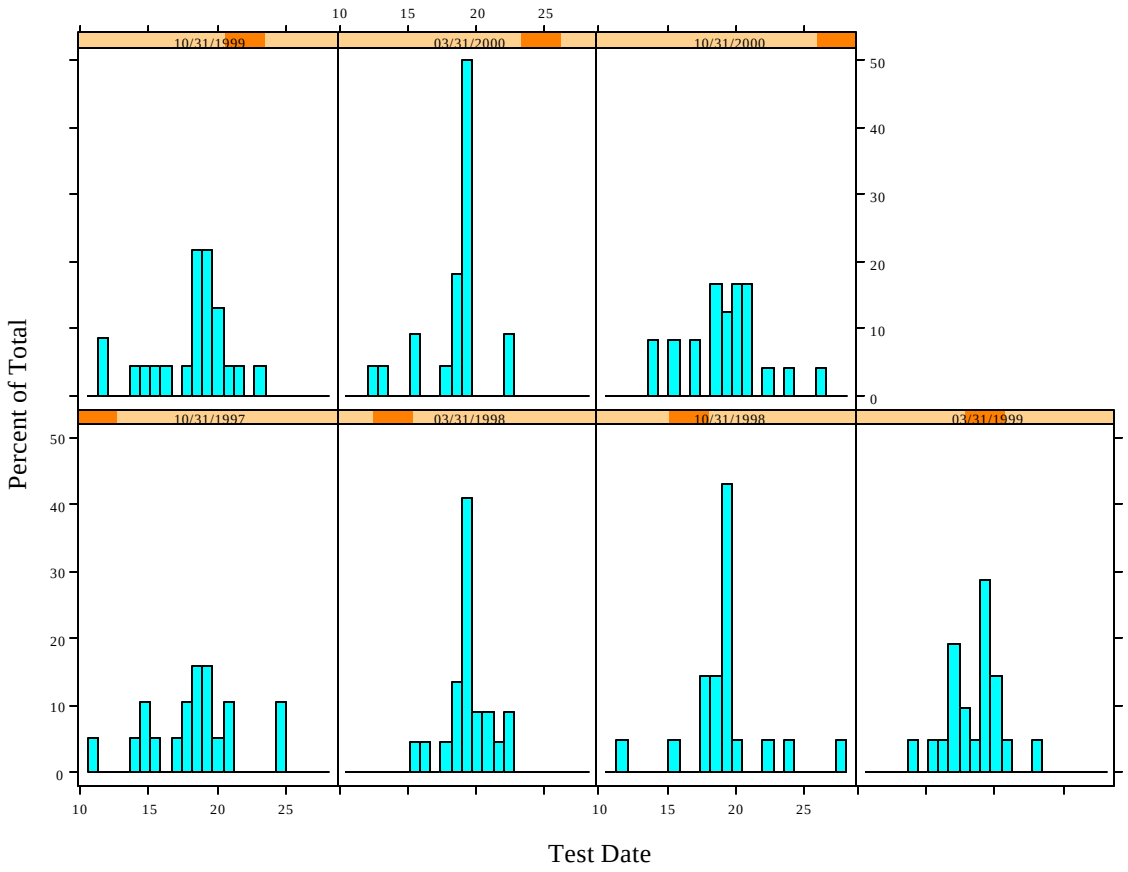


Figure B1: Frequency Histogram for *Daphnia magna* LC50 Estimates for Sample C12-1

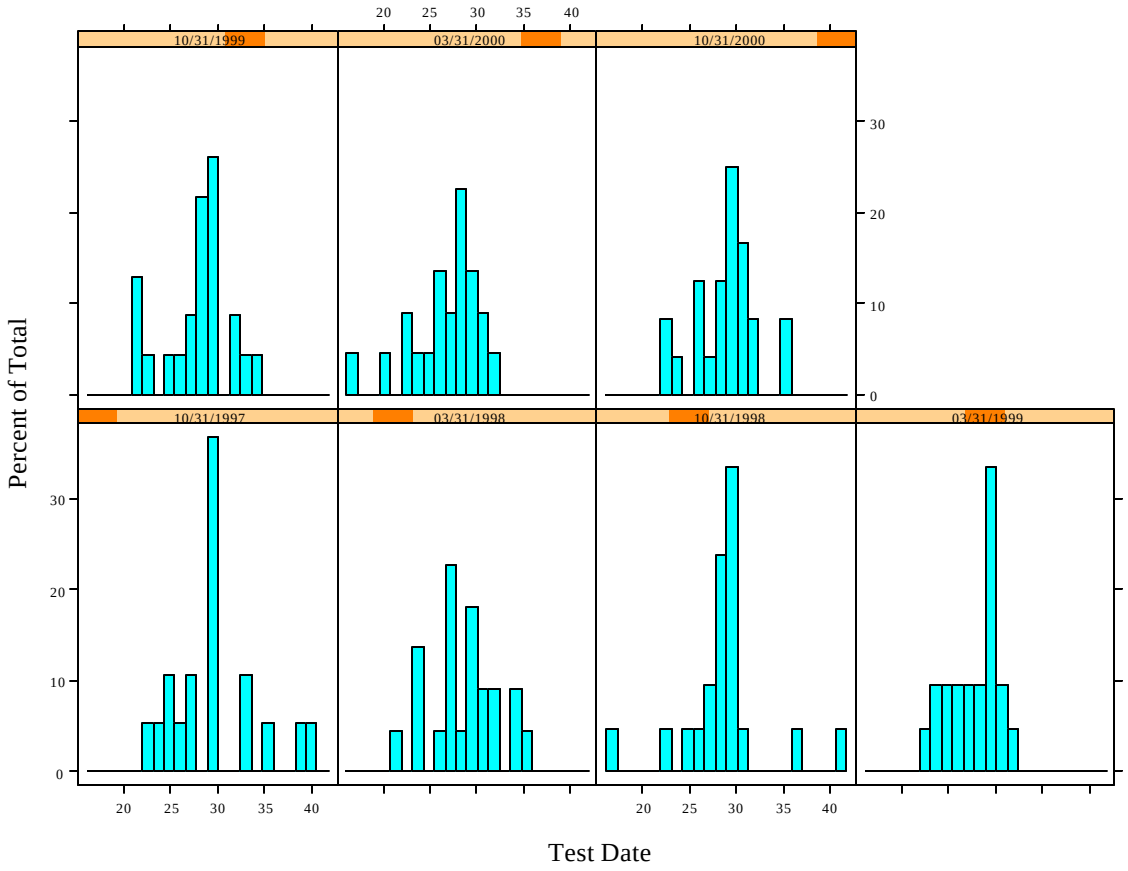


Figure B2: Frequency Histogram for *Daphnia magna* LC50 Estimates for Sample C12-2

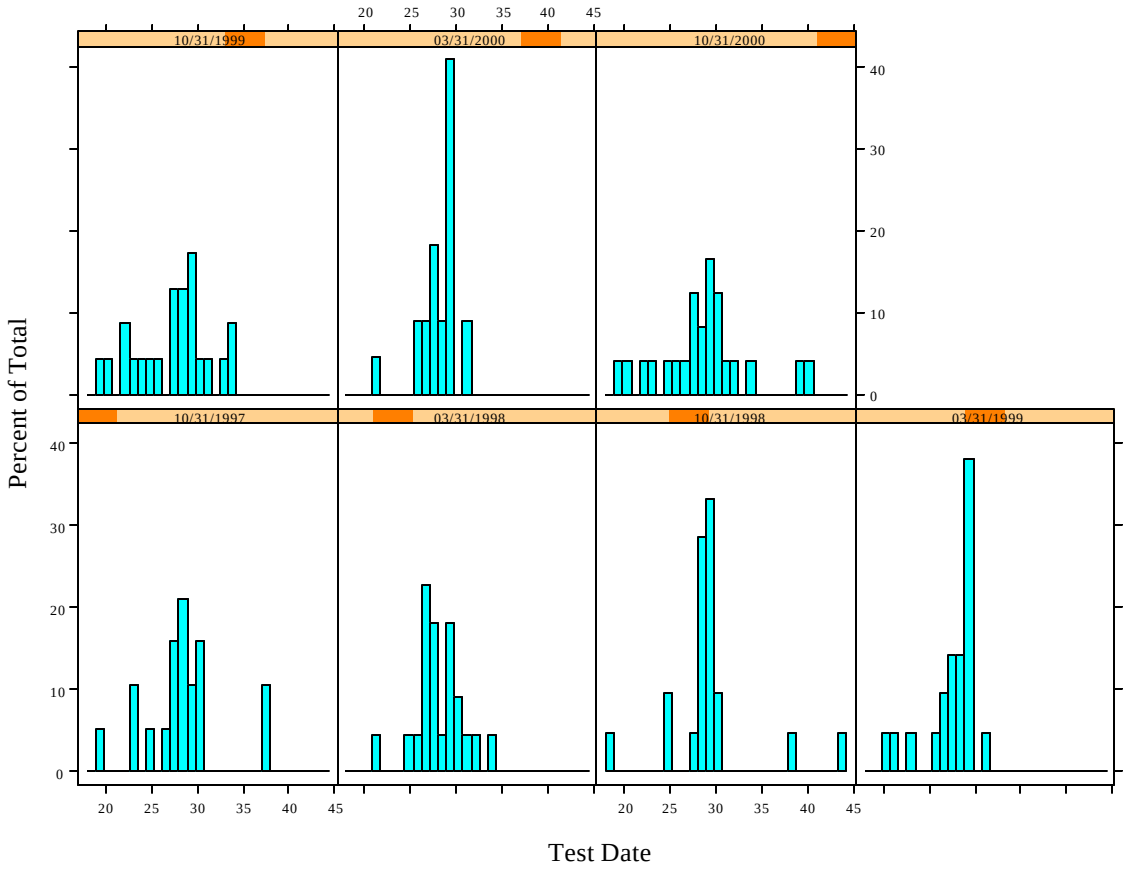
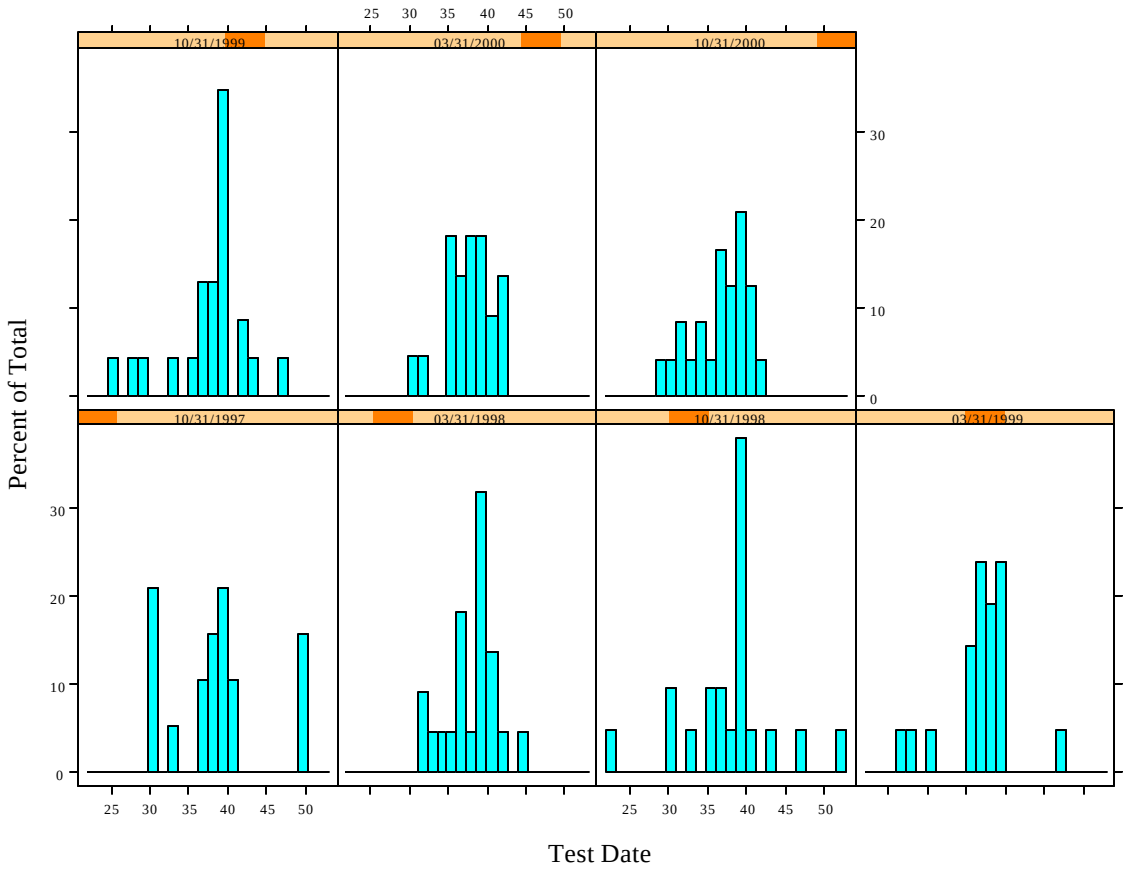


Figure B3: Frequency Histogram for *Daphnia magna* LC50 Estimates for Sample C12-3



FigureB4: Frequency Histogram for *Daphnia magna* LC50 Estimates for Sample C12-4

APPENDIX C

FREQUENCY HISTOGRAMS FOR RAINBOW TROUT REFERENCE TOXICANT DATA

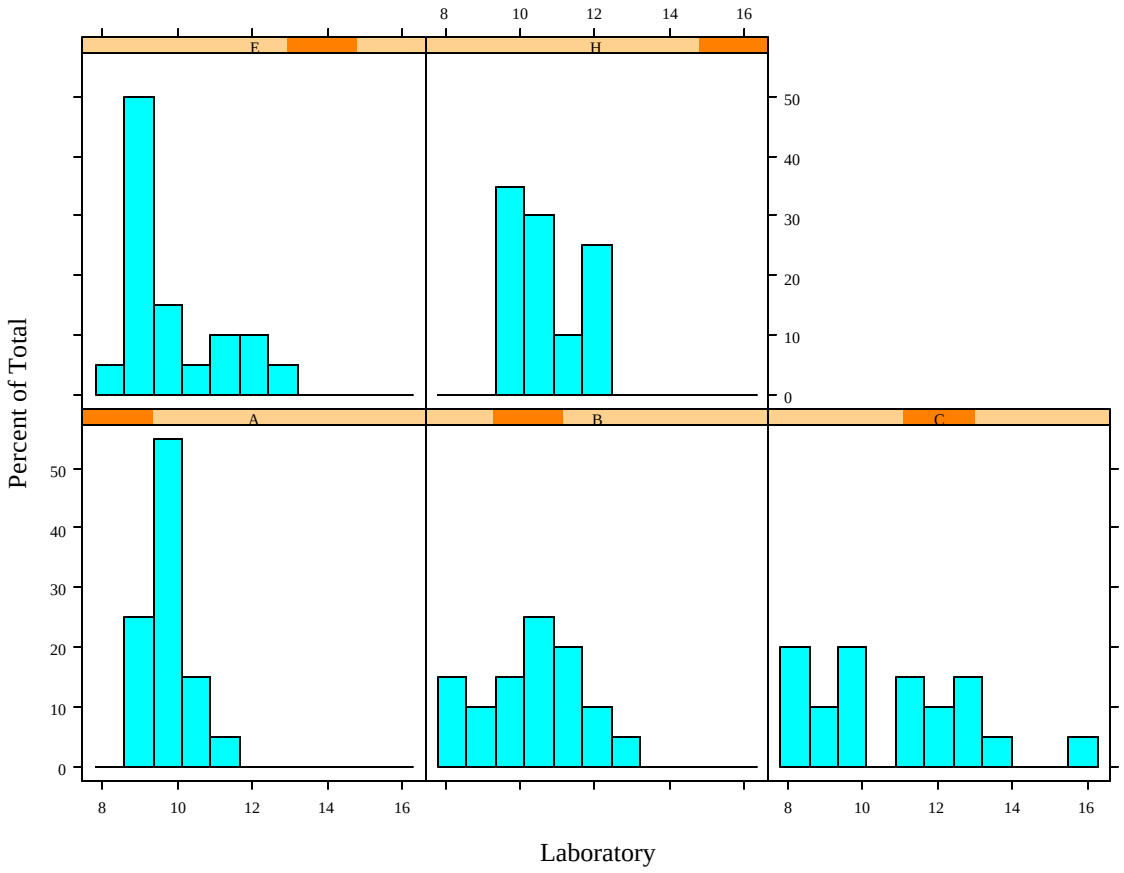


Figure C1: Frequency Histograms for Rainbow Trout Phenol Reference Toxicant Tests

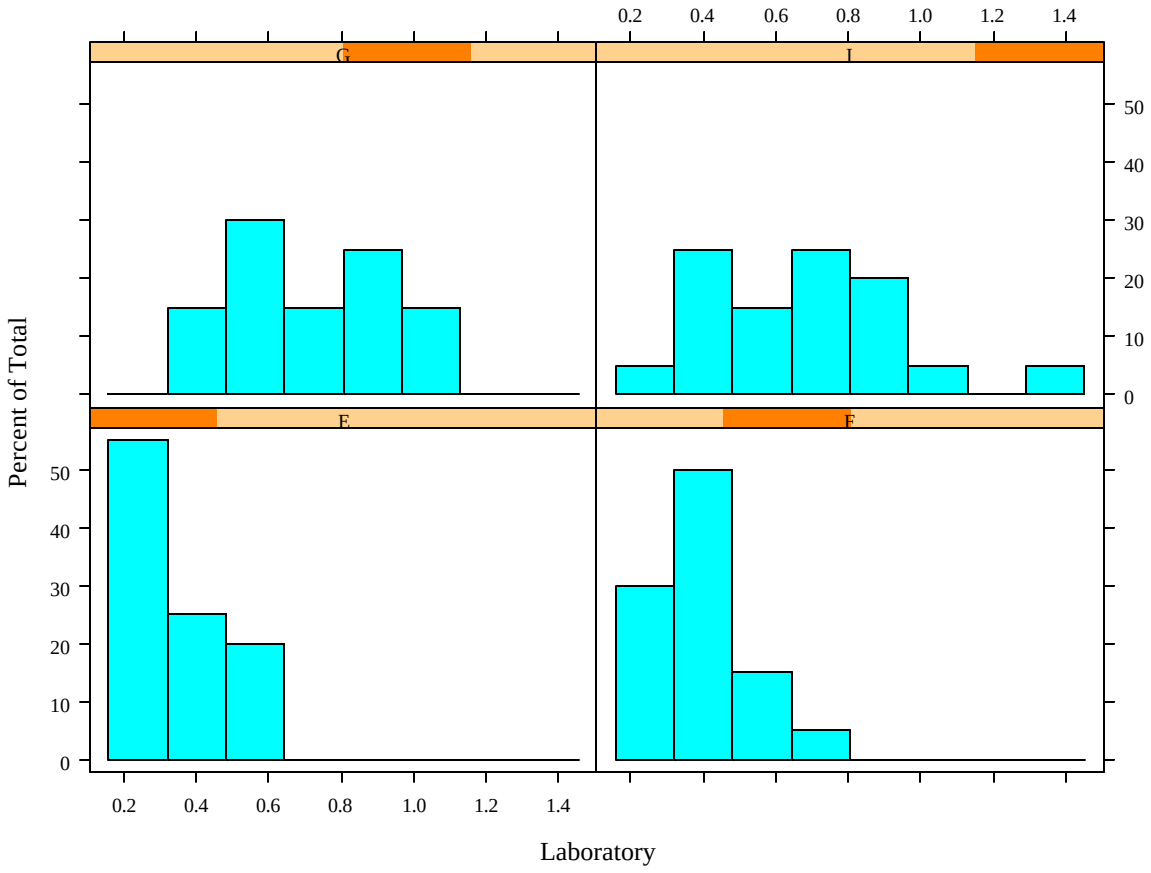


Figure C2: Frequency Histograms for Rainbow Trout Zn Reference Toxicant Tests

APPENDIX D

FREQUENCY HISTOGRAMS FOR *DAPHNIA MAGNA* REFERENCE TOXICANT DATA

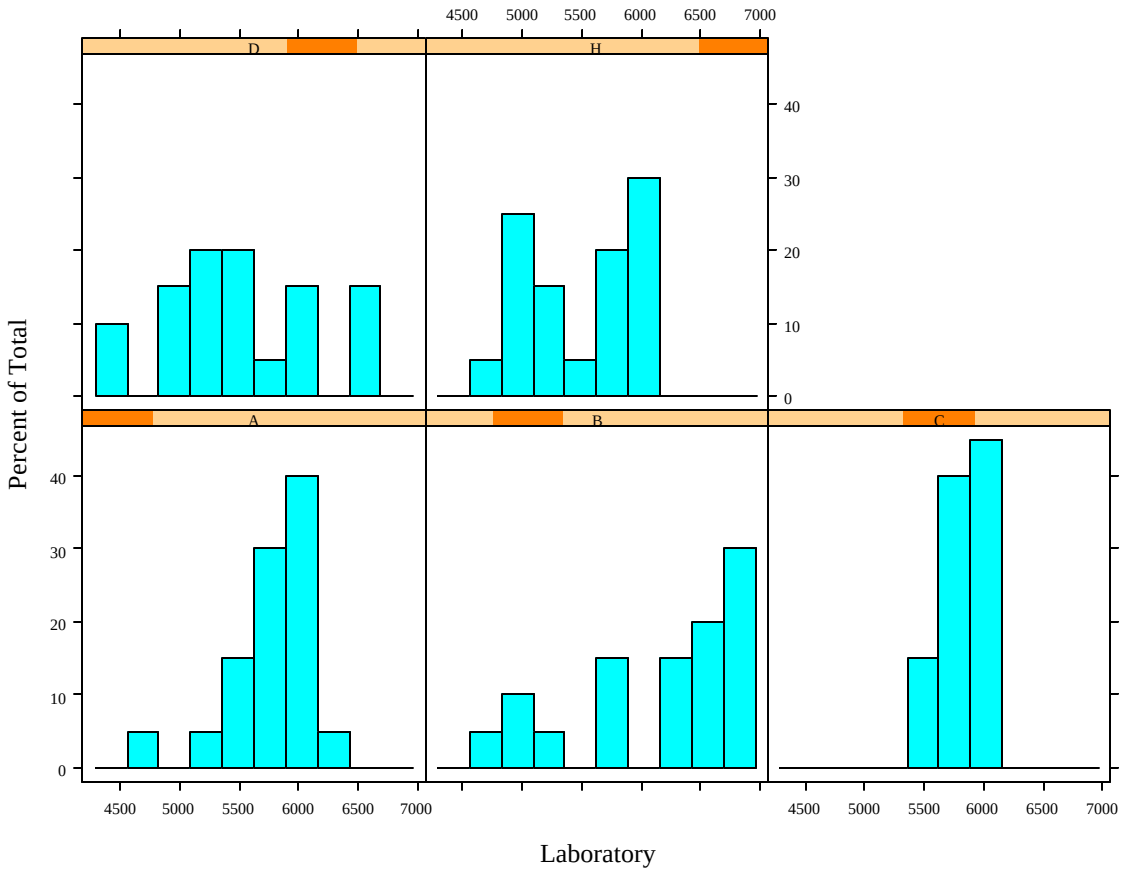


Figure D1: Frequency Histograms for *Daphnia magna* NaCl Reference Toxicant Tests

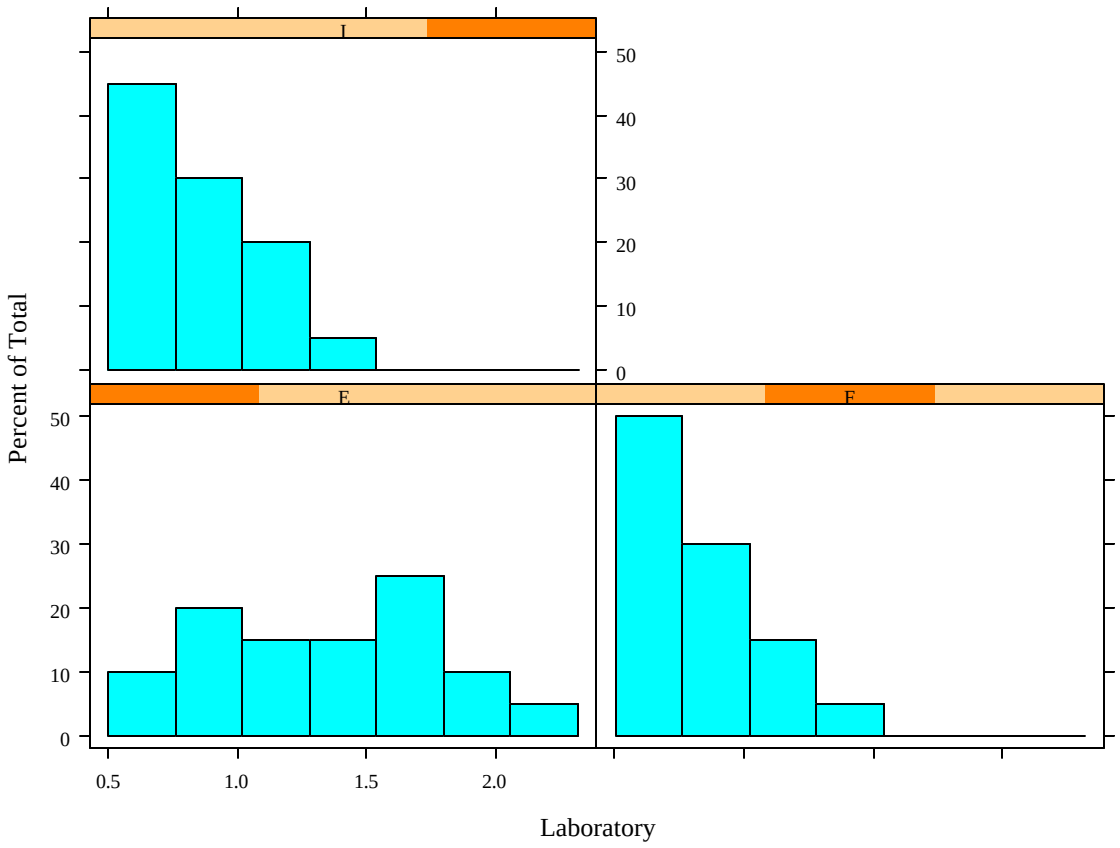


Figure D2: Frequency Histograms for *Daphnia magna* Zn Reference Toxicant Tests

APPENDIX D

TABLES OF FISHER EXACT TEST CRITICAL VALUES FOR COMPARISON OF TWO PROPORTIONS

Comparison of Two Proportions

It is often desirable to compare the results of two toxicity tests. The following table may be used to determine when the results of two toxicity tests, each with a sample size of 10 are significantly different from one another. Each cell contains the p-value for the Fisher exact test comparing the number of mortalities in test 1, with the number of mortalities in test 2. Tests with significantly different proportion mortality are highlighted.

An Example

If 8 organisms die in test 1, and 3 organisms die in test 2, the p-value for the Fisher exact test is 0.0698. Since this p-value is slightly larger than the traditionally accepted significance level of 0.05, the two tests results are not significantly different.

However, if 2 organisms die in test 2, then the p-value is 00230. Since this p-value is less than the traditionally accepted significance level of 0.05, the two tests results are significantly different.

Table of Fisher Exact Test[1,2] Critical Values for Comparison[3] of Two Proportions, $n_1=n_2=10$

Number of Mortalities in Test 2 \ Number of Mortalities in Test 1	0	1	2	3	4	5	6	7	8	9	10
0		1	0.4737	0.2105	0.0867	0.0325	0.0108	0.0031	0.0007	0.0001	0.0000
1	1		1	0.5820	0.3034	0.1409	0.0573	0.0198	0.0055	0.0011	0.0001
2	0.4737	1		1	0.6285	0.3498	0.1698	0.0698	0.0230	0.0055	0.0007
3	0.2105	0.5820	1		1	0.6499	0.3698	0.1789	0.0698	0.0198	0.0031
4	0.0867	0.3034	0.6285	1		1	0.6563	0.3698	0.1698	0.0573	0.0108
5	0.0325	0.1409	0.3498	0.6499	1		1	0.6499	0.3498	0.1409	0.0325
6	0.0108	0.0573	0.1698	0.3698	0.6563	1		1	0.6285	0.3034	0.0867
7	0.0031	0.0198	0.0698	0.1789	0.3698	0.6499	1		1	0.5820	0.2105
8	0.0007	0.0055	0.0230	0.0698	0.1698	0.3498	0.6285	1		1	0.4737
9	0.0001	0.0011	0.0055	0.0198	0.0573	0.1409	0.3034	0.5820	1		1
10	0.0000	0.0001	0.0007	0.0031	0.0108	0.0325	0.0867	0.2105	0.4737	1	

[1] The Fisher exact test was chosen on two counts. It provides exact probabilities when marginal frequencies are fixed. Also, a rule of thumb is that the chi-square approximation is not appropriate if marginal totals are less than 5, which frequently occurs in this table.

The alternative hypothesis for the Fisher exact test is that the two proportions are not equal.

[2] This test assumes that observations are independent which is not strictly the case for organisms within a test vessel.

[3] The results of this table may differ from tables constructed using a chi-squared test, particularly if Yates' continuity correction was not used.

In some toxicity tests, 3 replicates of 10 or 12 organisms are tested, thus the sample size for comparison of proportions is 30 or 36. The current method for comparing the results involves collapsing the data over the test vessels and comparing the results as if they were a single vessel of 30 or 36 organisms. The following tables provide p-values for the comparison of two proportions when sample sizes are 30 or 36.

However, other methods are available than can determine whether collapsing over tanks is valid and can perform a valid test of significance, if ignoring replicates is not appropriate.

Table[4] of Fisher Exact Test Critical Values for Comparison of Two Proportions, $n_1=n_2=30$

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
0	1	1	0.4915	0.2373	0.1124	0.0522	0.0237	0.0105	0.0046	0.0019	0.0008	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1	1	1	1	0.6120	0.3533	0.1945	0.1028	0.0523	0.0257	0.0122	0.0056	0.0025	0.0011	0.0004	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	0.4915	1	1	1	0.6707	0.4238	0.2542	0.1455	0.0797	0.0419	0.0211	0.0102	0.0048	0.0021	0.0009	0.0004	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3	0.2373	0.6120	1	1	1	0.7065	0.4716	0.2990	0.1806	0.1042	0.0575	0.0303	0.0153	0.0074	0.0034	0.0015	0.0006	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	0.1124	0.3533	0.6707	1	1	1	0.7306	0.5062	0.3334	0.2092	0.1253	0.0716	0.0391	0.0204	0.0101	0.0048	0.0022	0.0009	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.0522	0.1945	0.4238	0.7065	1	1	1	0.7480	0.5321	0.3604	0.2326	0.1432	0.0840	0.0470	0.0251	0.0127	0.0061	0.0028	0.0012	0.0005	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	0.0237	0.1028	0.2542	0.4716	0.7306	1	1	1	0.7611	0.5520	0.3817	0.2516	0.1581	0.0946	0.0539	0.0292	0.0150	0.0073	0.0033	0.0014	0.0006	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
7	0.0105	0.0523	0.1455	0.2990	0.5062	0.7480	1	1	1	0.7710	0.5675	0.3985	0.2668	0.1702	0.1033	0.0596	0.0326	0.0169	0.0082	0.0038	0.0016	0.0006	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	0.0046	0.0257	0.0797	0.1806	0.3334	0.5321	0.7611	1	1	1	0.7787	0.5796	0.4118	0.2789	0.1799	0.1102	0.0641	0.0352	0.0182	0.0089	0.0040	0.0017	0.0007	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
9	0.0019	0.0122	0.0419	0.1042	0.2092	0.3604	0.5520	0.7710	1	1	1	0.7847	0.5889	0.4220	0.2882	0.1872	0.1154	0.0673	0.0370	0.0191	0.0092	0.0041	0.0017	0.0006	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000
10	0.0008	0.0056	0.0211	0.0575	0.1253	0.2326	0.3817	0.5675	0.7787	1	1	1	0.7892	0.5959	0.4296	0.2949	0.1923	0.1188	0.0692	0.0379	0.0194	0.0092	0.0040	0.0016	0.0006	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000
11	0.0003	0.0025	0.0102	0.0303	0.0716	0.1432	0.2516	0.3985	0.5796	0.7847	1	1	1	0.7925	0.6010	0.4348	0.2993	0.1954	0.1205	0.0698	0.0379	0.0191	0.0089	0.0038	0.0014	0.0005	0.0001	0.0000	0.0000	0.0000	0.0000
12	0.0001	0.0011	0.0048	0.0153	0.0391	0.0840	0.1581	0.2668	0.4118	0.5889	0.7892	1	1	1	0.7948	0.6042	0.4379	0.3015	0.1964	0.1205	0.0692	0.0370	0.0182	0.0082	0.0033	0.0012	0.0004	0.0001	0.0000	0.0000	0.0000
13	0.0000	0.0004	0.0021	0.0074	0.0204	0.0470	0.0946	0.1702	0.2789	0.4220	0.5959	0.7925	1	1	1	0.7961	0.6058	0.4389	0.3015	0.1954	0.1188	0.0673	0.0352	0.0169	0.0073	0.0028	0.0009	0.0003	0.0001	0.0000	0.0000
14	0.0000	0.0002	0.0009	0.0034	0.0101	0.0251	0.0539	0.1033	0.1799	0.2882	0.4296	0.6010	0.7948	1	1	1	0.7965	0.6058	0.4379	0.2993	0.1923	0.1154	0.0641	0.0326	0.0150	0.0061	0.0022	0.0006	0.0001	0.0000	0.0000
15	0.0000	0.0001	0.0004	0.0015	0.0048	0.0127	0.0292	0.0596	0.1102	0.1872	0.2949	0.4348	0.6042	0.7961	1	1	1	0.7961	0.6042	0.4348	0.2949	0.1872	0.1102	0.0596	0.0292	0.0127	0.0048	0.0015	0.0004	0.0001	0.0000
16	0.0000	0.0000	0.0001	0.0006	0.0022	0.0061	0.0150	0.0326	0.0641	0.1154	0.1923	0.2993	0.4379	0.6058	0.7965	1	1	1	0.7948	0.6010	0.4296	0.2882	0.1799	0.1033	0.0539	0.0251	0.0101	0.0034	0.0009	0.0002	0.0000
17	0.0000	0.0000	0.0001	0.0003	0.0009	0.0028	0.0073	0.0169	0.0352	0.0673	0.1188	0.1954	0.3015	0.4389	0.6058	0.7961	1	1	1	0.7925	0.5959	0.4220	0.2789	0.1702	0.0946	0.0470	0.0204	0.0074	0.0021	0.0004	0.0000
18	0.0000	0.0000	0.0000	0.0001	0.0004	0.0012	0.0033	0.0082	0.0182	0.0370	0.0692	0.1205	0.1964	0.3015	0.4379	0.6042	0.7948	1	1	1	0.7892	0.5889	0.4118	0.2668	0.1581	0.0840	0.0391	0.0153	0.0048	0.0011	0.0001
19	0.0000	0.0000	0.0000	0.0000	0.0001	0.0005	0.0014	0.0038	0.0089	0.0191	0.0379	0.0698	0.1205	0.1954	0.2993	0.4348	0.6010	0.7925	1	1	1	0.7847	0.5796	0.3985	0.2516	0.1432	0.0716	0.0303	0.0102	0.0025	0.0003
20	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0006	0.0016	0.0040	0.0092	0.0194	0.0379	0.0692	0.1188	0.1923	0.2949	0.4296	0.5959	0.7892	1	1	1	0.7787	0.5675	0.3817	0.2326	0.1253	0.0575	0.0211	0.0056	0.0008
21	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0006	0.0017	0.0041	0.0092	0.0191	0.0370	0.0673	0.1154	0.1872	0.2882	0.4220	0.5889	0.7847	1	1	1	0.7710	0.5520	0.3604	0.2092	0.1042	0.0419	0.0122	0.0019
22	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0007	0.0017	0.0040	0.0089	0.0182	0.0352	0.0641	0.1102	0.1799	0.2789	0.4118	0.5796	0.7787	1	1	1	0.7611	0.5321	0.3334	0.1806	0.0797	0.0257	0.0046
23	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0006	0.0016	0.0038	0.0082	0.0169	0.0326	0.0596	0.1033	0.1702	0.2668	0.3985	0.5675	0.7710	1	1	1	0.7480	0.5062	0.2990	0.1455	0.0523	0.0105
24	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0006	0.0014	0.0033	0.0073	0.0150	0.0292	0.0539	0.0946	0.1581	0.2516	0.3817	0.5520	0.7611	1	1	1	0.7306	0.4716	0.2542	0.1028	0.0237
25	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0005	0.0012	0.0028	0.0061	0.0127	0.0251	0.0470	0.0840	0.1432	0.2326	0.3604	0.5321	0.7480	1	1	1	0.7065	0.4238	0.1945	0.0522
26	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0004	0.0009	0.0022	0.0048	0.0101	0.0204	0.0391	0.0716	0.1253	0.2092	0.3334	0.5062	0.7306	1	1	1	0.6707	0.3533	0.1124
27	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0003	0.0006	0.0015	0.0034	0.0074	0.0153	0.0303	0.0575	0.1042	0.1806	0.2990	0.4716	0.7065	1	1	1	0.6120	0.2373
28	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0004	0.0009	0.0021	0.0048	0.0102	0.0211	0.0419	0.0797	0.1455	0.2542	0.4238	0.6707	1	1	1	0.4915
29	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0004	0.0011	0.0025	0.0056	0.0122	0.0257	0.0523	0.1028	0.1945	0.3533	0.6120	1	1	1
30	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0003	0.0008	0.0019	0.0046	0.0105	0.0237	0.0522	0.1124	0.2373	0.4915	1	1

[4] Footnotes applying to Table of Fisher Exact Test[4,4] Critical Values for Comparison[4] of Two Proportions, $n_1=n_2=10$ also apply here.

Table[5] of Fisher Exact Test Critical Values for Comparison of Two Proportions, $n_1=n_2=36$

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	1	1	0.4930	0.2394	0.1145	0.0539	0.0249	0.0113	0.0051	0.0022	0.0009	0.0004	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000
1	1	1	1	0.6142	0.3570	0.1987	0.1065	0.0553	0.0278	0.0136	0.0065	0.0030	0.0013	0.0006	0.0002	0.0001	0.0000	0.0000	0.0000
2	0.4930	1	1	1	0.6737	0.4290	0.2603	0.1514	0.0847	0.0457	0.0238	0.0120	0.0059	0.0028	0.0013	0.0006	0.0002	0.0001	0.0000
3	0.2394	0.6142	1	1	1	0.7101	0.4782	0.3070	0.1887	0.1113	0.0632	0.0346	0.0182	0.0093	0.0046	0.0022	0.0010	0.0004	0.0002
4	0.1145	0.3570	0.6737	1	1	1	0.7350	0.5141	0.3434	0.2196	0.1348	0.0795	0.0451	0.0246	0.0130	0.0066	0.0032	0.0015	0.0007
5	0.0539	0.1987	0.4290	0.7101	1	1	1	0.7531	0.5414	0.3723	0.2454	0.1552	0.0942	0.0550	0.0309	0.0166	0.0086	0.0043	0.0020
6	0.0249	0.1065	0.2603	0.4782	0.7350	1	1	1	0.7668	0.5628	0.3957	0.2668	0.1727	0.1073	0.0640	0.0367	0.0202	0.0106	0.0054
7	0.0113	0.0553	0.1514	0.3070	0.5141	0.7531	1	1	1	0.7775	0.5798	0.4148	0.2848	0.1877	0.1187	0.0721	0.0420	0.0234	0.0125
8	0.0051	0.0278	0.0847	0.1887	0.3434	0.5414	0.7668	1	1	1	0.7861	0.5936	0.4304	0.2997	0.2003	0.1285	0.0791	0.0466	0.0263
9	0.0022	0.0136	0.0457	0.1113	0.2196	0.3723	0.5628	0.7775	1	1	1	0.7929	0.6047	0.4432	0.3121	0.2109	0.1368	0.0850	0.0505
10	0.0009	0.0065	0.0238	0.0632	0.1348	0.2454	0.3957	0.5798	0.7861	1	1	1	0.7985	0.6138	0.4537	0.3222	0.2196	0.1435	0.0898
11	0.0004	0.0030	0.0120	0.0346	0.0795	0.1552	0.2668	0.4148	0.5936	0.7929	1	1	1	0.8029	0.6210	0.4621	0.3303	0.2265	0.1488
12	0.0002	0.0013	0.0059	0.0182	0.0451	0.0942	0.1727	0.2848	0.4304	0.6047	0.7985	1	1	1	0.8065	0.6268	0.4687	0.3366	0.2318
13	0.0001	0.0006	0.0028	0.0093	0.0246	0.0550	0.1073	0.1877	0.2997	0.4432	0.6138	0.8029	1	1	1	0.8092	0.6312	0.4736	0.3412
14	0.0000	0.0002	0.0013	0.0046	0.0130	0.0309	0.0640	0.1187	0.2003	0.3121	0.4537	0.6210	0.8065	1	1	1	0.8113	0.6344	0.4771
15	0.0000	0.0001	0.0006	0.0022	0.0066	0.0166	0.0367	0.0721	0.1285	0.2109	0.3222	0.4621	0.6268	0.8092	1	1	1	0.8128	0.6365
16	0.0000	0.0000	0.0002	0.0010	0.0032	0.0086	0.0202	0.0420	0.0791	0.1368	0.2196	0.3303	0.4687	0.6312	0.8113	1	1	1	0.8136
17	0.0000	0.0000	0.0001	0.0004	0.0015	0.0043	0.0106	0.0234	0.0466	0.0850	0.1435	0.2265	0.3366	0.4736	0.6344	0.8128	1	1	1
18	0.0000	0.0000	0.0000	0.0002	0.0007	0.0020	0.0054	0.0125	0.0263	0.0505	0.0898	0.1488	0.2318	0.3412	0.4771	0.6365	0.8136	1	1
19	0.0000	0.0000	0.0000	0.0001	0.0003	0.0009	0.0026	0.0064	0.0142	0.0287	0.0537	0.0935	0.1528	0.2355	0.3442	0.4791	0.6376	0.8139	1
20	0.0000	0.0000	0.0000	0.0000	0.0001	0.0004	0.0012	0.0031	0.0073	0.0156	0.0307	0.0561	0.0962	0.1554	0.2377	0.3457	0.4798	0.6376	0.8136
21	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0005	0.0015	0.0036	0.0080	0.0167	0.0321	0.0577	0.0978	0.1567	0.2384	0.3457	0.4791	0.6365
22	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0006	0.0017	0.0039	0.0086	0.0174	0.0329	0.0585	0.0983	0.1567	0.2377	0.3442	0.4771
23	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0003	0.0007	0.0018	0.0042	0.0089	0.0178	0.0332	0.0585	0.0978	0.1554	0.2355	0.3412
24	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0003	0.0008	0.0019	0.0043	0.0090	0.0178	0.0329	0.0577	0.0962	0.1528	0.2318
25	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0003	0.0008	0.0020	0.0043	0.0089	0.0174	0.0321	0.0561	0.0935	0.1488
26	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0003	0.0008	0.0019	0.0042	0.0086	0.0167	0.0307	0.0537	0.0898
27	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0003	0.0008	0.0018	0.0039	0.0080	0.0156	0.0287	0.0505
28	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0003	0.0007	0.0017	0.0036	0.0073	0.0142	0.0263
29	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0003	0.0006	0.0015	0.0031	0.0064	0.0125
30	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0005	0.0012	0.0026	0.0054
31	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0004	0.0009	0.0020
32	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0003	0.0007
33	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002
34	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
35	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
36	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

[5] Footnotes applying to Table of Fisher Exact Test[5,5] Critical Values for Comparison[5] of Two Proportions, $n_1=n_2=10$ also apply here.

Table of Fisher Exact Test Critical Values for Comparison of Two Proportions, $n_1=n_2=36$ (Continued)

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.0009	0.0004	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	0.0026	0.0012	0.0005	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
7	0.0064	0.0031	0.0015	0.0006	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	0.0142	0.0073	0.0036	0.0017	0.0007	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
9	0.0287	0.0156	0.0080	0.0039	0.0018	0.0008	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
10	0.0537	0.0307	0.0167	0.0086	0.0042	0.0019	0.0008	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
11	0.0935	0.0561	0.0321	0.0174	0.0089	0.0043	0.0020	0.0008	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
12	0.1528	0.0962	0.0577	0.0329	0.0178	0.0090	0.0043	0.0019	0.0008	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
13	0.2355	0.1554	0.0978	0.0585	0.0332	0.0178	0.0089	0.0042	0.0018	0.0007	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
14	0.3442	0.2377	0.1567	0.0983	0.0585	0.0329	0.0174	0.0086	0.0039	0.0017	0.0006	0.0002	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000
15	0.4791	0.3457	0.2384	0.1567	0.0978	0.0577	0.0321	0.0167	0.0080	0.0036	0.0015	0.0005	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000
16	0.6376	0.4798	0.3457	0.2377	0.1554	0.0962	0.0561	0.0307	0.0156	0.0073	0.0031	0.0012	0.0004	0.0001	0.0000	0.0000	0.0000	0.0000
17	0.8139	0.6376	0.4791	0.3442	0.2355	0.1528	0.0935	0.0537	0.0287	0.0142	0.0064	0.0026	0.0009	0.0003	0.0001	0.0000	0.0000	0.0000
18	1	0.8136	0.6365	0.4771	0.3412	0.2318	0.1488	0.0898	0.0505	0.0263	0.0125	0.0054	0.0020	0.0007	0.0002	0.0000	0.0000	0.0000
19	1	1	0.8128	0.6344	0.4736	0.3366	0.2265	0.1435	0.0850	0.0466	0.0234	0.0106	0.0043	0.0015	0.0004	0.0001	0.0000	0.0000
20	1	1	1	0.8113	0.6312	0.4687	0.3303	0.2196	0.1368	0.0791	0.0420	0.0202	0.0086	0.0032	0.0010	0.0002	0.0000	0.0000
21	0.8128	1	1	1	0.8092	0.6268	0.4621	0.3222	0.2109	0.1285	0.0721	0.0367	0.0166	0.0066	0.0022	0.0006	0.0001	0.0000
22	0.6344	0.8113	1	1	1	0.8065	0.6210	0.4537	0.3121	0.2003	0.1187	0.0640	0.0309	0.0130	0.0046	0.0013	0.0002	0.0000
23	0.4736	0.6312	0.8092	1	1	1	0.8029	0.6138	0.4432	0.2997	0.1877	0.1073	0.0550	0.0246	0.0093	0.0028	0.0006	0.0001
24	0.3366	0.4687	0.6268	0.8065	1	1	1	0.7985	0.6047	0.4304	0.2848	0.1727	0.0942	0.0451	0.0182	0.0059	0.0013	0.0002
25	0.2265	0.3303	0.4621	0.6210	0.8029	1	1	1	0.7929	0.5936	0.4148	0.2668	0.1552	0.0795	0.0346	0.0120	0.0030	0.0004
26	0.1435	0.2196	0.3222	0.4537	0.6138	0.7985	1	1	1	0.7861	0.5798	0.3957	0.2454	0.1348	0.0632	0.0238	0.0065	0.0009
27	0.0850	0.1368	0.2109	0.3121	0.4432	0.6047	0.7929	1	1	1	0.7775	0.5628	0.3723	0.2196	0.1113	0.0457	0.0136	0.0022
28	0.0466	0.0791	0.1285	0.2003	0.2997	0.4304	0.5936	0.7861	1	1	1	0.7668	0.5414	0.3434	0.1887	0.0847	0.0278	0.0051
29	0.0234	0.0420	0.0721	0.1187	0.1877	0.2848	0.4148	0.5798	0.7775	1	1	1	0.7531	0.5141	0.3070	0.1514	0.0553	0.0113
30	0.0106	0.0202	0.0367	0.0640	0.1073	0.1727	0.2668	0.3957	0.5628	0.7668	1	1	1	0.7350	0.4782	0.2603	0.1065	0.0249
31	0.0043	0.0086	0.0166	0.0309	0.0550	0.0942	0.1552	0.2454	0.3723	0.5414	0.7531	1	1	1	0.7101	0.4290	0.1987	0.0539
32	0.0015	0.0032	0.0066	0.0130	0.0246	0.0451	0.0795	0.1348	0.2196	0.3434	0.5141	0.7350	1	1	1	0.6737	0.3570	0.1145
33	0.0004	0.0010	0.0022	0.0046	0.0093	0.0182	0.0346	0.0632	0.1113	0.1887	0.3070	0.4782	0.7101	1	1	1	0.6142	0.2394
34	0.0001	0.0002	0.0006	0.0013	0.0028	0.0059	0.0120	0.0238	0.0457	0.0847	0.1514	0.2603	0.4290	0.6737	1	1	1	0.4930
35	0.0000	0.0000	0.0001	0.0002	0.0006	0.0013	0.0030	0.0065	0.0136	0.0278	0.0553	0.1065	0.1987	0.3570	0.6142	1	1	1
36	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.0004	0.0009	0.0022	0.0051	0.0113	0.0249	0.0539	0.1145	0.2394	0.4930	1	1

APPENDIX E

LABORATORY SELECTION CHECKLIST

Checklist for Laboratory Qualifications

Parameter	Specification	Met Specifics? Y	N	NA
PERSONNEL				
Organizational and Management Structure:	Is there a clear and well-defined organization structure for the laboratory? Is this structure reflected in an organizational chart?	___ ___	___ ___	___ ___
Staff Qualifications:	Do staff have qualifications commensurate with their roles in the laboratory?	___	___	___
Training	Is there ongoing training program? Is training documented and are staff records up to date (may include performance based on PE or reference toxicant testing)?	___ ___	___ ___	___ ___
Quality Assurance Officer / Unit:	Does the laboratory have a Quality Assurance Officer or Unit? Is the Officer / Unit independent of laboratory work?	___ ___	___ ___	___ ___
	Are there accurate records kept for all laboratory equipment?	___	___	___
QUALITY MANAGEMENT SYSTEM				
Standard Operating Procedures (SOPs):	Does the laboratory have written, comprehensive SOPs? Are SOPs established for all procedures implemented in the laboratory? Are SOPs routinely and frequently updated? Are SOPs reviewed and signed by the QA Officer / Unit?	___ ___ ___ ___	___ ___ ___ ___	___ ___ ___ ___
	Does lab have an organizational chart?	___	___	___
FACILITIES				
Cold Storage	Does lab have sufficient facilities for cold storage of samples? Is storage area limited to storage of samples?	___ ___	___ ___	___ ___
Water Supply	Is an adequate supply of clean water available for holding, culturing and testing purposes? Is treatment of water supply required and, if so, are controls adequate (eg. Dechlorination system if on chlorinated water supply)?	___ ___	___ ___	___ ___
Temperature Control	Are there systems in place for temperature control (e.g. water baths, temperature control rooms, cabinets etc.)?	___	___	___

Parameter	Specification	Met Specifics? Y	N	NA
General Housekeeping procedures	Is laboratory generally well organized, neat and tidy, free of clutter?	___	___	___
Fish Holding Tanks	Are facilities adequate for volume of testing?	___	___	___
Separation of Culture and Testing Area	Is there separation of culture / holding and testing of organisms?	___	___	___
Organism Health Criteria:	Are test organisms obtained from reputable and registered suppliers?	___	___	___
	Are test organisms acclimated to lab conditions prior to testing?	___	___	___
	Are accurate records kept for organism acclimation?	___	___	___
	Are there stringent criteria for establishing organism / culture health?	___	___	___
Dilution Medium Quality:	Does the laboratory have established dilution medium quality criteria?	___	___	___
	Is the quality of dilution medium monitored routinely and frequently?	___	___	___
Statistical Methods / Software:	Are standard statistical methods used in the calculation of ecotoxicity test results?	___	___	___
	Are calculations and statistical outputs cross-checked for data entry and / or other potential errors?	___	___	___
	Are the methods / software validated and updated regularly?	___	___	___
Archiving:	Are all bench sheets, study reports, QA / QC data, and other documentation archived?	___	___	___
	Is there a security system in place to address access to archives (both hard copy and electronic format)?	___	___	___
QA/QC PROGRAM				
Quality Manual:	Does the laboratory have a Quality Manual outlining (in detail) the Quality System?	___	___	___
	Is the Quality Manual periodically updated to complement changes in laboratory procedures?	___	___	___
	Is the Quality Manual available for sponsor / client review?	___	___	___
Accreditation / Certification:	Does the laboratory maintain "second- or third-party" accreditations / certifications?	___	___	___
	Are certifications based on site audits? performance evaluation samples? management review?	___	___	___

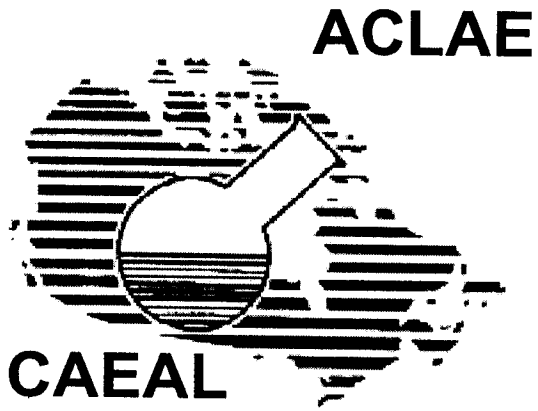
Parameter	Specification	Met Specifics? Y	N	NA
Interlaboratory Testing:	Does the laboratory participate in interlaboratory ("round-robin") testing?	___	___	___
	Do the results obtained compare favourably with other laboratories?	___	___	___
Internal / External Auditing:	Does the laboratory operation conduct internal audits as part of its QA / QC program?	___	___	___
	Are the results of these audits (including follow-up actions) available for sponsor / client review?	___	___	___
	Are the results of these audits (including follow-up actions) available for sponsor / client review?	___	___	___
	Does the laboratory permit / encourage external audits from regulatory personnel and / or clients?	___	___	___

APPENDIX F

DETAILED DESCRIPTIONS OF LABORATORY ACCREDITATION PROGRAMS

PROGRAM DESCRIPTION

SCC/CAEAL Laboratory Accreditation Program For Environmental Laboratories



Canadian Association for Environmental Analytical Laboratories (CAEAL) Inc.

Association canadienne des laboratoires d'analyse environnementale (ACLAE) inc.

Standards Council of Canada (SCC)

Conseil canadien des normes (CCN)

CAEAL Proficiency Testing Program For Environmental Laboratories

December 2000

Revision 5.1

SCC/CAEAL Laboratory Accreditation Program For Environmental Laboratories

CAEAL Proficiency Testing Program For Environmental Laboratories

PROGRAM DESCRIPTION

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1.0 Definitions

The following definitions apply.

Acceptable Deviation (AD) Value. Value defining the acceptable deviation of a reported value from the reference value. Acceptable Deviations are based on inter-laboratory 95% confidence limits or other appropriate criteria.

Accreditation. Formal recognition, by the SCC, of the competence of a laboratory to carry out specific functions.

Appendix. A unique matrix - test method combination that may contain more than one parameter.

Environmental Laboratory. A laboratory engaged in the measurement of biological, chemical, physical, or toxicological characteristics of either the receiving environment or discharges to the receiving environment.

Proficiency Testing. The determination of laboratory testing performance by means of inter-laboratory comparisons.

Proficiency Testing Recognition. Formal recognition, by CAEAL, of the proficiency of an environmental laboratory to carry out specific tests.

Proficiency Testing (PT) Sample. A characterized sample, having designated reference values, that is used in the evaluation of laboratory performance.

Recommended Actions. Corrective actions specified if the consequences of the deficiencies are not so serious as to potentially compromise the integrity of the testing. Necessary improvements that require more time to complete; they must be completed by the next regularly scheduled re-assessment.

Reference Value. Value assigned to a PT sample. This value may be based on any appropriate combination of design value, inter-laboratory consensus value, reference consensus value or direct comparison value.

Required Actions. Corrective actions specified if the observed deficiencies are deemed to potentially compromise the integrity of the testing (e.g. absence of necessary documentation, faulty facilities or equipment, inadequate staff performance, etc.). Must be corrected within 6 months of an initial assessment for accreditation or within 3 months of a re-assessment for continued accreditation.

Test. A unique combination of matrix, parameter and text method (e.g. Pb in water by ICP).

Test Group. One or more parameters in a specific sample matrix that is/are offered as a unique set of Proficiency Testing samples (designated as C-1, C-2, C-3, etc.).

2.0 Introduction

In June 1994, the Canadian Association for Environmental Analytical Laboratories (CAEAL) and the Standards Council of Canada (SCC) entered into an Accreditation Partnership Agreement to accredit environmental laboratories. This Program Description describes the process leading to the granting of accreditation as provided for in the SCC/CAEAL Accreditation Partnership Agreement. Section 3.0 describes the CAEAL Proficiency Testing Program; this section will be of interest to laboratories participating in only this program, as well as laboratories participating in the Accreditation Program.

CAEAL. CAEAL was formed in 1989 by the combined interests of public and private sector laboratories and is incorporated as a not for profit association. A principal objective of the association is to promote and maintain a high level of assurance in analytical test data. To this end, CAEAL offers proficiency testing and site assessment programs that are tailored to meet the specific needs of environmental laboratories.

The Proficiency Testing Program targets high volume testing in the major disciplines of inorganic chemistry, organic chemistry, toxicology, occupational health and microbiology. This program currently includes the following matrices: water, waste oil, soil/sediment and air collection media (e.g. quartz and cellulose acetate filters, and charcoal tubes). As of January 2001, Asbestos testing will also be included in the PT Program.

SCC. The SCC was established in 1970 by Parliament under the Standards Council of Canada Act (amended in 1996) to promote voluntary standardization in Canada, facilitate domestic and international trade, and further international co-operation in relation to standards. As a part of its overall mandate, the SCC represents Canada in international standards organizations such as the International Organization for Standardization (ISO) and the International Laboratory Accreditation Cooperation (ILAC). In addition, it accredits standards development organizations, certification organizations, quality system registrars, auditor course providers, auditor certifiers, and calibration and testing laboratories.

The Program for Accreditation of Laboratories - Canada (PALCAN) provides formal recognition of the competence of a laboratory to manage and perform specific tests or types of tests listed in the scope of accreditation approved by the Council. Accreditation is available for all types of tests, measurements and observations and is currently offered in the following fields of testing: Acoustics & Vibration, Biological, Calibration, Chemical, Electrical/Electronic, Ionizing Radiation, Mechanical, Nondestructive Evaluation, Optics & Optical Radiation, Physical, Thermal & Fire. Environmental testing is assigned, as appropriate, to the biological, chemical and physical fields of testing.

SCC/CAEAL Accreditation Partnership. The initial (1994) SCC/CAEAL Accreditation Partnership Agreement merged the environmental component of the SCC accreditation program with the CAEAL site assessment program to provide a single national program which, through its affiliation with the SCC, enjoys not only national but international recognition. The international recognition afforded will become increasingly important as the provisions of NAFTA, and other international trade agreements, are implemented. These provisions typically require suppliers of laboratory services to meet ISO/IEC 17025 requirements. Similarly, at the national level, there is an increasing trend for both government and private sector contracting policies to specify laboratory accreditation.

Under the terms of the SCC/CAEAL Accreditation Partnership Agreement, CAEAL carries out site assessments and operates a proficiency testing program. The granting and maintenance of accreditation is under the authority of the SCC on the recommendation of CAEAL. Reference to the SCC/CAEAL Accreditation Program throughout this document is made in this context. Accreditation itself is based on satisfactory participation in the site assessment program plus satisfactory participation in proficiency testing, where such testing is offered as part of the accreditation.

Documentation Sources. Documentation sources relevant to the SCC/CAEAL Accreditation Program for Environmental Laboratories include the following.

1. SCC/CAEAL Accreditation Partnership Agreement; January 2000.

2. Joint SCC-CAEAL Assessments; April 22, 1999.

3. Program for the Accreditation of Laboratories (PALCAN) Handbook, SCC QMS Document — D92.6; February 11, 2000.

4. CAN-P-1510D, Assessment Rating Guide, Standards Council of Canada; Interim I, March 24, 2000, to be updated in late 2000.

5. Rating Guide Appendix, Canadian Association for Environmental Analytical Laboratories; March 1997, to be updated in late 2000.

6. Program Description - SCC/CAEAL Laboratory Accreditation Program for Environmental Laboratories, Canadian Association for Environmental Analytical Laboratories and the Standards Council of Canada; November 2000.

7. Application Form - SCC/CAEAL Laboratory Accreditation Program for Environmental Laboratories; Canadian Association for Environmental Analytical Laboratories and the Standards Council of Canada; November 2000.

8. CAN-P-4D (ISO/IEC 17025), General Requirements for the Competence of Testing and Calibration Laboratories, Standards Council of Canada; March 2000.

Where there are inconsistencies among documentation sources (1) - (3), noted above, the provisions of the SCC/CAEAL Partnership Agreement shall prevail.

Scope of the SCC/CAEAL Accreditation Program. The SCC/CAEAL Accreditation Program for environmental laboratories applies to all tests associated with the measurement of chemical, radio-chemical, biological, toxicological and related physical characteristics of environmental samples (i.e. waste materials, air, water, soil, biological tissue, etc.). The related site assessments may, at the discretion of the SCC, include a limited amount of testing which is outside the environmental field (e.g. foods, pharmaceuticals, etc.). For such additional testing, accreditation requirements are based on SCC approved guidelines.

If only a small portion of the scope of testing is environmental testing, then the SCC application process applies with the further provision that, for the environmental component, (i) CAEAL proficiency testing requirements, where applicable, apply and (ii) the CAEAL Rating Guide Appendix applies to the assessment of test specific capabilities. Applicant laboratories for which only a small portion of the scope of testing is environmental testing should contact the SCC directly.

Standards Council of Canada
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Ottawa, Ontario K1P 6N7

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Email: jsomers@scc.ca

3.0 The Proficiency Testing Program

General. For laboratories participating in the SCC/CAEAL Accreditation Program, all tests appearing in the laboratory s Scope of Testing must be supported by Proficiency Testing (PT) in those cases where PT samples are offered by the CAEAL program.

In some cases, two (or more) identical PT test group subscriptions may be required to accommodate two (or more) different test methods (e.g. ICP/MS and ICP/OES, AA and ICP, GC/MS - headspace and GC/MS - purge + trap, IC and SIE, titration and autocolor, etc.).

Laboratories may also choose to be recognized by CAEAL for proficiency testing by participating only in the PT program. Tests available in the PT program are listed in the SCC/CAEAL Fee Schedule.

All laboratoriesparticipating in the Proficiency Testing Program must comply with the Proficiency Testing Related Policies, available on the CAEAL web site at http://www.caeal.ca.

Frequency. PT samples are sent to participating laboratories generally twice annually; the shipping schedule for each PT test group is noted in the Appendix to the SCC/CAEAL Fee Schedule. The samples are shipped directly to the laboratories by the reference laboratory that prepares the samples. With each sample shipment, laboratories receive detailed instructions for analysing samples and reporting results to CAEAL. Laboratories must analyze 4 distinct concentration levels per test, and forward results directly to CAEAL within 30 days.

Scoring System. Laboratory performance is evaluated, for each sample, against a reference value and an acceptable deviation (AD).

The reference value is the value assigned to a PT sample. This value may be based on any appropriate combination of design value, inter-laboratory consensus value, reference consensus value or direct comparison value.

For each sample, the deviation is calculated as the difference between the value reported by the laboratory and the reference value.

The AD value is the acceptable deviation of a reported value from the reference value. It is based on interlaboratory 95% confidence limits or other appropriate criteria, and corresponds to twice the target standard deviation, as identified in ISO Guide 43.

Since each PT study involves 4 separate samples of distinct concentrations for each test, it is necessary to calculate a composite PT score for each test to determine overall performance. The composite score is calculated by first assigning points for the performance on each sample as shown in the following table:

Z Score	Deviation/AD Value	Points Assigned
≤1.00	≤0.50	5
1.01-2.00	0.51-1.00	4
2.01-3.00	1.01-1.50	2
>3.00	>1.50	0

Note: z score = 2(deviation/AD Value)

The composite (or PT) score is then calculated as:

PT Score = (Total Points / No. of PT Samples) x (100 / 5)

Acceptable PT scores equal or exceed 70.

The following example data illustrates the calculation of a Proficiency Testing score.

Sample No.	Reference Value, mg/l	Reported Value, mg/l	Deviation mg/l	AD Value mg/l	Deviation / AD Value	Points Assigned
1	2.3	1.8	0.5	0.6	0.83	4
2	8.6	9.1	0.5	1.6	0.31	5
3	10.3	11.1	0.8	1.8	0.44	5
4	26.0	20.8	5.2	4.2	1.24	2

$$\text{PT Score} = \frac{16}{4} \times \frac{100}{5} = 80$$

Proficiency Testing Report. Approximately 6 weeks after the deadline for submission of results, the laboratory receives a preliminary report consisting of a confidential report on the individual laboratory s performance. The laboratory has the opportunity to review this preliminary report and provide any feedback to CAEAL (i.e. advise CAEAL of any transcription errors made by CAEAL). CAEAL then prepares and issues a final Proficiency Testing Report which contains both a confidential report on the individual lab s performance, and an inter-laboratory comparison report.

Note: As of December 1st 2000, CAEAL s Web Data Entry system will be available to all participating laboratories. This project is intended to eliminate transcription errors and the need for a Preliminary PT Report, resulting in a shorter turnaround time for the Final PT Report.

Notification of PT Recognition. CAEAL grants recognition for proficiency testing following a successful PT study. After each PT study, laboratories are notified in writing of any new tests for which CAEAL recognition has been granted. Annually, laboratories receive a Registration Status Report that lists all tests for which recognition has been granted. Written notices received after each PT study are used to update recognition status in the interval between receiving the annual Registration Status Report. Laboratories also receive a Certificate of Laboratory Proficiency, signed by the President of CAEAL, following their first successful PT study.

Suspension and Withdrawal. A laboratory that fails to achieve an acceptable PT score for a specific test is notified by CAEAL in writing (a Notice of Possible Suspension is issued). If the laboratory fails to achieve an acceptable score on the second successive set of samples, the laboratory receives written notice from CAEAL that recognition for the test in question is suspended (a Notice of Suspension is issued). If the laboratory is accredited, it receives written notice from the SCC that accreditation for the test in question is suspended. If the laboratory fails to achieve an acceptable PT score on the third successive set of PT samples, CAEAL recognition and SCC accreditation are withdrawn (a Notice of Withdrawal is issued by CAEAL and a notice and amended Scope of Testing are issued by the SCC).

Termination of proficiency testing recognition (or accreditation) does not preclude a laboratory from applying again at a later date. Such a re-application is evaluated under the same requirements and procedures applicable to every other applicant laboratory.

Appeals. Within 30 days of receiving a suspension or withdrawal notice for PT recognition, the laboratory has the right to appeal its case to the CAEAL Board in writing. The subsequent decision of the Board, based on evidence for review of the appeal by a duly constituted committee of the Board, is final.

Proficiency Testing for Asbestos Analysts (NEW)

Process. Asbestos PT samples are sent to participating laboratories twice annually, in January and June. The samples are shipped directly to the analysts by the Asbestos Quality Assurance Program (AQAP).

The analyst must analyze two PT samples: one asbestos reference (REF) slide with relocatable fields (prepared from chrysotile, amosite or field samples) and one asbestos filter wedge. For the REF slide, the analyst records the numbers and the positions of the fibres counted in the pre-designated fields. For the filter wedge, the analyst follows the NIOSH 7400 or the DMF/Euparal analytical method of fibre analysis and the fibre counting rule A of the NIOSH 7400 method. **The analyst returns the results and the slide to the reference laboratory within 30 days.**

Approximately six weeks after the deadline for submission of results, the analyst receives a preliminary report from the reference laboratory, including the fibre counting errors for each field. The analyst has two weeks to review this preliminary report and provide any feedback to the reference laboratory (i.e. advise the reference laboratory of any transcription errors made by the reference laboratory). The reference laboratory will submit the final results to the CAEAL office. CAEAL then prepares and issues a final Proficiency Testing Report, including the fibre counting errors for each field.

Scoring. For the REF slide, the reported fibres and their positions in individual designated fields are evaluated against their respective Verified Fibres ** and the errors are catalogued as: (1) sizing; (2) oversight; (3) identification of fibre by the aspect ratio; and (4) recording.

The PT score is expressed as a function of the number of errors and the number of verified fibres:

$$\text{PT Score} = \left(1 - \frac{\text{No. of errors}}{\text{No. of verified fibres}}\right) \times 100$$

One (1) point is assigned if the score is equal to or exceeds 50. Zero (0) points are assigned if the score is less than 50.

For the filter wedge, the reported fibre count result is compared with the performance limits that are calculated from the Reference Value and the Acceptable Deviation Value. One (1) point is assigned if the fibre count lies within the performance limits. Zero (0) points are assigned if the fibre count lies outside the performance limits.

The PT score is then calculated as follows:

$$\text{PT Score} = \frac{\text{Total points}}{\text{No. of PT samples}} \times 100$$

Acceptable PT scores equal or exceed 50.

Note: ** Verified fibres of the REF slide are based on the fibre counts, which may be any appropriate combination of inter-laboratory consensus value or value provided by the reference laboratory with demonstrated accuracy.

4.0 The Accreditation Process

Application. Laboratories may apply for accreditation by forwarding a completed application form to CAEAL. As part of the application process, applicant laboratories must agree to the terms and conditions of accreditation, attest to the availability of key documentation (e.g. quality manual and relevant test methods), and provide summary information on all tests for which accreditation is sought. This summary information is used to identify the Scope of Testing and includes information on the matrix, parameter, test method and test method reference.

The application package provided to all applicant laboratories includes the Application Form, Fee Schedule and Program Description. A complimentary copy of the Rating Guide (CAN-P-1510D) and the Rating Guide Appendix are forwarded to laboratories that submit a completed application for accreditation.

Once CAEAL has received an application, it ensures the application is complete and then immediately informs the SCC so that an official SCC accreditation file can be opened (or updated).

Subsequent to completing the original application, a laboratory may apply for additional tests up to **one month** before the scheduled site visit by updating the original application. Once an updated application has been received, the procedures cited above, for the original application, apply.

Proficiency Testing Participation. All tests appearing in the laboratory s Scope of Testing must be supported by ProficiencyTesting (PT) in those cases where PT samples are offered by the CAEAL program. In some cases, two (or more) identical PT test group subscriptions may be required to accommodate two (or more) different test methods (e.g. ICP/MS and ICP/OES, AA and ICP, GC/MS - headspace and GC/MS - purge + trap, IC and SIE, titration and autocolor, etc.).

Laboratories applying for SCC/CAEAL accreditation must pass at least one PT study before accreditation will be granted.

Rating Guide. The Rating Guide (CAN-P-1510D) is used to assess the management quality system. It is based on CAN-P-4D (ISO/IEC 17025), General Requirements for the Competence of Testing and Calibration Laboratories.

Rating Guide Appendix. Test specific issues are dealt with in the Rating Guide Appendix, which assesses specific information on the tests for which the laboratory is seeking accreditation (e.g. test method currency, validation and content, the availability and functioning of equipment and supplies, and the conduct of testing including record keeping practices, etc.).

Site Assessments. Starting with the initial assessment, assessments are carried out every 2 years. Prior to an assessment, the applicant laboratory is given the opportunity of vetting the assigned assessors, site assessment scheduling and scope of testing. In assigning assessors, CAEAL (i) avoids known commercial conflicts and (ii) matches assessor expertise with the testing to be assessed. The assigned schedules take into account any limitations noted by the laboratory at the time of application. The assigned scope of testing is based on the application information provided by the laboratory.

Qualified assessors perform the assessments by interviewing staff, examining laboratory records, reviewing technical documentation, and inspecting facilities, equipment and the conduct of laboratory testing. In all cases the assessment is made relative to specific requirements, and as a part of the assessment any significant deficiencies are noted and corrective actions identified. The prescribed corrective actions may be either non test-specific (Rating Guide) or test-specific (Rating Guide Appendix).

All corrective actions fall into one of two categories: (i) required actions or (ii) recommended actions. Required actions are specified if the observed deficiencies are deemed to potentially compromise the integrity of the testing (e.g. absence of necessary documentation, faulty facilities or equipment, inadequate staff performance, etc.). Recommended actions are specified if the consequences of the deficiencies are not so serious as to potentially compromise the integrity of the testing.

At the end of the assessment, the Assessment Team provides the laboratory with a copy of an assessment report that summarizes the results of the assessment and the associated corrective actions. Within 10 working days of the assessment the laboratory may provide to CAEAL, in writing, relevant supplementary information that could affect the need for corrective actions. A final Site Evaluation Report is issued to the laboratory by CAEAL.

The final Site Evaluation Report definitively identifies the corrective actions (or, in exceptional cases, recommends re-assessment). All required actions must be carried out within 6 months of an initial assessment and 3 months of re-assessments. Appropriate evidence of implementation (e.g. updated documentation, samples of records, purchase orders, photographs, etc.) must be provided to CAEAL. Implementation of required actions may be subject to on-site verification by CAEAL officials. Conformance with recommended actions is reviewed at the subsequent regularly scheduled re-assessment.

Evaluation and Approval. The CAEAL Advisory Panel, reviews and reports to the CAEAL Board of Directors on the results and information provided by both the proficiency testing and site assessment programs. The CAEAL Board then forwards recommendations on the granting and/or maintenance of accreditation to the SCC.

To be recommended for accreditation, a laboratory must conform with all the required non test-specific actions. The recommended scope of accreditation includes only those tests for which (i) there has been conformance with the test specific required actions and (ii) acceptable PT scores have been obtained where such testing is offered as a part of the accreditation.

Notification. The SCC Chair formally advises the laboratory of the decision as to whether or not accreditation has been granted. An approved scope of testing is issued to the laboratory at the time of accreditation, subsequent to each re-assessment, and in the event of scope changes due to extensions, suspensions or withdrawals.

Subsequent to each Proficiency Testing round, CAEAL will issue status letters which will indicate those tests for which CAEAL proficiency testing recognition has been granted, suspended or withdrawn. These status letters, where applicable, shall be used to update accreditation status.

Certificates of Accreditation. Certificates of Accreditation are issued by SCC on the recommendation of CAEAL. The wording appearing on the certificate is as follows:

> [Laboratory Name] having been assessed by the Canadian Association for Environmental Analytical Laboratories (CAEAL) Inc., under the authority of the Standards Council Of Canada (SCC), and found to comply with the requirements of the ISO/IEC Guide 25, the conditions established by the SCC and the CAEAL proficiency testing program, is hereby recognized as an
>
> ACCREDITED ENVIRONMENTAL LABORATORY
>
> for specific tests or types of tests listed in the scope of accreditation approved by the Standards Council of Canada.

Note: The wording on certificates will change to reflect ISO/IEC 17025 as of April 1, 2001.

Scope Extensions. Accreditation of additional tests in the interval between regularly scheduled site assessments proceeds as follows:

i) Tests that can be added to an existing appendix

The Assessments Manager and/or Advisory Panel shall assess factors such as the laboratory's technical abilities, experience, scope of testing and relative scale of the new activity with respect to the existing scope and a recommendation will be made to grant the scope extension or not. In the case of approval, the requested modifications to the scope of accreditation will be made. If the request is not approved and the laboratory wishes to pursue addition of these tests to the scope, an abbreviated site assessment will be required.

ii) Tests that require a new appendix

Tests that require a new appendix must be incorporated into the regularly scheduled site visit or an abbreviated site visit.

Surveillance Questionnaire. In the intervening year between biennial site assessments, laboratories must complete and submit by the due date a surveillance questionnaire. The questionnaire covers activities or changes related to the accredited scope of testing since the last site assessment.

Suspension and Withdrawal of Accreditation. Accreditation may be suspended, subsequent to its having been granted, if a laboratory: (i) fails to comply with the terms and conditions of accreditation, (ii) fails to carry out all required actions (either test specific or non-test specific) within the time period specified, or (iii) fails to successfully analyze two successive sets of PT samples for a specific test.

A laboratory that is found to be not in compliance with items (i) or (ii) above is notified in writing and requested to take the appropriate corrective action. If the laboratory does not initiate appropriate corrective action and so advise CAEAL, in writing, within 30 days of being notified, the SCC on the recommendation of CAEAL provides written notice that accreditation for the tests in question is suspended. If appropriate action is not taken within a further 30 days, accreditation is withdrawn.

A laboratory that fails to achieve an acceptable PT score for a specific test is notified by CAEAL in writing (a Notice of Possible Suspension is issued). If the laboratory fails to achieve an acceptable score on the second successive set of samples (i.e. item (iii) above occurs), the laboratory receives written notice from CAEAL that recognition for the test in question is suspended (a Notice of Suspension is issued), and written notice from the SCC that accreditation for the test in question is suspended. If the laboratory fails to achieve an acceptable PT score on the third successive set of PT samples, CAEAL recognition and SCC accreditation are withdrawn (a Notice of Withdrawal is issued by CAEAL and a notice and amended scope are issued by the SCC).

Appeal. Within 30 days of receiving a suspension or withdrawal notice, for whatever reason, a laboratory has the right to appeal its case to the SCC in writing. In such cases, the current SCC procedure for conducting appeals (CAN-P-15) is followed. The subsequent decision of the SCC based on evidence for review of the appeal by a duly constituted committee of the Council is final.

Termination of Accreditation. Accreditation is deemed to be terminated if it is either withdrawn or voluntarily relinquished. If a laboratory wishes for whatever reason to voluntarily relinquish its accreditation, either in whole or in part, it may do so by providing written notice to CAEAL copied to the SCC. In such cases, the current SCC procedure for termination of accreditation (CAN-P-15) is followed.

Re-application. Termination of accreditation, either in whole or in part, does not preclude a laboratory from applying for accreditation at a later date. Such a re-application is evaluated under the same requirements and procedures applicable to every other applicant laboratory.

Assessor Qualifications. Site assessments are conducted by a team of qualified professionals drawn mostly from member laboratories. All candidate assessors must participate in a formal training program to ensure fair and equitable application of the rating criteria used in the assessment process. The training program includes participation in an approved 36 hour course on the Assessment of Laboratory Quality Systems together with biennial (refresher) courses on the application of the Rating Guide (CAN-P-1510D).

5.0 Publicity Guidelines

Proficiency Testing

Laboratories that perform successfully in the CAEAL Proficiency Testing Program may claim on their company letterhead and advertisements that they are recognized as a participant in the CAEAL Proficiency Testing Program.

Accreditation

SCC/CAEAL Sponsored Publicity. The SCC and CAEAL publicize the accreditation of laboratories in several ways, including the following:

a) an official Certificate of Accreditation, for public display, is presented by the SCC to each laboratory following accreditation;

b) a Notice of New Accreditation or Voluntary Withdrawal of each affected laboratory, is published on the SCC web site at www.scc.ca;

c) accredited status is published in the accreditations database on the SCC web site;

d) press releases announcing the accreditation of laboratories, and general news items dealing with the laboratory accreditation program, will be released to the media from time to time;

e) other publicity programs may be developed to promote accreditation activities and increase public awareness of the program.

Recommended Practices for Accredited Laboratories. A significant benefit of SCC accreditation is that a laboratory may publicize its competence based on a nationally recognized accreditation program. SCC and CAEAL encourage such activities; however, certain restrictions apply to prevent misunderstanding about the significance of accreditation. A condition of accreditation is that the laboratory agrees to abide by these restrictions.

Laboratories may publicize their accredited status in several ways. The following may be used without approval from the Standards Council.

a) Accredited laboratories may include a statement on their company letterhead and advertisements as follows:

> Accredited by the Standards Council of Canada (SCC), in co-operation with the Canadian Association for Environmental Analytical Laboratories (CAEAL), for specific environmental tests listed in the scope of accreditation approved by the SCC.

An accredited laboratory that is part of a larger organization may use this statement on the organizational letterhead, provided that the accredited laboratory is identified by name immediately preceding or following the statement.

b) Reference to accredited status may be made in test reports that deal solely with tests covered under the terms of accreditation. The reference must read as follows:

> This laboratory is accredited by the Standards Council of Canada (SCC), in co-operation with the Canadian Association for Environmental Analytical Laboratories (CAEAL). The tests included in this report are within the scope of this accreditation.

Other Practices. The following may be used by accredited laboratories as alternatives to the statements listed in a) and b) above if permission is obtained first from the SCC:

> SCC/CAEAL accredited to ISO/IEC Guide 25 for specific tests. and/or
> SCC/CAEAL accredited to ISO/IEC Guide 25 for specific services.

Accredited laboratories that wish to promote their accredited status using either of these statements, or in any manner deviating from approved a) and b) above, may do so only with the approval of the SCC. Advice and assistance are available from Mr. Stephen Cross or Dr. Jim Somers at the SCC (613-238-3222 or jsomers@scc.ca)

Restrictions. The following restrictions apply to publicizing an accredited status.

a) Reference to the accredited status of a laboratory may not be part of any promotional endorsement of products or services, or be part of a claim of acceptability of data by certification organizations.

b) Similarly, in order to ensure against interpretation as part of a certification program, no statement or mark relating to laboratory accreditation may appear on any product, package or test report (except as allowed in the recommended practices for accredited laboratories).

c) Should accreditation be voluntarily relinquished by the laboratory, or withdrawn or suspended by the SCC, the laboratory must immediately cease issuing all reference to its former accredited status (for the affected tests). Upon reinstatement of its suspended or terminated accreditation, a laboratory may resume its publicity program.

CAEAL Logo. The CAEAL logo is a registered trademark and may not be used by laboratories.

6.0 Confidentiality

CAEAL Policy and Procedures. All CAEAL Board members, staff, members of the Advisory Panel, members of the Program Committee, and members of Assessment Teams are required to sign a confidentiality agreement that has the following elements.

1. Agreement to disclose to the CAEAL Board all involvement in personal or professional activities that would put the assessor in a position of a real or apparent conflict of interest with the performance of his/her duties as an assessor.

2. Agreement that CAEAL disclose to the applicant laboratory the assessor's involvement in any such activity, that in CAEAL's opinion, represents a real or apparent conflict of interest.

3. Agreement that if a finding of real or apparent conflict of interest is made that the assessor will absent himself/herself from deliberations, of either the CAEAL Board of Directors or the Advisory Panel, which relate to the application or evaluation of the applicant laboratory.

4. Agreement to respect and safeguard the confidentiality of all information attained on an applicant laboratory including documents provided by CAEAL and any information personally observed or obtained.

5. Agreement to return to CAEAL all documents relating to the application or evaluation of an applicant laboratory.

6. Agreement to recognize that the identity of the applicant laboratory is confidential until such time as formal recognition has been granted by the SCC.

As a further safeguard to confidentiality, the CAEAL Proficiency Testing Manager assigns a confidential code to each applicant laboratory before Proficiency Testing commences. This code is known only to the applicant laboratory, CAEAL staff and the Advisory Panel; all communication of proficiency testing data to the CAEAL membership, including the CAEAL Board of Directors, is by laboratory code.

Curriculum Vitae and signed confidentiality agreements for all assessors are kept on file by CAEAL and are available upon request.

SCC Policy. The SCC safeguards confidentiality of information disclosed in an application, documentation additional to the application, and proficiency testing or site assessment reports provided by CAEAL. The identity of an applicant remains confidential until accreditation is granted.

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Accreditation Programs - Calibration and testing Labs

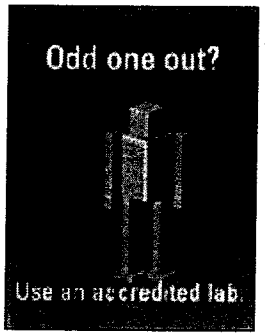
PALCAN
Program for Accreditation of Laboratories – CANADA

Labs accredited by the SCC under ISO/IEC Guide 25 are part of an internationally recognised program that demonstrates proof of their technical competence within a trusted ISO quality management system.

The formal recognition of technical competence in an SCC accreditation is a gateway to markets - used by leaders to gain confidence and trust from their clientele.

- History of PALCAN
- PALCAN Initiatives
- Criteria and Procedures for Accreditation
- Accredited Laboratories
- PALCAN Program Speciality Areas (PSA)
- Good Laboratory Practices (GLP)
- PALCAN Forums
- Links
- Canadian procedural documents (CAN-P)
- North American Calibration Cooperation (NACC)
- Accreditation and Recognition Notices

A new fee structure. **Update** on the Standards Council of Canada's, Program for the Accreditation of Laboratories-Canada (PALCAN)! PDF



You may request general information on the PALCAN accreditation program and a copy of the accreditation documentation through info@scc.ca

More detailed program information may be obtained from SPetersen@scc.ca.

Last updated 2001-05-10

Important Notices and Disclaimers

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Canada

History of PALCAN

The History of the Program for the Accreditation of Laboratories - Canada (PALCAN)

The National Accreditation Program for Testing Organizations (NAPTO) had its origins in 1979 as part of Canada's new National Standards System, operated by the Standards Council of Canada (SCC).

It was the third accreditation program to be launched. The SCC accredited the first two laboratories in June 1981. By 1984 there were 17 in the program, a number that rose to 45 by 1987.

NAPTO was developed to accept applications from a wide variety of Canadian laboratories - from commercial to governmental to those found as part of manufacturing plants and in universities. The size of the laboratory did not matter and all were equally welcome to apply for SCC accreditation. Fees were modest at that time because a large part of the SCC funding was available from government appropriation.

The accreditation program criteria that were developed were published in an SCC "standard publication", CAN-P-4. This document was based upon the international criteria contained in ISO/IEC Guide 25.

Over the years, the program grew steadily and expanded its coverage into all parts of Canada. With the growth came the need to expand the capacity of the SCC teams, and at that time SCC NAPTO staff was leading all visits.

In 1988 the SCC, in cooperation with the National Research Council (NRC), launched a program to accredit calibration laboratories. At that time the NRC provided the technical certification of the laboratories following international metrological standards while the SCC performed the quality assessments in accordance with ISO/IEC Guide 25. This joint program was known as the Calibration Laboratory Accreditation Service (CLAS).

In 1993, NAPTO was renamed the Program for the Accreditation of Laboratories - Canada and has been known ever since by the bilingual acronym PALCAN.

A major change to PALCAN took place in 1994 when an agreement was struck with the Canadian Association of Environmental Analytical Laboratories (CAEAL). The partnership that was formed initially brought several dozen laboratories into PALCAN and with the formation of the partnership came a different arrangement for the delivery of accreditation services to the environmental laboratories. The vast majority of the work was to be performed by CAEAL teams with the accreditation action being taken by the SCC in accordance with its accreditation program requirements. In cases where there were non-environmental aspects to the laboratory's program, the SCC managed that part of the assessment/reassessment process.

Continued PALCAN growth in the face of declining government appropriation led to a decision to have all accreditation programs recover their costs of providing services. In 1995 the then Executive Committee of the Standards Council called upon all conformity assessment programs to become fully self-sufficient with respect to revenue and to recover both direct and indirect costs.

Eighty percent of the PALCAN laboratories were small businesses and unable to sustain large fee increases. It was decided to amend the fee structure to reflect the scope of testing and the complexity of each laboratory's program. This created small increases in fees for small laboratories while the larger and more complex laboratories experienced proportionately larger increases in fees. It was agreed that PALCAN would be permitted to take a number of years to reach self-sufficiency, expected by the year 2000.

In the last six years, there have been a number of changes in PALCAN including the formation of Program Specialty Areas (PSAs) for specific program activities such as pesticide residues, drug abuse, environmental, fasteners, food, forensic and mineral analysis. See the article at http://www.scc.ca/palcan/psa_e.html, which provides additional information on this subject. It should be noted that a great deal of the effort leading to these new programs has been provided by volunteers - experts in the disciplines and industry sectors which initiated the requirement for these specialty programs.

Then in 1997 it was decided to augment the numbers of non-staff Team Leaders working in PALCAN by holding a Team Leader seminar. About three dozen existing and potential new Team Leaders were given training in the PALCAN process and the responsibilities of those

destined to lead audit teams visiting PALCAN clients. In addition, these Team Leaders were encouraged to seek certification as quality auditors from the National Quality Institute, Canada's certifier of quality system auditors.

PALCAN has been blessed with a broad range of very competent technical assessors, principally from governmental agencies, to add the technical competence required by ISO/IEC Guide 25 accreditation. The staff members are grateful for this support, normally provided at no cost to the SCC or the SCC's clients - apart from travel and living expenses.

Looking back, the evolution of PALCAN has been steady and positive. While there have been some growing pains, the SCC staff and volunteers have worked hard to work out the bugs and keep the program moving forward. At the end of fiscal year 1997/98, there were 208 accredited laboratories registered with PALCAN.

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Palcan initiatives

Program Specialty Areas

You will see from recent articles in CONSENSUS that PALCAN is responding to client requests and developing new Program Specialty Areas (PSAs) for such testing areas as mineral analysis and DNA testing. You will also have seen some information about maintaining current PSAs. This and other news reflects our determination to find new and more effective ways to meet all the needs of our clients through an improved service delivery process and streamlined procedures at our Ottawa offices.

The first laboratory visit as part of the Mineral Analysis PSA took place in February. The forensics program, of which DNA testing is a part, is to be launched in April/May of this year.

Another launch in April/May is the SCC's PALCAN Good Laboratory Practice (GLP) Compliance Recognition Program. This service will be initiated in cooperation with the Pest Management Regulatory Agency (PMRA), the regulatory agency responsible for this area. The SCC's PALCAN group will become a Compliance Monitoring Agency (CMA). The program's scope includes physical-chemical property, analytical, residue and toxicological or ecotoxicological studies. The SCC's GLP Recognition program will follow the conditions established by the Organization for Economic Cooperation and Development (OECD). The SCC-PALCAN program is being developed in such a way as to permit its expansion into other areas where GLP principles apply. In addition, it is expected that links will be established with other CMAs worldwide.

Information Services

Frequent visitors to our web site will know that not only are we providing the listing of all laboratories but also we have a search capability for all laboratory scopes available to our web site visitors. We currently plan to offer that information paid for, in part, by our new fee structure, as a service to our PALCAN members and to our laboratory customers as well.

A decision was made recently to make our program documents more readily available to those interested. Henceforth our PALCAN criteria and guidance documents will be available at no cost to registered visitors to the SCC web site. In due course the documents will be posted to the web site for the convenience of visitors.

Meeting with our Clients

Another initiative to note is our intention to organize meetings at least once a year with laboratory associations who represent PALCAN members. The purpose of these meetings would be to provide you, through your associations, an opportunity to discuss your needs as members of PALCAN and to provide us with an opportunity to share with you developments in the program. The SCC's Certification Organizations (COs) and Registration Organizations (ROs - both Quality and Environment) each have regular contact with SCC management as members of the Canadian Conformity Assessment Conference (CCAC) and we would like to offer you similar access to SCC management.

Team Leaders

At the end of April, the PALCAN staff organized and ran a three-day seminar for the existing Lead Assessors employed part-time by the SCC and invited thirty-two potential Team Leaders to attend. The seminar provided instruction in the methods used by SCC Program Officers in conducting accreditation visits to laboratories. The candidates were given the opportunity to address the group and at the end there were a series of interviews to provide candidates and PALCAN staff with a final opportunity to consider their suitability as Team Leaders to lead PALCAN visits in the future. Following the seminar, there was a comprehensive review of the contribution made by each candidate and a list of twenty candidates was compiled. These candidates will now be involved in on-the-job training, with the first half-dozen becoming qualified as PALCAN Team Leaders by the end of March, 1998. It is intended to continue to bring more Team Leaders into the PALCAN "pool" over the next 12 to 18 months. Our objective in doing this is to increase the capacity of PALCAN. We recognize that our response time needs improvement. We expect that later in 1998 our clients will notice a distinct improvement in the PALCAN services.

Quality Management and Continuous Improvement

We will continue to make improvements to our program as we adopt ISO 9001 at the SCC. The initial flow charting has been done and we have compiled a list of improvements to work on

initial flow-charting has been done and we have compiled a list of improvements to work on whenever we have an opportunity. PALCAN soon will have improved technological support services, designed to improve our turnaround time on visit reports and to facilitate the planning of the visits themselves along with process changes that will allow electronic balloting of assessment results. We expect the technological changes will take effect by April or May of 1998.

Auditor Certification

All PALCAN officers are now certified auditors in the National Quality Institute's new auditor certification program or those of equivalent organizations such as the Institute for the Registration of Certificated Auditors (IRCA) in the U.K. or the Registrar Accreditation Board (RAB) in the U.S. We consider that this is an important move that will acknowledge the competence of our Officers and provide a baseline for newcomers to our program. It is our intention to seek certification as an Auditor or Lead Auditor as a desirable qualification of Team Leaders who we will employ part-time in our program.

International

We are also providing information concerning our moves internationally that are intended to facilitate international acceptance of the testing conducted by our members. One such initiative concerns our preparations for an evaluation by an assessment team from the Asia Pacific Laboratory Accreditation Cooperation (APLAC). The APLAC team will conduct a pre-assessment the week of May 19th, 1998 and will return for the assessment before the end of calendar 1998.

The SCC has applied to join the peer review program that will result in an agreement with the European cooperation for Accreditation (EA). We are doing this because of the clear message that we are getting from some of our PALCAN clients regarding the difficulty they are encountering trying to test products for sale in the European Community. This program will require that Canada begin the process by participating in a number of intercomparison projects and plans are underway to see that this happens. The intercomparison work is being spearheaded by NRC, the SCC's partner in the program for accrediting calibration laboratories.

These are two of the exciting things happening that are designed to improve the business opportunities for members of PALCAN.

STANDARDS COUNCIL OF CANADA
CONSEIL CANADIEN DES NORMES

ACLAE
CAEAL

Accreditation Partnership Agreement

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PURPOSE

1. The purpose of this Agreement is to describe the responsibilities of both parties in a national program for the accreditation of environmental laboratories. This Agreement covers the technical and quality system assessment of environmental laboratories by the Canadian Association for Environmental Analytical Laboratories (CAEAL) and the accreditation, to ISO/IEC Guide 25 and future version thereof, of those assessed laboratories by the Standards Council of Canada (SCC). The accreditation is effected through the SCC's Program for the Accreditation of Laboratories – Canada (PALCAN).

DEFINITIONS

"ACCA", the Advisory Committee on Conformity Assessment, the SCC advisory committee that provides the SCC Council with advice on Conformity Assessment matters;

"CAEAL" the Canadian Association for Environmental Analytical Laboratories (Inc.), Suite 300, 265 Carling Avenue, Ottawa, Ontario, K1S 2E1;

"CAN-P-4" SCC's *General Requirements for the Accreditation of Calibration and Testing Organizations*, latest edition, ISO/IEC Guide 25 verbatim and future versions thereof;

"CAN-P-15" *Requirements And Procedures For Suspension And Withdrawal, Complaints, Appeals And Hearings;*

"CAN-P-1510" SCC's *Guidelines for Preparing an Application for Accreditation for Calibration and Testing Organizations*, latest edition;

"CAN-P-1515" SCC's *Conditions for the Accreditation of Calibration and Testing Organizations*, latest edition;

"CAN-P-1600" means the *Environmental Interpretive Document* that interprets CAN-P-4 for

environmental laboratories;

"EWG" the Environmental Working Group of the TG Laboratories;

ISO/IEC Guide 43 Parts 1 and 2, *Proficiency Testing by interlaboratory comparisons – Development and operation of proficiency testing schemes – Selection and use of proficiency testing schemes by laboratory accreditation bodies.* The SCC adoption is CAN-P-1543;

ISO/IEC Guide 58, *Calibration and Testing Laboratory Accreditation Systems - General Requirements for Operation and Recognition.* The SCC adoption is CAN-P-1558;

"NSS" the National Standards System, A grouping of Canadian volunteers and organizations which contribute to Canadian and international voluntary standards, and a variety of Canadian organizations concerned with standards development, promotion and implementation;

"Partnership", in the sense used in this Agreement, refers to a cooperative arrangement between the SCC and CAEAL to provide SCC accreditation to environmental laboratory clients for whom accreditation services are provided by CAEAL;

"SCC" means the Standards Council of Canada, whose office is located in the City of Ottawa; and,

"TG Laboratories", a task group of the SCC's Advisory Committee on Conformity Assessment (ACCA) made up of technical experts responsible for the technical competence of the PALCAN.

Note - In the context of this document, the term laboratory means environmental laboratory, unless otherwise specified.

PREAMBLE

2. It is the aim of the SCC-CAEAL partnership to deliver viable accreditation services known for their integrity, credibility, efficiency, quality of service and commitment to excellence. The partnership is committed to this aim and will conduct regular internal audits and program reviews to identify areas where improvements would be seen as beneficial.

3. The Standards Council of Canada (SCC) was established by Parliament under the Standards Council of Canada Act to foster and promote voluntary standardization in Canada. The SCC Act relates to the fields of construction, manufacture, production, quality, performance, and safety of buildings, structures, manufactured articles and products, and other goods, including components thereof, not expressly provided for by law, as a means of advancing the national economy, benefiting the health, safety, and welfare of the public, assisting and protecting consumers, facilitating domestic and international trade, and furthering international cooperation in relation to written standards.

4. Since 1981, SCC has been operating a program for the accreditation of calibration and testing laboratories in accordance with published criteria, conducting periodic on-site assessments of applicant and accredited laboratories and issuing certificates of accreditation. Through the operation of its Program, known as PALCAN, the SCC provides for the assessment of laboratories seeking accreditation to ISO/IEC Guide 25. SCC/PALCAN conducts the assessments of certain Program Specialty Areas, such as environmental and calibration laboratories, in collaboration with other organizations but retains full authority and responsibility for laboratory accreditation.
5. The Canadian Association for Environmental Analytical Laboratories (CAEAL) was formed in 1989 by the combined interests of government and commercial laboratories to maintain and

promote a high level of assurance in environmental analytical test data. CAEAL has initiated a performance evaluation (proficiency testing) program, on-site assessments and has issued certificates of accreditation.

6. Since June 20th, 1994, SCC has accredited environmental laboratories under an agreement with CAEAL, contributing towards providing Canada with a single national laboratory accreditation program. This partnership is consistent with the federal government's fiscal strategy of avoiding duplication of government-financed services and reduces administrative and financial obligations on Canadian laboratories.

7. Both organizations will continue to work to provide information and promote the utilization and acceptance of accredited laboratories in accordance with each organization's marketing strategies. Environmental laboratories are the clients of this partnership, and it is the goal of the partners to make the accreditation process as effective and efficient as possible. This will be achieved by seeking means to streamline the respective processes and by regular reviews aimed at achieving the most cost-effective accreditation program possible. Attention to the principle of continuous improvement will lend force to this approach by partners.

8. Both CAEAL and SCC are active on the international scene. CAEAL represents Canadian environmental laboratories on the NAtional Cooperation for Laboratory Accreditation (NACLA). SCC is responsible for ensuring the laboratory accreditation system in Canada meets the provisions of ISO/IEC Guide 58 and is equivalent to, and compatible with, the accreditation systems of other parties concerned, within North America and in other regions of the world. SCC is the Canadian signatory to the Memorandum of Understanding of the International Laboratory Accreditation Cooperation (ILAC). ILAC is a cooperation between accreditation bodies representing individual economies; in most cases there is one such body per economy. The objective of ILAC is to enable the establishment of mutual confidence between regional organizations and between participating accreditation bodies. To this end, ILAC has developed an Arrangement that will permit mutual recognition between regions. SCC is the leading Canadian organization providing accreditation services in the general area of conformity assessment. In keeping with the Action Plan of the Industry Portfolio established by the federal government to extend science and technology linkages internationally, the accreditation activities give Canada's trading partners confidence that Canadian testing activities are based on both a reliable measurement and a sound accreditation system. These activities adhere to international standards and guides and aid in reducing technical barriers to trade.

ELIGIBILITY

9. This Agreement applies primarily to the accreditation of environmental analytical testing laboratories as defined in the Environmental Interpretive Document, CAN-P-1600. Other related or accreditation activities would be by mutual agreement.

TERMS OF REFERENCE

10. SCC has authority for laboratory accreditation in Canada and as such is responsible for the granting and the maintenance of accreditation contingent upon eligibility of the laboratories for CAEAL recommendation. The CAEAL is responsible for all aspects of the environmental laboratory assessment including reassessment and surveillance of accredited laboratories. As

the national accreditation body, the SCC is responsible to ensure CAEAL is competent and complies with the applicable provisions of ISO/IEC Guide 58.

11. Both SCC and CAEAL comply with the applicable provisions of ISO/IEC Guide 58. International laboratory accreditation recognition bodies such as the Asia Pacific Laboratory Accreditation Cooperation (APLAC) assess this compliance. In addition, PALCAN conducts surveillance of the CAEAL program by performing an annual audit that includes on-site observation of one CAEAL assessment or reassessment visit. The audit plan will take into consideration any other relevant audits conducted by the aforementioned body and will be agreed to by the signatories to this Agreement in an exchange of correspondence.

12. All applicable fees will be determined on a yearly basis and confirmed by an exchange of correspondence between the signatories of this Agreement. CAEAL will determine the assessment and proficiency testing fees. SCC and CAEAL will negotiate the PALCAN annual laboratory fees and those for the audit of CAEAL. All applicable fees will be invoiced to applicant and accredited laboratories by CAEAL. Each Party to this Agreement will inform the other of any proposed change of fees at least three months before the change is due to take effect. In the case where CAEAL has provided a firm quotation for the assessment of a laboratory, a change in the SCC fees will not come into effect until the end of the period for which the quotation was cited providing the period does not exceed six months.

13. The conduct of any laboratory assessment or surveillance activity made pursuant to this Agreement is done in accordance with ISO/IEC Guide 58, and the PALCAN and CAEAL quality management systems. The purpose of these activities is to determine laboratory conformance with ISO/IEC Guide 25 and its successor document ISO/IEC 17025 and the relevant PALCAN and CAEAL documents.

14. The operation of the Proficiency Testing program for PT services administered by CAEAL will be entirely within the purview of CAEAL. The Parties will adhere to the criteria and procedures contained in ISO/IEC Guide 43 Parts 1 and 2, in its international form as adopted for use in PALCAN.

15. In order to assure uniformity and harmonization of approach with other laboratory accreditation activities, the SCC and CAEAL collaborate in the development of all applicable documentation. CAEAL retains custody of, and is responsible for, the development and maintenance of all CAEAL environmental interpretive documents. The CAEAL Board approves these documents. SCC retains custody of, and is responsible for, the development and maintenance of all PALCAN documents. The Task Group Laboratories Committee of the SCC approves these documents. Documents for which the SCC and CAEAL share responsibilities will be developed jointly by and shall require approval from both Parties.

16. Each Party to this Agreement ensures that the policies of the other are taken into consideration in developing Canadian policies, positions, and programs on national and international laboratory accreditation matters. SCC ensures CAEAL comments are taken into consideration in developing Canadian input into the revisions of relevant international standards and guides. Policy developments related to accreditation proposed by either Party will be provided to the other Party for review to ensure that those draft policies do not

conflict with program directions or priorities.

17. The Parties agree to cooperate and carry out marketing activities to promote the benefits of accreditation to accredited laboratories and the users of those laboratories' services, nationally and internationally. Each Party to this Agreement agrees to cover its own costs for such activities. Nothing in this Agreement is construed as restricting the right of SCC or CAEAL to advertise or otherwise market the SCC/CAEAL accreditation collaboration, including exclusive administrative references to PALCAN or CAEAL, provided that such advertisement or marketing proposal includes a statement identifying SCC as the authority for accreditation and CAEAL as the environmental laboratory assessment service and proficiency testing provider.

18. Nothing in the Agreement, by specific mention or omission, limits in any manner the right of the SCC to give consideration to proposals from other national or regional environmental analytical laboratory accrediting organizations. Any offers, approaches, etc. that would fundamentally change the nature of the partnership would be mutually discussed in order to arrive at a decision that would enhance the status and integrity of the SCC-CAEAL partnership.

OPERATIONAL

19. Applicant and accredited environmental laboratories deal with CAEAL on all technical and quality system matters. When an applicant laboratory requests accreditation for both environmental and other testing services, PALCAN and CAEAL will manage the assessment activities jointly. Such applicant and accredited laboratories deal with CAEAL as defined in the Joint SCC-CAEAL Assessments procedure. The procedure is maintained in the CAEAL quality management system.

20. **Application:**
    - 20.1 Either Party may provide a letter or brochure providing general information on the accreditation process to interested laboratories.
    - 20.2 Environmental laboratories wishing to apply for SCC accreditation are instructed to request application packages from CAEAL.
    - 20.3 Along with the detailed CAEAL documentation, the application package, available from CAEAL, includes an Application Form having the following information:
        - (i) A statement of intent that this is an application for SCC accreditation and CAEAL assessment;
        - (ii) The names and addresses of both Parties to this Agreement;
        - (iii) CAEAL as the official destination for completed applications for accreditation of environmental services and SCC for accreditation of all other testing services;
        - (iv) A brief statement of the responsibilities of both CAEAL and PALCAN;
        - (v) A statement, to be signed by the applicant, authorizing the full exchange of information between the Parties to this Agreement; and,
        - (vi) An agreement to abide by the conditions and requirements of SCC accreditation and CAEAL, to pay the required fees, and recognition that accreditation may be withdrawn on failure to comply with the above.

20.4 Where an application for accreditation or expansion of accredited scope includes subject areas other than environmental, CAEAL deals with the environmental activity portion of the application as described in this Agreement. CAEAL forwards a copy of the application to the SCC with a request that PALCAN deals with the assessment of all other testing activities and that a joint assessment be conducted. The SCC invoices costs relating distinctly to testing to the laboratory.

21. **Document Review:**

21.1 CAEAL acknowledges receipt of the application documentation and application fee, and informs SCC by copy of the Application Form and scope of measurement for which accreditation is sought, with a request for PALCAN to open an accreditation file. CAEAL reviews the application for completeness of information, identifying any deficiencies in the application and, where necessary, requests an appropriate response from the laboratory; a copy of the report is provided to PALCAN. When the application process and documentation are determined to be complete, and an official SCC accreditation file is opened, CAEAL commences the assessment process.

22. **Assessment:**

22.1 CAEAL conducts the on-site assessment(s) of the applicant laboratory for compliance with the requirements of ISO/IEC Guide 25 as defined in its Quality Management System documentation. A report will be provided to the laboratory identifying any deficiencies in the laboratory's implementation of its quality system and its operation, and requesting an appropriate response from the laboratory.

22.2 CAEAL appoints and provides their own assessors for environmental duties. The procedures for environmental assessors are in the CAEAL Quality Management System documentation.

22.3 Some assessors may be called upon to lead assessments or reassessments that have non-environmental components. These assessors will be provided with specific instructions dealing with the joint aspects of the visit. Both liaison officers will review these instructions before the visit. Assessors willing to lead non-environmental visits are subject to SCC/PALCAN familiarization training to help them conduct these visits to the satisfaction of all participants. Those assessors involved in annual assessments of CAEAL (and other Partners) will be competent to perform assessments to the level of ISO/IEC Guide 58 published by the SCC as CAN-P-1558.

22.4 CAEAL will arrange for the requisite reference laboratory services necessary for verification of the capability of an applicant or an accredited laboratory. This will be done through the CAEAL PT program to perform specified environmental analytical tests for which SCC accreditation is sought pursuant to this Agreement, recognizing that accreditation may be sought for tests outside the environmental analytical subject area.

22.5 CAEAL will determine the proper PT program with which each laboratory should comply and will monitor the proficiency of environmental laboratories.

22.6 The PT scores will be determined by the scoring rules of the CAEAL laboratory technical assessment program for environmental analytical testing appearing in the SCC/CAEAL Program Description and meeting the requirements of ISO/IEC Guide 43.

22.7 CAEAL will provide SCC and the laboratory with a copy of a statement that the laboratory meets the assessment and PT criteria for the specific tests for which accreditation is sought, from their successful participation in its assessment and PT process, attaching the detailed report.

23. CAEAL Recommendation and SCC Accreditation:

23.1 The application and the recommendation for accreditation will be processed for approval by the fastest possible means - within the limits of the SCC's process for the approval of accreditation applications.

23.2 Once SCC has accredited a laboratory, that laboratory will then be issued with a SCC Certificate of Accreditation along with the supporting documentation, which will list specific tests for which it is accredited. The scope of accreditation will be made available on the SCC website. The certificate will acknowledge the technical assessment by CAEAL and also will carry the CAEAL logo.

24. Surveillance and Reassessments:

24.1 The surveillance and reassessment process of the accredited laboratories follows a schedule agreed to between PALCAN and CAEAL.

24.2 SCC and CAEAL keep each other informed of all plans and progress in the accreditation process and coordinate activities as may from time to time be necessary. Details of the laboratory assessment activities are retained in the CAEAL files.

24.3 Any publicity statement approved by either Party and for the use of accredited laboratories is prepared in consultation with the other Party and includes appropriate references to SCC and CAEAL.

24.4 Each Party to this Agreement informs the other of any known failure of a SCC accredited laboratory in the performance of an accreditation requirement.

24.5 SCC and CAEAL may require an accredited laboratory to undergo reassessment at any time for cause. Under the terms of the published criteria, accreditation may be revoked, suspended, or withdrawn by SCC if the conditions under which accreditation was granted are not being met. SCC relies on CAEAL in deciding whether to withdraw full or partial accreditation based on technical and quality system deficiencies. The requirements and procedures for suspensions, withdrawal, appeals, complaints and hearings are contained in the SCC document CAN-P-15.

DIRECTORIES

25. SCC publishes on its web site, and regularly updates, a list of accredited calibration and testing laboratories, and the detailed scopes of the testing laboratories. Those without Internet access may obtain this information by contacting the SCC Information Services. CAEAL has authority for the written description of each accredited laboratory's testing capabilities, while ensuring that the descriptions respect any requirements imposed by Memoranda of

Understanding (MOUs), Mutual Recognition Agreements or Arrangements (MRAs), and Multilateral Agreements or Arrangements (MLAs) or similar documents to which SCC is signatory. These documents are published for information purposes and the official scope of accreditation is the document signed by the PALCAN Manager.

26. CAEAL may publish a directory of laboratories accredited under the terms of this Agreement.

LIAISON, INFORMATION EXCHANGE AND FINAL PROVISIONS

27. Subject to article 22.3, both Parties will ensure the protection of laboratories' confidential information or proprietary rights.

28. If either party is unable to fulfil its responsibilities under this Agreement, it must give prompt and adequate notice of its inability to the other Party within 30 calendar days.

29. One CAEAL representative will be invited to be a member of the TG Laboratories and one SCC representative will be invited to sit on the board of CAEAL. This will promote the harmonization of CAEAL policies with those of SCC. It also provides a direct opportunity for CAEAL to influence the direction and objectives of SCC's PALCAN.

30. SCC and CAEAL each have an appointed liaison officer responsible for implementing and monitoring the activities of both Parties in relation to this Agreement. The liaison officers will keep each other fully informed of all activities covered by this Agreement through regular meetings. However, either Party may bypass the other Party's liaison officer in cases where such action is necessary. The exercise of this alternative must be restricted to cases of emergency where one or other liaison officer is unavailable. In such an instance, the other Party's liaison officer must be informed as soon as possible. Each Party informs the other in advance of proposals or representations that it makes to international organizations, and keeps the other briefed on issues that are of current concern in the organizations in which each participates. The Executive Director of CAEAL and the Manager of PALCAN will meet at least quarterly to review issues of mutual interest.

31. Should there occur a complaint or dispute that cannot be immediately resolved by the Liaison Officers as part of the normal course of their responsibilities, the Signatories will address the matter by the appropriate SCC and CAEAL quality management system procedure. SCC is the final authority on the granting, suspension and withdrawal of accreditation.

32. The Parties to this Agreement encourage each other to inform other interested parties of the existence and content of this Agreement. Nothing in the Agreement is considered confidential and shall be fully accessible by any member of the public upon request.

33. The liaison officers for this Agreement will be identified by an exchange of correspondence between the signatories of this Agreement or their successors.

34. The Agreement will remain in effect until 2005-01-01 unless terminated by either Party upon three months written notice to the other party. The Agreement may be renewed when mutually agreed for further term(s) of 5 years.

35. Each of the parties shall only be liable for those acts and responsibilities as set out in this Agreement. Notwithstanding the above, it is expressly acknowledged by the parties that they shall not be liable to the other for any matters, direct or indirect, arising from a refusal to accredit, or withdrawal of accreditation and further including matters arising from the withdrawal or termination of participation for any reason from any organization, the activities of which may impact on the delivery of services under this Agreement.

36. Neither party may assign or transfer the rights or obligations under this agreement without the prior written consent of the other party. -

Signed in Ottawa on this ..... day of January, 2000.

Mr. Peter Clark Executive Director, Standards Council of Canada	**Mr. Jeffrey Pike** President, Canadian Association for Environmental Analytical Laboratories (Inc.)
Ms. Sandra Watson Corporate Secretary, Standards Council of Canada	**Ms. Denise LeBlanc** Vice President, Canadian Association for Environmental Analytical Laboratories (Inc.)

Programme d'accréditation des laboratoires d'analyse environnementale (PALAE)

Table des matières

- Clientèle visée
- Champs et domaines d'accréditation
- Tarification
- Laboratoires accrédités
- Documents disponibles
- Pour nous joindre

Le Centre d'expertise en analyse environnementale du Québec procède à l'accréditation des laboratoires privés, municipaux et institutionnels aux fins de l'application réglementaire. Le programme d'accréditation repose sur le pouvoir conféré au ministre à l'article 118.6 de la Loi sur la qualité de l'environnement du Québec (L.R.Q., chap. Q-2).

Le programme d'accréditation des laboratoires d'analyse environnementale (PALAE) est constitué d'un ensemble de *normes et d'exigences* régissant le processus de qualité pour les laboratoires. Il a été élaboré en 1984 dans le but de s'assurer de la qualité des analyses réalisées par les laboratoires accrédités pour la surveillance des eaux de consommation, des eaux souterraines, des eaux usées municipales et industrielles, des boues d'usines d'épuration, des sols contaminés, des déchets dangereux, des huiles usées et des rejets à l'atmosphère. L'objectif du programme est d'assurer et de maintenir un niveau de qualité analytique suffisamment élevé pour que la clientèle faisant appel à ces laboratoires puisse utiliser en toute confiance les renseignements analytiques ainsi produits.

Clientèle visée

Le programme s'adresse à tous les laboratoires privés, publics et parapublics oeuvrant dans le domaine de l'analyse environnementale au Québec. Ces laboratoires peuvent être de type commercial, industriel, municipal, gouvernemental ou institutionnel.

Les laboratoires accrédités sont reconnus par le ministre de l'Environnement selon les dispositions de l'article 118.6 de la Loi sur la qualité de l'environnement (L.R.Q., chap. Q-2) et conformément aux normes et exigences d'accréditation incluant celles du Guide ISO/CEI 25.

Champs et domaines d'accréditation

Le programme d'accréditation concerne l'expertise analytique relative à la chimie, la microbiologie et la toxicologie. Il s'applique en principe à tout paramètre analytique visé par la gestion environnementale. Le document DR-12-CDA présente les champs et domaines d'accréditation en vigueur.

L'opportunité d'élargir les secteurs d'application repose sur des éléments de faisabilité, de marché, de coût et de pertinence.

Pour nous joindre :

Centre d'expertise en analyse environnementale du Québec
Service de l'accréditation
1665, boul. Wilfrid-Hamel
Édifice 2, bureau 1.03
Québec (Québec) G1N 3Y7
Tél. : (418) 643-1301
Téléc. : (418) 528-1091
Courrier électronique : ceaeq.fra@menv.gouv.qc.ca

APPENDIX G

RAINBOW TROUT AND *DAPHNIA MAGNA* TEST METHOD CHECKLISTS FOR REVIEWING LABORATORY SOPS AND FOR ANALYST INTERVIEWS

TEST SPECIFIC CHECKLIST

Revised: December 2001

Acute Lethality Test Using *Daphnia magna* Spp. (GM)
Reference Method For Determining Acute Lethality Of Effluents To *Daphnia magna* (RM)
Note: Shaded text reflects Dec. 2000 method amendments

Parameter	Specification	Met Specifics Y	N	NA
Sample Preparation				
Filtering	Filtering of solids is not allowed **(Must RM)**	...	...	...
D.O. Measurement	D.O. to be measured in sample prior to test initiation **(Must RM)**	...	...	...
Pre-aeration	If 40 $ D.O. # 100 saturation, pre-aeration is not allowed **(Must RM)**	...	...	...
	If D.O. in the test sample is <40% or >100%, pre-aeration is only allowed for 30 min at a rate within the range of 25 to 50 mL/min"L **(Must RM)**	...	...	...
Conductivity	Measured after warming the effluent sample to room T/ and before any dilutions are made. If conductivity is # 100 :mhos/cm, sample hardness measured before starting the test **(Must RM & GM)**	...	...	...
Hardness Adjustment	If sample hardness < 25 mg/L, adjust to 25-30 mg/L following instructions in test method document **(Must RM)**	...	...	...
	If sample hardness < 25 mg/L, use either *D. pulex* or adjust hardness to 25 mg/L if still using *D. magna* **(GM)**	...	...	...
	Any sample adjusted for hardness thoroughly mixed and its hardness confirmed before use **(Must GM)**	...	...	...
pH Adjustment	No pH adjustment of sample or test solutions allowed **(Must RM)**	...	...	...
	No adjustment if pH of test solution is within range 6.0 - 8.5 **(GM)**	...	...	...
T/ Adjustment	Effluent sample and control/dilution water adjust to 20 ± 2/C before use **(Must RM)**	...	...	...
	No use of immersion heaters **(Must RM & GM)**; water bath recommended	...	...	...
Test Conditions				
Test Facility	Separate lab., test isolated from general disturbance **(Must RM)**	...	...	...
Test Type	Static **(Must RM)**	...	...	...
Test Duration	48h	...	...	...
Test T/	20 ± 2°C **(Must RM & GM)**	...	...	...
Light Quality	"Cool White" fluorescent	...	...	...
Light Intensity	400 - 800 lux at surface	...	...	...
Photoperiod	16 ± 1h light; 8 ± 1h dark and coincides with culture photoperiod **(Must RM)**	...	...	...
In-test pH	pH not to be adjusted during test **(Must RM)**	...	...	...
D.O. Range	40 - 100% air saturation **(GM)**	...	...	...
Aeration	No aeration during test **(Must RM)**	...	...	...
Vessel Size & Type	Glass or clear plastic of high quality **(Must RM & GM)**	...	...	...
	Identical for all test solutions; uncovered or loosely covered	...	...	...
	Do not contain leachable substances **(Must RM)**	...	...	...
	If volatiles suspected, parallel test with capped vessels can be run	...	...	...
	Position of the test vessels within the testing facility is randomized	...	...	...
Test Volume	$ 150 mL **(Must RM)**	...	...	...
	Identical volume in each test vessel **(Must RM)**	...	...	...
Renewal of Solution	None **(Must RM)**	...	...	...
Dilution/Control Water	Same as culture or acclimation water; ground, surface or dechlorinated municipal water, reconstituted water; D.O. 90 - 100% air saturation **(Must RM)**, hardness $ 25 mg/L	...	...	...
	Hardness within ± 20% of water used for culturing organisms **(Must RM)**	...	...	...
# Control/Test	One or more control(s) for each test conducted **(Must RM & GM)**	...	...	...
Vessel Labeling	Clearly labeled conc., date and start time **(Must RM)**	...	...	...
# Test Conc.	Multi conc. test: $5 plus one or more control(s) **(Must RM & GM)**	...	...	...
	Highest conc. full-strength effluent, successive conc. at least 50% strength of next highest conc. **(Must RM)**	...	...	...
	Single conc. test: 1 (100% test solution) plus control **(Must RM)**	...	...	...
# Replicates/Conc.	Multi conc. test: 1 vessel per conc., more may be used	...	...	...
	Single conc. test: minimum of 3 replicates and 30 daphnids for 100% sample and control **(Must RM)**	...	...	...

TEST SPECIFIC CHECKLIST

Revised: December 2001

Acute Lethality Test Using *Daphnia magna* Spp. (GM)
Reference Method For Determining Acute Lethality Of Effluents To *Daphnia magna* (RM)
Note: Shaded text reflects Dec. 2000 method amendments

Parameter	Specification	Met Specifics Y	N	NA
# Organisms/Vessel	Equal numbers of neonates to be introduced into each concentration including the control: minimum 10 per treatment for LC50 test, and 30 divided among a minimum 3 replicates for single-concentration test **(Must RM)**	...	...	...
	Sequential addition of daphnids to each test solution including control(s), and random order of adding daphnids to vessels	...	...	...
Organisms Loading Density	# 1 organism per 15 mL solution **(Must RM)**	...	...	...
Feeding Regime	No feeding during test **(Must RM)**	...	...	...
Vessel Cleaning	All containers and apparatus thoroughly cleaned and rinsed with control/dilution water before use **(Must RM)**	...	...	...
Substance Testing	Solvent control solution to be run, # 0.5 mL/L limit **(GM)**	...	...	...
Endpoint	Multi conc. test: Mortality (48h-LC50, 95% confidence limits) **(Must RM & GM)**	...	...	...
	Immobility (48h-EC50, 95% confidence limits) if appropriate	...	...	...
	Single conc. test: Mortality (% mortality at 48h) **(Must RM)**	...	...	...
EPS/RM 11 & 14 Amendments	Has the laboratory incorporated the Dec. 2000 Amendments into lab SOPs?	...	...	...
Observations & Measurements				
D.O. + pH + T/	At least at start and end of test in all test vessels **(Must RM & GM)**	...	...	...
Conductivity	At least at start of test in all test vessels **(Must RM & GM)**	...	...	...
Hardness	At least at start of test in controls and 100% test solution **(Must RM & GM)**	...	...	...
Appearance/Behaviour	As a minimum at end of test in all test vessels	...	...	...
Mortality	As a minimum at end of test in all test vessels (magnifying device recommended)	...	...	...
Test Organisms				
Source	Commercial supply houses or govn't laboratory; taxonomically verified	...	...	...
	All organisms used in a test are from the same culture **(Must RM & GM)**	...	...	...
Age	Neonates (# 24h old) **(Must RM)**	...	...	...
Lot #	Traceable to specific health monitoring daphnid(s) which represent(s) a known stock **(Must RM)**	...	...	...
Health Criteria	No ephippia present in the brood stock **(Must RM)**	...	...	...
	# 25% mortality of parental organisms during week before test	...	...	...
	Time to first brood # 12 days **(Must RM)**	...	...	...
	Females 2 - 5 weeks old to deliver an average of $15 neonates per brood **(Must RM)**	...	...	...
Health Monitoring daphnid(s)	Same age as brood stock and of known age **(Must RM)**	...	...	...
	Member(s) of same brood(s) used to create the brood stock **(Must RM)**	...	...	...
	Cultured under similar loading conditions and feeding rates as the brood stock **(Must RM)**	...	...	...
	Maintained for as long as the brood stock is being used to supply neonates as test organisms **(Must RM)**	...	...	...

TEST SPECIFIC CHECKLIST

Revised: December 2001

Acute Lethality Test Using *Daphnia magna* Spp. (GM)

Reference Method For Determining Acute Lethality Of Effluents To *Daphnia magna* (RM)

Note: Shaded text reflects Dec. 2000 method amendments

Parameter	Specification	Met Specifics Y	N	NA
Culture/Holding Conditions				
T/	20 ± 2°C for 2 weeks prior to organism use **(Must RM)**	...	...	...
pH	6.0 - 8.5	...	...	...
D.O.	60 - 100% air saturation	...	...	...
Hardness	Within 20% of that of control/dilution water, for $ 7 days before test; recommend 80 - 250 mg/L	...	...	...
Light Quality	"Cool White" fluorescent	...	...	...
Light Intensity	400 - 800 lux at surface	...	...	...
Photoperiod	16 ± 1h light; 8 ± 1h dark **(Must RM)**	...	...	...
Water Quality	Uncontaminated ground, surface or dechlorinated municipal water, reconstituted water: TRC # 0.002 mg/L **(Must RM)**	...	...	...
Monitoring	T/, D.O., pH, daily for each brood stock culture vessel	...	...	...
Holding Volume/Flow	Daphnids thinned to 20/L weekly	...	...	...
Feeding	One algae species minimum **(Must RM)**	...	...	...
	Two algae species recommended with possible yeast, trout chow and/or Cerophyll supplement. Vitamin B_{12} and selenium be routinely added to culture water	...	...	...
	Feeding regime is such that daphnid health criteria are met	...	...	...
Cleaning	Water replaced weekly; minimal handling of daphnids	...	...	...
QA/QC				
Acceptability Criteria	Test invalid if > 10% of control daphnids (combined replicates) die or exhibit overt, stressed behaviour (eg: immobility) or if > 2 of the control organisms in any test vessel exhibit either of these responses **(Must RM)**	...	...	...
	Same water to be used for culturing/holding and control/dilution water **(Must RM)**	...	...	...
Reference Toxicant	Conducted upon preparation of a new batch of daphnids for possible use	...	...	...
	Within 14 days before or after a toxicity test **(Must RM)**	...	...	...
Warning Chart	Prepared for each reference toxicant using LC50 results and continually updated **(Must RM)**	...	...	...
	Within acceptable warning limits (± 2 SD on log scale) **(Must RM)**	...	...	...
Sample Handling				
Containers	Non-toxic materials for sample and transport containers, new containers or thoroughly rinsed used containers **(Must RM)**	...	...	...
T/ measurement	Upon receipt of sample(s) at laboratory, effluent t/ to be measured and recorded	...	...	...
Holding Time	Test to be initiated within 5 days after sampling **(Must RM)**	...	...	...
	Recommend test initiation within 3 days after sampling	...	...	...
Holding Conditions	Held in the dark in full sealed container(s) at 4 ± 2/C in refrigerated facility (or at 20 ± 2/C if test to be initiated the next day) **(Must RM)**	...	...	...
	Sample be kept from freezing **(Must RM)**	...	...	...
Volume Recommended	$ 2 L for single and multi conc. tests	...	...	...
Labeling	Include at least sample type, source, date and time of collection and name of sampler(s) **(Must RM)**	...	...	...
Subsample Mixing	Content of each container to be agitated thoroughly prior to preparing test solutions **(Must RM & GM)**	...	...	...
Sample Aliquots	Aliquots (sub-samples) to be combined **(Must RM & GM)**	...	...	...

TEST SPECIFIC CHECKLIST

Revised: December 2001

Acute Lethality Test Using *Daphnia magna* Spp. (GM)
Reference Method For Determining Acute Lethality Of Effluents To *Daphnia magna* (RM)
Note: Shaded text reflects Dec. 2000 method amendments

Parameter	Specification	Met Specifics Y	N	NA
Test Report				
	Have lab SOPs been updated to indicate amended requirement that all toxicity tests initiated (finished or not) are to be reported? **(Must RM)**	...	...	...
Sample Data	Name and location of effluent generator **(Must RM)**	...	...	...
	Date and time of sampling **(Must RM)**	...	...	...
	Type of sample **(Must RM)**	...	...	...
	Brief description of sampling point **(Must RM)**	...	...	...
	Sampling method **(Must RM)**	...	...	...
	Person providing **(GM)** / collecting **(Must RM)** sample	...	...	...
Test Organism	Species **(Must RM)**	...	...	...
	Most recent estimates of time to first brood, average number of neonates per brood (i.e. second and all subsequent broods) and % mortality during the 7-d period prior to test **(Must RM)**	...	...	...
Test Facilities	Name and city of testing laboratory **(Must RM)**	...	...	...
	Person(s) performing test and verifying results **(Must RM)**	...	...	...
Test Type and Method	Test type and method (e.g., single-concentration test) **(Must RM)**	...	...	...
	Description of any deviations from one or more "must" requirements in test method **(Must RM & GM)**	...	...	...
Test Conditions	Date and time for start of definitive test **(Must RM)**	...	...	...
	pH, T/, D.O., and conductivity of unadjusted undiluted effluent prior to test solutions preparation **(Must RM)**	...	...	...
	Confirmation of no pH adjustment **(Must RM)**. If both pH-adjusted and non-adjusted tests are run, indication of pH adjustment procedure **(Must RM)**	...	...	...
	Indication of any adjustment of effluent hardness **(Must RM)**	...	...	...
	If hardness adjusted, measurements of sample hardness before and after adjustment **(Must RM)**	...	...	...
	Indication of any aeration of sample or test solutions (rate, time) prior introduction of daphnids **(Must RM)**	...	...	...
	Conc. and volumes tested (including controls) and indication of any replication **(Must RM)**	...	...	...
	D.O., pH and T/ for each test solution (including controls) at the start and end of the test **(Must RM)**	...	...	...
	Conductivity for each test solution (including controls) at the start of the test **(Must RM)**	...	...	...
	Hardness on 100% effluent and control solutions at the start of the test **(Must RM)**	...	...	...
	# of neonates per vessel; mL of solution per daphnid **(Must RM)**	...	...	...
Test Results	# of dead or immobile daphnids in each test solution (including controls) at 48h	...	...	...
	Single conc. test: # of daphnids dead (or immobilized if death cannot be confirmed) in each of three replicate effluent solutions and each of three replicate control solutions at 48h **(Must RM)**; Mean value representing % dead (or immobilized) for combined 3 replicates of each of the effluent and control solutions **(Must RM)**	...	...	...
	Multi conc. test: 48h-LC50 (or 48h-EC50 if immobilization used) with 95% confidence limits (if statistically achievable) **(Must RM)**; Statistical method (eg: log-probit, moving average etc) on which result is based **(Must RM)** or LT50 **(GM)**	...	...	...
	Most recent 48h-LC50 (with 95% confidence limits) for reference toxicant(s) **(Must RM)**	...	...	...
	Chemical(s) used for reference toxicant(s), date test initiated (within 14 days of test using same culture of daphnids as in test), historical geometric mean LC50 and warning limits (± 2SD) **(Must RM)**	...	...	...

Parameter	Specification	Met Specifics Y	N	NA
Info Kept On-File	Do lab SOPs indicate that the information on Section 8.2 of the EPS 1/RM/14 method must be kept on file for 5 years? **(Must RM)** . . . . . For details of this information, see EPS 1/RM/14, section 8.2.	...	...	...

TEST SPECIFIC CHECKLIST

Revised: December 2001

Acute Lethality Test Using Rainbow Trout (GM)

Reference Method For Determining Acute Lethality Of Effluents To Rainbow Trout (RM)

5 Pages

Note: Shaded text reflects Dec. 2000 method amendments

Parameter	Specification	Met Specifics Y	N	NA
Sample Preparation				
Filtering	Filtering of solids is not allowed **(Must RM)**	...	...	...
Pre-aeration	All test solutions and controls for 30 min at a rate of 6.5 ± 1 mL/min@L **(Must RM)**. Second period if D.O. in highest test concentration is < 70% or > 100% (pre-aeration continued at 6.5 ± 1mL/min@L^{-1} until D.O. is 70 - 100% or 90 min, whichever is shorter) **(Must RM)**	...	...	...
Temp. Adjustment	No use of immersion heaters **(Must RM & GM)**; water bath recommended	...	...	...
pH Adjustment	No pH adjustment of sample or test solutions allowed **(Must RM)**	...	...	...
	No adjustment if pH of test solution is within range of 5.5 to 8.5 **(GM)**	...	...	...
Test Conditions				
Facility	Tests isolated from general disturbance **(Must RM)**	...	...	...
Test Type	Static **(Must RM)**	...	...	...
Duration	96h	...	...	...
Temperature	15 ± 1°C **(Must RM)**	...	...	...
Lighting	Full spectrum fluorescent; 100 - 500 lux at surface; same as that defined for acclimation **(Must RM)**	...	...	...
Photoperiod	16 ± 1h light; 8 ± 1h dark **(Must RM)** (preferably with 15-30 min transition)	...	...	...
In-test pH	pH not to be adjusted during test **(Must RM)**	...	...	...
D.O. Range	70 - 100% air saturation	...	...	...
Aeration	6.5 ± 1 mL/min@L throughout test period **(Must RM)**	...	...	...
Vessel Size & Type	Covered if necessary and identical for all test solutions **(Must RM)**	...	...	...
	Glass, plexiglas®, polyethylene, acrylic, polypropylene or polyethylene-lined **(Must RM)**	...	...	...
	Liners to be discarded after use **(Must RM)**	...	...	...
Test Volume	Depth of $ 15cm **(Must RM & GM)**	...	...	...
	Identical in all test solutions and well mixed before use **(Must RM & GM)**	...	...	...
Renewal of Solution	None **(Must RM)**	...	...	...
Dilution/Control Water	Same as holding and acclimation water	...	...	...
	Uncontaminated ground, surface or dechlorinated municipal water	...	...	...
	D.O. 90-100% air saturation **(Must RM)**	...	...	...
	Same water used for controls and test solutions preparation **(Must RM & GM)**	...	...	...
# Control/Test	One or more control(s) for each test conducted **(Must RM & GM)**	...	...	...
	Use of control solution and its fish for only one toxicity test and/or one effluent sample **(Must RM)**	...	...	...
Vessel Labelling	Clearly labelled conc., date and start time **(Must RM)**	...	...	...
# Test Conc.	Multi conc. test: $ 5 plus one or more controls **(Must RM)**	...	...	...
	Highest conc. full-strength effluent, successive conc. at least 50% strength of next highest conc. **(Must RM)**	...	...	...
	Single conc. test: 1 (100% test solution) plus control **(Must RM)**	...	...	...
	Randomized position of test concentrations within testing facility	...	...	...
# Replicates/Conc.	Only 1 vessel per conc. required, however more may be used	...	...	...
# Organisms/Vessel	Minimum 10 fish per test concentration for single-concentration and LC50 tests **(Must RM)**	...	...	...
Fish handling	Equal number into each solution **(Must RM)**	...	...	...
	Healthy fish taken randomly from the acclimation tanks **(Must RM)**	...	...	...
	Handling and transfer procedure done in such as way as to minimize stress	...	...	...
Loading Density	Random order for adding fish to each test solution	...	...	...
	# 0.5g/L, as determined by the mean wet weight of control fish at end of test **(Must RM & GM)**	...	...	...
Removal of Dead	Daily after observations **(Must RM)**	...	...	...
Feeding Regime	No feeding 16h before start of test; nor during test **(Must RM & GM)**	...	...	...
Vessel Cleaning	All test vessels, measurement devices, stirring equipment and fish transfer pails thoroughly cleaned and rinsed with control/dilution water before use **(Must RM)**	...	...	...
Chemical Testing	Solvent control solution to be run, # 0.5 mL/L limit **(GM)**	...	...	...

TEST SPECIFIC CHECKLIST

Revised: December 2001

Acute Lethality Test Using Rainbow Trout (GM)

Reference Method For Determining Acute Lethality Of Effluents To Rainbow Trout (RM)

5 Pages

Note: Shaded text reflects Dec. 2000 method amendments

Parameter	Specification	Met Specifics Y	N	NA
Endpoint	Multi conc. test: Mortality (LC50-96h, 95% confidence limits) **(Must RM)**	...	...	...
	Single conc. test: Mortality (% mortality at 96h) **(Must RM)**	...	...	...
EPS 1/RM/13 Amendments	Have Dec 2000 amendments been incorporated into Standard Operating Procedures (SOPs)?	...	...	...
Observations & Measurements				
D.O., pH, Temperature	At least at start and end of test in all test vessels **(Must RM & GM)**	...	...	...
Conductivity	At least at start of test in all test vessels **(Must RM & GM)**	...	...	...
Appearance/Behaviour	Daily in all test vessels	...	...	...
Mortality	Daily in all test vessels	...	...	...
	All dead fish recorded and removed **(Must RM)**	...	...	...
Control fish Length & Weight	Mean fork length and mean wet weight of control fish at end of test **(Must RM & GM)**	...	...	...
Test Organism				
Source	One hatchery certified "disease-free" of known diseases, with an ongoing health monitoring and certification program	...	...	...
Age	Swim-up fry or fingerling	...	...	...
Size	Mean weight 0.3 to 2.5 g **(Must RM)**	...	...	...
	Length of largest fish not to be more than twice that of smallest in the same test	...	...	...
Population	All fish used in a test are derived from the same population and source **(GM)**	...	...	...
Acclimation	Record of arrival date	...	...	...
	Fish acclimated to test conditions for a period of at least 2 weeks prior to use in test at 15 ± 2°C **(Must RM & GM)**	...	...	...
	Rate of change # 3°C/day **(GM)**	...	...	...
	Acclimation period immediately preceding fish use in a test **(Must RM)**	...	...	...
Test Fish disposal	Surviving fish used in the test to be disposed in a humane manner at end of test (e.g., overdosing with anaesthesic such as tricaine methanesulphonate) **(Must RM)**	...	...	...
Culture/Holding Conditions				
Temperature	4 - 18°C	...	...	...
pH	6.0 - 8.5	...	...	...
D.O.	80 - 100% air saturation	...	...	...
Lighting	Full spectrum fluorescent	...	...	...
Photoperiod	100 - 500 lux at surface	...	...	...
	For at least 2 weeks before a test, constant 16 ± 1h light; 8 ± 1h dark **(Must RM)**	...	...	...
Water Quality	Preferably with a 15 to 30 min transition period	...	...	...
	Uncontaminated ground, surface or dechlorinated municipal drinking water; Total Residual Chlorine # 0.002 mg/L; Unionized ammonia # 0.02 mg/L, nitrite # 0.06 mg/L	...	...	...
Monitoring	Temperature, D.O., pH monitored daily; ammonia and nitrite monitored weekly; total residual chlorine monitored as a minimum weekly (if using dechlorinated municipal drinking water)	...	...	...
	Water flow monitored daily or weekly; individual wet weights determined at regular intervals from $10 fish removed randomly from each holding tank	...	...	...
	Dead and moribund fish removed immediately **(Must GM)**	...	...	...
	Mortality monitored and recorded 5 days/week minimum **(Must RM & GM)**	...	...	...
	Cumulative rate of mortality <2% during 7-day period preceding test **(Must RM & GM)**	...	...	...

TEST SPECIFIC CHECKLIST

Revised: December 2001

Acute Lethality Test Using Rainbow Trout (GM)

Reference Method For Determining Acute Lethality Of Effluents To Rainbow Trout (RM)

5 Pages

Note: Shaded text reflects Dec. 2000 method amendments

Parameter	Specification	Met Specifics Y	N	NA
	If cumulative mortality is 2 to 10%. acclimation be extended for at least an additional 7 days and until cumulative 7-d mortality rate of <2% is achieved in the 7 day period preceding test **(Must RM & GM)**	...	...	...
	Cumulative mortality > 10% per week during any 7-d period makes the group of fish unacceptable for future use if deaths are caused by disease or aquatic contaminants **(Must RM & GM)**	...	...	...
Volume/Flow of water	$ 1.0 L/10 g of fish; $ 1.4 L/g fish per day **(Must RM)**	...	...	...
Feeding	At least once a day with standard commercial food pellet; 1 - 5% of wet body weight per day; as recommended by manufacturer	...	...	...
Cleaning	Siphoning of debris to eliminate buildup; tanks are to be disinfected and thoroughly rinsed with holding/acclimating water prior to introducing a new batch of fish (disinfectants such as those containing chlorinated or iodophore compounds or n-alkyldimethylbenzylammonium chloride should be used)	...	...	...
Disease	If chemically treated for disease, fish not to be used for 2 weeks thereafter **(Must RM)**	...	...	...
QA/QC				
Validity Criterion	Test is invalid if > 10% of control fish (combined data if replicates used in test) die or exhibit atypical/stressed behaviour **(Must RM & GM)**	...	...	...
Reference Toxicant	Reagent-grade phenol and/or zinc sulphate; LC50-96h (mg/L) determined	...	...	...
	Performed under the same conditions and using the same control/dilution water than the effluent test **(Must RM)**	...	...	...
	Performed at least once during each calendar month when an effluent is tested, and upon acclimation of a new batch of fish **(Must RM)**	...	...	...
	Fish used come from the same group used in effluent test **(Must RM)**	...	...	...
	Stock solution of phenol to be made on day of use; zinc stored in dark at pH 3-4 **(Must RM)**	...	...	...
	Concentrations in stock solution to be measured chemically and used to calculate LC50 if different ($20%) from nominal concentrations	...	...	...
Warning Chart	Prepared for each reference toxicant using LC50 results and continually updated **(Must RM)**	...	...	...
	LC50-96h is acceptable if within warning limits (± 2 SD on log scale)	...	...	...
	All calculations based on log concentrations **(Must RM)**	...	...	...
Sample Handling				
Containers	Containers for storage/transport made of non-toxic materials **(Must RM & GM)**	...	...	...
	New or thoroughly cleaned/rinsed if used containers **(Must RM & GM)**	...	...	...
Volume Recommended	Single conc. test: $ 25 L ; Multi conc. test: $ 50 L	...	...	...
Labelling	Include at least sample type, source, date and time of collection and name of sampler(s) **(Must RM)**	...	...	...
T/ measurement	Upon receipt of sample(s) at the laboratory, effluent t/ to be measured and recorded	...	...	...
Holding Time	Test to be initiated within 5 days after sampling **(Must RM & GM)**	...	...	...
	Recommend test initiation within 3 days after sampling	...	...	...
Holding Conditions	Held in the dark at 4 ± 2/C for a brief period in full and sealed container(s) and in a refrigerated facility: or held in full sealed container(s) at 15 ± 1/C overnight if test to be started the next day **(Must RM)**	...	...	...
	Sample be kept from freezing **(Must RM)**	...	...	...
Sub-samples	Content of each sample container to be thoroughly agitated and combined prior to use **(Must RM & GM)**	...	...	...
Sample Aliquots	Samples thoroughly agitated prior to use for preparing aliquots **(Must RM & GM)**	...	...	...

TEST SPECIFIC CHECKLIST

Revised: December 2001

Acute Lethality Test Using Rainbow Trout (GM)

Reference Method For Determining Acute Lethality Of Effluents To Rainbow Trout (RM)

5 Pages

Note: Shaded text reflects Dec. 2000 method amendments

Parameter	Specification	Met Specifics Y	N	NA
Test Report	Have lab SOPs been updated to indicate amended requirement that all toxicity tests initiated (finished or not) are to be reported? **(Must RM)**	...	...	...
Sample Data	Name and location of effluent generator **(Must RM)**	...	...	...
	Date and time of sampling **(Must RM & GM)**	...	...	...
	Type of sample **(Must RM & GM)**	...	...	...
	Brief description of sampling point **(Must RM)**	...	...	...
	Sampling method **(Must RM & GM)**	...	...	...
	Person collecting sample **(Must RM & GM)**	...	...	...
Test Conditions	Test type and method (e.g., single-concentration test) **(Must RM & GM)**	...	...	...
	Indication of any deviation from any must requirements **(Must RM & GM)**	...	...	...
	Name and city of testing laboratory **(Must RM & GM)**	...	...	...
	Test species **(Must RM & GM)**	...	...	...
	Person(s) performing test and verifying results **(Must RM)**	...	...	...
	Date and time for start of definitive test **(Must RM & GM)**	...	...	...
	pH, Temperature, D.O., and conductivity of unadjusted undiluted effluent prior to test solutions preparation **(Must RM)**	...	...	...
	Confirmation of no pH adjustment **(Must RM)**	...	...	...
	If both pH-adjusted and non-adjusted tests are run, indication of pH adjustment procedure **(Must RM)**	...	...	...
	Indication of pre-aeration of test solutions (rate, time) prior introduction of fish and rate of aeration throughout test **(Must RM & GM)**	...	...	...
	Concentrations and volumes tested (including controls) and indication of any replication **(Must RM)**	...	...	...
	D.O., pH and Temperature for each test solution (including controls) at the start and end of the test; Conductivity for each test solution (including controls) at the start of the test **(Must RM & GM)**	...	...	...
Fish density (lenght/weight)	# of fish per vessel **(Must RM & GM)**	...	...	...
	Estimated loading density (g/L); mean fork length of control fish at the end of the test, with range; mean wet weight of control fish **(Must RM & GM)**	...	...	...
Results	% mortality in fish stock tank from which test fish are taken, recorded for a minimum of 5 of the 7-d period preceding test **(Must RM)**	...	...	...
	# of mortalities in each test solution (and controls) at 96h **(Must RM & GM)**	...	...	...
	# of control fish showing atypical/stressed behaviour **(Must RM & GM)**	...	...	...
	Mean % mortality in solutions of effluent and control water if test conducted with replicates **(Must RM)**	...	...	...
	Mean # of control fish showing atypical/stressed behaviour if replicates used for control **(Must RM)**	...	...	...
	Multi conc. test: LC50-96h (with 95% confidence limits, if statistically achievable) or LT50 **(GM)** and statistical method (eg: log-probit, moving average etc) on which result is based **(Must RM & GM)**	...	...	...
	Most recent LC50-96h (with 95% confidence limits) for reference toxicant(s) **(Must RM & GM)**	...	...	...
	Chemical(s) used for reference toxicant(s), date test initiated (within one month of test using the same population from which test fish were selected), historical geometric mean LC50 and warning limits (± 2SD) **(Must RM & GM)**	...	...	...

TEST SPECIFIC CHECKLIST

Revised: December 2001

Acute Lethality Test Using Rainbow Trout (GM)

Reference Method For Determining Acute Lethality Of Effluents To Rainbow Trout (RM)

5 Pages

Note: Shaded text reflects Dec. 2000 method amendments

Parameter	Specification	Met Specifics Y	N	NA
Info Kept On-File	Do lab SOPs indicate that the information on Section 8.2 of the EPS 1/RM/13 method must be kept on file for 5 years? **(Must RM)** . . . . . For details of this information, see EPS 1/RM/13, section 8.2.	...	...	...

APPENDIX H

RAINBOW TROUT AND *DAPHNIA MAGNA* TEST REPORT CHECKLISTS

REPORT ASSESSMENT SHEET FOR ACUTE LETHALITY *DAPHNIA MAGNA* TEST

(Revised: March 2002; May 1996 and December 2000 Amendments incorporated)

EFFLUENT SAMPLE IDENTIFICATION

Client Name/Location:_____ Sampling Date/Time:_____

Testing Lab Name/Location:_____ Test Type:____ Pass/Fail or LC50 (circle one)

INSTRUCTIONS FOR COMPLETION OF ASSESSMENT SHEET

- Column one of the table lists reporting and method requirements. Reporting requirements are specified in regular type, and method requirements are indicated in **bold** type.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided.
- If data meet the method requirements specified, mark under the Y in the third table column; if the method requirements specified are not met, indicate under the N. Items which have not associated method requirement have been hard-coded with an X in the "Not Applicable" (NA) column.

REPORTING AND METHOD REQUIREMENTS	Reported Data		Met Requirements		
Effluent	Y	N	Y	N	NA
Sample type (eg: whole effluent, final effluent etc.)	...	...			.X.
Description of sampling point	...	...			.X.
Sampling method (eg: grab, composite etc.)	...	...			.X.
Person collecting sample	...	...			.X.
Test Facilities and Conditions					
Test type and method **(amended May 1996 and December 2000; EPS 1/RM/14)**	...	...	...	...	
Species of test organism **(≤ 24h old *Daphnia magna*)**	...	...	...	...	
Date/time for test start **(test initiated ≤ 5 days after termination of sampling)**	...	...	...	...	
Person performing test/Person verifying results	...	...			.X.
Physical characteristics of whole (100%) effluent prior to test dilution preparation:					
▸ pH **(no pH adjustment allowed)** and confirmation of no adjustment	...	...	...	...	
▸ If both pH-adjusted and non-adjusted tests were run, indication of pH adjustment procedure	...	...			.X.
▸ Temperature **(no use of immersion heaters)**	...	...	...	...	
▸ Dissolved oxygen (D.O.)	...	...			.X.
▸ Conductivity **(if effluent sample conductivity ≤ 100 µmhos/cm, sample hardness measured before starting the test)**	...	...	...	...	
▸ Hardness **(if hardness of effluent sample < 25 mg/L, adjust to 25 ± 1 mg/L; effluent sample hardness confirmed after adjustment)**	...	...	...	...	
Indication if effluent sample pre-aerated prior to introduction of daphnids:					
▸ Rate of pre-aeration **(25 - 50 mL/min·L^{-1})**	...	...	...	...	
▸ Length of pre-aeration **(if D.O. of effluent sample < 40% or > 100%, pre-aeration is only allowed for ≤ 30 min; test initiated at this point regardless of whether D.O. of 40 - 100% saturation was achieved)**	...	...	...	...	
# of test dilutions and volumes tested (including controls) and indication of replication:					
▸ Pass/Fail test **(100% effluent and a control; volume ≥150 mL; identical volume in all vessels; 3 replicates for each of the effluent and controls solutions)**	...	...	...	...	
▸ LC50 test **(≥ 5 conc. plus one or more controls; highest conc. 100% effluent, each subsequent conc. at least 50% of the strength of the next highest conc.; volume ≥150 mL; identical volume in all vessels)**	...	...	...	...	
Physical characteristics of dilutions (and controls) during test:					
▸ pH **(measured at least at start and end of test in all vessels)**	...	...	...	...	
▸ D.O. **(measured at least at start and end of test in all vessels; control water 90-100% of air saturation at test initiation)**	...	...	...	...	
▸ Temperature **(measured at least at start and end of test in all vessels; 20 ± 2°C)**	...	...	...	...	
▸ Conductivity **(measured at start of test in all vessels)**	...	...	...	...	
▸ Hardness **(measured at start of test in 100% effluent and control solutions; control water hardness within 80 to 250 mg/L)**	...	...	...	...	
Indication of culture health prior to use in test:					
▸ For brood stocks, most recent estimates of time to first brood **(daphnids ≤ 12d old at delivery of first brood)**	...	...	...	...	
▸ Most recent estimates of average # of neonates per brood **(i.e., second and all subsequent broods; and % mortality during the 7 d period prior to a test) (females 2 - 5 weeks old deliver an average of ≥ 15 neonates per brood)**	...	...	...	...	
# of neonates per test vessel **(≥ 10 daphnids per vessel)**	...	...	...	...	
Organism loading density **(≥ 15 mL of solution per daphnid)**	...	...	...	...	

Results					
# of dead or immobile daphnids in each sample dilution (including the controls) at 48h **(test is invalid if > 10% of control daphnids** (combined data if replicates used) **die or exhibit overt, stressed behaviour** (eg: immobility) **or if > 2 control organisms in any vessel exhibit either of these responses)**	...	...	...	...	
Pass/Fail test:					
▸ # of dead (or immobilized if mortality can not be confirmed) daphnids in each of 3 replicate effluent and control solutions at 48h	...	...			.X.
▸ Mean value representing % dead (or immobilized) for combined 3 replicates of each of the effluent and control solutions	...	...			.X.
LC50 test: 48h-LC50 (or 48h-EC50 if immobilization used) with 95% confidence limits (if statistically achievable) and statistical method (eg: log-probit, moving average etc) on which result is based	...	...			.X.
Reference toxicant data:					
▸ Most recent 48h-LC50 (with 95% confidence limits) for reference toxicity test(s) **(within the warning limits of the historic reference toxicant mean)**	...	...	...	...	
▸ Reference Chemical(s) used for reference toxicant	...	...			.X.
▸ Date reference toxicant test initiated **(conducted within 14 days of the date when effluent was tested)**	...	...	...	...	
▸ Historic reference toxicant geometric mean LC50 and warning limits (± 2 SD)	...	...			.X.

REPORT ASSESSMENT SHEET FOR ACUTE LETHALITY RAINBOW TROUT TEST

(Revised: March 2002; May 1996 and December 2000 Amendments incorporated)

EFFLUENT SAMPLE IDENTIFICATION

Client Name/Location:_____ Sampling Date/Time:_____

Testing Lab Name/Location:_____ Test Type:____Pass/Fail or LC50 (circle one)

INSTRUCTIONS FOR COMPLETION OF ASSESSMENT SHEET

- Column one of the table lists reporting and method requirements. Reporting requirements are specified in regular type, and method requirements are indicated in **bold** type.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided.
- If data meet the method requirements specified, mark under the Y in the third table column; if the method requirements specified are not met, indicate under the N. Items which have not associated method requirement have been hard-coded with an X in the "Not Applicable" (NA) column.

REPORTING AND METHOD REQUIREMENTS	Reported Data		Met Requirements		
Effluent	Y	N	Y	N	NA
Sample type (eg: whole effluent, final effluent etc.)	...	...			.X.
Description of sampling point	...	...			.X.
Sampling method (eg: grab, composite etc.)	...	...			.X.
Person collecting sample	...	...			.X.
Organisms Holding					
Species of test organism (***Oncorhynchus mykiss***)	...	...	...	...	
% mortality of fish in stock tank(s) from which test fish are taken, as recorded daily (or, as a minimum, for 5 of the 7 d spanning a week) for the 7 d period immediately preceding the test **(< 2% mortality)**	...	...	...	...	
Test Facilities and Conditions					
Test type and method **(amended May 1996 and December 2000;EPS 1/RM/13)**	...	...	...	...	
Date/time for test start **(test initiated ≤ 5 days after termination of sampling)**	...	...	...	...	
Person performing test/Person verifying results	...	...			.X.
Physical characteristics of whole (100%) effluent prior to test dilution preparation:					
▸ pH **(no pH adjustment allowed)** and confirmation of no adjustment	...	...	...	...	
▸ If both pH-adjusted and non-adjusted tests were run, indication of pH adjustment procedure	...	...			.X.
▸ Temperature **(no use of immersion heaters)**	...	...	...	...	
▸ Dissolved oxygen (D.O.)	...	...			.X.
▸ Conductivity or salinity	...	...			.X.
Indication if effluent sample pre-aerated prior to introduction of fish:					
▸ Rate of pre-aeration **(6.5 ± 1 mL/min·L^{-1})**	...	...	...	...	
▸ Length of pre-aeration **(all sample dilutions and controls pre-aerated for 30 min; if D.O. in the highest test conc. (whole effluent) is < 70% or > 100% of air saturation at the end of the 30 min pre-aeration period, then pre-aeration to continue at the same rate for the lesser of 90 min or until 70 - 100% saturation is achieved; test initiated at this point regardless of whether D.O. of 70 - 100% saturation was achieved)**	...	...	...	...	
▸ Method and rate of aeration throughout test **(all sample dilutions including controls aerated at a rate of 6.5 ± 1 mL/min·L^{-1} throughout the test)**	...	...	...	...	
# of test dilutions and volumes tested (including controls):					
▸ Pass/Fail test **(100% effluent and a control; solution depth ≥15 cm; identical volume in all vessels)**	...	...	...	...	
▸ LC50 test **(≥ 5 conc. plus one or more controls; highest conc. 100% effluent, each subsequent conc. at least 50% of the strength of the next highest conc.; solution depth ≥15 cm; identical volume in all vessels)**	...	...	...	...	
Physical characteristics of dilutions (and controls) during test:					
▸ pH **(measured at least at start and end of test in all vessels)**	...	...	...	...	
▸ D.O. **(measured at least at start and end of test in all vessels; control water 90-100% of air saturation at test initiation)**	...	...	...	...	
▸ Temperature **(measured at least at start and end of test in all vessels; 15 ± 1°C)**	...	...	...	...	
▸ Conductivity or salinity **(measured at start of test in all vessels)**	...	...	...	...	
# of fish per test vessel:					
▸ Pass/Fail test **(minimum 10; equal number per test vessel)**	...	...	...	...	
▸ LC50 test **(minimum 10; equal number per test vessel)**	...	...	...	...	
Estimated organism loading density (≤ **0.5 g/L**; calculated by multiplying the average weight of fish by the # of fish per test vessel and dividing by the test volume (in litres))	...	...	...	...	

Mean (± SD) fork length & mean wet weight of individual control fish **(at end of test)** together with the range of the values measured & sample size.....	....	....	...	....	
Results					
# of fish mortalities in each sample dilution (including controls) at 96h **(test is invalid if > 10% of control fish die or if > 10% of control fish show atypical/stressed behaviour)**.....	...	...	...	...	
mean mortality rate in solutions of effluent and control water (if a pass/fail or LC50 test is performed using replicate solutions); mean # of control fish showing atypical/stressed behaviour, if replicate control solutions	...	...			.X.
Pass/Fail test: % mortality in 100% effluent and control solutions at 96h.....	...	...			X.
LC50 test: 96h-LC50 (with 95% confidence limits) and statistical method (eg: log-probit, moving average, etc) on which result is based.....	...	...			.X.
Reference toxicant data:					
▸ Most recent 96h-LC50 (with 95% confidence limits) for reference toxicity test(s) **(within the warning limits of the historic reference toxicant mean)**	...	...	...	.....	
▸ Reference Chemical(s) used for reference toxicity test(s)	...	...			X.
▸ Date reference toxicant test initiated **(conducted within one calendar month of the date when effluent was tested and for every batch of acclimated fish; using the same batch of fish as that used in the effluent test)**.....	...	...	...	...	
▸ Historic reference toxicant geometric mean LC50 and warning limits (± 2 SD).....	....	.....			.X.