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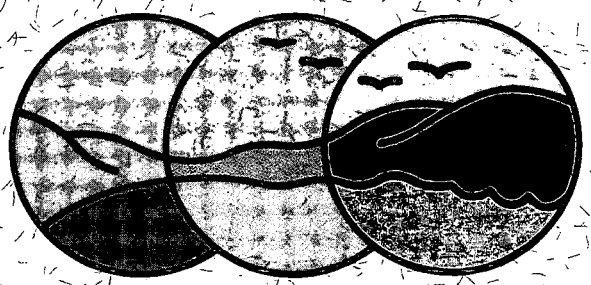
A Review of Whole Organism Bioassays for Assessing the Quality of Soil, Freshwater Sediment, and Freshwater in Canada

C. Keddy, J.C. Greene, and M.A. Bonnell

The National
Contaminated Sites
Remediation Program



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EVALUATION AND INTERPRETATION BRANCH
OTTAWA, ONTARIO 1994

(Disponible en français sur demande)

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Prepared for the

CCME Subcommittee on Environmental Quality Criteria

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Preface

This document contains background information related to the development of the Canadian Council of Ministers of the Environment's (CCME) Canadian Environmental Quality Criteria for Contaminated Sites in support of the National Contaminated Sites Remediation Program (NCSRP).

This report has been reviewed by the Conservation and Protection Service of Environment Canada, and approved for publication. This approval does not necessarily signify that the contents reflect the views and policies of Environment Canada. Mention of trade names or commercial prodects does not constitute recommendation or endorsement for use.

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Ron Freyberg (Environmental Quality Management Inc., Cincinnati) carried out the statistical review of the bioassays that were evaluated, and prepared Appendix D. Morris Schnitzer (Agriculture Canada, Ottawa) provided comments on reference soils for toxicity testing in Canada.

Abstract

As part of the effort to clean up contaminated sites in Canada under the National Contaminated Sites Remediation Program, whole organism bioassays were identified as one tool for assessing soil, freshwater sediment, and freshwater quality at contaminated sites. Current Canadian and international biological test methods were reviewed and evaluated to select tests for inclusion in assessment test batteries for the media above. A two-tiered approach was used to evaluate individual tests at both the screening and definitive assessment levels. Individual tests were categorized as "usable," "prototype," or "developmental" for each level. A usable set of tests that do not require further work was found for all three media considered. Additional effort required to elevate the prototype tests to assessment battery candidates was identified. The final test composition of current and future assessment batteries also involved consideration of trophic level representation, sensitivity, reproducibility, and Canadian relevance. Future priorities for contaminant assessment with biological organisms were identified for each medium, including upgrading and developing new whole organism tests, multispecies testing, assessing contaminant impacts on microbial processes, and in situ testing.

Résumé

Dans le contexte des efforts de nettoyage des lieux contaminés du Canada, déployés dans le cadre du Programme national d'assainissement des lieux contaminés, on a identifié les biotests sur les organismes entiers comme un outil d'évaluation des sols, des sédiments déposés en eau douce, et de la qualité des eaux douces dans les lieux contaminés. On a revu et évalué les procédés des tests biologiques employés au Canada et ailleurs pour choisir les tests qui seront inclus dans les séries de tests réalisés à des fins d'évaluation des milieux susmentionnés. On a utilisé une méthode à deux niveaux pour évaluer chacun des tests à la fois au niveau de la détection et au niveau de l'évaluation définitive. On a placé chacun des tests dans l'une des catégories «utilisable», «prototype» ou «de développement» pour chaque niveau. On a trouvé un ensemble utilisable de tests qui ne nécessitent pas d'autres recherches pour chacun des trois milieux considérés. On a déterminé les travaux supplémentaires nécessaires pour élever les tests prototypes au niveau des tests susceptibles d'être inclus dans la série de tests d'évaluation. On a aussi tenu compte dans la composition finale des séries de tests d'évaluation actuelles et futures, de la représentation des niveaux trophiques, de leur sensibilité, de leur reproductibilité et de leur pertinence dans le contexte canadien. On a défini les priorités futures du point de vue de l'évaluation des contaminants au moyen d'organismes biologiques dans chaque milieu, notamment le perfectionnement et la mise au point de nouveaux tests sur des organismes entiers, les tests effectués sur plusieurs espèces, l'évaluation de l'incidence des contaminants sur les processus microbiens, et les tests in situ.

A Review of Whole Organism Bioassays for Assessing the Quality of Soil, Freshwater Sediment, and Freshwater in Canada

1.0 INTRODUCTION

The Canadian Council of Ministers of the Environment initiated the National Contaminated Sites Remediation Program (NCSRP) for the remediation of high priority contaminated sites in Canada (CCME 1991). The use of bioassays, as described below, will provide a consistent and scientifically defensible basis for the remediation program.

1.1 Importance of Bioassays in Contaminated Site Remediation

Living organisms integrate the effects of positive and negative chemical impacts experienced during growth because they respond to the biologically active components contained in complex chemical waste. Bioassays provide a more direct measure of environmentally relevant toxicity of contaminated sites than do chemical analyses because the results are an integration of all environmental variables and contaminants. They can be used to identify the most toxic areas, thereby helping to prioritize sites for more thorough evaluation, including the direction of chemical analysis. Bioassay endpoints are quantitative measures of toxicity. They complement biological surveys that describe communities of organisms present in the field (U.S. EPA 1989, 1990) and chemical analyses (Chapman 1992, Mount et al. 1986b) that provide information on the nature of the contaminants at a site, the magnitude of the remediation problem, and potential methods of treating the site.

Plant and animal communities are diverse; their members differ in their sensitivity to toxicants. Thus a battery of bioassays that reflects different trophic levels, rather than single species assays, is typically used in toxicity evaluation (Dutka 1991, Greene et al. 1989, Slooff et al. 1983, Peterson et al. 1985, Miller et al. 1985). This approach will provide the broadest picture of site contamination from a biological perspective. There is ample evidence that the toxicity of contaminated sites, particularly when contaminant mixtures are present, can only be properly assessed using a battery of test species (Munawar et al. 1989, Giesy and Hoke 1989, Burton 1991), and numerous batteries have been proposed (Reynoldson and Day 1993, Giesy and Hoke 1989, Greene et al. 1989, Weber et al. 1989, IJC 1988). A battery with a variety of test species representing different trophic levels and varied habitats will provide a range of sensitivities that one hopes will represent those of the field organisms.

In the NCSRP, bioassays can be used to derive national criteria, prioritize contaminated sites (and areas within sites) for remediation, establish site-specific remediation objectives, and determine when remediation goals have been reached. To use bioassays within the NCSRP, a critical review of available bioassays is first required to identify those useful to the program.

1.2 Review Objectives

The primary objective of this review is to critically evaluate bioassays for soil, freshwater sediment, and freshwater and recommend a suite of ecologically relevant bioassays suitable for assessing the hazard of contaminants to organisms at contaminated sites in Canada.

This objective is met by

1. conducting a comprehensive literature search on toxicological bioassays
2. identifying potential tests for use in contamination assessment
3. evaluating test suitability
4. selecting batteries of tests for assessing water, sediment, and soil quality

The identification of needs for further work to correct weaknesses in tests, or of opportunities to develop new tests from current research follows naturally from critical test evaluation.

1.3 Review Scope

This review of international literature covers bioassays for contaminant assessment of soil, freshwater sediment, and freshwater. Environment Canada has already reviewed in detail and has prepared (Environment Canada 1992a, 1992b, 1991, 1990a, 1990b, 1990d, 1990e) or is preparing (Environment Canada 1992c) protocols for several aquatic bioassays. The tests from which these protocols were derived were not re-examined. They were accepted as appropriate versions for Canadian environments. The main emphasis of the review is on freshwater sediment and soil testing, areas that have received less attention. We further focus our efforts on whole organism, single species, and acute and chronic tests but not those concerning mutagenicity, genotoxicity, or bioaccumulation (as the ecological interpretation of observed effects is uncertain), or multispecies testing (see 8.4.2). Only organisms likely to be in direct contact with the contaminated medium for a substantial portion of their life span are considered. This excludes consideration of tests with species that may be secondarily affected such as avian species (e.g., Anonymous 1985) and honey bees (e.g., Thomas et al. 1983, Federal Biological Institute for Agriculture and Forestry 1990, Great Britain Ministry of Agriculture 1986). Birds, for example, could be affected by consuming contaminated earthworms.

Our consideration of bacterial tests does not include multispecies tests that are used to assess microbial processes such as litter decomposition, carbon mineralization, and nitrogen transformations. There is a vast literature on soil processes (Baath 1989) and some aquatic and terrestrial tests are under development (e.g., phosphatase activity, arylsulphatase activity, microbial biomass, glutamic acid degradation; C^{14}-acetate, C^{14}-chloroform, C^{14}-benzoate, and C^{14}-chlorophenol mineralization) in the Netherlands (D. de Zwart, Nat. Inst. of Public Health and Environmental Protection, pers. comm.). As well, some process tests have already been adopted by international standards organizations (e.g., OECD 1984e). Laboratory tests for assessing the effects of pesticides are reviewed in Anonymous (1989). A thorough evaluation of tests, similar to this review, should be carried out to identify tests relating to processes that are currently usable, exist as prototypes, or are under development and desirable for inclusion in a Canadian test battery for toxicological assessment. While the ecological importance of microbial processes cannot be ignored, review of this immense topic is beyond the scope of this report on whole organism toxicity testing.

1.4 Historical Background

In response to concerns about the role of biological tests, requirements for in-house aquatic testing capabilities, the need for national consistency in biological testing, and the need to keep pace with testing regimes of other environmental protection agencies, Environmental Protection prepared a document covering recommendations for aquatic biological tests (Sergy 1987). Based on this work, Environment Canada began to develop several aquatic protocols (Environment Canada 1992a, 1992b, 1992c, 1991, 1990a, 1990b, 1990d, 1990e). This review, emphasizing soil and sediment testing, complements the work on aquatic testing assessment and test development.

Under the NCSRP, environmental quality criteria for assessing site contamination have been developed. They include interim assessment and remediation criteria for various uses of soil and water at contaminated sites (CCME 1991). The interim assessment criteria are approximate background levels or analytical limits for various chemicals in soil and water. Interim remediation criteria for soil provide guidance for cleanup and have been developed for agricultural, residential/park land and commercial/industrial land uses. The interim assessment criteria and the remediation criteria for soil have been established based on a critical review of existing environmental quality criteria currently used by various agencies worldwide. Remediation criteria have been established for various uses of water including aquatic life, irrigation, livestock watering, and drinking water. These criteria have been adopted from the Canadian Water Quality Guidelines (CCREM 1987) and the Guidelines for Canadian Drinking Water Quality (Health and Welfare Canada 1989).

This document gives some information on the relative toxicity of many compounds and mixtures of compounds to organisms inhabiting soil, freshwater sediment, and freshwater. It provides the basis for conducting a standardized series of biological tests that will generate needed biological effect information for deriving ecological effect-based environmental quality criteria, evaluating hazards to organisms on a site-specific basis, and evaluating the effectiveness of cleanup in a post cleanup assessment under the NCSRP.

1.5 Report Use

1.5.1 General

This report provides batteries of tests using terrestrial and freshwater organisms for the assessment of soil, freshwater sediment, and freshwater quality that can be used under the NCSRP to provide

- data required for deriving national environmental quality criteria
- an indication of the environmental quality of a site

- guidance for determining the need for further site investigation
- guidance for determining when remedial action is required
- verification of the adequacy of cleanup
- the basis for establishing site-specific objectives
- the basis for developing legally enforceable standards

It highlights weaknesses in existing test methods and provides direction for further effort to refine and develop test batteries useful to the program.

1.5.2 Application of Recommended Bioassays to Site Assessment and Remediation

The tests recommended in the batteries can be applied in two ways. First, they can be used to derive biologically based criteria for compounds where none exist or where the data supporting them are weak. Second, they can be used to assess the relative importance of contaminated sites for remediation action, to establish site-specific remediation objectives, to assess the effectiveness of the remediation practices implemented, and to determine when the objectives have been met.

The batteries recommended in this report contain two sets of tests to be used in a two-tiered approach to site assessment. The set of preliminary screening tests would be used to determine the relative toxicity of sites or relative toxicity within a site (extent of contamination, identification of most highly contaminated areas) on a coarse scale using less expensive tests with organisms covering a breadth of ecological roles. The set of definitive tests would then be used to further refine the limits of toxic contamination and establish site-specific objectives. Tests in this second tier would expand upon the variety of biological roles and organism development stages considered and have a longer duration than the preliminary screening tests. The tests found to be most sensitive to contaminants on the site would be used to periodically assess the effectiveness of the remediation techniques used and to determine when the site objectives were met.

The recommendations for test batteries are generic in nature. Some flexibility in test selection should be maintained, depending on the particular site history. In cases where the contaminants are known, test selection can be contaminant driven. For example, where herbicides are the major contaminant on a site, tests with plants would be most appropriate, and the tests outlined in this document could be expanded to cover several species rather than doing additional tests with animals.

A general discussion of the approach to using bioassays in site remediation and an example can be found in Athey et al. (1987) and Thomas et al. (1983).

1.6 Report Organization

Following this introduction are the definitions and abbreviations used in the report. Then the methods for identifying tests for consideration in the batteries are described, followed by the three-stage evaluation process used to select battery tests. The test identification and evaluation results, followed by comments on the tests selected and priorities for further work, are presented separately for each medium (soil, freshwater sediment, water). By integrating the priorities for each medium, priorities for further work under the NCSRP are established. The report concludes with new prospects for bioassays, literature cited, and literature reviewed. An appendix of contacts made during this review is included.

2.0 DEFINITIONS AND ABBREVIATIONS

Note: *All definitions are given in the context of this report and might not be appropriate in another context.*

acceptability criteria — a standard for the negative control that must be achieved within the test period to allow for the toxicity test results to be accepted.

accuracy — the ability to predict actual effects.

acute — within a short period in relation to the life span of the organism: would be of the order of some minutes for bacteria and usually days for fish.

acute lethality, acute toxicity — causing death of the test organisms within a short period of exposure to a test material.

aquatic — growing or living in water.

bioassay — a test that determines the relative strength of a substance by comparing its effect on a test organism with that of a standard preparation (negative control).

bioluminescence — a phenomenon of light emitted from living organisms as a result of their biochemical activities, usually enzymatic.

blank — used interchangeably with the term *control* (q.v.).

chemical — any element, compound, formulation, or mixture of a substance that might enter the aquatic environment through spillage, application, or discharge. Examples of chemicals that are applied to the environment are insecticides, herbicides, fungicides, sea lamprey larvicides, and agents for treating oil spills.

chronic — occurring during a relatively long-term period of exposure, usually a significant portion of the life span of the organism such as 10% or more. For cladocerans, chronic is typically defined as continuing until three broods are produced.

chronic toxicity — long-term effects that are related to changes in such things as metabolism, growth, reproduction, survival, or availability to survive.

chronic value — the geometric mean of the NOEC and LOEC in tests that have a chronic exposure.

compliance — in accordance with government licensing or regulatory requirements.

conductivity — a numerical expression of the ability of an aqueous solution to carry an electric current. This ability depends on the concentrations of ions in solution, their valence and mobility, and on the solution's temperature. Conductivity in freshwater is normally reported in the SI unit of millisiemens per metre, or as µmho/cm (1 mS/m = 10 µho/cm). Conductivity is a standard method for measuring *salinity* (q.v.), with a result that is usually read off as g/kg or parts per thousand.

contamination — the process of making soil, sediment, or water impure or unfit for use by the introduction of unwholesome or undesirable elements and compounds.

control — see *negative control.*

criterion — a standard on which a judgment or decision may be based.

deionized water — water that has been purified to remove ions from solution by being passed through resin columns or a reverse osmosis system.

diluent — the standard water used for dilution of test material in the Microtox test; see also *dilution water.*

dilution water — the water used to dilute a test material in order to prepare different concentrations for a toxicity test. The standard dilution water used in the Microtox test is a specific formulation of saline water called diluent.

dispersant — a substance that reduces the surface tension between water and a hydrophobic substance (e.g., oil), thereby facilitating the dispersal of the hydrophobic material throughout the water as an emulsion.

distilled water — water that has been passed through a distillation apparatus of borosilicate glass or other material to remove impurities.

EC_{50}/EC_{50} — the median effective concentration, i.e., the concentration of material in water that is estimated to cause a specified effect in 50% of the individuals exposed to that concentration. The effect could be lethal but is usually sublethal. EC_{50}, like LC_{50}, refers to a quantal effect since each exposed individual must be categorized as either showing the effect or not showing it. The effect must be specified and often also the exposure time, for example, "the 2-month EC_{50} for reproductive failure" or "the EC_{50} for avoidance reactions." The term does not apply to a per cent reduction in some rate or process in an organism or a group of organisms.

effluent — any liquid waste (e.g., industrial, municipal) discharged to the aquatic environment.

elutriate — an aqueous solution obtained after adding water to a solid material (e.g., sediment, tailings, drilling mud, dredge spoil), shaking the mixture, then centrifuging or filtering it or decanting the supernatant.

emulsifier — a substance that aids the fine mixing (in the form of small droplets) within water of an otherwise hydrophobic substance.

endpoint — the variables (e.g., time, reaction of the organisms) that indicate the termination of a test. Endpoint also means the measurement(s) or value(s) derived that characterize the results of the test (lethal concentration, LC_{50}, etc.).

field validation — the process of comparing laboratory toxicity test results with measurements of naturally occurring species, populations, or communities to look for similar negative effects caused by toxic substances released into the environment.

FIFRA — Federal Insecticide, Fungicide and Rodenticide Act, under which pesticides are registered in the United States and under which the U.S. Environmental Protection Agency provides its testing requirements for registration.

genotoxicity — an adverse effect manifested in the genome (e.g., mutation, chromosomal damage).

hardness — the concentration of cations in water that will react with a sodium soap to precipitate an insoluble residue. In general, hardness is a measure of the concentration of calcium and magnesium ions in water, and is expressed as mg/L calcium carbonate or equivalent.

ICp — the inhibiting concentration for a (specified) per cent effect. It represents a point estimate of the concentration of test material that would cause a designated per cent impairment in a quantitative biological function such as light production by bacteria or growth of fish relative to the control. This term should be used for any toxicological test that measures a change in rate, such as reproduction, growth, or respiration. (The term median effective concentration [EC_{50}] is not appropriate in tests of this kind because it is limited to quantal measurements, i.e., an estimate that 50% of the individual organisms that were exposed to that concentration would show a particular effect, while the other 50% would not.)

interlaboratory testing — the process of many laboratories performing a standard toxicity test using the same toxic chemical or a shared environmental sample and comparing the consistency, reproducibility, and statistical quality of the results.

interstitial water — the water within a wet sediment (or similar material) that surrounds the solid particles. The amount of interstitial water is calculated and expressed as the percentage ratio of the weight of water in the sediment to the weight of the wet sediment.

intralaboratory testing — the process of one laboratory performing a standard toxicity test several times while testing a toxic chemical or environmental sample and comparing the consistency, reproducibility, and statistical quality of the results.

LC_{50}/LC_{50} — the median lethal concentration, i.e., the concentration of material in water that is estimated to be lethal to 50% of the test organisms exposed to that concentration. The LC_{50} and its 95% confidence limits are usually derived by statistical analysis of mortalities in several test concentrations after a fixed period of exposure. The duration of exposure must be specified (e.g., 7-day LC_{50}).

leachate — water or wastewater that has percolated through a column of soil or solid waste within the environment.

lethal — causing death by direct action. Death is usually defined as the cessation of all visible signs of movement or other activity.

LOEC — the lowest-observed-effect concentration. This is the lowest concentration of test material to which organisms are exposed that causes adverse effects on the organism that are detected by the observer and are statistically significant.

luminescent — emitting light, caused by other than high temperature.

lux — a unit of illumination based on units per square metre. One lux = 0.0929 foot-candles, and one foot-candle = 10.76 lx.

lyophilized — freeze-dried under a vacuum; applied to the bacteria used in the Microtox test, as received from the supplier.

marine water — seawater in or from the ocean, sea, or inshore location where there is no appreciable dilution by natural freshwater derived from land drainage.

monitoring — the routine (e.g., daily, weekly, monthly, quarterly) checking of quality, or collection and reporting of information. In the context of this report, it means either the periodic (routine) checking and measurement of certain biological or water-quality variables, or the collection and testing of samples of effluent, leachate, elutriate, or marine/estuarine receiving water for toxicity.

negative control — a treatment in an investigation or study that duplicates all the conditions and factors that might affect the results of the investigation except the specific condition that is being studied. In an aquatic toxicity test, the control must duplicate all the conditions of the exposure treatment(s) but must contain no test material. The control is used to determine the absence of measurable toxicity due to basic test conditions (e.g., quality of the dilution water, health, or handling of test organisms).

neonate — a newly born or newly hatched individual (first-instar daphnid <24 h old).

NOEC — the no-observed-effect concentration, the highest concentration of test material to which organisms are exposed that does not cause any observed and statistically significant adverse effects on the organism.

organism — an individual constituted to carry on the activities of life by means of organs separate in function but mutually dependent.

per cent (%) — a concentration expressed in parts per hundred parts. One per cent represents one unit or part of material (e.g., effluent, leachate, elutriate, or receiving water) diluted with water to a total of 100 parts. Concentrations can be prepared on a volume-to-volume or weight-to-weight basis and are expressed as the percentage of test material in the final solution.

pH — the negative logarithm of the activity of hydrogen ions in gram equivalents per litre. The pH value expresses the degree or intensity of both acidic and alkaline reactions on a scale from 0 to 14, with 7 representing neutrality, numbers less than 7 signifying increasingly acidic reactions, and numbers greater than 7 indicating increasingly basic or alkaline reactions.

pore water — see *interstitial water.*

positive control — a standard chemical used to measure the sensitivity of the tested organisms in order to establish confidence in the toxicity data obtained for a test material. In most instances, a toxicity test is performed with a reference toxicant to assess the sensitivity of the organisms at the time the test material is evaluated, and the precision of results obtained by the laboratory for that chemical.

photoperiod — the duration of illumination within a 24-h day.

precision — the variation in the analysis of identical samples.

precipitation — the formation of a solid (i.e., precipitate) that comes from a solution.

pre-treatment — treatment (e.g., dilution) of a sample before testing its toxicity.

protocol — a toxicity test describing required procedures for performance of scientific experiment.

receiving water — natural surface water (e.g., in a river) that has received a discharged waste, or is about to receive such a waste (e.g., it is just upstream or up-current from the discharge point). Further descriptive information must be provided to indicate which meaning is intended.

reconstituted water — deionized or glass-distilled water to which reagent-grade chemicals have been added. The resultant synthetic freshwater is free from contaminants and has the desired pH and hardness characteristics.

reference sediment — a natural sediment used to assess localized sediment conditions exclusive of the specific contamination of concern.

reference soil — a natural soil used to assess localized soil conditions exclusive of the specific contamination of concern.

reference toxicant — see *positive control.*

remediation — concerned with correction and cleanup of chemically contaminated environmental sites.

ruggedness — the measure of whether or not a given test responds to a wide variety of variables such as test volume, lighting regime, organism loading density.

salinity — the total amount of solid material, in grams, dissolved in 1 kg of aqueous solution. For seawater, salinity is determined after all carbonates have been converted to oxides, all bromide and iodide have been replaced by chloride, and all organic matter has been oxidized. Salinity can also be measured directly using a salinity/

conductivity meter or other means. The normal unit would be g/kg, or the approximate equivalent of that, parts per thousand (‰).

sediment — a particulate material that has been transported to, and deposited at the bottom of, a body of water.

sensitivity — the capacity of a toxicity test to respond to a toxicant. The lower the level required to elicit a response, the more sensitive is the test.

static — describes toxicity tests in which test solutions are not renewed during the test.

static renewal — a toxicity test in which test solutions are renewed (replaced) periodically during the test, usually at the beginning of each 24-h period of testing. Synonymous terms are semistatic renewal, static replacement, and batch replacement.

stock solution — a concentrated aqueous solution of the material to be tested. Measured volumes of a stock solution are added to dilution water to prepare the required strengths of test solutions.

sublethal — detrimental to a living organism but below the level that directly causes death within the test period.

surfactant — a surface-active substance (e.g., detergent) that when added to a nonaqueous liquid, decreases surface tension and facilitates dispersion of materials in water.

surrogate — a test organism, or population of organisms, cultured under laboratory conditions to substitute in toxicity testing for indigenous organisms, communities, or populations.

toxicity — the inherent potential or capacity of a material to cause adverse effects on living organisms. The effect could be lethal or sublethal.

test, prototype — a test that has met all of the "must" criteria (Sec. 3.2.1) and <88% of the "want" criteria (Sec. 3.2.2) or a test that is missing "must" criteria but scored ≥88% for the "want" criteria.

test, under development — any test that did not meet the "must" criteria (Sec. 3.2.1) and scored <88% for "want" criteria (Sec. 3.2.2).

test, usable — a test that meets the three "must" criteria (Sec. 3.2.1) and scored ≥88% for "want" criteria (Sec. 3.2.2).

terrestrial — relating to land as distinct from water or air.

TSCA — Toxic Substances Control Act, under which the U.S. Environmental Protection Agency outlines its testing requirements for the registration of chemicals other than pesticides.

toxicity identification evaluation — a systematic sample pre-treatment (e.g., pH adjustment, filtration, aeration) followed by tests for toxicity. This evaluation is used to identify the agents that are primarily responsible for toxicity in a complex mixture. The toxicity test can be lethal or sublethal.

toxicity test — a determination of the effect of a material on a group of selected organisms, under defined conditions. An aquatic toxicity test usually measures either (a) the proportions of organisms affected (quantal), or (b) the degree of effect shown (graded or quantitative), after exposure to specific concentrations of chemical, wastewater, receiving water, or liquid derived from sediment or similar solid material.

turbidity — the extent to which the clarity of water has been reduced by the presence of suspended or other matter that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample. It is generally expressed in terms of nephelometric turbidity units.

upstream water — surface water (e.g., in a stream, river, lake, estuary, or marine water body) that is not influenced by the effluent (or other test material) by virtue of being removed from it in a direction against the current or sufficiently far across the current.

wastewater — a general term that includes effluents, leachates, and elutriates.

3.0 METHODS

The methods used for assessing tests in this document can be applied to re-evaluate currently available tests and new tests involving whole organisms, as well as tests in other areas that require review, such as genotoxicity and processes.

The methods by which we arrived at the recommended batteries of biological tests and priorities for work with tests for each medium are summarized, with details provided in the following sections.

Potentially suitable tests for inclusion in the batteries were identified, as described in 3.1, and then evaluated in a two-step selection process. The first step was an assessment of methodology completeness (Fig. 1), using the Kepner-Tregoe approach (Kepner and Tregoe 1965) described by Stanley Industrial Consultants (1992), which involved

1) a preliminary screening based on 'must' criteria, ones that are *essential* for a viable test (3.2.1)

2) a detailed evaluation based on 'want' criteria, ones that are *desired* in a test (3.2.2)

From this initial assessment, preliminary priorities for further work were determined (3.5.1).

The second step involved consideration of other relevant available information on the tests such as trophic level, sensitivity, reproducibility, field validation, and applicability to the Canadian environment (3.3). This additional information was used to revise preliminary priorities and was taken into account in selecting tests for the batteries and in determining priorities for further work for the medium as a whole (3.5.2).

Based on the results of the two steps, recommendations are made for a battery of tests that are currently usable for preliminary site screening and definitive testing. Recommendations are also made concerning tests that should be added to these batteries pending upgrading of test methodologies (filling the gaps identified in 3.2.1 and 3.2.2).

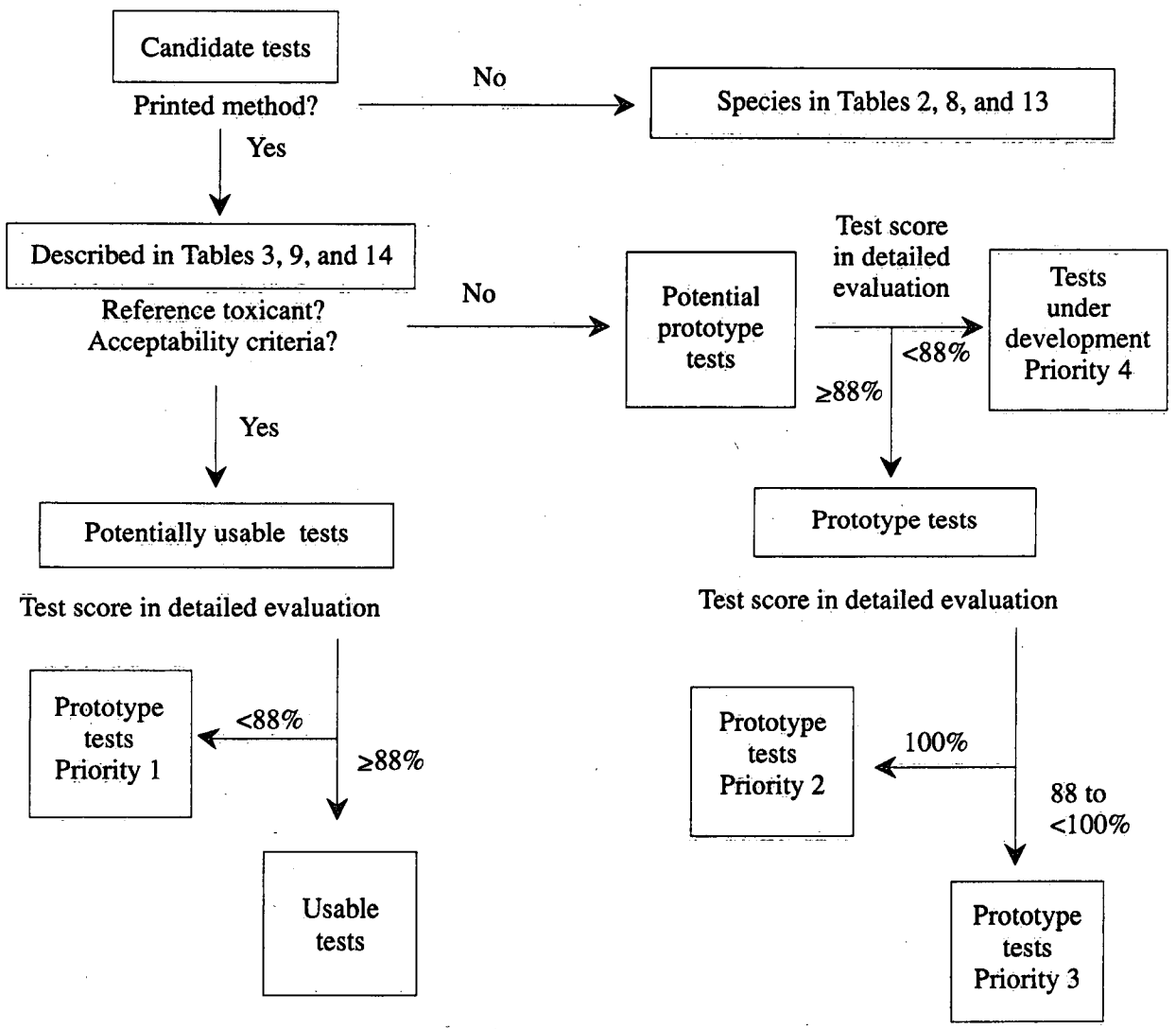

Figure 1. A diagrammatic representation of the test evaluation and priority establishment process for biological tests reviewed (see 3.2 and 3.5 for additional details). Usable tests are considered sufficiently complete not to require further work.

3.1 Identification of Potentially Suitable Tests

Potential biological tests for toxicity assessment and relevant literature were identified through contact with agencies that develop standardized toxicity tests (e.g., International Standards Organization, American Society for Testing and Materials, Organization for Economic Cooperation and Development), with researchers involved with toxicity testing around the world (App. A), and through review of recent issues of journals publishing articles on toxicity testing and existing bioassay reviews (Garric et al. 1991, Analex 1990).

3.2 Step 1 Assessment — Test Methodology

3.2.1 Preliminary Test Assessment Using Kepner-Tregoe Approach

In the preliminary screening, each of the tests obtained as indicated in 3.1 was evaluated against the 'must' criteria (as shown in Fig. 1). Three features that any biological test must have are

1) a readily available method in print

2) a reference toxicant

3) acceptability criteria.

Printed Test Method

Printed test methods include tests found as indicated in 3.1 that had written descriptions prepared by recognized provincial/state, national, and international standards organizations, and also tests reported in the literature that were clearly published specifically as test methods (as opposed to a simple report of the results of a toxicity test with a biological organism).

Reference Toxicant

A reference toxicant is a chemical that is toxic to the test organism and is used to provide a measure of the reproducibility of a toxicity test method. Variations in the results of a test conducted with the same reference toxicant, under standard testing conditions, are used to trigger test method evaluation (Environment Canada 1990c). It is not sufficient to name an appropriate reference toxicant. The method should also provide the toxic concentration values (e.g., EC_{50}) expected under the test conditions described. Ideally, the associated 95% confidence interval for the expected values should be provided as well, but no test lost points for absence of confidence intervals (CI). Few tests provided this level of detail.

Acceptability criteria

Acceptability criteria are levels of measurable characteristics of organism health that if not met invalidate a test. Acceptability criteria are used to assess the health of the test organism under a test's standardized control conditions and in the absence of toxicants.

3.2.2 Detailed Evaluation Using Kepner-Tregoe Approach

The assessment using the 'must' criteria (3.2.1) split the potentially usable tests (3.1) into two groups — those with and without the 'must' criteria (Fig. 1). The tests in each of these groups were further evaluated in detail using the 'want' criteria to provide direction for further work required on test methods (3.5).

The inclusion of the 'want' criteria in a test method increases its replicability by reducing the variability of results that are not due to variation in toxicity and increases its utility as a test standard. A definition of each of these criteria and the weightings used (in brackets) in the detailed evaluation are given below. When the term adequate is used below it means that sufficient detail is provided to replicate the test condition/method. Finally, the method for scoring tests is described.

Species (1)

At least one test organism has to be identified to species. No point is scored in tests listing only genera.

Endpoint (1)

The endpoint is the variable measured (e.g., total no. young, growth in dry weight) over a specified period of time. At least one measurable endpoint has to be specified.

Organism Selection (1)

It is important to indicate additional characteristics of the test organism (such as weight, age, size, and variety) that will influence the results of the test.

Number of Organisms, Replicates/Treatment (1)

The variability in test results, and therefore test sensitivity, depends in part on the number of replicates and organisms used per replicate. A test should specify the minimum number of replicates and the number of organisms/replicates required to give reliable values for endpoints.

Observation Frequency (1)

Observation frequency refers to the times during the test at which observations or measurements of

effects on the test organisms are to be made and when the test is to be terminated.

Volume Test Solution/Solid (1)

It is important to provide some indication of the amount of test solution or solid that is required to support the test organisms. It may be defined in terms of loading factors (e.g., ≤0.5 g/L/day over 4 days, Environment Canada 1990d), relative volumes of container and solution (Environment Canada 1992a), or a specific volume (Greene et al. 1989). It is also useful to know test substance volume to determine the minimum amount of chemical or contaminant that will be needed to perform a test.

Volume Test Vessel (1)

The test vessel used influences the surface area of the test medium and should be tailored to avoid stress on the test organism.

Test substance preparation and addition to test vessel (2)

The details of sample preparation should, for example, cover how to collect samples in the field and store them (if necessary), how to prepare the test substance (e.g., pesticide preparation according to label instructions, elutriate preparation methods), and how to add the test substance to the medium (e.g., test soil and artificial soil). These details may be presented within the test, or the test may provide a reference that contains the necessary details. Not all the details mentioned above apply to every test. A test will score 2 points if adequate details are provided on how to prepare the substance to be tested and how to add it to the test vessel. Adequate information on either topic receives 1 point.

Organism Culture, Handling (1)

The conditions under which organisms are maintained before testing are included under culture. By allowing organisms to acclimate (become physiologically adapted to a particular level of one or more environmental variables) to the environmental test conditions (in the absence of a toxicant), variability in test results not due to toxicological action will be reduced. Both the environmental conditions under which the organisms are to be maintained before testing as well as the duration of the maintenance period should be indicated.

For tests during which the organisms must be handled to make the required observations (e.g., earthworm, Greene et al. 1989), details should be provided to ensure that this is carried out in a consistent way and that damage to the organisms is minimized. If either culture or organism handling (when applicable) is not addressed in a test, no points are scored.

Environmental Conditions (3)

The physical environmental conditions under which the test should be performed (in some cases also maintained) and, as a corollary, the conditions under which it should not be used, should be provided. The conditions specified should take into account the organism's tolerance range and ensure that the test provides data on toxicity, not tolerance to other environmental variables. Specified conditions should include temperature, light (intensity and photoperiod where applicable), pH, and dissolved oxygen (where applicable). Tests providing levels of all relevant major environmental variables score 3. Scores of 2, 1, and 0 are given when one variable, two variables, and more than two variables are not provided, respectively.

Medium Definition, Manipulation (2)

The composition and preparation of growth media (e.g., nutrient solutions) and toxicant dilution media (e.g., reconstituted water, artificial soil) used in a test should be described in sufficient detail to be readily duplicated. As well, the procedure for medium replacement, if required, should be specified. If the media are undefined/insufficiently defined or replacement procedures (when applicable) are not addressed in a test, 1 point is scored.

Statistical Analysis (2)

Interpretation of the test observations typically requires comparisons to be made between the results for the control treatment (without toxicant) and treatments with varied concentrations of toxicant as well as comparisons among the results for treatments that vary in toxicant concentration. Methods that provide no, little, or only general guidance on the statistical analysis of the associated data have a serious deficiency.

A test scored 2 points for statistical analysis when it indicated which statistical tests should be used under which circumstances. Ideally, tests should also provide examples of result analysis (e.g., Environment Canada 1992a), but no points were deducted for the absence of examples. The statistical component of a test is considered only partially complete and the test scores 1 point if test names are provided without indicating under which conditions they should be applied or if any of the statistical tests recommended are incorrect (Biesinger et al. 1987). Graphic interpolation without the use of statistics can be considered a useful check of the results of statistical analysis but should only be used in endpoint calculations as a last resort when more quantitative techniques cannot be used. A test was

given no points for statistical analysis when this method was the only one proposed.

Negative Control

The negative control conditions for a test are the test conditions without toxicant. While it is essential that a toxicity test include this control for result interpretation, it was not used in the scoring scheme because every test necessarily has these conditions.

Test Scores

For each test, the points awarded for each criterion were summed to obtain a score out of 17, which was converted to a percentage. Two examples will illustrate the application of the scoring scheme. At one end of the spectrum is Environment Canada's 48-h *Daphnia* spp. test (Environment Canada 1990b) (Table 14 and App. C). This test provided acceptability criteria and referred to a document that provided information on expected reference toxicant levels and scored 100% under the detailed evaluation. On the other hand, the APHA test methods for shrimp (APHA 1989) (Table 14) scored 59%. No points were given for organism selection, culture, and handling; test conditions (only partially specified); medium definition; and reference toxicant. It is not surprising that this test scored poorly as it is written as a general purpose methodology and is more of a guidance document than a protocol. While some flexibility of method is useful, too much disqualifies a document for consideration as applicable to a national program.

The scores for each test considered are provided in the tables describing the tests for each medium, and the rationale for the scores is provided in Appendix B.

3.3 Step 2 Assessment — Test Application

Tests with or without 'must' criteria that scored ≥88% in the detailed evaluation (3.2.2) were further assessed in step 2. For the second step of the selection process, additional information concerning test application was obtained from the literature and from personal communication with those with firsthand experience with the tests and test organisms. This included a consideration of trophic level, test sensitivity, reproducibility, and ecological relevance and potential for Canadianization. Any available information on field validation of tests was noted but not used to evaluate tests (explained under Field Validation below).

In some cases, sources of information below (e.g., reproducibility) were identified, but the effort involved in providing it was considered beyond the scope of this contract.

3.3.1 Criteria Considered

Trophic Level

There is no single species that has been shown to consistently be most sensitive to contaminants. Members of plant and animal communities differ in their sensitivity to toxicants. Before statements on the toxicity to biological organisms can be made, information is required on the effects on a battery of species. In selecting the tests for inclusion in a battery, it is important to involve a variety of species representing different trophic levels so that, one hopes, the results will be representative for field organisms.

Sensitivity

Test sensitivity is determined in part by organism sensitivity (Peterson et al. 1985, Miller et al. 1985, Santelmann 1977, Blum and Speece 1990, van Leeuwen 1990). The organism should show response to a range of contaminants (including pure chemicals, compounds, and mixtures of contaminants). Test conditions (Peterson et al. 1989; van Straalen and Dennemen 1989) and other factors also affect test sensitivity.

Reproducibility

As mentioned in section 3.2.1, a reference toxicant is a 'must' criterion for a test, but its specification is not a guarantee of low variability of test results. Repeated use of a reference toxicant provides a measure of the expected reproducibility of a test. The results of inter- and intralaboratory tests with the same reference toxicant or the same test substance are discussed. The greater the variability in results obtained using a single sample, the lower our confidence in result interpretation. A maximum value of 30% has been recommended by Environment Canada as an acceptable coefficient of variation (CV) for an endpoint obtained from a test with a reference toxicant. Results for contaminants can vary considerably. For example, in the 96-h rainbow trout test, CVs are typically higher for metals than for other compounds (K. Doe, Environment Canada, pers. comm.).

Field Validation

Toxicity tests are meant to be used as tools to assess toxic effects but not to predict precise effects in the field. Given that lab tests are intended as surrogates for field tests, which cannot be standardized because of inherent variation (e.g., diurnal light, fluctuations in temperature and water flow, grazing, and predation), it is not surprising that little information is available on comparative studies of laboratory tests and field observations. However, the U.S. EPA has demonstrated that chronic toxicity test endpoints can be

positively correlated with effects on community structure in the field (e.g., Mount et al. 1986a, Mount and Norberg-King 1986). These researchers found positive correlations between whole effluent toxicity tests using *Ceriodaphnia dubia* and fathead minnows (*Pimephales promelas*) in the lab and benthic and fish community structure in the field. Thomas et al. (1986) demonstrated a similar relationship between laboratory and field tests for lettuce seed germination in contaminated soil and field assessments of plant cover at the same site. Based on conclusions from these studies one can assume that the demonstration of toxicity in laboratory tests can be used to predict potential effects in the field. Where field validation information is available for a test, it is described.

Ecological Relevance and Potential for Canadianization

Ideally, tests would be conducted with key organisms representative of communities and conditions in Canada that are most likely to be affected by the contaminant in question. On the other hand, laboratory toxicity testing has been driven by the need for organisms that are readily available and easy to culture and defined substrates that enhance test standardization. Tests have been fine-tuned for particular species. Adapting a test to make it more ecologically relevant or Canadianizing it (adapting it to Canadian conditions, using species important in Canadian ecosystems) does not simply involve substituting species, but requires considerable expenditure with no guarantee of successful development and utility. Therefore, in general, opportunities for Canadianization of tests not currently recommending use of native organisms are considered minimal. Development of new tests beginning with native organisms, however, would be more worthwhile.

Another aspect of ecological relevance taken into account in establishing the test batteries for each medium is the appropriate application of tests. For example, while aquatic tests theoretically could be used to assess toxicity of soil using leachates, they would not be appropriate when the soil contaminants are unlikely to leach into surface water. More appropriate tests would be those using organisms that depend on the soil directly such as earthworms and vascular plants.

3.4 Test Batteries

For each medium, two batteries of tests are recommended. The usable battery contains tests that are considered usable based on the evaluation scores (3.5.1, Fig. 1). An augmented battery is also recommended for future consideration as a replacement of the usable battery. The augmented battery includes modified tests from the usable battery and additional tests.

Test selection for the batteries is based on the results of the methodology evaluation (3.2), additional information on test applicability (3.3), and the purpose of performing the tests.

The batteries of tests recommended are to be used in contaminated site assessment and remediation in Canada. A two-tiered approach, which has been used in other places (e.g., Slooff 1985), seems most appropriate and most effort effective. Preliminary screening would first be carried out to determine the relative toxicity of sites or relative toxicity within a site (extent of contamination, identification of most highly contaminated areas) using rapid, less expensive tests (cost is considered to be roughly proportional to the amount of contaminated substance required for the test, the duration of a test, and the size of the test organism). Acute tests and short-term chronic tests are appropriate for the screening set of tests in the battery. At the same time, some breadth of ecological roles should be maintained at the screening level.

Once a general impression of site contamination has been obtained from the screening tests, definitive tests that cover a greater variety of biological roles, are more sensitive (effects are produced at lower concentrations), and have a longer duration could then be used to further refine the limits of toxic contamination and establish site objectives. The definitive tests found to be most sensitive to contaminants on the site would be used to periodically assess the effectiveness of the remediation techniques used and to determine when the site objectives were met.

There is also a need for some degree of flexibility in the selection of tests for batteries, depending on the knowledge of site characteristics. In cases where contaminants are known, test selection can be contaminant driven. For example, where herbicides are the major contaminant on a site, tests with plants would be most appropriate, and the tests outlined in this document could be expanded to cover several species rather than doing additional tests with animal species. A general discussion of the approach to the use of bioassays in site remediation and an example can be found in Athey (1987) and Thomas et al. (1983).

Based on the two-tiered approach to site assessment and remediation suggested above, a set of screening tests and a set of definitive tests are recommended for each of the usable and augmented batteries.

3.5 Priorities for Further Work

For each medium, priorities for further work on individual tests described were initially established based on the results of the Step 1 evaluation procedure (3.2) and subsequently re-evaluated using the

information obtained in the Step 2 evaluation (3.3). The need for this work on individual tests was integrated with the need for further work relevant to several tests and work concerning medium testing in general (e.g., test medium preparation). Finally, priorities for further work for each of the three media were integrated to provide a list of priorities for work for the National Contaminated Sites Remediation Program (7.0).

3.5.1 Priorities for Further Work on Tests Evaluated

Priorities for further work based on the assessment of tests through the 'must' and 'want' criteria reflect the amount of effort required to make the test sufficiently complete for routine use for contaminated site assessment (Fig. 1). A test is considered to be sufficiently complete when it has all the 'must' criteria (3.2) and ≥88% of the 'want' criteria (3.3). The greater the amount of effort required to bring a test to completion, the lower its priority for effort.

Tests were allotted to three major groups — usable, prototype, and under development, according to the amount of effort required to make them complete — and are defined below.

Usable

The group of tests meeting the 'must' criteria were divided into two groups based on the 'want' criteria — those that scored ≥88% and those that scored <88%. The first group of tests was considered immediately usable for assessment of contaminated sites and not to require immediate further effort. The tests in this group were considered eligible for use in the usable battery.

Prototype

Prototype tests include tests with all the 'must' criteria and <88% of the 'want' criteria and those missing 'must' criteria but having ≥88% of the 'want' criteria. Many tests evaluated fell into the prototype category. Tests in this group are considered priorities for further work.

Under Development

Tests that did not meet the 'must' criteria and had a score of <88% for the 'want' criteria were allotted to this category. These tests were either very poorly described or still under development and initially considered of lowest priority for further work.

The initial priorities for further work, assigned above, were revised based on information in 3.3. For example, where two tests in the same trophic level were considered equally complete, but one was shown to be less sensitive (based on literature available and personal communications to date), re-evaluation would result in assigning the latter a lower priority for further work. As a second example, consider two tests that differ in their level of completion (test 1 > test 2) and represent different trophic levels. The priority of test 2 (less complete) would be raised if a third test existed (as complete or more complete than test 1) for the same trophic level as test 1. In this case, the emphasis is put on broadening the trophic spectrum of the battery rather than effort required to complete a test.

3.5.2 Priorities for Further Work by Medium

The priorities for work in each medium integrate the re-evaluated priorities for individual tests reviewed (3.5.1) as well as other needs for work related to several tests and testing within the medium in general (e.g., test medium preparation, reference substrate determination, statistical guidance).

3.5.3 Priorities for the National Contaminated Sites Remediation Program

To provide the program with a broader perspective on priorities for further work, priorities for all three media were integrated (7.0). In assessing program priorities, the urgency with which tests are required was taken into account. For example, numerous tests are currently available for a number of aquatic organisms representing several trophic levels. For the other media (soil, sediment), particularly sediment, there is a paucity of tests; clearly this is an area that should be given high priority. As well, the degree of need for fulfilment of test requirements was also taken into account. For example, needs shared by many tests, such as the need for a designated reference sediment or a standard artificial reference sediment, were considered of higher priority than the needs of single tests.

4.0 ASSESSMENT AND RECOMMENDATIONS FOR SOIL TESTS

While soil leachates or elutriates could always be made and have often been used to assess soil toxicity via water quality tests with aquatic organisms, the main focus of soil quality testing should concern soil dependent organisms. Generally, water quality tests are appropriate for surface water adjacent to or overlying contaminated soil.

In this section, however, we included one aquatic test for assessing groundwater quality. The algal test using *Selenastrum capricornutum* with soil leachates or elutriates (Environment Canada 1992c, Lower and Sutton 1987) was selected because this species exhibited a toxic response to the largest proportion (85%) of 185 soil and sediment elutriates and water and waste-

water samples that were toxic to a three-species (the alga, *Daphnia magna*, *Photobacterium phosphoreum*) test battery (Greene and Barich 1991). (*Photobacterium phosphoreum*, although commonly used for toxicity assessment, showed a toxic response to only 36% of these samples and only 8 [4%] were not toxic to either the alga or the daphnid.)

4.1 Identification of Potentially Suitable Tests

The results of the literature review of organisms used in soil testing are summarized in two tables. Those for which the test methods meet the first criterion considered essential for retaining the test for further evaluation of suitability (appropriate printed test method, see 3.2.1 and 4.2.1) are found in Table 1. Table 2 lists the organisms for which test methods do not meet this criterion and are not considered further.

Forty-seven species from nine major groups of organisms were identified in connection with soil toxicity testing (Tables 1 and 2). Of these groups, algae (1 sp.), vascular plants (24 spp.), earthworms (4 spp.), and springtails (1 sp.) had appropriate printed test methods.

4.2 Step 1 Assessment — Test Methodology

4.2.1 Preliminary Assessment

The tests identified as indicated in 4.1 were first evaluated according to three criteria that are considered essential for a complete test method (acceptable printed method, acceptability criteria, reference toxicant; see 3.2.1 for definition and importance of criteria). The methodologies of 17 tests that met the first criterion are summarized in Table 3 while the methods for Environment Canada's algal test are described in Table 14 (in 6.2.1).

Only two tests (algal growth inhibition—Lower and Sutton 1987; earthworm survival—ISO 1991a) satisfied the second and third criteria. Seven tests provided acceptability criteria but only names of reference toxicants with no indication of expected toxicity values for them under test conditions (algal growth inhibition—Environment Canada 1992c; earthworm survival—OECD 1984d, Eirkson et al. 1987, Greene et al. 1989, ISO 1991a; seed germination—Greene et al. 1989; root elongation—Greene et al. 1989). Four tests provided acceptability criteria but mentioned no reference toxicants (seedling emergence—ASTM 1990e, OECD 1984b; springtails—OECD 1990, ISO 1991d; earthworm reproduction—ISO 1991b), and six tests provided neither acceptability criteria nor a reference toxicant (seed germination—ASTM 1990f, Holst and Ellwanger 1982 [FIFRA], U.S. EPA 1985c [TSCA]; plant growth—Holst and Ellwanger 1982 [FIFRA]; seedling growth—U.S. EPA 1985d [TSCA]; flower production—Lower 1990).

4.2.2 Detailed Evaluation

Eighteen tests with written methods (Table 3; Environment Canada's algal test, Table 14) were further assessed in terms of the 12 'want' criteria (described in 3.2.2) that are valuable but not as important as the 3 'must' criteria.

Test scores ranged from 53% to 100%, as shown by the bold number at the top of the columns in Tables 3 and 14. The rationale for these scores is provided in tables B-1 and B-3 (App. B). Detailed comments on statistical analysis for some of the tests can be found in Appendix D. The results are summarized below:

- 5 tests scored 100%

  earthworm survival, U.S. EPA (Greene et al. 1989)
  earthworm survival, U.S. FDA (Eirkson et al. 1987))
  seedling emergence, U.S. EPA (Greene et al. 1989)
  root elongation, U.S. EPA (Greene et al. 1989)
  algal growth inhibition (Environment Canada 1992c)

- 6 tests scored ≥88% and <100%

  94% - algal growth inhibition, U.S. EPA (Lower and Sutton 1987)
  94% - springtail survival, reproduction (OECD 1990)
  96% - earthworm survival (ISO 1991a)
  94% - earthworm survival (OECD 1984d)
  88% - seedling emergence (ASTM 1990e)
  88% - earthworm reproduction (ISO 1991b)

- 7 tests scored <88%

  82% - root elongation (ASTM 1990f)
  71% - seed germination (U.S. EPA 1985c)
  71% - seedling growth (U.S. EPA 1985d)
  65% - flower production (Lower 1990)
  59% - seedling emergence (OECD 1984b)
  53% - seedling emergence, U.S. EPA (Holst and Ellwanger 1982)
  53% - plant growth, U.S. EPA (Holst and Ellwanger 1982)

The results of this evaluation are further discussed and interpreted in terms of priorities for future work in sections 4.5, 4.6, and 4.7.

Table 1

Species with test methods (for assessing soil quality) from recognized standards organizations and the literature

(ASTM = American Society for Testing and Materials, EC = Environment Canada, ISO = International Standards Organization, OECD = Organization for Economic Cooperation and Development, USEPA = United States Environmental Protection Agency)

Organism group	Species	Organization/ Reference	Test type
Algae			
	Selenastrum capricornutum	EC, Lower and Sutton (1987)	chronic, growth, reproduction
Terrestrial vascular plants			chronic tests
	Allium cepa	USEPA	seed germination, root elongation
		Holst and Ellwanger (1982)	seedling growth
		USEPA	
		Holst and Ellwanger (1982)	seed germination, seedling emergence
	Avena sativa	OECD	seedling emergence, growth
		USEPA	seed germination, root elongation
		Holst and Ellwanger (1982)	seedling growth
		USEPA	
		Holst and Ellwanger (1982)	seed germination, seedling emergence
	Brassica alba *B. campestris* var. *chinensis* *B. napus* *B. rapa*	OECD	seedling emergence, growth
	B. oleracea	USEPA	seed germination, root elongation
		Holst and Ellwanger (1982)	seedling growth
		USEPA	
		Holst and Ellwanger (1982)	seed germination, seedling emergence
	Cucumis sativa	ASTM, Holst and Ellwanger (1982)	seed germination, seedling emergence
		ASTM, USEPA	seed germination, root elongation
		Holst and Ellwanger (1982)	seedling growth
		USEPA	
	Daucus carota	USEPA	seed germination, root elongation
		Holst and Ellwanger (1982)	seedling growth
		USEPA	
		Holst and Ellwanger (1982)	seed germination, seedling emergence
	Glycine max	USEPA	seed germination, root elongation
		Holst and Ellwanger (1982)	seedling growth
		USEPA	
		Holst and Ellwanger (1982)	seed germination, seedling emergence

Table 1 (continued)

Organism group	Species	Organization/ Reference	Test type
Terrestrial vascular plants (continued)			
	Lactuca sativa	ASTM, USEPA, Greene et al. (1989)	seed germination, root elongation
		ASTM, Greene et al. (1989), Holst and Ellwanger (1982)	seed germination, seedling emergence
		OECD	seedling emergence, growth
		Holst and Ellwanger (1982), USEPA	seedling growth
	Lepidium sativum	OECD	seedling emergence, growth
		USEPA	seed germination, root elongation
		Holst and Ellwanger (1982), USEPA	seedling growth
		Holst and Ellwanger (1982)	seed germination, seedling emergence
	Lolium perenne	OECD	seedling emergence, growth
	Lycopersicon esculentum	USEPA	seed germination, root elongation
		Holst and Ellwanger (1982), USEPA	seedling growth
		Holst and Ellwanger (1982)	seed germination, seedling emergence
	Oryza sativa	OECD	seedling emergence, growth
	Phaseolus aureus	OECD	seedling emergence, growth
	Raphanus sativa	ASTM	seed germination, seedling emergence
		ASTM	seed germination, root elongation
		OECD	seedling emergence, growth
	Sorghum bicolor	OECD	seedling emergence, growth
	Tradescantia spp.	ASTM	flower production
	Trifolium ornithopodioides	OECD	seedling emergence, growth
	T. pratense	ASTM	seed germination, root elongation
		ASTM	seed germination, seedling emergence
	Triticum aestivum	ASTM	seed germination, root elongation
		ASTM	seed germination, seedling emergence
		OECD	seedling emergence, growth
	Vicia sativa	OECD	seedling emergence, growth
	Zea mays	USEPA	seed germination, root elongation
		Holst and Ellwanger (1982)	seedling growth
		USEPA	
		Holst and Ellwanger (1982)	seed germination, seedling emergence

Table 1 (continued)

Organism group	Species	Organization/ Reference	Test type
Earthworms			
	Eisenia andrei	ISO, Greene et al. (1989)	acute, survival
		ISO	chronic, reproduction
	E. foetida	ISO, OECD	acute, survival
		Eirkson et al. (1987)	chronic, survival
		ISO	chronic, reproduction
	Lumbricus terrestris	Eirkson et al. (1987)	chronic, survival, growth
	L. rubellus	Eirkson et al. (1987)	chronic, survival, growth
Springtails (Collembola)			
	Folsomia candida	OECD	chronic, reproduction, survival, offspring emergence

Table 2

Organisms that have been used in the assessment of soil quality but for which tests have not yet been prepared by recognized standards organizations or published in the literature

Organism group	Species	Organization/reference
Vascular plants	*Arabidopsis* sp.	Ratsch (1989)
	Panicum miliaceum	Wang and Elseth (1990)
	Phaseolus vulgaris	Keddy et al. (1991)
Protozoa	*Colpoda cucullus*	de Zwart pers. comm.
Nematodes	*Plectus parientus*	de Zwart pers. comm.
Isopods	*Porcellis scaber*	de Zwart pers. comm.
	Trichoniscus pusillus	
Diplopods	*Glomeris marginata*	de Zwart pers. comm.
	Cylindroiulus sylvarus	
Earthworms	*Allolobophora caliginosa*	van Gestel (1991d)
	A. chlorotica	
	A. rosea	
	A. tuberculata	
	Dendrobaea rubida	
	Octochaetus pattoni	
	Pheretima posthuma	
Predatory mites	*Platynothrus peltifer*	de Zwart pers. comm.
	Adoristes ovatus	
Springtails	*Orchesella cincinata*	de Zwart pers. comm.

Table 3

Brief descriptions of tests evaluated for assessing soil quality

The percentage at the column head is the test score (see 3.2, 4.2, and Table B-1) and reflects methodology completeness (NS= not specified). Additional details are provided in Appendix C.

	Lower and Sutton (1987) 94%	ASTM (1990f) 88%	ASTM (1990e) 88%	OECD (1984b) 59%
Test type	alga, chronic, growth, reproduction, static	vascular plants, acute, seed germination, root elongation	vascular plants, acute, seed germination, seedling emergence	vascular plants, acute, seedling emergence, growth
Application	soil contaminants transported to surface/groundwater, elutriate	soil/sediment contaminants in elutriate	contaminants/ chemicals incorporated into whole soil/sediment	soil incorporated, solid/liquid chemical substances
Species	*Selenastrum capricornutum*	lettuce, radish, red clover, wheat, cucumber	see left	16 candidate spp.
Endpoints	cell concentration, EC_{50}	root length, EC_{50}	seedling emergence (1 cm above soil), EC_{50}	seedling emergence above soil, plant weight, EC_{50}
Organism selection	ATCC 22662	seed sizing	seed sizing	seed sizing
No. organisms + replicates	$1\times10^4 \pm 1\times10^2$ cells/mL, 2 reps, 3 conc.	5 seeds, 3 reps, geometric series, min. 3 conc.	40 seeds, 3 reps, at least 5 test conc.	5 seeds, 4 reps, randomized block, 3 conc.
Observation frequency	0, 96 h	120 h ± 30 min	120 h	14 d after 50% control seeds germinated
Volume test vessel	125mL	100x15mm petri dishes	150x15mm petri dishes	NS
Volume test substance	125g soil all reps, max 50mL (100%) elutriate/rep	20mL elutriate/rep	100g test soil/rep	NS
Test substance preparation	1 soil elutriate: 4 diluent water (volume)	NS	wt test soil:wt diluting sand, 20-mesh sand, 85% WHC	incorporate chemical in sand, mix sand with soil, particle size given

Table 3 (continued)

	Lower & Sutton, alga	ASTM, germination	ASTM, emergence	OECD, emergence
Culture, handling	handling	untreated seeds	untreated seeds	water as needed
Conditions (light, temp, pH, etc.)	4300±430 lm 24±2°C, pH 6-10	dark, 24±2°C	48h dark, then 72h 16h light:8h dark, 4300±430 lm, 24±2°C	pH 5.0—7.5, temp, light, humidity not specified
Acceptability criteria	inhibition must be shown in reference toxicant; cell counts for negative controls within 80% of each other and 1 rep must have ≥ 1×10^6 cells	NS	control germination at least 80%	control germination at least 80%
Medium defn., manipulation	macro, micro nutrients	type III reagent grade deionized water	see left	soil with <3% OM, particles <20µm are 10-20%
Negative control	growth medium	deionized water	deionized water in sand	absence of test substance
Reference toxicant	toxic effects should be shown at 0.074 mg/L $ZnCl_2$	NS	NS	NS
Statistical analysis	regression, EC_{50}	mean, SD per conc.; regression EC_{50}	mean, SD per conc.; regression, EC_{50}	mean/conc., LC_{50} emergence, EC_{50} plant weight
Organism availability	easily	easily	easily	easily

	Holst and Ellwanger (1982) 53%	**Holst and Ellwanger (1982) 53%**	**Lower (1990) 65%**	**OECD (1990) 94%**
Test type	vascular plants, acute, seed germination, seedling	vascular plants, acute, growth	vascular plant, chronic, flower production	springtail, chronic, survival, reproduction, offspring emergence
Application	pesticide toxicity, chemical applied to soil, sand, filter paper	pesticide toxicity, see left	whole contaminated soil, leachates, elutriates	testing of chemicals in whole artificial soil

Table 3 (continued)

	Holst and Ellwanger, seed germination	Holst and Ellwanger, plant growth	Lower, flowering	OECD, springtails
Species	soybean, corn, root crop + 7 others	see left	*Tradescantia* hybrid	*Folsomia candida*
Endpoints	seed germination (5mm long radicle), seedling emergence EC_{25}, EC_{50}	growth, morphology, development, EC_{25}, EC_{50}	flower stalks and blooming flowers	adult survival, offspring number, NOEC, LOEC
Organism selection	NS	plants 1-4 wk postemergent	clone 4430 commonly used	10-14 day old juvenile
No. organisms + replicates	10 seeds, 3 reps, 5 test conc.	5 plants, 3 reps, 5 test conc.	NS	10 animals, 4 reps, at least 5 conc.
Observation frequency	5 days germination, weekly for emergence	weekly for at least 2 wk	daily (one flower produced/day)	4 wk
Volume test vessel	NS	NS	NS	100mL glass containers
Volume test substance	NS	NS	NS	30g wet wt soil
Test substance preparation	according to manufacturer	according to manufacturer	saturate soil with Hoagland's solution	blending aqueous test substance with soil
Culture, handling	seeds may be surface sterilized	NS	culture details	rearing and feeding details, counting procedures
Conditions (light, temp, pH, etc.)	optimal for test species, growth chamber	optimal for test species; growth chamber, greenhouse, field	12-22°C, <100µE cool white fluorescent, 16h light:8h dark, high soil moisture	20±2°C, 400-800 lx; 16h light: 8h dark/ continuous light
Acceptability criteria	NS	NS	NS	adult survival > 90%, 100 instars/control vessel

Table 3 (continued)

	Holst and Ellwanger, seed germination	Holst and Ellwanger, plant growth	Lower, flowering	OECD, springtails
Medium defn., manipulation	filter paper/sand/ standardized soil treated with chemical for germination, the latter 2 for emerg.	soil	natural/artificial soil	soil composition (10% peat, 20% kaolin clay, 1% $CaCO_3$, 69% quartz sand)
Negative control	medium without toxicant	medium without toxicant	soil without toxicant	soil without toxicant
Reference toxicant	NS	NS	NS	NS
Statistical analysis	confidence intervals with probability	confidence intervals with probability	NS	concentration means, differences with control
Organism availability	easily	easily	easily	European suppliers

	Greene et al. (1989) 100%	Greene et al. (1989) 100%	Greene et al. (1989) 100%	ISO (1991a) 94%
Test type	earthworm, acute, survival	vascular plant, acute, seed germination, seedling emergence	vascular plant, acute, seed germination, root elongation	earthworm, acute, survival
Species	*Eisenia andrei* (J. Greene pers. comm.)	lettuce (butter crunch)	lettuce (butter crunch)	*Eisenia foetida/ E. andrei*
Application	toxicity of whole natural soil, hazardous wastes	toxicity of whole natural soil, hazardous wastes	aqueous wastes, elutriates from solid wastes	toxic substances incorporated into artificial soil
Endpoints	survival, EC_{50}	no. seedlings 1cm above soil surface, EC_{50}	root length, EC_{50}	survival, LC_{50}, NOEC
Organism selection	>60 d old, with clitellum, 300-500mg, same culture	1 seed size, 1 seed lot, untreated	1 seed size, 1 seed lot, untreated	worms at least 2 mo. old, with clitellum, 300-600mg
No. organisms + replicates	10 worms, 3 reps, at least 5, preferably 7 test conc.	40 seeds, 3 reps, at least 5, preferably 7 test conc.	5 seeds, 3 reps, at least 5, preferably 7 test conc.	10 worms, 4 reps, geometric series of 5 conc.

Table 3 (continued)

	Greene et al., earthworm	Greene et al., seedling emergence	Greene et al., root elongation	ISO, earthworm
Observation frequency	7, 14 d	120 h	120 h	7, 14 d
Volume test vessel	1-pt glass canning jars	150x15mm plastic petri dish bottom half in 30× 30cm plastic bags	100×15mm glass petri dish	1-2L glass container not tightly closed
Volume test	200g test soil/rep	100g/rep	4mL/rep	500g dry wt soil/rep
Test substance preparation	blend test soil with artificial soil, hydrate to 75% WHC	blend test soil with artificial soil, hydrate to 85% WHC	dilute test solution with deionized water with artificial soil	blend liquid test substance (dissolved in water/volatile solvent)/ other test substance (mixed in 10g sand), hydrate to 40-60% WHC
Culture, handling	rearing methods	seed storage methods	seed storage methods	breeding methods
Conditions (light, temp, pH, etc.)	540-1080 lx, continuous, 20±2°C, pH 4-10	4300±430 lx, 48h dark then 16h light: 8h dark, 24±2°C, pH 4-10	dark, 24±2°C, pH 4-10	400-800 lx, 20±2°C, pH 6±0.5
Acceptability criteria	90% control survival	90% control survival	90% control germination	90% control survival and biomass maintenance
Medium defn., manipulation	artificial soil (10% 2.36 mm screened peat, 20% colloidal kaolinite clay, 70% grade 70 silica sand)	artificial soil (20-mesh washed silica sand); cover sand is 16-mesh sand passed 20-mesh sieve	Whatman No. 3 filter paper, synthetic soft water	artificial soil (10% sphagnum peat, 20% kaolinite clay, 69% quartz sand >50% size 0.05-0.2 mm, 1% $CaCO_3$)
Negative control	artificial soil	artificial soil	deionized water	artificial soil with deionized/distiller water
Reference toxicant	sodium dodecyl-sulfate, sodium penta-chlorophenate, cadmium chloride, LC_{50} 2-chloracetamide =35.0mg/kg (J. Greene pers. comm.)	sodium dodecyl-sulfate, sodium penta-chlorophenate, cadmium chloride, LC_{50} 2-chloracetamide =10.4mg/kg (J. Greene pers. comm.)	sodium dodecyl-sulfate, sodium penta-chlorophenate, cadmium chloride, 480 mg/L sodium fluoride will inhibit root growth by 35-65% (J. Greene pers. comm.)	LC_{50} chloroacetamide= 30-100mg/kg

Table 3 (continued)

	Greene et al., earthworm	Greene et al., seedling emergence	Greene et al., root elongation	ISO, earthworm
Statistical analysis	means with 95% CI, moving average angle/ probit analysis for LC_{50} when possible	means with 95% CI, moving average angle/ probit analysis for LC_{50} where possible	means with 95% CI, moving average angle/ probit analysis for EC_{50} where possible	mean per cent mortality, LC_{50} with 95% CI using Litchfield and Wilcoxin test, no methods for NOEC
Organism availability	easily	easily	easily	easily

	ISO (1991b) 88%	OECD (1984d) 94%	Eirkson et al. (1987) 100%
Test type	earthworm, chronic, reproduction	earthworm, acute, survival	earthworm, acute/ chronic, survival, growth
Application	chemicals in artificial soil	chemicals in artificial soil/ filter paper	chemicals in artificial soil
Species	*Eisenia foetida/ E. andrei*	*Eisenia foetida*	*Lumbricus terrestris/ L. rubellus/Eisenia foetida*
Endpoints	survival, cocoon production, hatch-ability, juveniles/ cocoon, LC_{50}, EC_{50}, NOEC	survival, LC_{50}	survival, body weight, LC_{50}, EC_{50}
Organism selection	at least 2 mo. old, with clitellum, 250-600mg; batches of 10 worms differ by <1 g	at least 2 mo. old, with clitellum, 300-600mg	*L. terristris* mature, with clitellum, 8-30cm long; other species 5-12cm long
No. organisms + replicates	10 worms, 4 reps, geometric series of 5 conc.	1 worm, 10 reps, geometric series of 5 conc.	10 worms, 4 reps, geometric series of 5 conc.
Observation frequency	21 d for mortality, cocoon production, 5 wk hatchability, juveniles	14 d for soil; 48h, 72h optional for contact test	7, 14, 21, 28 d for survival, wt at start and end
Volume test vessel	1L glass container <15cm diam, loosely covered with lids	1L glass container with lid for soil; glass vial 8cm long 3cm diam for contact test	2.5L glass container, diameter 1:2 height
Volume test substance	500g test soil/rep	750g wet wt test soil/rep; 1mL test solution for contact	2kg soil/rep *L. terrestris*, 1kg other species

Table 3 (continued)

	ISO, *Eisenia* reproduction	OECD, *E. foetida* survival	Eirkson et al., *L. terrestris*
Test substance preparation	blend liquid test substance (dissolved in water/volatile solvent)/ other test substances	see left, hydrate to moisture content 35% with deionized water	defined slurry of test substance, water, food mixed with soil and food mixture
Test substance preparation	mixed in 10g sand into artificial soil, hydrate to 50-55% WHC	filter paper moistened with test substance diluted with deionized water	
Culture, handling	methods for 7-d preconditioning	breeding methods; methods for pre-test gut voiding for contact test	acclimation in test soil
Conditions (light, temp, pH, etc.)	400-800 lx, 20±2°C, pH 5.5±0.5	400-800 lx continuous light, 20±2°C, pH 6±0.5 for soil; dark for contact test	400-800 lx continuous, *L. terrestris* 13±2°C, other species 20±2°C, pH 6±0.5
Acceptability criteria	90% control survival, reproduction of treatments ≤ control	90% control survival	90% control survival
Medium defn., manipulation	artificial soil (10% 1.0mm sphagnum peat, 20% kaolin clay, 70% quartz sand, KCl)	artificial soil (10% sphagnum peat finely ground, 20% kaolin clay, 70% sand >50% particles 50-200μ)	artificial soil (see OECD soil) + distilled water to 25% dry wt; 50g rabbit feces/kg soil
	for cocoon substrate use <0.5 mm peat + 1% 0.5mm cow dung	80-85g/m^2, 0.2 mm thick, medium-grade filter paper	
Negative control	artificial soil with deionized water	artificial soil/ filter paper with deionized water	soil with water
Reference toxicant	NS	chloracetamide	chloracetamide
Statistical analysis	LC_{50}, EC_{50}, NOEC, no methods proposed	plot dose-response relationship and LC_{50} with confidence limits; probit analysis acceptable	references cited for statistical analysis
Organism availability	easily	easily	easily

Table 3 (continued)

	USEPA (1985c) 71%	USEPA (1985d) 71%
Test type	vascular plants, acute, seed germination, root elongation	vascular plants, acute, seedling growth
Application	chemicals applied to sand/glass beads	chemicals applied to plants growing hydroponically/in glass beads
Species	tomato, cucumber, lettuce, soybean, cabbage, oats, perennial ryegrass, onion, carrot, corn	see left
Endpoints	EC_{10}, EC_{50} for seed germination, root length	EC_{10}, EC_{50} for weight & length of roots and shoots
Organism selection	seed sizing	uniform seedlings
No. organisms + replicates	10 seeds, 3 reps, 6 concentrations	10 seedlings, 3 reps, 5 concentrations
Observation frequency	end of test (65% of control seeds germinated with roots 20mm)	end of test (14 d after 50% seeds germinated)
Volume test vessel	200mm petri dishes	NS
Volume test substance	NS	NS
Test substance preparation	dilution with deionized water	dilution with Hoagland nutrient medium

Table 3 (continued)

	USEPA, seed germination	US EPA, seedling growth
Culture, handling	NS	seed germination conditions
Conditions (light, temp, pH, etc.)	dark, 25±1°C	350μE/m^2·s^{-1} at 400-700nm, 16h light: 8h dark, 25±3°C d, 20±3°C night, RH 70-90%, CO_2 350 ppm
Acceptability criteria	NS	NS
Medium defn., manipulation	deionized water	Hoagland nutrient medium
Negative control	see above	see above
Reference toxicant	NS	NS
Statistical analysis	NS	NS
Organism availability	easily	easily

4.3 Step 2 Assessment — Test Application

For each test that scored ≥88% in the detailed evaluation (4.2.2), additional information on trophic level, test sensitivity, test reproducibility, field validation, and ecological relevance is provided.

4.3.1 Algal Test

A method specifically designed for testing solutions collected from hazardous chemical waste sites was published in 1983 by Porcella. The test, without modification, was republished in Greene et al. (1989). The test was also published by Lower and Sutton (1987). More recently, Environment Canada has supported the development of a microplate technique. Development of the technique is near completion and a draft protocol has been circulated for review (Environment Canada 1992c).

Trophic Level

Algae are natural inhabitants of water and are an extremely important group of plant organisms. Through their photosynthetic activity they help to provide the oxygen necessary for the survival of animal species found in the aquatic environment. Algae contribute to the purification of streams, lakes, and estuaries, and also serve as the basis of the food chain within the aquatic ecosystem.

Sensitivity

Sensitivity of the green alga *Selenastrum capricornutum*, relative to organisms other than algae, is shown in tables 4 and 15. These tables show that this alga was less sensitive to 19 nonpesticide organic compounds than *Daphnia magna* and more sensitive to heavy metals and insecticides than *Photobacterium phosphoreum*, *D. magna*, earthworms, and terrestrial vascular plants. The alga is less sensitive than vascular plants to numerous herbicides. Tests with 21 herbicides using radish, barley, beans, and *S. capricornutum* showed that the alga was most sensitive to 11 and that an alga cannot be used as a surrogate for evaluating toxicity to vascular plants (Garten and Frank 1984).

Concerning effluents and waters contaminated with a mixture of chemicals, *S. capricornutum* was more sensitive to pulp and paper effluent than *P. phosphoreum* and rainbow trout and more sensitive to 11 industrial effluents (e.g., paper mill, textile dyeing, oil refinery, leather tanning) than *D. magna*. The alga was less sensitive to creosote-contaminated water and sediment elutriates than *D. magna* and *P. phosphoreum*, respectively. *Selenastrum capricornutum* was less sensitive than *P. phosphoreum* and more sensitive than the rotifer *Brachionus calyciflorus*, the nematode *Panagrellus redivivus*, and *D. pulex* to elutriates from river sediment (Sloterdijk et al. 1989).

To tests performed on leachates or elutriates from sanitary landfills and soil containing heavy metals, pesticides, polynuclear aromatic hydrocarbons, herbicides, insecticides, and neurotoxins, *S. capricornutum* was more sensitive than *P. phosphoreum* and *D. magna*. It was also more sensitive than tests with earthworms and lettuce using solid soil from the same sites.

When the results of tests with 326 water, waste, and sediment/soil elutriates were examined, *S. capricornutum* responded to the toxic constituents contained in the samples more often than did *D. magna* or *P. phosphoreum* (Greene and Barich 1991). *Photobacterium phosphoreum* responded to only 36% of the samples that were toxic to either *S. capricornutum* or *D. magna*, or both. The information above indicates that *S. capricornutum* is sensitive to a variety of toxic substances in water and sediment/soil elutriates and soil leachates. In many cases, it shows greater sensitivity than do numerous other organisms.

Lewis (1990) shows that the relative sensitivity of algal species to the same toxicant can vary by more than 2000 times (disodium hydrogen arsenate, 13 spp.). As well, the toxicity of one group of compounds to one species of alga may vary from two (nonionic surfactants, *Microcystis aeruginosa*) to more than 100 times (organic acids, *M. aeruginosa*). In a comparison of *S. capricornutum* and *Chlorella vulgaris* with 21 herbicides, the former was most sensitive to all but two (Garten and Frank 1984).

Reproducibility

Table 17 shows that both the microplate and flask methods typically show good reproducibility with coefficients of variation of less than 30%.

Ecological Relevance

Algal tests for soil toxicity testing, using leachates or elutriates, are relevant for assessing groundwater toxicity. When there is concern about the potential for surface water contamination due to the close proximity of contaminated soil, additional aquatic tests recommended in section 6 could be employed.

4.3.2 Earthworm Tests

Tests for earthworms (*Eisenia* spp.) have been available from the OECD since 1984 (OECD 1984d). That initial test was adopted by the ISO and U.S. EPA with only minor variations (e.g., % soil hydration). More recently, protocols for assessing not only survival but reproduction have been developed (ISO 1991b). The

Table 4

Relative sensitivity of organisms used (in tests reviewed in this document) for assessing soil quality

The lower the number, the higher the sensitivity. The endpoints listed correspond with the organisms tested in order from left to right. In the first study, for example, the endpoint for the *Eisenia* test was survival, the endpoint for the vascular plants was root elongation, and the endpoint for the *Photobacterium* test was luminescence after 30 minutes. These endpoints apply to the three substances tested. Unless indicated, soil tests with terrestrial species are conducted with whole soil. Due to limited space, only vascular plant studies involving groups of organisms in addition to plants are included in this table. Table 5 provides further sensitivity information for plants.

(Ei= *Eisenia foetida*, Cu= cucumber, Le= lettuce, Mi= millet, Ra= radish, Ri= rice, P= *Photobacterium phosphoreum*, S= *Selenastrum capricornutum*, Dm= *Daphnia magna* (= *D. pulex* for last 2 studies), F= fathead minnow, R= rainbow trout, L= *Lemna minor*, d= deionized water extract, e= elutriate, rt elong= root elongation (120-h test), sa= sodium acetate extract)

Test Substance	Endpoint	Species												Reference
		Ei	Cu	Le	Mi	Ra	Ri	P	S	Dm	F	R	L	
Heavy metals	14 d LC50	7	6	4	-	5	-	3	1	2	-	-	-	Miller et al.
Herbicides	IC50 rt elong	5	1	1	-	1	-	3	2	4	-	-	-	(1985)
Insecticides	IC50 rt elong	3	-	-	-	-	-	4	1	2	-	-	-	
	IC50 rt elong													
	30 min IC50													
	96 h IC50 growth													
	48 h LC50													
Heavy metal	14 d LC_{50}	4	-	4	-	-	-	3	1	2	-	-	-	Thomas et al.
Pesticide e	IC_{50} rt elong	2	-	2	-	-	-	2	1	1	-	-	-	(1986)
PAH contam-	30 min IC_{50}	2	-	5	-	-	-	3	1	4	-	-	-	
inated soils	96 h IC_{50} growth													
	48 h LC_{50}													
Toxic	14 d LC_{50}	3	-	3	-	-	-	3	1	2	-	-	-	Barich et al.
soil e	EC_{50} seed germ													(1987)
	30 min IC_{50}													
	96 h IC_{50} growth													
	48 h LC_{50}													

Table 4 (continued)

Test Substance	Endpoint	Species												Reference
		Ei	Cu	Le	Mi	Ra	Ri	P	S	Dm	F	R	L	
Metal effluent	EC_{50} seed germination	-	3	2	4	-	1	-	-	-	-	-		Wang and Keturi (1990)
Phenolic compounds	IC_{50} rt elong	-	2	2	1	-	-	-	-	-	-	-	-	Wang (1986)
9 waste d elutriates sa	IC_{50} rt elong	-	-	3	-	-	-	3	1	2	-	-	-	Peterson et al. (1989)
	30 min IC_{50}	-	-	4	-	-	-	3	1	2	-	-	-	
	96 h IC_{50} growth													
	48 h LC_{50}													
21 herbicides	shoot biomass NOEC	-	-	-	1	-	-	2	-	-	-	-		Garten and Frank (1984)
	96 h growth NOEC													
Sanitary landfill leachate	5 min IC_{50}	-	-	-	-	-	-	2	1	3	4	-	-	Plotkin and Ram (1984)
	13 d IC_{50} chl *a*													
	48 h LC_{50}													
	96 h LC_{55}													
326 samples water, wastes, soil/sediment elutriates	30 min IC_{50}	-	-	-	-	-	-	3	1	2	-	-	-	Greene and Barich (1991)
	96 h IC_{50} growth													
	48 h LC_{50}													
Sanitary landfill leachate	48+96 h LC_{50}	-	-	-	-	-	-	-	-	2	-	1	-	Atwater et al. (1983)
	96 h LC_{50}													

latter is based on the OECD method with some modification in pH for cocoon production. The U.S. FDA test (Erikson et al. 1987) with *Lumbricus terrestris* (and other species) uses essentially the same conditions as the OECD test, but the worms are fed.

Trophic Level

Earthworms contribute in many ways to soil structure. They incorporate decaying organic matter into the soil and turn it over, mixing it with other fractions and enhancing the decomposition and mineralization processes. Burrowing worms increase the moisture-holding capacity of the soil and stimulate aeration and drainage. Earthworms are an important food source for amphibians, reptiles, birds, and small mammals (van Gestel 1991e).

Sensitivity

Few comparative studies involving earthworms have been conducted. Table 4 shows that *Eisenia foetida* is less often sensitive than the aquatic organisms *Selenastrum capricornutum* and *Daphnia magna*. It was more sensitive to heavy metals and herbicides than vascular plants (root elongation) when both were tested in whole soil. When compared to lettuce root elongation in soil elutriates, the earthworm was equally or more sensitive to a variety of contaminants. *Eisenia foetida* showed variable sensitivity relative to *Photobacterium phosphoreum*.

For 23 chemicals, Heimbach (1988) reports an acceptable correlation ($r^2 = 0.65$) between 14-day LC_{50}s for *Eisenia* spp. (*foetida* and *andrei*) in artificial soil and those of *Lumbricus terrestris* in a natural soil substrate. *Eisenia foetida* appeared to be less sensitive than *L. terrestris* for pesticides with low LC_{50}s. The validity of using two different soil types in this comparison is questionable.

In a literature review of relative toxicity of pesticides (14-day EC_{50}s) to earthworms, van Gestel (1991d) found that *L. terrestris* was at least 47 times more sensitive than *E. andrei* and *E. foetida* to benomyl, but different temperatures and substrates were used for each worm species. In 90-day chronic tests, *L. terrestris* was more sensitive to this pesticide than *Aporrectodea* spp.

A study recently initiated by Environment Canada that involves both *L. terristris* and *E. foetida* under identical soil conditions will provide useful data on relative sensitivities (see Ecological Relevance below).

Reproducibility

When used to test the toxicity of natural gas plant sludges, the U.S. EPA 14-day test with *E. andrei* showed high reproducibility both within (CV = 2.5%, 5.5%) and among laboratories (5.5%, 14.0%) (Table 6). Intralaboratory testing with chloracetamide gave a CV of 16.2% for the LC_{50} (J. Greene, pers. comm.).

Field Validation

In a study involving 12 different pesticides, toxicity to earthworms in the field (21 sites) was compared to 14-day LC_{50}s (standardized by estimated environmental concentrations) for *Eisenia foetida* obtained in the laboratory. The correlation between laboratory results and reduction in abundance of earthworms in the field was very good ($r^2 = 0.74$, $n = 29$) (Heimbach 1988). Based on a literature review, van Gestel (1991d) found the LC_{50} for benomyl (based on *E. andrei, E. foetida, L. terrestris*) in laboratory studies to range from 0.4 to 27 mg/kg, while field concentrations that resulted in ≥50% reduction of earthworm populations varied from 1.6 to 28.6 mg/kg (two different sets of literature were used in the comparison). In the case of carbofuran, laboratory LC_{50} values ranged from 0.6 to >64 mg/kg while a ≥50% reduction in field populations occurred between 1.4 and 16 mg/kg.

No information was found on the relationship between lab tests using site soils and observations of the effects of site contamination on field communities. An on-site field testing method using *L. terrestris* has been carried out, but the results were not compared to field observations (Callahan et al. 1991).

Ecological Relevance

The natural habitat of the species traditionally used in toxicity testing (*Eisenia foetida, E. andrei*) is compost, rather than soil (Fender 1985). *Lumbricus terrestris*, among the most common species in arable soils in Canada, might be considered a more appropriate organism for soil quality assessment. At the present time, however, this species is difficult to culture and must be purchased. This could lead to supply problems and the need for a taxonomist to verify the species each time it arrived in the laboratory. A study is now under way to determine the relative sensitivities of *E. foetida* and *L. terrestris* to four priority pesticides in artificial soil and natural soils. The interactions of species with type of soil and of chemical with type of soil will also be assessed and the suitability of the traditional test with *E. foetida* to Canadian environmental conditions will be determined (R. Kent, State of the Environment Reporting, Environment Canada, pers. comm.).

4.3.3 Springtail Test

A draft springtail test was prepared for the OECD (1990), based on testing carried out in the Netherlands; it has since been adopted as a draft test method by the ISO (1991d). Further work is being done on this group

in the Netherlands (D. de Zwart, Nat. Inst. of Public Health and Environmental Protection, Bilthoven, pers. comm.).

Trophic Level

Springtails (Collembola) are minute primitive insects without wings that live in soil, leaf litter, decaying wood, and fungi. Their populations sometimes number several million per acre, and they are important as scavengers in the decomposition process (Borror and White 1970).

Sensitivity, Reproducibility

No information was found on the sensitivity of springtails relative to other organisms or on the reproducibility of tests using these organisms. Data on intralaboratory reproducibility for the springtail *Folsomia candida* will be presented in a manuscript currently being prepared, and a European interlaboratory test is being considered (N.M. van Straalen, Free Univ. of Amsterdam, pers. comm.).

Ecological Relevance

Springtails are abundant organisms in soil and are important as decomposers. The genus *Folsomia* occurs in Canada, but not the test species.

4.3.4 Terrestrial Vascular Plant Tests

Tests for assessing toxicity using seedling emergence (Holst and Ellwanger 1982, Greene et al. 1989, ASTM 1990e, OECD 1984b), root elongation (Greene et al. 1989, ASTM 1990f, U.S. EPA 1985c), and seedling growth (Holst and Ellwanger 1982, U.S. EPA 1985d, OECD 1984b) have been developed. The majority of testing appears to have involved the use of root elongation tests (Table 5a).

Trophic Level

Plants play a critical role in terrestrial ecosystems in nutrient cycling, primary production, and as food and habitat for other organisms. They make up 99.9% of the biomass of the planet and about 20% of the total number of species (Keddy et al. 1991). Tropical forests, the largest terrestrial contributors to global net primary production (170 kg x 10^9 dry tonnes carbon/yr), produce 49.5 x 10^9 t/yr. Temperate forests produce 24.5 x 10^9 t/yr (Whittaker 1975).

Sensitivity

Table 4 shows the sensitivity of terrestrial vascular plants to toxic substances relative to other types of test organisms. For heavy metals, plant root elongation was less sensitive than were *P. phosphoreum*, *S. capricornutum* or *D. magna*, but it was more sensitive than earthworms (Miller et al. 1985). For herbicides, all plant species tested were more sensitive than *S. capricornutum* and earthworms (Miller et al. 1985). Testing of soil elutriates and leachates showed that lettuce seed (root elongation) was equally sensitive as or less sensitive than *P. phosphoreum*, *S. capricornutum* or *D. magna* (Barich et al. 1987, Thomas et al. 1986, Peterson et al. 1989) and *Eisenia foetida* (Thomas et al. 1986).

Table 5 shows the relative sensitivity of plant species to various toxicants. Generally, lettuce seems to be more sensitive than other terrestrial species tested while wheat seems to be least sensitive (Table 5a). Limited studies with aquatic plant species indicate that lettuce may be less sensitive to waterborne contaminants than rice but more sensitive than Japanese millet (Table 5b).

Reproducibility

When the test methods specified in the U.S. EPA seed germination test (Greene et al. 1989) were applied using chloracetamide and lettuce (Table 6), an intralaboratory coefficient of variation of 16.2% for the EC_{50} was found, based on three tests. For the same test, intralaboratory CVs with lettuce were 20% (n = 15) for heavy metals and 10% (n = 20) for herbicides (Thomas et al. 1986). The same test was also used by three laboratories to assess toxicity of three natural gas plant sludges (Novak 1990). For barley, intralaboratory CVs for EC_{50}s of 2.7 to 8.7% (sample 2) and 2.0 to 20.2% (sample 3) were reported. Reported CVs for cucumber were 4.0-34.6% (sample 1) and 9.8-15.4 (sample 2).

Interlaboratory root elongation tests, using the glass plate/aquaria test design, were conducted with seven laboratories, ten toxic chemicals, and five plant species (Ratsch 1983). Coefficients of variation for control replicates varied from 9-44% (one lab) to 23-27% (one lab) for the five species. Within a species, laboratory CVs ranged from 9-27% (cucumber) to 14-37% (radish). An ANOVA showed that there were no significant differences among laboratories for estimated EC_{50}s for six chemicals for five species.

In an intralaboratory test repeated four times with different concentrations of zinc, nickel, and copper, there was no difference at the 95% level in the reduction in root length for flowering Chinese cabbage among the four trials in 11 out of 12 treatments (Cheung et al. 1989). Elongation in one of the zinc treatments differed from the other three concentrations because of the low variability.

In an intralaboratory test with tomato seeds, a CV of 27% was obtained for the number of germinated seeds using 50 µg/g of sodium pentachlorophenate, while a CV of 16% was obtained with a concentration of

Table 5a

Relative sensitivity of vascular plants to toxic compounds and effluents

The lower the number, the greater the sensitivity. (Ba= barley, Ca= cabbage, Co= corn, Cu= cucumber, Mi= millet, Ra= radish, Rc= red clover, Sb= soybean, Wh= wheat; Le= lettuce, a common test species for comparison)

		Species										
Substance	**Endpoint**	Le	Rc	Ra	Cu	Mi	Ca	Ba	Wh	Co	Sb	**Reference**
Herbicides	IC_{50} root elongation	1	1	1	1	-	-	-	2	-	-	Miller et al. (1985)
Heavy metals	IC_{50} root elongation	1	2	3	4	-	-	-	5	-	-	Miller et al. (1985)
2 metals	EC_{50} root elongation	1	2	2	2	-	-	-	3	-	-	Ratch (1983)
Methane arsonic acid		2	1	3	4	-	-	-	5	-	-	
Monuron		2	1	4	3	-	-	-	5	-	-	
2,4-D		3	1	2	4	-	-	-	5	-	-	
Sodium fluoride		5	3	4	1	-	-	-	2	-	-	
26 chemicals	shoot and root length	1	-	2	-	-	-	-	-	-	-	Gorsuch et al. (1990)
Metal effluent	EC_{50} seed germination	1	-	-	2	-	3	-	4	-	-	Wang and Keturi (1990)
Phenolics	IC_{50} root elongation	2	-	-	2	1	-	-	-	-	-	Wang (1986)
Heavy metals	IC_{50} root elongation	1	-	-	3	2	-	-	-	-	-	Wang (1987a)
Organics		2	-	-	3	1	-	-	-	-	-	
Phenolics	IC_{50} root dry weight	-	-	1	-	1	-	-	-	-	-	Wang (1985)

Table 5a (continued)

Substance	Endpoint	Species										Reference
		Le	Rc	Ra	Cu	Mi	Ca	Ba	Wh	Co	Sb	
Heavy metals	IC_{50} root elongation	-	-	1	-	-	2	4	-	-	3	Cheung et al. (1989)
131,596 chemicals	% chemicals causing 100% mortality	-	-	3	-	-	-	-	-	2	1	Kenaga (1981)
Heavy machinery effluent	seeds germinated	-	-	-	1	3	4	-	2	-	-	Wang and Williams (1988)

Table 5b

Relative sensitivity of vascular plants to toxic compounds and effluents

The lower the number the greater the sensitivity. (Lm= *Lemna minor*, Lp= *L. perpusila*, Lg= *L. gibba*, Sp= *Spirodela polyrhiza*, floating aquatics; Ri= rice, rooted aquatic; Jm= Japanese millet, wetland species; Le= lettuce, a common test species for comparison)

		Species							
Substance	**Endpoint**	Lm	Lp	Lg	Sp	Ri	Le	Jm	**Reference**
Chromium	IC_{50} frond number	1	-	-	2	-	-	-	Wang (1990b)
Raw coal distillate		1	2	3	-	-	-	-	
Fuel oil		2	1	3	-	-	-	-	
Ammonia	% inhibition root biomass (Ri), no. fronds (Lm) (renewal method)	1	-	-	-	2	-	-	Wang (1991)
Industrial wastewater	% inhibition root biomass (Ri, Le), no. fronds (Lm)	1	-	-	-	2	3	-	Wang (1990a)
Metal effluent	EC_{50} seed germination	-	-	-	-	1	2	3	Wang and Keturi (1990)
Heavy machinery effluents	seeds germinated	-	-	-	-	1	-	2	Wang and Williams (1988)

Table 6

Reproducibility of tests for soil quality using soil-dependent organisms

Where more than one coefficient of variation (CV) or more than one range of CVs is given for a test, each corresponds to a different sample. (A= intralaboratory test [1 laboratory], E= interlaboratory test [a single CV for an E test is for the mean endpoint among laboratories; a range of CVs for an E test indicates CVs for individual laboratories that conducted the test], p.c. = personal communication, sp= species)

Organism	Test Method	Endpoint	Substance	CV	Type	Reference
Barley	120 h (whole sediment) Greene et al. (1989)	EC_{50} seed germination	natural gas plant sludge	2.7-8.7% 2.0-20.2%	E	Novak (1990)
Cucumber	120 h (whole sediment) Greene et al. (1989)	EC_{50} seed germination	natural gas plant sludge	4.0-43.6% 9.8-15.4%	E	Novak (1990)
Lettuce	120 h (standard sand) Greene et al. (1989)	EC_{50} seedling emergence	2-chloroacetamide	18.1%	A	J. Greene p.c.
Terrestrial plants (4 spp.)	115 h (aqueous sample) (Porcella 1983)	EC_{50} seed germination	heavy metals herbicides	20% 10%	A	Miller et al. (1985)
Tomato	96 h (aqueous sample) Lower et al. 1987)	% seeds germinated	sodium penta-chlorophenate	16% (100µg/g) 27% (5µg/g) 177% (1µg/g)	A	Lower et al. (1987)
Red clover Lettuce Wheat Cucumber Radish	115 h (aqueous sample) (Porcella 1983)	IC_{50} root elongation	control replicates	14-25% 7-23% 10-22% 9-27% 14-37%	E	Ratsch (1983)
Eisenia andrei	14 d (artificial soil) (Greene et al. 1989)	LC_{50}	natural gas plant sludge	14.0%, 5.5% 5.5% 2.2%	E A A	Novak (1990)
Eisenia andrei	14 d (artificial soil) (Greene et al. 1989)	LC_{50}	2-chloroacetamide	16.2%	A	J. Greene p.c.

100 µg/g (Lower et al. 1987). The precision for early seedling growth using 5 µg/g was 23%, while it was 177% when a concentration of 1 µg/g was used. EC_{50} values for seed germination based on 20 determinations ranged from 50 to 80 µg/g sodium pentachlorophenate. For early seedling growth (20 determinations), EC_{50} values ranged from 24.7 to 45.3 µg/g.

Field Validation

Based on an general analysis of EC_{50}s (endpoint variables not distinguished) obtained from the PHYTOTOX database for vascular plant species, it was shown that on average there was a 1.8 ± 0.4 (95% CI) fold difference between EC_{50}s calculated using greenhouse and field data (Fletcher et al. 1990). As well, taxonomic differences among plants had a greater influence on response to chemical treatment than did test condition (laboratory vs. field).

No site-specific references to the relationship between laboratory tests with terrestrial plants and the condition of field communities were found.

Ecological Relevance

The species used in the terrestrial plant toxicity tests prepared to date (Table 3) are all crops. If crop species are to be used, they should at least reflect the common crops grown in Canada. Other tests are to be described as part of the ASTM tests (ASTM 1990e, 1990f), which allow for the use of other types of seeds including those from native species.

4.4 Usable Battery

The following tests are considered eligible for inclusion in the usable battery because they meet all of the 'must' criteria (3.2.1) and at least 88% of the 'want' criteria (3.2.2, 4.2.2):

- algal growth inhibition test, U.S. EPA (Lower and Sutton 1987)
- earthworm survival (ISO 1991a)

Once information is added on expected values for reference toxicants that were produced during test development but not included in the printed test description (tables 3, 14, and App. C), the following tests also become eligible for conclusion in the usable battery:

- algal growth inhibition test (Environment Canada 1992c)
- seedling emergence, U.S. EPA (Greene et al. 1989)
- root elongation, U.S. EPA (Greene et al. 1989)
- earthworm survival, U.S. EPA (Greene et al. 1989)

Two trophic levels are represented by the soil-dependent test organisms for which tests are currently usable.

4.4.1 Screening Tests

The six tests identified above as currently usable for soil toxicity testing are of relatively short duration. The tests measure acute effects with the exception of the algal test, which measures chronic effects. All are considered candidates for the set of screening tests. The relative merits of each are discussed by major organism group, and conclusions concerning the most appropriate tests are drawn below.

To summarize, the following tests are recommended for screening: seedling emergence using lettuce and radish (Greene et al. 1989), earthworm survival using *Eisenia andrei* (Greene et al. 1989), and algal growth inhibition using *Selenastrum capricornutum* (Environment Canada 1992c). The application of these tests is shown in Figure 2.

Algal Test

Algal testing is included as a soil test to assess the toxicity of groundwater. (The rationale for selecting this test is provided in section 4.0). When contamination of nearby surface water is of concern as well as soil contamination, tests in the aquatic batteries (see 6.4), not considered routinely appropriate for soil toxicity assessment, should be considered.

The algal test (Lower and Sutton 1987) was included in the soil testing section of this report because it was specifically written as a 'soil' toxicity test. It is merely a minor adaptation of a common aquatic algal test that can be useful for assessing the toxicity of leachates or elutriates.

It is recommended that the flask soil test with algae (Lower and Sutton 1987) be replaced by the microplate test described in the water quality battery (modified for use with sediment elutriate) for the same reasons that it was recommended over the water quality flask test (see 6.4.1).

Seedling Emergence Test

Tests for seedling emergence and root elongation (Greene et al. 1989) are considered currently usable. These methods, unlike those of the OECD or ISO (designed for testing individual substances), were prepared specifically for the assessment of whole contaminated soil.

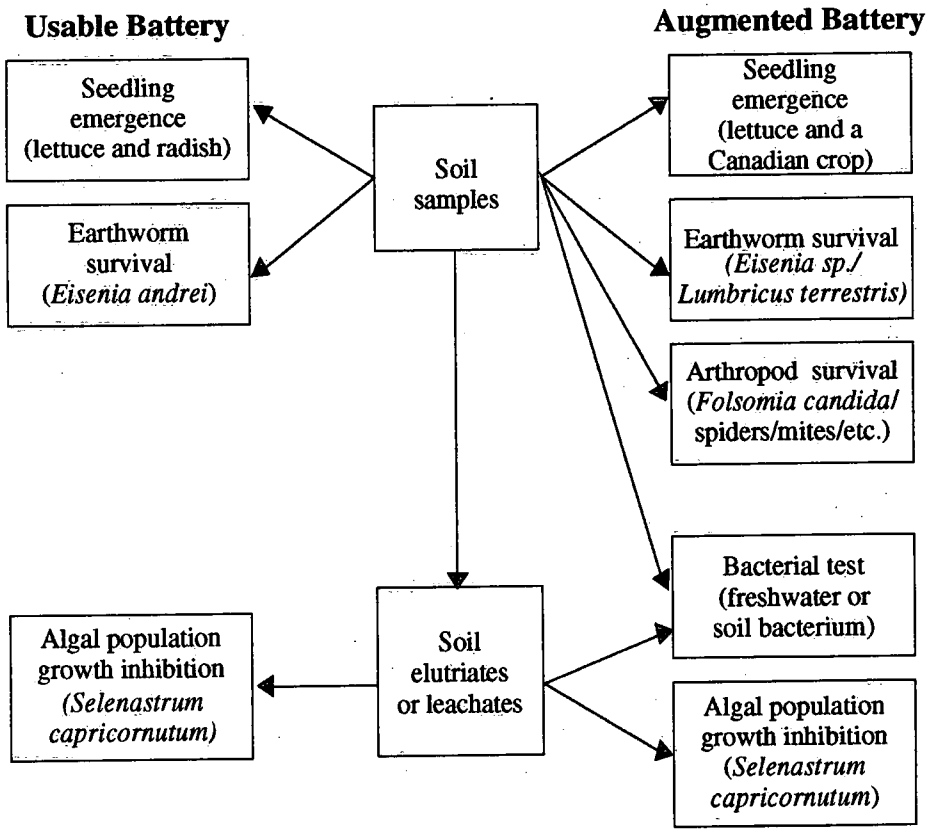

Figure 2. Screening tests recommended for the usable and augmented batteries for soil quality assessment (see 4.4.1 and 4.4.3.1 for additional details).

The seedling emergence test exposes the seed to total available toxic constituents in the soil, while the root elongation test exposes the seeds to the water soluble constituents eluted from the soil. The seedling emergence test is, therefore, likely to demonstrate greater sensitivity than the root elongation test if non-water-soluble toxic constituents are present in the soil. Seedling emergence tests are therefore recommended for the screening battery. The root elongation test could be conducted if toxic constituent mobility, caused by precipitation events, was of specific interest.

Only one species (lettuce) is specified in the printed test method although the methodology has been applied to show toxicity and adequate reproducibility (CV 2.8%-43.6%, Table 6), using several species (barley, cucumber — Novak 1990; radish — J. Greene pers. comm.). The ASTM draft guidelines (ASTM 1990d, 1990e) are based directly on the methods of Greene et al. (1989) and indicate that both lettuce and radish are recommended as the minimal test species. These guidelines also indicate that the test methods are valid for cucumber, red clover, and wheat.

If a single species is to be tested, it should be lettuce because it is often more sensitive to a variety of substances than other species tested (Table 5a) and because it is a standard test species for which a significant historical toxicity database is available for comparative purposes. However, it is not an important commercial crop in Canada.

The soil used as a diluent in the seedling emergence test is sand. The potential for using the same artificial soil as is recommended for the earthworm test could be considered. The appropriateness of the artificial soil in the test method in relation to Canadian soil conditions is discussed further in relation to the earthworm test in section 4.7.

Earthworm Test

The only earthworm tests currently usable are the 14-d tests using *Eisenia andrei* and *E. foetida*, proposed by the U.S. EPA (Greene et al. 1989) and the ISO (1991a). They are similar except for the test medium. The first uses site soil diluted with artificial soil while the second is designed for testing liquid substances incorporated into artificial soil. Both artificial soils are essentially the same in composition. The U.S. EPA test with *E. andrei* is selected for the battery as it is standardized, iterations of it are used around the world, reproducibility is good (CVs range from 2.2% to 16.2%, Table 6), and it is designed for the assessment of contaminated sites.

The species recommended has a substantial history of toxicity testing but is not native to Canada and typically inhabits compost rather than soil. (The use of *Lumbricus terrestris*, a soil-inhabiting species native to Canada, for soil toxicity testing is discussed in 4.3.2). The relevance of the standard soil used in the recommended test to Canadian soil conditions is discussed in Section 4.7.

4.4.2 Definitive Tests

As with the current screening battery (4.4.1), options for the definitive soil toxicity assessment battery are currently limited to tests using an alga (Environment Canada 1992c; Lower and Sutton 1987), vascular plants (seedling emergence, root elongation—Greene et al. 1989) and the 14-d earthworm survival test using *Eisenia andrei* (Greene et al. 1989) or *E. foetida* (ISO 1991a). For the reasons provided in 4.4.1, the algal test of Environment Canada and the seedling emergence and earthworm tests of Greene et al. (1989) are recommended for the definitive battery at this time (Fig. 3).

4.4.3 Recommendations for Augmenting the Usable Battery

4.4.3.1 Screening Tests

The screening tests in the usable battery include an algal growth inhibition test, a seedling emergence test with vascular plants, and a survival test using *Eisenia* (Greene et al. 1989). These tests represent only two trophic levels in the soil ecosystem. Missing are organisms that forage on the soil surface (e.g., spiders, mites) and bacteria that mediate microbial processes. It is recommended that the set of screening tests be augmented to include tests with algae (Environment Canada 1992c), bacteria, vascular plants (seedling emergence, Greene et al. 1989), arthropods, and earthworms (*Eisenia*, Greene et al. 1989/*Lumbricus terrestris*), as shown in Figure 2. Only additions or changes to the set of screening tests described under the usable battery are discussed below. See 4.4.1 for a discussion of the tests retained from the usable battery.

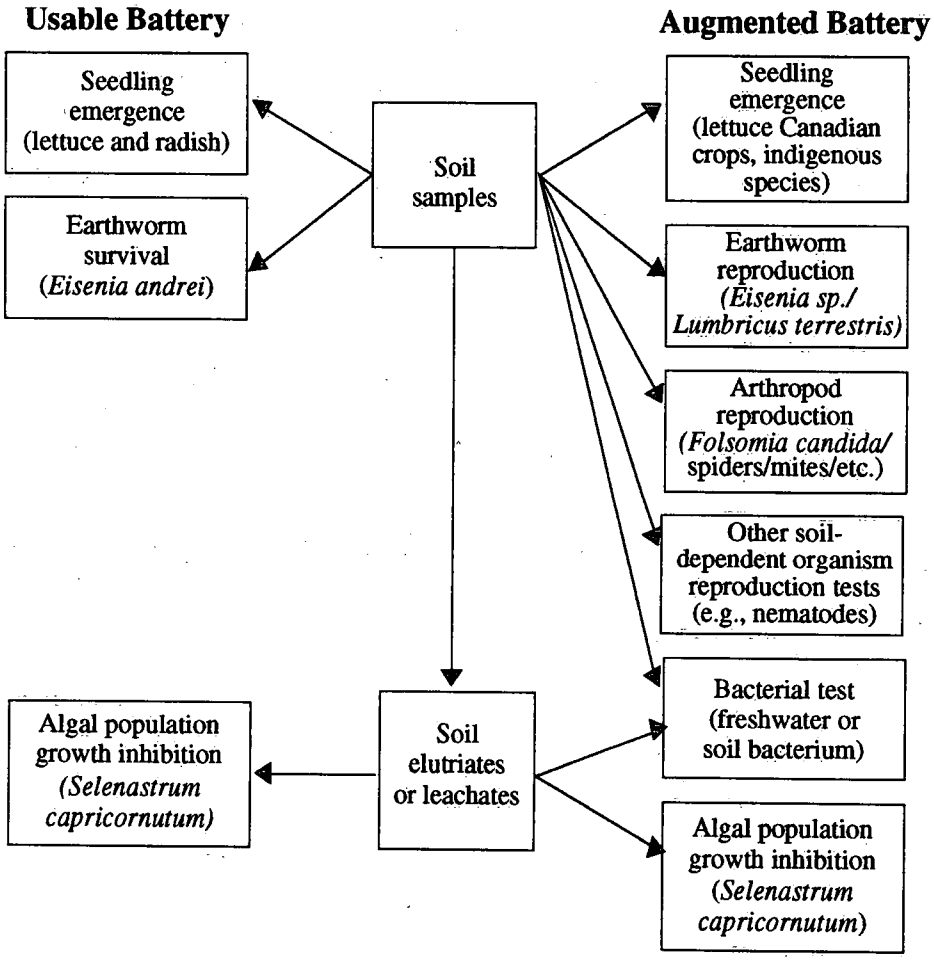

Figure 3. Definitive tests recommended for the usable and augmented batteries for soil quality assessment (see 4.4.2 and 4.4.3.2 for additional details).

Bacterial Test

Ideally, soil toxicity to bacteria should be examined using a representative soil bacterium or freshwater bacterium and the soil as a medium. An aquatic test using elutriates or leachates and the marine bacterium *Photobacterium phosphoreum* has been used for assessing soil toxicity and shown to be variably sensitive (Table 4). A solid-phase test using this bacterium has been developed by Microbics Corporation (1992a, b). The appropriateness of using a marine bacterium, however, should be assessed (see section 7). The Toxi-chromotest™ using *Escherichia coli* has shown variable sensitivity to contaminants (Table 16). Neither test is recommended at this time, pending the results of comparative testing and resolution of test design deficiencies.

Seedling Emergence Test

It is recommended that lettuce be retained in the vascular plant test because of its sensitivity and historical toxicity testing database and that at least one more species be added. The second species should be of a different family and an economically significant crop species. While wheat is an important crop in Canada, it often showed lower sensitivity than other species (Table 5a) and is not recommended as the second test species. An analysis of information on the relative sensitivity of other crops to a variety of substances will indicate which species are most promising as test organisms. The analysis by Kenaga (1981) in Table 5a, which looked at thousands of substances, suggests that soybean and corn would be good candidates.

Earthworm Test

Environment Canada is sponsoring a study to determine the relative sensitivity of the earthworms *Eisenia foetida* (not native to Canada, a compost-inhabiting worm) and *Lumbricus terrestris* (native to Canada, a soil-inhabiting worm). If the native species is shown to be significantly more sensitive than the compost worm and the drawbacks associated with using it as a test species (4.3.2) can be overcome, a test method should be developed for using *L. terrestris* to assess contaminated soil.

Arthropod Test

A short-term variant of the springtail test (ISO 1991d, OECD 1990) to assess survival may be appropriate for a screening battery. Tests for other arthropods including a second species of springtail, an orbatid mite, two species of diplopoda, and two species of isopoda are being developed in the Netherlands (D. de Zwart, pers. comm.). A test has been developed for assessing the effects of pesticide residues on spiders (Aukema et al. 1990) that might form a basis for developing a test for soil toxicity.

4.4.3.2 Definitive Tests

Definitive tests in the usable battery (4.4.2) are short term except for the algal test. There is a need to broaden the trophic representation and increase test duration. Those in the augmented battery address toxic effects on chronic survival and reproduction and involve additional soil-dependent organisms. Figure 3 shows the definitive tests recommended for the augmented battery. Only additions or changes to the set of definitive tests described under the usable battery are discussed below. See 4.4.2 for a discussion of the tests retained from the usable battery.

Bacterial Test

See discussion in 4.4.3.1.

Seedling Emergence Test

The seedling emergence test in the usable battery is retained, but the species used should reflect sensitive Canadian crops or keystone species of native plant communities that are significant to the region or site(s) in question (see 4.7).

Earthworm Test

The earthworm reproduction test using *E. foetida* or *E. andrei* being developed by the ISO (1991b) may prove to be appropriate to replace the 14-d earthworm survival test recommended in the usable battery. The comparative testing program for *E. foetida* and *L. terrestris*, sponsored by Environment Canada (see 4.3.2), will provide some insight into the utility of developing and using a chronic test with *L. terrestris* as an alternative test in the augmented battery.

Arthropod Reproduction Test

The reproductive test with springtails using *Folsomia candida* (ISO 1991d, OECD 1990) may be appropriate for the augmented battery. For a discussion of other possibilities for tests with arthropods, see 4.4.3.1.

Other Soil-Dependent Organism Tests

Tests for other soil-dependent organisms are being developed in the Netherlands, including a terrestrial nematode (*Plectus parientus*) and a mole (D. de Zwart, pers. comm.).

4.5 Prototype Tests

Of the 18 tests evaluated in this review, 9 initially fell into the prototype category. Of these, 4 were promoted to the usable category for the reasons described

in 4.4. The remaining 5 test prototypes (tests missing 'must' criteria but having a score of ≥88% for 'want' criteria; see 3.2.2) are listed below along with the work required to make them usable.

- earthworm survival, *L. terrestris* (expected reference toxicant value), U.S. FDA (Eirkson et al. 1987)
- earthworm survival, *Eisenia foetida* (expected reference toxicant level) (OECD 1984a)
- earthworm reproduction, *Eisenia foetida/andrei* (reference toxicant) (ISO 1991b)
- seedling emergence (reference toxicant) (ASTM 1990e)
- springtail (reference toxicant) (OECD 1990)

Examination of these prototype tests shows that only the first, third, and last are different from those already considered usable (4.4) and thus are considered of priority for further attention. For the springtail test and the earthworm reproduction test, which are draft tests and not yet widely applied, it is unlikely that much information is currently available on their reproducibility and sensitivity.

Based on the scores for these tests (4.2.2) and the methods outlined in Figure 1, initial priorities for further work on these tests are as follows:

Priority 2 (score 100%)

earthworm survival, *L. terrestris*

Priority 3 (score 88 - <100%)

springtail, *Folsomia candida*
earthworm reproduction, *E. foetida/andrei*

4.6 Tests under Development

The seven tests listed below did not meet the 'must' criteria, scored <88% for the 'want' criteria, and are considered of the lowest priority for concern (Fig. 1) at this point in time. Five of these are old tests for which newer versions are usable (e.g., TSCA seed germination), and the fourth test is derived from the usable test of Greene et al. (1989).

- seedling emergence (OECD 1984b)
- seedling emergence U.S. EPA (FIFRA) (Holst and Ellwanger 1982)
- seed germination (TSCA) (U.S. EPA 1985c)
- root elongation (ASTM 1990f)
- plant growth, U.S. EPA (FIFRA) (Holst and Ellwanger 1982)
- seedling growth (TSCA) (U.S. EPA, 1985d)
- flower production, ASTM (Lower 1990)

4.7 Priorities for Assessing Soil Quality with Bioassays

In this section, priorities for work required to meet the needs of the National Contaminated Sites Remediation Program related to the assessment and remediation of soils in Canada are described, beginning with the work of highest priority. Priority work required to upgrade prototype tests reviewed to usable tests (4.5) is integrated with additional areas of work considered essential for implementing the recommended test batteries. For a discussion of the rationale for identifying these tasks as priority items, see sections 3.5, 4.4, and 4.5.

1) Identify sensitive terrestrial plant species most suitable for soil toxicity testing in Canada

The need to use terrestrial plants that are of economic importance to Canada as agricultural crops or are keystone species of native plant communities was identified in Section 4.4.1. To identify the species most appropriate for the screening and definitive tests a detailed review should be carried out to cover candidate species, relative sensitivity to toxicants (levels that result in toxicity, frequency of toxic response), test reproducibility, seed sources, and the identification of needs for research to support the recommendations for test species inclusion in the batteries. Lettuce should be retained as a universal test species for comparative purposes and considered comparable to the laboratory white rat.

2) Determine the species of earthworm to be used in testing

Eisenia foetida, used in many bioassays, can be found worldwide in its specific habitat of manure piles, compost heaps, and soils with a high proportion of organic matter (Fender 1985). *Eisenia andrei*, however, inhabits the drier parts of manure piles inhabited by *E. foetida* and is often most abundant in or below the soil contact zone (Fender 1985). *Eisenia andrei* is used in the U.S. EPA earthworm survival test that was recommended for the usable battery. "CERL has used *andrei* as its test organism for the last two years" (p. 45, Sec. A.5.6.4, Greene et al. 1989). The use of *Lumbricus terrestris*, a common soil-inhabiting species in Canada, is currently being examined by Environment Canada (see 4.4.1, R. Kent, pers. comm.).

The decision to replace *Eisenia* by *L. terrestris* in the screening set of tests should depend on the results of this work as well as comparison of other differences between these species, such as culturing abilities (see 4.3.2). The assessment of relative sensitivity should cover both the levels that result in a specific endpoint (LC_{50}) and the duration of exposure. For example, the results from approximately 40 unpublished tests with *E. andrei* demonstrated that the LC_{50} results for 7- and 14-d exposures were the same (J. Greene, pers. comm.). The assessment should be made under at least two sets of conditions — those optimal for *E. foetida*/*E. andrei* and those optimal for *L. terrestris*.

3) Develop tests for additional groups of soil-dependent organisms

Only two trophic levels are represented in the current screening and definitive test batteries. Thus there is an urgent need for tests with additional soil-dependent species, particularly for the definitive test battery, for which no chronic tests are considered usable (4.4.2).

Among the prototype tests, a high priority is completion of the one using springtails (*Folsomia candida* — OECD 1990, ISO 1991d), which are very abundant and ecologically important decomposers, and its adaptation for soil testing in Canada. Work is currently under way in the Netherlands on tests involving predatory mites, an isopod, a diplopod, another species of springtail, a mole, and a nematode (D. de Zwart, pers. comm.). These tests are scheduled for completion in 1993 (D. de Zwart, pers. comm.). They should be considered for adoption and expansion of the definitive test battery.

Another area that should be examined is the adaptation of tests involving soil-dwelling organisms and aqueous solutions of single chemicals for use with soil samples or soil elutriates. The recent test with erigonid and linyphiid spiders (Aukema et al. 1990) is an example.

To further guide the development of tests for additional groups of soil-dependent organisms, there should be a thorough evaluation of the ecological importance of potential test organisms, which would include those for which tests are in preparation and groups not currently under investigation. With this information and available data on sensitivity, ease of acquisition and culture, candidate organisms and tests could be identified for soil testing in Canada. Following test development, comparative testing to examine relative sensitivities and reproducibility would be required in the context not only of pure compounds but also in mixtures found in samples obtained from contaminated sites. Canadian laboratories, in cooperation with those in the United States, should be encouraged to develop test methods and evaluate them through intralaboratory and interlaboratory testing programs.

4) Develop a reproductive test for earthworms

Chronic tests for any soil-dependent organism are currently unavailable for use in a definitive soil test battery. The current protocol for testing earthworm survival is a moderately insensitive soil test. The use of reproductive endpoints might improve sensitivity. If the results of the comparative sensitivity study involving *E. foetida* and *L. terrestris* show that *Eisenia* is not significantly less sensitive than *L. terrestris*, the draft reproductive test for *E. andrei*/*E. foetida* (ISO 1991b) could be used as a basis for developing a Canadian test. If *L. terrestris* is shown to be significantly more sensitive, the development of a comparable test for reproduction using this species would be more appropriate.

Concerning reproductive tests, a study of nine chemicals showed that the sensitivity of reproductive endpoints for *E. andrei* varied within and between chemicals (van Gestel 1991c). For example, the weekly number of cocoons per worm was a more sensitive measure of cadmium toxicity than was the number of juveniles per fertilized cocoon while, for chromium, the weekly number of juveniles per worm was the most sensitive endpoint. For the nine chemicals, LC_{50}s and NOECs differed by factors of 5 (pentachlorophenol) to 100 (cadmium).

The importance of a pre-test acclimation period to control soil to stimulate reproductive activity for *E. andrei* was indicated by van Gestel (1991b). It was also shown that cocoon production (OECD artificial soil) was reduced at pH $\geq$7 and optimal at 20°C, at a moisture content of 85% (exceeds field capacity). The results indicate that standardization of reproductive tests must include strict adherence to pH limits and that a pH (5.0-6.0) lower than that indicated by OECD (6.0 ± 0.5) would be better for reproduction tests. The moisture content for the OECD acute test (55%) is much lower than the optimal for reproductive tests while that for the screening test (75%, Greene et al. 1989) is closer to this level.

5) Prepare a handbook for statistical guidance

A weakness of many of the tests reviewed was inadequate statistical guidance. The need for a handbook on statistical guidance is common to all three media and is discussed in section 7.

6) Re-evaluate bacteria for soil toxicity testing

The screening battery should have a test where the bacterium is in the soil. The marine bacterium *Photobacterium phosphoreum* has been widely used to assess the toxicity of soil elutriates, but its relevance as

a surrogate for soil or freshwater bacteria is questionable (see 7.0).

7) Determine a set of standard substrates for use in Canadian soil toxicity tests

Standard substrates are required for soil toxicity tests to serve as a negative control and diluent. Differences in the composition of artificial soils can affect the results of toxicity tests (van Gestel 1991a). For example, 14-d tests with the earthworm *E. andrei* using three soils, including artificial soil (OECD 1984d), showed that EC_{50} values differed between soils of the same pH and between soils identical except for pH, illustrating the importance of soil characteristics as well as substance in determining toxicity (van Gestel 1991a). It is therefore important to adopt a standard soil (or soils) for testing. This could be the artificial soil already defined (Greene et al. 1989), an artificial soil defined for Canada, or a natural soil as discussed below.

The artificial soil used in the recommended earthworm test is an international standard. The results of tests using this soil may be comparable to an immense database for numerous substances, but are the results relevant to soil conditions in Canada? The composition of the recommended artificial soil is not typical of the average agricultural soil in Canada in two major respects.

While the average Canadian agricultural soil contains about 20% clay (M. Schnitzer, Agriculture Canada, pers. comm.), micaceous or smectitic clay minerals, not kaolinite clay minerals, dominate (35%). Altering the type of clay minerals present in the soil changes the surface area, which controls the concentration of inorganic and organic contaminants that can be absorbed and changes the cation exchange capacity, which determines how many metal or organic cations can interact with the clay mineral. Smectite is many times more active in both respects than kaolinite. To better represent the clay mineral content of Canadian soils a mixture of 10% kaolinite and 10% smectite, rather than 20% kaolinite, should be investigated as a potential substitute for the standard artificial soil.

The second condition that is not typical of soils is the form of the organic matter. In soils, most of the organic matter has been humified. This is the conversion by microbes (or chemically) of plant and animal residues to complex polymeric substances with large numbers of oxygen-containing functional groups with large surface areas (M. Schnitzer 1978). *Sphagnum* peat has not undergone these reactions. Since the average Canadian agricultural soil contains about 5% organic matter (M. Schnitzer, pers. comm.), the artificial soil could be made more comparable to Canadian soils, in terms of organic matter, by substituting 5% mature plant and animal compost for the 10% peat.

Rather than using an artificial soil as a diluent, reference soils could also be used which would be representative of those found in Canada. Considering the variability of Canadian soils, more than one standard soil should be considered. Two reference soils, representing a sandy loam (e.g., Bainsville, 20% clay, 5% organic matter) and a clay loam (e.g., Melfort, 45-50% clay, 10% organic matter), are recommended for use in Canada. They are described in detail by Schnitzer and Schuppli (1989).

The suggested modifications to the standardized artificial soil formula must be thoroughly evaluated by rigorous testing and comparison to the internationally accepted standard artificial soil prior to their inclusion in a Canadianized standard testing protocol. A most important factor is to determine that the earthworm could successfully grow and reproduce in the "experimental" soil matrix. The merits, development, and use of Canadian artificial soils are not addressed by this publication and they have not been addressed to any level of detail under the NCSRP.

The use of the artificial soil in Greene et al. (1989) and three natural Canadian soils for earthworm testing is being assessed by Environment Canada (R. Kent, pers. comm.; see 4.3.2).

The standard soil(s) chosen will be used in all tests involving soil-dwelling animals. Its adoption in the seedling emergence test as well would provide test consistency.

8) Prepare standard methods for the collection, storage, and preparation of soil samples

The use of appropriate standard techniques for obtaining test samples is critical for correct interpretation and comparability of the results of the biological tests conducted to assess site contamination. Such a manual should cover soil samples as well as elutriate and leachate preparation.

While general guidance on the use of leachates and elutriates is given in Environment Canada's protocols for toxicity testing (e.g., Environment Canada 1992b), more specific detail (leaching agent, soil : water ratio, etc.) concerning their preparation is required to improve test standardization.

The most immediate toxic and subtoxic fractions of substances are those soluble in water. Some guidance for elutriate preparation is given in Daniels et al. (1989). A standardized soil/sediment elution procedure has been published (Greene et al. 1989). Elutriates are prepared by adding 1 mL of water to 4 g (dry wt.) of soil or sediment. The mixtures are eluted (end over end) in the dark at 20 ± 2°C for 48 h. The duration of extraction described in this method appears to be

excessively long and is in need of evaluation (J. Greene, pers. comm.).

Factors affecting the preparation of water-soluble fractions of oils by the slow stirring method have been evaluated (Maher 1986). The toxicity of leachates is discussed by Epler et al. (1980). Testing with *Photobacterium phosphoreum* and *Daphnia magna* showed that the sensitivity of bioassays depends on the methods used to obtain leachates (Calleja et al. 1986).

The U.S. EPA (Greene et al. 1989) has published guidance on appropriate measures for the packaging and shipping of hazardous chemical wastes. Sample collection was not addressed. Recently, the ASTM (1985, 1987a, 1990g) published three standard practices aimed at providing proper guidance for sampling solids and groundwater. In each of the aquatic test methods prepared by Environment Canada, sample transportation and storage is addressed, and in the more recent tests (e.g., Environment Canada 1992a), sample collection is also briefly discussed.

9) Prepare a manual for field sampling guidance

A manual for designing field sampling schemes is required to ensure that the collection techniques (point 8 above) are applied appropriately (see 7.0).

5.0 ASSESSMENT AND RECOMMENDATIONS FOR FRESHWATER SEDIMENT TESTS

Until the recent development of tests using sediment-dependent organisms, sediment elutriate or pore water was used to assess sediment toxicity by means of water quality tests with water column organisms. Generally, water quality tests are most appropriate for water overlying the contaminated sediment.

In this section, however, we consider one aquatic test for assessing sediment toxicity to plants. In the absence of tests with rooted aquatic plant species, the algal test using *Selenastrum capricornutum* (Environment Canada 1992c) is considered for sediment toxicity assessment. This test

Table 7

Species with test methods (for assessing sediment quality) from recognized standards organizations and the literature

(ASTM= American Society for Testing and Materials, EC= Environment Canada)

Organism group	Species	Organization/ Reference	Test type
Algae	*Selenastrum capricornutum*	EC (1992c)	chronic, growth, reproduction
Amphipods	*Hyalella azteca*	ASTM (1990b)	chronic, survival, growth
Oligochaetes	*Lumbriculus variegatus*	Phipps et al. (1991)	acute, chronic, survival
	Tubifex tubifex	ASTM (draft)	chronic, survival, reproduction
Mayflies	*Hexagenia* spp.	Bedard et al. (1992)	acute, survival; chronic, survival, growth
	Hexagenia spp.	Bedard and Henry (1992)	
Midges	*Chironomus riparius*	ASTM (1990b)	chronic, survival, growth
	C. tentans	ASTM, Bedard et al. (1992).	chronic, survival, growth

was selected because this species exhibited a toxic response to the largest proportion (85%) of 185 soil and sediment elutriates, water, and wastewater samples that were toxic to a three-species (the alga, *Daphnia magna*, *Photobacterium phosphoreum*) test battery (Greene and Barich 1991). (*Photobacterium phosphoreum*, although commonly used for toxicity assessment, showed a toxic response to only 36% of these samples and only 8 (4%) were not toxic to either the alga or the daphnid.)

5.1 Test Methods and Candidate Organisms

The results of the literature review of organisms used in sediment testing are summarized in two tables. Those meeting the first criterion considered essential for retaining the test for further evaluation of suitability (appropriate printed test method, see 3.2.1 and 5.2.1) are found in Table 7. Table 8 lists the organisms for which test methods do not meet this criterion and are not considered further.

Nineteen sediment-dependent species from eight major groups of organisms were identified in connection with sediment toxicity testing (tables 7 and 8). Of these groups, algae (1 sp.), amphipods (1 sp.), oligochaetes (2 spp.), mayflies (2 spp.), and midges (2 spp.) had appropriate printed test methods.

5.2 Step 1 Assessment — Test Methodology

5.2.1 Preliminary Assessment

The tests identified in 5.1 and the algal test (Environment Canada 1992c) were first evaluated according to three criteria that are considered essential to a complete test method (acceptable printed method, acceptability criteria, reference toxicant; see 3.2.1 for definition and importance of criteria). The methodologies of the eight sediment tests that met the first criterion are summarized in Table 9. The algal test is described in Table 14.

Table 8

Organisms that have been used in the assessment of sediment quality but for which tests have not yet been prepared by recognized standards organizations or published in the literature

Organism group	Species	Organization/reference
Vascular plants	*Cyperus esculentus*	Folsom and Price (1989)
	Potamogeton pectinatus	Ailstock et al. (1991)
Oligochaetes	*Limnodrilus hoffmeisteri*	Wiederholm et al. (1987)
	L. claparedeanus	
	L. udekemianus	
	Potamothrix hammonienis	
	Stylodrilus heringianus	Keilty and Landrum (1990)
Amphipods	*Pontoporeia affinis*	Wiederholm et al. (1987)
	Simocephalus vetulus	Sloterdijk et al. (1989)
Isopods	*Asellus communis*	Prater and Anderson (1977)
Snails	*Juga plicifera*	Nebeker et al. (1986)
	Lithoglyphus virens	
	Physa gyrina	

Table 9

Brief descriptions of tests evaluated for assessing sediment quality

The percentage at the column head is the test score (see 3.2, 5.2, and Table B-2) and reflects methodology completeness (NS= not specified). Additional details are provided in Appendix C

	ASTM (1990b) 82%	ASTM (1990b) 82%	ASTM (1990b) 82%	Bedard and Henry (1992) 59%
Test type	amphipod, chronic, survival, growth, static/flow-through	midge larva, chronic, survival, growth, emergence, static/ flow-through	midge larva, chronic, survival, growth, emergence, static/ flow-through	burrowing mayfly, chronic, survival, growth, static/ flow-through
Application	toxicity of whole contaminated/ spiked sediment	toxicity of whole contaminated/ spiked sediment	toxicity of whole contaminated/ spiked sediment	toxicity of whole contaminated/ spiked sediment
Species	*Hyalella azteca*	*Chironomus tentans*	*Chironomus riparius*	*Hexagenia* spp.
Endpoints	survival, growth, reproduction, LC_{50}, EC_{50}	larval emergence, growth, survival; adult emergence LC_{50}, EC_{50}	larval emergence, growth, survival; adult emergence LC_{50}, EC_{50}	nymph survival, weight
Organism selection	2nd/3rd instars, 2-3mm long	2nd instars, from 3 separate egg cases	1st instars/ 3 d old larvae	<10mm long (young), 150-d old for 10-d and 7-d survival test
No. organisms + replicates	100 amphipods in 20L aquaria, at least 2 reps or 20 amphipods in 1L beakers, 4 reps	20, 25, 100 larvae for 2L, 3L, 20L containers; no. reps not specified	50, 130 for 1L, 13L containers; reps not specified	10 for 1L or 1.8L glass container, 5-10 for 23×6.4×16cm container, min. 3 reps
Observation frequency	≤ 10d short-term test, approx. 30 d for reproduction	day 10-14 for growth survival; day 20-25 daily counts of adults	day 10-14 for growth, survival; approx. day 30 for adult emergence	7/10 day survival; 21 day growth, survivorship
Volume test vessel	20L or 1L	2L or 3L, 20L	1L, 13L	1L, 1.8L or 23×6.4×16 cm
Volume test substance	200 mL (2cm deep) in 1L containers, 2-3cm in 20L containers	(2L) 2cm sediment + 1.5L water or (3L) 100g sediment + 2L water or (20L) 2-3cm sediment + 15cm water	(1L) 200mL sediment + 800mL water or (13L) 2L sediment + 11L water	(1L) 200mL sediment + 800mL water or (1.8L) 325mL sediment + 1300mL water or (23 6.4x16cm) 5cm sediment + 1000mL water sediment
Test substance preparation	field collection, spiking, addition to test chamber	field collection, spiking, addition to test chamber	field collection, spiking, addition to test chamber	field collection, addition to test chamber
Culture, handling	explicit details, feeding regime	explicit details	explicit details, culturing not easy	brood stock preparation, egg storage, handling

Table 9 (continued)

	ASTM, *Hyalella*	ASTM, *C. tentans*	ASTM, *C. riparius*	Bed. & Hen., Hexagenia spp.
Conditions (light, temp, pH, etc.)	538 lx, 16h light: 8h dark, 20-25°C	light as left, 20-23°C	light as left, 20-22°C	natural photoperiod/ 16h light: 8h dark, 17-22°C, O_2 1-10ppm in water (static test)
Acceptability criteria	control survival > 80%, temp variation ± 3°C	control survival > 70%, temp variation ± 3°C	see left	control survival >80%
Medium defn., manipulation	sediment characterization (pH, total organic carbon content, % sand silt, clay, % water content), details for static/flow-through systems, overlying water, feeding regime	see left	see left	NS
Negative control	reference sediment that is nontoxic characterization as for test sediment	see left	see left	NS
Reference toxicant	NS	NS	NS	NS
Statistical analysis	LC_{50}, EC_{50} with 95% CI, several methods cited	see left	see left	NS
Organism availability	easily	easily	easily	moderate

	ASTM (draft) 82%	Bedard et al. (1992) 88%	Bedard et al. (1992) 88%	Phipps et al. (1991) 71%
Test type	oligochaete, chronic, survival, reproduction, static	burrowing mayfly, acute survival, chronic, growth, static	midge larva, chronic, survival, growth, static	oligochaete, growth, reproduction, chronic, static-renewal/flow-through
Application	toxicity of whole contaminated sediment, spiked sediment	toxicity of whole sediment	whole sediment	sediment elutriates, pore water
Species	*Tubifex tubifex*	*Hexagenia* spp.	*Chironomus tentans*	*Lumbriculus variegatus*
Endpoints	adult survival, cocoons produced, % hatch cocoons, total young, cocoons/adult, young/cocoon, young/ adult	% survival, fresh weight	% survival, fresh weight	survival, dry weight, reproduction

Table 9 (continued)

	ASTM (draft) 82%	Bedard et al. (1992) 88%	Bedard et al. (1992) 88%	Phipps et al. (1991) 71%
Organism selection	sexually mature	3-4 mo old, average wt 5mg	10-12 d old, second instar, average wt < 1mg	NS
No. organisms + replicates	4 worms, 5 reps, concentrations depend on study	10 nymphs, 3 reps	15 larvae, 3 reps	10 worms, 8 reps
Observation frequency	every 2-4 d for 28 d	daily for 10 d survival, 21 d growth	daily for 10 d	10-28 d
Volume test vessel	250mL glass beaker	1.8L (11.5×11.5× 14.5cm)	see left	300mL beaker
Volume test substance	100mL sediment, 100mL water	325mL sediment, 1300mL water	see left	100mL sediment, 100-150mL water
Test substance preparation	field collection, addition to test chamber	field collection storage, addition to chamber	see left	spiking, addition to test chamber
Culture, handling	culture initiation, worm transfer	rearing methods, handling	see left	collection, culture, acclimation, feeding, handling
Conditions (light, temp, pH, etc.)	dark, 23±1°C	20±2°C, 16h light:8h dark, fluorescent light	see left	cool white fluorescent, 16h light:8h dark, < 25°C, >60% DO
Acceptability criteria	reference toxicant results within 2 SD of mean of 20 reference tests; control production of young within 1 SD of long term data	85% survival in control	75% survival in control	NS
Medium defn., manipulation	soil characterization (pH, organic carbon content, particle size distribution, % water content); sieving to remove large microfauna	site sediment characterization	see left	site sediment characterization
Negative control	uncontaminated control sediment (organic content >12%, 70-90% sand, 7-22% silt, 4-7% clay)	uncontaminated control sediment	see left	reference sediment that is nontoxic, characterization as for test sediment

Table 9 (continued)

	ASTM (draft) *Tubifex*	Bedard et al., *Hexagenia* spp.	Bedard et al., *C. tentans*	Phipps et al., *Lumbriculus*
Reference toxicant	NS	NS	NS	NS
Statistical analysis	NS	One-way ANOVA, comparative t-tests	see left	NS
Organism availability	easily	moderate	easily	easily

While the algal test, after the addition of reference toxicant information from the literature, satisfied the remaining two criteria, no test with sediment-dwelling organisms satisfied both of them. Seven of the tests provided acceptability criteria but no reference toxicant: *Hyalella azteca* (ASTM 1990b); *Chironomus tentans* and *C. riparius* (ASTM 1990b); *Tubifex tubifex* (ASTM draft); *Hexagenia* spp. and *Chironomus tentans*, Ontario Ministry of the Environment (Bedard et al. 1992); *Hexagenia* spp. (Bedard and Henry 1992); and one, *Lumbriculus variegatus*, U.S. EPA (Phipps et al. 1991), had neither.

5.2.2 Detailed Evaluation

The nine tests with written methods (Tables 9, 14) were further assessed in terms of the 12 'want' criteria (described in 3.2.2) that are valuable but not as important as the three 'must' criteria.

Test scores ranged from 59% to 100%, as shown by the bold number at the top of the columns in Table 9 and Table 14 (algal test). The rationale for these scores is provided in tables B-2 and B-3, Appendix B. The results are summarized below:

1 test scored 100%

algal growth (Environment Canada 1992c)

2 tests scored ≥88% and <100%

88% - *Chironomus tentans*, OMOE (Bedard et al. 1992)
88% - *Hexagenia* spp., OMOE (Bedard et al. 1992)

6 tests scored <88%

82% - *Hyalella azteca* (ASTM 1990b)
82% - *Chironomus tentans* (ASTM 1990b)
82% - *Chironomus riparius* (ASTM 1990b)
82% - *Tubifex tubifex* (ASTM draft)
71% - *Lumbriculus variegatus*, U.S. EPA (Phipps et al. 1991)
59% - *Hexagenia* spp. (Bedard and Henry 1992)

The results of this evaluation are further discussed and interpreted in terms of priorities for future work in sections 5.5, 5.6, and 5.7.

5.3 Step 2 Assessment — Test Application

Because so few tests scored ≥88% under the detailed evaluation (5.2.2), the threshold score for consideration under Step 2 was lowered to ≥80% for sediment tests. Additional information on trophic level represented, test sensitivity, test reproducibility, field validation, and ecological relevance is provided for all tests scoring ≥80% under the detailed evaluation.

5.3.1 Algal Test

A method specifically designed for testing solutions collected from hazardous chemical waste sites was published in 1983 by Porcella. The test, without modification, was republished in Greene et al. (1989). More recently Environment Canada has supported the development of a microplate technique. Development of the technique is near completion and a draft protocol has been circulated for review (Environment Canada 1992c).

Trophic Level

Algae are natural inhabitants of water and are an extremely important group of plant organisms. Through their photosynthetic activity they help to provide the

oxygen necessary for the survival of animal species found in the aquatic environment. Algae contribute to the purification of streams, lakes, and estuaries, and also serve as the basis of the food chain within the aquatic ecosystem.

Sensitivity

Sensitivity of the green alga *Selenastrum capricornutum* relative to organisms other than algae, is shown in tables 10 and 15. These tables show that this alga was less sensitive to 19 nonpesticide organic compounds than *Daphnia magna* and more sensitive to heavy metals and insecticides than *Photobacterium phosphoreum* and *D. magna*.

Concerning effluents and waters contaminated with a mixture of chemicals, *S. capricornutum* was more sensitive to pulp and paper effluent than *P. phosphoreum* and rainbow trout and more sensitive to 11 industrial effluents (e.g., paper mill, textile dyeing, oil refinery, leather tanning) than *D. magna*. The alga was less sensitive to creosote-contaminated water and sediment elutriates than *D. magna* and *P. phosphoreum*, respectively. *Selenastrum capricornutum* was less sensitive than *P. phosphoreum* and more sensitive than the rotifer *Brachionus calyciflorus*, the nematode *Panagrellus redivivus* and *D. pulex* to elutriates from river sediment.

In tests performed on leachates or elutriates from sanitary landfills and soil containing heavy metals, pesticides and polynuclear aromatic hydrocarbons, herbicides, insecticides and neurotoxins, *S. capricornutum* was more sensitive than *P. phosphoreum* and *D. magna*.

When the results of tests with 326 water, waste, and sediment/soil elutriates were examined, *S. capricornutum* responded to the toxic constituents contained in the samples more often than did *D. magna* or *P. phosphoreum*. *Photobacterium phosphoreum* responded to only 36% of the samples that were toxic to either *S. capricornutum* or *D. magna*, or both.

The information above indicates that *Selenastrum capricornutum* is sensitive to a variety of toxic substances in water and sediment/soil elutriates and soil leachates. In many cases, it shows greater sensitivity than do numerous other aquatic organisms.

Lewis (1990) shows that the relative sensitivity of algal species to the same toxicant can vary by more than 2000 times (disodium hydrogen arsenate, 13 spp.). As well, the toxicity of one group of compounds to one species of alga may vary from two (nonionic surfactants, *Microcystis aeruginosa*) to more than 100 times (organic acids, *M. aeruginosa*). In a comparison of *S. capricornutum* and *Chlorella vulgaris* with 21 herbicides, the former was most sensitive to all but two (Garten and Frank 1984).

Reproducibility

Table 17 shows that both the microplate and flask methods typically show good reproducibility with coefficients of variation of less than 30%.

Ecological Relevance

The alga is a surrogate plant species for plant species rooted in the sediment. The correlation between the responses of these two groups of plants is likely to vary depending upon the toxicants in the sediment, but both are primary producers. When the overlying water is to be assessed for toxicity, additional aquatic tests recommended in section 6 could be employed.

5.3.2 Amphipod Test

A test for assessing sediment toxicity using *Hyalella azteca* has been prepared through ASTM (1990b). Borgmann and Munawar (1989) and Borgmann et al. (1989) also provide information on test procedures using *H. azteca*.

Trophic Level

Hyalella azteca is an epibenthic detritivore that dwells on the sediment surface and feeds on algae and detritus (Borgmann et al. 1989). It is the principal prey of many fish, birds, and larger invertebrate species. This species has a wide tolerance to sediment grain size and is found in many surface waters.

Sensitivity

The sensitivity of *Hyalella azteca* relative to other test organisms is variable, as shown in Table 10. It was more sensitive than *Lumbriculus variegatus*, *Ceriodaphnia dubia*, and the fathead minnow to lake sediment pore water and elutriates. For contaminated lake sediments, *H. azteca* was as sensitive as *D. magna* (Munawar et al. 1989) and less sensitive than *Chironomus riparius* (Ingersoll and Nelson 1990). It was as sensitive to harbour sediment as *D. magna* and *C. dubia*, or less so. *Hyalella azeca* was equally sensitive to cadmium-spiked sediments as *D. magna*, but less sensitive to copper-spiked sediments than *Chironomus tentans* and *D. magna*. Sensitivity was significantly different in static and flow-through tests (Ingersoll and Nelson 1990).

Table 10 shows that *Hyalella azteca* was generally more sensitive to toxic sediments or aqueous derivatives than *D. magna* and less sensitive to toxic sediment than both *Chironomus* species.

Table 10

Relative sensitivity of organisms used (in tests reviewed in this document) for assessing sediment quality

The lower the number, the higher the sensitivity. The endpoints listed correspond with the organisms tested in order from left to right. In the second study, for example, the endpoint for the 30-day *C. riparius* test was emergence while the endpoint for the 21-day *D. magna* test was reproduction. As well, the LC_{50} was determined for both species in 48-hour tests. Unless specified, tests with sediment invertebrates are conducted with whole sediment.

(Cr= *Chironomus riparius*, Ct= *C. tentans*, Ha= *Hyalella azteca*, He= *Hexagenia limbata* [=*H. bilinata* in second study], L= *Lumbriculus variegatus*, P= *Photobacterium phosphoreum*, S= *Selenastrum capricornutum*, D= *Daphnia magna* [=*D. pulex* in second last study], C= *Ceriodaphnia dubia*, F= fathead minnow, R= rainbow trout, e= elutriate, f= flowthrough, pw= pore water, w= water, s= sediment)

Test Substance		Endpoint	Species Cr	Ct	Ha	He	L	P	S	D	C	F	R	Reference
Lake sediment	f	29 d emergence 29 d survival	1	-	2	-	-	-	-	-	-	-	-	Ingersoll and Nelson (1990)
Waterborne selenium		30 d emergence 21 d reprod. 48 h LC_{50}	2 2	- -	- -	- -	- -	- -	- -	1 1	- -	- -	- -	Ingersoll et al. (1990)
Lake sediment		10 d LC_{50} 168 h LC_{50}	-	1	-	2	-	-	-	-	-	-	-	Giesy et al. (1990)
Lake sediment	pw1 pw2	10 d LC_{50} 168 h LC_{50} 15 min IC_{50} 48 h LC_{50}	- -	3 2	- -	1 1	- -	4 3	- -	2 4	- -	- -	- -	Giesy et al. (1990)
Copper-spiked sediment		10 d LC_{50} 10 d LC_{50} 48 h LC_{50}	-	2	3	-	-	-	-	1	-	-	-	Cairns et al. (1984)
Cadmium-spiked sediment		96 h/10d LC_{50} 48 h LC_{50}	-	1	-	-	-	-	2	-	-	-	-	Nebeker et al. (1986)

Table 10 (continued)

Test Substance		Endpoint	Species Cr	Ct	Ha	He	L	P	S	D	C	F	R	Reference
Pond sediment		15 d larval surv. 10 d survival	-	1	-	-	-	-	-	2	-	-	-	Nebeker et al. (1988)
		25 d emergence 10 d survival	-	1	-	-	-	-	-	2	-	-	-	
Lake sediment	pw e	96 h LC_{50} 96 h LC_{50} 48 h LC_{50} 96 h LC_{50} larvae	- -	- -	1 1	- -	4 4	- -	- -	- -	2 2	3 2	- -	Ankley et al. (1991)
Lake sediment		28 d survival 48 h survival	-	-	1	-	-	-	-	1	-	-	-	Munawar et al. (1989)
Harbour sediment		48 h survival	-	-	2	-	-	-	-	2	1	-	-	Burton et al. (1989)
Harbour sediment	e	48 h growth 48 h survival 48 h survival 48 h survival	-	-	4	-	-	-	3	2	1	-	-	Burton et al. (1989)
Metal-contaminated sediment		10 d survival 48 h survival	-	-	-	2	-	-	-	1	-	-	-	Malueg et al. (1984)
Waterborne hexachloro-ethane smoke combustion products		48 h LC_{50} w 48 h LC_{50} 96 h LC_{50} 96 h LC_{50}	-	-	-	4	-	-	-	2	-	3	1	Fisher et al. (1990)

Table 10 (continued)

Test Substance		Endpoint	Species											Reference
			Cr	Ct	Ha	He	L	P	S	D	C	F	R	
Creosote contaminated sediment	e	30 min IC_{50} 96 h IC_{50} growth 48 h LC_{50}	-	-	-	-	-	1	2	3	-	-	-	Athey et al. (1989)
River sediment	e	15 min IC_{50} 3 h ^{14}C uptake 48 h survival	-	-	-	-	-	1	2	3	-	-	-	Sloterdijk et al. (1989)
326 samples water, wastes, soil/sediment elutriates		30 min IC_{50} 96 h IC_{50} growth 48 h LC_{50}	-	-	-	-	-	3	1	2	-	-	-	Greene and Barich (1991)

Reproducibility

In clean sediments from near-shore regions of the Great Lakes, coefficients of variation for amphipod tests were 6.9 ± 3.9% for survival and 15.0 ± 10.2% for growth (K. Day, NWRI, pers. comm).

Field Validation

The U.S. EPA in Duluth is initiating field validation studies for *H. azteca* (Phipps et al. 1991).

Ecological Relevance

Hyalella azteca is an amphipod that is found in Canada. It typically dwells on the surface and tolerates a wide range of sediment grain sizes. Ingersoll and Nelson (1990) observed no reduction in survival or growth in the laboratory with sediment ranging from >90% silt- and clay-sized particles to 100% sand-sized particles.

5.3.3 Midge Tests

Chironomus spp. have been recommended as routine whole sediment and interstitial water test species (Nebeker et al. 1984, Dwyer et al. 1991, Reynoldson and Day 1993).

Trophic Level

The larvae of the midges *Chironomus tentans* and *C. riparius* dwell in tubes built in the sediment. They often make up a large portion of the benthic biomass and are important in the cycling of residues into and from the sediment. They are important in the diets of young and adult fish and ducks (ASTM 1990b).

Sensitivity

The sensitivity of *Chironomus* spp. relative to other test organisms is shown in Table 10. *C. riparius* was less sensitive to waterborne selenium than *Daphnia magna*. Growth of *C. riparius* has been shown to be correlated with *P. phosphoreum* effect concentrations, *Hexagenia limbata* and *D. magna* response, benthic community health, and has discriminated areas of contamination (Burton 1991).

For contaminated pond sediments, *C. tentans* was more sensitive than *D. magna* (solid phase) when survival was used as the endpoint. Reproductive tests for *D. magna* and emergence tests with *C. tentans* (Nebeker et al. 1988) ranked all three lakes tested in the same order according to relative toxicity. In tests using sediment pore water, *C. tentans* was shown to be less sensitive than *H. limbata* and more sensitive than *P. phosphoreum*. With whole sediment tests, however, *C. tentans* was more sensitive than *H. limbata* (Giesy et al. 1990). The sensitivity of *C. tentans* to contaminated sediment was similar to that of *P. phosphoreum* when weight gain rather than LC_{50} was used for the midge.

Reproducibility

In clean sediments from near-shore regions of the Great Lakes, coefficients of variation for *C. riparius* were 13.1 ± 5.2% for survival and 10.7 ± 5.3% for growth (K. Day, pers. comm.). For the test with *C. tentans* prepared by Bedard et al. (1992), CVs for 36 samples of contaminated sediment ranged from 15 to 88% (mean = 62%) for mortality as an endpoint and from 8 to 19% (mean = 13%) for growth as an endpoint (D. Bedard, Ontario Ministry of the Environment, pers. comm., Table 11).

Field Validation

The U.S. EPA in Duluth is initiating field validation studies for *C. tentans* (Phipps et al. 1991).

Ecological Relevance

Chironomids are widely distributed in freshwater sediments during their larval stage of development (Giesy and Hoke 1989). *Chironomus tentans* is a common midge found in mid-continental Canada. The larvae occur in sediments with a range of physical compositions, and growth is enhanced for coarser substrates where >80% is sand (Bedard et al. 1992). *Chironomus riparius* is also indigenous to Canada and tolerates a wide range of particle sizes. Ingersoll and Nelson (1990) observed no reduction in survival or growth in the laboratory with sediment ranging from >90% silt- and clay-sized particles to 100% sand-sized particles. Burrowing into the sediment to build a case, chironomid larvae are in close proximity to the sediment and they are exposed to contaminants in the interstitial and overlying waters (Bedard et al. 1992).

5.3.4 Mayfly Tests

A test for *Hexagenia* spp. (a mixture of *H. limbata* + *H. rigida*) (Bedard et al. 1992) has been prepared and general test methods for the genus are in preparation (Bedard and Henry 1992).

Trophic Level

Burrowing mayfly nymphs are deposit feeders, ingesting mud, detritus, and organic matter (Bedard et al. 1992). They are a common food source for fish (Hanes et al. 1990).

Sensitivity

As Table 10 shows, *Hexagenia bilineata* was less sensitive than *Daphnia magna*, fathead minnows, and rainbow trout to hexachloroethane smoke combustion

Table 11

Reproducibility of tests for freshwater sediment quality using benthic invertebrates

Multiple coefficients of variation (CV) or ranges of CVs for a test are for different samples analyzed. (A= intralaboratory test, E= interlaboratory test, p.c.= personal communication)

Organism	Test Method	Endpoint	Substance	CV	Type	Reference
Chironomus riparius		survival growth	50 clean sediments from Great Lakes	13.1±5.2% 10.7±5.3%	A	K. Day p.c.
Chironomus tentans	10 day Bedard et al. (1992)	% survival larval weight	36 sediment samples	15-88% 8-19%	A	D. Bedard p.c.
Hexagenia spp.	10 day Bedard et al. (1992)	% survival nymph weight	36 sediment samples	27-180% 9-24%	A	D. Bedard p.c.
Hexagenia spp.		survival growth	50 clean sediments from Great Lakes	3.4±3.4% 9.6±5.3%	A	K. Day p.c.
Tubifex tubifex	28 day ASTM (draft)	young/adult	control sediment sediment sample	9.6% 5.5% 5.6% 6.5%	A	Reynoldson et al. (1991)
Hyalella azteca		survival growth	50 clean sediments from Great Lakes	6.9±3.9% 15.0±10.2%	A	K. Day p.c.

products. *Hexagenia limbata* was more sensitive to copper-spiked sediment than *chironomus tentans*. *H. limbata* was less sensitive than *D. magna* to metal-contaminated sediment. *H. limbata* was found to be less sensitive to whole contaminated sediments than *C. tentans* (Giesy et al. 1990). When pore water from these toxic sediments was used, *H. limbata* was found to be more sensitive than *C. tentans*, *D. magna*, and *Photobacterium phosphoreum*. Reynoldson and Day (1993) reported that *H. limbata* was less sensitive than *D. magna* and less highly correlated with the field distributions of benthic organisms.

Reproducibility

In clean sediments from near-shore regions of the Great Lakes, CVs for tests with *Hexagenia* spp. were 3.4 ± 3.4% for survival and 9.6 ± 5.3% for growth (K. Day, pers. comm.). For the test of Bedard et al. (1992), CVs for 36 samples of contaminated sediment ranged from 27 to 180% (mean = 103%) for mortality as an endpoint and from 9 to 24% (mean = 16%) for growth as an endpoint (D. Bedard, pers. comm., Table 11).

Field Validation

No information on field validation was found.

Ecological Relevance

Hexagenia nymphs are often found in soft, fine-textured, and organically rich sediments. They are found in U-shaped tubes and are continuously exposed to sediment, pore water, and overlying water (Bedard and Henry 1992). They may play a significant role in contaminant transfer from sediments to other trophic levels (e.g., fish).

5.3.5 Tubificid Oligochaete Test

A draft test using *Tubifex tubifex* has been prepared for ASTM (Reynoldson and Day 1993) based on work by ASTM (draft).

Trophic Level

T. tubifex forms dense colonies in organically rich sediments. It is frequently a major component of benthic invertebrate communities in freshwater and estuarine sediments throughout the world and is an extremely important link in the aquatic food chain. It feeds by ingesting sediment particles and is thus directly exposed to contaminants both through feeding and bodily contact (Wiederholm et al. 1987).

Sensitivity

There are conflicting reports on the sensitivity of oligochaetes to contaminants. Correlations between high levels of heavy metals and the absence of oligochaetes has been found, but they also have been shown to be one of the most metal tolerant invertebrates. Aquatic oligochaetes, particularly the Tubificidae, have been shown to be fairly sensitive to specific chemical contaminants, particularly metals, and some organics in whole-sediment toxicity tests (Bailey and Liu 1980, McMurty 1984). Field observations show that *T. tubifex* is fairly tolerant and does occur in contaminated sediments (Reynoldson and Day 1993).

Reproducibility

Sediments from two sites were tested at least six times with five replicates over a period of several months (Reynoldson et al. 1991). From this total of 90 sediment comparisons, only two pairs showed differences.

For a control sediment using five replicates, the coefficient of variation for young/adult was 9-10% while values of 5.5% and 6.5% were obtained for three contaminated sediments (Table 11). The magnitude of the CV depends on the endpoint chosen. With 15 replicates, the CV for young/adult was about 6%, while it was 0.8 to 1% for the number of cocoons produced.

Field Validation

No studies comparing the results of toxicological tests and organism communities in the field were found.

Ecological Relevance

Tubificid oligochaetes are found as a major component of benthic communities in freshwater sediments across Canada and throughout the world. Living in and feeding from sediments, tubificid worms are directly exposed to sediment contaminants. Although tubificids are generally considered one of the sediment-dwelling organisms more tolerant to contamination (Reynoldson and Day 1993), they have been shown to be fairly sensitive to metals and some organics (ASTM draft).

5.4 Usable Battery

The science of sediment testing is far behind that of water or soil. Under the criteria laid out in 3.2, none of the tests described is considered eligible for inclusion in the usable battery, because they all lack reference toxicants and their expected toxic values. This is a serious weakness in a test method. The recommendations for tests under the usable battery are made in anticipation that appropriate reference toxicant data will be available within the year (D. Bedard, pers. comm.; K. Day, pers. comm.), the time frame for implementing the recommendations of this report.

If reference toxicants are not available when it comes time to implement the battery recommended in this report, there will be two options. One will be to carry out the tests on a provisory basis, with only a negative control(s). This uncontaminated sediment could be either a sediment in which the test species is known to grow well (natural or artificial, already used routinely in a laboratory) or a reference sample taken from an uncontaminated area of the site being assessed. The absence of reference toxicants does not prevent the test from being conducted, but it reduces QA/QC for the test and confidence in the interpretation of the results. The other alternative is to implement surrogate batteries of aquatic tests covering a wide trophic range (using elutriates or pore water) that have previously been used in sediment toxicity testing and are considered currently usable (e.g., alga, *Daphnia*, bacterium; see sections 6.4.1, 6.4.2, and below).

5.4.1 Screening Tests

It is important to include sediment-dwelling organisms in the battery rather than to conduct tests with only aquatic water column organisms using sediment elutriates or pore water. The comparative work of Ankley et al. (1991), for example, shows that assessments that rely on elutriates to predict the toxicity of whole sediments may protect pelagic organisms but will likely underestimate toxicity to benthic communities.

The selection of screening tests for the usable battery is discussed below by organism. To summarize, the following tests are recommended: amphipod survival using *Hyalella azteca* (ASTM 1990b), midge survival using *Chironomus tentans* (Bedard et al. 1992), mayfly survival using *Hexagenia* spp. (Bedard et al. 1992), and algal growth inhibition using *Selenastrum capricornutum* (Environment Canada 1992c). The application of these tests is shown in Figure 4.

Tubifex tubifex is a species easily cultured in the laboratory, and the test proposed has demonstrated good reproducibility (5.3.5). Its tolerance for contaminants (Reynoldson and Day 1993) and survival in virtually all types of sediment (K. Day, pers. comm.) indicates that a definitive test involving reproduction (5.4.4.2) would be more appropriate than a screening test using survival as an endpoint.

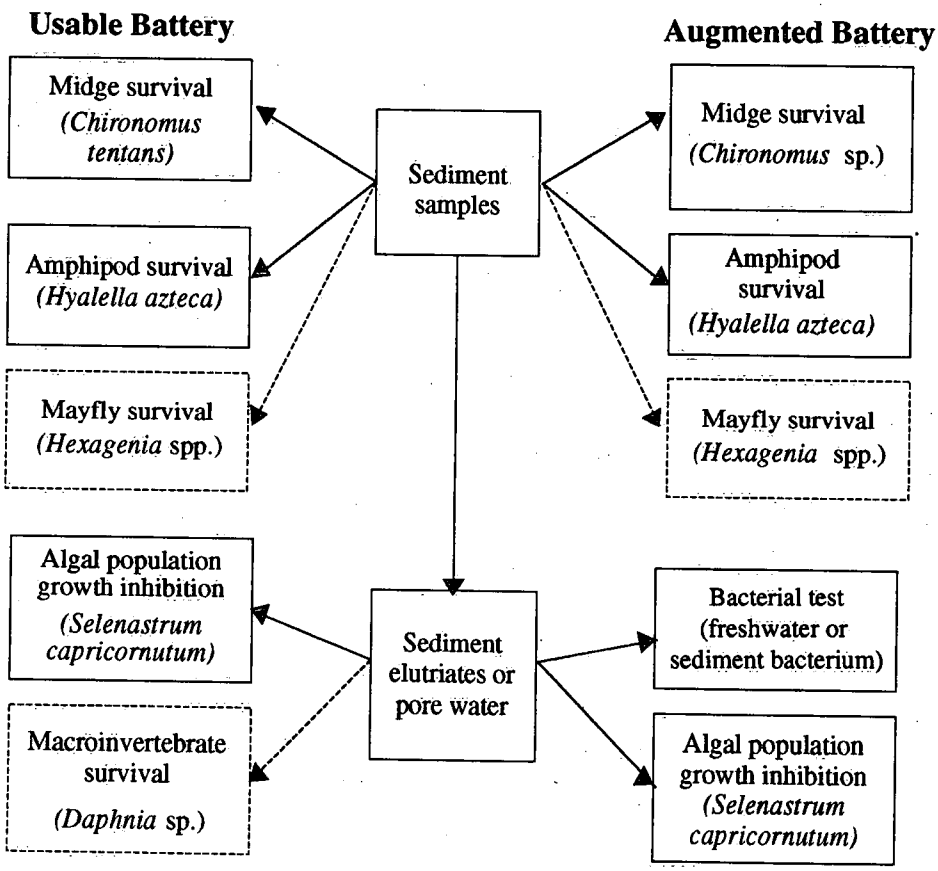

Figure 4. Screening tests recommended for the usable and augmented batteries for sediment quality assessment (see 5.4.1 and 5.4.4.1 for additional details).

Dashed lines occur around the *Hexagenia* test in Figure 4 because it is recommended for sites where this genus would typically form a significant component of the benthic biomass but not for all sites.

In the event that reference toxicant data cannot be found for the *Hyalella* test, the 48-h test with *Daphnia* spp., recommended as a water quality screening test (6.4.1), is suggested as a surrogate test (dashed lines in Fig. 4). Detailed method descriptions are provided in Appendix C.

Algal Test

In the absence of a test using a rooted aquatic plant, an aquatic plant test is recommended for sediment testing. The algal test with *Selenastrum capricornutum*, discussed in 6.2 and 6.3.2, is recommended over a test with *Lemna* spp. because the algal test requires of a smaller amount of test sediment, less space, and is shorter (4 vs. 7 d). No information on the relative sensitivity of these two tests was found. Neither test species can be expected to predict the toxicity of whole sediment to rooted aquatic plants, but the tests will provide an indication of the potential for toxicity from the water soluble constituents in the sediment and pore water.

Amphipod Test

Hyalella azteca, indigenous to the sediment of Canadian lakes, differs from the other benthic test organisms being considered because it dwells on the sediment surface rather than beneath it. This and its potential for applicability to sediments with a relatively wide range of particle sizes and demonstrated varied sensitivity support its inclusion in the usable battery. Tests running from <10 to >30 d have been described and many laboratories run a short-term survival test of 14 days because it is convenient for time scheduling. The U.S. Environmental Protection Agency has recently commissioned the development of a protocol for a 10-d survival test (C. Ingersoll, U.S. Fish and Wildlife Service, pers. comm.). To improve regulatory harmonization, a 10-d survival test is recommended (ASTM 1990b) as a screening test in the usable battery.

Midge Test

Two survival tests for *C. tentans* (Bedard et al. 1992, ASTM 1990b) and one for *C. riparius* (ASTM 1990b) were reviewed. Both species are easily cultured, have short generation times, have a relatively wide tolerance to particle size, have demonstrated sensitivity to sediment contaminants, and have shown good reproducibility (Table 11). The advantage of using *C. riparius* is that younger, and likely more sensitive, individuals can be used in the test. Using either species would support regulatory harmonization with the United States given that the U.S. Environmental Protection Agency recently commissioned the development of protocols for 10-d survival tests with both species (K. Day, pers. comm.). The test description for *C. tentans* by Bedard et al. (1992) is more standardized than the ASTM (1990b) guideline for *C. riparius*, and the former is most widely used in North America (C. Ingersoll, pers. comm.). The 10-d test with *C. tentans* (Bedard et al. 1992) is thus recommended for the usable battery. It is for this test that reference toxicants are being investigated (D. Bedard, pers. comm.). The use of either species should be considered for the usable battery when the U.S. EPA protocols become available.

Mayfly Test

Hexagenia spp. have demonstrated some degree of sensitivity to sediment contaminants, and reproducibility of the test method is good (5.3.4). The 10-d survival test described by Bedard et al. (1992) using *Hexagenia* spp. (*H. limbata* + *H. rigida* mixture) is not recommended as a general screening test (hence the dashed lines in Fig. 4), but is more appropriate where *Hexagenia* forms a significant element of the benthic fauna.

Hexagenia cannot be cultured in the laboratory. Inconsistent rearing results are a potential problem with this test. Once eggs are removed from cold storage, 30 days are required to establish a culture (J. Ciborowski, Univ. of Windsor, Windsor, Ont., pers. comm.). In the event of high mortality of eggs, considerable delays in carrying out tests could arise. As well, because the species of adults collected in the field (to obtain eggs for rearing test organisms) cannot be identified, it is likely that most collections of eggs will be composed of a mixture of species (e.g., *H. limbata* and *H. rigida*, J. Ciborowski, pers. comm.). While both species appear to have similar physical and chemical requirements, this has not been experimentally demonstrated, and no comparative testing concerning sensitivity has been carried out.

Daphnid Test

Daphnids are planktonic microcrustaceans that live in the water column. In the absence of reference toxicant information for the *Hyalella azteca* test, the 48-h test with *Daphnia magna* (Environment Canada 1990b), which is discussed in detail in sections 6.2, 6.3.4, and 6.4.1, could be considered a surrogate. It has often, but not always, been shown to be more sensitive than a variety of other water column and sediment-dwelling species when exposed to solid toxic sediment, elutriates, and pore water (tables 10, 15).

5.4.2 *Definitive Tests*

Many of the screening tests conducted in the usable battery can be adapted for the definitive set of

tests by extending the time over which the test runs and adjusting the endpoints. The definitive tests selected for the usable battery are discussed below by organism.

To summarize, the following tests are recommended as definitive tests for the usable battery: amphipod survival, growth, and sexual maturation using *Hyalella azteca* (ASTM 1990b); midge survival using *Chironomus tentans* (Bedard et al. 1992); mayfly survival using *Hexagenia* spp. (Bedard et al. 1992); and algal growth inhibition using *Selenastrum capricornutum* (Environment Canada 1992c). The application of these tests is shown in Figure 5.

In the event that reference toxicant data cannot be found for the *Hyalella* test, the chronic test of reproduction with *Ceriodaphnia dubia* recommended for the water quality definitive battery (6.4.2) is suggested as a surrogate test (dashed lines in Fig. 5). Dashed lines occur around the *Hexagenia* spp. survival test in Figure 5 because it is recommended only for sites where this genus would typically form a significant component of the benthic fauna (see discussion in 5.4.1). Detailed method descriptions are provided in Appendix C. The reproductive test with *Tubifex tubifex* (ASTM draft) is considered more appropriate as a definitive test in the augmented battery (5.4.4.2).

Algal Test

See discussion in 5.4.1.

Amphipod Test

A chronic test using *Hyalella azteca* that is carried out over 28 days to observe survival, growth, and sexual

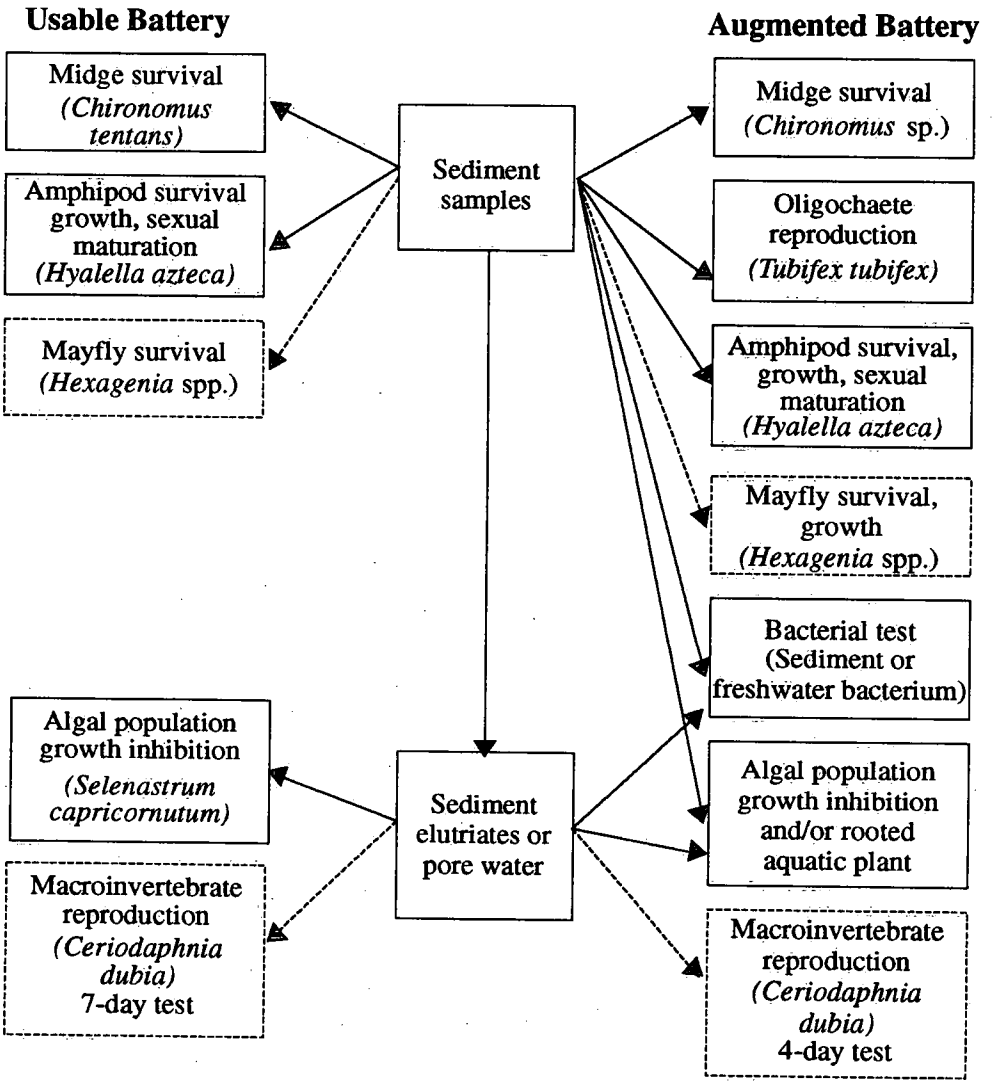

Figure 5. Definitive tests recommended for the usable and augmented batteries for sediment quality assessment (see 5.4.2 and 5.4.4.2 for additional details).

maturation is recommended (ASTM 1990b). It has been shown that this test is 25 to 30% more sensitive than a 14-d test (C. Ingersoll, pers. comm.).

Midge Test

Bedard et al. (1992) do not describe a long-term (>10 day) test for *Chironomus tentans*. Guidance for long-term tests is provided by ASTM (1990b) for *C. tentans* and *C. riparius* to determine the effects on emergence. Most laboratories have, however, discontinued the use of emergence tests because monitoring emergence daily over a 2-wk time period from the start of emergence is too labour-intensive (K. Day, NWRI, pers. comm., 1992). For this reason, the survival test described under screening tests is retained in the definitive set of tests.

Mayfly Test

Where *Hexagenia* species are a significant component of the benthic community, the 10-d survival screening test is also recommended for the definitive set of tests. Although a definitive 21-d survival and growth test with *Hexagenia* spp. has been described by Bedard et al. (1992), it is recommended as a definitive test for the augmented rather than the usable battery because of its highly variable nature (5.4.4.2). Bedard et al. (1992) suggest using dry weight as a measure of growth while head width and body length have also been used (Ciborowski et al. 1991).

Daphnid Test

Daphnids are planktonic microcrustaceans that live in the water column. In the absence of reference toxicant information for the chronic *Hyalella azteca* test, the 7-d test with *Ceriodaphnia dubia* (Environment Canada 1992a), which is discussed in detail in sections 6.2, 6.3.5, and 6.4.2, could be considered a surrogate. It has often, but not always, been shown to be more sensitive than a variety of other water column and sediment-dwelling species when exposed to solid toxic sediment, elutriates, and pore water (tables 10, 15). *Ceriodaphnia* is chosen rather than *Daphnia magna*, which was used in the screening battery, because the test requires only 7 days (Environment Canada) or 4 days (Oris et al. 1991) rather than 21 days. The Environment Canada test with *Ceriodaphnia* is described in Table 14 and in Appendix C.

5.4.3 Recommendations for Sediment Test Batteries from the Literature

Several reviews of bioassays for sediment toxicity testing have resulted in recommendations for sediment test batteries. Reynoldson and Day (1993) recommend using either *Chironomus riparius* or *C. tentans* as well as *Hyalella azteca* in the battery. In their screening battery, Giesy and Hoke (1989) recommend inclusion of tests using *Photobacterium phosphoreum*, *Selenastrum capricornutum*, *C. tentans*, and *Daphnia magna* (48-h). Following screening, they recommend the 7-d fathead minnow and *Ceriodaphnia dubia* tests.

Burton (1991) discussed available tests but concludes that the optimal test battery depends on the study objectives and does not propose a specific list of tests for sediment quality evaluation. The International Joint Commission (IJC 1988) suggests the following tests be used to assess contaminated sediments: *P. phosphoreum*, algal photosynthesis, *D. magna* life cycle; either *Hexagenia limbata*, *C. tentans*, or *H. azteca*, and the fathead minnow.

5.4.4 Recommendations for Augmenting the Usable Battery

5.4.4.1 Screening Tests

For the augmented battery, it is recommended that three screening tests from the usable battery (5.4.1) be retained (amphipod survival using *Hyalella azteca*, ASTM 1990b; algal growth inhibition using *Selenastrum capricornutum*, Environment Canada 1992c; mayfly survival using *Hexagenia* spp., Bedard et al. 1992), one be modified to include other members of the genus (midge survival using *Chironomus* sp.) and one be added (bacterial test, species and test to be determined). The application of these tests is shown in Figure 4. Only additions or changes to the set of screening tests described under the usable battery are discussed below. See 5.4.1 for a discussion of the tests retained from the usable battery.

Midge Test

If further testing indicates that *C. riparius* is significantly more sensitive than *C. tentans* across a wide spectrum of contaminated samples, then consideration should be given to using the former in a screening test. The methodology for the survival test of Bedard et al. (1992) is recommended for *C. tentans*. For *C. riparius*, appropriate modifications of this test methodology or the methodology in ASTM (1990b) could be considered. Further evaluation of the relative sensitivity of these two species (section 5.7) may be useful in determining the species to test.

Freshwater Bacterial Test

Ideally the screening tests should include a test representing bacteria that are critical in sediment processes. Sediment toxicity to bacteria should be assessed with tests conducted in the sediment.

An aquatic test using elutriates or pore water and the marine bacterium *Photobacterium phosphoreum* has often been used for assessing freshwater sediment elutriate toxicity and shown to be variably sensitive (Table 15). See section 7.0 for a discussion of the appropriateness of this species in a freshwater sediment test battery. The Toxi-chromotest™ using *Escherichia coli*, a freshwater bacterium, has also shown variable sensitivity to contaminants (Table 16). Neither test is recommended for the bacterial test, pending the results of comparative testing (7.0) and the resolution of test design deficiencies.

5.4.4.2 Definitive Tests

It is recommended that the augmented battery contain definitive tests adopted from the usable battery (5.4.2) including amphipod survival, growth, and sexual maturation using *Hyalella azteca* (ASTM 1990b), midge survival using *Chironomus* sp., and algal growth inhibition using *Selenastrum capricornutum* (Environment Canada 1992c). It is recommended that consideration be given to shortening the duration of the daphnid test with *Ceriodaphnia dubia* (as a surrogate for the *Hyalella* test, see below). Extension of the *Hexagenia* spp. test to incorporate growth as an endpoint is discussed. An oligochaete reproduction test and a bacterial test using a freshwater or sediment inhabiting species are added to the usable battery. Testing with rooted aquatic plants, provided appropriate species are identified, is also recommended as an addition to the usable battery. The application of these tests is shown in Figure 5. Only changes or additions to the set of definitive tests described under the usable battery are discussed below. See 5.4.2 for a discussion of the tests retained from the usable battery.

Mayfly Test

A longer-term test with *Hexagenia* spp. is desirable to incorporate test endpoints in addition to survival. A 21-day test has been described by Bedard et al. (1992). Other researchers to date, however, have found great variability in measures of growth, which would necessitate hundreds to thousands of replicates at any given site to detect the effects of contaminants (K. Day, pers. comm.).

Rooted Aquatic Plant Test

A test with rooted aquatic plants is recommended in addition to (when the algal screening test is shown to be sensitive), or as an ecologically more relevant (5.3.1) alternative to, the algal test in the usable battery. Several tests with rooted aquatic plants are being developed, and comparative data on sensitivity to contaminants for these tests is required before one can be selected for the test battery (see 7.0).

Tubificid Oligochaete Test

For the 28-d reproduction test with *Tubifex tubifex* (ASTM draft) data are missing on sensitivity relative to other species and to toxic contaminants. It is thus considered more appropriate for inclusion in the augmented than in the usable battery.

Daphnid Reproduction Test

The replacement of the 7-d *Ceriodaphnia dubia* reproduction test in the current definitive battery (5.4.2) by a 4-d test (Oris et al. 1991; see 6.2, 6.3.5, 6.4.3.2, Table 14) as a surrogate for the *Hyalella azteca* survival, growth, and sexual maturity test could be considered if reference toxicant information for the latter test was unavailable.

5.5 Prototype Tests

Of the eight tests using sediment-dependent organisms that were evaluated in this review, the two listed below (along with the work required to make them usable) were identified as prototypes (missing 'must' criteria but having a score of ≥88% for 'want' criteria; see 3.2.1, 3.2.2).

Chironomus tentans, OMOE (reference toxicant, specified safe pH range, complete statistics, Bedard et al. 1992)
Hexagenia spp., OMOE (see test above, Bedard et al. 1992)

According to Figure 1, these tests are priority 2. In anticipation of provision of the missing details within the time frame for review of this document (D. Bedard, pers. comm.), they were considered eligible for inclusion in the usable battery (5.4.1, 5.4.2).

5.6 Tests under Development

The following six tests were initially considered under development, scoring <88% for the 'want' criteria and having a priority of 4 for attention. The work required to complete four of them is described in brackets.

Hyalella azteca (reference toxicant, provide more specific culture vessel and substance volumes, specify safe pH range, complete statistics) (ASTM 1990b)
Chironomus tentans (see test above) (ASTM 1990b)
C. riparius (see test above) (ASTM 1990b)
Tubifex tubifex (reference toxicant, specify pH, provide statistical methods) (ASTM draft)
Hexagenia spp., ASTM (Bedard and Henry 1992)
Lumbriculus variegatus (Phipps et al. 1991)

Examination of these tests shows that the OMOE test with *C. tentans* (prototype test, 5.5) and the ASTM test with this organism are similar. Given the greater degree of completeness and standardization of the former, the latter is not considered a priority for work.

The last two tests scored less than 80% for the 'want' criteria and are not considered to be priority concerns at this time. The first test is a general draft guideline based on the second test in the group of prototypes. A test protocol for the last species is being developed for the U.S. Environmental Protection Agency (C. Ingersoll, pers. comm.). Upon completion it should be evaluated as a candidate for the usable battery using the approach outlined in this document.

Given the likelihood that work currently in progress would elevate the remaining three tests to the usable category within the time frame for review of this document, they are considered of highest priority for attention within the category. These tests were also considered as candidates for the usable battery (5.4.1, 5.4.2).

5.7 Priorities for Assessing Sediment Quality with Bioassays

In this section, priorities for work required to meet the needs of the National Contaminated Sites Remediation Program (NCSRP) related to the assessment and remediation of freshwater sediment in Canada are described, beginning with the work of highest priority. Priority work required to upgrade tests reviewed to usable tests (5.5., 5.6) is integrated with additional areas of work considered essential for implementing the recommended test batteries. For a discussion of the rationale for identifying these tasks as priority items, see sections 3.5, 5.4, 5.5, and 5.6.

1) Prepare standard methods for sediment collection, storage, and sample preparation

This need is being addressed by a contract currently being carried out for Commercial Chemicals Branch (R. Scroggins, Industrial Programs Branch, Environment Canada, pers. comm.). ASTM (1990d) has recently prepared a standard guide for the collection, storage, characterization, and manipulation of sediments for toxicological testing.

2) Determine suitable reference toxicants for tests with benthic organisms

Suitable reference toxicants and expected toxicity values are required for toxicity tests with *Hyalella azteca*, *Chironomus tentans*, *C. riparius*, *Hexagenia* spp., and *Tubifex tubifex*. Some experimental work with cadmium and copper reference toxicants is being conducted by the Ontario Ministry of the Environment (Bedard et al. 1992), but the results are as yet unavailable.

3) Develop a standardized sediment(s) for toxicity testing

A standard reference sediment(s) would be useful for preparing sediment dilutions for testing sediment toxicity and as a substrate that could be used for testing individual compounds (utility of reference toxicants) and determining national environmental quality criteria.

There are two options for standardized sediments. One is to designate an uncontaminated natural sediment as the standard sediment for each test while the other is to develop an artificially composed sediment. The number of standard sediments required depends on the similarity of the requirements of the test organisms. The standard sediment used in a test must support good performance of the test organism. Both sediments should be appropriate for testing sediment-dependent organisms found in Canada. The advantages of an artificial soil are that it does not have to be transported, and composition can be defined and strictly controlled.

An artificial soil has been developed and used in testing the toxicity of sediments to *Hexagenia limbata* and *H. rigida* (Ciborowski et al. 1991; Hanes et al. 1990; J. Ciborowski, pers. comm.). The applicability of this standard sediment for testing other benthic test species and rooted aquatic plants, and the potential for making minor modifications so that it is suitable for other species has yet to be investigated (J. Ciborowski, pers. comm.).

Walsh et al. (1990a, 1991) have begun to address the suitability of artificial sediments for use in tests with emergent wetland plants and a variety of sediment-dependent organisms including submerged plants, crustaceans, toads, and fish (Walsh et al. 1990b). These sources could serve as a basis for developing the required standard sediments for the test battery.

4) Develop a test with rooted aquatic plants

Rooted plants exert significant control over the physical, chemical, and biological characteristics of wetland communities. These primary producers are sources of detritus and provide food and shelter for other organisms. Their roots and rhizomes stabilize sediment. Aquatic plants are sinks for toxicants that are taken up by the roots and translocated to other parts of the plant. The absence of a test with rooted aquatic plants from the sediment battery is a major gap in trophic level representation.

Several species have been examined as candidates for a rooted aquatic test plant. For example, the U.S. Army Engineer Waterways Experiment Station has

prepared a test using the sedge *Cyperus esculentus* (widespread in the eastern half of Canada) for examining the potential mobility of contaminants from dredged material into the environment through plant uptake (Folsom and Price 1989). Experimental culturing conditions for sago pond weed, *Potamogeton pectinatus* (widespread across Canada), have been described in preparation for using the plant for toxicity assessment (Ailstock et al. 1991).

Walsh et al. (in press) have used *Echinochloa crusgalli* var. *crusgalli* (a grass, widespread across Canada, but not particularly a species of wetlands) and *Sesbania exultata* (legume, not indigenous to Canada) to examine the toxicity to seedling growth of a variety of effluents in artificial sediments.

A 14-d test for sediment toxicity to the growth of roots and shoots of *Hydrilla verticillata*, not indigenous to Canada, is also being developed (Klaine 1991). Aquatic species that produce numerous vegetative propagules and have short life spans, such as annual shoreline plants, are ideal candidates.

5) Re-evaluate bacteria for freshwater sediment toxicity testing

The screening battery, at least, should have a test representing bacteria that are critical in sediment processes. The marine bacterium *Photobacterium phosphoreum* has been widely used to assess the toxicity of sediment elutriates, but its relevance as a surrogate for freshwater bacteria is questionable (see 7.0).

Recently, the Toxi-chromotest™ with *Escherichia coli* has been applied directly to sediments, rather than to sediment elutriates, and shown to be sensitive to sediment toxicity (Kwan and Dutka 1992). This technique requires further comparative testing.

6) Prepare a handbook for statistical guidance

A weakness of many of the tests reviewed was inadequate statistical guidance. The need for a handbook on statistical guidance is common to all three media and is discussed in section 7.0.

7) Examine the relative sensitivity of *Chironomus* species

C. tentans was selected as the current test species because a more standardized printed test method was available for it (Bedard et al. 1992) than for *C. riparius* (ASTM 1990b). In the test with *C. riparius*, the individuals used are younger than those in the *C. tentans* test. For this reason, the former test may be more sensitive. The literature reviewed in this evaluation showed both species to be variably sensitive to contaminants. More detailed investigations of existing comparative data would elucidate the relative sensitivity of these two species and the need for additional experimental work.

8) Prepare a manual for field sampling guidance

A manual for designing field sampling schemes is required to ensure that the collection techniques (point 1 above) are applied appropriately (see 7.0).

6.0 ASSESSMENT AND RECOMMENDATIONS FOR FRESHWATER TESTS

6.1 Test Methods and Candidate Organisms

The results of the literature review of organisms used in aquatic testing are summarized in two tables. Those meeting the first criterion considered essential for retaining the test for further evaluation of suitability (appropriate printed test method, see 3.1) are found in Table 12. Table 13 lists the organisms for which test methods do not meet this criterion and are not considered further.

One hundred and nineteen aquatic species from 22 major groups of organisms were identified in connection with aquatic toxicity testing (tables 12 and 13). Of these, bacteria (5 spp.), algae (26 spp.), invertebrates (30 spp.), amphibians (2 genera), fish (25 spp.), and vascular plants (1 sp.) had appropriate printed test methods.

6.2 Step 1 Assessment — Test Methodology

6.2.1 Preliminary Assessment

The tests identified in 6.1 were first evaluated according to three criteria that are considered essential to a complete test method (acceptable printed method, acceptability criteria, reference toxicant; see 3.2.1 for definition and importance of criteria).

Considering the large number of aquatic tests that met the first criterion, and the emphasis on soil and sediment testing in this report, a 25-test subsample that had appropriate printed methods was further evaluated according to the second and third criteria, and subsequently in Step 2. These tests are briefly described in Table 14.

Table 12

Species with test methods (for assessing water quality) from recognized standards organizations and the literature

(ALTA ENV= Alberta Environmental Centre, APHA= American Public Health Association, ASTM= American Society for Testing and Materials, EC= Environment Canada, EEC= European Economic Community, ISO= International Standards Organization, OECD= Organization for Economic Cooperation and Development, OMOE= Ontario Ministry of the Environment, SNCI= Swedish National Chemicals Inspectorate, USEPA= United States Environmental Protection Agency)

Organism group	Species	Organization/ Reference	Test type
Bacteria	*Bacillus cereus*	Thomson et al. (1986)	chronic, dehydrogenase activity
	Escherichia coli	Orgenics Ltd. (1985)	chronic, enzyme synthesis (Toxi-chromotest™)
	Pseudomonas putida	ISO	chronic, growth
	Photobacterium phosphoreum	EC, Microbics (1992a,b)	chronic, luminescence
	Spirillum volutans	Dutka (1991)	chronic, motility
Algae	*Anabaena flos-aquae*	APHA, ASTM, Holst and Ellwanger (1982), SNCI	chronic, growth, reproduction
	Aphanizomenon flos-aquae	SNCI	
	Asterionella formosa	SNCI	
	Bumilleriopsis filiformis	SNCI	
	Chlamydomonas dysosmos	SNCI	
	C. reinhardtii	SNCI	
	Chlorella vulgaris	ASTM, EEC, Holst and Ellwanger (1982), OECD, USEPA	
	C. emersonii	SNCI	
	Cryptomonas pyrenoidifera	SNCI	
	Cyclotella spp.	APHA, SNCI (*C. cryptica*)	
	Diatoma elongata	SNCI	
	Microcystis aeruginosa	APHA, ASTM	
	Monoraphidium contortum	SNCI	
	M. pusillum	SNCI	
	Navicula spp.	APHA, ASTM (*N. pelliculosa*)	
	Nitzchia spp.	APHA	
	Pediastrum spp.	SNCI	
	Phormidium luridum	SNCI	
	Raphidonema longiseta	SNCI	
	Scenedesmus obtusiusculus	SNCI	
	S. quadricauda	TSCA	
	S. subspicatus	ASTM, EEC, ISO, OECD	
	Selenastum capricornutum	APHA, ASTM, EC, EEC, Greene et al. (1989), Holst and Ellwanger (1982), ISO, OECD, USEPA, SNCI	
	Staurastrum gracile	SNCI	
	Synechococcus leopoliensis	SNCI	
	Synedra spp.	APHA	

Table 12 (continued)

Organism group	Species	Organization/ Reference	Test type
Protozoa	*Colpidium campylum*	Dive et al. (1989)	chronic, growth, reproduction
	Tetrahymena vorax	Gilron et al. (1991)	acute, chemostatic behavioural response
Metazoa	*Brachionus calyciflorus*	Anonymous (1990a)	acute, survival
	B. rubens	Snell and Persoone (1989)	acute, survival
Flatworms	*Dugesia tigrina*	ASTM (1980)	acute effects
Nematodes	*Panagrellus redivivus*	Samoiloff (1990)	survival, growth, maturation, fitness
Oligochaetes	*Limnodrilus hoffmeisteri*	APHA	acute, chronic, survival
	Branchiuria sowewrbyi		
	Stylodrilus heringianus		
Amphipods	*Gammarus lacustris*	ASTM, ALTA ENV, APHA	acute, survival, other effects
	G. fasciatus	APHA, ASTM	
	G. pseudolimnoeus	APHA, ASTM	
	Hyalella azteca	APHA, ASTM	acute, chronic, survival, other effects
	Pontoporeia affinis	APHA	
Isopods	*Ceriodaphnia dubia*	ASTM, EC, Oris et al. (1991), Weber et al.(1989)	acute, chronic, survival, reproduction
	Daphnia magna	APHA, ASTM, EC, EEC, Greene et al. (1989), ISO, OECD (*Daphnia* spp.), OMOE, USEPA	acute, survival, mobility
	D. magna	Biesinger et al. (1987), ISO, OECD	chronic, reproduction
	D. pulex	APHA, ASTM, EC, Greene et al.(1989)	acute, survival, mobility
	D. pulicaria	ASTM	acute, survival
Shrimp	*Mysis relica*	APHA	acute, chronic, survival, reproduction
	Palaemonetes cummingi		
	P. kadiakensis		
Crayfish	*Orconectes* spp.	APHA (*O. rusticus*), ASTM	acute, mobility
	Cambarus spp.	APHA, ASTM	
	Procambarus spp.	ASTM	
	Pacifastacus leniusculus		
Mosquitos	*Wyeomyia Smithii*	ASTM	acute, mobility

Table 12 (continued)

Organism group	Species	Organization/ Reference	Test type
Stoneflies	*Hesperoperla lycorias*	APHA	acute, survival
	H. pacifica	APHA	
	Pteronarcys californica	APHA, ASTM (*P.* spp.)	
	P. dorsata	APHA	
Mayflies	*Baetis* spp.	ASTM	acute, survival
	Ephemerella subvaria	APHA, ASTM (*E.* spp.)	acute, survival
	Hexagenia bilineata	APHA	acute, survival
	H. limbata		
	H. rigida		
Caddisflies	*Brachycentrus americanus*	APHA	acute, survival
	B. occidentalis		
	Clistoronia magnifica		
Snails	*Physa integra*	ASTM	acute effects
	P. heterostropha		
	Amnicola limosa		
Amphibians	*Rana* spp.	ASTM	acute, survival
	Bufo spp.		
Fish	Alewife	APHA	acute/chronic
	Threadfin shad		
	Lake herring		
	Lake whitefish		
	Mountain whitefish		
	Rainbow trout	APHA, ASTM, EC, EEC, OECD, OMOE	acute, survival (4 d)
		OECD	acute, survival (14-28 d)
		EEC	acute, growth rate (28 d)
		APHA, ASTM, OECD,	chronic, early life stages
		ASTM	acute, survival
	Coho salmon	APHA, ASTM	acute, survival
	Salmon spp.	APHA, ASTM	chronic, early life stages
	Brook trout	ASTM	acute, survival
	Trout	APHA, ASTM	chronic, early life stages
	Goldfish	APHA, ASTM	acute, survival

Table 12 (continued)

Organism group	Species	Organization/ Reference	Test type
Fish (continued)	Fathead minnow	APHA, ASTM, EEC, OECD, OMOE	acute, survival
		EC	chronic, larval growth
		APHA, ASTM, OECD	chronic, early life stages
		OECD, OMOE	chronic, survival (14-28 d)
	Shiners	APHA	acute, chronic survival
	Channel catfish	ASTM	acute, survival
		ASTM	chronic, early life stages
	Bluegill	APHA, ASTM, EEC, OECD,	acute, survival
		ASTM	chronic, early life stages
		OECD	acute survival (14-28 d)
	Green sunfish	ASTM	acute, survival
	Northern pike	APHA, ASTM	chronic, early life stages
	Bass	APHA	acute, chronic survival
	White sucker	APHA, ASTM	chronic, early life stages
	Common carp	APHA, EEC, OECD	acute, survival
		OECD	acute survival (14-28 d)
	Red killifish	EEC, OECD	acute, survival
		OECD	acute, survival (14-28 d)
	Guppy	APHA, EEC, OECD	acute, survival
		OECD	chronic, survival (14-28 d)
	Yellow perch	APHA	acute, chronic survival
	Golden orfe	EEC	acute, survival
	Zebra fish	EEC, ISO, OECD	acute, survival
		OECD	acute, survival (14-28 d)
		OECD	chronic, early life stages
	Ricefish	OECD	chronic, early life stages
Floating vascular plants	*Lemna gibba*	ASTM, Holst and Ellwanger (1982), USEPA	chronic, growth, reproduction

Table 13

Organisms that have been used in the asssessment of water quality but for which tests have not yet been prepared by recognized standards organizations or published in the literature

Organism group	Species	Organization/reference
Bacteria	*Salmonella typhimurum*	Epler et al. (1980)
Algae	*Chlorella pyrenoidosa*	Slooff et al. (1983)
	Navicula seminulum	Payne and Hall (1979)
	Scenesdesmus pannonicus	Slooff et al. (1983)
Flatworms	*Dugensia dorotocephela*	Ewell et al. (1986)
Nematodes	*Caenorhabditis elegans*	Williams and Dusenbery (1990)
Leeches	*Dina dubia*	Metcalfe et al. (1988)
	Erpobdella punctata	
	Helobdella stagnalis	
	Haemapsos marmorata	Metcalfe and Hayton (1989)
Mollusks	*Anodonta imbecilis*	Keller and Zam (1991)
	Elliptio complanata	Metcalfe and Hayton (1989)
Pillbugs	*Caecidotea intermedia*	Ewell et al. (1986)
	Ascellua intermedius	
Amphibians	*Xenopus laevis*	Dawson et al. (1988)
Vascular plants	*Lemna minor*	Wang (1990b)

Table 14

Brief descriptions of tests evaluated for assessing water quality

The percentage at the column head is the test score (see 3.2, 6.2, and Table B-3) and reflects methodology completeness (NS= not specified). Additional details are provided in Appendix C

	ISO (1991c) 82%	Orgenics Ltd. (1985) (Toxi-chromotest™) 71%	Environment Canada (1991), Microbics (1992a,b) 100%	Dutka (1991) 77%
Test type	bacterium, chronic, cell multiplication	bacterium, chronic, enzyme activity	bacterium, luminescence, static	bacterium, motility, inhibition
Application	water, wastewater, water soluble substances	water, chemicals, pharmaceuticals, food additives	chemicals, leachates, receiving waters, elutriates	water and effluents
Species	*Pseudomonas putida*	*Escherichia coli*	*Photobacterium phosphoreum*	*Spirillum volutans*
Endpoints	growth inhibition, IC_{10}, IC_{50}, NOEC	inhibition of beta-galactosidase induction; MIC_{20}	luminescence, IC_{50}, IC_{20}	90% inhibition of reversing motility (MEC90)
Organism selection	Berlin 33/2 strain, DSM 50026	K12 OR85, rough mutant	NRRL B-11177 from Microbics Corp.	ATTC 19554
No. organisms + replicates	optical density of bacterial suspension (formazin turbidity units=TE/F); 3 reps	2.5 x 10^8 cells/mL, 2 reps	1 vial bacterial reagent, at least 4 concentrations, 2 reps, other reps not required	1 mL of over-night 30°C culture; 2 control reps, 1 rep of ≤5 test conc.
Observation frequency	16 ±1 h	1.5 h	before test (0); 5, 15, or 30 min after addition of test sample	0 and 2 h
Volume test vessel	250 mL Erlenmeyer flasks	200 µL microtiter plate wells	wells of standard Microtox Analyzer	chemically clean microscope slide
Volume test substance	90 mL	100 µL	2 mL/rep	1.0 mL
Test substance preparation	sample storage 2-4°C up to 2 d	not required	sample storage; dilution with Microtox diluent/ other uncontaminated	a variety of methods proposed

Table 14 (continued)

	ISO, *Pseudomonas*	Orgenics Ltd., *Escherichia*	EC, *Photobacterium*	Dutka, *Spirillum*
Test substance preparation			water with 2% salinity	
Culture, handling	grow in culture medium 1 d before starting test	rehydrated, freeze-dried	reconstitution of bacteria	stock culture maintenance
Conditions (light, temp, pH, etc.)	21±1°C; pH not adjusted	37°C	15±0.3°C, pH 6.0-8.5; pre-test aeration if DO <40%/ >100% saturation	25°C
Acceptability criteria	≥100× increase of initial inoculum conc.	NS	repeat test if luminescence </> 50% for all test concentrations; reference toxicant within 2 SD of mean; IC_{50} must be based on interpolated data	no motility loss in neg. control; 90% motility loss in pos. control within 2.0 h
Medium defn., manipulation	defined medium	LB medium reaction mixture	reconstitution solution	defined medium slide application
Reference toxicant	3,5-dichlorophenol, 13.7mg/L mean IC_{10}, 21.4mg/L mean IC_{50}	mercuric chloride	phenol (5min IC_{50}= 13-26mg/L @ 15°C), zinc sulphate (5min IC_{50}= 1.4-1.7mg Zn/L @ 15°C), sodium lauryl sulphate (5min mean IC_{50}= 1.3mg/L), potassium dichromate	mercuric chloride, ≥ 1.2 ppm Hg^{++} in 0.8mL water
Negative control	nutrient medium; uninoculated solutions for colour/turbidity correction	reaction medium	Microtox reagent control; colour/ turbidity correction	Kriegs medium formulation
Statistical analysis	effect formula, graphical interpolation	simple effect formula, graphical interpolation	IC_{50} or IC_{20} with 95% CI; graphical estimate/least square regression; computer program provided	graph
Organism	easily	easily	easily	easily

Table 14 (continued)

	Thomson et al. (1986) 65%	Environment Canada (1992c) 100%	ASTM (1990c) 82%	Anonymous (1990a) 88%
Test type	bacterium, chronic, dehydrogenase activity, static	alga, chronic, growth, reproduction, microplate, static	alga, chronic, growth, reproduction, static, flask	rotifer, acute, survival, static
Application	toxicity of chemicals, receiving water, wastewater	toxicity of chemicals, effluent, receiving water, leachates, elutriates	toxicity of chemicals	toxicity of chemicals, effluent
Species	*Bacillus cereus*	*Selenastrum capricornutum*	*S. capricornutum* *Microcystis aeruginosa* *Anabaena flos-aquae* *Navicula pelliculosa*	*Brachionus calyciflorus*
Endpoints	resazurin reduction, EC_x	cell concentration, IC_{50}, LOEC, NOEC	cell concentration, chlorophyll *a*, EC_{50}	survival LC_{50}
Organism selection	activated sewage sludge isolate	strain ATCC 22662/ UTEX 1648/ UTCC 37, culture 4-7 d old, exponentially growing	culture in log phase, growth	0-2 h old
No. organisms + replicates	spectrophotometer at 610 nm, reps NS	10 000 cells/mL, min. 3 reps, 9 conc. dilution factor 3	2×10^4 cells/mL except *M. aeruginosa* at 5×10^4 cells/mL; dilution factor 0.6, 5 conc., at least 3 reps	10 rotifers, 3 reps, 5 conc. with 50% dilution
Observation frequency	0.5 h	72 h	24, 48, 72, 96 h for growth rate/area under growth curve analysis; 96 h for final cell conc. analysis, EC_{50}	24 h
Volume test vessel	12 mL glass centrifuge tubes	220µL well microplate	glass flask, test solution volume < 50% flask for tests with shaker, <20% without shaker; depends on sp.	24-well microplate equivalent to Corning 2580
Volume test substance	5mL/rep	5mL test substance	see above	1 mL/rep
Test substance preparation	NS	dilution of substance with reagent, receiving or upstream water	stock solution preparation, deionized/distilled water as diluent	sample dilution preparation with hatching medium

Table 14 (continued)

	Thomson, *Bacillus*	EC, alga	ASTM, algae	Anonymous, rotifer
Culture, handling	grow culture at 21°C on rotary shaker for 18 h; dilute cell density OD to 2.0 at 625 nm	culture methods	reference cited for culture details	cyst hatching
Conditions (light, temp, pH, etc.)	21±1°C, pH 7.0	continuous, cool-white fluorescent, 4.0 Klx, 60μE/m^2·s^{-1}, 24±2°C; pH 6.5-8.5	continuous cool-white fluorescent 60 μE/m^2·s^{-1} for green algae + diatoms, half for blue-greens 24±2°C except for *N. pelliculosa* at 20±2°C	dark, 25°C, pH 7.5, hardness 80-100mg $CaCO_3$/L, alkalinity 60-70 mg/L
Acceptability criteria	NS	growth in control not statistically different from quality control microplate; control yield > 16× in 72 h; evaporation <10%; pH of controls <±1.5; reference toxicant within 3 SD of mean for toxicant	light variability ≤15%, at least 10^5 cell/mL in control, temperature variation between highest and lowest < 4°C	90% control survival
Medium defn., manipulation	defined medium	enrichment medium	macronutrient solution	hatching medium
Reference toxicant	NS	potassium dichromate (IC_{50} = 129.7μg/L, 94.2-166.6 = 95%CI), zinc chloride (IC_{50}= 52.6μg/L, 31.9-72.7= 95%CI), copper sulphate 65.7μg/L, 60.7-70.7= 95%CI), phenol (IC_{50}= 63.1μg/L, 18.8-104.4= 95%CI) (St. Laurent et al. 1992)	NS	potassium chromate (every 10-15 tests)
Negative control	nutrient medium	reagent water alone; reagent water + enrichment medium; dilution water?	dilution water, solvent control	hatching medium
Statistical analysis	effect formula	IC_{50} with 95% CI, linear regression	EC_{50} with 95% CI with linear regression, based on area under growth curve, daily cell conc., or final cell conc.	graphical interpolation by eye

Table 14 (continued)

	Thomson, *Bacillus*	EC, alga	ASTM, algae	Anonymous, rotifer
Organism availability	moderate	easily	easily	easily
	Samoiloff (1990) 76%	**Snell and Persoone (1989) 94%**	**Biesinger et al. (1987) 88%**	**OECD (1991a) 82%**
Test type	nematode, chronic, survival, growth, static	rotifer, acute, survival, static	daphnid, chronic, reproduction, static-renewal	daphnid, chronic, reproduction, static-renewal
Application	water samples, effluents, aqueous, methanolic organic extracts of sediments, soils, sludges	toxicity of chemicals, effluents	toxicity of chemicals, leachates	toxicity of chemicals
Species	*Panagrellus redivivus*	*Brachionus rubens*	*Daphnia magna*	*Daphnia magna*
Endpoints	survival, growth, maturation, fitness	survival LC_{50}, NOEC	survival, young/female, length, LOEC, NOEC, EC_{50}/LC_{50}	offspring/female, LOEC, NOEC
Organism selection	strain bq-1, J2 stage	0-2 h old, female	<24 h old, female	<24 h old, clone 5, parental stock same age (21±7 d), not 1st brood
No. organisms + replicates	10 nematodes, 10 reps	10 rotifers, at least 6 reps, concentrations not specified	1 daphnid, 10 reps, 5 conc., ≥ 0.5 dilution factor	1 daphnid, 10 reps, at least 5 conc.
Observation frequency	96 h	24 h	3 times/week for 3 wk	3 times/week for 3 wk
Volume test vessel	2.5 mL cups	24 well microplate	100 mL beaker	NS
Volume test substance	1-0.1 mL sample/rep, depending upon extraction procedure	1 mL test solution/well	80 mL medium/rep	100 mL medium/rep
Test substance preparation	dilution with M9-Y growth medium	dilution with synthetic fresh water	dilution with reagent water, identical to culture water; pH adjustment may be required	dilution with culture medium

Table 14 (continued)

	Samoiloff, nematode	Snell & Persoone, *B. rubens*	Biesinger, *Daphnia*	OECD, *Daphnia*
Culture, handling	culturing, nematode transfer	cyst storage, hatching	brood culture, algal culture	culture conditions similar/same as test conditions
Conditions (light, temp, pH, etc.)	19-25°C	dark, 25°C, pH 7.4-7.8, hardness 100mg/L $CaCO_3$, alkalinity 60-70mg/L, rotation at 12 rev/h	16 h light, 30-100 ft-c, 20±2°C, pH 6.8-8.5, hardness, alkalinity, no aeration, DO 90-100% at start	12-16 h light, 1000 lx, 18-22±1°C, no aeration
Acceptability criteria	>40% control nematodes become adults, control survival >80%, no microbial growth	90% control survival	adult survival 90% during 14 d before test, 80% control survival, 40 young/female in control for 21 d	90% control survival, ≤60 offspring/female with CV mean no.≤25%, DO >2mg/L and ≥80% initial conc.,pH change ≤1
Medium defn., manipulation	M9 buffer, cholesterol solution, yeast suspension for growth medium	hatching medium (EPA 1985 medium)	food preparation, solution changed and food added 3 times/wk	Elendt medium, food preparation, solution changed and food added 3 times/wk
Reference toxicant	NS	sodium pentachlorophenate (LC_{50}= 0.5-0.7mg/L)	sodium pentachlorophenate	NS
Negative control	growth medium, solvent control	hatching medium	dilution water, leaching agent control, DO control	test medium + food solvent control
Statistical analysis	chi-square to compare control and 1 conc., no method for series of concentrations	probit analysis	ANOVA and Dunnett's many-one t or Bonferoni t for survival NOEC, LOEC; trimmed Spearman-Karber for LC_{50}; reproduction use outlier detection test and equality and multiple comparison test as above or Kruskal-Wallis rank-sum-based procedure for LOEC, NOEC reproduction	Dunnett's and William's test for NOEC, LOEC
Organism availability	easily	easily	easily	easily

Table 14 (continued)

	Environment Canada 1990b) 100%	Environment Canada (1992a) 100%	American Public Health Association et al. (1989) 59%	Alberta Environmental Centre (1989) 76%
Test type	daphnid, acute, survival, static	daphnid, chronic, survival, reproduction, static-renewal, 7-d	shrimp, acute + chronic survival, reproduction, static-renewal/ flow-through	amphipod, acute survival, static
Application	toxicity of chemicals, effluents, receiving water, elutriates, leachates	see left	water, wastewater	water
Species	*Daphnia magna* *D. pulex*	*Ceriodaphnia dubia*	*Mysis relicta* *Palaemonetes cummingi* *P. kadiakensis*	*Gammarus lacustris*
Endpoints	survival LC_{50}, mobility EC_{50}	survival LC_{50}, offspring produced, EC_{50}, NOEC, LOEC	acute-larval survival LC_{50}, life cycle hatching success, larval survival, egg production, EC_{50}	survival LC_{50}
Organism selection	<24 h old, from middle broods (females 2-4 wk)	<24 h old females, within 8d of same age	larvae of specified age or adults in same condition	animals of same size range
No. organisms + replicates	at least 10 daphnids, ≤ 1/15mL, 2/3 reps, 5 conc., 50% dilution factor	1 daphnid, ≥ 10 reps, 5 conc., 50% dilution factor	> 20 individuals/rep for all tests, 25 females to start life cycle test, 2/3 reps, 5 conc.	at least 10 animals, loading factors of < 0.5g/L > 17°C, < 0.8g/L ≤ 17°C
Observation frequency	0, 48 h	daily until 60% control daphnids have produced 3 broods (7±1 d)	daily for short term tests	0.5, 1, 2, 4, 24 h (24 h for opaque solutions)
Volume test vessel	150-250 mL glass beakers/inert plastic bags except for chemicals	30 mL plastic cup, glass beaker/test tube	glass jars/aquaria; 1L capacity is one possibility	NS
Volume test substance	determined by density requirement	≥15 mL test solution	loading rate 0.1g/L	determined by loading factors

Table 14 (continued)

	EC, *Daphnia*	EC, *Ceriodaphnia*	APHA, shrimp	Alta., *Gammarus*
Test substance preparation	dilution water hardness within 20% culture medium; diluent depends on test purpose	see left; surface water diluent filtered through 60µm plankton net	diluent depends on test purpose	dechlorinated water is diluent
Culture, handling	organism transfer, observation methods, culture methods	see left	general culture methods for macroinvertebrates	field collection, culture maintenance
Conditions (light, temp, pH, etc.)	cool white fluorescent ≤ 800 lx at surface, 16h light: 8h dark, 20±2°C, pH 6-8.5, hardness 80-250mg/L *D. magna*, 10-250mg/L *D. pulex* for natural water; 80-100 mg/L and 40-48mg/L, respectively for reconstituted water; DO 90-100% at start, no aeration	cool white fluorescent, ≤ 600 lx at surface, 16h light: 8h dark, 25±1°C, pH 6.0-8.5 hardness not specified, but culture and control hardness should be within 20%; DO 40-100% throughout test, 90-100% at start; no aeration	light, temperature, pH similar to field conditions/known preferred conditions; general for macroinvertebrates: wide spectrum fluorescent light, 16h light: 8h dark, DO > 60% for coldwater species, > 40% for warmwater species	16 h light: 8h dark, room temp. (about 20°C)
Acceptability criteria	90% control survival/ mobility, if chemical conc. at end ≤ 20% start, flow-through system required	60% control daphnids produce 3 broods within 9 d, control survival >80%, control reproduction >15 young/ daphnid; health criteria for culture too	90% control survival in short-term tests	90% control survival
Medium defn., manipulation	numerous diluent waters depending on test purpose	see left; food recommendations, medium changed daily	general recommendations for macroinvertebrate feeding	diluent
Reference toxicant	zinc sulphate, sodium chloride, potassium dichromate, LC_{50}s in Environment Canada (1990c) determined within 14 d of test	zinc sulphate, sodium chloride (LOEC survival=1246mg/L), phenol, potassium chromate (LOEC survival= 0.125mg/L), cadmium chloride (NOEC survival= 0.03mg/L) (Eco-Research 1991) determined within 14 d of test	NS	NS
Negative control	dilution water, hardness ± 20% culture medium; solvent control	dilution water, hardness ± 20% culture medium; solvent control	diluent water + food if required	diluent water

Table 14 (continued)

	EC, *Daphnia*	EC, *Ceriodaphnia*	APHA, shrimp	Alta., *Gammarus*
Statistical analysis	LC_{50}/EC_{50} with 95% CI, probit analysis, moving average, binomial methods; trimmed Spearman-Karber not recommended; calculation example provided	LC_{50} as left; NOEC, LOEC using TOXSTAT computer program and Williams' test	LC_{50} with 95% CI using probit, logit, moving average, Litchfield-Wilcoxon method; one-way ANOVA to assess significance of differences, Student-Newman-Keuls test, Duncan's new multiple range test, Dunnett's test to assess differences between control and treatments	LC_{50}, trimmed Spearman-Karber procedure
Organism availability	easily	easily	?	?

	Oris et al. (1991) 100%		Oris et al. *Ceriodaphnia*
Test type	daphnid, chronic, reproduction, static, 4-d	**Conditions** (light, temp, pH, etc.)	28 lx, cool white fluorescent light, 16h light: 8h dark, 25±1°C, pH 8.81, hardness 57.07 mg $CaCO_3$, alkalinity 81.00 mg $CaCO_3$
Application	toxicity of chemicals		
Species	*Ceriodaphnia dubia*		
Endpoints	mean total young/female, LOEC, NOEC, ChV	**Acceptability criteria**	control mean total young/female > 13, control survival ≥90%
Organism selection	52 h old	**Medium defn., manipulation**	reconstituted water
No. organisms + replicates	1 daphnid, 10 reps, 4 conc.	**Reference toxicant**	phenol (IC_{50}= 5.3-5.8mg/L), 2,4-D (IC_{50}= 81.8-86.8mg/L)
Observation frequency	4 d	**Negative control**	reconstituted water
Volume test vessel	30 mL cups	**Statistical analysis**	Fisher's exact test for LOEC, NOEC survival; ANOVA, Dunnett's test for reproduction LOEC, NOEC; nonparametric monotonic smoothing technique for EC_{50} reproduction
Volume test substance	15 mL		
Test substance preparation	reconstituted water is diluent		
Culture, handling	feeding, culture	**Organism availability**	easily

Table 14 (continued)

	ASTM (1980) 71%	ASTM (1990a) 76%	ASTM (1988) 88%	Environment Canada (1992b) 100%
Test type	crayfish, acute, static/flow-through	mosquito, acute, mobility, static	fish, chronic, early life-stage, flow-through	fish, chronic, larval growth, static-renewal
Application	toxicity of a chemical/known mixtures	toxicity of chemicals, effluents	toxicity of a chemical/known mixture	toxicity of chemicals, receiving water, leachates, elutriates
Species	*Orconectes* sp. *Cambarus* sp. *Procambarus* sp. *Pacifastacus leniusculus*	*Wyeomyia Smithii*	*Oncorhynchus* sp. *Salmo* sp. *Salvelinus* sp. *Esox lucius* *Pimephales promelas* *Catostomus commersoni* *Ictalurus punctatus* *Lepomis macrochirus*	*Pimephales promelas*
Endpoints	mobility EC_{50}	mobility, EC_{50}	survival LC_{50}, weight, length EC_{50}, LOEC, NOEC	survival EC_{50}, LOEC, NOEC
Organism selection	from same source, about the same size, immature stages, not bearing eggs	nonbiting form, second instar	newly fertilized embryos (≤ 48 h after fertilization), except for salmonids (96 h) from at least 3 females	larval minnows hatched ≤ 24 h
No. organisms + replicates	at least 10 crayfish for static, 20 for flow-through, at least 2 reps, 5 conc. ≥ 60% dilution factor	10 larvae, at least 2 reps, 5 conc., 50% dilution factor	20-60 embryos depending upon species, at least 2 reps, 5 conc., 50% dilution factor	10 larvae, 3 reps, 5 conc., 50% dilution factor
Observation frequency	every 24 h to 96 h	24, 48 h	daily observation for at least 30 d (depends on species)	daily, survival, swimming behaviour; dry weight day 7

Table 14 (continued)

	ASTM, crayfish	ASTM, mosquito	ASTM, fish	EC, fathead minnow
Volume test vessel	to accommodate test substance volume; horizontal dimension ≥ 1.5 × horizontal dimension of crayfish	NS	NS	vessel diameter = depth test solution
Volume test substance	loading ≤ 0.8g/L ≤ 17°C, < 0.5g/L > 17°C	NS	loading ≤ 0.5g/L of solution passing through chamber/day	depth ≥ 3 cm with approximately same diameter, volume ≥ 250 mL
Test substance preparation	dilution water depends on test objectives	dilution water depends on test objectives	see left	see left
Culture, handling	general holding + acclimation information for aquatic organisms	colony maintenance, feeding	holding, feeding, handling	culturing, breeding, feeding, handling
Conditions (light, temp, pH, etc.)	light not specified, 17°C for first 3 genera, 17°C for last species; DO 60-100% saturation during first 48 h, 40-100% for remainder for static test; 60-100% for flow-through	16h light: 8h dark, 150ft-c, 27±2°C	light, temp given for each species, DO 60-100% saturation	≤ 500 lx at surface, 16h light: 8h dark, 25±1°C daily mean, extremes 23-27°C, pH 6.5-8.5, DO 40-100%
Acceptability criteria	90% control crayfish mobile; 1 treatment other than control affected < 37% crayfish + 1 treatment affected > 63% crayfish	90% control mobility	60-80% control survival depending upon species	80% control survival/typical swimming, control weight ≥ 250 µg, minimum significant difference in weights ≤ 20% mean control dry weight
Medium defn., manipulation	hardness, alkalinity, pH, conductivity should be measured; COD desirable	hardness, alkalinity, pH, conductivity, DO, TDS should be measured	hardness, alkalinity, pH, conductivity should be measured	hardness, conductivity pH, DO, temperature, should be measured daily solution renewal
Reference toxicant	NS	NS	NS	sodium chloride, phenol, zinc, LC_{50}s in Environment Canada (1990c), test monthly

Table 14 (continued)

	ASTM, crayfish	ASTM, mosquito	ASTM, fish	EC, fathead minnow
Negative control	dilution water	dilution water, performance control for effluent (10 reps in culture water)	dilution water + food	dilution water + food
Statistical analysis	EC_{50} with 95% CI, probit, moving average, Litchfield-Wilcoxon, binomial, sample EC_{50} calculations	EC_{50} with 95% CI, see left	EC_{50} with 95% CI, LOEC, NOEC; ANOVA, Williams'/ Shirley's/Dunnett's/ Tukey's tests; probit, logit	LC_{50}, EC_{50}, LOEC, NOEC: ANOVA, Williams'/Dunnett's/ Bonferroni t-test, Steel's many-one rank/Wilcoxon rank sum test; probit, not trimmed Spearman-Karber method
Organism availability	easily	variable	variable	easily

	Environment Canada (1990a) 100%	Holst and Ellwanger (1982)76%	USEPA (1985b) 88%	ASTM (1991) 88%
Test type	fish, 96 h acute, survival, static	floating vascular plant, chronic, growth, reproduction static	floating vascular plant, chronic, growth, reproduction, static-renewal	floating vascular plant, chronic, growth, reproduction, static
Application	toxicity of chemicals, effluents, leachates, elutriates	toxicity of pesticides	toxicity of chemicals	toxicity of a chemical/known mixtures
Species	*Oncorhynchus mykiss*	*Lemna gibba*	*Lemna gibba*	*Lemna gibba*
Endpoints	survival LC_{50}, behaviour/appearance, EC_{50}	no. plants, fronds, EC_{50}, LOEC, NOEC	frond number, growth rate, per cent survival EC_{10}, EC_{50}, EC_{90}	biomass (no. plants, fronds, dry weight), NOEC, EC_{50}
Organism selection	swim-up fry/fingerlings, mean weight 0.3-5g	strain G3, 3-frond plants	strain G3, 4-frond plants from cultures < 2 wk old	strain G3, 3/4-frond plants so total > 12 <16 fronds/rep
No. organisms + replicates	10, 2/3 reps for chemicals, 7-10 conc.	5 plants, 3 reps, 5 conc. in geometric series ≤ 2-fold	3 plants, 7 reps, 5 conc. to cover EC_{10} to EC_{90}	3-5 plants, at least 3 reps, 5 conc. dilution factor 0.6
Observation frequency	24, 48, 72, 96 h	at least every 3 d for 14 d	day 0, 3, 6, 7	day 0, 7

Table 14 (continued

	EC, rainbow trout	Holst & Ell., *Lemna*	USEPA, *Lemna*	ASTM, *Lemna*
Volume test vessel	depth≥ 15 cm	see below	250 mL beaker (container:medium is 5:3)	glass 250 mL beaker, 200 mL flat-bottomed test tube, 200/500 mLrlenmeyer flask
Volume test substance	solution depth ≥ 15cm, to accommodate loading of ≤ 0.5 g/L over 4 d	vessel size:medium is 5:2	150 mL	container:medium is 5:2
Test substance preparation	dilution water depends on test purpose	diluent is growth medium	diluent is growth medium	diluent is growth medium
Culture, handling	holding, acclimation, handling, feeding	NS	acclimation	acclimation
Conditions (light, temp, pH, etc.)	≤ 500 lx at surface, 16h light: 8h dark, full-spectrum fluorescent, 15±1°C, pH 5.5-8.5, DO 70-100%, aeration ≤ 7.5 mL/min/L	5 Klx, warm white fluorescent, 25± 2°C, pH 5.0±1	400 ± 50μE/m^2·s^{-1}, continuous light, 25±2°C, pH 4.8-5.2	620-6700 lx, continuous warm fluorescent light, 25±2°C
Acceptability criteria	control survival 90%	NS	NS	control frond number ≥ 5 times that at test start; light intensity varied >15%, highest and lowest temp differed by >4°C
Medium defn., manipulation	dilution water	M type Hoagland's medium without EDTA or sucrose	Hoagland's medium without chelating agents or sucrose; replace on day 3 and 6	M type Hoagland's medium without EDTA/sucrose or 20X-AAP medium
Reference toxicant	phenol, zinc sulphate, LC_{50}s in Environment Canada (1990c), monthly	NS	NS	NS
Negative control	diluent	growth medium	growth medium, solvent control	growth medium, solvent control

Table 14 (continued)

	EC, rainbow trout	Holst & Ell., *Lemna*	USEPA, *Lemna*	ASTM, *Lemna*
Statistical analysis	LC_{50} with 95% CI, probit, moving average, binomial methods; trimmed Spearman-Karber method not recommended	EC_{50}, LOEC, NOEC, references cited	means, SD for end-points, concentration-response curve with 95% CI, goodness-of-fit determination, EC_{10}, EC_{50}, EC_{90}	EC_{50} with 95% CI using linear/non-linear regression; use outlier detection procedures, tests of heterogeneity, pair-wise comparison techniques to determine NOEC
Organism availability	easily	easily	easily	easily

For tests written to apply to a large number of species (e.g., ASTM 1980, APHA 1989), the method for one test species was selected for further evaluation as representative of the completeness of the test as a whole. Aquatic tests from other agencies that are identical or very similar in terms of species, development stage selected, and duration to those already prepared by Environment Canada were not evaluated. They were reviewed and considered in the context of evaluating the Canadian protocols.

The following tests met all the 'must' criteria:

Photobacterium phosphoreum (Environment Canada 1991)

Pseudomonas putida (ISO 1991c)

Spirillum volutans (Dutka 1991)

Brachionus rubens (Snell and Persoone 1989)

Daphnia spp., 48-h (Environment Canada 1990b)

Ceriodaphnia dubia, 4-d (Oris et al. 1991)

Fathead minnow larva (Environment Canada 1992b)

Rainbow trout (Environment Canada 1990a)

The following tests had inadequate information on reference toxicants but met the acceptability criteria:

Selenastrum capricornutum (Environment Canada 1992c)

Algal growth (ASTM 1990c)

Brachionus calyciflorus, Rotoxkit™, (Anonymous 1990a)

Panagrellus redivivus (Samoiloff 1990)

D. magna, reproduction (Biesinger et al. 1987)

D. magna, reproduction (OECD 1991a)

C. dubia, 7-d (Environment Canada 1992a)

Shrimp (APHA et al. 1989)

Gammarus lacustris (Alberta Environmental Centre 1989)

Crayfish (ASTM 1980)

Wyeomyia smithii (ASTM 1990a)

Fish, early life-stage (ASTM 1988)

Lemna gibba (ASTM 1991)

The following tests had neither acceptability criteria nor adequate reference toxicant information:

Escherichia coli, Toxi-chromotest™ (Orgenics Ltd. 1985)

Bacillus cereus, resazurin reduction (Thomson et al. 1986)

Lemna gibba, U.S. EPA (FIFRA) (Holst and Ellwanger 1982)

L. gibba, U.S. EPA (TSCA) (U.S. EPA 1985b)

6.2.2 Detailed Evaluation

The 25 tests with appropriate written methods (Table 14) were further assessed in terms of the 12 'want' criteria (described in 3.2.2) that are valuable but not as important as the three 'must' criteria.

Test scores ranged from 59% to 100%, as shown by the bold number at the top of the columns in Table 14. The rationale for these scores is provided in Table B-3 (App. B). The results are summarized below:

7 tests scored 100%

Photobacterium phosphoreum (Environment Canada 1991)

Selenastrum capricornutum (Environment Canada 1992c)

Daphnia spp. (Environment Canada 1990b)

Ceriodaphnia dubia, 4-d (Oris et al. 1991)

C. dubia, 7-d (Environment Canada 1992a)

Fathead minnow larva (Environment Canada 1992b)

Rainbow trout (Environment Canada 1990a)

6 tests scored ≥ 88% and <100%

88% - *Brachionus rubens* (Snell and Persoone 1989)

88% - *B. calyciflorus*, Rotoxkit™ (Anon. 1990a)

88% - *Daphnia magna*, reproduction (Biesinger et al. 1987)

88% - Fish, early life-stage (ASTM 1988)

88% - *Lemna gibba* (ASTM 1991)

88% - *Lemna gibba*, U.S. EPA (TSCA) (U.S. EPA 1985b)

12 tests scored <88%

82% - *Pseudomonas putida* (ISO 1991c)

82% - Algal growth (ASTM 1990c)

82% - *Daphnia magna*, reproduction (OECD 1991a)

77% - *Spirillum volutans* (Dutka 1991)

76% - *Gammarus lacustris* (Alberta Environmental Centre 1989)

76% - *Panagrellus redivivus* (Samoiloff 1990)

76% - *Wyeomyia smithii* (ASTM 1990a)

76% - *Lemna gibba*, U.S. EPA (FIFRA) (Holst and Ellwanger 1982)

71% - *Escherichia coli*, Toxi-chromotest™ (Orgenics Ltd. 1985)

71% - Crayfish (ASTM 1980)

65% - *Bacillus cereus* (Thomson et al. 1986)

59% - Shrimp (APHA 1989)

The results of this evaluation are further discussed and interpreted in terms of priorities for future work in sections 6.5, 6.6, and 6.7.

6.3 Step 2 Assessment — Test Application

For each of the types of tests that scored ≥ 88% in the detailed evaluation (6.2.2), as well as two tests that met all three 'must' criteria but scored <88% (*Pseudomonas putida*, *Spirillum volutans*; see Table B-3 for point loss), additional information on trophic level represented, test sensitivity, test reproducibility, field validation, and ecological relevance is provided.

6.3.1 Bacterial Tests

There is a strong need to standardize bacterial tests, and efforts are being made towards these goals under the sponsorship of ISO (1990, 1991c) and other standards organizations. First, the importance of bacteria is described, followed by information on sensitivity, reproducibility, field validation, and ecological relevance for each test.

Trophic Level

Bacteria are involved primarily in the mineralization of organic substrates and in the recycling of mineral nutrients. Their activities are essential to self-purification processes in the environment. Many enzyme and bacterial growth tests have been developed for monitoring or screening toxicants in water and effluent discharges. Most of these are rapid, relatively reproducible, and inexpensive. Bacteria appear to be sensitive sensors of chemical toxicity; they respond relatively quickly to changes in their environment. However, little information is available on comparative studies of short-term bacterial assays for estimating the impact of toxicants on the aquatic environment (Dutka and Bitton 1986).

6.3.1.1 *Photobacterium phosphoreum*

The basic protocol (Microtox™) was originally marketed by Microbics Corp. in 1978 (Environment Canada 1991). It has been used extensively for freshwater toxicity testing. This test has been adopted as an official test in Quebec. Alberta has prepared method guidelines and British Columbia has produced a guidance document. A standard operating procedure has been prepared by the U.S. EPA and Germany has prepared a draft standard method (Environment Canada 1991). More recently, Microbics Corporation (1992a, 1992b) has developed a solid-phase protocol for testing sediment and soil toxicity.

Sensitivity

Bulich (1986) reviews the literature on the aquatic test. A data bank of Canadian test results is maintained (Kaiser and Ribo 1988). Munkittrick et al.(1991) showed that *P. phosphoreum* was about as sensitive to pure organic compounds as fathead minnows, trout, and *Daphnia* when lethality tests were used but was less sensitive to inorganic toxicants and pesticides.

Table 15 provides further information on the sensitivity of *P. phosphoreum* relative to other test organisms. This bacterium was less sensitive to a variety of compounds than *Daphnia magna* and rainbow trout, but more sensitive than *Spirillum volutans* (Qureshi et al. 1982, Indorato et al. 1983). With respect to heavy metals and insecticides, *P. phosphoreum* was less sensitive than *D. magna* and *Sel. capricornutum* (Miller et al. 1985). Concerning herbicides, it was more sensitive than *D. magna* but less sensitive than *S. capricornutum* and vascular plants (Miller et al. 1985). It was more sensitive to heavy metals but less sensitive to insecticides than earthworms (Miller et al. 1985). *Photobacterium phosphoreum* was less sensitive to pond water contaminated with herbicides, insecticides, and neurotoxicants than *Sel. capricornutum*, but more sensitive than *D. magna*. It was less sensitive than *D. magna* to river water contaminants, but more sensitive than *Spir. volutans*. *Photobacterium phosphoreum* was more sensitive to elutriates from river sediment than was *Sel. capricornutum* and *Brachionus calyciflorus* (rotifer) (Sloterdijk et al. 1989).

Generally, *P. phosphoreum* is less sensitive to contaminated water, effluents, and sediment and soil elutriates than *Sel. capricornutum* (Greene and Barich 1991, Miller et al. 1985, Peterson et al. 1987, Peterson et al. 1989, Greene et al. 1988, Plotkin and Ram 1984, Blaise et al. 1987).

Studies have shown *P. phosphoreum* to be relatively sensitive to some samples exhibiting toxicity to freshwater fish and invertebrates (Ankley et al. 1990b). However, the organism can be quite insensitive to others (Ankley et al. 1990a, Calleja et al. 1986, Qureshi et al. 1982, Chang et al. 1981). One explanation for its low correlation among toxicity tests is that the *P. phosphoreum* is used to test freshwater samples that must be osmotically adjusted to a final concentration of 2% sodium chloride. Salts, such as sodium chloride, can influence the bioavailability of toxicants in water samples. In response to this concern, the substitution of 20.4% sucrose for osmotic adjustment was evaluated (Hinwood and McCormick 1987). In single chemical experiments *P. phosphoreum* was more sensitive to zinc and cadmium and nearly two orders of magnitude more sensitive to ammonia when tested with sucrose rather than sodium chloride.

Photobacterium phosphoreum tested with sodium chloride was sensitive to 14 effluents, of which 10 were also toxic to *Ceriodaphnia dubia* and 7 were toxic to fathead minnows (Ankley et al. 1990b). Four samples toxic to the bacterium were not toxic to *C. dubia* or the fathead minnow. Fifteen-minute IC_{20}s for *P. phosphoreum* were significantly lower than 48-h LC_{50}s for *C. dubia* for 5 effluents, higher for 3 effluents, and not different for 29 effluents. Relative to 96-h LC_{50}s for the fathead minnow, IC_{20}s for the bacterium were lower for 7 effluents, higher for 9 effluents, and no different for 24 effluents. The remainder of the 44 test effluent results could not be compared because confidence intervals were not available.

The relative sensitivity of *P. phosphoreum* compared to other bacterial tests is shown in Table 16. It was more sensitive to many compounds than the activated sludge respiration test, the glucose mineralization test, the oxygen consumption test, and the resazurin reduction test.

Reproducibility

The reproducibility and variability of the test method developed by Microbics Corporation (1992a, b) is reviewed in Environment Canada (1991), which

Table 15

Relative sensitivity of water-dwelling organisms used (in tests reviewed in this document) for assessing water quality

The lower the rank, the lower the endpoint, the higher the sensitivity. Unless indicated in the table by asterisks, test endpoints are listed below. Due to limited space, only water-dwelling test species are included. Comparative studies showing the sensitivity of these organisms relative to other organisms that have been used in water quality assessment are found in Tables 4, 6, 10, and 16.

(P= *Photobacterium phosphoreum*, 15 min IC50; Sv= *Spirillum volutans*, 2 h IC_{90}; S= *Selenastrum capricornutum* (bottle test except for Blaise et al.= microplate); Dm= *Daphnia magna* (Atwater et al., Eco-Research and U. de Québec, Sloterdijk et al.= *D. pulex*), 48 h LC_{50}; C= *Ceriodaphnia dubia* (Kovacs and Ferguson= *C. affinis*), 7 d survival; F= fathead minnow, 96 h LC_{50}; R= rainbow trout, 96 h LC_{50}; L= *Lemna minor*)

(d= deionized water extract, f= food, fem= female, ft= flow-through, e= elutriate, emb= embryo, pw= pore water, s= static, sa= sodium acetate extract, w= water, #= relative sensitivity based on no. stations where toxic effect observed, ##= sensitivity is rank assigned by reference based on endpoints)

Test Substance	Endpoint	Species P	Sv	S	Dm	C	F	R	L	Reference
7 chemicals		1	2	-	-	-	-	-	-	Dutka et al. (in press)
River sediment elutriate #	3 h ^{14}C uptake* 48 h survival**	1	-	2*	3**	-	-	-	-	Sloterdijk et al. (1989)
7 effluents	5 min IC_{50}*	1*	-	-	1	-	-	2	-	Qureshi et al. (1982)
Lake sediment (1)		2	-	-	1	-	-	-	-	Giesy et al. (1990)
Pore water (2)		1	-	-	2	-	-	-	-	
44 effluents	15 min IC_{20}*	1*	-	-	-	1	1	-	-	Ankley et al. (1990b)
Effluent receiving water	? min IC_{50}*	2*	3	-	1	-	-	-	-	Dutka et al. (1989)
Sediment elutriate	? min IC_{50}*	2*	3	-	1	-	-	-	-	Dutka et al. (1989)
Natural gas plant sludges		2	-	1	3	-	-	-	-	Novak (1990)
		2	-	1	2	-	-	-	-	
		2	-	3	1	-	-	-	-	

Table 15 (continued)

Test Substance	Endpoint	Species P	Sv	S	Dm	C	F	R	L	Reference
Sanitary landfill leachate	5 min IC_{50}* 13 d IC_{50} chl *a***	2*	-	1**	3	-	4	-	-	Plotkin and Ram (1984)
Pulp & paper mill effluent	?? min IC_{50}*	2*	-	1	-	-	-	3	-	Blaise et al. (1987)
Herbicide + insecticide contaminated pond water	30 min IC_{50}*	2*	-	1	3	-	-	-	-	Peterson et al. (1985)
Pesticide	5/15/30 min IC_{50}*	2*	-	-	1**	-	-	-	-	Calleja et al. (1986)
Electroplating leachates	24 h EC_{50}**	2*	-	-	1**	-	-	-	-	
11 compounds	5 min IC_{50}* 5 min IC_{90}**	3*	4**	-	2	-	-	1	-	Qureshi et al. (1982)
326 samples water, soil/ sediment elutriates, wastes	30 min IC_{50}*	3*	-	1	2	-	-	-	-	Greene and Barich (1991)
soil leachate	30 min IC_{50}*	3*	-	1	2	-	-	-	-	Barich et al. (1987)
9 waste elutriates d	30 min IC_{50}*	3*	-	1	2	-	-	-	-	Peterson et al. (1989)
sa		3*	-	1	2	-	-	-	-	
Heavy metal	30 min IC_{50}*	3*	-	1	2	-	-	-	-	Thomas et al. (1986)
Pesticide		2*	-	1	1	-	-	-	-	
PAH soil elutriates		2*	-	1	3	-	-	-	-	
Creosote-contaminated w	? min IC_{50}*	3*	-	2	1	-	-	-	-	Athey et al. (1989)
water, sediment e		1*	-	2	3	-	-	-	-	

Table 15 (continued)

Test Substance		Endpoint	Species P	Sv	S	Dm	C	F	R	L	Reference
Heavy metals		30 min IC_{50}*	3*	-	1	2	-	-	-	-	Miller et al. (1985)
Herbicides			2*	-	1	3	-	-	-	-	
Insecticides			3*	-	1	2	-	-	-	-	
11 industrial effluents		96 h LC_{50}*	-	-	1	2*	-	-	-	-	Walsh et al. (1982)
19 nonpesticide organics			-	-	2	1	-	-	-	-	LeBlanc (1984)
Complex effluent		7 d LC_{50}* 7 d larval LC_{50}**	-	-	-	1	2*	3**	-	-	Pontasch et al. (1989)
Diflubenzuron		30 d emb-larval LC_{50}*	-	-	-	1	-	2*	-	-	Nebeker et al. (1983c)
11 metals			-	-	-	1	-	2	-	-	LeBlanc (1984)
Silver nitrate		21 d EC_{50}*	-	-	-	1*	-	2	3	-	Nebeker et al. (1983b)
Industrial effluents	##		-	-	-	1	-	2	3	-	Eco-Research and l'Université de Québec (1991)
Sanitary landfill leachate		48+96 h LC_{50}*	-	-	-	1*	-	-	1	-	Atwater et al. (1983)
Harbour sediment	e	48 h survival*	-	-	-	2*	1*	-	-	-	Burton et al. (1989)
Pyrethroid insecticides		48 h LC_{50}*	-	-	-	2	1*	-	-	-	Mokry and Hoagland (1990)
Silver nitrate	s		-	-	-	2f	-	1	3	-	Nebeker et al. (1983b)
	ft		-	-	-	-	-	1	2	-	
Hexachoroethane smoke combustion products			-	-	-	2	-	3	1	-	Fisher et al. (1990)

Table 15 (continued)

Test Substance		Endpoint	Species P	Sv	S	Dm	C	F	R	L	Reference
Endosulfan insecticide	s		-	-	-	3	-	1	2	-	Nebeker et al. (1983a)
	ft		-	-	-	-	-	2	1	-	
Pulp & paper mill effluent		48 h LC_{50}*	-	-	-	3	2*	-	1	-	Kovacs and Ferguson (1990)
Effluent receiving water		7 d larval survival*	-	-	-	-	1	1*	-	-	Mount et al. (1985)
Effluent receiving water		as above	-	-	-	-	1	1	-	-	Norberg-King and Mount (1986)
Fertilizer plant effluent		7 d survival LOEC*	-	-	-	-	1*	1**	-	-	Norberg-King and Mount (1986)
		7 d larval survival LOEC**									
		7 d young/fem LOEC*	-	-	-	-	1*	1**	-	-	
		7 d larval weight LOEC**									
Effluent receiving water		7 d larval survival*	-	-	-	-	2	1*	-	-	Mount et al. (1986a)
Effluent receiving water		as above	-	-	-	-	1	1	-	-	Mount et al. (1984)
Water treatment effluent		7 d survival LOEC*	-	-	-	-	1*	2**	-	-	Mount et al. (1985)
		7 d larval survival LOEC**									
		7 d young/fem LOEC*	-	-	-	-	1*	2**	-	-	
		7 d larval weight LOEC**									
Sewage treatment plant effluent		as above	-	-	-	-	1	1	-	-	Mount et al. (1984)
		as above	-	-	-	-	1	2	-	-	
Chemical plant effluent		as above	-	-	-	-	1	1	-	-	Mount et al. (1984)
		as above	-	-	-	-	2	1	-	-	

Table 15 (continued)

Test Substance		Endpoint	Species P	Sv	S	Dm	C	F	R	L	Reference
Waste treatment effluent		7 d NOEC*	-	-	-	-	1*	1**	-	-	Stewart et al. (1990)
Coal yard effluent		7 d larval NOEC**	-	-	-	-	1*	2**	-	-	
Sewage treatment effluent			-	-	-	-	1*	2**	-	-	
Lake	e	96 h larval LC_{50}*	-	-	-	-	1	1*	-	-	Ankley et al. (1991)
sediment	pw		-	-	-	-	1	2	-	-	
Industrial effluents	##	7 d survival NOEC* 7 d larval NOEC** 96 h ATP stress NOEC***	-	-	-	-	2*	1**	3***	-	Eco-Research and l'Université de Québec (1991)
Coke plant effluents		7 d survival LOEC*	-	-	-	-	2*	1**	-	-	Mount et al. (1985)
		7 d larval survival LOEC**									
		7 d young/fem LOEC*	-	-	-	-	2*	1**	-	-	
		7 d larval weight LOEC**									
Refinery effluent		as above	-	-	-	-	2	1	-	-	Mount et al. (1984)
		as above	-	-	-	-	1	2	-	-	
Refinery effluent		as above	-	-	-	-	2	1	-	-	Norberg-King and Mount (1986)
		as above	-	-	-	-	2	1	-	-	
Copper		ChV	-	-	-	-	3	1	-	2	Taraldsen and Norberg-King (1990)
Herbicide			-	-	-	-	2	-	-	1	
Refinery effluent			-	-	-	-	2	2	-	1	
Oil treatment effluent			-	-	-	-	1	2	-	3	

Table 16

Relative sensitivity of bacteria to toxicants

(P= *Photobacterium phosphoreum*, Sv= *Spirillum volutans*, ASR= activated sludge respiration test, ASTTC= activated sludge TTC test, OXY= oxygen consumption test, GM= glucose mineralization test, TC= Toxi-chromotest™, *= not toxic at 1g/L, **= not toxic at 100mg/L)

Test Substance	Species or Test Method										
	P Environment Canada (1991)	Sv Dutka (1991)	ASR ISO (1983)	ASTCC Ryssov-Nielsen (1975)	OXY Retuna et al. (1989)	GM Retuna et al. (1989)	Resazurin Thomson et al. (1986) isolate	Resazurin Thomson et al. (1986) *E. coli*	Resazurin Thomson et al. (1986) isolate	Resazurin Thomson et al. (1986) *E. coli*	TC Reinhartz et al. (1987)
Copper	1	1	-	-	-	-	-	-	-	-	-
Zinc	2	1	-	-	-	-	-	-	-	-	-
Mercury	1	2	-	-	-	-	-	-	-	-	-
Arsenate	1	2	-	-	-	-	-	-	-	-	-
Cyanide	2	1	-	-		-	-	-	-	-	-
Ammonia, total	2	1	-	-	-	-	-	-	-	-	-
Ammonia, un-ionized	2	1	-	-	-	-	-	-	-	-	-
Phenol	1	2	-	-	-	-	-	-	-	-	-
Styrene	1	2	-	-	-	-	-	-	-	-	-
Chloroform	1	2	-	-	-	-	-	-	-	-	-
1,2-dichloroethane (Qureshi et al. 1982)	1	2	-	-	-	-	-	-	-	-	-
3,5-dichlorophenol	1	2	3	4	-	-	-	-	-	-	-
Cetyl trimethyl ammonium chloride	1	2	3	4	-	-	-	-	-	-	-
Sodium lauryl sulfate	1	2	4	3	-	-	-	-	-	-	-
Phenol	1	2	3	4	-	-	-	-	-	-	-
Copper	2	3	4	1	-	-	-	-	-	-	-
Mercury	1	2	4	3	-	-	-	-	-	-	-
Zinc (Casseri et al., n.d.)	1	3	2	4	-	-	-	-	-	-	-
Copper	1	-	2	-	-	-	-	-	-	-	-
3,5-dichlorophenol (Retuna et al. 1986)	1	-	2	-	-	-	-	-	-	-	-

Table 16 (continued)

Test Substance	Species or Test Method										
	P Environment Canada (1991)	Sv Dutka (1991)	ASR ISO (1983)	ASTCC Ryssov-Nielsen (1975)	OXY Retuna et al. (1989)	GM Retuna et al. (1989)	Resazurin Thomson et al. (1986) isolate	Resazurin Thomson et al. (1986) *E. coli*	Resazurin Thomson et al. (1986) isolate	Resazurin Thomson et al. (1986) *E. coli*	TC Reinhartz et al. (1987)
9 chemical wastes, 15 metal & inorganic compounds (Jones and Greene 1991)	2	-	-	-	-	-	-	-	-	-	1
Copper	1	-	-	-	3	2	-	-	-	-	-
Zinc	1	-	-	-	3	2	-	-	-	-	-
Cadmium	1	-	-	-	2	3	-	-	-	-	-
3,5-dichlorophenol	1	-	-	-	2	3	-	-	-	-	-
Chromium	2	-	-	-	1	3	-	-	-	-	-
Diethylamine	1	-	-	-	3	2	-	-	-	-	-
Dodecylbenzene sulfonic acid	1	-	-	-	3	2	-	-	-	-	-
Benzene	1	-	-	-	2	3*	-	-	-	-	-
Malathion	1	-	-	-	2**	2**	-	-	-	-	-
Atrazine (Retuna et al. 1989)	1	-	-	-	2**	2**	-	-	-	-	-
Copper (Greene et al. 1985)	1	-	-	-	-	-	2	3	4	5	-

provides the following information. It was noted that variation in cuvette geometry and transfer volumes may each contribute 1% to light reading uncertainty. Testing of 236 samples by Environnement Québec showed that a minimum of 17% and a maximum of 83% light inhibition could be quantified with statistical significance, while the detection limit was 12%.

For a series of 81 tests with the reference toxicant sodium lauryl sulphate, the CV in inhibition was 18%. The CVs for the three lots of bacteria used ranged from 6% to 10%. Variation attributed to different technicians and different analyzers used for the 81 samples was not significant. Work with eight organic chemicals showed that the overall mean deviation of replicates from the mean IC_{50} was 10%. Average CVs of 2% to 30% are typical except for metal tests, where the average CV was 60%.

Ecological Relevance

The ecological relevance of using this marine bacterium to test freshwater toxicity is questionable. Its natural habitat is sea water or the surface and alimentary tract of some marine fishes and the luminous organs of some fish and cephalopods. Optimum NaCl concentration is usually 3.0%, but growth occurs in nutrient media with 0.5% to 5.0% NaCl. There is no growth without NaCl (Holt 1984). In testing freshwater, the addition of salt or sucrose to the test solution is necessary. How can we be confident that the toxicity of this mixture reflects the toxicity of freshwater to freshwater bacteria?

6.3.1.2 *Escherichia coli* (Toxi-chromotest™)

An extensive compilation and comparison of the various microbial and animal toxicity bioassessment methods was assembled by Liu and Dutka (1984). At that time, the conclusion was that the most useful microbial test was Microtox™ using *P. phosphoreum*. Since then a new standardized microbial test procedure, the Toxi-chromotest™, appeared on the market (Orgenics Ltd. 1985, Reinhartz et al. 1987). The Toxi-chromotest™ is based on a mutant strain of *Escherichia coli*. Toxicants can easily penetrate the rough lipopolysaccharide cell wall and inhibit the de novo synthesis of the inducible enzyme beta-galactosidase. The test has a colorimetric endpoint. It is performed in microplates and read using the widely available microtitration plate photometers (ELISA Readers).

Sensitivity

Toxicity of nine hazardous chemical waste-site samples and 15 organic and metal compounds were evaluated using *E. coli* (Toxi-chromotest™) and *P. phosphoreum* (Microtox™) (Jones and Greene 1991, Table 16). The Toxi-chromotest™ demonstrated sensitivity equal to or greater than that of *P. phosphoreum* in 69% of the samples.

Comparative toxicity assessment of 128 samples from a contaminated chemical manufacturing site was performed using the green alga *Selenastrum capricornutum*, *Daphnia magna*, and the Toxi-chromotest ™ (J. Greene, pers. comm.). Seventy per cent of the samples demonstrated toxicity to one or more organisms. The Toxi-chromotest™, however, identified only 3% of the samples as containing toxic constituents.

Reproducibility

For six metals, coefficients of variation ranged from 16% to 64% in intralaboratory testing (Table 17).

Ecological Relevance

Escherichia coli is a common freshwater bacterium.

6.3.1.3 Pseudomonas putida

There are a large number of microbial assays for chemical toxicity in aquatic environments based on the measurement of growth inhibition. In 1991 a German standard (NAW 1991) was published. Simultaneously, the ISO (1991c), working on a draft protocol, concluded that *P. putida* was a suitable organism for representing heterotrophic organisms in freshwater.

Sensitivity

No information was found on the sensitivity of *P. putida* relative to other test organisms.

Reproducibility

An international round-robin test was carried out with participation of 21 laboratories in 1989 (ISO 1991c). An EC_{50} value for 3,5-dichlorophenol of 21.4 mg/L with a CV of 23% was established using this procedure. The corresponding CV for the IC_{10} was 31.8% (Table 17).

Ecological Relevance

Pseudomonas putida is representative of destruent freshwater bacteria. They are single-celled straight or curved rods that are motile by polar flagella. Their metabolism is respiratory, and they are able to use H_2 or CO as energy sources (Holt 1984).

6.3.1.4 *Spirillum volutans*

Spirillum volutans is a large aquatic bacterium that is readily visible under low magnification (Dutka 1991). It has a fascicle of flagella at each end that, under normal conditions, form oriented revolving cones

Table 17

Reproducibility of tests for freshwater quality

Multiple coefficients of variation (CV) or ranges of CVs for a test are for different samples analyzed. Where more than one CV or more than one range of CVs is given for a test, each corresponds to a different sample. (A= intralaboratory test [1 laboratory], E= interlaboratory test [a single CV for an E test is for the mean endpoint among laboratories, a range of CVs for an E test indicates CVs for individual laboratories that conducted the test], p.c. = personal communication)

Organism	Test Method	Endpoint	Substance	CV	Type	Reference
Photobacterium phosphoreum	5-minute exposure Microbics Corporation (1992) Environment Canada (1991)	IC_{50} bioluminescence	natural gas plant sludge	1.6-100.2% 1.8-41.2% 3.9-43.0%	A	Novak (1990)
	15-minute exposure	IC_{50}		1.9-115.6% 0.2-13.2% 5.4-40.9%		
	5-minute exposure (method above)	IC_{50}		<20%	E	Eco-Research & l'Université de Québec (1991)
	5-minute exposure 15-minute exposure 30-minute exposure 60-minute exposure	IC_{50}	copper	67.77% 46.43% 26.09% 25.00%	A	Greene et al. (1985)
Pseudomonas putida	ISO (1991c)	IC_{50} IC_{10}	3,5-dichlorophenol	23% 31.8%	E	ISO (1991c)
Escherichia coli	Toxi-chromotest™ (Orgenics Ltd., 1985)	IC_{50}	copper aluminum cadmium mercury lead vanadium	18% 64% 59% 39% 39% 16%	A	J. Clarke p.c. (1992)
Selenastrum capricornutum	96 h microplate Environment Canada (1992c)	IC_{50} growth	industrial effluent	<20% <20% <20% <20% <20% >20%	E	Eco-Research & l'Université de Québec (1991)

Table 17 (continued)

Organism	Test Method	Endpoint	Substance	CV	Type	Reference
Selenastrum capricornutum (cont.)		IC_{50}	natural gas	16.8%	A	Novak (1990)
			plant sludge	19.3%		
				21.1%		
		IC_{50}	control	8.7%	A	Thellen et al. (1989)
		IC_{50}	cadmium	24.3%		
		IC_{50}	phenol	34.9%		
	96-h flask USEPA (1987c)	effect of 50% effluent	industrial effluent	<20%	E	Eco-Research & l'Université de Québec (1991)
				<20%		
				<20%		
				<20%		
				>20%		
Brachionus calyciflorus	24 h Rotoxkit	IC_{50} growth	copper sulphate	49.1%	E	Persoone et al. (1990)
			all laboratory tests	15-20%	A	G. Persoone p.c.
		LC_{50}	5 pesticides	8.37-17.47%	A	Ferrando and Andreu-Moliner (1991)
Brachionus calyciflorus	48 h	IC_{50}, NOEC growth, LC_{50}	9 chemicals	20-30%	A	Snell et al. (1990)
				10-20%		
Daphnia pulex	48 h Environment Canada (1990b)	IC_{50}	industrial effluent	<20%	E	Eco-Research & l'Université de Québec (1991)
				<20%		
				<20%		
				<20%		
				<20%		
				>20%		
				>20%		

Table 17 (continued)

Organism	Test Method	Endpoint	Substance	CV	Type	Reference
Daphnia pulex (cont.)	48 h Environment Canada (1990b)	LC_{50}	sodium dodecyl sulphate, sodium penta-chlorophenate, cadmium	43.8% 35.7% 20.9%	A	Lewis and Weber (1985)
Daphnia magna	48 h Environment Canada (1990b)	LC_{50}	natural gas plant sludge	2.1-6.7% 3.2-5.1% 7.5-14.1%	A	Novak (1990)
D. magna	48 h Environment Canada (1990b)	LC_{50}	sodium dodecyl sulphate, sodium penta-chlorophenate, cadmium	28.9% 10.4% 72.4% 7.5-14.1%	A	Lewis and Weber (1985)
	48 h Gersich et al. (1986)	LC_{50}	7 chemicals	0.57-6.08%	A	Gersich et al. (1986)
	28 d (Parkhurst et al. 1981)	% survival broods/female young/brood young/female	acridine (0.2mg/L)	0-15.4% 7.7-28.8% 11.2-33.0% 9.3-39.7%	A	Parkhurst et al. (1981)
Ceriodaphnia dubia	7 d USEPA (nd)	LOEC reproduction	industrial effluent	<20% <20% >20% >20% >20%	E	Eco-Research & l'Université de Québec (1991)
		LOEC survival		<20% <20% <20% <20% <20% <20% >20%		

Table 17 (continued)

Organism	Test Method	Endpoint	Substance	CV	Type	Reference
C. dubia	7 day USEPA (1985f)	IC_{50} young production	sodium chloride	29%	E	Anderson and Norberg-King (1991)
	7 day	LC_{50}	sodium lauryl	7.8%	A	Cowgill et al. (1990)
		IC reproduction	sulphate	1.4%		
	7 day USEPA (n.d.)	LC_{50}	6 substances	30-38%	E	EPRI (1989)
		IC_{50} reproduction		29%-39%		
Fathead minnow	7 day larval growth and survival USEPA (n.d.)	LC_{50}	pentachlorophenate	43.7%	E	De Graeve et al. (1991)
		LC_{50}	potassium dichromate	24.1%		
		% survival	control	12.7%		
		weight	control	52.0%		
		LC_{50}	refinery effluent	31.3%	E	
		LC_{50}	refinery effluent	25.6%		
		LC_{50}	utility waste	37.5%		
		IC_{50} weight	refinery effluent	40.4%	E	
		IC_{50} weight	refinery effluent	22.4%		
		IC_{50} weight	utility waste	61.9%		
		LOEC survival	industrial effluents	<20%	E	Eco-Research & l'Université de Québec (1991)
				<20%		
				<20%		
		LOEC growth		<20%		
				<20%		
				<20%		
	7 day USEPA (1985f)	IC_{50} weight	hexavalent chromium	31%	E	Anderson and Norberg-King (1991)

Table 17 (continued)

Organism	Test Method	Endpoint	Substance	CV	Type	Reference
Rainbow trout	96 h Eco-Research & l'Université de Québec (1991)	LC_{50}	industrial effluents	<20% <20% <20% <20% <20% <20%	E	Eco-Research & l'Université de Québec (1991)
	96 h	LC_{50}	sodium lauryl sulphate	16.3%	E	K. Doe p.c.

allowing the bacterium to move forward and reverse directions at will. During the reversing process the polar fascicles reorient simultaneously. This bioassay is based on observing a decrease in reversing motility of 90% of the test cells, which is considered a positive effect (Trevor 1986). If a sample is toxic but contains nonlethal levels of toxicants, *S. volutans* loses coordination, as both fascicles try to assume the head or tail orientation, thus preventing normal bacterial motion (Boudre and Kreig 1974).

Sensitivity

Comparisons of toxicity for 11 chemical compounds (metals, arsenate, cyanide, ammonia, phenol, styrene, chloroform, and 1,2-dichloroethane) showed that *S. volutans* was least sensitive relative to rainbow trout, *Daphnia magna*, and *Photobacterium phosphoreum* (Qureshi et al. 1982) (Table 15). For seven chemicals, *S. volutans* was less sensitive than *P. phosphoreum* (Indorato et al. 1983). Testing of river water also showed *S. volutans* to be insensitive relative to *P. phosphoreum* and *D. magna* (Dutka et al. 1989).

Reproducibility

No information was found on reproducibility of this test.

Ecological Relevance

Spirillum volutans is a bacterium that is common in polluted and stagnant freshwater. These organisms have a strictly respiratory metabolism with oxygen as the terminal electron acceptor. Optimum temperature is 30°C with no growth at 10 or 45°C (Holt 1984).

6.3.2 Algal Tests

Many regulatory and standards organizations use flask algal bioassays for testing the toxicity of chemicals (e.g., U.S. EPA, Greene et al. 1989; Weber et al. 1989; ASTM 1990c; ISO 1987; EEC, Anonymous 1988). Environment Canada (1992c) has adopted the microplate technique. The Swedish National Chemicals Inspectorate uses the microplate technique in its manual for routine growth inhibition tests with 20 freshwater species (Blanck and Bjornsater 1989; Table 12).

Given the emphasis of this document on soil and sediment, it is beyond its scope to do a complete review of relative toxicity of algal species, which has recently been done (Swanson 1989). Additional information below pertains only to *S. capricornutum*, the test species in Environment Canada's (1992c) algal test.

Trophic Level

Algae are the primary carbon-fixing organisms in aquatic environments and are thus an indispensable link between solar radiation, the complex solution of chemicals in the water, and all aquatic animals and man, whose existence is dependent on the oxygen evolved in photosynthesis. Algae produce an estimated 50% to 90% of the world's oxygen supply (Round 1984). In freshwater ecosystems, particularly larger lakes, algae are more important than vascular plants in terms of primary production (Wetzel 1975).

Sensitivity

The sensitivity of *S. capricornutum* relative to organisms other than algae is shown in Table 15. It shows that this alga was less sensitive to 19 non pesticide organic compounds than *Daphnia magna* and more sensitive to heavy metals and insecticides than *P. phosphoreum*, *D. magna*, earthworms, and terrestrial vascular plants. It is less sensitive than vascular plants to numerous herbicides. Tests with 21 herbicides using radish, barley, beans, and *S. capricornutum* showed that the alga was most sensitive to 11 and that an alga cannot be used as a surrogate for evaluating toxicity to vascular plants (Garten and Frank 1984).

Concerning effluents and waters contaminated by a mixture of chemicals, *S. capricornutum* was more sensitive to pulp and paper effluent than *P. phosphoreum* and rainbow trout (*Oncorhynchus mykiss*) and more sensitive to 11 industrial effluents (e.g., paper mill, textile dyeing, oil refinery, leather tanning) than *D. magna*. The alga was less sensitive to creosote contaminated water and sediment than *D. magna* and *P. phosphoreum*, respectively. *Selenastrum capricornutum* was less sensitive to elutriates from river sediment than was *P. phosphoreum* but more sensitive than the rotifer *Brachionus calyciflorus* (Sloterdijk et al. 1989).

For leachates or elutriates from sanitary landfills and soil containing heavy metals, pesticides, polynuclear aromatic hydrocarbons, herbicides, insecticides, and neurotoxins, *S. capricornutum* was more sensitive than *P. phosphoreum*, and *D. magna*. It was also more sensitive than tests with earthworms and lettuce using solid soil from the same sites.

When the results of tests with 326 water, waste and sediment/soil elutriates were examined, *S. capricornutum* and *D. magna* were more sensitive than *P. phosphoreum*. *S. capricornutum* is sensitive to a variety of toxic substances in water and sediment/soil elutriates and soil leachates. In numerous cases, it shows greater sensitivity than do numerous other test organisms.

Lewis (1990) showed that the relative sensitivity of algal species to the same toxicant can vary by more than 2000 times (e.g., disodium hydrogen arsenate, 13 spp.). As well, the toxicity of one group of compounds to one species of alga may vary from two (nonionic surfactants, *Microcystis aeruginosa*) to more than 100 times (organic acids, *M. aeruginosa*). In a comparison of *S. capricornutum* and *Chlorella vulgaris* with 21 herbicides, the former was most sensitive to all but two (Garten and Frank 1984).

Reproducibility

In a round robin microplate test involving three laboratories, six technicians, and 204 tests, the overall control coefficient of variation was 8.7% (Thellen et. al. 1989). Coefficients of variation for cadmium and phenol 96-h IC_{50}s were 24.3% and 34.9%, respectively. Algal assay technique whether standardized or 'in house' had no effect on the toxicity results. Other tests using either the flask or microplate technique (Table 17) showed good reproducibility.

Field Validation

The biological relevance of laboratory algal toxicity tests is largely undefined and in need of investigation. Because of the rapid regeneration of algae it is necessary to integrate toxicological principles with ecological factors such as adaptation and compensation to better understand the significance of reductions in algal growth observed in laboratory tests (Lewis 1990). Field validation studies have shown that laboratory-derived single species data for pure chemicals are comparable to those derived for natural algal communities under more natural conditions (Indorato et al. 1983).

Ecological Relevance

Selenastrum capricornutum is a freshwater algal species that is not indigenous to Canada. The genus is, however, indigenous to the North American continent. It has a long history of toxicological testing.

6.3.3 Rotifer Tests

Extensive research has been conducted over the last decade in Belgium and Florida to develop bioassay methods that begin with the dormant stages of test organisms, such as rotifers (Persoone et al. 1990). Tests were identified that use two species of rotifers (Rotoxkit™, Anonymous 1990a; Snell and Persoone 1989). Standardized toxkits are available for one species (*Brachionus calyciflorus*), tests are routinely conducted by private laboratories (e.g., Bio-Response Systems 1990; S. Goudey, HydroQual, Calgary, pers. comm.), and the ASTM has recently prepared a standard guide for acute toxicity testing with rotifers (G. Persoone, State Univ. of Ghent, Belgium, pers. comm.), which was unavailable for review.

Brachionus rubens was the species around which the rotifer test was developed. The species *B. calyciflorus* was adopted as the test species for use in the toxkits subsequently prepared (G. Persoone, pers. comm.). With the provision of reference toxicant data, unavailable during this review but currently in preparation, rotifer tests will be considered as candidates for addition to the usable battery (6.4.3).

Trophic Level

Rotifers are zooplankton that filter feed on phytoplankton and bacteria and at times exert grazing pressure that exceeds that of the larger crustacean zooplankton. They are a significant food for many larval fish, planktivorous adult fish, and several invertebrate predators (Anonymous 1990a).

Sensitivity

Chronic testing (NOEC endpoint) with *Brachionus calyciflorus* (48-h instantaneous growth rate) has shown that it is about three times more sensitive to PCP than *Daphnia* (total young per female, 7-d test) and *Ceriodaphnia* (total young per female, 7-d test) (Snell et al. 1990). The rotifer is less sensitive than the other two to cadmium. A study with river sediment elutriates showed that *Photobacterium phosphoreum, Selenastrum capricornutum, Panagrellus redivivus*, and *Daphnia magna* were more sensitive than *B. calyciflorus* based on the number of samples resulting in a toxic response (Sloterdijk et al. 1989).

Reproducibility

In a round-robin test involving 170 laboratories using *Brachionus calyciflorus*, a 24-h LC_{50} test (Anonymous 1990a) was carried out with copper sulphate (Persoone et al. 1990) (Table 17). The coefficient of variation for this endpoint was 49.1%. Sixty per cent of the tests were successful. Failures were due to excessive mortality in the controls (24%), hatching problems (13%), and other reasons (3%). High mortality and low hatching success were attributed to longer than expected shipment times and inappropriate storage methods by participants. Excessive control mortality was found in some cases where neonate age specified in the procedure was exceeded. Neonates that were too old starved. The toxicity of the reference toxicant was found to decrease after a number of months, introducing further variability of the results. It is clear from this study that strict adherence to the standard operational procedure is essential.

Based on the results of this study, new toxkits have been prepared and are available commercially. Further

research on the drying of cysts has been conducted following the interlaboratory study. The viability and hatching success of cysts remains constant and can be guaranteed for at least six months. Within-laboratory CVs of 15% to 20% are routinely obtained with this new version of the test (G. Persoone, pers. comm.).

Coefficients of variation for r values (growth rate) for the 48-h test with *B. calyciflorus* range from 20% to 30% (Snell et al. 1990).

Ecological Relevance

In freshwater, rotifers often account for the major fraction of zooplankton biomass at certain times of the year. Ten species in the genus *Brachionus* have a cosmopolitan distribution and are found in diverse aquatic habitats on all continents (Anonymous 1990a).

An extensive database exists on the biology of this group. The rotifer life cycle is well-defined and the factors regulating it reasonably well understood. Several aspects of rotifer behaviour have been examined closely and the systematics of the group well described.

6.3.4 Daphnia *Tests*

Daphnia magna is a traditional and most widely used organism for assessing aquatic toxicity (e.g., EEC 1989; OECD 1984c, 1991a; Environment Canada 1990b, 1990e; Peltier and Weber 1985; Calleja et al. 1986; Greene et al. 1989). Tests have been prepared for assessing lethality and effects on reproduction. As well, tests have been prepared for *D. pulex*, which tolerates a wider range of hardness than *D. magna* (Environment Canada 1990b).

Trophic Level

Daphnids are planktonic microcrustaceans that feed at the surface of sediments and in the water column. They are a major component of the freshwater zooplankton and form a significant portion of the diet of numerous fish species (Environment Canada 1990b, 1990e).

Sensitivity

Table 15 shows the sensitivity of *D. magna* with respect to numerous other test organisms. For the individual compounds listed in this table, and pulp and paper effluent, *D. magna* appears to be less sensitive than fathead minnows or rainbow trout (Nebeker et al. 1983b, Fisher et al. 1989, Qureshi et al. 1982, Nebeker et al. 1983a). Fish were less sensitive to other effluents (Pontasch et al. 1989, Qureshi et al. 1982). *Daphnia magna* was more sensitive to a variety of compounds (Qureshi et al. 1982), heavy metals and insecticides (Miller et al. 1985), herbicide-insecticide-neurotoxin contaminated pond water (Miller et al. 1985), and pesticide and electroplating leachates (Calleja et al. 1986) than *Photobacterium phosphoreum*. With some heavy metals, herbicides, and insecticides, *D. magna* is less sensitive than *S. capricornutum* (Miller et al. 1985). Two natural gas plant sludges were found to be more toxic to *D. magna* than *S. capricornutum* and *P. phosphoreum*, while *D. magna* was less sensitive than these organisms to a third sludge (Novak 1990).

Concerning soil leachates or elutriates, *D. magna* was more sensitive to most while *P. phosphoreum* was more sensitive to a few. Toxicity testing with 326 samples of water, wastes and soil/sediment elutriates, *D. magna* generally is more sensitive than *P. phosphoreum* and less sensitive than *S. capricornutum* (Greene and Barich 1991).

Daphnia magna was less sensitive than *Ceriodaphnia dubia* to harbour sediment elutriates.

A comparison of 24-h LC_{50} tests with six compounds showed that the LC_{50} for *D. magna* were lower than that of *Brachionus rubens* (rotifer) for sodium pentachlorophenate, malathion, copper sulphate, and cadmium chloride, while those of the daphnid were lower for sodium dodecyl sulphate and free ammonia (Snell and Persoone 1989).

In a survey of the literature Vaishnav and Korthals (1990) showed that the 48-h EC immobilization for *D. magna* was lower than the 96-h LC_{50} for fathead minnows for acetone, ethanol, methanol, and phenol, while it was higher for 2-propenol, 1-octanol, 1-heptanol, and 1-hexanol. For seven out of eight compounds including metals, insecticides, phosphate esters, polynuclear aromatics, and herbicides, the literature showed that *D. magna* was more sensitive than *Chironomus riparius* or *C. plumosus* (Ingersoll et al. 1990; Table 10). All tests used first instar midges.

In a study where 96-h LC_{50}s were determined simultaneously for 12 chemicals, *D. magna* was more sensitive to all of them than a flatworm, snail, fathead minnow, pillbug, sideswimmer, and segmented worm (Ewell et al. 1986).

A comparison of 96-h LC_{50}s for *D. magna* from the literature with experimental results for the nematode *Caenohabditis elegans* (Williams and Dusenbery 1990) showed that *D. magna* was more sensitive to cadmium, silver, copper, mercury, zinc, and arsenic, but less sensitive to lead and nickel.

In summary, literature cited in Table 15 and elsewhere illustrate that *Daphnia magna* often shows greater sensitivity to compounds and contaminated water and soils than non-daphnid test species.

Testing with *D. pulex* is less common. Based on 48-h LC_{50} values, it has been shown to be equally sensitive to sodium dodecyl sulphate and sodium pentachlorophenate as *D. magna*, but less sensitive to cadmium (Lewis and Weber 1985). In acute tests with industrial effluents (Eco-Research and l'Université de Québec 1991), *D. pulex* was more sensitive than fathead minnows and rainbow trout. In chronic tests, *D. pulex* was more sensitive than rainbow trout, but less sensitive than fathead minnow embryos or larvae.

Correlation and regression analysis for 48-h LC_{50}s for *D. magna* vs. fathead minnows ($r^2 = 0.92$, n = 29) and rainbow trout ($r^2 = 0.86$, n = 40) showed that there is a good relationship between the relative sensitivity of *D. magna* and the two fish (Doherty 1983).

Reproducibility

The reproducibility of the 48-h *Daphnia* test is generally good but varies with the type of substance. For example, coefficients of variation are typically higher for metals (K. Doe, Envir. Protection, Environment Canada, pers. comm.). Given the wide use of this test, there is considerable information on its reproducibility, a selection of which is provided in Table 17. Coefficients of variation ranged from 0.57% to 72.4%. For long-term reproductive tests, CVs from 0% to 39.7% are reported.

Field Validation

In a toxicity test with chlorothalonil, a fungicide used to protect crops from potato blight, the 48-h LC_{50} for *D. magna* was determined in the laboratory and compared to effects on caged endemic species (waterboatmen, clams, caddisfly larvae, beetle, midge larvae, scud) and rainbow trout as well as the composition of the natural benthic fauna in ponds sprayed with the fungicide (Ernst et al. 1991). The LC_{50} of *D. magna* not fed during the test was lower than the concentration in all ponds and only one of the six caged invertebrates (*Chironomus* sp.) exhibited <50% survival (the authors attribute this to damage during inspection of cages) and caged rainbow trout showed no mortality. Changes in total numbers of benthic invertebrates over the duration of three spray events reflect, in part, emergence that confounded effects due to fungicide application. These results indicate poor correlation between laboratory toxicity and field toxicity for caged invertebrates and fish. Reduction of exposure to available chlorothalonil through physical and chemical processes probably contributed to the poor correlation.

In a study of lake sediments, no relationship between relative toxicity to *D. magna* (in an Anderson-Prater type recirculating test apparatus) and benthic macroinvertebrate density and number of taxa was obvious (Malueg et al. 1984).

Ecological Relevance

Daphnids, forming a major component of the freshwater zooplankton, are widely distributed across Canadian water bodies in a variety of habitats (Environment Canada 1990b).

Daphnia magna is principally a lake dweller and is restricted to waters in northern and western North America with a hardness greater than 150 mg/l (as $CaCO_3$) (Peltier and Weber 1985). It has a long history of toxicity testing. *D. pulex* is mainly a pond dweller, but is also found in lakes and tolerates a wide range of water hardness (Environment Canada 1990b).

6.3.5 Ceriodaphnia *Tests*

Tests have been developed by Environment Canada (1992a), Oris et al. (1991), ASTM (1989), and the U.S. EPA (Weber et al. 1989). This species is one of two (fathead minnow being the other) being used to assess the appropriateness and utility of whole effluent testing under the Complex Effluent Toxicity Testing Program of the U.S. Environmental Protection Agency (Mount et al. 1986b).

Trophic Level

Ceriodaphnia dubia is a microcrustacean smaller than *Daphnia* and has a shorter life cycle. It feeds on phytoplankton and bacteria and is a major component of the freshwater zooplankton. *Ceriodaphnia* form a significant portion of the diet of numerous fish species (Environment Canada 1990b, 1992a).

Sensitivity

The sensitivity of *Ceriodaphnia dubia* to toxic materials relative to other test organisms is shown in Table 15. It was more sensitive to pyrethroid insecticides (Mokry and Hoagland 1990), pulp and paper mill effluent (Kovacs and Ferguson 1990), and sediment elutriates (Burton et al. 1989) than *Daphnia magna*, but less sensitive to a complex effluent (Pontasch et al. 1989). In relation to fathead minnows, *C. dubia* was more sensitive in four, equally sensitive in two, and less sensitive in three effluent-containing waters or effluents.

An analysis of the toxicity of 44 effluent samples (mining operations, industrial, waste treatment facilities, oil treatment processing plants) showed that overall, the sensitivity (average rank out of three) of *C. dubia* was similar to that of fathead minnows and *Photobacterium phosphoreum* (Ankley et al. 1990). *Ceriodaphnia dubia* and fathead minnow tests both indicated 18 effluents to be toxic while *P. phosphoreum* indicated only 14. With harbour sediment elutriates, *C. dubia* was more

sensitive than *D. magna* and *Hyalella azteca* (solid phase test) (Burton et al. 1989).

Testing with effluent from an airplane maintenance company that contained chromium showed that *C. dubia* (48-h LC_{50}) was as sensitive as the mussel *Anodonta imbecilis* (96-h LC_{50}) (Keller and Zam 1991).

Reproducibility

In a study involving 11 laboratories, the U.S. EPA 7-d survival and reproduction test was evaluated using four effluents and two reference toxicants (Nebeker et al. 1984). For survival, differences in NOECs averaged 2.9 concentrations (almost fourfold) while differences in NOECs for reproduction averaged 4.1 concentrations (more than sixfold). The overall mean interlaboratory coefficients of variation for the eight tests ranged from 30% to 38% for the LC_{50} and 29% to 39% for the EC_{50} for reproduction (Table 17).

In intralaboratory testing with sodium lauryl sulphate (SLS) for three non-consecutive tests, the CV for the LC_{50} was 7.8% while the EC_{50} for reproduction was 1.4% (Persoone et al. 1989). The small amount of variation was in part due to the use of pure SLS, minimal handling, and the use of a mixed algal diet. For industrial effluent, CVs for survival LOECs were typically less than those for reproduction LOECs.

Field Validation

The efficacy of the 7-d *C. dubia* test for predicting the effects of copper on field enclosure communities (water and sediment suspended in plastic bags) in uncontaminated ponds was studied (Burton and Stemmer 1988). *Ceriodaphnia dubia* showed decreased toxicity to copper at higher concentrations, which *Daphnia magna* also experienced in the field enclosures. Rotifers, copepods, and the benthic community, however, showed a decrease in density with increasing concentrations of copper.

Toxicity of a river that receives overland flow and groundwater discharge from a site contaminated with organic chemicals was assessed using a 7-d test with *C. dubia* (Burmaster et al. 1991). Daphnid survival corresponded well to numbers of benthic invertebrates while net increase in individuals did not.

A comparative study of the results of 7-d *C. dubia* tests with benthic macroinvertebrate populations for 43 instream wastes of municipal treatment works showed high correlation between toxicity to *C. dubia* and invertebrate community degradation (Eagleson et al. 1990).

For a stream receiving several effluents, sewer overflow, and effluent from a creosote treatment operation, *C. dubia* (solid-phase sediment tests) showed lower (by three times) survival in the laboratory than in in-situ field tests (Sasson-Brickson and Burton 1991).

The toxicity of water at several stream and landfill sites was assessed using 7-d *C. dubia* tests (Burton and Stemmer 1988). Correlations between toxicity and biological responses were significant for diatom diversity and number of diatom species.

Toxicity of water in a river receiving effluent from a municipal sewage treatment plant, a refinery, and a chemical company was assessed using the 7-d *C. dubia* reproduction test under the U.S. EPA Complex Effluent Toxicity Testing Program (Mount et al. 1984). Water downstream from these effluent sources was toxic to *C. dubia*, and there was a corresponding reduction in the number of algae and benthic species. There was no relationship between toxicity to *C. dubia* and the number and diversity of fish species. The biological impact ended at the same stations as those having no toxicity in the laboratory tests. Laboratory toxicity tests thus reflected the biological effects (except on fish) in the receiving water. (Toxicity testing with fathead minnows also did not reflect fish community changes.)

Under the same program as the site above, toxicity testing of a river receiving effluent from a municipal sewage treatment plant, a fertilizer plant, and a refinery was carried out using 7-d *Ceriodaphnia* tests (species uncertain) (Norberg-King and Mount 1986). There was poor correlation between per cent reduction in reproduction with per cent reduction in in-stream numbers of macroinvertebrates and fish.

The toxicity of river water receiving effluent from two coke plants and a municipal sewage treatment plant was determined under the program above using 7-d *C. dubia* tests (Mount et al. 1985). A reduction in young production was correlated with a reduction in the number of zooplankton species but poorly related to numbers of benthic and fish species.

A portion of the Ohio River receiving effluent from industrial facilities and steel mills was assessed for toxicity using 7-d *C. dubia* tests (Mount et al. 1986a). The per cent reduction in young production gave the same profile as the per cent reduction in macroinvertebrate species richness.

In the study of a river that receives discharges from 11 diverse chemical and industrial facilities, 7-d tests with *C. dubia* showed that the correlation between reproduction and zooplankton species diversity over 125 km was significant ($p \leq 0.005$) (Mount and Norberg-King 1986). There was no pattern to the number of macroinvertebrates and so no comparison with toxicity was possible.

Along a 60-km stretch of the Naugatuck River, the impacts of effluent from industries and municipal treatment plants was assessed using 7-d *C. dubia* tests (Mount et al. 1986b). The correlation between toxicity data and numbers of periphyton, macroinvertebrate, and fish (but not zooplankton) species was significant ($p \leq 0.05$).

Ecological Relevance

Ceriodaphnia dubia is an important link in many aquatic food chains. It has a wide hardness tolerance and is found in lakes, ponds, and slow sections of streams and rivers throughout North America (Environment Canada 1992a).

6.3.6 Fathead Minnow Tests

The fathead minnow is one of two species (the other being *Ceriodaphnia dubia*) being used to assess the appropriateness and utility of whole effluent testing under the Complex Effluent Toxicity Testing Program of the U.S. Environmental Protection Agency (Mount et al. 1986b). It is recommended for testing by ASTM (1988), APHA (1989), OECD (1991b, 1991c), EEC (Anonymous 1984), U.S. EPA (Greene et al. 1989, Weber et al. 1989), and Environment Canada (1992b). Tests have been developed for many life stages.

Trophic Level

Fathead minnows are primarily omnivorous and provide food for other fish and birds. They occur in a wide range of habitats from brooks to ponds to lakes and are tolerant of high temperature, turbidity, and low oxygen concentrations (U.S. EPA 1985e). These minnows are often used as bait and are easily cultured.

Sensitivity

The sensitivity of fathead minnows to toxic materials relative to other test organisms is shown in Table 15. It is more sensitive to silver nitrate (Nebeker et al. 1983b) and insecticides (Nebeker et al. 1983a), and less sensitive to other compounds (Fisher et al. 1989), metals (Pickering 1980), a complex effluent (Fisher et al. 1989) and a sanitary landfill leachate (Plotkin and Ram 1984) than *Daphnia magna*.

With respect to industrial effluents, fathead minnow tests with embryos and larvae were more sensitive than ATP tests with rainbow trout (Eco-Research and l'Université de Québec 1991). An analysis of the toxicity of effluent samples from mining operations, industrial and waste treatment facilities, and oil treatment processing plants showed that overall, the sensitivity (average rank out of three) of fathead minnows was similar to that of *Ceriodaphnia dubia* and *Photobacterium phosphoreum* (Ankley et al. 1990b). Fathead minnow and *C. dubia* tests both indicated 18 effluents were toxic while *P. phosphoreum* indicated only 14 to be toxic.

Fathead minnows are more sensitive to some compounds than rainbow trout, and less sensitive than *Selenastrum capricornutum* and *P. phosphoreum* to a sanitary landfill leachate (Plotkin and Ram 1984). Fathead minnows were equally sensitive to or more sensitive than *C. dubia* to effluent receiving waters (Mount et al. 1984, Mount et al. 1985, Mount et al. 1986a, Norberg-King and Mount 1986).

A study with metal-contaminated sediment extracts (four locations, four pH extracts) showed that the 6-d EC_{50} for malformation for the fathead minnow was lower (by 1.1 to 6.4 times) than the 4-d EC_{50} for malformation of frog embryo-larvae in all cases, regardless of extract pH. The relative sensitivities were the same for the reference toxicant zinc sulphate (Dawson et al. 1988). Six-day LC_{50}s for the fathead minnow were higher than the EC_{50}s for malformation, but still lower than the malformation EC_{50}s for frog embryo-larvae.

In a survey of the literature, Vaishnav and Korthals (1990) showed that the 96-h LC_{50} for fathead minnows was lower for 1-heptanol, 2-propenol, 1-octanol, and 1-hexanol than for the 48-h EC_{50} (immobilization) for *D. magna*, but higher for acetone, ethanol, methanol, and phenol.

Correlation and regression analysis for 96-h LC_{50}s for fathead minnows vs. rainbow trout ($r^2 = 0.85$, $n = 31$) and *D. magna* ($r^2 = 0.86$, $n = 40$) for over 20 compounds in the literature shows that there is a good relationship between relative sensitivity to fathead minnows and *D. magna* and rainbow trout (Doherty 1983).

Reproducibility

Reproducibility data for fathead minnow tests are provided in Table 17. The 7-d fathead minnow larval-survival test was conducted in 10 laboratories in the United States using two reference toxicants, two effluents, and the waste stream from a power plant. Inter-laboratory coefficients of variation of 24.1% to 43.7% for LC_{50} larval survival and 22.4% to 88.0% for IC_{50} growth were obtained (De Graeve et al. 1991). For controls, the CV for survival was 12.7% and for growth was 52.0%. For nine laboratories in California, a CV of 31% was obtained (Anderson and Norberg-King 1991). With industrial effluents, CVs of less than 20% were reported for tests involving two or three laboratories (Eco-Research and l'Université de Québec 1991).

Field Validation

A study of effluent from a secondary wastewater treatment plant showed that survival of embryo-larval

fathead minnows in 8-d static-renewal tests (conducted on site) were highly correlated with the number of fish species ($r^2 = 0.85$) and number of invertebrate taxa ($r^2 = 0.92$) for six sites downstream of the effluent source and two controls (Birge et al. 1989). The LC_{50} was 55.8% while field concentrations of 53% and 60% resulted in 86% and 69% survival, respectively.

Toxicity in a river that receives overland flow and groundwater discharge from a site contaminated with organic chemicals was assessed using a 7-d test with fathead minnows (Burmaster et al. 1991). The minnows were tested in a container of spring water connected to a second container with water over sediment between which there was continuous water flow. There was no correlation between number of benthic invertebrate taxa and survival (which remained at over 90%) or weight gain.

Toxicity of water in a river receiving effluent from a municipal sewage treatment plant, a refinery, and a chemical company was assessed using the 7-d larval fathead minnow test under the U.S. EPA Complex Effluent Toxicity Testing Program (Mount et al. 1984). There was no apparent relationship between toxicity to larval fathead minnows and number of fish species or total number of fish. Downstream from the refinery and chemical plant, both algal and benthic communities were severely altered but these effects were not reflected in fish toxicity. In this case, laboratory fathead minnow toxicity tests appeared to be a poor predictor of biological effects.

Under the same program as the site above, toxicity testing of a river receiving effluent from a municipal sewage treatment plant, a fertilizer plant, and a refinery was carried out using 7-d larval fathead minnow tests (Norberg-King and Mount 1986). There was poor correlation between per cent reduction in weight with per cent reduction in instream numbers of macroinvertebrates and fish. When fathead minnow results were combined with per cent reduction in *Ceriodaphnia* sp. reproduction, there was a high correlation between maximum per cent increase in toxicity and maximum per cent decrease in fauna (highest value among zooplankton, macroinvertebrates, or fish).

The toxicity of river water receiving effluent from two coke plants and a municipal sewage treatment plant was determined under the program above using 7-d larval fathead minnow tests (Mount et al. 1985). Per cent reduction in weight was correlated with per cent reduction in the number of fish species but poorly related to per cent reduction in numbers of benthic and macroinvertebrate species.

A portion of the Ohio River receiving effluent from industrial facilities and steel mills was assessed for toxicity using 7-d fathead minnow tests (Mount et al. 1986a). The per cent reduction in fish weight did not correspond to per cent reductions in macroinvertebrate diversity.

In the study of a river that receives discharges from 11 diverse chemical and industrial facilities, 7-d toxicity tests with fathead minnows showed that there was no correlation between per cent increase in toxicity and per cent decrease in zooplankton taxa (Mount and Norberg-King 1986). There was no pattern to the number of macroinvertebrates and so no comparison was possible with toxicity.

Along a 60-km stretch of the Naugatuck River, the impacts of effluent from industries and municipal treatment plants was assessed using 7-d fathead minnow tests (Mount et al. 1986b). The correlation between toxicity data and numbers of periphyton, macroinvertebrate, and fish (but not zooplankton) species was moderate.

Ecological Relevance

The fathead minnow is widely distributed in North America east of the Rockies (Peltier and Weber 1985).

6.3.7 Rainbow Trout Tests

Rainbow trout (*Oncorhynchus mykiss*; formerly *Salmo gairdneri* Kendall 1988) are easily cultured and of commercial value. A substantial database of toxicological information has been built up from the use of this species as a standard cool-water test fish (OECD 1984a, Weber et al. 1989, Environment Canada 1990a). The rainbow trout has become the world's standard cool-water fish for freshwater pollution studies and research in aquatic toxicology.

Trophic Level

Rainbow trout are carnivores that feed on aquatic insects. They are, in turn, prey for larger fish, and mammals such as raccoons and man.

Sensitivity

The relative sensitivity of rainbow trout to other test organisms is shown in Table 15. Rainbow trout were more sensitive to 11 diverse compounds (metals, arsenate, cyanide, ammonia, phenol, styrene, chloroform, 1,2-dichloroethane) than *Photobacterum phosphoreum*, *Spirillum volutans*, and *Daphnia magna*; more sensitive to combustion toxicants (Fisher et al. 1989) than *D. magna*, or fathead minnows; less sensitive to silver nitrate than fathead minnows or *D. magna*; less sensitive to seven other effluents (Qureshi et al. 1982) than *D. magna* and *P. phosphoreum*; and as sensitive as *D. magna* to a sanitary landfill leachate (Atwater et al. 1983). Rainbow trout are more sensitive to pulp and

paper mill effluent than *D. magna* and *Ceriodaphnia dubia* (Kovacs and Ferguson 1990) but less sensitive than *P. phosphoreum* and *Selenastrum capricornutum* (Blaise et al. 1987). Rainbow trout were less sensitive to industrial effluents than *D. pulex* and fathead minnows (Eco-Research and l'Université de Québec 1991).

Correlation and regression analysis for 96-h LC_{50}s for rainbow trout vs. fathead minnows (r^2 = 0.851, n = 31) and *D. magna* (r^2 = 0.860, n = 40) for over 20 compounds in the literature shows that the relative sensitivities of these organisms are highly correlated (Doherty 1983).

Reproducibility

Table 17 shows the 96-h test with rainbow trout to be reproducible with interlaboratory tests resulting in CVs of less than 20%. As with *Daphnia magna*, CVs can be expected to vary with the type of substance tested with higher CVs reported for metals (K. Doe, pers. comm.).

Field Validation

In a toxicity test with chlorothalonil, a fungicide used to protect crops from potato blight, the 96-h LC_{50} for rainbow trout was determined in the laboratory and compared to effects on caged endemic species (waterboatmen, clams, caddisfly larvae, beetle, midge larvae, scud) and rainbow trout as well as on the composition of the natural benthic fauna in ponds sprayed with the fungicide (Ernst et al. 1991). The laboratory-derived LC_{50} was 2.5 times lower than the lowest concentration in all ponds but only one of the six caged invertebrates (*Chironomus* sp.) exhibited <50% survival (the authors attribute this to damage during inspection of cages) and caged rainbow trout showed no mortality. Changes in total numbers of benthic invertebrates over the duration of three spray events reflect in part emergence that confounded effects due to fungicide application. These results indicate poor correlation between laboratory toxicity and field toxicity for caged invertebrates and fish. Deduction of exposure to available chlorothalonil through physical and chemical processes probably contributed to the poor correlation.

When evaluated using toxicity tests, water-soluble cationic polymers were highly toxic to fish, but their use has not been associated with adverse effects on fish populations (Goodrich et al. 1991).

Ecological Relevance

Rainbow trout are native to western North America, mostly west of the Rocky Mountains, although this fish species now frequents waters of all Canadian provinces as a result of intentional or unintentional releases. It thrives in most cool freshwater bodies. The species has been introduced around the world and now is probably the most widespread of the salmonids (Environment Canada 1990a).

6.3.8 Aquatic Vascular Plant Tests

Test methods for *Lemna gibba* have been prepared by the U.S. EPA (1985b) and ASTM (1991). Comparative testing with other vascular plants has largely been done with *L. minor*, a plant that is widespread throughout North America. Testing in some laboratories has also been carried out with *L. paucicostata* (S. Goudey, pers. comm.).

Trophic Level

Lemna gibba is a floating vascular plant that provides food for waterfowl and shelter and support for small aquatic invertebrates.

Sensitivity

Tables 5b and 15 provide information on the sensitivity of *Lemna* relative to other test organisms. These tables show that the majority of the comparative data for *Lemna* are for *L. minor* rather than the test species, *L. gibba*. As well, comparisons with rooted aquatic plants (rice) or terrestrial plants are as common as comparisons with other floating plants (*L. gibba*, *Spirodella polyrhiza*). Most testing with floating vascular plants has been done on single species and few comparative toxicity data exist.

Lemna minor was more sensitive to copper than *Ceriodaphnia dubia* but less sensitive than the fathead minnow (Taraldsen and Norberg-King 1990). *L. minor* was more sensitive to herbicide effluent than *C. dubia*, more sensitive to refinery effluent than *C. dubia* or fathead minnows, and less sensitive than these organisms to oil treater effluent (Taraldsen and Norberg-King 1990).

Concerning metal toxicity, the literature indicates that *L. minor* is more sensitive to nickel and cadmium than a variety of fish (Wang 1987c). It was shown to be less sensitive than rye grass to seven metals, more sensitive than millet to six metals, and more sensitive than fish to three metals (Wang and Elseth 1990).

For an industrial effluent (Wang 1990a) and another complex effluent (Wang and Williams 1990), *L. minor* was more sensitive than lettuce, rice, cabbage, and millet. *L. minor* was as sensitive as rice to ammonia in a static system but less sensitive in a flow-through system (Wang 1991). *L. minor* was more sensitive to raw coal distillate, and fuel oil, than *L. gibba*. It was more sensitive to chromium than *S. polyrhiza*.

Reproducibility

A ring test (interlaboratory test) is currently under way in Europe to evaluate a duckweed test using *Lemna minor* (E. Bjornestad, Water Quality Institute, Horsholm, Denmark, pers. comm.). Intralaboratory testing showed good repeatability in relation to the doubling time of *L. minor* in control cultures (CVs = 8% and 9%, Wang 1991). The mean doubling time in control culture for *L. minor* over 61 tests was 1.9 days and ranged from 1.3 to 2.8 days (Wang 1990b).

Information on the reproducibility of tests with *L. gibba* was not found.

Field Validation

No information was found on the relationship between toxicity to *Lemna* and effects of toxic material on field communities.

Ecological Relevance

Several species of duckweed, which vary in form, are native to Canada. *L. minor*, for example, floats on the surface, and *L. trisulca* remains submerged. These species occur in ponds and along the margins of lakes and slow-moving rivers. *Lemna gibba*, for which a test method exists, is not native to Canada. *Lemna* grows and reproduces fast relative to other vascular plants (Taraldsen and Norberg-King 1990).

6.4 Usable Battery

The following tests are considered eligible for inclusion in a current test battery because they meet all the 'must' criteria (3.2.1) and ≥88% of the 'want' criteria (3.2.2, 6.2.2):

Photobacterium phosphoreum (Environment Canada 1991)

Brachionus rubens (Snell and Persoone 1989)

Daphnia magna and *D. pulex*, 48-h (Environment Canada 1990b)

Ceriodaphnia dubia, 4-d (Oris et al. 1991)

Fathead minnow, larval growth (Environment Canada 1992b)

Rainbow trout, 96-h (Environment Canada 1990a)

By adding information on expected values for reference toxicants (not originally included in the test descriptions) that has become available through test development and application or was obtained from the literature, the following tests scoring ≥88% can also be considered eligible for inclusion in the usable battery:

Selenastrum capricornutum (Environment Canada 1992c)

Ceriodaphnia dubia, 7-d (Environment Canada 1992a)

Five trophic groups are represented by the test organisms for which tests are currently usable.

6.4.1 Screening Tests

The relative merits of the eight tests identified above as eligible for inclusion in test batteries for assessing water quality are discussed and conclusions concerning the most appropriate tests are drawn below.

To summarize, the following screening tests are recommended for the usable battery: algal growth inhibition using *Selenastrum capricornutum* (Environment Canada 1992c), macroinvertebrate survival using *Daphnia* spp. (Environment Canada 1990b), and a bacterial test using *Photobacterium phosphoreum* (Environment Canada 1991). The last test is included because it is currently widely applied in water quality assessment, and information on freshwater bacterial tests is insufficient to consider any usable (see below). The application of these tests is shown in Figure 6 and detailed descriptions are provided in Appendix C.

The 24-h test with *Brachionus rubens* is eligible for consideration in the screening battery. Experimental work with this species was used as a basis for developing the Rotoxkit™ (Anonymous 1990a), which uses *B. calyciflorus*. While the test reported for the former species is sound, it is the latter species for which the most up-to-date methods are being prepared. The Rotoxkit™ rotifer test was not considered usable because reference toxicant data were unavailable during the current bioassay evaluation process. These data are currently in preparation, and once complete, this test will be considered as a candidate for addition to the usable battery.

The 7-d larval fathead minnow test (Environment Canada 1992b) was considered for inclusion at the screening level as it represents a trophic level in addition to those tests already selected for the screening battery, but it was rejected. *Daphnia magna* was considered a sufficiently adequate surrogate test species for the fathead minnow at the screening level since a high correlation was found between LC_{50}s for fathead minnows (96-h tests) and *D. magna* (48-h tests) ($r^2 = 0.92$) (Doherty 1983) and a moderate correlation was found between NOECs for fathead minnows (7-d test) and *D. magna* (48-h test) ($r^2 = 0.62$) (Giesy and

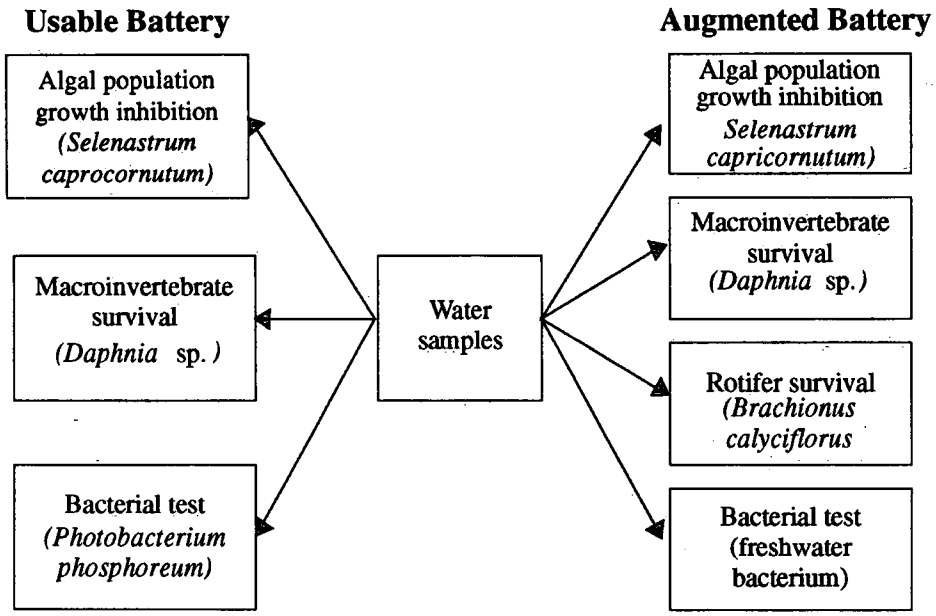

Figure 6. Screening tests recommended for the usable and augmented batteries for water quality assessment (see 6.4.1 and 6.4.3.1 for additional information).

Hoke 1989). The *D. magna* test was similarly considered a surrogate for the rainbow trout test since the correlation between LC_{50}s was high ($r^2 = 0.86$) (Doherty 1983).

Bacterial Test

The screening battery should have a representative bacterial test. The test with *Photobacterium phosphoreum* is the only bacterial test that is currently considered usable. It requires very small sample volumes, the test organisms require no maintenance, and the method is highly standardized with kits available for purchase. This marine bacterium has often been used for assessing freshwater toxicity and shown to be variably sensitive (see 6.3.1.1 and tables 15, 16). It showed a toxic response to only 8 of 185 soil and sediment elutriate, water and waste samples that were not toxic to *Daphnia magna* or *Selenastrum capricornutum* (Greene and Barich 1991). The test is very easy to conduct and the low cost (after equipment purchase) encourages its use. There should, however, be concern over the apparent disregard for the influence on toxicity as a result of adding the required salt or sugar solutions to the test sample and the appropriateness of using a marine bacterium for freshwater testing (see 7.0).

Algal Test

Algae are important primary producers in the aquatic environment and should be represented in a screening test battery. A test with *Selenastrum capricornutum* is recommended because it has demonstrated sensitivity over many other species with many toxic samples.

Two 96-h tests using *S. capricornutum* are available to determine the effects of water quality on algae. Testing with four reference toxicants and nine herbicides using the microplate method (Environment Canada 1992c) and the flask method (ASTM 1990c) showed that there was good concordance between the methods for all but one herbicide (St. Laurent et al. 1992). Thus the microplate assay is an appropriate alternative to the flask test. The microplate method is recommended over the flask method because less sample volume is required, the time required for glassware washing is less, and a larger number of samples can be run per unit of time. As well, there is a greater potential for automation of the microplate system.

There are several disadvantages of the microplate system relative to the flask test. Volatile substances may affect the growth of algae in other wells, instrumentation must be calibrated more often, and accurate pipetting is required since volumes are small and the initial cost for equipment is higher.

Daphnid Test

A 48-h acute test with *Daphnia magna* or *D. pulex* and 7-d and 4-d survival and reproductive tests with *Ceriodaphnia dubia* can be considered. All three species are indigenous to Canada and easily identified,

show sensitivity to toxicants, and give reproducible test results. The 48-h test with *D. magna* or *D. pulex* is recommended for the screening battery. A test with *Daphnia* is recommended over the test with *C. dubia* because it is considerably shorter and therefore less costly. Selection of the *Daphnia* species used for testing should take into account the nature of the water sample collected. *Daphnia pulex* tolerates a wider range of water hardness, and based on limited data, appears similar to *D. magna* in sensitivity to toxicants. In favour of the use of *D. magna* is the large database built on toxicity assessment.

6.4.2 Definitive Tests

Chronic tests are the focus of definitive tests, but the test options are rather limited, as shown in 6.4. The set of definitive tests recommended for the usable battery includes two of the tests from the screening set (algal growth inhibition using *Selenastrum capricornutum*, Environment Canada 1992c; bacterial test using *Photobacterium phosphoreum*, Environment Canada 1991). A daphnid reproductive test using *Ceriodaphnia dubia* (Environment Canada 1992a) replaces the screening survival test with *Daphnia* spp. and a fish test using either the fathead minnow (larval growth and survival, Environment Canada 1992b) or rainbow trout survival (Environment Canada 1990a) is added to broaden the trophic spectrum considered. The applications of the tests for the current definitive battery are shown in Figure 7 and are discussed below. See Appendix C for detailed test descriptions.

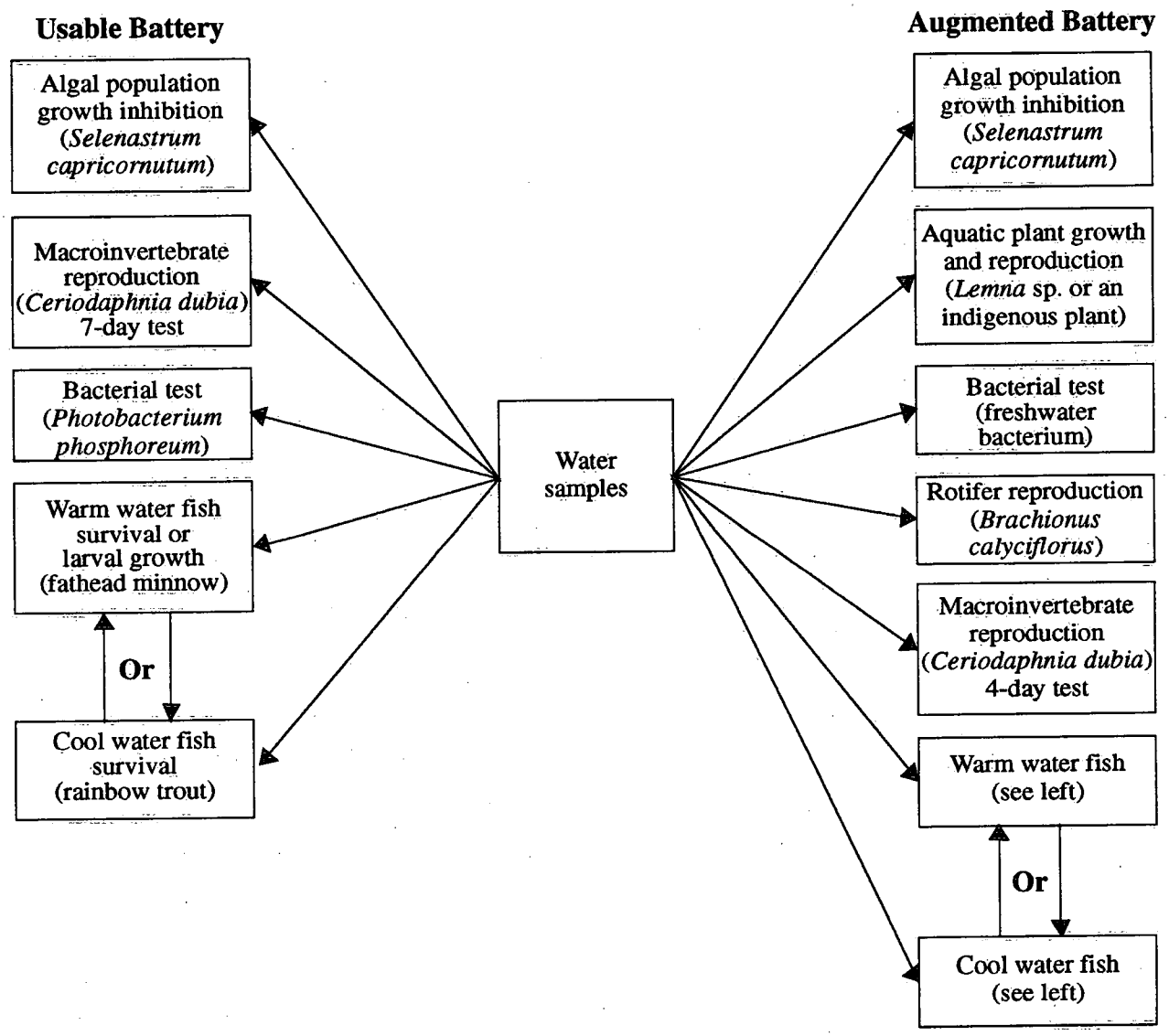

Figure 7. Definitive tests recommended for the usable and augmented batteries for water quality assessment (see 6.4.2 and 6.4.3.2 for additional details).

Algal Test

See Screening Tests (6.4.1).

Daphnid Test

Ceriodaphnia dubia rather than *Daphnia magna* is recommended as a test species for a reproductive test because it has a shorter generation time and shows comparable sensitivity (6.3.5). Chronic reproductive tests can be carried out in only 4 or 7 days, rather than the 21 days required for *Daphnia* reproduction tests (Biesinger et al. 1987, OECD 1991a).

A 4-d (Oris et al. 1991) and a 7-d (Environment Canada 1992a) test have been proposed for assessing toxic effects on the reproduction of *Ceriodaphnia dubia*. No significant differences between the 4-d and 7-d test were obtained for IC_{50}s and chronic values for 12 chemicals (Oris et al. 1991). For the current battery, the 7-d test is recommended because it has demonstrated sensitivity to both individual substances and complex effluents (6.3.5) while data on the latter are absent for the 4-d test.

Bacterial Test

See Screening Tests (6.4.1).

Fish Test

Both fathead minnow tests (larval survival and growth, Environment Canada 1992b) and rainbow trout tests (96-h survival, Environment Canada 1990a) show variable relative sensitivities to toxic substances (Table 15). One fish test is recommended for definitive testing. The rainbow trout assay might be more appropriate for evaluating cool waters.

6.4.3 Recommendations for Augmenting the Usable Battery

6.4.3.1 Screening Tests

It is recommended that the augmented battery include the algal growth inhibition test with *Selenastrum capricornutum* (Environment Canada 1992c) and the *Daphnia* spp. survival test (Environment Canada 1990b). A freshwater species is recommended to replace *Photobacterium phosphoreum* used in the current battery (6.4.1). A 24-h test with the rotifer *Brachionus calyciflorus* (Anonymous 1990a) is recommended to expand trophic level representation. Figure 6 shows the application of these tests. Only additions or changes to the set of screening tests described under the usable battery are discussed below. See 6.4.1 for a discussion of the tests retained from the usable battery.

Algal Test

Plants are represented by the algal test (see 6.4.1) rather than a *Lemna* test (ASTM 1991, U.S. EPA 1985b) for two reasons. The sensitivity of *S. capricornutum* relative to other test organisms is known and shown to be high with respect to numerous toxicants (Table 15), while the relative sensitivity of *Lemna* (tables 5b, 15), particularly to contaminant mixtures, is less well known. Secondly, the duration of the algal test is shorter (3 d) than the *Lemna* test (7 d).

Rotifer Test

A 24-h test with *Brachionus calyciflorus* is recommended. With the provision of reference toxicant data, unavailable during this review but currently in preparation, this test will be considered as a candidate for addition to the usable battery. The use of standardized toxkits (Anonymous 1990a), which are relatively inexpensive, would result in high test standardization. A second advantage of tests with rotifers is that there is no need to maintain cultures to obtain test organisms as they come from cysts, which can be stored for long periods of time (Persoone et al. 1990). The potentials and limitations of the Rotoxkit™ are presently being determined in parallel with the test using *Photobacterium phosphoreum* as a limited battery to screen the toxicity of hundreds of effluents, well waters, solid wastes, and sediments in a large biomonitoring program in Belgium sponsored by the Commission of European Communities (G. Persoone, pers. comm.). ASTM is currently in the process of adopting a standard guide for rotifer testing (G. Persoone, pers. comm.).

Bacterial Test

Escherichia coli and *Pseudomonas putida* (freshwater bacteria) are candidates for inclusion in the augmented battery. *Photobacterium phosphoreum* (6.4.1) should be used until complete test evaluation data and comparative information are available on the sensitivity of it and a variety of freshwater species. *Spirillum volutans* is not considered a candidate for the future test battery because all three studies providing information on its relative sensitivity (Table 15) indicated that it was the least sensitive species in the battery tested.

6.4.3.2 Definitive Tests

It is recommended that the augmented battery include the algal growth inhibition test with *Selenastrum capricornutum* (Environment Canada 1992c) and a fish test using either the fathead minnow larval growth and survival (Environment Canada 1992b) or rainbow trout survival (Environment Canada 1990a) that were part of the usable battery. Consideration of aquatic plants is expanded to growth and reproduction of vascular plants

in a test using *Lemna* spp. (e.g., ASTM 1991, U.S. EPA 1985b) or another indigenous aquatic species. A chronic 48-h test with the rotifer *Brachionus calyciflorus* (Snell et al. 1990) is recommended to expand trophic level representation. A freshwater bacterium is recommended to replace *Photobacterium phosphoreum* in the usable battery. Replacement of the 7-d reproductive test using *Ceriodaphnia dubia* (Environment Canada 1992a) in the usable battery (6.4.2) with the 4-d test (Oris et al. 1991) would save time and therefore cost. The application of the definitive tests recommended for the augmented battery is shown in Figure 7. Only additions or changes to the set of definitive tests described under the usable battery are discussed below. See 6.4.2 for a discussion of the tests retained from the usable battery.

Algal Test

The test with *Selenastrum capricornutum* recommended for the current usable battery (6.4.2) is also recommended for the augmented battery. Consideration should be given to testing species from more than one class of algae (e.g., diatoms, blue-green) to improve their use as an indicator of the effects of toxic constituents in water on algae (Lewis 1990). The sensitivity and feasibility of using other species is currently being investigated by the Saskatchewan Research Council (C. Boutin, Canadian Wildlife Service, pers. comm.).

Aquatic Vascular Plant Test

Since the toxicity of compounds to algae does not necessarily reflect toxicity to aquatic vascular plants, testing with *Lemna* spp. (preferably indigenous) or other indigenous aquatic plant is recommended in addition to the algal test. A test (ASTM 1991, U.S. EPA 1985b) is described for *L. gibba*, but all the sensitivity and reproducibility data found in the literature were for *L. minor* (6.3.8). A proposal for a test using *L. minor* is being prepared for the American Public Health Association (Anonymous 1991). When the gaps in the *L. gibba* test are filled, it will be considered usable (see 6.5). It should be used until standard test methods for *L. minor*, which is indigenous to Canada, are available.

Rotifer Test

Snell et al. (1990) report coefficients of variation of 20% to 30% for growth rate in 48-h tests with *B. calyciflorus*. They indicated that this test was about three times more sensitive to pentachlorophenol than *Daphnia magna* (total young/female, 7-d test) and *Ceriodaphnia* (total young/female, 7-d test). With the provision of reference toxicant data, unavailable during this review but currently in preparation, a 48-h reproduction test will be considered as a candidate for addition to the usable battery. ASTM is currently in the process of adopting a standard guide for rotifer testing (G. Persoone, pers. comm.). Additional information on the sensitivity of *B. calyciflorus* relative to other test species would help to further clarify its utility as an addition to the usable battery.

Bacterial Test

See 6.4.3.1.

Daphnid Test

If further comparative testing with the 4- and 7-d tests using *Ceriodaphnia dubia* and complex effluents shows the shorter test to be equally sensitive (as was the case for 12 individual chemicals, Oris et al. 1991), the 4-d test should be adopted as it will increase productivity and decrease costs.

Fish Test

A variety of chronic tests (Environment Canada 1992b, ASTM 1988, Eco-Research and L'Université de Québec 1991) are available for assessing toxicity to fish, but the data are too limited to assess the relative sensitivity of these tests. Until additional testing of these methods and species is conducted with more substances and complex toxic wastes, no test can be recommended to replace the fathead minnow larval growth and survival test (Environment Canada 1992b) or the rainbow trout test (Environment Canada 1990a) proposed for the usable battery.

6.5 Prototype Tests

Of the 25 tests evaluated in this review, 9 initially fell into the prototype category. The first 2 below, initially classified as prototypes because they lacked an expected value for the reference toxicant, were promoted to the usable category when the required values were provided through additional literature review. The remaining 7 prototypes either had all the 'must' criteria and scored <88% for the 'want' criteria or were missing 'must' criteria and had a score of ≥ 88% for 'want' criteria (see 3.2.1, 3.2.2). They are listed below along with the work required to make them usable (see Table B-3, App. B).

Selenastrum capricornutum (Environment Canada 1992c)

Ceriodaphnia dubia (Environment Canada 1992a)

Pseudomonas putida (complete statistics, specify conditions, ISO 1991c)

Spirillum volutans (complete statistics, specify conditions, Dutka 1991)

Brachionus calyciflorus, Rotoxkit™ (reference toxicant, complete statistics, Anonymous 1990a)

Daphnia magna, reproduction (correct statistical errors, reference toxicant, Biesinger et al. 1987)

Fish, early life-stage (reference toxicant, complete conditions, provide test vessel size, ASTM 1988)

Lemna gibba (complete conditions, complete statistics, reference toxicant, ASTM 1991)

Lemna gibba (complete statistics, reference toxicant, acceptability criteria, U.S. EPA 1985b)

The test with *Spirillum volutans* is considered of low priority for further work as the available comparative studies indicate it is a relatively insensitive organism. The reproductive test with *D. magna* is of low priority because the usable test with *Ceriodaphnia dubia* is considered a less time consuming but a sensitive and reproducible surrogate test for daphnids. The use of tests with the early life stages of fish species in addition to the fathead minnow test in the usable battery (6.4.1, 6.4.2) would be applicable in specific cases where toxicity to fish is of primary interest. For general testing purposes, use of the fathead minnow test alone will suffice. Further work on the fish early life stage test is therefore of low priority. Vascular plants are not currently represented by a usable test. Of the two tests with *L. gibba*, the first is recommended for further work as it is most complete.

Based on the considerations above, the scores for these tests (6.2.2) and the methods outlined in Figure 1, initial priorities for further work on these tests are as follows:

Priority 1 (all 'must' criteria, score <88%)

Pseudomonas putida

Priority 3 (missing some 'must' criteria, score 88-100%)

Brachionus calyciflorus

Lemna gibba (ASTM 1991)

6.6 Tests under Development

The 10 tests listed below did not meet the 'must' criteria, scored <88% for the 'want' criteria, and are not considered to be high priority concerns at this time:

Selenastrum capricornutum (ASTM 1990c)

Daphnia magna, reproduction (OECD 1991d)

Gammarus lacustris (Alberta Environmental Centre 1989)

Panagrellus redivivus (Samoiloff 1990)

Wyeomyia smithii (ASTM 1990a)

Lemna gibba (Holst and Ellwanger 1982)

Escherichia coli, Toxi-chromotest™ (Orgenics Ltd. 1985)

Crayfish (ASTM 1980)

Bacillus cereus (Thomson et al. 1986)

Shrimp (APHA 1989)

When further work is done on these tests (Table B-3, App. B), priority should be given to those representing trophic levels not covered by the recommended batteries. Tests 1, 2, and 6 concern organisms for which prototype tests have already been identified. Little information is available on the sensitivity of the second last test (Table 16) and therefore its utility relative to other bacterial tests (6.3.1).

6.7 Priorities for Assessing Freshwater Quality with Bioassays

In this section, priorities for work required to meet the needs of the National Contaminated Sites Remediation Program related to the assessment and remediation of freshwater in Canada are described beginning with the work of highest priority. Priority work required to upgrade prototype tests reviewed to usable tests (6.5) is integrated with additional areas of work identified during the review. For a discussion of the rationale for identifying these tasks as priority items, see sections 3.5, 6.4, and 6.5.

1) Conduct comparative testing with freshwater bacteria

A marine bacterium, *Photobacterium phosphoreum*, is currently widely used as a surrogate species for testing the toxicity of freshwater. The adequacy of this surrogate species requires evaluation (see 7.0).

2) Conduct tests to obtain/collate data from comparative testing with rotifers

The potentials and limitations of the Rotoxkit™ are presently being determined in parallel with the test using *Photobacterium phosphoreum* as a limited battery to screen the toxicity of hundreds of effluents, well waters, solid wastes, and sediment in a large biomonitoring program in Belgium. The study is sponsored by the

Commission of European Communities (G. Persoone, pers. comm.).

Snell et al. (1990) report coefficients of variation of 20% to 30% for growth rate in 48-h tests with *Brachionus calyciflorus*. They indicated that this test was about 3 times more sensitive to pentachlorophenol than that using *Daphnia magna* (total young/female, 7-d test) and *Ceriodaphnia* (total young/female, 7-d test). ASTM is currently in the process of adopting a standard guide for rotifer testing (G. Persoone, pers. comm.).

Additional information on the sensitivity of *B. calyciflorus* relative to other test species would help to further clarify its utility as an addition to the usable battery.

3) Describe a protocol for testing *Lemna* species native to Canada

Several *Lemna* species, including *L. minor* and *L. trisulca*, are found in Canada. A test using *L. minor* under preparation for the American Public Health Association (Anonymous 1991) could serve as a basis for a Canadian test. Considerable work has been done on the influence of EDTA on metal toxicity to *L. trisulca* (Huebert and Shay 1991). The implications of this for nutrient media preparation for a *L. minor* protocol should be assessed. The use of soil as a nutrient source has been suggested (Taraldsen and Norberg-King 1990). Wang (1990b) indicates that the doubling time for control cultures of *L. minor* is 1.9 d.

4) Determine a reference toxicant for the *Lemna gibba* test

In the temporary absence of a protocol for a *Lemna* species indigenous to Canada, the ASTM (1991) test using *L. gibba* could be made usable by providing a reference toxicant and an expected value. The chromate ion, suggested for *L. minor* by Wang (1987b), appears to be useful as a reference toxicant for aquatic phytotoxicity tests. Sodium chloride is also a potential reference toxicant (Taraldsen and Norberg-King 1990). Chlorophyll *a* and frond number were correlated endpoints (Taraldsen and Norberg-King 1990).

5) Prepare a handbook for statistical guidance

A weakness of many of the tests reviewed was inadequate statistical guidance. The need for a handbook on statistical guidance is common to all three media and is discussed in section 7.0.

6) Evaluate the relative sensitivity of additional algal species

Using single species, determine whether tests with diatoms and blue-green algae would contribute significant new toxicity information (in addition to that provided by *Selenastrum capricornutum*) when performed as part of a test battery. Some comparative testing is under way with a variety of algal species and pesticides at the Saskatchewan Research Council (C. Boutin, pers. comm.).

7) Determine the relative sensitivity of the 7-d *Ceriodaphnia* test and the proposed 4-d test

A reproductive test only 4 days long has been proposed (Oris et al. 1991) that begins with more mature females than the 7-d test. This shortens considerably the duration of the test, bringing it close to that of the less sensitive *D. magna* 48-h acute toxicity test that is commonly used.

8) Develop new tests

While usable tests cover several trophic levels, the development and application of definitive tests for additional groups of organisms would broaden the information base and improve the assessment of toxicity to field organisms. Organisms that could be considered candidates are included in the tests identified as under development (6.6), as could other organisms in Table 13.

Two new tests with ciliate protozoa are being developed. The one using *Colpidium campylum* is being revised following the results of a recent workshop (Dive et al. 1989, 1990), and the test involving *Tetrahymena vorax* is being evaluated (Gilron et al. 1991). They could be considered for augmenting the proposed test batteries.

New bioassays that have excellent potential for standardization are being developed beginning with the resting phases of organisms. For organisms with short life spans, that means that chronic tests, considered to be more sensitive measures of toxicity than acute tests, can be completed over a very short time. Aside from rotifers, the shortest chronic test currently widely employed is the 7-d *Ceriodaphnia* test.

A toxkit (Streptoxkit™) is now available based on the cysts of the anostracan crustacean *Sterptocephalus proboscideus* (Anonymous 1990b) and should be evaluated for inclusion in the usable battery. *Daphnia* toxkits (Daphtoxkit™) are being developed (G. Persoone, pers. comm.), based on the ephippia.

A second new test is currently under development for *Daphnia* (Aqua Survey 1991). *Daphnia magna* eats a fluorogenically tagged substrate and fluorescence is used as a measure of contaminant effect. The bioassay has very strong possibilities for use as a *D. magna* pretest since it can be performed in under two hours.

An interlaboratory and intralaboratory comparison study was performed in 1991 (J. Fischer, Johns Hopkins Univ., pers. comm.). Intralaboratory coefficients of variation ranged from 4.7% to 47.1%, with a mean CV of 23.1%. Interlaboratory results for 16 sets resulted in a 43.3% CV. The "blind" copper standard toxicant resulted in an average EC_{50} of 0.11 mg/L for the 16 laboratories. Concurrently performed 48-h *D. magna* tests resulted in an LC_{50} of 0.082 mg/L copper.

A new bacterial test has been developed in England as a rapid biocide test (ECHA 1991). A dip-slide (plastic stick with a dot of bacteria mixed with growth indicator dye) is exposed to the test fluid, and the level of colour indicates the relative toxicity of the fluid. The utility of this technique for toxicity assessment should be evaluated.

9) Prepare standard methods for the collection of water samples

In the earlier test methods Environment Canada (1990a, 1990b) did not address sample collection. In the more recent methods, Environment Canada (1992a, 1992b) provides information on container type and a sampling schedule, but no details on appropriate or standard techniques for sample collection. Standardizing collection methods will increase the comparative value of the test results.

10) Prepare a manual for field sampling guidance

A manual for designing field sampling schemes is required to ensure that the collection techniques (point 9 above) are applied appropriately (see 7.0).

7.0 TOP PRIORITIES FOR THE NATIONAL CONTAMINATED SITES REMEDIATION PROGRAM

Among the three media — freshwater, freshwater sediment, and soil, 20 different needs were identified related to developing test batteries for contaminated site assessment. Because of limited time and resources, it is important to put all these needs in perspective for the National Contaminated Sites Remediation Program. The needs identified as a result of this review are listed below in approximate order of priority. They are ordered based on the current level of testing possible in each medium, test utility for the batteries, the effort required to make tests usable, and the number of media having similar needs.

Of the needs listed below, 5 are already being addressed and therefore not considered priorities for attention. Attention should focus on the remaining 15 needs, identified in bold type. For descriptions of the needs particular to a medium, see the appropriate section indicated in brackets after each need.

Generally, the greatest needs are in the area of sediment testing, the most recent medium to receive attention. There is a long history of aquatic testing and many tests are currently available. The lack of detailed, appropriate statistical guidance is a major gap identified in many tests.

1. Determine reference toxicants for benthic invertebrate tests (in progress, 5.7)

2. **Identify a standard sediment(s) for benthic invertebrate tests** (in progress only for *Hexagenia*, 5.7)

3. **Develop a test for rooted aquatic plants** (4.7)

4. **Select sensitive terrestrial plant species for seedling emergence test** (4.7)

5. **Prepare a handbook providing statistical guidance for battery tests** (4.7, 5.7, 6.7)

While some test methods (e.g., Environment Canada 1992c) did provide a detailed discussion on statistical analysis and interpretation of data (even more in-depth coverage, as described below, would be useful), a weakness of many of the tests reviewed was inadequate statistical guidance.

Statistical analyses have been developed and promoted for quantal biological measurements such as number of young produced and number of gravid females, and to a lesser extent, for nonquantal biological measurements such as weight of young produced. Although excellent recommendations for statistical analyses can be found in Weber et al. (1989), further guidance is needed that will detail the applicability of each statistical method to the specific biological measurement being made during the test. Quantal toxicity tests for macroinvertebrates, fish, and earthworms, for example, are well suited for probit analysis and/or logistic regression, whereas these statistical methods are not appropriate for the nonquantal toxicity tests with algae and bacteria.

Computer simulation studies should be considered to evaluate the performance of the different statistical procedures for various bioassays. Simulation studies would be useful to evaluate the effects of different aspects of experimental designs including replication, within- and between-concentration variability, the number of test concentrations, and violated model assumptions (e.g., the use of normal-theory procedures when the data are not normally distributed) on the sensitivity and power of a statistical procedure to determine significant effects. Dunnett's test could be compared, under various controlled conditions, to

Williams' test to determine when one test performs better than the other and the magnitude of the difference in performance.

The formats of many of the tests are identical and require the same statistical considerations. Rather than approaching statistical guidance on a test-by-test basis, a statistical 'cookbook' should be written to provide guidance on the available statistical procedures applicable to specific experimental designs. Procedures such as probit analysis, logistic regression, and Steel's test are not widely documented and are generally difficult for the layperson to understand. A complete and thorough statistical reference should be developed that, at a minimum, (1) discusses the advantages and dis-advantages of hypothesis tests and point estimation; (2) presents the logic behind each method in an easily understandable fashion; (3) gives a thorough discussion of the assumptions associated with each method, tests for the assumptions, and the consequences of violating the assumptions; (4) provides detailed and annotated examples using each method; (5) discusses the importance of other statistical issues such as randomization and independence in the design of bioassay tests; and (6) provides methods for detecting outlying observations and how to handle suspected outliers. Computer software should also be developed as a companion product to the statistical reference. The software should be user-friendly and menu-driven to provide the user with a means to implement the documented statistical procedures.

The statistical guidance given for interpreting the data from tests with non-target plants, as required for pesticide registration in Canada (Boutin et al. 1992), provides an example of the type of document required.

6. Select species of earthworm for soil testing (in progress, 4.7)

7. **Re-evaluate bacterial species for testing** (4.7, 5.7, 6.7)

   The extensive use of the marine bacterium *Photobacterium phosphoreum* to assess the toxicity of freshwater and freshwater sediment pore water and elutriates does not confirm its utility for representing freshwater bacteria, but reflects the low cost, ease with which the test can be carried out, and the test's high degree of standardization and reproducibility. The influence on toxicity as a result of adding the required salt or sugar solutions to the test sample and the appropriateness of using a marine bacterium for freshwater testing should be addressed.

   Comparative experimental studies are required on the relative responses of freshwater bacteria such as *Escherichia coli* and *Pseudomonas putida* and the marine bacterium *Ph. phosphoreum* to single substances, organic and inorganic compounds, and complex effluents. If *P. phosphoreum* consistently overestimates *or* underestimates sediment toxicity to freshwater bacteria, it is not providing useful information for remediation. Freshwater species showing the greatest sensitivity (IC_{50} values) and ability to detect toxicity should be considered for use in the test batteries.

8. **Complete and examine soil tests for organisms other than terrestrial plants and earthworms** (4.7)

9. **Pending the results of item 6 above, develop a test for earthworm reproduction** (4.7)

10. **Develop a test for the floating aquatic plant *Lemna minor*** (6.7)

11. **Determine a reference toxicant for the *Lemna gibba* test** (6.7)

12. Evaluate the relative sensitivity of algal species other than *Selenastrum capricornutum* (6.7)

13. **Conduct comparative testing with *Chironomus* species** (5.7)

14. **Conduct comparative testing with 7- and 4-day *Ceriodaphnia* tests** (6.7)

15. **Conduct comparative testing with additional aquatic test organisms** (6.7)

16. Prepare standard methods for the collection, storage, and manipulation of test sediment (in progress, 5.7)

17. **Prepare standard methods for the collection, storage, and manipulation of test soil** (4.7)

18. **Prepare standard methods for the collection of water samples** (6.7)

19. Select a standard soil, or standard soil set, for whole soil toxicity tests (in progress for earthworms, 4.7)

20. **Prepare a manual for sampling guidance** (4.7, 5.7, 6.7)

8.0 THE FUTURE FOR CONTAMINANT ASSESSMENT WITH BIOLOGICAL ORGANISMS

8.1 The Need to Maintain State-of-the-Art Knowledge

The test batteries recommended for the assessment of soil, freshwater sediment, and freshwater

quality in this report are based on state-of-the-art knowledge of toxicity testing. As research progresses, better tests, new test organisms, and other endpoints will be discovered. It is important that Environment Canada remain abreast of the changes in this field so that its criteria and objectives reflect current knowledge of toxicity testing. While there is always some lag between the state-of-the-art toxicological research and its implementation, care should be taken not to get into the trap of hanging onto familiar tests with reams of historical data when newer, more useful and cost-effective tests may be available.

A periodic review of the state-of-the-art toxicity test methods and comparison to the current test batteries would be worthwhile. To remain current in the field of toxicity testing, representatives of Environment Canada should develop better communication links with individuals and organizations in the United States and Europe. This does not mean simply attending the occasional relevant scientific conference but also speaking or writing regularly to informed individuals, exchanging documents, and visiting with other agencies. As well, Environment Canada should foster collaboration in test development to split costs and ensure that the tests being developed are relevant to Canadian conditions. Within Environment Canada, knowledge about international developments related to toxicity testing is spread among a variety of branches among which communication appears to be limited. In summary, better communication both within Environment Canada and between Environment Canada and other agencies involved with contamination assessment is required if the Eco-Health Branch is to maintain a state-of-the-art level of knowledge of international toxicity assessment methods.

8.2 Alternative Test Endpoints

The extension of testing following the removal of a toxicant and the use of the potential for recovery may be a more realistic endpoint than LC_{50}s. The endpoint would be the concentration at which there is no net change in the measured variable after exposure but which permits establishment of normal levels when the organism is returned to a noncontaminated medium. Proposals for this type of testing have been made for algae (Payne and Hall 1979) and algae and *Lemna* (Hughes et al. 1988).

8.3 In Situ Tests

The use of on-site tests (in situ and using mobile laboratories) is increasing, with the advantages that the test substance does not have to be transported and that conditions represent exactly those in the field. For example, earthworms (*Lumbricus terrestris*) were used to field test the toxicity of a hazardous waste site (Callahan et al. 1991). The reproducibility of such tests could be low due to the natural variation of environmental factors in time and space that influence survival and growth other than the contaminant. As well, natural predation and recovery of animals are potential problems.

In-situ toxicity testing has been carried out with leeches (Metcalf and Hayton 1989) to examine biomonitoring and bioaccumulation. Fathead minnows, *Daphnia magna* and *Ceriodaphnia dubia* have been used to assess in-situ sediment toxicity (Skalski et al. 1990a, Skalski et al. 1990b).

An apparatus for in-situ toxicity tests with prolarval and yearling striped bass has been described that may be useful for other fish species (Ziegenfuss et al. 1990).

For many effluents tested, on-site or off-site toxicity data did not appear to be significantly different (U.S. EPA 1985e). The major consideration was practicality. Cost should be weighed against data needs to make the choice for on- or off-site testing. If it is not considered important to the analysis of toxic impact, off-site testing (which is cheaper and can result in the generation of more data) is as acceptable as on-site testing.

8.4 Assessment Beyond Whole Organisms and Freshwater

This document has dealt with the use of whole-organism tests for the development of criteria for assessing the quality of freshwater, freshwater sediment, and soil. Whole-organism tests for aquatic and sediment-dwelling marine species, tests at the sub-organism level (e.g., mutagenicity, genotoxicity) and tests at the supra-organism level (processes, multi-species testing) should be evaluated in the manner presented in this report.

8.4.1 Methods for Assessing Impacts on Microbial Processes

There is a vast literature on soil processes and some aquatic and terrestrial tests are under development (e.g., phosphatase activity, arylsulphatase activity, microbial biomass, glutamic acid degradation; C^{14}-acetate, C^{14}-chloroform, C^{14}-benzoate, and C^{14}-chlorophenol mineralization) in the Netherlands (D. de Zwart, Nat. Inst. of Public Health and Envir. Protection, Bilthoven, pers. comm.). As well, some process tests have already been adopted by international standards organizations (e.g., OECD 1984e). A thorough evaluation of tests, similar to this review, should be carried out to identify tests relating to processes that are currently usable, prototypes, or under

development and desirable for inclusion in a Canadian test battery for soil assessment.

8.4.2 Multispecies Testing

Since microcosms are more closely related to multispecies communities in the field than is any particular species, the predictive capability of toxicity tests using them may be enhanced. With just two species tested together (Malueg et al. 1983), *Daphnia magna* and *Hexagenia limbata*, toxicity of sediments was greater to *D. magna* when *H. limbata* was present. Several methods for multispecies toxicity testing have been proposed (ASTM 1987b, Taub 1989, U.S. EPA 1987a and b, Leffler 1984). These tests require more effort than single species tests. Microcosms should be evaluated against multitrophic single species toxicity tests and indigenous community studies to determine the differences and similarities in results.

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Appendix A

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Appendix A

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Appendix B

Rationales for Test Scores in Detailed Evaluation

Appendix B

Rationales for Test Scores in Detailed Evaluation

Table B-1 Rationale for scores of tests for assessing soil quality described in Table 3

Test	Reference	Point loss rationale	Point loss	Total point loss	Final score	
Algal growth (*Selenastrum capricornutum*)	Lower and Sutton (1987)	Incomplete statistical guidance	1	1	16	(94%)
Seed germination Root elongation (5 species)	ASTM (1990f)	No details for elutriate prep	1	3	14	(82%)
		pH unspecified	1			
		Incomplete statistical guidance	1			
Seed germination Root elongation (10 species)	USEPA (1985c)	Substance volume unspecified	1	5	12	(71%)
		Seed pretreatment unspecified	1			
		pH unspecified	1			
		No statistical guidance	2			
Seed germination Root elongation (lettuce)	Greene et al. (1989)			0	17	(100%)
Seedling emergence (lettuce)	Greene et al. (1989)			0	17	(100%)
Seedling emergence (5 species)	ASTM (1990e)	pH unspecified	1	2	15	(88%)
		Incomplete statistical guidance	1			
Seedling emergence (16 candidate species)	OECD (1984b)	Substance volume unspecified	1	7	10	(59%)
		Vessel size unspecified	1			
		Seed pretreatment unspecified	1			
		Light, temp unspecified	2			
		No statistical guidance	2			

Table B-1 (Cont.)

Test	Reference	Point loss rationale	Point loss	Total point loss	Final score	
Seedling emergence (10 species)	Holst and Ellwanger (1982)	No seed sizing	1	8	9	(53%)
		Vessel volume unspecified	1			
		Substance volume unspecified	1			
		Light, temp, pH unspecified	3			
		No statistical guidance	2			
Seedling growth (10 species)	USEPA (1985d)	Vessel volume unspecified	1	5	12	(71%)
		Substance volume unspecified	1			
		pH unspecified	1			
		No statistical guidance	2			
Plant growth (10 species)	Holst and Ellwanger (1982)	Vessel volume unspecified	1	8	9	(53%)
		Substance volume unspecified	1			
		No culture details	1			
		Light, temp, pH unspecified	3			
		No statistical guidance	2			
Flower production (*Tradescantia* sp.)	Lower (1990)	No no. organisms/replicates	1	6	11	(65%)
		Test vessel volume unspecified	1			
		Substance volume unspecified	1			
		pH unspecified	1			
		No statistical guidance	2			
Springtail survival and reproduction (*Folsomia candida*)	OECD (1990)	Incomplete statistical guidance	1	1	16	(94%)
Earthworm survival (*Eisenia andrei*)	Greene et al. (1989)			0	17	(100%)

Table B-1 (Cont.)

Test	Reference	Point loss rationale	Point loss	Total point loss	Final score	
Earthworm survival (*Eisenia foetida*)	OECD (1984d)	Weak statistical guidance	1	1	16	(94%)
Earthworm survival (*Eisenia foetida*/ *E. andrei*)	ISO (1991a)	No statistical guidance for NOEC	1	1	16	(94%)
Earthworm reproduction (species above)	ISO (1991b)	No statistical guidance	2	2	15	(88%)
Earthworm survival (*Lumbricus terrestris*)	Eirkson et al. (1987)			0	17	(100%)

Table B-2. Rationale for scores of tests for assessing freshwater sediment quality described in Table 9.

Test	Reference	Point loss rationale	Point loss	Total point loss	Final score	
Hyallella azteca (10 day survival, ~ 30 day reproduction)	ASTM (1990b)	Defined but variable vessel and substance volume	1	3	14	(82%)
		pH unspecified	1			
		Incomplete statistical guidance	1			
Chironomus tentans (10 day survival, ~ 25 day adult emergence)	ASTM (1990b)	As above		3	14	(82%)
Chironomus riparius (10 day survival, ~ 30 day adult emergence)	ASTM (1990b)	As above		3	14	(82%)
Hexagenia spp. (7/10 day survival, 21 day growth)	Bedard and Henry (1992)	Defined but variable vessel and substance volume	1	7	10	(59%)
		Species unspecified	1			
		pH water unspecified	1			
		No medium information	2			
		No statistical guidance	2			
Chironomus tentans (10 day survival, growth)	Bedard et al. (1992)	pH unspecified	1	2	15	(88%)
		Incomplete statistical guidance	1			
Hexagenia spp. (10 day survival, 21 day growth)	Bedard et al. (1992)	As above		2	15	(88%)

Table B-2 (Cont.)

Test	Reference	Point loss rationale	Point loss	Total point loss	Final score	
Tubifex tubifex (28 day survival, reproduction)	ASTM (draft)	pH unspecified No statistical guidance	1 2	3	14	(82%)
Lumbriculus variegatus	Phipps et al. (1991)	No organism selection criteria Inadequate test substance prep pH unspecified No statistical guidance	1 1 1 2	5	12	(71%)

Table B-3. Rationale for scores of tests for assessing water quality described in Table 14.

Test	Reference	Point loss rationale	Point loss	Total point loss	Final score	
Pseudomonas putida	ISO (1991c)	Light unspecified No statistical methods	1 2	3	14	(82%)
Toxi-chromotest™ (*Escherichia coli*)	Orgenics Ltd. (1985)	pH unspecified No test substance prep details No statistical guidance	1 2 2	5	12	(71%)
Photobacterium phosphoreum	Microbics (1992a,b), Environment Canada (1991)	(light not specified but controlled in analyzer)	0	0	17	(100%)
Spirillum volutans	Dutka (1991)	Light, pH unspecified No statistical guidance	2 2	4	13	(77%)
Dehydrogenase activity (*Bacillus cereus*)	Thomson et al. (1986)	No. replicates unspecified No test substance prep details Light unspecified No statistical guidance	1 2 1 2	6	11	(65%)
Algal growth (*Selenastrum capricornutum*, microplate)	Environment Canada (1992c)		0	0	17	(100%)
Algal growth (*S. capricornutum*, flask)	ASTM (1990c)	pH unspecified Vague statistical guidance	1 1	3	14	(82%)
Rotoxkit (24h) (*Brachionus calyciflorus*)	Anonymous (1990a)	No statistical methods (graphic interpolation by eye)	2	2	15	(88%)

Table B-3 (Cont.)

Test	Reference	Point loss rationale	Point loss	Total point loss	Final score	
Brachionus rubens	Snell and Persoone (1989)	Microplate well size unspecified	1	2	15	(88%)
Daphnia magna (48 h)	Biesinger et al. (1987)	Inappropriate statistical guidance	2	2	15	(88%)
Daphnia magna (21 days)	OECD (1991a)	Vessel volume unspecified pH unspecified Vague statistical guidance	1	3	14	(82%)
Daphnia magna/ *D. pulex* (48 h)	Environment Canada (1990b)		0	0	17	(100%)
Ceriodaphnia dubia (7 day)	Environment Canada (1992a)		0	0	17	(100%)
Ceriodaphnia dubia (4 day)	Oris et al. (1991)		0	0	17	(100%)
Shrimp	APHA (1989)	Vague organism selection Vessel size unspecified Temp, pH unspecified Vague test substance prep Vague feeding details Incomplete statistical guidance	1 1 2 1 1 1	7	10	(59%)
Gammarus lacustris	Alberta Environmental Centre (1989)	Vague organism selection Vessel volume unspecified pH unspecified Inadequate statistical guidance	1 1 1 1	4	13	(76%)

Table B-3 (Cont.)

Test	Reference	Point loss rationale	Point loss	Total point loss	Final score	
Panagrellus redivivus	Samoiloff (1990)	pH, light unspecified Inappropriate statistical guidance	2 2	4	13	(76%)
Crayfish	ASTM (1980)	Vague organism selection Vague culture methods Light, pH unspecified Incomplete statistical guidance	1 1 2 1	5	12	(71%)
Wyeomia smithii	ASTM (1990a)	Vessel volume unspecified Substance volume unspecified pH unspecified Incomplete statistical guidance	1 1 1 1	4	13	(76%)
Fish early life stage	ASTM (1988)	Vessel volume unspecified pH unspecified	1 1	2	15	(88%)
Fathead minnow larval growth	Environment Canada (1992b)	(volume test vessel determined by restrictions on substance volume)	0	0	17	(100%)
Rainbow trout (96 h)	Environment Canada (1990a)		0	0	17	(100%)
Lemna gibba	USEPA (1985b)	No statistical guidance	2	2	15	(88%)
Lemna gibba	Holst and Ellwanger (1982)	Vague vessel volume Acclimation unspecified No statistical guidance	1 1 2	4	13	(76%)
Lemna gibba	ASTM (1991)	pH unspecified Vague statistical guidance	1 1	2	15	(88%)

Appendix C

Method Details for Tests in the Usable Batteries for Assessing Soil, Freshwater Sediment and Freshwater Quality

Appendix C

Method Details for Tests in the Usable Batteries for Assessing Soil, Freshwater Sediment and Freshwater Quality

Summary of the U.S. Environmental Protection Agency Seedling Emergence Soil Test Using Lettuce (Greene et al. 1989)

Test type	Acute, static
Temperature	24 ± 2°C
pH	≥4 but ≤10
Light intensity/quality Photoperiod	4300 ± 430 lx fluorescent light Initial 48 h in the dark, followed by 16:8 h light:dark
Soil moisture	85% of the water holding capacity
Test chamber size	150-mm wide by 15-mm high petri dish bottom placed in a 12" × 12" polyethylene resealable bag
Test soil volume	100 g
Breeding stocks	*Lactuca sativa*
Age of test organisms	Seeds
No./container; No. replicates	40; 3 replicates
Feeding	No nutrients added
Test duration	120 h
Neg. control/dilution soil Acceptability criteria	20-mesh washed silica sand ≥90% germination
Positive control/reference toxicant Mean LC_{50}, and CV	2-chloroacetamide 10.4 mg/kg; 3 tests resulted in a CV of 18.1%
Statistics	Trimmed Spearman-Karber (Hamilton et al. 1977); probit (Finney 1971)
Endpoints	LC_{50}
Reproducibility	No data found

Summary of the Recommended Test Conditions for the *Eisenia andrei* Earthworm Survival Test (Greene et al. 1989)

Test type	Acute, static
Temperature	20 ± 2°C
pH	≥4 but ≤10
Light intensity/quality	540-1080 lx ambient laboratory light
Photoperiod	Continuous
Soil moisture	75% of the water-holding capacity
Test chamber size	1 pint jar
Test soil volume	200 g
Breeding stocks	*Eisenia andrei* (J. Greene, pers. comm.)
Age of test organisms	>60 d with an individual weight of 300-500 mg
No./container; No. replicates	10; 3 replicates
Feeding	Do not feed
Test duration	14 d
Neg. control/dilution soil	Artificial soil (formula provided)
Acceptability criteria	≥90% survival at the end of 14 d
Positive control/ reference toxicant	2-chloroacetamide applied to 100% artificial soil
Mean LC_{50}, and CV	35 mg/kg, 3 tests resulted in a CV of 16.2% (J. Greene, pers. comm.)
Statistics	Trimmed Spearman-Karber (Hamilton et al. 1977); probit (Finney 1971)
Endpoints	LC_{50}
Reproducibility	No data found

Summary of the Survival and Growth Test Using *Hexagenia* spp. (Bedard et al. 1992)

Test type	Acute, chronic, static
Temperature	20 ± 2°C
pH	Not stated
Light intensity/quality Photoperiod	Ambient fluorescent light 16 h light:8 h dark
Test chamber size	1.8L (11.5x11.5x14.5cm)
Test water and sediment volumes	1300mL water, 325mL sediment
Egg stocks	Available from J. Ciborowski (U. of Windsor, Windsor, Ont.)
Age of test organisms	Nymphs 3-4 mo old with average weight of 5mg
No./container; No. replicates	10; 3 replicates
Feeding	Do not feed
Test duration	10 d for acute test, 21 d for chronic growth test
Neg. control Acceptability criteria	Clean sediment capable of supporting normal growth 85% survival in control
Positive control/ reference toxicant Mean LC_{50}, and CV	 The use of cadmium and copper as reference toxicants is being examined Not stated
Statistics	Comparative t-tests, one-way ANOVA
Endpoints	LC_{50} for per cent survival, EC_{50} for fresh weight
Reproducibility	For a 10-d test with 36 sediments, CVs were 27-180% for survival and 9-24% for nymph weight (D. Bedard, Ont. Min. Environ., pers. comm.); for 50 clean sediments from the Great Lakes, CVs were 3.4±3.4% for survival and 9.6±5.3% for growth (K. Day, NWRI, pers. comm.)

Summary of the Survival and Growth Test Using *Chironomus tentans* (Bedard et al. 1992)

Test type	Chronic, static
Temperature	20 ± 2°C
pH	Not stated
Light intensity/quality Photoperiod	Ambient fluorescent light 16 h light:8 h dark
Test chamber size	1.8L (11.5x11.5x14.5cm)
Test water and sediment volumes	1300mL water, 325mL sediment
Egg stocks	Stock cultures maintained by N. Collins, U. of Toronto; J. Giesy, Michigan State U.; federal agencies
Age of test organisms	10-12 d old larvae, second instar, average weight < 1mg
No./container; No. replicates	15; 3 replicates
Feeding	Daily with a mixture of Ceraphyll™ and Tetra Conditioning Food™
Test duration	10 d
Neg. control Acceptability criteria	Clean sediment capable of supporting normal growth 75% survival in control
Positive control/ reference toxicant Mean LC_{50}, and CV	The use of cadmium and copper as reference toxicants is being examined Not stated
Statistics	Comparative t-tests, one-way ANOVA
Endpoints	LC_{50} mortality, EC_{50} growth
Reproducibility	For a 10-d test with 36 sediments, CVs were 15-88% for survival and 8-19% for larval weight (D. Bedard, pers. comm.)

Summary of the Survival, Growth and Sexual Maturation Test Using *Hyalella azteca* (ASTM 1990b)

Test type	Chronic, static/flow-through
Temperature	20-25°C
pH	Not stated
Light intensity/quality Photoperiod	538 lx 16 h light:8 h dark
Test chamber size	1L or 20L aquaria
Test water and sediment volumes	200mL (2cm deep) in 1L containers, 2-3cm in 20L containers
Stocks	Stock cultures are maintained by some federal agencies
Age of test organisms	Second/third instar, 2-3mm long
No./container; No. replicates	100 in 20L aquaria, 2 replicates; 20 in 1L beakers, 4 replicates
Feeding	2-3 times weekly with rabbit pellets mixed with water
Test duration	<10-30 d
Neg. control Acceptability criteria	Clean sediment capable of supporting normal growth 80% survival in control
Positive control/ reference toxicant Mean LC_{50}, and CV	Not stated
Statistics	Probit, moving average, Spearman-Karber, Litchfield-Wilcoxon methods
Endpoints	LC_{50} mortality, EC_{50} growth, sexual maturation
Reproducibility	For 50 clean sediments from the Great Lakes, CVs were 6.9±3.9% for survival and 15.0.±10.2% for growth (K. Day, pers. comm.)

Summary of the Test Using Luminescent Bacteria (Environment Canada 1991)

Test type	15-60 min, static
Temperature	15±0.3°C
pH, DO	6.0-8.5, preaeration
Light intensity/quality Photoperiod	Not applicable
Test chamber size	Wells of Microtox Analyzer Model 2055 or automated Model 500
Test sample volume	2mL/replicate
Stocks Corp.	*Photobacterium phosphoreum*, strain NRRL B-11177, from Microbics
Age of test organisms	Not applicable
No./container; No. replicates	2 replicates per concentration
Culturing	Lyophilized 'bacteria reagent' is stable for 1 yr at -20°C; bacteria are brought back to living state (reconstituted reagent) by adding liquid (Recon) and bringing them to a suitable temperature
Test duration	5-60 min
Neg. control Acceptability criteri	Bacterial reagent Luminescence must be </> 50% for all test concentrations; reference toxicant within 2 SD of mean
Positive control/ reference toxicant Mean LC_{50}, and CV	Phenol, zinc, potassium dichromate, sodium lauryl sulphate Range (5 min, 15°C) IC_{50} phenol= 13-36mg/L, zinc sulphate= 1.4-7mgZn/L; sodium lauryl sulphate mean= 1.3mg/L
Statistics	Regression analysis
Endpoints	IC_{50} or IC_{20} at 5 and 15 min
Reproducibility	CVs for copper IC_{50} for 5, 15, 30, 60 min= 68%, 46%, 26%, 25% (Greene et al. 1985); CVs for natural gas plant sludge for IC_{50}= 1.6-100.2% (5 min), 0.2-115.6% (15 min) (Novak 1990)

Summary of the Growth Inhibition Test Using the Freshwater Alga *Selenastrum capricornutum* (Environment Canada 1992c)

Test type	Chronic, static, microplate
Temperature	24 ±2°C
pH	6.0-9.0
Light intensity/quality	4.0 Klx cool white fluorescent
Photoperiod	Continuous
Test chamber size	96-well microplates, volume of each well is approximately 250 µL
Test solution volume	20 µL/well
Breeding stocks	*Selenastrum capricornutum* ATCC 22662 can be obtained from government, private laboratories, or the American Type Culture Collection in Rockville, Md.
Age of test organisms	4-7 d old cells in log phase
No./container; No. replicates	Initial density of 10 000 cells/mL; 3 replicates/test concentration
Feeding	Test takes place in enriched nutrient medium
Aeration	No aeration but constantly shaken at 100 rpm or manually shaken twice daily
Test duration	72 h
Neg. control/dilution medium	Nutrient medium (formula provided)
Acceptability criteria	Coefficient of variation in the controls is ≤20%; control yield >16x
Positive control/ reference toxicant	Phenol, zinc chloride, potassium dichromate
Mean IC_{50}, and CV	Phenol= 63.1µg/L, 18.8-104.4 95%CI; $ZnCl_2$=52.6µg/L, 31.9-72.7 95%CI; $K_2Cr_2O_7$=129.7µg/L, 94.2-166.6 95%CI (St. Laurent et al. 1992)
Statistics	Arcsin transformation; linear regression
Endpoints	IC_{50}
Reproducibility	3 tests with phenol resulted in a CV of 12.7%, 3 tests with zinc chloride resulted in a CV of 22% (D. St-Laurent, St. Lawrence Centre, Environment Canada, pers. comm.); 11 tests with chromium resulted in a CV of 9.2% (Weber et al. 1989)

Summary of the Acute Lethality Test Using *Daphnia magna* (Environment Canada 1990b)

Test type	Static, acute
Temperature	20 ±2°C
pH; DO; hardness	6.0-8.5; >5.5 mg/L; ≥80 mg/L
Light intensity/quality Photoperiod	<800 lx cool white fluorescence at the water surface 16:8 h, light:dark
Test chamber size	150-250 mL beakers
Test solution volume	Loading density of 1 daphnid/15 mL
Breeding stocks	*Daphnia magna*; commercial biological supply houses and government laboratories
Age of test organisms	≤ 24-h old neonates
No./container; No. replicates	10 per container; "Replicates of each test concentration may be employed if desired."
Feeding	None
Aeration	Do not aerate
Test duration	48 h
Negative control Acceptability criteria	Uncontaminated ground, surface, or municipal water or reconstituted water <10% mortality
Positive control/ reference toxicant LC_{50}, and CV (1990c)	 Sodium chloride, zinc sulphate, potassium dichromate Not stated; LC_{50} for Zn with varying hardness in Environment Canada
Statistics	Probit analysis; Spearman-Karber method, binomial
Endpoints	LC_{50} or EC_{50} and 95% C.I.
Reproducibility—CV for EC_{50}s	Sodium pentachlorophenate: 10 tests resulted in a CV of 10% (Lewis and Weber 1985). Phenol: 5 tests resulted in a CV of 4.9% (U.S. EPA 1980). 4-chlorophenol: 13 tests over 6 mo resulted in a CV of 25%; 6 tests resulted in a CV of 21.7% (Environment Canada 1990e). Cadmium: 8 tests resulted in a CV of 72.4% (Lewis and Weber 1985); 4 tests resulted in a CV of 20% (Thomas et al. 1986). Copper: 4 tests resulted in a CV of 10% (Thomas et al. 1986).

Summary of the Test of Larval Growth and Survival Using Fathead Minnows (Environment Canada 1992b)

Test type	Chronic, sublethal, static-renewal
Temperature 27°C	Daily mean 25 ±1°C with extreme fluctuations within the range of 23-
pH, DO	6.5-8.5; 40-100% saturation
Light intensity/quality Photoperiod	≤500 lx, full spectrum fluorescent lights 16:8 h, light:dark
Test chamber size	500 mL
Test solution volume	250 mL
Breeding stocks	*Pimephales promelas.* Available from commercial biological supply houses and from government laboratories.
Age of test organisms	≤24-h larval fish
No./container; No. replicates	10; 3 replicates required, 4 replicates recommended
Feeding	2 to 3 times per day with brine shrimp nauplii. Do not feed during final 12 h of the test
Aeration	Do not aerate
Test duration	7 d
Neg. control/dilution medium Acceptability criteria	Reconstituted deionized water or noncontaminated well water ≤20% mortality in 7 d
Positive control/ reference toxicant Mean IC_p and LC_{50}, and CV	 Sodium chloride; phenol; zinc Not stated; LC_{50} for Zn with varying hardness in Environment Canada (1990c)
Statistics	Probit analysis, not trimmed Spearman-Karber method
Endpoints	NOEC; LOEC; IC_p for growth and mortality; if appropriate, LC_{50} at selected times(s)
Reproducibility	Sodium pentachlorophenate: 10 labs gave CV of the LC_{50} of 44% (DeGraeve 1991); 10 tests resulted in CV of IC_{50} of 21% (Environment Canada 1990e). Cadmium: 5 tests gave CV of LC_{50}s of 62% (Weber et al. 1989) Chromium: combined data for 10 labs and two days showed total intralaboratory variability of 26% (DeGraeve et al. 1991).

Summary of the Acute Lethality Test Using Rainbow Trout *Oncorhynchus mykiss* (Environment Canada 1990a)

Test type	Acute, static
Temperature	15 ±1 °C
pH; DO	5.5-8.5; ≥70% saturation
Light intensity/quality Photoperiod	≤500 lx, full-spectrum fluorescent lights 16:8 h light:dark
Test chamber size	Can be jars or aquaria depending on size and number of fish
Test solution volume 4 d	Minimum depth of 15 cm with a loading rate based on ≤0.5 g/L over
Breeding stocks	*Oncorhynchus mykiss*; available from commercial biological supply houses and from government laboratories
Age of test organisms	Swim-up fry or fingerlings with a mean weight of 0.3 to 5 g
No./container; No. replicates	10 fish may be divided between two or more vessels to accommodate the loading density
Feeding	Do not feed for 24 h before start of the test or during the test
Aeration	Aerate at ≤7.5 mL/min/L throughout the test
Test duration	96 h
Negative control Acceptability criteria	Reconstituted deionized water or noncontaminated well water ≤10% mortality in 96 h
Positive control/ reference toxicant Mean IC_{10} and IC_{50}, and CV	Phenol; zinc Not stated; LC_{50} for Zn with varying hardness in Environment Canada (1990c)
Statistics	Probit analysis; trimmed Spearman-Karber method is not recommended
Endpoints	LC_{50} and 95% confidence limits; or in a single-concentration test an LT_{50}
Reproducibility	4-chlorophenol: 2 tests resulted in a CV of 20%; 10 tests in 6 laboratories resulted in a CV of 38% (Walker 1988); 19 tests resulted in a CV of 13.6%; and another 68 tests resulted in a CV of 17.3% (Environment Canada 1990c). Sodium pentachlorophenate: 71 tests conducted over 4 yr resulted in a CV of 22% (Environment Canada 1990c). Cadmium: 5 tests resulted in a CV of 59% (U.S. EPA 1980).

Appendix D

Statistical Analysis of Usable and Prototype Tests

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Statistical Analysis of Usable and Prototype Tests

Comments on Reference Documents

Specific comments on the statistical procedures used in each of the provided protocols are given separately for each reference.

Computer Software

Four computer software programs are currently available from U.S. EPA. One program analyzes toxicity data from the *Ceriodaphnia dubia* survival and reproduction test, and another program analyzes toxicity data from the fathead minnow (*Pimephales promelas*) larval survival and growth test. Also available are a Dunnett's test program and a probit analysis program. These computer programs can be obtained by writing

Environmental Monitoring and Support Laboratory
United States Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, OH 45268

The Statistical Analysis Software (SAS) system is also available for statistical analysis. SAS is an industry standard for statistical analysis and can be used to conduct the majority of the procedures recommended for toxicity data. SAS is probably the most powerful and comprehensive statistical package available and contains specific programs for analysis of variance, probit analysis, linear and nonlinear regression, Wilcoxon Rank Sum test, Student's t-test, and many others. SAS programs can be written to conduct procedures such as Steel's Many-to-One test. Unfortunately, SAS is quite expensive and requires a significant amount of memory to run on a personal computer, some programming skills, and generally a significant time to learn. Nevertheless, SAS is highly recommended.

Conclusions

(1) There are a variety of statistical methods available to analyze toxicity data. The appropriateness of each method depends primarily on the experimental design of the bioassay and the validity of the assumptions associated with each statistical method. It is not a simple task to develop a standardized set of statistical procedures that are globally applicable to all bioassay tests, and it is unlikely that a roomful of statisticians could agree on such a set of procedures.

(2) Several of the methods that were reviewed provided very general or little to no guidance on the statistical analysis of the associated data. This is a serious deficiency.

(3) Hypothesis testing procedures were typically presented in the NOEC/LOEC framework for comparing test concentrations to a control. In this case, Dunnett's test, Williams' test, Steel's Many-to-One test, and the Wilcoxon Rank Sum test with the Bonferroni adjustment are all applicable procedures. However, there may be instances where the test groups do not represent various concentrations of a single wastewater or chemical, and comparisons to a control are not the only comparison of interest. For example, surface water from different sampling locations relative to a hazardous waste site may be collected. In this case, differences between the specific locations, as well as to a control, may be of interest, and

Dunnett's, Williams', and Steel's procedures are no longer appropriate, and other multiple comparison procedures should be used (e.g., Tukey's mean separation procedure). This is simply another example of how the appropriate statistical analysis depends on the objectives of the bioassay test.

Recommendations

(1) Computer simulation studies should be considered to evaluate the performance of the different statistical procedures for various bioassay procedures. Simulation studies would be useful to evaluate the effects of different aspects of experimental designs, including replication, within- and between-concentration variability, the number of test concentrations, and violated model assumptions (e.g., the use of normal-theory procedures when the data are not normally distributed) on the sensitivity and power of a statistical procedure to determine significant effects. The different statistical procedures could also be compared by using computer simulation. Dunnett's test could be compared, under various controlled conditions, to Williams' test to determine when one test performs better than the other and the magnitude of difference in performance.

(2) A statistical "cookbook" should be written to provide guidance on the available statistical procedures applicable to specific experimental designs. Procedures such as probit analysis, logistic regression, and Steel's test are not widely documented and are generally difficult for the layperson to understand. A complete and thorough statistical reference should be developed that, at a minimum, (1) discusses the advantages and disadvantages of hypothesis tests and point estimation; (2) presents the logic behind each method in an easily understandable fashion; (3) gives a thorough discussion of the assumptions associated with each method, tests for the assumptions, and consequences of violating the assumptions; (4) provides detailed and annotated examples using each method; (5) discusses the importance of other statistical issues such as randomization and independence in the design of bioassay tests; and (6) provides methods for detecting outlying observations and handling suspected outliers. Computer software should also be developed as a companion product to the statistical reference. The software should be user-friendly and menu-driven to provide the user with a means to implement the documented statistical procedures.

Protocols for Short-Term Toxicity Screening of Hazardous Waste Sites
(Greene et al. 1989)

(1) In general, the data analysis section is well written and complete.

(2) It is my understanding that the Litchfield-Wilcoxon test is simply a hand-calculation estimation procedure for probit analysis. Therefore, I recommend removing this method from the list of possible procedures for calculating the LC_{50} and EC_{50}.

(3) I also recommend removing the reference to the binomial method. The U.S. EPA has removed this method from its toxicity test methods (U.S. EPA 1991).

(4) It is also my understanding that Mr. Jim Dryer is no longer U.S. EPA Cincinnati contact for the computer programs.

(5) On page 14, the reference to comparing EC_{50}s and LC_{50}s by using a two-sample t-test is misleading. The appropriateness of comparing LC_{50}s depends on the method used to calculate them. A t-statistic is probably not appropriate for LC_{50}s calculated by probit analysis because a t-statistic is not used to calculate the confidence intervals (they are actually called fiducial intervals). Therefore, I recommend removing this sentence.

Ontario Ministry of the Environment
Laboratory Sediment Biological Testing Protocol
(Bedard et al. 1992)

(1) In general, the data interpretation section is too vague. If this document is to be used as a guidance document, this section must be expanded to provide a more detailed statistical analysis approach.

(2) The assumptions of the ANOVA, Dunnett's test, and Tukey's test should be clearly stated. These assumptions should be formally verified. Specific tests for verifying the assumptions should be identified (e.g., Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variance). Williams' test could also be used as an alternative to Dunnett's test.

(3) Reference is made to performing the analysis on the logarithmic scale. There are other transformations that may be appropriate to satisfy model assumptions. More discussion is needed on transformations (different types, purpose, etc.).

(4) If the model assumptions are not reasonable, nonparametric procedures should be recommended. Steel's Many-to-One test should be used for comparisons with a control. The Kruskall-Wallis (KW) test should be used for comparisons between sediments followed by a multiple comparison procedure based on the KW rank sums.

(5) The appropriateness of the statistical method depends on whether or not there is replication. If there is no replication, the statistical methods are not appropriate.

(6) How are the endpoints calculated? Are average or individual weights used? More detail is needed about the test endpoints.

(7) More discussion is needed on the Spearman Rank Correlation analysis in order to assess the usefulness of this method.

(8) The calculation of the coefficient of variation (CV) should be presented. Is the CV calculated for each sediment and each site? More discussion is needed on the use of CVs.

Outline for ASTM Standard Guide for Conducting Chronic Sediment Bioassays with the Freshwater Oligochaete Tubificid Worm, *Tubifex tubifex* Muller 1774

(ASTM draft)

(1) A variety of responses are defined: survival of adults, the number of cocoons produced, per cent hatch of cocoons, total young produced, the ratio of cocoons to adult, the ratio of young to cocoon, and the ratio of young to adult. However, no statistical analyses are recommended and no specific experimental design is given.

A detailed section on data analysis should be presented based on the specific experimental design and objectives of the bioassay.

In general, if comparisons are made to a control, Dunnett's or Williams' test may be appropriate. Data transformations may be necessary depending on the specific response used in the analysis. Steel's test or Wilcoxon's Rank Sum test with the Bonferroni adjustment can be used if the assumptions associated with the parametric procedures are not reasonable. For comparisons between sediments, a one-factor analysis of variance followed by Tukey's mean separation procedure can be used. The Kruskal-Wallis test is a nonparametric alternative to the one-factor analysis of variance.

Standard Guide for Conducting Sediment Toxicity Tests
Freshwater Invertebrates
ASTM E 1383-90 (ASTM 1990b)

(1) The methods recommended to determine the LC_{50} or EC_{50} and the associated 95% confidence intervals are the most common methods and are all appropriate. In general, I rank these methods in the following order of preference: probit analysis, Spearman-Karber, and the graphical method. The Spearman-Karber, moving average, and moving average angle methods are similar in that they share similar assumptions: if one of these methods cannot be used, none of them can be used. The Spearman-Karber method is easy to do by hand and is therefore the preferred procedure.

(2) I do not recommend the binomial method. The U.S. EPA has removed this method from its toxicity test methods (U.S. EPA 1991).

(3) A good point is made in section 16.4: field sites cannot be statistically compared unless the sites are independently replicated.

(4) The ANOVA F-test is an appropriate technique for testing overall differences between concentrations or field sites. This is a general test for differences among the test and control concentrations (or field sites). The F-test, however, does not identify where the specific differences occur. Multiple comparisons should be used to identify specific pairwise differences.

(5) The procedures recommended for the comparison of each test concentration (or field site) with the control are all appropriate methods depending on the specific experimental design and test objectives. Since there is no specific experimental design or specific objectives, a single method cannot be recommended.

(1) The graphical method for determining the IC_{50} should be used only as a last resort when more quantitative techniques (e.g., regression analysis) cannot be used. For example, regression analysis cannot be used when the per cent inhibition is either 0% or 100% for all test chambers. The graphical method is a qualitative and subjective approach and should not be used as the primary method for determining the IC_{50}. The graphical method is more appropriate as either a screening tool, a qualitative check of the results from a statistical estimation procedure, or when the per cent inhibition is either 0% or 100% for all test chambers.

(2) The specific model assumptions for linear and nonlinear regression analyses should be verified (e.g., normality and homogeneity of variance). Since the response in the analysis is a percentage, an arcsine transformation may be necessary to stabilize the variance.

(3) The section that describes methods for determining the NOEC (14.3) is extremely vague. Since the ASTM method identifies neither a specific experimental design nor a single goal for the bioassay, a myriad of statistical methods are presented. The appropriateness of the statistical test depends on the experimental design and the specific objectives of the bioassay; therefore it is difficult to comment on the appropriateness of each method. Contingency tables may or may not be appropriate depending on the specific experimental design. Appropriate tests for comparing a series of concentrations to a control are Dunnett's or Williams' procedures, or Steel's nonparametric Many-to-One test. The procedures take into account the total number of comparisons that will be made.

This section also suggests reporting the power of the statistical test. This is not an easy and straightforward calculation and would almost always require the assistance of a statistician or sophisticated computer software. The minimum detectable difference is sufficient to indicate the sensitivity of the test.

The specific response to use in the determination of the NOEC is not specified. Should the per cent inhibitions be used or the actual increase in biomass?

This section recommends that the data be evaluated for outliers and heterogeneity (of variance). It should also recommend that the data be evaluated for normality and, more generally, that specific model assumptions associated with selected statistical analysis method be verified.

User's Guide: Procedures for Conducting *Daphnia magna* Toxicity Bioassays
EPA/600/8-87/011 (Biesinger et al. 1987)

(1) The reproduction and growth analysis is conducted on only those daphnids that survive to the end of the test. Although this is not a shortcoming of the test, another school of thought uses the data from all daphnids in the test, regardless of whether they survive to the end of the test. In that case, the effect on growth would be a function of the mortality effect as well. This is not a criticism, only mentioned as a sidenote.

(2) Presumably, the goal of the statistical analysis on the mortality, reproduction, and growth data is to detect a statistically significant difference regardless of the direction of the difference (e.g., either a significant increase or decrease in length). This is presumed because of the use of the term "statistically significant effect concentration" rather than using LOEC. It seems unusual that such an approach is used. Would increased reproduction or increased length be of interest? It seems that a one-sided (rather than two-sided) test would be more appropriate. That is, an increase in mortality, a decrease in reproduction, or a decrease in length appear to be the more appropriate effects of interest. A two-sided test is also inferred in the discussion of confidence intervals.

(3) *Survival* — Dunnett's procedure and the Bonferroni t-test procedure are not appropriate for the experimental design of this test. Since there is one daphnid in each beaker and 10 beakers for each test concentration, Fisher's Exact test is the appropriate statistical method for analysis. Fisher's test provides a conservative test for the equality of any two survival proportions, assuming only the independence of the individual responses. This assumption is satisfied because there is only one daphnid in each beaker.

Although the recommended procedures are not appropriate, I will comment on the text describing them. First, a small-sample transformation is recommended, but nothing is suggested for larger samples. Second, the one-way analysis of variance (ANOVA) is only used to determine a pooled estimate of variability, not for determining the equality of proportions. Dunnett's procedure tests for equality of proportions and is performed regardless of the results of the ANOVA F-test. Finally, the Bonferroni t-test procedure should only be used when there is not equal replication; otherwise, Dunnett's test should be used. Also, the assumptions associated with Dunnett's test and the Bonferroni t-test should be formally verified.

(4) I could not comment on the methods for determining the LC_{10} and LC_{50} because I did not have a copy of the acute toxicity manual. The trimmed Spearman-Karber is appropriate only if the associated assumptions are reasonable. For example, the Spearman-Karber method requires a symmetric tolerance distribution.

(5) *Reproduction and Length* — The method suggests that if an outlier is detected, the analysis should be conducted with and without the suspected value, but makes no recommendations with respect to which analysis to use if different conclusions are reached.

The assumptions of Dunnett's test (normality and homogeneity of variance) should be formally verified, not simply by examining scatterplots.

The Bonferroni t-test procedure should only be used when there is not equal replication; otherwise, Dunnett's test should be used. The Kruskall-Wallis procedure is not the most appropriate test. Steel's Many-to-One test (if there is equal replication and 4 or more replicates) or the Wilcoxon Rank Sum test with the Bonferroni adjustment (if there is unequal replication) are the more appropriate nonparametric alternatives to Dunnett's test.

(6) *Confidence Intervals and After-the-Fact Power Calculations* — Power calculations are not easy to understand nor easy to calculate. After-the-fact power calculations typically require the assistance of a statistician or sophisticated computer software. Although there is nothing wrong with after-the-fact power calculations, they should not be required. A confidence interval is a sufficient descriptive measure to indicate the sensitivity of the test.

Biological Test Method: Acute Lethality Test Using *Daphnia* spp. Environment Canada (1990b)

The following comments are the same as those presented for the rainbow trout method (EPS 1/RM/9) because the texts in the data analysis sections are nearly identical. They are only noted again for completeness.

(1) The data analysis section is good and discusses appropriate methods for determining the LC_{50} and the associated 95% confidence intervals. In general, I rank these methods in the following order of preference: probit analysis, Spearman-Karber, and the graphical method. The Spearman-Karber, moving average, and moving average angle methods are similar in that they share similar assumptions: if one of these methods cannot be used, none of them can be used. The Spearman-Karber method is easy to do by hand and is therefore the preferred procedure.

(2) I recommend removing the reference to the binomial method. The US EPA has removed this method from its toxicity test methods (U.S. EPA (1991).

(3) I am not familiar with the Litchfield (1949) method and therefore cannot comment on its use.

(4) A probit analysis program is also available from the U.S. EPA.

Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*
Environment Canada (1992a)

(1) *Mortality* — The method states that the analysis should begin with "a check of normality and homogeneity of data." The phrase "homogeneity of data" is misleading. Homogeneity of data can be interpreted as meaning that the data are the same from one concentration to another, whereas the assumption that should be verified is the homogeneity *of variance* within and between the test concentrations.

The methods described in the discussion of TOXSTAT are appropriate for the experimental design of this test and are consistent with U.S. EPA guidance. However, I cannot endorse TOXSTAT in general because I have not reviewed the documentation or the software.

The choice of whether or not to use Williams' test over Dunnett's test depends on the validity of the assumptions associated with each test. Williams'test is a more powerful and more sensitive test than Dunnett's test *if* the assumptions associated with Williams' test are reasonable and appropriate. Williams' test assumes, a priori, that you expect the data to be monotonically increasing or monotonically decreasing. If this assumption is violated, Williams' test may or may not be better than Dunnett's test.

(2) *Reproduction* — The recommended methods for evaluating the reproduction data are the same as those for the mortality data, which are appropriate for the experimental design of the test.

The approach for the reproduction data is an "all data" approach. That is, if a female dies during the test, the actual number of young produced before death is used in the analysis. It is appropriately noted that reproduction effect incorporates both the mortality and reproductive effects.

(3) The chronic value is highly dependent on the experimental design of the bioassay. Since the chronic value is simply the geometric mean of two of the test concentrations, there are only as many possible estimates as there are test concentrations. The chronic value is also dependent on sample size and, in turn, the power of the statistical test to detect a significant difference between test concentrations thus defining the NOEC and LOEC. There is also no easy method for summarizing the variability associated with the chronic value or for constructing confidence intervals.

It is my understanding that the use of a chronic value has been eliminated from all of the U.S. EPA bioassay tests.

(4) In the discussion of a single-concentration test, the method states that no particular nonparametric test has become standard practice. In fact, the Wilcoxon Rank Sum test is the nonparametric counterpart to the standard two-sample t-test. This would be the most logical test to use if the assumptions associated with the parametric t-test are not valid.

(5) In footnote 'x' on page 26, the second paragraph begins "If the data are regular...." What are *regular* data? I assume that the authors are implying that the data must follow a normal distribution. The use of the term "regular" is, in fact, irregular.

The last paragraph of this footnote states that nonparametric tests are less powerful than parametric tests when the data are in fact normally distributed. This is true, but the nonparametric procedures are not necessarily *extremely* less powerful than their parametric counterparts in this situation.

It should also be noted that at least four replicates are required to use Steel's nonparametric Many-to-One test.

Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows
Environment Canada (1992b)

(1) *Mortality and Growth* — The method states that the analysis should begin with "a check of normality and homogeneity of data." The phrase "homogeneity of data" is misleading. Homogeneity of data can be interpreted as meaning that the data are the same from one concentration to another, whereas the assumption that should be verified is the homogeneity *of variance* within and between the test concentrations.

The methods described in the discussion of TOXSTAT are appropriate for the experimental design of this test and are consistent with U.S. EPA guidance. However, I cannot endorse TOXSTAT in general because I have not reviewed the documentation or the software.
The choice of whether or not to use Williams' test over Dunnett's test depends on the validity of the assumptions associated with each test. Williams' test is a more powerful and more sensitive test than Dunnett's test *if* the assumptions associated with Williams' test are reasonable and appropriate. Williams' test assumes, a priori, that you expect the data to be monotonically increasing or monotonically decreasing. If this assumption is violated, Williams' test may or may not be better than Dunnett's test.

(2) The chronic value is highly dependent on the experimental design of the bioassay. Since the chronic value is simply the geometric mean of two of the test concentrations, there are only as many possible estimates as there are test concentrations. The chronic value is also dependent on sample size and, in turn, the power of the statistical test to detect a significant difference between test concentrations thus defining the NOEC and LOEC. There is also no easy method for summarizing the variability associated with the chronic value or for constructing confidence intervals.
It is my understanding that the use of a chronic value has been eliminated from all of the U.S. EPA bioassay tests.

(4) In the discussion of a single-concentration test, the method states that no particular nonparametric test has become standard practice. In fact, the Wilcoxon Rank Sum test is the nonparametric counterpart to the standard two-sample t-test. This would be the most logical test to use if the assumptions associated with the parametric t-test are not valid.

(5) In footnote 'v' on page 32, the second paragraph begins "If the data are regular . . ." What are *regular* data? I assume that the authors are implying that the data must follow a normal distribution. The use of the term "regular" is, in fact, irregular.

The last paragraph of this footnote states that nonparametric tests are less powerful than parametric tests when the data are in fact normally distributed. This is true, but the nonparametric procedures are not necessarily *extremely* less powerful than their parametric counterparts in this situation.

Biological Test Method: Acute Lethality Test
Using Rainbow Trout
Environment Canada (1990a)

(1) The data analysis section is good and discusses appropriate methods for determining the LC_{50} and the associated 95% confidence intervals. In general, I rank these methods in the following order of preference: probit analysis, Spearman-Karber, and the graphical method. The Spearman-Karber, moving average, and moving average angle methods are similar in that they share similar assumptions: if one of these methods cannot be used, none of them can be used. The Spearman-Karber method is easy to do by hand and is therefore the preferred procedure.

(2) I recommend removing the reference to the binomial method. The U.S. EPA has removed this method from its toxicity test methods (U.S. EPA 1991).

(3) I am not familiar with the Litchfield (1949) method and therefore cannot comment on its use.

(4) A probit analysis program is also available from the U.S. EPA.

Biological Test Method: Growth Inhibition Test
Using the Freshwater Alga *Selenastrum capricornutum*
Environment Canada (1992c)

(1) I am unsure of the effect on the estimation of the IC_{50} by trimming the upper and lower 16% of the data and performing the regression only on the data falling within the 16-84% range, especially given that the data have been transformed by using an arcsine transformation. The reason for trimming is presumably to estimate only the linear portion of the dose-response curve. A more detailed discussion would be helpful.

(2) The reason for using the arcsine transformation is to help minimize the inherent heterogeneity of variance associated with analyzing proportions or percentages. The interpretation of the inverse prediction, however, is questionable. The response that is estimated is not the concentration that shows a 50% reduction in growth, but rather the concentration where the arcsine square-root is equal to 50. The y-axis on the graph in Figure 2 does not represent per cent inhibition.

Biological Test Method: Toxicity Test Using Luminescent Bacteria (*Photobacterium phosphoreum*) Environment Canada (1991)

(1) This test method recommends a graphical procedure for determining the IC_{50}. Appropriately, the test method recommends using the graphical procedure as a check of the reasonableness of a mathematically determined IC_{50}. The graphical method is also useful as an exploratory tool for detecting data anomalies.

(2) I am not familiar with the Microbics software and therefore cannot comment on the statistical methods used to determine the IC_{50}. Section 4.5.2 suggests that the Microbic software uses regression analysis to calculate the IC_{50}. Regression analysis is appropriate for this type of data.

Standard Guide for Conducting Static 96-h Toxicity Tests With Microalgae
ASTM E 1218-90 (ASTM 1990c)

(1) The graphical method for determining the IC_{50} should only be used as a last resort when more quantitative techniques (e.g., regression analysis) cannot be used. For example, regression analysis cannot be used when the per cent inhibition is either 0% or 100% for all test chambers. The graphical method is a qualitative and subjective approach and should not be used as the primary method for determining the IC_{50}. The graphical method is more appropriate as either a screening tool, a qualitative check of the results from a statistical estimation procedure, or when the per cent inhibition is either 0% or 100% for all test chambers.

(2) The specific model assumptions for linear and nonlinear regression analyses should be verified (e.g., normality and homogeneity of variance). Since the response in the analysis is a percentage, an arcsine transformation may be necessary to stabilize the variance.

(3) The section that describes methods for determining the NOEC (14.3) is extremely vague. Since the ASTM method identifies neither a specific experimental design nor a single goal for the bioassay, a myriad of statistical methods are presented. The appropriateness of the statistical test depends on the experimental design and the specific objectives of the bioassay; therefore it is difficult to comment on the appropriateness of each method. Contingency tables may or may not be appropriate depending on the experimental design. Appropriate tests for comparing a series of concentrations to a control are Dunnett's or Williams' procedures, or Steel's nonparametric Many-to-One test. The procedures take into account the total number of comparisons that will be made.

This section also suggests reporting the power of the statistical test. This is not an easy and straightforward calculation and would almost always require the assistance of a statistician or sophisticated computer software. The minimum detectable difference is sufficient to indicate the sensitivity of the test.

The specific response to use in the determination of the NOEC is not specified. Should all three responses (standing crop, growth rate, area under the growth curve) be used?

This section recommends that the data be evaluated for outliers and heterogeneity (of variance). It should also recommend that the data be evaluated for normality and, more generally, that specific model assumptions associated with selected statistical analysis method be verified.

Finally, no recommendations are made regarding the significance levels at which to perform the statistical analyses. It is general practice to select a significance level of 0.05 for the statistical comparisons and a significance level of 0.01 to test model assumptions.

(4) The appropriateness of the recommended analysis on the per cent inhibition using each of the three responses (standing crop, growth rate, area under the growth curve) is questionable and would require a more thorough review. I am not familiar with the use of growth rate and area under the growth curve or with how such responses should be treated in a statistical analysis. Therefore, I cannot really comment on the appropriateness of the analyses.

(5) In Section 14.1.2.2, the definition of N_1 appears to be wrong. I believe it should be "N_1 = biomass at time t_1."

The Nematode Toxicity Assay Using *Panagrellus redivivus*
(Samoiloff 1990)

(1) The test organisms should be randomly placed in the replicate cups to avoid situations where the organisms are placed serially by concentration into the cups. That is, the control cups should not be filled first, followed by the test concentration cups.

(2) It is not clear whether multiple dilutions are run or if a single test concentration will be tested.

(3) A number of endpoints are defined but they do not appear to be used in the statistical analysis.

(4) The appropriateness of the chi-square analysis is highly questionable. There is not sufficient information about the chi-square tests to allow a thorough understanding of its use. Clearly, a good deal of information regarding variability among the replicates is lost by pooling all the replicate data together into the proposed chi-square analysis.

The critical chi-square value of 5 appears to be associated with a one-degree-of-freedom test at an alpha level of 0.025. No discussion of how this critical value was determined is provided.

The appropriate statistical analysis will incorporate the variability among the cups and use all the information of the test and will depend on the specific design of the test (i.e., multiple dilutions or single concentration). For the survival data, it appears that classical comparison procedures (e.g., t-test, Dunnett's) might be appropriate.

I recommend that other statistical methods be evaluated for analyzing these particular data.

Determination of the Inhibitory Effect of Water Constituents on Bacteria (*Pseudomonas* Cell Multiplication Inhibition Test) (ISO 1991c)

(1) A graphical method is the only procedure suggested to determine the IC_{50}. The graphical method for determining the IC_{50} should be used only when more quantitative techniques (e.g., regression analysis) cannot not be used. For example, regression analysis cannot be used when the per cent inhibition is either 0% or 100% for all test chambers. The graphical method is a qualitative and subjective approach and should not be used as the primary method for determining the IC_{50}. The graphical method is recommended as either a screening tool, a qualitative check of the results from a statistical estimation procedure, or when the per cent inhibition is either 0% or 100% for all test chambers. I highly recommend establishing a statistical method (e.g., regression analysis) for determining the IC_{50}.

(2) Transforming the data to the log scale is not a necessary first step. The data should first be plotted on the original scale; log transformations can be used to linearize the data, if necessary.

(3) In general, the language used to describe the graphical method is complex and, in my opinion, is not easy for a layperson to understand. The method could be described in much simpler terms.

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Appendix E

Additional Literature Reviewed

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