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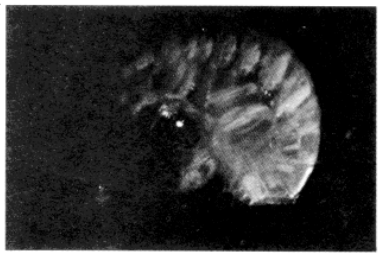

Biological Test Method: Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods

Report EPS 1/RM/26
December 1992
Including October 1998 Amendments

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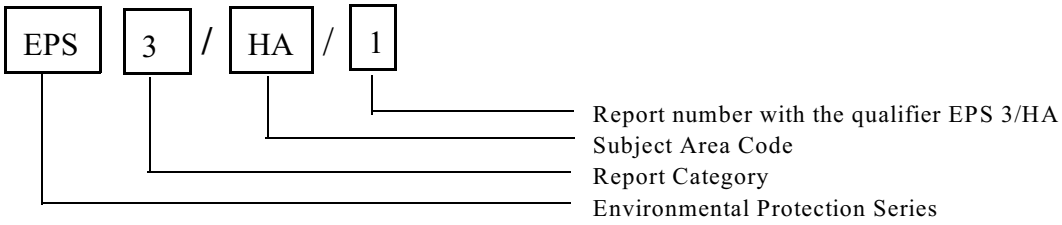

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Abstract

Methods recommended by Environment Canada (EC) for performing static 10-day tests for sediment toxicity, using one or more of the following species of marine or estuarine sediment-burrowing amphipods, are described in this report: Amphiporeia virginiana, Corophium volutator; Eohaustorius estuarius; Eohaustorius washingtonianus; Foxiphalus xiximeus; Leptocheirus pinguis; *and* Rhepoxynius abronius. *The biological endpoint for the test is percent mortality at Day 10. Additional (sublethal) endpoints that measure the percentage of surviving amphipods that emerge from the sediment at Day 10 (i.e., avoidance response), and/or the ability of the amphipods to rebury when transferred to control sediment, can also be determined using this test. The test is performed in 1-L glass vessels, with 175 mL of solid-phase sediment and 750 mL of overlying seawater.*

General or universal conditions and procedures are outlined for preparing for and undertaking the test. Additional conditions and procedures are stipulated that are specific to the intended use of the test (e.g., assessment of samples of field-collected sediment, sludge or other solid waste, or chemical introduced to sediment). Included are: instructions on collecting, identifying, and transporting test organisms; sorting and handling procedures; holding and acclimation conditions; sample transport and storage; test facility requirements; procedures for preparing test sediments and test initiation; specified test conditions; appropriate observations and measurements; endpoints; methods of calculation; the use of reference toxicants; and test validation.

Résumé

Le présent rapport décrit les méthodes recommandées par Environnement Canada pour l'exécution d'essais statiques d'une durée de 10 jours visant à évaluer la toxicité de sédiments chez une ou plusieurs des espèces suivantes d'amphipodes fouisseurs marins ou estuariens : Amphiporeia virginiana, Corophium volutator, Eohaustorius estuarius, Eohaustorius washingtonianus, Foxiphalus xiximeus, Leptocheirus pinguis *et* Rhepoxynius abronius. *Le résultat biologique de l'essai est le pourcentage de mortalité au 10^{e} jour. Cet essai permet aussi de déterminer des résultats additionnels (sublétaux) tels que le pourcentage des amphipodes survivants qui émergent des sédiments au 10^{e} jour (c.-à-d. qui ont une réaction d'évitement) ou le pourcentage de ceux qui sont capables de s'enfouir dans un sédiment de contrôle après y avoir été transférés. L'essai est effectué dans des récipients de verre d'une capacité de 1 L, avec 175 mL de sédiments en phase solide recouverts de 750 mL d'eau de mer.*

On expose dans le présent rapport des conditions et méthodes générales se rapportant à la préparation et la réalisation de l'essai. On y précise aussi d'autres conditions et méthodes particulières qui dépendent de l'objectif de l'essai (p. ex., évaluation d'échantillons de sédiments prélevés sur le terrain, de boues ou d'autres déchets solides, ou encore de produits chimiques ajoutés à des sédiments). Le lecteur y trouvera des instructions concernant : le prélèvement, l'identifation et le transport des organismes soumis à l'essai; le tri et la manipulation des échantillons; la détention et l'acclimatation des organismes soumis à l'essai; le transport et le stockage des échantillons; les installations d'essai; la préparation des sédiments d'essai et la mise en route des essais; les conditions prescrites pour les essais; les observations et mesures appropriées; les résultats des essais; les méthodes de calcul; l'utilisation de produits toxiques de référence; et la validation des résultats des essais.

Foreword

*This one of a series of **recommended methods** for measuring and assessing the aquatic biological effects of toxic substances. Recommended methods are those that have been evaluated by Environmental Protection Service (EPS), and are favoured:*

- *for use in Environment Canada aquatic toxicity laboratories;*
- *for testing that is contracted out by Environment Canada or requested from outside agencies or industry;*
- *in the absence of more specific instructions, such as are contained in regulations; and*
- *as a foundation for the provision of very explicit instructions as might be required in a regulatory protocol or standard reference method.*

The different types of tests included in this series were selected on the basis of their acceptability for the needs of programs for environmental protection and management carried out by Environment Canada. These reports are intended to provide guidance and to facilitate the use of consistent, appropriate, and comprehensive procedures for obtaining data on the toxic effects of samples of chemical, effluent, elutriate, leachate, receiving water, or, where appropriate, sediment or similar solid substance.

Mention of trade names in this report does not constitute endorsement by Environment Canada; other products with similar value are available.

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List of Abbreviations and Chemical Formulae

°C . . . . . degree(s) Celsius
Cd . . . . . cadmium
Cl . . . . . chloride
cm . . . . . centimetre(s)
d . . . . . day(s)
DO . . . . . dissolved oxygen (concentration)
EC50 . . . . . median effective concentration
Eh . . . . . oxidation-reduction potential
g . . . . . gram(s)
h . . . . . hour(s)
H_2O . . . . . water
kg . . . . . kilogram(s)
L . . . . . litre(s)
LC50 . . . . . median lethal concentration
m . . . . . metre(s)
mg . . . . . milligram(s)
min . . . . . minute(s)
mL . . . . . millilitre(s)
mm . . . . . millimetre(s)
rpm . . . . . revolutions per minute
SD . . . . . standard deviation
SI . . . . . International System of Units
TM(™) . . . . . Trade Mark
µg . . . . . mirogram(s)
µm . . . . . micrometre(s)
> . . . . . greater than
< . . . . . less than
≥ . . . . . greater than or equal to
≤ . . . . . less than or equal to
± . . . . . plus or minus
% . . . . . percentage or percent
‰ . . . . . parts per thousand

Terminology

Note: all definitions are given in the context of the procedures in this report, and may not be appropriate in another context.

Grammatical Terms

Must is used to express an absolute requirement.

Should is used to state that the specified condition or procedure is recommended and ought to be met if possible.

May is used to mean "is (are) allowed to".

Can is used to mean "is (are) able to".

Might is used to express the possibility that something could exist or happen.

General Technical Terms

Acclimation means to become physiologically adjusted to a particular level of one or more environmental factors such as temperature. The term usually refers to controlled laboratory conditions.

Compliance means in accordance with governmental permitting or regulatory requirements.

Conductivity is 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 is normally reported in the SI unit of millisiemens/metre, or as micromhos/centimetre (1 mS/m = 10 µmhos/cm).

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

Monitoring is the routine (e.g., daily, weekly, monthly, quarterly) checking of quality, or collection and reporting of information. It means either the periodic (routine) checking and measurement of certain biological or water-quality variables, or the collection and testing of samples of sediment for toxicity.

Percentage (%) is a concentration expressed in parts per hundred parts. With respect to test substances, 10% represents ten units or parts of substance diluted with sediment or water to a total of 100 parts. Depending on the test substance, concentrations can be prepared on a weight-to-weight, weight-to-volume, or volume-to-volume basis, and are expressed as the percentage of test substance in the final sediment mixture or solution.

pH is 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 greater acidic reactions, and numbers greater than 7 indicating increasingly basic or alkaline reactions.

Pretreatment means treatment of a sediment sample, or portion thereof, before exposure of amphipods.

Salinity is the total amount of solid substance, in grams, dissolved in 1 kg of water. It 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 (see APHA *et al.*, 1989). It is usually reported in grams per kilogram (g/kg) or parts per thousand (‰).

Spiking refers to the addition of a known amount of chemical to a clean, control sediment. After the addition of the chemical, the sediment is mixed thoroughly to evenly distribute the chemical throughout the sediment.

Terms for Test Substances

Chemical is any element, compound, formulation, or mixture of a chemical substance that might be mixed with, deposited in, or found in association with sediment or water.

Clean seawater is seawater that does not contain concentrations of toxicants that cause discernible distress to the test organisms or reduce their survival in 10-day assays.

Clean sediment is sediment that does not contain concentrations of toxicants that cause discernible distress to the test organisms or reduce their survival in 10-day assays.

Control is 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 substance. The control is used to determine the absence of measurable toxicity due to basic test conditions (e.g., salinity, temperature, health of test organisms, or effects due to handling of test organisms).

Control/dilution water is the water used to dilute a test chemical or reference toxicant, for a seawater-only control, or as test water in a 10-day sediment toxicity test.

Control sediment is clean sediment, taken from the site where the test organisms were collected (or, for cultured organisms, a sample of sediment identical to that used for the culture). This sediment must contain no test substance, and must enable an acceptable survival rate for the test organisms during the 10-day period of exposure. It is usually a sample of sieved sediment obtained from the amphipod-collection site when the test organisms are collected.

Dechlorinated water is a chlorinated water (usually municipal drinking water) that has been treated to remove chlorine and chlorinated compounds from solution.

Deionized water is fresh water that has been purified by passing it through resin columns or a reverse osmosis system.

Dilution water is the water used to prepare specific concentrations of a reference toxicant or other test chemical (seawater-only exposure).

Distilled water is water that has been passed through a distillation apparatus of borosilicate glass, or other material, to remove impurities.

Elutriate is an aqueous solution obtained by adding water to a solid substance (e.g., sediment, tailings, drilling mud, dredge spoil), shaking the mixture, then centrifuging or filtering it or decanting the supernatant.

Estuarine water is brackish seawater from a coastal body of ocean water that is measurably diluted with fresh water derived from land drainage.

Marine water is seawater in, or obtained from, the ocean, sea, or inshore location where there is no appreciable dilution by natural fresh water derived from land drainage.

Pore water is the water occupying space between sediment particles. The amount of pore water is expressed as a percentage of the wet sediment, by weight.

Reconstituted seawater is fresh water to which hypersaline brine has been added in a quantity that provides the seawater salinity (and pH) required for holding/acclimating organisms and for use in the test.

Reference sediment is a field-collected sample of presumably clean (uncontaminated) sediment, selected for properties (e.g., particle size, compactness, total organic content) representing sediment conditions that closely match those of the sample(s) of test sediment except for the degree of chemical contaminants. It is often selected from a site that is uninfluenced or minimally influenced by the source(s) of contamination but within the general vicinity of the site(s) where samples of test sediment are collected.

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

Resuspended sediment is sediment that has been mixed vigorously with seawater and allowed to settle.

Sediment is a natural particluate substance that has been transported to, and deposited at the bottom of, a body of water. The term can also describe a substrate that has been experimentally prepared, and into which the test organisms can burrow.

Solid-phase sediment is the whole, intact sediment used to expose the amphipods. It is not a form or derivative of the sediment such as an elutriate or a resuspended sediment.

Spiked control sediment is control sediment that has been spiked with a specific amount of a reference toxicant to achieve a specific homogenous concentration in the sediment.

Spiked sediment is control, reference, or other clean sediment to which a test substance (such as a chemical, a mixture of chemicals, drilling mud, contaminated dredge spoil, or sludge) has been added and then mixed thoroughly through the sediment.

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

Substance is a particular kind of material having more or less uniform properties.

Test sediment is a field-collected sample of solid-phase sediment that is taken from a site thought to be contaminated with one or more chemicals, and is intended for use in the 10-day test with amphipods. In some instances, the term might also apply to any sediment sample (including control and reference sediment) used in the test.

Test water is the seawater placed over the layer of sediment in the test vessels. It is also the water used to manipulate the sediment, if necessary (e.g., for wet sieving), and the control/dilution water for seawater-only tests with reference toxicants.

Toxicity Terms

Acute means within a short period (seconds, minutes, hours, or a few days) in relation to the life span of the test organism.

Acute toxicity is a discernible adverse effect (lethal or sublethal) induced in the test organisms within a short period of exposure (in this instance, $\leq$10 days) to a test substance.

EC50 is the median effective concentration. That is, the concentration of a substance in the sediment (e.g., mg/kg or percent by weight) or water (e.g., mg/L) that is estimated to cause a discernible sublethal toxic effect to 50% of the test organisms. The EC50 and its 95% confidence limits are usually derived by statistical analysis of an observed sublethal response (e.g., emergence, or inhibition of reburial in control sediment) for several test concentrations, after a fixed period of exposure. The duration of exposure must be specified (e.g., 10-day EC50).

Endpoint is the variable(s) (i.e., time, reaction of the organisms, etc.) that indicate(s) the termination of a test, and the measurement(s) or derived value(s) that characterize the results of the test (e.g., EC50, LC50).

Flow-through describes tests in which solutions in test vessels are renewed continuously by the constant inflow of a fresh solution, or by a frequent intermittent inflow.

LC50 is the median lethal concentration, i.e., the concentration of a substance in the sediment (e.g., mg/kg) or water (e.g., mg/L) that is estimated to be lethal to 50% of the test organisms. The LC50 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., 10-day LC50).

Lethal means causing death by direct action. Death of amphipods is defined as the cessation of all visible signs of movement or other activity (e.g., absence of a pleopod twitch).

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

Sublethal means detrimental to the amphipod, but below the level that directly causes death within the test period.

Sublethal concentration is a concentration of test substance that does not cause death under the defined test conditions.

Sublethal effect is an adverse effect on an organism, below the level that directly causes death within the test period.

Toxicity is the inherent potential or capacity of a substance to cause adverse effects on living organisms.

Toxicity test is a determination of the effect of a substance on a group of selected organisms of a single species (e.g., *Rhepoxynius abronius*) 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 a specific test substance (e.g., a sample of sediment).

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Introduction

1.1 Background

Aquatic toxicity tests are used within Canada and elsewhere to measure, predict, and control the discharge of substances that might be harmful to indigenous aquatic life, and to determine and monitor their toxic effects in the receiving environment (water and sediment). Because no single test method or test organism can be expected to satisfy a comprehensive approach to environmental conservation and protection, the Inter-Governmental Aquatic Toxicity Group (Appendix A) proposed a set of aquatic toxicity tests that would be broadly acceptable, and would measure different toxic effects using different test substances. Samples of chemical, effluent, receiving water, or sediment and organisms representing different trophic levels and taxonomic groups are used in these tests (Sergy, 1987). A 10-day test for measuring the acute lethality of sediment, using the marine infaunal amphipod *Rhepoxynius abronius* (Swartz *et al*., 1985a), was one of several "core" aquatic toxicity tests that was selected in 1987 to be standardized sufficiently to help meet Environment Canada's testing requirements. Recently, Environment Canada's regional laboratories (Appendix B) have examined the sensitivity and performance of 10-day sediment assays using *R. abronius* and other species of marine or estuarine infaunal amphipods common to Canadian coastal waters.

Universal procedures for conducting acute tests for sediment toxicity, using one or more species of sediment-burrowing amphipods found within Canadian coastal (Atlantic, Pacific, or Arctic) waters, are described in this report. Also presented are specific sets of test conditions and procedures, required or recommended when using the test for evaluating different types of substances. Figure 1 gives a general picture of the universal procedures covered in this report, as well as the procedures specific to testing samples of field-collected sediment or similar solid substance, chemicals, or chemical-sediment mixtures.

The biological test method presented in this report is based largely on the 10-day *R. abronius* test for marine sediment toxicity developed by the United States Environmental Protection Agency (USEPA), (Swartz *et al*., 1985a), and the ensuing documents prepared by the American Society for Testing and Materials (ASTM, 1991a, b) and USEPA (1990; 1994a). It has been developed after a review of specific procedural variations indicated in existing U.S. and Canadian "methodology" documents for 10-day amphipod assays (see Appendix C), and a review of subject-related reports and publications available to the authors (McLeay and Sprague, 1991). The October 1998 amendments herein are included in keeping with procedural improvements since 1992 and to ensure compatibility with Environment Canada's related Reference Method for measuring sediment toxicity using marine or estuarine amphipods (EC, 1998a).

The biological endpoint for the test is percent survival at Day 10. Additional (sublethal) endpoints that measure the percentage of surviving amphipods that emerge from the

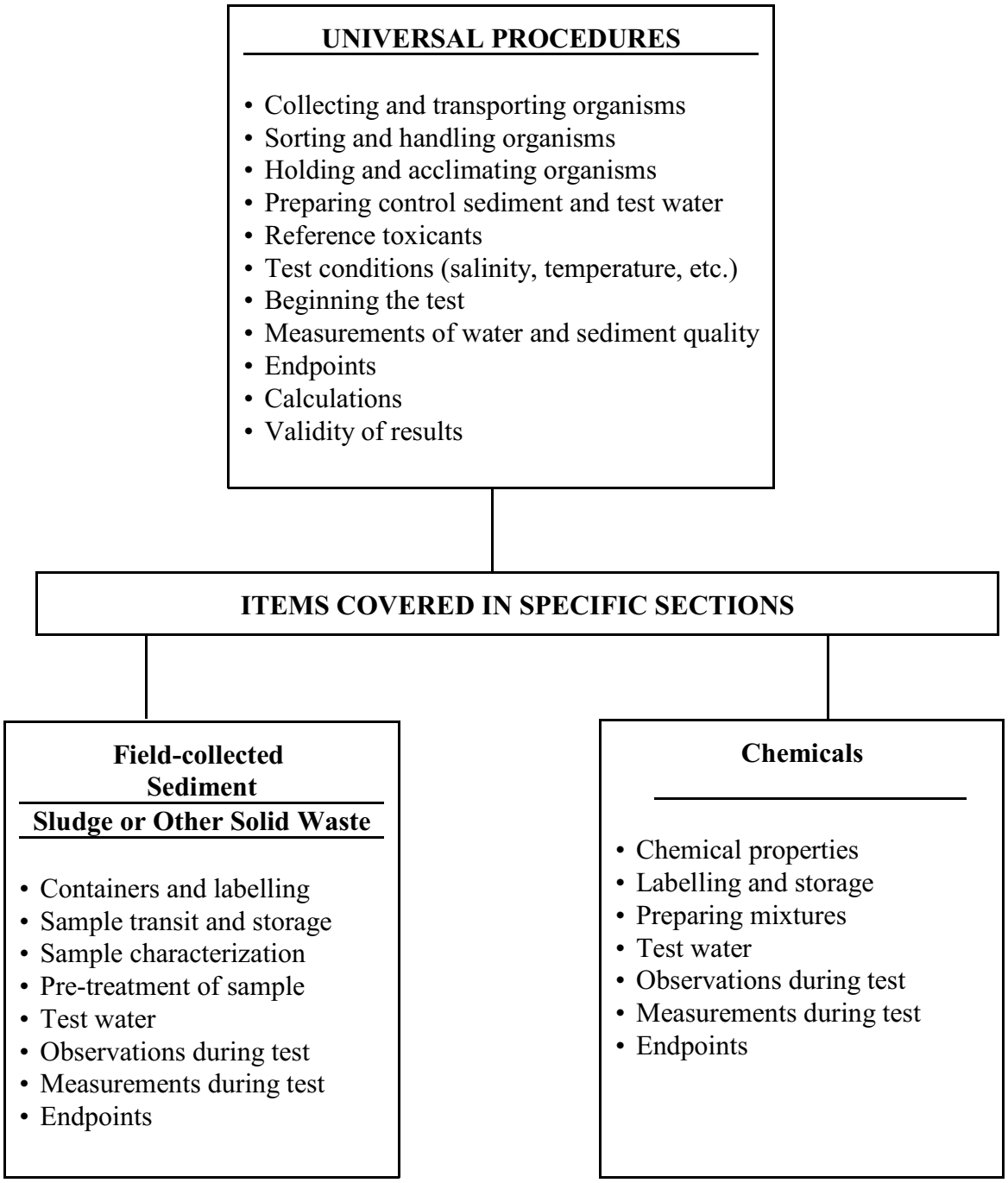

Figure 1 Diagram of Approach Taken in Delineating Test Conditions and Procedures Appropriate for Various Types of Materials

sediment at Day 10 (i.e., avoidance response), and/or the ability of the amphipods to rebury when transferred to control sediment, can also be determined using this test. The test is intended to evaluate the toxicity of samples of marine or estuarine sediment, of chemical, industrial or municipal sludge, or of similar solid wastes being considered for discharge to the marine or estuarine environment. Other (related) tests and test organisms (e.g., ASTM, 1991c), which use freshwater sediments or substances, are appropriate to evaluate the toxicity of freshwater sediment to indigenous aquatic life.

When these procedures were formulated, an attempt was made to balance scientific, practical, and cost considerations, and to ensure that the results would be accurate and precise enough for the majority of situations in which they would be applied. The authors assume that the user has a certain degree of familiarity with aquatic toxicity tests. Explicit instructions that might be required in a regulatory protocol are not provided, although this report is intended as a guidance document useful for that and other applications.

1.2 Historical Use of Test

Sediment assays with appropriate test organisms, including amphipods, are becoming widely recognized and accepted as effective tools to determine the biological significance of the contamination found in coastal sediments. Amphipods are an abundant component of benthic communities in estuarine and marine environments, and are a primary source of food for certain species of whales and for many species of birds, fish, and larger invertebrates. Although some species of amphipods are predators of smaller benthic invertebrates, many burrowing or tube-dwelling amphipods ingest sediment particles and are exposed directly to sediment-associated contaminants. According to ASTM (1991a): "The ecological importance of amphipods, their wide geographical distribution, ease of handling in the laboratory, and their sensitivity to contaminated sediments make them an appropriate sediment toxicity test organism."

In 1985, USEPA scientists reported a standardized 10-day test for sediment toxicity using the marine infaunal amphipod, *Rhepoxynius abronius* (Swartz *et al*., 1985a). This test, with *R. abronius* or other species of marine or estuarine amphipods, has been used extensively by U.S. researchers and regulatory authorities for evaluating the spatial and temporal distribution of contaminants in marine sediments (Ott *et al*., 1982; Swartz *et al*., 1982, 1985b, 1986; Long, 1983; Chapman and Becker, 1986; Kemp *et al.*, 1986). Additional applications include its use as part of permitting programs to determine the acceptability of dredged substances and other solid wastes for marine disposal (Swartz *et al*., 1984; Chapman, 1986; Scott *et al.*, 1990a); and its ability to measure the toxicity of specific chemicals when mixed with and absorbed to marine sediments (Swartz *et al*., 1985c; Kemp and Swartz, 1988; Plesha *et al.*, 1988; Swartz *et al*.,1988; Ditsworth *et al*., 1990; Swartz *et al*., 1990; Word and Ward, 1991).

The 10-day test for marine sediment toxicity using *R. abronius* is presently the most well-defined of all sediment toxicity tests. An interlaboratory comparison of the test using five laboratories and seven marine sediments found acceptable agreement in the derived toxicity data (Mearns *et al*., 1986). Comparative evaluations of the sensitivity of this test, relative to sediment elutriate or solid-phase assays with other species of marine invertebrates [e.g., tests for

fertilization success and development of sea urchins; developmental assays with blue mussels; bacterial luminescence assays (Microtox™); and tests with oyster larvae or polychaetes] indicate that the 10-day test with *R. abronius* is among the most sensitive (Williams *et al.,* 1986; Long and Buchman, 1989; Pastorok and Becker, 1989; Long *et al*., 1990; Tay *et al*., 1991; Chapman *et al*., 1991).

If used in conjunction with chemical measurements of sediment samples and benthic marine surveys for *in-situ* biological effects, the 10-day amphipod test can provide a spatial or temporal determination of contaminant-induced degradation of the marine environment (Long and Chapman, 1985; Chapman *et al*., 1986, 1987, 1991; Cross *et al*., 1991; Dexter, 1991). A number of studies have shown that laboratory results with *R. abronius* and field-collected sediments are positively correlated with field observations of amphipod density and species richness (Swartz *et al*., 1982, 1985b; Swartz, 1987). More extensive field-validation studies have generally found significant decreases in the richness and abundance of macrobenthic communities at stations where sediment was acutely toxic to *R. abronius* or other species of infaunal amphipods and contained high concentrations of chemical contaminants (Swartz *et al*., 1985b, 1986; Chapman, 1986; Swartz, 1987; Becker *et al*., 1990); although exceptions have been reported (Scott, 1991; Shimek *et al*., 1991).

The methodology of Swartz *et al.* 1985a), with minor modifications, has been adopted as a standard test for evaluating the toxicity of marine or estuarine sediment by the American Society for Testing and Materials (ASTM, 1991a) and by scientests and regulators at the USEPA (DeWitt *et al.,* 1990a; Scott *et al.,* 1990a; USEPA, 1990). Within Canada, the test has been used for several years by certain researchers (e.g., Chapman *et al.,* 1982; Chapman and Fink, 1983) and, more recently, has been studied by investigators at Environment Canada's regional laboratories[1] (McLeay *et al.*, 1989, 1991; Nicol and Doe, 1990; Paine and McPherson, 1991a, b; Tay *et al.*, 1991). Canadian researchers familiar with the test have recommended its routine use for measuring and evaluating the toxicity of marine sediments, or solid wastes proposed for marine discharge (Konasewich *et al.*, 1986; Chapman, 1988; Cross *et al.*, 1991).

1.3 Species of Amphipods Studied and Recommended

The laboratory performance and sensitivity of several species of marine or estuarine sediment-burrowing amphipods besides *R. abronius* have been studied using the 10-day test method standardized by Swartz *et al*. (1985a). Scientists in the U.S., including those associated with the USEPA Environmental Research Laboratory at Narragansett, RI, are now using the marine phoxocephalid, *Rhepoxynius hudson*[2] (Scott *et al*., 1990a, b) as well as the euryhaline, tube-dwelling amphipod *Ampelisca abdita*[3] (Breteler *et al*., 1989; Long and Buchman, 1989; Long *et al*., 1990; Scott *et al*., 1990a, b) for sediment toxicity tests. The tube-dwelling corophioidean amphipod,

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[1] Addresses for these laboratories are provided in Appendix B.

[2] Found in coastal waters from southern Maine to North Carolina (Bousfield, 1973).

[3] Found on the east coast from Maine to south-central Florida and the eastern Gulf of Mexico (Mills, 1964; Bousfield, 1973), and, on the west coast, in San Francisco Bay (Long *et al*., 1990).

Grandidierella japonica[4], has been used recently in the southwestern United States (California) for solid-phase sediment assays (Nipper *et al*., 1989; Carr and Chapman,1990). *Eohaustorius washingtonianus*[5], a marine, somewhat euryhaline, haustoriid amphipod species (Bosworth, 1976), has been used occsionally in seawater-only assays (Meador *et al*., 1991), in sediment assays (Ott *et al.,* 1982), and in recent tests by Canadian researchers (Paine and McPherson, 1991a, b) A related euryhaline amphipod, *Eohaustorius estuarius*[6], has been studied more extensively in 10-day sediment assays (DeWitt *et al*., 1989; Pastorok and Becker, 1989), and has been included in recent multiple-species tests by Canadian scientists (Paine and McPherson, 1991a,b). Canadian investigators have also appraised the laboratory performance and sensitivity of the west-coast phoxocephalid amphipod, *Foxiphalus xiximeus*[7] (Paine and McPherson, 1991a,b) and the following east-coast species: *Corophium volutator*[8] (Nicol and Doe, 1990; Mcleay *et al*; 1991; Tay *et al*., 1991; Paine and McPherson, 1991a, b); *Amphiporeia virginiana*[9] (Doe and Wade, 1991; Paine and McPherson, 1991a, b); and *Leptocheirus pinguis*[10] (Paine and McPherson, 1991b).

The American Society for Testing and Materials has recommended the following species of marine or estuarine amphipods for use with 10-day sediment assays: *Ampelisca abdita, Eohaustorius estuarius, Grandidierella japonica,* and *Rhepoxynius abronius* (ASTM, 1991a). Region 10 of the USEPA (Seattle, WA) requires that *R. abronius* or *E. estuarius* be used for 10-day amphipod assays with Puget Sound sediments (USEPA, 1990).

A number of marine or estuarine species of sediment-burrowing amphipods, common to Canadian coastal waters, are recommended in this report as suitable for use in static, 10-day assays with samples of field-collected sediment, chemically spiked sediment, sludge, or other solid wastes. The recommended species (*A. virginiana, C. volutator, E. estuarius, E. washingtonianus, F. xixmeus, L. pinguis*, and *R. abronius*) have been chosen because of their distribution in Canadian coastal (Atlantic, Pacific, Arctic) waters, species-dependent range of salinity tolerance and applicability to estuarine and/or marine locales, known collection sites and ease of collection and handling, seasonal availability, adaptability to laboratory conditions, high survival under control conditions, sensitivity to

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[4] Found in California from Long Beach to San Francisco Bay (ASTM, 1991a).

[5] Found on the west coast of North America from Oregon to SE Alaska (Bousfield, 1990a).

[6] Found in western North America from central California to the Queen Charlotte Islands, B.C. (ASTM, 1991a; Bousfield, 1990a).

[7] Found in western North America from southern California to southeastern Alaska and the Aleutian Islands (Bousfield, 1990a).

[8] Estuarine, tube-dwelling corophiid species found along the North Atlantic coast of Europe and, in North America, only in the Bay of Fundy, south to Yarmouth, N.S. and Casco Bay, Maine (Bousfield, 1973).

[9] Marine pontoporeiid amphipod, found from eastern Nova Scotia to North Carolina (Bousfield, 1973).

[10] Tube-dwelling, corophioidean, amphipod, found in estuaries on the east coast of North America from southern Labrador to Virginia (Bousfield, 1973).

contaminated sediments, low sensitivity to natural sediment variables, and current use by North American and other investigators.

Gammaridean amphipods (i.e., members of the Suborder Gammaridea) comprise nearly 85% of the known species of marine or estuarine amphipods, including all of those recommended for use in this test (see Section 2.1). The basic body parts of gammaridean amphipods are illustrated in Appendix D. Known information regarding the geographical distribution, habitat, life cycle, anatomy, appearance, behaviour, tolerance to natural environmental variables, and tolerance to contaminants, is summarized for each species in Appendices E to K. As research progresses, this list of suitable test organisms might be modified.

Test Organisms and Acclimation

2.1 Species

The following species of marine or estuarine infaunal amphipods are recommended for use in this test:

Pacific/Arctic

Eohaustorius estuarius
Eohaustorius washingtonianus
Foxiphalus xiximeus
Rhepoxynius abronius

Atlantic/Arctic

Amphiporeia virginiana
Corophium volutator
Leptocheirus pinguis

Selection of one or more of these seven species for use in particular study must take into consideration the known or anticipated physicochemical characteristics of the test material (e.g., sediment grain size, porewater salinty, and porewater ammonia concentration) together with the known tolerance limits of the candidate species to these characteristics. In particular, the species selected should be tolerant of the grain size and porewater salinity of the test material, and the investigator(s) should ensure that this is the case for meaningful test results.

Appendices E to K provide useful information on the known tolerance limits of each species to "non-contaminant" variables for test materials including salinity and grain size. Appendices D to G in EC (1998a) should be consulted for more recent information specific to *R. abronius*, *E. washingtonianus, E. estuarius*, and *A. virginiana*.

The investigator should be aware that *F. xiximeus* and *L. pinguis* have only received limited testing using this assay, and that their tolerance limits to non-contaminant variables including salinity and grain size are poorly understood. Both *A. virginiana* and *E. washingtonianus* have proven very sensitive to temperature shock and/or handling (Doe and Wade, 1991; Paine and McPherson, 1991a,b); thus extra care and attention should be taken during the collection, transport, and acclimation of these two species. The species of test organisms should be confirmed by a qualified taxonomist familiar with identifying marine or estuarine amphipods.

2.2 Life Stage and Size

Large immature or young mature amphipods should normally be used for the test. All individuals from a collection site should be as uniform as possible in age and size. Size ranges for each species appropriate for the test are identified in Appendices E to K. Mature females, evidently bearing embryos, should not be used; neither should particularly large individuals (i.e., greater than the maximum size recommended in Appendices E to K) because they might be senescent.

2.3 Source

All amphipods used in a test must be derived from the same population and source. Test organisms are usually those recently obtained

from a wild population in a clean area, although it might prove possible to culture certain species or hold them for a prolonged period in the laboratory. The former source is recommended until it can be demonstrated that the sensitivity of cultured or laboratory-reared animals to reference toxicant(s) or contaminated sediment is not changed appreciably, because of suboptimal culturing procedures and/or conditions, from the sensitivity of recently collected specimens (Robinson *et al*., 1988; McLeay *et al*., 1989).

2.4 Collection, Handling, and Transport

Depending on species, season, and/or collection site, amphipods to be used in this test can be collected subtidally using a benthic grab (e.g., Smith-McIntyre or van Veen) or a small biological dredge, or intertidally using a shovel. If a dredge is used, a short haul (≤10 m) will minimize damage to the animals (Swartz *et al*., 1985a). Collect at least one-third more individuals than are required for the test. The collection site chosen should be one for which the presence of abundant organisms of the correct size and age has been demonstrated previously, and the species confirmed taxonomically (e.g., Bousfield, 1973; Barnard and Barnard, 1982).

The salinity and temperature of surface and bottom seawater at the collection site should be measured and recorded. Containers used to transport amphipods are usually those used to hold and acclimate the organisms at the laboratory. Suitable containers with sealable lids include plastic food containers or plastic pails. At the collection site, a minimum 2- to 4-cm (or thicker) layer of sieved (0.5- to 1.0-mm mesh screen)[11] sediment from the place where the animals are collected should be placed in the bottom of the container(s). Water from the collection site is then added to form a layer of ≥2 cm of overlying water. Amphipods sieved from other aliquots of the collection site sediment should then be transferred gently to the container(s). Numbers added to each container should be counted and recorded. The density of amphipods in each container should not exceed 1 amphipod/cm^2.

An additional portion of sediment from the amphipod-collection site should be collected, sieved (0.5- to 1.0-mm screen), and delivered to the laboratory for use as control sediment in the sediment toxicity test, and for physicochemical analyses (Section 3.4).

Depending on transport conditions and time, it might be necessary during transit to chill the contents of the transport container(s) (ice pack), maintain a cool temperature (cooler), and/or aerate the overlying seawater. All apparatus and containers used for collecting, sieving, and transporting the organisms and sediment must be clean and made of nontoxic materials (see Subsection 2.5.2). These must be used only for handling and transporting live animals and control sediment. The containers and other collection apparatus should be cleaned and rinsed with distilled water, deionized water, dechlorinated laboratory water, reconstituted seawater, or natural seawater from the collection site or an uncontaminated source.

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[11] Sieve size should be slightly smaller than the minimum size of amphipods to be used in the test (see Appendices E to K for acceptable size ranges for each species).

Handling of amphipods in the field and the laboratory should be minimized. Organisms should be handled gently by the slow agitation of a sieve immersed in seawater, or by using a wide-bore pipette[12]. Sieved organisms should be submersed in seawater at all times. Handling should be done carefully and quickly to minimize stress to the animals. Amphipods that are dropped, injured, or contact dry surfaces must be discarded.

2.5 Holding and Acclimation

Table 1 provides a summary checklist of recommended conditions and procedures for holding and acclimating amphipods in the laboratory.

2.5.1 Sorting and Holding Organisms

Sieving and sorting of amphipods in the laboratory, upon their receipt or at any time preceding the day that the test is started, is not recommended since this procedure can unduly stress the animals. Upon receipt of field-collected animals at the laboratory, the quality (i.e., temperature, salinity, dissolved oxygen, and pH) of the overlying water in one or more of the containers holding field-collected animals and sediment from the collection site should be determined and recorded. Any dead organisms observed on the surface of the sediment should be counted and removed, together with any debris evident.

To minimize disturbance, it is recommended that amphipods be held and acclimated in the container(s) used to collect and transport them. Alternatively, the sediment (and burrowed amphipods therein) within the collection container(s) could be gently removed without sieving, and transferred to a larger holding/acclimation chamber. This latter procedure is useful if it is considered necessary to reduce crowding of animals (i.e., to assure a density of ≤ 1 amphipod/cm^2; Section 2.3) and increase surface area.

During the holding and acclimation period, amphipods should be held unfed in a minimum 2- to 4-cm (or deeper) layer of sieved sediment from the collection site. Water overlying this sediment should be at least 2 cm deep. If the duration of the holding/acclimation period exceeds two days, the overlying water should be replaced continuously or periodically (i.e., daily or every second day) with air-saturated, fresh seawater adjusted to the required temperature and salinity.

2.5.2 Facilities

Amphipods should be held and acclimated to test conditions in a laboratory facility. The air supply should be free of detectable odours and dust. Ideally, this facility should be isolated from the test facility to reduce the possibility of the amphipods being exposed to volatiles released from contaminated sediments.

Holding containers should be placed in one of the following: a tank or trough with flowing seawater; a large aquarium (e.g., 60 to 100 L) containing reconstituted seawater or natural, clean seawater held under static conditions; a smaller aquarium (e.g., 20 to 40 L) containing seawater held under semi-static conditions (e.g., with daily renewal of 50% of the seawater); or a separate room with the appropriate temperature and lighting conditions. The seawater in which holding

[12] A disposable glass pipette with the delivery end cut off and fire-polished to provide an opening of 5 mm is suitable for transfers, unless amphipods are too large.

Table 1 Checklist of Recommended Conditions and Procedures for Holding and Acclimating Amphipods

Source of amphipods	–	collected subtidally or intertidally from clean sediment
Life stage	–	juveniles or young adults
Holding amphipods	–	no sieving or sorting of amphipods until the day that the test is started; normally hold in transport container(s); density $\leq$1amphipod/cm^2, hold in sieved (0.5 to 1.0 mm) collection-site sediment with $\geq$2 cm overlying water
Holding sediment	–	collection-site (control) sediment, 2 to 4 cm in depth; sieved (0.5 to 1.0 mm) in the field
Holding seawater	–	reconstituted or clean natural seawater
Acclimation conditions	–	salinity of seawater same as that for overlying seawater in test; temperature normally 15 ± 2 °C; dissolved oxygen 90 to 100% of air saturation. Temperature and salinity measured daily during initial period of adjustment ($\leq$3 °C/day, $\leq$5 g/kg · d); thereafter, measure and record temperature, salinity, pH, and dissolved oxygen at the beginning and end of the remaining period of acclimation, as a minimum
Lighting	–	constant, overhead illumination (fluorescent or equivalent broad spectrum); 500 to 1000 lux adjacent to surface of overlying water in holding/acclimation containers
Feeding	–	none
Duration of acclimation	–	2 to 10 days, once temperature and salinity adjusted to that of the test water
Selection of test organisms	–	remove and discard inactive amphipods that have emerged from sediment or do not bury at any time during the holding/acclimation period; on the day of the test, select amphipods that are active and apparently healthy, and which have an appearance and behaviour typical of that species (see Appendices E to K); discard any animals that appear or behave atypically

containers are submersed should be aerated (see Subsection 2.5.7).

All containers and accessories that might contact the organisms, control sediment, and seawater during sorting, handling, holding, and acclimation must be clean, rinsed, and made of nontoxic materials (e.g., glass, stainless steel, Nalgene™, nylon, porcelain, polyethylene, polypropylene, fibreglass). Materials such as copper, zinc, brass, galvanized metal, lead, and natural rubber must not come in contact with this apparatus and equipment, or with samples of control, reference or test sediment, seawater, or test vessels.

2.5.3 Lighting

The facility in which amphipods are held and acclimated should be brightly lit and provide light intensity adjacent to the surface of the overlying water of 500 to 1000 lux. Fluorescent (or equivalent broad-spectrum) lighting should be used; lighting must be constant and continuous throughout the holding/acclimation period.

2.5.4 Water

Depending on the nature and intent of the test (see Sections 5 and 6), amphipods may be held and acclimated using either an uncontaminated supply of natural seawater, or reconstituted seawater.

Reconstituted seawater should be prepared by adding hypersaline brine (HSB) to a suitable fresh water, in quantities sufficient to provide the desired salinity. The use of HSB derived from an uncontaminated source of natural seawater is recommended (EC, 1997). Hypersaline brine may also be prepared using commercially available dry ocean salts (e.g., Instant Ocean™) or reagent-grade salts (i.e., "modified GP2"; see Table 2 in USEPA, 1994b or USEPA, 1995). However, any HSB which is prepared using commercial sea salt or reagent-grade salts must be filtered ($\leq$1 µm), aerated overnight, and then capped and stored in the dark at 4 ± 2 °C for at least one week before use (EC, 1997). Reconstituted seawater should be filtered ($\leq$5 µm) shortly before use to remove suspended particles, and should be used within 24 h of filtration (USEPA, 1994a).

Laboratory personnel must be able to demonstrate that they can meet the species-specific criterion for a valid test (see Section 4.2) using control sediment and aged artificial seawater as the overlying (test) water, before artificial seawater is used to prepare HSB or test water. If ocean salts are used to prepare HSB, the suitability and consistency of these salts should also be verified by testing, since some investigators feel that specific batches of sea salt can produce unwanted toxic effects or sequester test substances.

Hypersaline brine may be prepared by concentrating seawater (natural or, less desirably, artificial) by freezing or evaporation. Once prepared, its salinity should be 90 ± 1 g/kg (EC, 1997). If prepared by freezing at -10 to -40 °C for $\geq$6 h and collect the HSB under the ice when it reaches a salinity of 90 ± 1 g/kg. If prepared by evaporation, heat the seawater in a non-corrosive, non-toxic container at $\leq$40 °C while aerating it, until the desired salinity (i.e., 90 ± 1 g/kg) is achieved (USEPA, 1994b; USEPA, 1995; EC, 1997). HSB may be added to natural seawater, fresh water, distilled water, or deionized water, to increase the salinity to the level desired for testing. Guidance in Environment Canada (1997) should be followed when preparing, aging, and storing HSB.

Sources of water used for preparing reconstituted seawater may be deionized

water or distilled water, or an uncontaminated supply of dechlorinated municipal drinking water, natural surface water, or groundwater. If municipal or natural freshwater sources are used, this water should also be monitored and assessed for water quality. Analyses of such variables as total residual chlorine, pH conductivity, suspended solids, dissolved oxygen, total dissolved gases, temperature, ammonia nitrogen, nitrite, pesticides, and metals are recommended.

Dechlorinated municipal water is a less desirable source from which to prepare reconstituted seawater because the dechlorination is often incomplete and chlorine is highly toxic to aquatic organisms. If a municipal drinking water source is used to prepare reconstituted seawater, effective dechlorination must be assured. The use of activated carbon (bone charcoal) filters and subsequent ultraviolet radiation (Armstrong and Scott, 1974) is suitable for this purpose. Aging the water in an aerated holding tank might be of further benefit. Thiosulphate or other chemicals should not be used for dechlorination, unless applied in trace amounts for "polishing" carbon-filtered water or in response to spikes of chlorine in municipal waters. The use of thiosulphate or other chelating agents for dechlorinating water is inadvisable as these chemicals might sequester toxic substances.

Depending on source, seawater should be used for holding/acclimating amphipods (and as overlying seawater in the test) should be filtered ($\leq$5 µm) shortly before use to remove suspended particles and organisms. Water that might be contaminated with pathogens should be treated shortly before use by further filtration ($\leq$0.45 µm) and/or ultraviolet sterilization (ASTM, 1991a). If stored, natural seawater should be held at the test temperature (normally 15 ± 2 °C) or cooler, and used within a few days. Reconstituted water should be filtered and/or sterilized and used within 24 h of preparation.

2.5.5 Salinity

The salinity of the seawater used for acclimating amphipods should be the same as the salinity of the overlying seawater used in the sediment toxicity test. Upon arrival in the laboratory, amphipods should be adjusted from the ambient salinity to this salinity, by changing the salinity in the holding container at a rate no greater than 5 g/(kg · d). Thereafter, amphipods should be acclimated to the salinity of the test water for a minimum of 2 days before testing. The choice of the appropriate salinity for the test water depends on a number of considerations, including the salinity of the pore water of the test sediments, the range of salinity tolerance for the test species (see Appendices E to K), and the test objectives. The salinity of the pore water of each test sediment should be known before the sediment toxicity test is initiated, and should be within the tolerance limits of the test organisms.

2.5.6 Temperature

When the amphipods are being acclimated to test conditions, the temperature of the sediment and overlying water/seawater within the holding/acclimation container should not be changed at a rate greater than 3 °C per day. Once the acclimation/test temperature (normally 15 ± 2 °C, except for *A. virginiana* (see Section 4.2) is achieved, amphipods must be acclimated to the test temperature for a minimum of 2 days before the test is started. An incubator, temperature-regulated room, or a water bath with temperature control may be used to regulate temperature within this range.

2.5.7 Dissolved Oxygen

The dissolved oxygen content of the seawater overlying the holding sediment should be 90 to 100% of the air-saturation value. Gentle aeration of the seawater, using filtered, oil-free compressed air, should be provided to maintain this level of dissolved oxygen.

2.5.8 Acclimation and Feeding

Field-collected amphipods must be acclimated to test conditions for a minimum of 2 days and a maximum of 10 days before testing. Temperature and salinity should be monitored at least daily during the initial period when the amphipods are being adjusted to the conditions of the test water. Thereafter, temperature, salinity, pH, and dissolved oxygen content of the seawater in the holding/acclimation containers should be measured and recorded at least at the start and end of the remaining acclimation period, and preferably daily. Amphipods are not to be fed during their period of acclimation to test conditions, nor during the test.

2.5.9 Selection of Test Organisms

Amphipods in the holding/acclimation containers should be checked daily. Individuals that remain emerged from the sediment, or those that emerge from the sediment and appear dead or inactive when gently prodded, should be discarded.

On the day of the test, amphipods should be sieved from the sediment within the holding/acclimation containers (see Section 4.1). Only those animals that are of a suitable size/range, active and apparently healthy, and which have an appearance and behaviour typical of that species (see Sections 2.2 and Appendices E to K) are to be used in a sediment toxicity test or a reference toxicity test.

Test System

3.1 Facilities

The test may be performed in a water bath, environmental chamber, or equivalent facility with temperature control. This facility should be well ventilated, and isolated from physical disturbances that might affect the test organisms. It is desirable to isolate the test facility from the acclimation area. Dust and fumes within the test facility should be minimized. Construction materials should be nontoxic (see Subsection 2.5.2).

3.2 Lighting

All test vessels should receive full-spectrum (e.g., fluorescent or equivalent broad-spectrum) illumination from directly overhead, at an intensity sufficient to provide 500 to 1000 lux adjacent to the surface of overlying water in test chambers. Illumination should be as uniform as possible for all test chambers; and must be continuous throughout the test period (Swartz *et al*., 1985a).

3.3 Test Vessels

One-litre glass containers (beakers or wide-mouthed jars) with internal diameter of approximately 10 cm are to be used as test vessels. Before each use, all test vessels and other glassware should be washed with laboratory detergent, followed by three distilled water rinses, a rinse in 10% nitric acid, at least two rinses in distilled water, and at least two rinses with test water (ASTM, 1991a; Paine and McPherson, 1991a). Each vessel should have a cover (e.g., a watchglass or a plastic lid) to reduce the possibility of contamination of the contents and to minimize evaporation.

3.4 Control Sediment

The portion of control sediment obtained from the amphipod-collection site for the toxicity test, and for particle size and chemical analyses, should be placed in a sealed container of nontoxic material, and stored in darkness at 4 ± 2 °C until required. This sediment should be sieved through an 0.5-mm screen to remove small amphipods and other organisms. A portion of the test water, previously adjusted to the test temperature (normally 15 $\pm$ 2 °C) and the required salinity (frequently 28 ± 2 g/kg) and aerated to ensure a dissolved oxygen value 90 to 100% saturation (Section 3.5), should be used for this sieving. Water overlying the sieved control sediment should be allowed settle for at least 4 h (preferably overnight) to recover sediment fines (i.e., silt/clay fraction, <0.063 mm) before it is decanted and discarded. If control sediment is to be used at the completion of the test for determining the ability of surviving amphipods to rebury, the required portion should be resealed and refrigerated.

A subsample of the control sediment must be analyzed for at least the following: for whole sediment—percent very coarse-grained sediment (i.e., particles >1.0 mm), percent sand (>0.063 to 2.0 mm), percent silt (>0.004 to 0.063 mm), percent clay (<0.004 mm), percent water content, and total organic carbon content; for pore water—salinity, pH, and ammonia (total and un-ionized). Other analyses could include: for whole

sediment—total inorganic carbon, cation exchange capacity, acid volatile sulphides, biochemical oxygen demand, chemical oxygen demand, metals, synthetic organic compounds, oil and grease, and petroleum hydrocarbons; for pore water—metals, synthetic organic compounds, and hydrogen sulphide (ASTM, 1991a, b; Burton, 1991; Chapman, 1991; EC, 1994; USEPA, 1994a). Recommended procedures for collecting pore water are described in Environment Canada (1994) and should be followed here.

3.5 Test and Control/Dilution Water

Depending on the test design and intent (see Sections 5 and 6), test water (i.e., water overlying sediment in the test) and control/dilution water (i.e., water used to prepare dilutions of test chemicals and as control water in seawater-only exposures with reference toxicants) may be reconstituted seawater (Subsection 2.5.4) or an uncontaminated supply of natural seawater.

Natural or reconstituted seawater can be adjusted to the required salinity (see Subsection 2.5.5 and Sections 5 and 6) by the addition of hypersaline brine (if too brackish), or distilled or deionized water (if too saline). Guidance regarding its preparation is provided in Subsection 2.5.4.

Test and control/dilution water should be adjusted to the test temperature (normally 15 ± 2 °C) before use. The dissolved oxygen content of the water should be 90 to 100% of the air-saturation value for the test temperature. As necessary, the required volume of water should be aerated vigorously (oil-free compressed air passed through air stones) immediately before use, and its dissolved oxygen content checked to confirm that 90 to 100% saturation has been acheived.

Universal Test Procedures

Procedures described in this section apply to all the tests of particular samples of sediment, solid waste, or chemical described in Sections 5 and 6. All aspects of the test system described in Section 3 must be incorporated into these universal test procedures. The summary checklist of recommended conditions and procedures in Table 2 describes both universal procedures and procedures for testing specific types of test substances.

4.1 Beginning the Test

Each test vessel placed within the test facility must be clearly coded or labelled to identify the test substance/concentration, date, and time the test was started. The vessels should be positioned for easy observation of amphipods. Each set of replicate treatments should be positioned randomly within the test facility.

On the day preceding the test, each sample of test sediment or similar solid material (including control and reference sediments) should be mixed thoroughly[13] to provide a homogeneous mixture consistent in colour, texture, and water content (see Sections 5.3 and 6.2). Immediately following mixing, a 175-mL aliquot should be added to each replicate test vessel[14]. A minimum of five laboratory replicates per treatment must be established[15] (see Sections 5.1 and 6.2).

[13] Any liquid that has separated from the sample during transport and storage must be remixed within the sample.

[14] This is sufficient to provide a layer on the vessel bottom with a uniform depth of approximately 2 cm.

[15] For sediment toxicity tests using samples of field-collected sediment (Section 5), the number of stations to be sampled at a study site and the number of replicate samples per station will be specific to each study. This will involve, in most cases, a compromise between logistical and practical constraints (e.g,, time and cost) and statistical considerations. Environment Canada (1994) should be consulted for guidance with respect to the sampling design, including the recommended minimum number of field replicates.

For sediment toxicity tests using multiple concentrations of chemicals spiked in sediment (Section 6), a minimum of five replicate test chambers is normally required for each concentration (treatment) including each control treatment (i.e., a negative control using ≥5 aliquots of control sediment; plus in some instances ≥5 aliquots of a solvent control). For non-regulatory tests, the number of replicates per treatment may be reduced (see section 6.2). For a reference toxicity test (Section 4.5), the number of replicates per treatment (concentration) may be reduced, or replication eliminated altogether.

Increasing the number of replicates per treatment will increase the power to detect a certain percent reduction in treatment response (e.g., ≥20% reduction in mean survival) relative to a reference or control treatment. It is always prudent to include as many replicates in the test design as is economically and logistically manageable. Advice and explanations related to the number of replicates per treatment and the related power of the test are found in Environment Canada (1994, 1998b) and USEPA (1994a).

In certain tests, one or more additional replicates per treatment might be set up for monitoring sediment chemistry (e.g., porewater salinity or hydrogen sulphide content). Toxicity data are not collected from these additional replicates due to the destructive nature of sediment sampling and analyses.

Table 2 Checklist of Recommended Test Conditions and Procedures

Test type	–	static, 10-day, duration[16]
Test vessel	–	1-L glass beaker or jar, internal diameter approximately 10 cm
Control sediment	–	clean sediment, usually from the site where test organisms were collected; sieved through 0.5-mm screen using test water; volume and depth in test vessels, 175 mL and ~2 cm
Test water	–	clean seawater, natural or reconstituted; salinity and temperature, same as overlying water during acclimation period; dissolved oxygen, 90 to 100% saturation; test water placed above a 175-mL layer of sediment in test vessel (to 750-mL mark) the day before the test is initiated and made up to 950 mL after amphipods are introduced
Aeration	–	aerate water in each test vessel overnight before start of test, and throughout test; aeration continuous and gentle (e.g., 2 to 3 bubbles/s)
Lighting	–	constant overhead illumination (fluorescent or equivalent broad spectrum); 500 to 1000 lux adjacent to surface of overlying water in test vessels
Amphipods	–	juveniles or young adults, 3 to 10 mm length (depending on species); normally 20 per test vessel
Number of replicates	–	recommend ≥5 field replicates, each a discrete (i.e., different) sample from the same location; must be ≥5 laboratory replicates for each field replicate; must be ≥5 replicates per concentration (treatment) if multi-concentration test with contaminant-spiked sediment performed for regulatory purposes
Feeding	–	none
Observation	–	daily or less frequently (e.g., Monday to Friday, each day), each test vessel, for air flow and amphipods floating at surface of test water or test solution; at termination of test for percent survival in each test vessel and, if sublethal endpoints included in test, numbers of survivors emerged and/or numbers of survivors not reburying in control sediment within 1 h
Measurements of overlying water	–	≥3 times/week (non-consecutive days), each treatment, for temperature and DO; start and end of test, each treatment, for salinity, pH, and ammonia
Endpoints	–	for each treatment, mean (± SD) percentage of amphipods that survived the 10-day exposure; optional endpoints based on percent emergence at ≤10 days and/or percent reburial success of survivors at test end

[16] Special situations (e.g., volatile or unstable test chemicals in seawater overlying sediment; or test sediment with high oxygen demand) might require the use of flow-through or static replacement test, or a modified test duration.

Reference toxicant test	–	either a 4-day static, seawater-only LC50 using cadmium; or a 10-day test with spiked control sediment using copper, cadmium, or fluoranthene
Test validity	–	mean 10-day survival in control sediment must be ≥85% for *E. washingtonianus;* ≥80% for *A. virginiana* and ≥90% for all other species

Field-collected Sediment or Similar Particulate Material

Transport and storage	–	if >7 °C, cool to 1 to 7 °C (ice or frozen gel packs); transport in dark at 1 to 7 °C (preferably 4 ± 2 °C); store in dark at 4 ± 2 °C; test should start within 2 weeks and must start within 6 weeks
Reference sediment	–	one or more samples required for tests with field-collected sediment; taken from sites presumed to be clean but in the general vicinity of those where test sediments are collected; ideally, particle size and organic content within the range of test sediments
Sample characterization	–	for whole sediment—at least percent very coarse-grained sediment (i.e., particles >1.0 mm), percent sand (>0.63 to 2.0 mm), percent silt (>0.004 to 0.063 mm), percent clay (<0.004 mm), percent water content, and total organic carbon; for pore water—at least salinity, pH, and ammonia (total and un-ionized)
Preparation of sample	–	sample normally homogenized and not wet-sieved; special circumstances might rule against mixing sample (e.g., concerns with altering anaerobic sediment) or might require wet-sieving using test water (e.g., substance considered for ocean dumping, or research investigation)
Test water	–	clean seawater, natural or reconstituted

Chemical

Characterization	–	information required concerning water solubility, vapour pressure, stability, biodegradability, and purity
Solvent	–	test water is the preferred solvent; if an organic solvent used, must include a solvent control
Preparation of mixtures	–	procedure depends on test design and test objectives; might include one or more chemical concentrations mixed in control or test sediment, or specific chemical concentrations added to the test water overlying control sediment; chemical-sediment mixtures may be prepared manually or by mechanical agitation as slurries or dry mixtures
Concentration	–	desirable to measure at beginning and end of exposure in high, medium, and low strengths
Test and dilution water	–	reconstituted seawater if a high degree of standardization is required; otherwise clean, natural seawater is acceptable

The sediment added to each vessel should be smoothed either by using a spatula or by tapping the vessel against the side of the hand. To minimize the disruption of sediment when test water is added, a disk cut from polyethylene, nylon, or Teflon™ sheeting (recommended thickness 4 to 6 mil) to fit the inside diameter of the test vessel, can be placed on the sediment surface (ASTM, 1991a). Test water (or, depending on the test, a test solution) is then added to the 750-mL mark on the side of the vessel. The disk should be removed[17], and rinsed with test water between replicates of a treatment. A separate disk should be used for each treatment.

The overlying seawater in each test vessel (including the controls) must be aerated overnight before the test amphipods are introduced, and throughout the duration of the test. Compressed air, previously filtered so as to be free of oil, should be bubbled through a glass or plastic pipette and attached plastic tubing (aquarium supply). The tip of the pipette should be suspended 2 to 4 cm above the surface of the sediment layer. Air flow to each test vessel must be gentle (e.g., 2 to 3 bubbles/s), and should not disturb the surface of the sediment. The rate of air flow should be adjusted as required to maintain a dissolved oxygen concentration in the overlying water of at least 90% saturation (USEPA, 1994a).

The toxicity test should be initiated the next day, by distributing 20 amphipods to each test vessel. Approximately one-third more amphipods than are required for the test should be sieved from the control sediment in the holding container(s), and transferred to a sorting tray. The additional animals allow the selection of healthy (active) individuals.

Active amphipods should be removed from the sediment in the holding container (Subsection 2.5.1) using an 0.5-mm or larger-sized sieve (depending on the size of amphipods to be used in test; see Appendices E to K). Individuals should be selected randomly using a transfer pipette or other suitable device, and be distributed sequentially among dishes containing $\leq$150 mL of test water (or, in certain instances, a specific test solution) until each dish contains twenty individuals. The number of amphipods in each dish should be verified by recounting (Swartz *et al*., 1985a; ASTM, 1991a).

Because replicate treatments are used, amphipods should be added at the same time to each set or block of test vessels representing each treatment, following a randomized block design. Amphipods should be added to the test vessels by gently pouring the water and amphipods from the sorting dish into the test vessel. Any amphipods remaining in the dish should be gently washed into the test vessel using test water. The water level in the test vessel should be brought up to the 950-mL mark (sediment plus liquid), the disk removed, the vessel covered, and the aeration continued.

The amphipods should be allowed 1 h to bury into the test substrate and control sediment. During this time, they should be observed carefully. Depending on species,[18] any

[17] A length of nylon monofilament line (or nontoxic equivalent) should be attached to the disk so that it can be removed after the overlying seawater is added.

[18] For tests using *Corophium volutator* or *Amphiporeia virginiana*, amphipods should only be replaced within the initial hour of the test if they are observed to be moribund or dead, or if their appearance or behaviour is atypical for the species.

amphipods that have not buried within 1 h should be replaced, unless they are observed to repeatedly burrow into the sediment and immediately emerge in an avoidance response to the test substrate. Amphipods displaying this avoidance behaviour during the initial hour of the test should not be replaced, i.e., they are to comprise the 20 test organisms in the vessel.

4.2 Test Conditions

- The test is to be static (no replacement of sediment or overlying solution during the test).
- Test duration is 10 days.
- The test must be conducted at a temperature of 15 ± 2 °C except for *A. virginiana*, which is tested at 10 ± 2 °C[19].
- Control and test sediments are to be a uniform, 175-mL layer, approximately 2 cm in thickness.
- Solutions overlying sediment must be aerated gently (e.g., 2 to 3 bubbles/s) and continuously, without disturbing sediments.
- Lighting is to be constant, overhead illumination (fluorescent or equivalent broad spectrum); 500 to 1000 lux adjacent to surface of overlying water in test vessels.
- No supplementary feeding is to be provided during the test.
- For a valid sediment toxicity test, mean 10-day survival in control sediment must be ≥85% for *E. washingtonianus*; ≥80% for *A. virginiana*; and ≥90% for all other species.

4.3 Test Observations and Measurements

During the 10-day test period, each test vessel must be checked regularly to confirm that the airflow to the overlying solution is uninterrupted and not excessive, and to note if amphipods are swimming in the overlying solution or floating at the water surface. Amphipods caught in the surface film should be gently pushed down into the water using a glass rod or pipette. Animals that appear to be dead should not be removed (Swartz *et al*., 1985a; Chapman and Becker, 1986).

The test is terminated after 10 days of exposure. For any test intended to determine if sediment exposure causes an emergence response (see Section 4.4), the number of surviving amphipods completely or partially out of the sediment (either on the sediment surface, swimming in the overlying water, or floating at the water surface) should be recorded for each test vessel prior to disturbance and sieving.

The contents of each test vessel must be sieved through a 1.0-mm (or smaller) screen to remove the test organisms and determine if they are dead or alive. Additional test water with a salinity and temperature within two units of that used in the test should be used for this sieving. Material retained on the screen should be washed into a sorting tray using clean test water, and the total number of live and dead amphipods recorded.

These species frequently do not rebury in control or other clean sediment for several hours after their disturbance and transfer.

[19] Studies at Environment Canada's Atlantic Regional laboratory have indicated that 10 ± 2° C is the preferred test temperature for this species (Doe and Wade, 1991).

Amphipods that are inactive but not obviously dead should be observed using a low-power dissecting miroscope or hand-held magnifying glass. Animals are considered to be dead if they fail to show any movement (such as a pleopod twitch) in response to gentle prodding. Animals that are missing are presumed to have died and are counted as dead organisms in the calculations (Section 4.4).

In some instances, it might be desirable to determine the effect of prior (10-day) exposure to test sediment on the ability of surviving amphipods to rebury in control sediment (Swartz *et al*., 1985a; USEPA, 1990; ASTM, 1991a)[20]. If this is the intent, amphipods surviving the test should be transferred to containers holding a 2-cm layer of control sediment (previously adjusted to the test temperature and sieved through an 0.5-mm screen using test water) and an overlying layer ($\geq$2 cm) of test water. The number of surviving amphipods that are unable to rebury in control sediment within 1 h is recorded for each test vessel.

Test measurements must be made in at least one test vessel representing each treatment. The temperature of the overlying water must be measured at the beginning of the test and thereafter at least three times per week (e.g., Mondays, Wednesdays, Fridays) on non-consecutive days until test completion. More frequent (i.e., daily) measurements of temperature are recommended. Additionally, it is recommended that the temperature of any water bath used, and/or of the air in a temperature-controlled room or chamber used for the test, be recorded continuously.

For at least one test vessel representing each treatment, the concentration of dissolved oxygen in the overlying water must be measured at the beginning of the test, and thereafter at least three times/week (e.g., Mondays, Wednesdays, Fridays) on non-consecutive days until test completion. More frequent (e.g., daily; USEPA, 1994a) measurements are recommended and should be performed for sediments having a high oxygen demand that depresses the dissolved oxygen of the overlying water below 90% saturation. A probe and calibrated dissolved oxygen (DO) meter is recommended for these measurements. The probe must be inspected carefully after each reading to ensure that organisms are not adhered to it, and must be rinsed in deionized or distilled water between samples to minimize cross-contamination. The position of the tip of the pipette in each test vessel and the rate of aeration should be checked frequently and routinely (e.g., daily) throughout the test.

If at any time during the test the air flow to one or more test chambers is observed to have stopped, the dissolved oxygen concentration in the overlying water must be measured and then the air flow re-established at a gentle rate. Any DO readings that have fallen below 60% saturation (USEPA, 1994a) must be included in the test-specific report.

The salinity and pH of the overlying water must be measured at the beginning and end of the test in at least one test chamber representing each treatment. Additionally, ammonia concentrations in the overlying water must be measured (total ammonia; see for example APHA *et al*., 1989) and calculated (un-ionized ammonia; Bower and Bidwell, 1978) at the beginning and end of

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[20] Using this approach, toxicity data can be analyzed in relation to "effective mortality", i.e. the sum of dead individuals plus surviving individuals that are unable to rebury. However, in most instances, surviving amphipods are able to rebury in control sediment.

the test in at least one test chamber representing each treatment. Salinity and pH may be measured using probes and calibrated meters. Ammonia may be measured using an ion-specific electrode or by extracting an aliquot of the overlying water for this analysis. As with DO measurements, any probe inserted in a test chamber must be inspected carefully immediately after each reading, and rinsed in deionized or distilled water between samples. For measurements of ammonia requiring sample aliquots, samples of overlying water must be taken just before the addition of test organisms and upon completion of the test. On each occasion, no more than 10% of the volume of the overlying water in a test chamber should be removed for this purpose. A pipette should be used carefully to remove water from a depth of about 1 to 2 cm above the sediment surface. The pipette should be checked to ensure that no amphipods are removed during the collection of aliquots of overlying water for ammonia analyses.

4.4 Test Endpoints and Calculations

For all tests, the following endpoint must be calculated for each treatment:

- the mean (± SD) percentage of amphipods that survived the 10-day exposure (i.e., percent survival at Day 10)

Depending on the study design and test objectives, two additional biological endpoints might also be calculated:

- the mean (± SD) percentage of amphipods, surviving the 10-day exposure, that emerged from each solid-phase test substance in ≤ 10 days

- the mean (± SD) percentage of surviving amphipods that did not rebury in control sediment upon termination of the exposure

Numbers of amphipods found to be dead, missing, or alive in each test vessel at Day 10 are determined and recorded. Missing individuals are assumed to have died and disintegrated during the test, and are to be included in calculating the percent survival for each replicate treatment. The mean (± SD) percent survival for replicate treatments (normally a minimum of $n = 5$) is then calculated. Means and standard deviations for any sublethal-effect data to be appraised (i.e., percentage of surviving amphipods emerged from sediment at Day 10; percentage of survivors not reburying in control sediment at test end) are also calculated for replicate treatments. The mean values for the replicates of each test sediment are then compared statistically with corresponding values for amphipods held in reference or control sediments under otherwise identical conditions.

The objective of a sediment toxicity test is to quantify contaminant effects on groups of test organisms exposed to field-collected sediment or similar dredged material, or laboratory-spiked sediments, and to determine whether these effects are statistically different from those occurring in a control or reference sediment. Various statistical procedures can be used to assess the results of the sediment toxicity test. The options, rationale for choice, and methods of calculation are discussed in depth in reports by the United States Environmental Protection Agency (1994a) and Environment Canada (1998b). The choice of statistical treatment depends on the test and study designs and, in particular, whether tests used replicate samples of sediment or multiple concentrations of test substances. Section 5.6 provides guidance on statistical endpoints and calculations for sediment toxicity tests

with samples of field-collected sediment or similar dredged material. Section 6.5 provides guidance on statistical endpoints and calculations for multi-concentration tests using sediment spiked experimentally with a particular chemical or chemical mixture under investigation.

4.5 Reference Toxicant

The routine use of a reference toxicant is necessary to assess, under standardized test conditions, the relative sensitivity of the population of organisms used to study the toxicity of test sediments and the precision and reliability of data produced by the laboratory personnel for that reference toxicant, under standardized test conditions (Environment Canada, 1990a; 1995). When determining the toxicity of samples of test material, a reference toxicity test must be performed on each batch of field-collected amphipods used for testing. A 4-day static LC50, using a range of concentrations of cadmium (as cadmium chloride) in seawater only (no sediment present), may be used (McLeay *et al*., 1989, 1991; USEPA, 1990; ASTM, 1991a; Paine and McPherson, 1991a, b). Alternatively, control sediment may be spiked with copper, cadmium, or fluoranthene, and used to determine a 10-day LC50 (Environment Canada, 1995).[21] Any test with the reference toxicant should be initiated within one day of starting the 10-day assay with test material(s), and is normally started on the same day.

When conducting a seawater-only, 4-day LC50 with cadmium or another reference toxicant, the test is performed in 1-L glass beakers or jars, with ≥800 mL of test solution and a minimum of 10 amphipods per test chamber. Unless otherwise described, all applicable conditions and procedures for preparing for and undertaking the test must be identical to those defined in Sections 2, 3, 4, and 6 of this report, except that sediment is not added to the test chambers and replicates are not required for each test concentration. One distinction is that, unlike the sediment toxicity test which requires continuous overhead illumination of test chambers, the reference toxicity test is to be performed in the dark (USEPA, 1994a). This can be achieved by covering test chambers with opaque material (e.g., aluminum foil), or by undertaking the test in a separate, enclosed testing facility where the lights are left off. A second distinction is that, unlike the sediment toxicity test, which requires gentle aeration of the overlying water throughout the test, the solutions of cadmium or water (control) in the test chambers are not aerated since the concentrations of dissolved oxygen that are present in each test solution (including the controls) are adequate to satisfy the oxygen requirements of the test organisms. Each test chamber is covered to minimize contamination and losses due to evaporation.

If a reference toxicity test is performed with cadmium, copper, or fluoranthene spiked in control sediment, the procedures given in Environment Canada (1995) and Section 6 apply.

Criteria used to select appropriate reference toxicants for this test might include the following:

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[21] Spiking control samples or other clean sediment with copper, cadmium, fluroanthene, or other chemicals is proving useful for solid-phase reference toxicity tests with spiked control sediment (Cairns *et al*., 1984; Ditsworth *et al*., 1990; Yee *et al*., 1992; Environment Canada, 1995). Such an approach should see increased use, especially once research establishes standardized formulated ("artificial") sediment appropriate for the particular species of amphipods being used (Environment Canada, 1995).

- chemical readily available in pure form,
- stable (long) shelf life of chemical,
- can be interspersed evenly throughout clean substrate,
- good dose-response curve for test organism,
- stable in aqueous solution,
- minimal hazard posed to user,
- concentration easily analyzed with precision,
- known influence of salinity on toxicity of chemical to test organism, and
- known influence of pH on toxicity of chemical to test organism.

The same type (i.e., natural or reconstituted seawater), source, and pretreatment of test water (i.e., the control/dilution water if a 4-day seawater-only test; or the overlying water used in a 10-day test with chemical-spiked sediment) should be used for each reference toxicity test method and a single species of test organisms. Salinity of this test water must be 28 ± 2 g/kg, and should be the same for each reference toxicity test performed with a particular species at each test facility. The test water must be temperature adjusted (i.e., 10 ± 2 °C if *A. virginiana*; 15 ± 2 °C if another species) and aerated as required to achieve a dissolved oxygen content of 90 to 100% saturation, before it is used to prepare test solutions or as overlying water in a toxicity test with a reference chemical spiked in control sediment. The temperature of the solution (4-day seawater-only test) or overlying water (10-day test with chemical in control sediment) in each test vessel should be measured daily, and must be measured at the beginning and end of the test. Mean daily temperature during the test must be 15 ± 2 °C for each test species except *A. virginiana*, for which the mean daily temperature must be 10 ± 2 °C. Dissolved oxygen, salinity, and pH in each test vessel must be measured at the beginning and end of the test.

For 4-day seawater-only tests using *R. abronius, E. estuarius, C. volutator, F. xiximeus, L. pinguis*, or *A. virginiana*, the results of the reference toxicity test are only valid and acceptable if control survival at 96 h is ≥90%. Given the historical performance of *E. washingtonianus* in 4-day seawater-only tests (Fennell, 1998), the results of a 4-day seawater-only test with cadimum are only valid and acceptable if, for this species only, the control survival at 96 h is ≥85%. For 10-day tests using a reference chemical spiked in control sediment, the species-specific criterion for a valid test given in Section 4.2 apply.

Numerous studies have reported the acute lethal tolerance of marine or estuarine amphipods to cadmium, using seawater-only tests (Swartz *et al*., 1985c; DeWitt *et al.,* 1989; McLeay *et al*., 1989, 1991; Nicol and Doe, 1990; Tay *et al*., 1991; ASTM, 1991a; Paine and McPherson 1991a,b). Toxicity tests using control sediment spiked with cadmium, copper, or fluoranthene have also been performed (see Cairns *et al*., 1984; Swartz *et al*., 1990; Environment Canada, 1995).

Pertinent reports by Environment Canada provide guidance on the selection, performance, and use of *water only* (Environment Canada, 1990a) or *spiked-sediment* (Environment Canada, 1995) reference toxicity tests. Laboratory personnel unfamiliar with such tests are advised to

consult these reports before preparing for or conducting them.

It is the responsibility of laboratory personnel to demonstrate their ability to obtain consistent, precise results with a reference toxicant before definitive sediment assays are conducted using this biological test method for measuring sediment toxicity. To meet this responsibility, the laboratory personnel should initially determine their intralaboratory precision, expressed as percent coefficient of variation (% CV), by performing five or more reference toxicity tests with different batches of test organisms of the same species, using the recommended reference toxicant(s) and the procedures and conditions defined herein. This should be conducted to gain experience with the test procedure, and as a point of reference for future tests (USEPA, 1994a).

While routinely performing this reference toxicity test with each batch of field-collected amphipods of the same species, laboratory personnel should continue to follow the same procedure. Once sufficient data are available (EC, 1990a), LC50s derived from these tests must be plotted successively on a species-specific warning chart, and examined to determine whether the results are within ± 2 SD of values obtained in previous tests using the same species, reference toxicant, and test procedure. A separate warning chart must be prepared and updated for each species of marine or estuarine amphipod used with this sediment toxicity test method. The warning chart should plot logarithm of concentration on the vertical axis against date of the test or test number on the horizontal axis. Each new LC50 for the reference toxicant must be compared with established limits of the chart; the LC50 is acceptable if it falls within the warning limits. All calculations of mean and standard deviation should be made on the basis of log(LC50).

The logarithm of concentration (including LC50) should be used in all calculations of mean and standard deviation, and in all plotting procedures. This simply represents continued adherence to the assumption by which each LC50 was estimated based on logarithms of concentrations. The warning chart may be constructed by plotting the logarithmic values of the mean and ± 2 SD on arithmetic paper, or by converting them to arithmetic values and plotting those on the logarithmic scale of semi-log paper. If it were demonstrated that the LC50s failed to fit a log-normal distribution, an arithmetic mean and SD might prove more suitable. The mean of the available values of log(LC50), together with the upper and lower warning limits (± 2 SD), should be recalculated with each successive LC50 until the statistics stabilize (EC, 1990a, 1995, 1998b; USEPA, 1994a).

If a particular LC50 fell outside the warning limits, the sensitivity of the test organisms and the performance and precision of the test would be suspect. Since this might occur 5% of the time due to chance alone, an outlying LC50 would not necessarily mean abnormal sensitivity of the batch of test organisms or unsatisfactory precision of toxicity data. Rather, it would provide a warning that there might be a problem. A thorough check of all acclimation and test conditions and test procedures should be carried out. Depending on the findings, it might be necessary to repeat the reference toxicity test, or to obtain a new batch of field-collected organisms for evaluating the toxicity of the samples of test material (together with a new reference toxicity test using the new batch of test organisms).

Results that remained within the warning limits might not necessarily indicate that a laboratory was generating consistent results. Extremely variable data for a reference toxicant would produce wide warning limits; a new data point could be within the warning limits but still represent undesirable variation in test results. A coefficient of variation of no more than 30%, and preferably 20% or less, is suggested as a reasonable limit by Environment Canada (1990a, 1995).

Specific Procedures for Testing Field-collected Sediment or Similar Solid Substance

This section gives particular instructions for preparing and testing samples of field-collected sediment or similar solid substance. These instructions are in addition to the procedures listed in Section 4.

Detailed guidance for the collection, handling, transport, storage, and analyses of field-collected sediment is given in Environment Canada (1994). This guidance document should be consulted and followed, in addition to the guidance provided here, when collecting samples of sediment and preparing them for toxicity tests with estuarine or marine amphipods.

5.1 Sample Collection

Samples of marine or estuarine sediment collected for assessment of their acute toxicity to one or more species of marine or estuarine infaunal amphipods can be taken from a number of designated sites on a routine (e.g., quarterly, semi-annually, or annually) basis for monitoring and compliance purposes, or during field surveys of sites for spatial definition of sediment quality. One or more sites should be sampled for reference (presumably clean) sediment during each field collection.

For certain monitoring and regulatory purposes, multiple replicates (i.e., separate samples from different grabs or cores taken at the same site) should be taken at each sampling station, including one or more reference stations (ASTM, 1991a; Chapman, 1991; Swartz, 1991; Environment Canada, 1994). Each of these field replicates must be tested for its acute toxicity to amphipods, using five or more test vessels per replicate sample. For certain other purposes (e.g., preliminary or extensive surveys of the spatial distribution of toxicity), the survey design might include only one sample from each station, in which case the sample would normally be homogenized and split between five or more test vessels (i.e., laboratory replicates). The latter approach precludes any determination of mean toxicity at a given sampling location (station), but allows a statistical comparison of toxicity of each sample with the control, and also if desired, a comparison among the test samples (stations), using appropriate statistical tests.

Sites for collecting reference sediment should be sought where the geochemical properties of the sediment, including grain size characteristics, are similar to those at the site(s) where samples of test sediment are collected. Ideally, reference sediment should be collected from a site uninfluenced by the source(s) of contamination but within the general vicinity of the site(s) where samples of test sediment are taken. Preliminary surveys to assess the toxicity and geochemical properties of sediment within the region(s) of concern and at neighbouring sites are useful for selecting appropriate sites at which to collect reference sediment. It is recommended that reference sediment from more than one site be collected to increase the likelihood of a good match with grain size and other physicochemical characteristics of the test sediments.

Samples of municipal or industrial sludge (e.g., sewage sludge, dewatered mine tailings, or sludge from an industrial clarifier or settling pond) can be collected to assess their acute toxicity in marine or estuarine environments to infaunal amphipods or other sensitive benthic life. Other solid wastes (e.g., drilling mud residue from offshore platforms) can also be taken for toxicity and chemical evaluation.

Procedures used for sample collection (i.e., core, grab, dredge, or composite) will depend on the study objectives and the nature of the solid waste. To sample sediment, a benthic grab or core should be used, rather than a dredge, to minimize disruption of the sample. Care must be taken to minimize loss of fines during sample collection. If the sample is obtained using a grab sampler, glass cores should be used to collect a sample from the surficial 2 cm, or desired, layer of the sediment. This can be achieved if the grab can be opened from the top to expose the surface of the undisturbed sediment. The sample should be transferred to a clean glass or plastic sample container (ASTM, 1991a; Environment Canada, 1994).

The same collection procedure should be used at all field sites sampled. The types of sediment collection devices and the advantages and disadvantages of various sediment-collection methods and apparatus have been reviewed recently (ASTM, 1991b; Mudroch and MacKnight, 1991; Environment Canada, 1994).

5.2 Sample Labelling, Transport, Storage, and Analyses

Containers for transport and storage of samples of field-collected sediment or similar solid substance must be made of nontoxic material. The containers must either be new, or thoroughly cleaned and then rinsed with clean water.

Each sample container should be filled completely, to exclude air. Immediately after filling, each sample container must be sealed, and labelled or coded. Labelling and an accompanying record made at this time must include at least a code which can be used to identify the sample and identify its type, source, precise location, replicate number, and date of collection. This record should also include the name and signature of the sampler(s).

Upon collection, warm (>7 °C) samples should be cooled to between 1 and 7 °C with regular ice or frozen gel packs, and kept cool (4 ± 3 °C) in darkness throughout transport (EC, 1994). As necessary, gel packs, regular ice, or other means of refrigeration should be used to assure that sample temperatures range within 1 to 7 °C during transit. Upon arrival at the laboratory, the sample temperature and date of receipt must be recorded. Samples to be stored for future use must be held in airtight containers and in darkness at 4 ± 2°C (EC, 1994). Any air headspace in the storage container should be purged with nitrogen gas, before capping tightly (EC, 1994). Samples must not freeze or partially freeze during transport or storage, and must not be allowed to dry (ASTM, 1991a, b; EC, 1994). It is recommended that samples of sediment or similar particulate material be tested as soon as possible after collection. The sediment toxicity test should begin within two weeks of sampling (Chapman, 1988; ASTM, 1991a,b), and preferably within one week; the test must start no later than six weeks after sample collection[22].

[22] The toxicity and geochemistry of contaminated sediments from Hamilton Harbour were reported to change with storage for longer than 1 week, although

In the laboratory, each sample of field-collected sediment should be thoroughly mixed (Section 5.3), and representative subsamples taken for physicochemical characterization. Each sample (including all samples of control and reference sediment) must be characterized by analyzing subsamples for at least the following constituents: for whole sediment–percent very coarse-grained sediment (i.e.,particles >1.0 mm), percent sand (>0.063 to 2.0 mm), percent silt (>0.004 to 0.063 mm), percent clay (<0.004 mm), percent water content, and total organic carbon content; for pore water–salinity, pH, and ammonia (total and un-ionized). Other (optional) analyses described in Section 3.4 for control sediment should also be considered and applied as appropriate. Unless indicated otherwise, identical analyses should be performed with subsamples representative of each replicate sample of field-collected sediment (including reference sediment) taken for a particular survey of sediment quality, together with one or more subsamples of control sediment.

5.3 Preparing Test Substance

With the exception of control sediment, samples of field-collected test sediment (including reference sediment) must not be wet-sieved because this removes contaminants present in the pore water or loosely adsorbed to particles (ASTM, 1991a; Environment Canada, 1994). If it is necessary to remove indigenous organisms or debris, samples may be press-sieved (without added water) through a 1- or 2-mm mesh stainless steel screen (USEPA, 1994a). Alternatively, these may be removed using forceps or a gloved hand.

Unless research or special study objectives dictate otherwise, each sample of field-collected test material should be homogenized in the laboratory before use. Sample homogenization has been specified in existing guideline documents (USEPA, 1990; ASTM, 1991a; Environment Canada, 1994). However, mixing can affect bioavailability and might not be desirable for all purposes.

To achieve a homogeneous sample, it might be necessary to transfer that portion of the sample required for biological and chemical testing to a bowl or other mixing chamber. The sample should be stirred using a nontoxic device (e.g., plastic spoon or spatula) until its texture and colour are homogenous (Chapman, 1988). Mixing conditions, including duration and temperature, must be standardized for each sample included in a test. After mixing, subsamples of the test substance required for chemical analyses must be removed, combined, and thoroughly mixed to ensure that they are representative of the substance to be used in the toxicity test. If concern exists regarding the effectiveness of sample mixing, subsamples of the sediment within a mixing container should be taken and analyzed separately to determine homogeneity.

When the grain size or porewater salinity of any sample of test material is beyond the range known to be tolerated by the species of

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the data supporting that statement were not provided (Brouwer *et al*., 1990). Testing within 2 weeks conforms with current standardization in U.S. procedures (ASTM, 1991a,b). A maximum permissible storage time of 6 weeks was included in interim guideline procedures for measuring the toxicity of sediment samples (Environment Canada, 1990b, c) in view of practical difficulties for shorter times, including the time required if initial chemical analyses are to be performed. A recent study by Outhoudt *et al.* (1991) indicates that the toxicity of samples of freshwater sediment did not differ significantly when stored at 4 °C for periods of 7 to 112 days.

amphipod intended for use in the test, another test organism which is tolerant of these physicochemical characteristics must be used (see Section 2.1 and Appendices E to K). Wet-sieving of samples of field-collected sediment to adjust the salinity of the pore water is not permitted for any test performed for regulatory compliance monitoring purposes, and is not recommended for other routine monitoring[23].

For certain research investigations, wet-sieving sediment samples (or a portion thereof) might be appropriate. For instance, the effect of the salinity of pore water on sample toxicity might be examined by conducting a series of 10-day assays at different salinities of pore water. Other (research) situations where wet-sieving of test substances might be performed include tests of sediments known to be anaerobic, or tests to examine the relationship between certain sediment fractions (particle sizes) and sample toxicity. Procedures described in Section 3.4 for sieving control sediment can be used as a guideline. Comparative toxicity testing using sieved versus unsieved sediment might, in some cases, be necessary to discern the effect of wet-sieving on sample toxicity and on the associated chemical constituents of the sediment.

5.4 Test Water

For tests with field-collected sediment or similar solid substance, the seawater introduced to test vessels should normally be from the same source as that used for acclimating amphipods and for sieving the control sediment (Section 3.5). This may be the laboratory's supply of clean, natural seawater, or reconstituted seawater. For certain applications, the experimental design might require the use of seawater taken from the site where test sediments were collected. Subsection 2.5.4 provides guidance regarding the preparation and analysis of test water.

5.5 Test Observations and Measurements

A qualitative description of each field-collected test substance should be made when the test is being set up. This might include observations of sample colour, texture, and homogeneity, and the presence of plants, animals, and tracks or burrows of animals. Any changes in appearance of the test substance or overlying seawater during the test, or upon its termination, should be noted and reported.

Measurements of the quality of each sample of test material must be made as described in Section 5.2. The quality of the overlying water in at least one replicate test vessel representing each treatment must be monitored as described in Section 4.3.

5.6 Test Endpoints and Calculations

Environment Canada (1998b) as well as USEPA (1994a) should be consulted for detailed guidance on appropriate statistical analyses for samples of field-collected sediment.

For each sample of field-collected test material (including control and reference sediment), the percent survival of amphipods at Day 10 must be calculated for each test vessel. The mean (± SD) percent survival for the laboratory replicates of each sample must

[23] This procedure results in the loss of interstitial water and the possible loss of contaminants that could be biologically available and thus contribute to sample toxicity.

also be calculated, and compared for significant differences due to treatment.

In many instances, measurements of sublethal responses of amphipods to field-collected sediments or similar material are not required because these secondary endpoints frequently show little if any sample toxicity not evident from the acute survival data. Unless stated otherwise in regulatory protocols or guidelines, tests performed for regulatory or compliance monitoring purposes need not measure sublethal endpoints. An exception is for regulatory or compliance monitoring tests using *Corophium volutator*, in which case the percentage of surviving amphipods emerged from sediment at Day 10 should be determined for each replicate treatment.

If measured, sublethal-effect data (i.e., percentage of surviving amphipods emerged, percentage of surviving amphipods not reburying in control sediment upon completion of the 10-day exposure) should be treated statistically as indicated herein.

Test data determined using laboratory replicates of a suitable reference sample should be used for comparative purposes whenever possible (Paine and McPherson, 1991b; USEPA, 1994a). Sometimes the reference sediment might be unsuitable for comparison because of toxicity or atypical physicochemical characteristics. In such cases, it would be necessary to compare the test sediments with the control sediment. Results for control sediment will assist in distinguishing contaminant effects from noncontaminant effects caused by such things as particle size. Regardless of whether the reference sediment or control sediment is used for the statistical comparisons, the results from control sediment must be used to judge the validity and acceptability of the test (Section 4.2).

The statistical procedures and interpretation of the results should be appropriate to the experimental design and study intent (see USEPA 1994a; EC 1998b; and EC 1998c for further guidance). Using this biological test method, pairwise comparisons of survival data for each test treatment are normally made against survival data derived for a particular reference or control sediment. Initially, all data should be tested for normality using the *Shapiro-Wilk's test*, and for homogeneity of variance (Eisenhart *et al*., 1947; Sokal and Rohlf, 1969 using *Bartlett's test* or other suitable test (USEPA, 1994a). These and other statistical procedures are included in the methods of "TOXSTAT"; a series of statistical programs on computer disk which can be purchased by contacting WEST, Inc. (2003 Central Avenue, Cheyenne, WY, USA). Instructions for use accompany the TOXSTAT programs on disk.

Survival data which pass the tests for normality and homogeneity of variance should be treated by a pairwise comparison of the results for each test treatment versus the results for the reference or control treatment (see earlier discussion). A one-tailed *Student's t-test* (Steel and Torrie, 1960) should be used for this purpose. If a set of data cannot meet the requirements for normality and homogeneity of variance, an arcsine-square root transformation should be applied, followed by retesting for both (USEPA, 1994a). If the transformed data do not meet the assumption of normality, nonparametric statistics such as the *Wilcoxon Rank Sum Test* (USEPA, 1994a) or other suitable tests can be applied. If the transformed data meet the assumption of normality, Bartlett's test or *Hartley's F test* should be used to test the homogeneity of variance assumption. Failure of the homogeneity of variance assumption leads to the use of a modified one-tailed Student's *t*-

test, with adjusted degrees of freedom (USEPA, 1994a). Transformed data which meet the requirements for both normality and homogeneity of variance should be treated by a straightforward pairwise comparison using a one-tailed Student's *t*-test. For comparative tests intended to define spatial variations in sediment toxicity using multiple samples, an analysis of variance (ANOVA), followed by Dunnett's test, Williams' test (Williams, 1971, 1972), or other suitable procedure for multiple comparisons (USEPA, 1994a; EC, 1998b) should be undertaken following the necessary arcsine transformations to determine if the endpoint values for different treatments differ significantly.

Specific Procedures for Testing Chemicals Introduced to Sediment

This section gives particular instructions for preparing and testing sediment spiked with a specific test chemical or mixture of chemicals under investigation. These instructions are in addition to the procedures listed in Section 4. Environment Canada (1994, 1995) reports provide more detailed instructions and recommendations for preparing and testing spiked sediment, and should be consulted for further guidance.

Further testing and standardization of procedures for preparing test mixtures (Section 6.2) might be required before this assay is applied to evaluate specific chemical-sediment mixtures for regulatory purposes.

6.1 Sample Properties, Labelling, and Storage

Information should be obtained on the properties of the chemical to be tested, including water solubility, vapour pressure, chemical stability, dissociation constants, and biodegradability. Where aqueous solubility is in doubt or problematic, acceptable procedures used previously for preparing aqueous solutions of the chemical should be obtained and reported. Other available information such as structural formula, degree of purity, nature and percentage of significant impurities, presence and amounts of additives, and n-octanol:water partition coefficient, should be obtained and recorded.

Chemical containers must be sealed and coded or labelled (chemical name, supplier, date received, person responsible for testing) upon receipt. Storage conditions (e.g., temperature, protection from light) are frequently dictated by the nature of the chemical. Standard operating procedures for chemical handling and storage must be followed.

6.2 Preparing Test Mixtures

To test chemicals, a multiconcentration test to determine the 10-day median lethal concentration (LC50) for chemical-sediment mixtures should usually be performed. For this purpose, at least five test concentrations plus a control are normally prepared. An appropriate geometric dilution series may be used, in which each successive concentration of chemical in the sediment is at least 50% of the previous concentration (e.g., 10 mg/kg, 5 mg/kg, 2.5 mg/kg, 1.25 mg/kg, 0.63 mg/kg)[24]. Test concentrations may also be selected from other appropriate logarithmic dilution series (see Appendix L). To select a suitable range of lethal concentrations, a preliminary or range-finding test, which covers a broader range of test concentrations, may be conducted.

Tests intended to evaluate the toxicity of mixtures of one or more chemicals in control sediment for federal registration or other regulatory purposes must be set up using a minimum of five replicates for each test concentration and each control sediment to be

[24] Chemical concentrations in sediment are normally calculated and expressed as µg/g or mg/kg dry weight. In some cases, concentrations in interstitial water might also be measured and expressed as mg/L (Swartz *et al*., 1985c; 1988).

included in the assay (see footnote 15, Section 4.1). The number of replicates per treatment could be reduced or eliminated altogether for range-finding tests and, depending on the expected variance among test vessels within a treatment, could also be reduced or eliminated for non-regulatory screening assays or research studies.

It is recommended that mixtures of spiked sediment be aged for four weeks before starting a test, in keeping with a common practice (USEPA, 1994a; Environment Canada, 1995). Although many studies with spiked sediment have been started within a few hours or days of preparing the mixtures, such short and variable time periods might not be long enough for equilibration of the chemicals mixed in control sediment. A consistent four-week period of aging a mixture before initiating a toxicity test would provide some standardization for intra- and interlaboratory comparisons of results for tests with spiked sediment. Once prepared, each mixture should be placed in a suitable, sealed (with no air space) container, and stored in the dark at 4 ± 2 °C (Section 5.2) for four weeks before use.

The method to be used is contingent on the study objectives and the nature of the test chemical. In most instances, a chemical-sediment mixture is prepared by making up a stock solution of the chemical and then mixing one or more measured volumes into control sediment (Swartz *et al*., 1985c; 1988; ASTM, 1991a). Chemical concentrations in sediment are normally calculated and expressed as µg/g or mg/kg dry weight (Swartz *et al*., 1985c; 1988). The preferred solvent for preparing stock solutions is filtered seawater at the test salinity (ASTM, 1991a). The source of this seawater may be reconstituted or natural seawater (Subsection 2.5.4), and should be identical to the test water used as overlying seawater (Sections 3.5 and 4.1).

For chemicals that do not dissolve readily in seawater, stock solutions may be prepared using the generator-column technique (Billington *et al*., 1988; Shiu *et al*., 1988) or, less desirably, by ultrasonic dispersion[25]. Other techniques, developed for preparing aqueous stock solutions of slightly soluble substances without the use of organic solvents, may also be employed (ASTM, 1991a). Alternatively, an organic solvent may be used. Triethylene glycol has been recommended because of its low toxicity to aquatic organisms, low volatility, and high ability to dissolve many organic chemicals (ASTM, 1991a). Other solvents, such as methanol, ehtanol, or acetone, may be used to prepare stock solutions of organic chemicals, although they might contribute to sample toxicity, alter sediment properties, or be lost from the test substance due to their volatility. Surfactants should not be used (ASTM, 1991a).

If an organic solvent is used, the test must be conducted using both a clean sediment control and a sediment control containing solvent. For this purpose, a solvent-control sediment must be prepared that contains the concentration of solubilizing agent that is present in the highest concentration of the test chemical-sediment mixture. Solvents should be used sparingly as they might contribute to the toxicity of the prepared test sediment.

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[25] Ultrasonic dispersion is not a preferred technique. The ultrasonics could result in variations in the biological availability of the chemical, and thus in its toxicity, because of the production of droplets differing in size and uniformity. Droplets might also migrate toward the surface of the overlying seawater during the test.

Measured volumes of stock solution should be mixed with the control sediment in a manner that results in a homogeneous distribution of the chemical throughout the sediment. Mixing may be by hand (e.g., using a clean spatula or glass rod), or with the aid of a mechanical stirring or mixing device (e.g., Ditsworth *et al*., 1990). Alternatively, the chemical can be coated on the walls of a flask and an aqueous slurry (control sediment and seawater) added. The flask contents are then mixed by agitation. Another alternative is to add a measured volume of the stock chemical solution directly to a slurry of the control sediment in seawater, agitate the mixture, and allow it to settle (ASTM, 1991a,b). Other methods of mixing might prove to be acceptable provided the chemical is shown to be evenly distributed in the sediment. Mixing conditions, including solution:sediment ratio, mixing and holding time, and mixing and holding temperature, must be standardized for each treatment included in a test. If necessary, subsamples of the mixture can be analyzed to determine the degree of mixing and homogeneity.

Based on the objectives of the test, it might be desirable to determine the effect of substrate characteristics (i.e., particle size, organic content) on the acute lethal toxicity of chemical-sediment mixtures. The influence of sediment particle size on chemical toxicity could be measured by conducting concurrent 10-day multiconcentration tests with a series of mixtures comprised of the test chemical mixed in differing fractions (particle sizes) of sieved control sediment. The degree to which the organic content of sediment can modify chemical toxicity could be examined by performing concurrent multiconcentration tests using different chemical-sediment mixtures prepared with a series of organically enriched control sediments (Swartz *et al*., 1985c). Separate controls should be prepared and tested for each sediment fraction or organically enriched sediment mixture used in these tests.

Tests could be required to measure the acute lethal toxicity, to one or more species of marine or estuarine infaunal amphipods, of one or more concentrations of specific chemicals introduced to the test vessel as a dilute seawater solution overlying the sediment. Procedures for preparing test concentrations could vary depending on the objectives of the study. One approach would be to add the test solutions(s) to replicate vessels containing a 2-cm layer of control or other (e.g., field-collected) sediment, with no disturbance or subsequent mixing of the sediment and test solution(s). A second approach would require the test solution(s) introduced to the test vessels to be agitated for a predetermined time in the presence of the sediment, before the test organisms are introduced. Sediment-chemical interactions might differ appreciably depending on the approach taken, and could result in a markedly different test result. Unless specified otherwise, the temperature and salinity of each test solution should be as described in Section 6.3. Control solutions, including replicate seawater controls and, if a solvent is used, replicate seawater/solvent mixtures containing the highest concentration of solvent used in any test solution, must be prepared and treated identically.

6.3 Test and Control/Dilution Water

The water used for preparing stock or test solutions of chemicals and as test water in 10-day assays with chemical-sediment mixtures should normally be clean seawater with a temperature of 15 ± 2 °C and a salinity

of 28 ± 2 g/kg[26]. The source of this water may be reconstituted seawater or natural marine water (Subsection 2.5.4, Section 3.5). Reconstituted seawater is recommended if a high degree of standardization is required (e.g., when the measured toxicity of the chemical-sediment mixture is to be compared and assessed relative to toxicity data derived at a number of test facilities for this and/or other chemicals).

6.4 Test Observations and Measurements

A qualitative description of each chemical-sediment mixture and of the overlying seawater should be made when the test is being established. This might include observations of the colour, texture, and homogeneity of each chemical-sediment mixture, and observations of the colour and opacity of the overlying seawater. Any change in appearance of the test mixture or overlying seawater during the test, or upon its termination, should be recorded. Measurements of the quality of each chemical-sediment mixture being tested (including the control sediment), and of the overlying seawater, should be made and recorded as described in Sections 3.4, 4.3, and 5.2.

If analytical capabilities permit, it is recommended that stock solutions, pore water[27], chemical-sediment mixtures (bulk dry-weight analyses), and test solutions (if studied) be analyzed to determine exact chemical concentrations to which the amphipods are exposed. When chemical strengths are to be measured, sample aliquots should be taken, as a minimum, from the high, medium, and low test concentrations at the beginning and end of the test. These aliquots should be preserved, stored, and analyzed according to the best proven methodologies available for determining the concentration of the particular chemical in aqueous solution or adsorbed to sediment.

Unless there is good reason to believe that the chemical measurements are not accurate, toxicity results for any test in which concentrations are measured should be calculated and expressed in terms of those average measured concentrations determined for both the whole sediment (µg/kg or mg/kg, dry weight) and the pore water (µg/L or mg/L). In cases where concentrations of chemical added to the overlying seawater are being tested, results should again be expressed as the average measured concentrations determined for the sediment and the pore water, although average chemical concentrations measured for the test solutions overlying sediment should also be calculated and reported.

6.5 Test Endpoints and Calculations

In most instances, the endpoints for tests conducted with chemical-sediment mixtures will include a 10-day LC50 (based on percent mortalities), together with any 10-day EC50s that might be calculated based on

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[26] Based on the study design, objectives, and other considerations, another salinity might be more appropriate. For example, a salinity typical of that at a particular receiving water being studied could be used in the test. If *A. virginiana* is to be used as the test organism, a test temperature of 10 ± 2 °C is required (see Section 4.2).

[27] Sediment pore water can be isolated using several methods, although centrifugation or squeezing generally yield the best recovery (ASTM, 1991b). Guidance provided in Environment Canada (1994) should be followed when collecting pore water.

observations of percent emergence and/or percent reburial in control sediment at the end of the test. The primary endpoint is the 10-day LC50; determination of secondary (sublethal-effect) endpoints might or might not be part of the study objectives, or might not be possible to calculate from the data derived from the study.

If a suitable range of concentrations of chemical-sediment mixtures are studied (Section 6.2), the amphipod-mortality data derived for each test concentration can be used to calculate the 10-day median lethal concentration (LC50) and its 95% confidence limits. To estimate an LC50, mortality data at 10 days are combined for all replicates at each concentration. If mortality is not $\geq$50% in at least one concentration, the LC50 cannot be estimated. If there is no mortality at a certain concentration, that information is used as an effect of 0% mortality. However, if successive concentrations yield a series of 0% mortalities, only one such value should be used to estimate the LC50, and that value should be the highest concentration of the series, i.e., the zero-effect that is "closest to the middle" of the distribution of data. Similarly, if there were a series of successive complete mortalities at the high concentrations in the test, only one value of 100% effect would be used. Only the one "closest to the middle", i.e., the 100% effect at the lowest concentration, would be used. Use of only one 0% and one 100% effect applies to data analysis by computer program or by hand plotting on a graph. The use of additional values of 0% and/or 100% might distort the estimate of LC50.

Various computer programs may be used to calculate this test endpoint. Stephan (1977) developed a program to estimate LC50s that uses probit, moving average, and binomial methods, and adapted it for the IBM-compatible personal computer. This program in the BASIC language is recommended, and is available on diskette[28] from Environment Canada (address in Appendix B). An efficient microcomputer program for probit analysis is also available from Hubert (1987), and other satisfactory computer and manual methods (APHA *et al*., 1989; USEPA, 1985) may be used. Programs using the trimmed Spearman-Kärber method (Hamilton *et al*., 1977) are available for personal computers but are not recommended because divergent results might be obtained by operators who are unfamiliar with the implications of trimming ends of the dose-response data.

The recommended program of Stephan (1977) provides estimates of LC50 and confidence limits by each of its three methods, if there are at least two partial mortalities in the set of data. For smooth or regular data, the three results will likely be similar, and values from the probit analysis should be taken as the preferred ones and reported. The binomial estimate might differ somewhat from the others, and this estimate should only be used as a last resort. If the results do not include two partial mortalities, only the binomial method functions, and it can be used to provide a best estimate of the LC50 with conservative (wide) confidence limits.

Any computer-derived LC50 should be checked by examining a plot, on logarithmic-probability scales, of percent mortalities at Day 10 for the various test concentrations (APHA *et al*., 1989). Any major disparity between the estimated LC50 derived from this plot and the computer-derived LC50 must be resolved.

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[28] Through the courtesy of Dr. Charles E. Stephan (U.S. Environmental Protection Agency, Duluth, Minnesota).

A manual plot of mortality-concentration data to derive an estimated LC50 is illustrated in Figure 2. In this hypothetical example, there were 100 amphipods (five replicates of 20 organisms per concentration) tested at each of five concentrations. This figure was based on concentrations of 1.8, 3.2, 5.6, 10, and 18 mg chemical/kg sediment (dry weight) that caused mortalities of 0, 20, 40, 90, and 100% of the test amphipods exposed to the respective concentrations for 10 days. The line was fitted by eye. The concentration expected to be lethal to 50% of the amphipods can be read by following across from 50% (broken line) to the intersection with the fitted line, then down to the horizontal axis to estimate the LC50 (5.6 mg/kg).

When fitting a line such as that in Figure 2, relatively more emphasis should be assigned to points that are near 50% mortality. Logarithmic-probability paper ("log-probit", as in Figure 2) can be purchased in good technical bookstores, or ordered through them.

Computer programs gave estimates that were very similar to the graphic one for the regular set of data in Figure 2. The LC50s (and 95% confidence limits) were:

Probit analysis of Hubert (1987):	5.56	(4.28 to 7.21)
Stephan (1977)		
method probit:	5.58	(4.24 to 7.37)
moving average:	5.58	(4.24 to 7.33)
binomial:	6.22	(1.8 to 10)
Spearman-Kärber method 0% trim:	5.64	(4.38 to 7.26)
(Hamilton *et al*., 1977)		
10% trim:	5.73	(4.34 to 7.58)
20% trim:	5.95	(4.34 to 9.80)

The binomial method did not estimate confidence limits, but selected two concentrations from the test as outer limits of a range within which the true confidence limits would lie.

Sublethal-effect data derived from multiconcentration tests can be analyzed to calculate median effective concentrations (EC50s) and their 95% confidence limits. Separate EC50s should be determined for each of the sublethal responses quantified (i.e., percentage of surviving amphipods emerged from sediment at Day 10; percentage of survivors not showing reburial in control sediment at the termination of the test). Statistical procedures for the calculation of these endpoints are the same as those described for LC50s.

If both a clean sediment control and a solvent control are used in a 10-day sediment toxicity test, the mean (± SD) percent survival and any sublethal-effect endpoints (Section 4.4) determined for each control should be compared statistically. Student's t-test (Steel and Torrie, 1960) may be applied for this comparison. If a statistically significant difference in any endpoint is found between the two controls, only the solvent control may be used to meet the species-specific criterion for a valid test (see Section 4.2). If no statistically significant difference is found, the data from both controls may be pooled to meet the acceptability of the test (ASTM, 1991a).

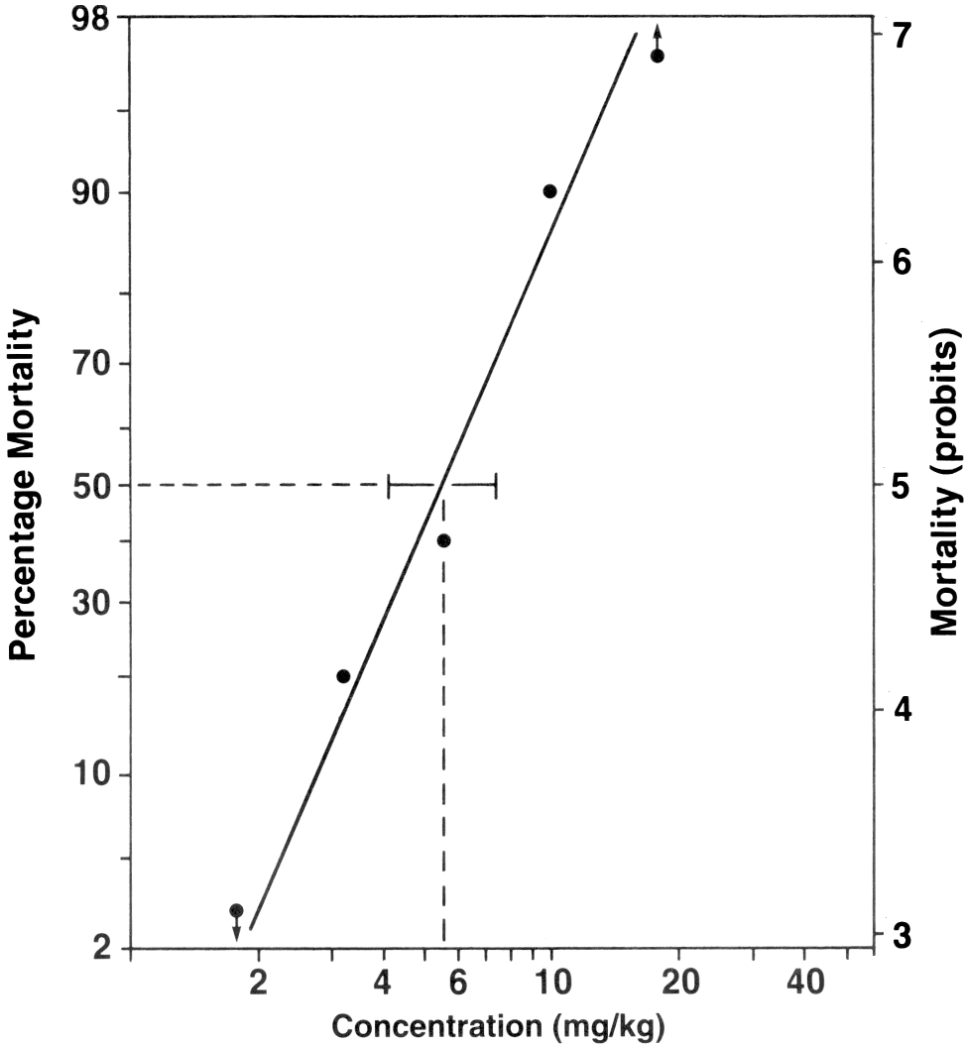

Figure 2 Estimating a Median Lethal Concentration by Plotting Mortalities on Logarithmic-probability Paper

Reporting Requirements

The test report should describe the materials and methods used, as well as the results. The reader should be able to establish from the report whether the conditions and procedures rendered the results acceptable for the use intended.

Procedures and conditions that are common to a series of ongoing tests (e.g., routine toxicity tests for monitoring and compliance purposes) and consistent with specifications in this report may be referred to by citation or by attachment of a general report that outlines standard laboratory practice. For the various reporting requirements identified here as bullets in Sections 7.1 to 7.7 inclusive, those that relate to test-specific information must be included in the individual test report. Procedural information that reflects "standard" laboratory practice in the performance of this biological test method may be restricted to the general report.

Each test-specific report must indicate if there has been any deviation from any of the "must" requirements delineated in Sections 2 to 6 of this biological test method, and if so, provide details on the deviation. Specific monitoring programs or related test protocols might require selected items (e.g., program- or protocol-specific procedures and/or conditions) in the test report, or might designate certain procedural-specific information as "data to be held on file". Details pertinent to the conduct and findings of the test, which are not conveyed by the test report or general reports, should be kept on file by the laboratory so that the appropriate information can be provided if an audit of the test is required.

7.1 Test Substance

- Sample type (e.g., chemical, field-collected sediment, sludge or other solid waste), source, and description; sampling location, method, and schedule; specifics regarding nature, appearance and properties, volume and/or weight).
- Information on labelling or coding of the test substance.
- Details on manner of sample collection, transport, and storage (e.g., core or benthic grab sample, description of container, temperature of sample upon receipt and during storage).
- Identification of person(s) who collected and/or provided the sample.
- Dates for sample collection, receipt at test facility, and start and end of definitive test.

7.2 Test Organisms

- Species, source, and date of collection.
- Description of procedures used to sort, identify, and handle the amphipods.
- Description of holding and acclimation conditions (facilities; lighting; seawater source and quality; water pretreatment; water exchange rate and density of amphipods in holding containers; salinity; temperature and dissolved oxygen during holding and acclimation; acclimation period).

- Daily percentage of amphipods that emerged from the control sediment during the holding/acclimation period.

- Average total body length (with range and sample size) of individual amphipods used in the test.

7.3 Test Facilities and Apparatus

- Name and address of test laboratory.

- Name of person(s) who performed the test.

- Description of systems for providing lighting and regulating temperature within test facility.

- Description of test vessels and lids (size, shape, type of material) and aeration system and apparatus.

- Description of procedure used to clean or rinse apparatus.

7.4 Control Sediment and Test Water

- Type and source of control sediment and test water.

- Type and quantity of any chemical(s) added to test water.

- Sampling and storage procedures and conditions for the control sediment.

- Pretreatment of control sediment (e.g., sieving, settling of sieved fines) and test water (e.g., temperature and salinity adjustments, degassing, aeration rates, and duration).

- Measured quality of control sediment (Section 3.4) and test water (Section 3.5) before and/or at commencement of toxicity test.

7.5 Test Method

- Brief mention of experimental method used if standard (e.g., as per this report).

- Design and description if specialized procedure (e.g., sieving of field-collected test sediment, preparation of chemical-sediment or solids-sediment mixtures) or modification of standard method.

- Procedures used for mixing or otherwise manipulating test sediments before use; time interval between preparation and testing.

- Procedure used for preparing stock and/or test solutions of chemicals.

- Procedures used for preparing and testing reference toxicant (e.g., spiked control sediment).

- Methods used for chemical analyses of test substance, prepared sediment mixtures, porewater, and test water. Details concerning sampling, and sample preparation and storage, before chemical analysis.

- Use of preliminary or range-finding test.

- Frequency and type of observations made during test

7.6 Test Conditions

- Number of replicate test vessels for each treatment; test concentrations (if applicable).

- Depth, weight, and volume of sediment and water in test vessels.

- Number of organisms per test vessel.

- Light intensity adjacent to surface of overlying water in test vessel.

- Statement of aeration rate and manner of application to test vessels, before and during the test.

7.7 Test Results

- Any chemical or physical measurements (e.g., chemical concentration, salinity, pH, Eh, dissolved oxygen, particle size distribution, organic content, temperature) made on control and reference sediments; test substances; pore water; and, if used, control/dilution water; before and during the test.

- Appearance of control and reference sediments, test substances, and overlying seawater, and changes noted during test.

- Results for range-finding test (if conducted).

- Biological endpoints (e.g., mean ± SD for percentage of amphipods that survived the 10-day exposure, mean ± SD for percentage of survivors emerged from sediment in ≤10 days, mean ± SD for percentage of survivors not reburying in control sediment at end of test) calculated for each test sediment or test concentration, together with the results of any statistical comparisons.

- Any LC50s and EC50s (including the associated 95% confidence limits) determined for test chemicals or reference toxicants, including the statistical method used for their calculation.

- The results for tests with any reference toxicant(s) performed during the toxicity test, together with the geometric mean value (± 2 SD) for the same reference toxicant(s) as derived at the test facility in previous tests.

- Specification as to whether results are based on measured or nominal concentrations of the test substance.

- Results of any other observations or analyses made on the samples of reference and control sediment and test substance(s) (e.g., faunal tracks, presence or fauna or detritus, geochemical analyses).

- Anything unusual about the test, problems encountered, remedial measures taken.

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[a] A BASIC computer program for calculating LC50 is available from the Aquatic Toxicology Section, Pacific Environmental Science Centre, 2645 Dollarton Highway, North Vancouver, BC, V7H 1V2, by providing a formatted computer diskette.

Procedural Variations for Tests of Sediment Toxicity Using Marine or Estuarine Amphipods, as Described in Canadian and U.S. Methodology Documents*

1. Species and Life Stage

Document	Test Species	Life Stage
Swartz *et al.,* 1985a	*R. abronius*	mature, 3- to 5-mm length
Chapman & Becker 1986	*R. abronius*	mature, 3- to 5-mm length
DeWitt *et al.,* 1989	*E. estuarius*	3- to 5-mm total length
Envir. Can. 1990b	*R. abronius*	NI[a]
Envir. Can. 1990c	*R. abronius*	NI
Envir. Can. 1990c	*A. abdita*	NI
USEPA 1990	*R. abronius*	mature, 3- to 5-mm length [b]
USEPA 1990	*E. estuarius*	mature, 3- to 5-mm length [b]
ASTM 1991a	*R. abronius*	3- to 5-mm total length [c]
ASTM 1991a	*E. estuarius*	3- to 5-mm total length [c]
ASTM 1991a	*A. abdita*	juvenile, or female adult
ASTM 1991a	*G. japonica*	immature, 3- to 6-mm length [b]

[a] NI = not indicated.
[b] Females carrying embryos should not be used.
[c] Very large, mature individuals should not be used because they may be senescent.

2. Requirement for Taxonomic Identification of Species

Document	Identification of Species
Swartz *et al.,* 1985a	NI
Chapman & Becker 1986	confirmed by qualified taxonomist, representative specimens archived
Envir. Can. 1990b	NI
Envir. Can. 1990c	NI
USEPA 1990	confirmed by qualified taxonomist, representative specimens archived
ASTM 1991a	confirmed by qualified taxonomist

* Based on documents available to the authors as of July 1991.

3. Holding Conditions Before Testing

Document	Sediment Samples	Amphipods[a]
Swartz *et al*., 1985a	4 °C, dark, ≤5 d	in lab. for 3 to 4 d
Chapman & Becker 1986	4 °C, dark, ≤14 d	in lab. for 4 to 10 d
Envir. Can. 1990b	4 °C, dark, ≤42 d	NI
Envir. Can. 1990c	4 °C, dark, ≤42 d	NI
USEPA 1990	4 °C, dark, ≤14 d	in lab. for 4 to 14 d
ASTM 1991a	4 ± 3 °C, dark, ≤14 d	in lab. for 2 to 14 d

[a] Held in sieved (0.5-mm) control sediment, with overlying seawater aerated and adjusted to temperature, salinity, and pH conditions to be used in test.

4. Use of Reference Sediment(s)

Document	Reference Sediment Recommended or Required
Swartz *et al.,* 1985a	yes, if test sed. >50% clay or >35% gravel
Chapman & Becker 1986	yes, if test sed. >50% clay or >35% gravel
Envir. Can. 1990b	NI
Envir. Can. 1990c	NI
USEPA 1990	yes, if test sediment >50% silt and clay, or >35% gravel
ASTM 1991a	yes, if test sediment has grain size or organic content which exceeds tolerance range of species

5. Salinity of Pore Water of Test Sediments

Document	Measured	Adjusted
Swartz *et al.,* 1985a	NI	no
Chapman & Becker 1986	yes, start + end	yes, if necessary
Envir. Can. 1990b	NI	NI
Envir. Can. 1990c	NI	NI
USEPA 1990	yes, start + end	normally, no [a]
ASTM 1991a	yes	no

[a] For tests with *R. abronius,* where estuarine dredged material is designated for disposal in the marine environment, interstitial salinities below 25 g/kg could require adjustment upward.

6. Type and Treatment of Seawater Used in Tests

Document	Recommended type and treatment of seawater
Swartz *et al.,* 1985a	filtered 0.45 µm; salinity adjust as necessary with deionized water, clean oceanic water or sea salts; covered and stored at test temperature; used within 2 d
Chapman & Becker 1986	as in Swartz *et al.,* 1985a
Envir. Can. 1990b	NI
Envir. Can. 1990c	NI
USEPA 1990	as in Swartz *et al.,* 1985a; if renconstituted, used within 2 d
ASTM 1991a	natural or reconstituted; if natural, filtered ≤5µm, UV sterilized or filtered 0.45 µm if pathogens, covered and stored at 4 ± 3 °C and intensively before use, aged 1 to 2 weeks

7. Test Vessels and Materials

Document	Vessel	Amount of Sediment	Amount of Seawater (mL)
Swartz *et al.,* 1985a	1-L glass beaker[a] with 10-cm ID	2-cm layer (~175 mL)	~775
Chapman & Becker 1986	1-L glass beaker[a] with 10-cm ID	2-cm layer (~175 mL)	~775
Envir. Can. 1990b	1-L glass jar	200 mL	800
Envir. Can. 1990c	NI	NI	NI
USEPA 1990	1-L glass beaker[a] with 10-cm ID	2-cm layer (~175 mL)	~775
ASTM 1991a	1-L glass beaker[a] with 10-cm ID[b]	2-cm layer (~175 mL)	~775

[a] Covered with 11.4-cm diameter watchglass.

[b] Quart-sized glass canning jars with a narrow mouth are frequently used in tests with *A. abdita*.

8. Test Type, Amphipods Per Vessel, and Replicates

Document	Test Type	Amphipods Per Vessel	Number of Replicates
Swartz *et al.,* 1985a	static	20	1 to 5 [a]
Chapman & Becker 1986	static	20	1 to 5 [b]
Envir. Can. 1990b	static	20	5
Envir. Can. 1990c	NI	NI	NI
USEPA 1990	static	20	1 to 5 [b]
ASTM 1991a	static [c]	20 [d]	1 to 5 [b]

[a] Five control replicates are included regardless of whether the test sediments are replicated.
[b] Five replicates of control and reference sediments are included regardless of whether the test sediments are replicated.
[c] Flow-through tests are sometimes conducted with *A. abdita.*
[d] Tests with *A. abdita* normally use 20 to 30 individuals per test vessel.

9. Temperature, Salinity, and Aeration During Test

Document	Temperature (˚C)	Salinity (g/kg)	Aeration
Swartz *et al.,* 1985a	15	25	minimal, by pipette at ≥2 cm from sediment
Chapman & Becker 1986	15 ± 1	28 ± 1	as above
Envir. Can. 1990b	15 ± 1	NI	NI
Envir. Can. 1990c	NI	NI	NI
USEPA 1990	15 ± 1	28 ± 1[a]	minimal, by pipette at ≥2 cm from sediment
ASTM 1991a	15 ± 3[b]	2 to 38[c]	minimal, to maintain DO ≥90% without disturbance of sediment

[a] Maintain at 28 ± 1 g/kg for tests with *R. abronius.* For tests with *E. estuarius,* maintain at the ambient salinity of the interstitial water at the collection site.
[b] *Rhepoxynius abronius* and *E. estuarius* are tested normally at 15 °C, and *G. japonica* at 15 to 19 °C. *Ampelisca abdita* is normally tested at 20 °C, although this species has been tested at 8 to 25 °C.
[C] Standard test salinities are 28 g/kg for *R. abronius,* 2 to 28 g/kg for *E. estuarius,* 20 to 35 g/kg for *A. abdita,* and 30 to 35 g/kg for *G. japonica.*

10. Lighting Conditions and Test Duration

Document	Lighting	Test Duration (days)
Swartz *et al.,* 1985a	constant by overhead lights	10
Chapman & Becker 1986	constant by normal room lighting	10
Envir. Can. 1990b	continous	10
USEPA 1990	constant by overhead lights	10
ASTM 1991a	continous, ≥100 lux at surface of sediment	10

11. Time Allowed for Burial at Test Initiation

Document	Time for Burial
Swartz *et al.,* 1985a	1 h[a]
Chapman & Becker 1986	1 h
Envir. Can. 1990b, c	NI
USEPA 1990	1 h
ASTM 1991a	5 to 10 min[b]

[a] After 1 h, amphipods that have not buried are removed and replaced.

[b] Amphipods showing repeated burrowing and emergence (i.e., avoidance response to sediment) are not replaced. For tests with *A. abdita,* amphipods should be given 1 h to burrow into the sediment before replacement (unless avoidance is evident).

12. Monitoring Quality of Overlying Seawater and Sediment During Test

Document	Variables Monitored and Frequency
Swartz *et al.,* 1985a	water daily, for temperature, pH, DO; can monitor sediment for Eh
Chapman & Becker 1986	water daily, for temperature, pH, DO; can monitor sediment for Eh
Envir. Can. 1990b	water quality measured daily
USEPA 1990	daily for temperature; can monitor DO, pH, salinity, Eh; salinity of pore water at start and end; measure sediment moisture, grain size, and total organic carbon
ASTM 1991a	DO if air flow interrupted; temperature hourly or daily maximum and minimum; perhaps test water for pH; salinity of test and pore water at least at start; sediment Eh and pH at start and end of test

13. Biological Endpoints

Document	Survival/Mortality	Emergence	Reburial [a]
Swartz *et al.,* 1985a	at Day 10	daily	at Day 10
Chapman & Becker 1985	at Day 10	daily	not done
Envir. Can. 1990b	at Day 10	daily	at Day 10
USEPA 1990	at Day 10[b]	daily	at Day 10[c]
ASTM 1991a	at Day 10	at least daily	at Day 10[c]

[a] Ability of amphipods surviving 10-day exposure to rebury in control sediment within 1 h.

[b] Mortality is the primary biological endpoint; emergence and reburial success could also be measured.

[c] Toxicity data can be analyzed in relation to "effective mortality", i.e., the sum of dead individuals plus those survivors that are not able to rebury.

14. Reference Toxicant

Document	Chemical	Required?	Test Type
Swartz *et al.,* 1985a	NI	no	NI
Chapman & Becker 1986	$CdCl_2$ or NaPCP	yes	96-h LC50 [a]
ASTM 1990a	fluoranthene	yes[b]	96-h LC50 [a]
Envir. Can. 1990b	NI	yes	NI
USEPA 1990	$CdCl_2$, $AgCl_2$	yes?[c]	96-h LC50 [a]
ASTM 1991a	$CdCl_2$	yes	96-h LC50 [a]

[a] Amphipods exposed in clean, filtered seawater without sediment.

[b] Only indicated for tests with *A. abdita*.

[c] A positive control is recommended.

15. Requirements for Valid Test

Document	Control Mortality	Other Requirements
Swartz *et al.,* 1985a	≤10%	NI
Chapman & Becker 1986	≤10%	NI
Envir. Can. 1990b	≤10%	no deviation from specified test conditions
USEPA 1990	≤10%	≤20% mortality in each control vessel
ASTM 1991	≤10%	≤10% of controls show signs of disease or stess; ≤20% mortality in each control vessel[a]

[a] A test should usually be considered invalid if one or more of the following occurred; all test vessels were not identical; treatments were not randomly assigned; organisms were not randomly distributed; positive or solvent controls were not included; all test animals were not from the same population, were not all of the same species or of acceptable quality; amphipods were held >2 weeks; organisms were not acclimated to test temperature and salinity for at least 48 h; DO in test vessel(s) <60%; temperatures and/or DO not measured; and observers had knowledge of the treatment of sediment in test vessels.

Appendix D

Illustration of Basic Body Parts for Gammaridean Amphipods

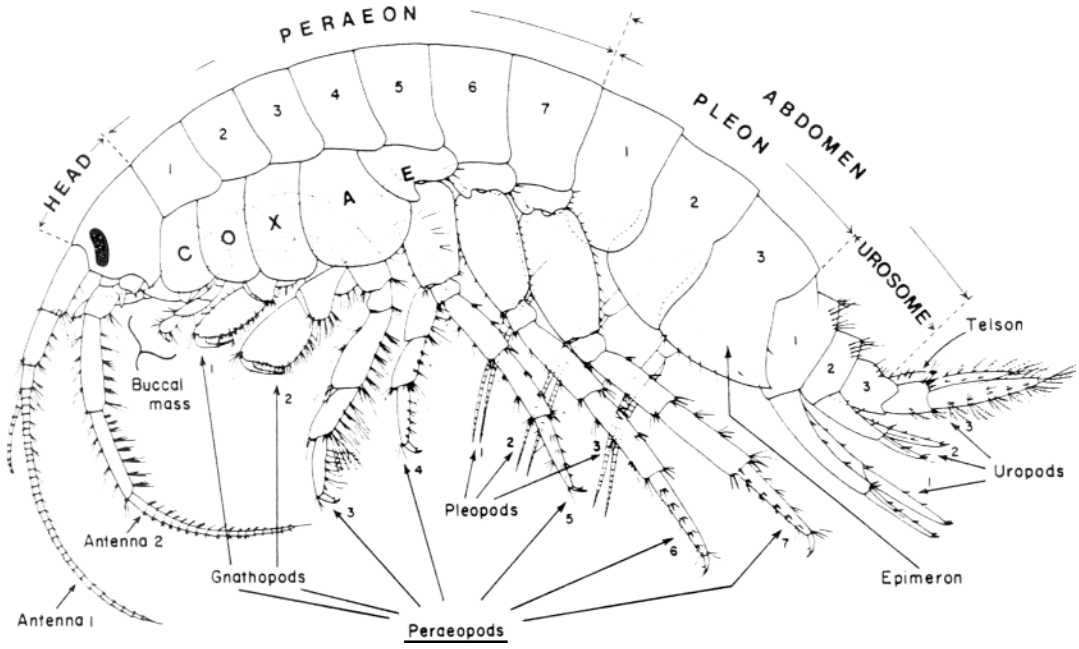
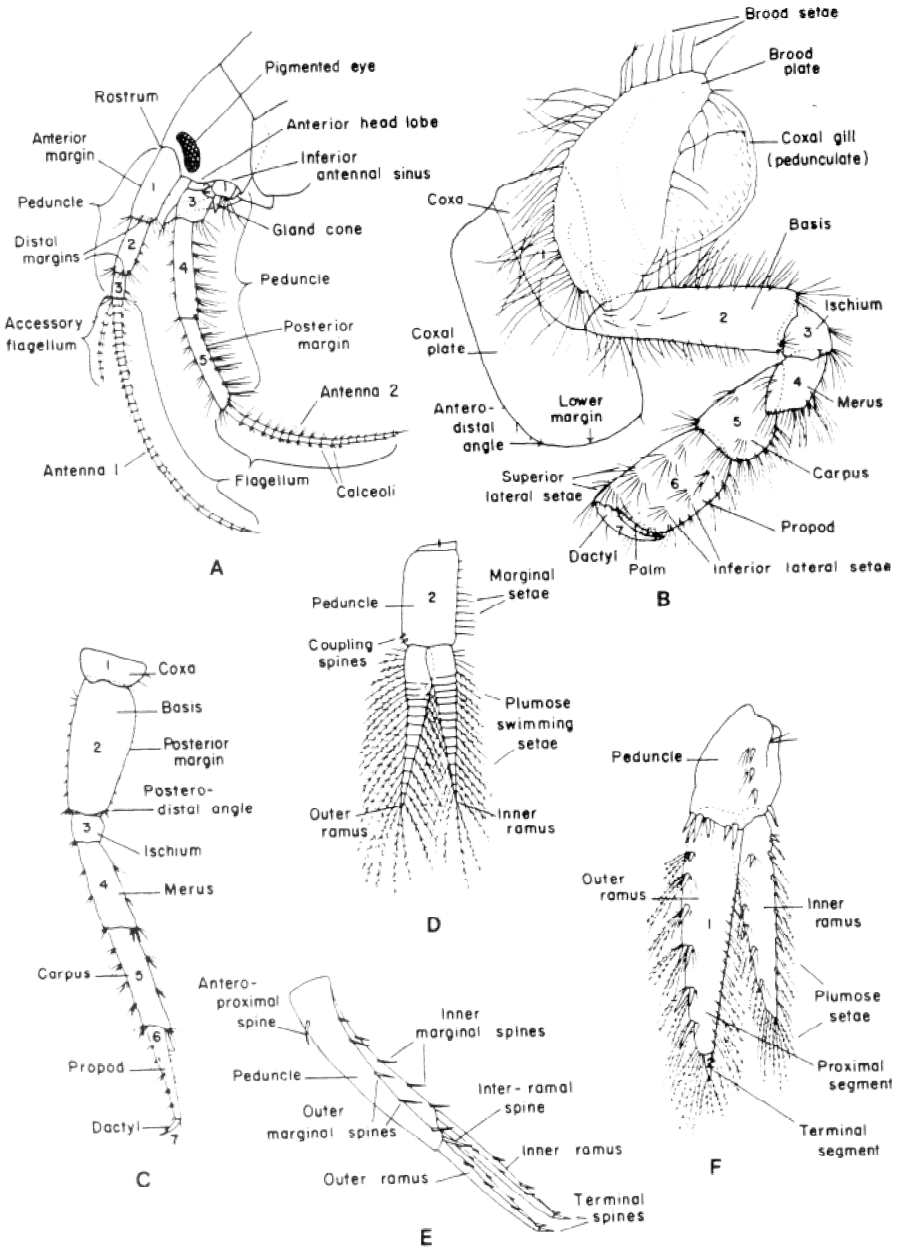


Figure D.1 Basic Gammaridean Amphipod (Lateral View) [a]

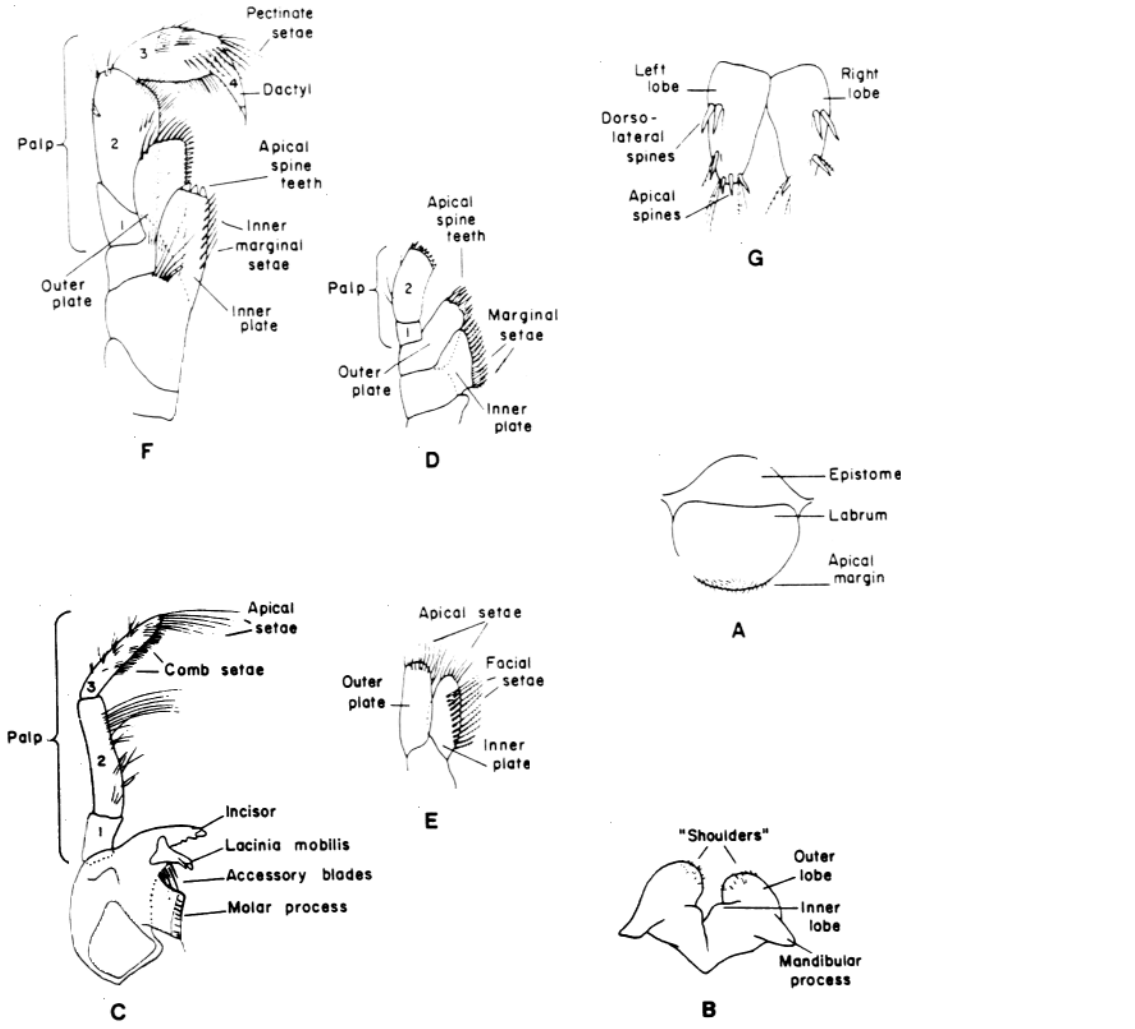
[a] Reproduced form Bousfield (1973), with permission.



A: Head region **B:** Gnathopod 2 **C:** Peraeopod 7 **D:** Pleopod
E: Uropod 1 **F:** Uropod 3

Figure D.2 Basic Gammaridean Body Appendages [a]

[a] Not to scale. Reproduced from Bousfield (1973), with permission.



A: Upper lip **B:** Lower lip **C:** Mandible **D:** Maxilla 1
E: Maxilla 2 **F:** Maxilliped **G:** Telson

Figure D.3 Basic Gammaridean Mouthparts and Telson [a]

[a] **Not to scale. Reproduced from Bousfield (1973), with permission.**

Appendix E

Amphiporeia virginiana (Shoemaker)

Distinctive Taxonomic Features

Amphiporeia virginiana is a member of the gammaridean family Pontoporeiidae. This family lacks rosturm, but has "pseudorostrum" formed from enlarged and approximated basal segments of weakly biramous antennae. The family is also distinguished by strongly fossorial (burrowing) appendages, subchelate gnathopods (often sexually dissimilar), basic mouthparts, and strongly dissimilar sexes (males smaller and with cup-calceolate antennae) (Bousfield, 1990b).

This species (Figure E.1) differs from other members of the genus *Amphiporeia* by its small size, narrow coxa 1, relatively small bases of peraeopods 6 and 7, and its narrow segment 5 of peraeopod 5. Additional distinctions are its elongate outer ramus of uropod 3, and greatly broadened maxilliped palp, segment 3 (Bousfield, 1990b) (see Appendix D).

Species Distribution

Amphiporeia virginiana is found from eastern Nova Scotia (Guysborough Co.), south along the Gulf of Maine, to the Middle Atlantic states and North Carolina. It has been recorded along the outer shores of Nantucket and Martha's Vineyard. Collection sites within close proximity to Halifax, N.S. include Martinique Beach, Marie-Joseph Beach, Clam Harbour Beach, and Cole Harbour Beach (Bousfield, 1990b).

Ecological Requirements

This species is found predominantly on surf-sand beaches, at midwater to slightly subtidal levels. *Amphiporeia virginiana* is often concentrated at freshwater stream outflows over sand flats. At Martinique Beach, N.S., where this species has been collected for laboratory studies, measurements of seabed water quality have shown a salinity of 31 g/kg and pH of 7.8 to 8.0 (Paine and McPherson, 1991a,b). During the months of March, April, June, September, and November, seawater temperatures at this intertidal collection site have ranged from 2 to 17˚C (Doe and Wade, 1991; Paine and McPherson, 1991a,b). Sediment particle sizes at the collection site have ranged from 0.063 to 1.0 mm, with the majority (80 to 87%) 0.13 to 0.25 mm (Paine and McPherson, 1991a,b).

Life Cycle and Age Class for Tests

The life cycle of *A. virginiana* has not been studied, although field collections indicate that it has an annual life cycle (one brood per year). Ovigerous females are evident from April to July (Bousfield, 1990b). Juveniles or adults, measuring 2- to 5-mm total length, should be used in the toxicity test. Very large, mature individuals (i.e., >5 mm) should not be used because they might be senescent.

Laboratory Testing and Tolerance

Based on the distribution of *A. virginiana* at locales ranging from freshwater outflows of beaches with salinities of 31 g/kg (Bousfield, 1990b; Paine and McPherson, 1991a,b), this species is thought to be tolerant of a wide range of salinities. However, recent laboratory studies found reduced 10-day survival rates for amphipods of this species held in seawater with salinities of ≤20 g/kg; whereas, 100% survival occurred in salinities of 25 g/kg or 30 g/kg (Wade and Doe, 1992).

These data indicate that *A. virginiana* is a desirable test species when studying the acute toxicity of sediment samples with pore-water salinities of 25g/kg or higher.

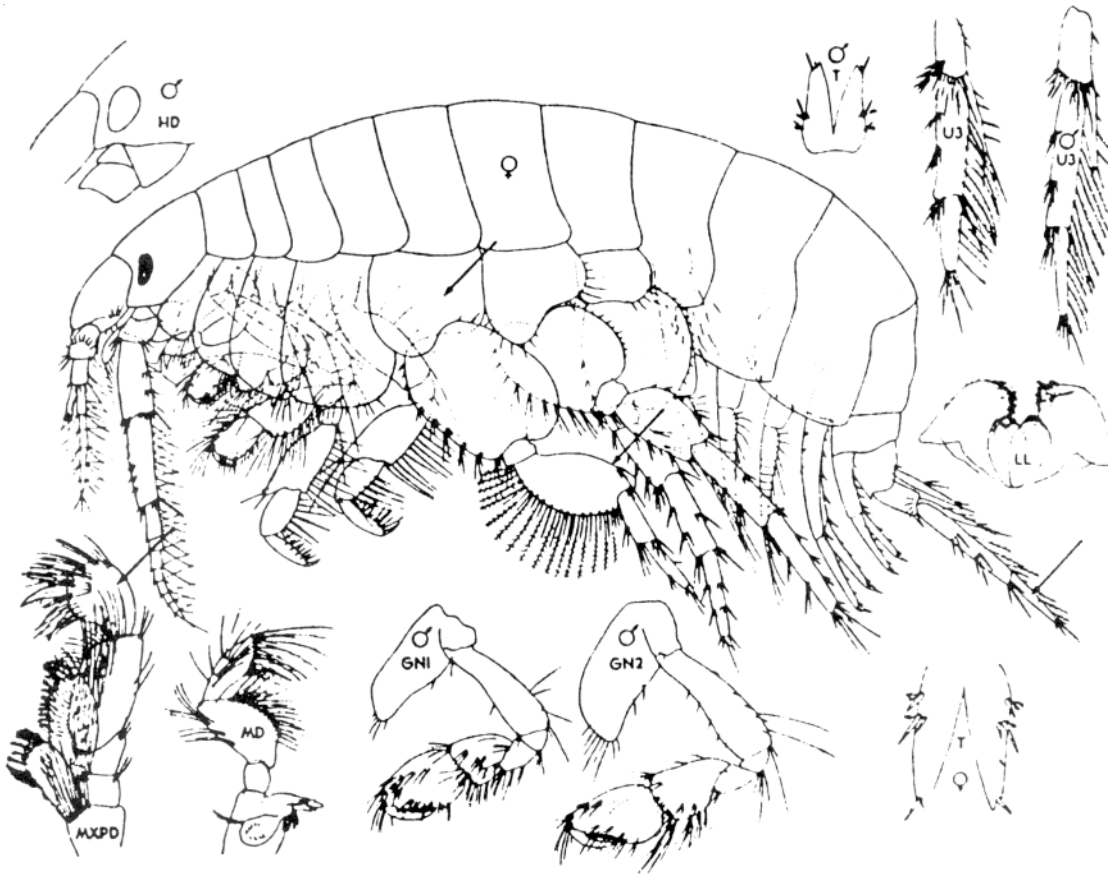
Limited data are available regarding the effect of sediment particle size or organic enrichment on the acute (10-day) survival of this species. Comparative tests by three laboratories, using coarse (97% of particles, 0.13 to 0.5 mm) and fine (30% of particles, ≤0.06 mm; 63%, 0.06 to 0.13 mm) reference sediments, showed survival rates similar to those for the control sediment (Paine and McPherson, 1991a). This preliminary finding suggests that the acute survival of *A. virginiana* in the laboratory is not influenced markedly by sediment particle size.

The acute survival of *A. virginiana* in 10-day tests with control sediment, and in 4-day seawater-only tests with cadmium chloride, has been studied using acclimation and test temperatures of 5 °C, 10 °C, and 15 °C (Doe and Wade, 1991; Paine and McPherson, 1991a, b). At 5 °C and 10 °C, 10-day survival rates in control sediment ranging from 93 to 98% have been found, whereas groups of amphipods from the same collection have shown survival rates as low as 66% when acclimated to and tested at 15 °C (Doe and Wade, 1991). Results to date indicate that acceptable control survival (≥ 90%) can be expected for this species if acclimated gradually to 10 °C (or 5 °C) and tested at this temperature; this might not be the case at 15 °C, especially when ambient temperatures are seasonally cold (Doe and Wade, 1991).

Comparative tests with *A. virginiana and R. abronius* suggest that, overall, the two species are similarly sensitive to contaminated sediment or cadmium (reference toxicant) (Doe and Wade, 1991). However, more studies are required to confirm the sensitivity of *A. virginiana* to contaminated sediment or reference chemical(s), relative to *R. abronius* or other species of marine or estuarine amphipods.

Amphiporeia virginiana usually reburies quickly when transferred from control sediment to other clean sediment or returned to control sediment, although some active individuals might remain swimming for periods of 1 h or longer following their disturbance. If disturbed, this animal often darts out of the sediment and swims quickly. When sieved, it often curls up, and will float on the surface of the water, although the animal frequently swims rapidly to the bottom. *Amphiporeia virginiana* has been described as a white, crescent-shaped amphipod, or on that is transparent, greyish in colour, with small red eyes (Paine and McPherson, 1991a, b).

Appendix G of Environment Canada (1998a) should be consulted for additional and more recent information on the grain-size tolerance limits for *A. virginiana*. This appendix also provides information on the tolerance of this species to ammonia in “water only” and “spiked sediment” tests, its known tolerance limits for porewater salinity, its historical control performance, and historical LC50s for “water only” reference toxicity tests with cadmium.



Legend:

GN	gnathopod	**T**	telson
HD	head	**U**	uropod
LL	lower lip	♂	male
MD	mandible	♀	female
MXPD	maxilliped		

Figure E.1 Taxonomic Illustration of *Amphiporeia virginiana* [a]

[a] Reproduced from Bousfield (1973), with permission.

Corophium volutator (Pallas)

Distinctive Taxonomic Features
As a member of the gammaridean family Corophiidae, distinctive features of *C. volutator* include: elongate, depressed, isopod-like body and small coxal plates; strongly sexually dimorphic, claw-tipped antenna 2 (large and heavy in males); peraeopods 3 and 4 with cement glands ducting thorough dactyls; peraeopods 5 and 6 short, reversed, 7 elongate, normal; pleopod peduncles very broad; uropod 3 very short, 1-branched; telson a small plate (Bousfield, 1990b).

This species is distinguished from other North American Atlantic corophiids by the following combination of characters (Bousfield, 1973, 1990b)(see Figure F.1): its large size (males up to 15 mm, including antennae); unfused urosomal segments; peraeopods 3 and 4, segment 5 normal (not very small); and by gnathopod 2, hind margin of dactyl (claw) smooth, lacking teeth (Bousfield, 1973, 1990b).

Species Distribution
Corophium volutator is distributed in mud and muddy-sand on both sides of the North Atlantic. In European coastal waters, this species of amphipod is widely distributed (McLusky, 1968; Peer *et al.*, 1986; Roddie and Kedwards, 1991). In North America, *C. volutator* has been found only in the Gulf of Maine; widely throughout the Bay of Fundy to about Yarmouth, N.S., and south to Casco Bay, Maine. The species is especially common along the south shore and upper portion of Minas Basin, Bay of Fundy, at the following locations: Kingsport; Evangeline Beach; Walton Beach; Burntcoat Head; Maitland (Bousfield, 1990b).

Ecological Requirements
Corophium volutator lives intertidally in the mud of estuarine mud flats, salt-marsh pools, and brackish ditches. The species resides from the low-water mark to almost the mean high-water level (Bousfield, 1973), at a maximum density exceeding 60 000/m^2 (Yeo, 1977). Animals usually form U-shaped tubes that extend to a maximum of 10 cm below the surface of the sediment (Hart, 1930; Meadows, 1964), although the species is occasionally taken planktonically (Bousfield, 1973). *Corophium volutator* is particularly abundant on mud flats during late July and August, where the species forms a major food component of migrating shorebirds (Peer *et al*., 1986; Bousfield, 1990b).

Corophium volutator inhabits sediments that are predominantly silt or clay (37% silt or clay, McLuskey 1967; $\leq$0.04 mm, Hawkins, 1985). Animals of this species are found on both sides of the North Atlantic in mud and muddy sand (Peer *et al*., 1986). *Corophium volutator* is especially abundant in sheltered conditions, and specimens are not found in heavily contaminated sediments, nor in sulphide mud blackened by excessive organic detritus or sand without a plentiful supply of detritus (McLusky, 1967). They tend to be more numerous in tidal pools than on well-drained portions of mudflats (Peer *et al*., 1986). The animals are believed to be selective deposit feeders, feeding mainly on diatoms, microalgae, and bacteria associated with sediment particles <0.06 mm (Peer *et al*., 1986).

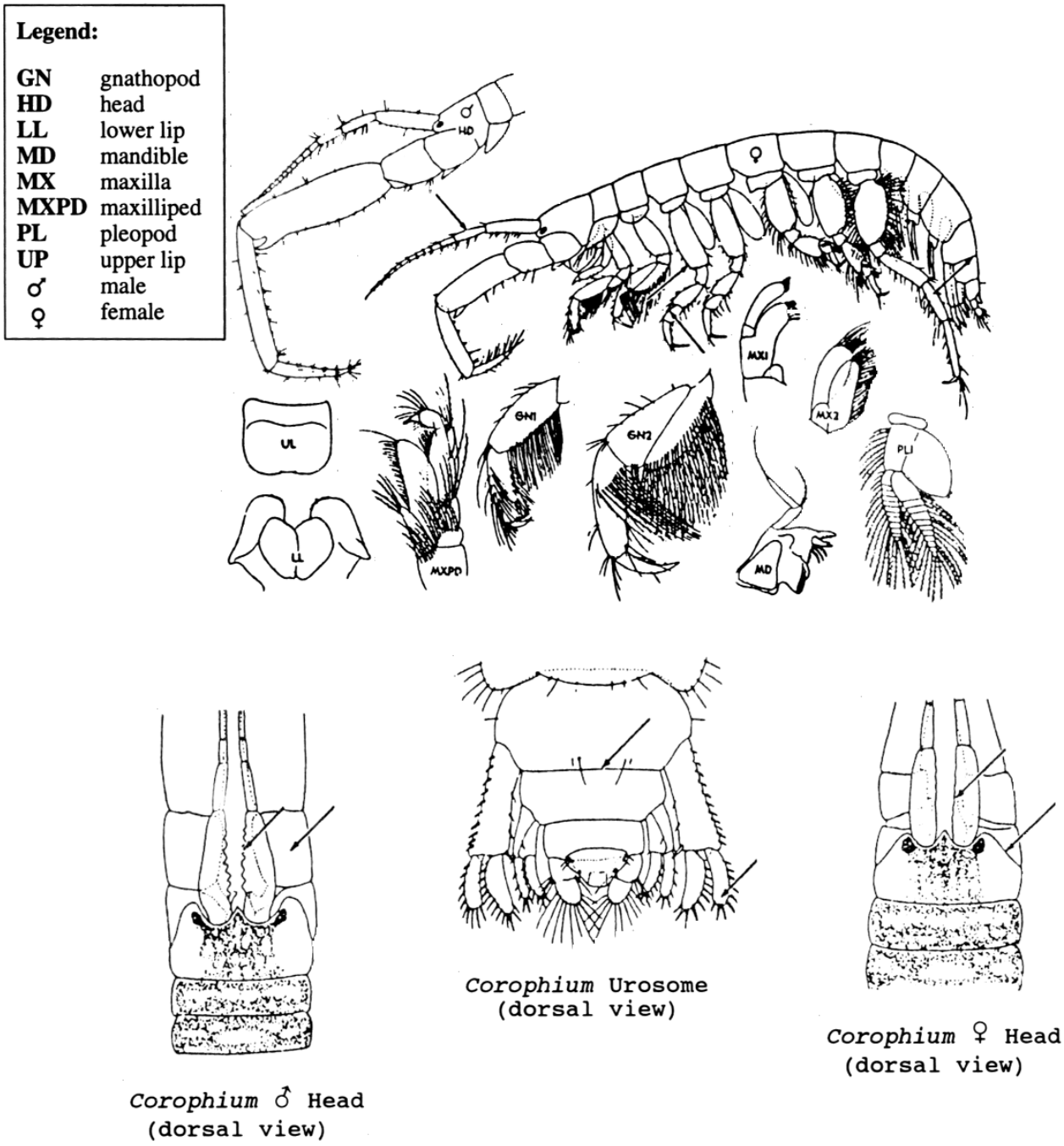


Figure F.1 Taxonomic Illustration of *Corophium volutator* [a]

[a] Reproduced from Bousfield (1973), with permission. See Appendix E for abbreviations.

This species is found over a wide range of salinities, although they are most frequently reported in estuarine waters, or seas such as the Baltic that have a reduced salinity (McLusky, 1967). *Corophium volutator* has been found in reduced numbers at areas where salinities are frequently between 2 g/kg and 5 g/kg; 2 g/kg appears to be a critical minimum salinity controlling its distribution (McLusky, 1968). The species resides in intertidal sediment with ambient temperatures of 2 to 20 °C (McLusky, 1968).

Life Cycle and Age Class for Tests

The life history and reproductive biology of *C. volutator* have been studied (Peer *et al*., 1986). The species has an annual life cycle. In the Bay of Fundy, densities are low from January to May, presumably due to ice scouring. Young are generally released in late May, and the population becomes bimodal in June. By late July or early August, the overwintering population disappears. Densities increase markedly from June to September (Fish and Mills, 1979; Peer *et al.,* 1986).

Corophium volutator attains a maximum body size, including antennae, of 12 to 15 mm (Peer *et al*., 1986; Paine and McPherson, 199lb). Juvenile or adult animals, 4- to 10-mm total length, are available year-round except when ice scours the beaches during winter months (Nicol and Doe, 1990; McLeay *et al*., 1991; Paine and McPherson, 1991a,b). Populations within this size range should be used for the toxicity test. Larger individuals should not be used because they might be senescent.

Laboratory Testing and Tolerance

Laboratory studies have demonstrated that *C. volutator* has a broad salinity tolerance. If supplied with clean sediment with a high mud content, the species can survive salinities ranging from 2 to 50 g/kg (McLusky, 1967). Growth was shown to be maximum at 15 g/kg, and only slightly lower at 4 g/kg and 31 g/kg, but progressively reduced below 4 g/kg (McLusky, 1967). Oxygen consumption rates were unaffected by a broad range of salinities (McLusky, 1969). Experiments indicated that a salinity range of 10 to 30 g/kg was preferred to higher or lower salinities (McLusky, 1970).

Ten-day mortality studies using a variety of sediments show little effect of sediment grain size or organic content on survival rates for this species. Comparative tests with *C. volutator* by three laboratories, using coarse (97% of particles, 0.13 to 0.5 mm) and fine (30% of particles, ≤0.06 mm; 63%, 0.06 to 0.13 mm) reference sediments, showed similarly high (>90%) survival rates for either sediment (Paine and McPherson, 1991a). For test sediments with up to 72% mud (silt and clay; ≤0.06 mm), survival rates as high as 98% have been recorded (Paine and McPherson, 1991b). High (≥90%) survival rates were also noted for sediment samples with concentrations of total volatile residue up to 102 g/kg total volatile residue (Paine and McPherson, 1991b).

When acclimated to and tested at 15 ± 2 °C, 10-day survival rates for *C. volutator* in control sediment are commonly ≥95% (Nicol and Doe, 1990; Paine and McPherson, 1991a,b; Tay *et al.,* 1991). Comparative tests indicate that this species is generally less sensitive to contaminated sediments than *R. abronius* or certain other species of marine or estuarine amphipods that do not inhabit tubes (Nicol and Doe, 1990; Paine and McPherson, 1991a,b; Tay *et al*., 1991). However, the 10-day survival rate for *C. volutator* can be markedly reduced by contaminated sediments

(Paine and McPherson, 1991a,b.; Tay *et al*., 1991) and the species can be useful in identifying the spatial distribution of contaminant gradients (Roddie and Kedwards, 1991). Four-day seawater-only tests with *C. volutator* and *R. abronius* indicated that the relative sensitivity of these two species to each of five chemicals varied depending on the chemical (Nicol and Doe, 1990).

The species is a brownish colour, with distinct antennae and darkened bands on the body dorsum. *Corophium volutator* does not float when sieved, but is very easy to collect due to its size. The animal curls up and stretches when swimming. Unlike other species of estuarine or marine amphipods studied, *Corophium volutator* generally does not bury quickly when transferred to control or other clean sediment, rendering this species unsuitable for a 1-h test for reburial success at the end of a 10-day study (Paine and McPherson, 1991a). However, once buried in clean sediment, the species normally remains buried during the acclimation and test periods (Paine and McPherson, 1991a). Emergence from contaminated sediments is common for this species (McLeay *et al*., 1991; Paine and McPherson, 1991a); this behaviour can make the overlying seawater turbid and prevent observations of numbers emerged.

Although less sensitive to contaminated sediments than *R. abronius* and certain other species of free-burrowing, estuarine or marine amphipods, *C. volutator* is useful for assessing the acute toxicity of sediments that have salinities of pore water ranging from 2 to >28 g/kg.

Eohaustorius estuarius (Bosworth)

Distinctive Taxonomic Features

This species of estuarine amphipod is a member of the gammaridean family Haustoriidae. *Eohaustorius estuarius* and other members of this family have the following characteristics (Bosworth, 1973) (see Figure G.1): body short, very broad, urosome deflected beneath pleosome; basal segments of antennae and peraeopods strongly broadened and spinose, adapted for burrowing in sand; mouthparts of filter-feeding type; gnathopods slender, unlike, simple or minutely cheliform; peraeopods 3 to 7 lacking dactyls; pleopods powerfully modified; uropod rami linear (not falcate); coxal gills lacking on P7; brood plates medium broad.

The species is distinguished from other haustoriids by the following combination of characters (Bosworth, 1973; see Figure G.1): peraeopods 3 and 4 strongly differing in form and size; peraeopod 7, basis smooth behind, lacking proximal cusp; peraeopod 5, outer face of segments 4 and 5, and margins, nearly lacking spine clusters; peraeopod 7 segment 6, posterior margin with only 1 to 2 spine clusters.

Species Distribution

Eohaustorius estuarius is distributed along the west coast of North America, ranging from central British Columbia, south to at least central California (ASTM, 1991a). It has been found on beaches of the Queen Charlotte Islands, but has not been taken in southeastern Alaska (Bosworth, 1973; Bousfield, 1990a, b). Suitable collection sites exist on the west coast of Vancouver Island (e.g., at McKenzie Beach or Long Beach, Wickaninnish Bay).

Ecological Requirments

This free-burrowing species of amphipod is found on protected and semiprotected beaches, from mid-winter level to shallow subtidal, within the upper 10 cm of sediment (ASTM, 1991a; Bousfield, 1990b). On open coasts, *E. estuarius* occurs in beds of freshwater streams flowing onto the beach, and in sand banks in estuaries, above the level of other regional eohaustoriids (*E. sawyeri* and *E. washingtonianus*) (Bousfield, 1990b). Peak densities occur near the mouths of streams and rivers where salinity of pore water ranges between 15g/kg and 25 g/kg, although this species has been found in estuarine streams where salinities of pore water regularly drop below 10 g/kg (DeWitt *et al*., 1989).

Eohaustorius estuarius inhabits clean, medium-fine sand with some organic content. Sediment particle sizes at collection sites have ranged from 0.06 to 2.0 mm, with the preponderance 0.13 to 0.25 mm (Paine and McPherson, 1991a,b). Collection-site salinities and temperatures have ranged from 1 to 8 g/kg and from 8 to 13 °C; respectively.

Life Cycle and Age Class for Tests

Eohaustorius estuarius appears to have an annual life cycle, with gravid females abundant in intertidal sediments from February through July (DeWitt *et al*., 1989; ASTM, 1991a). However, because juveniles are found throughout most of the year, reproduction might occur year-round (DeWitt *et al*., 1989). Large juvenile and adult animals, 3- to 5-mm total length, should be used for the toxicity test because they are available year-round and are easily handled

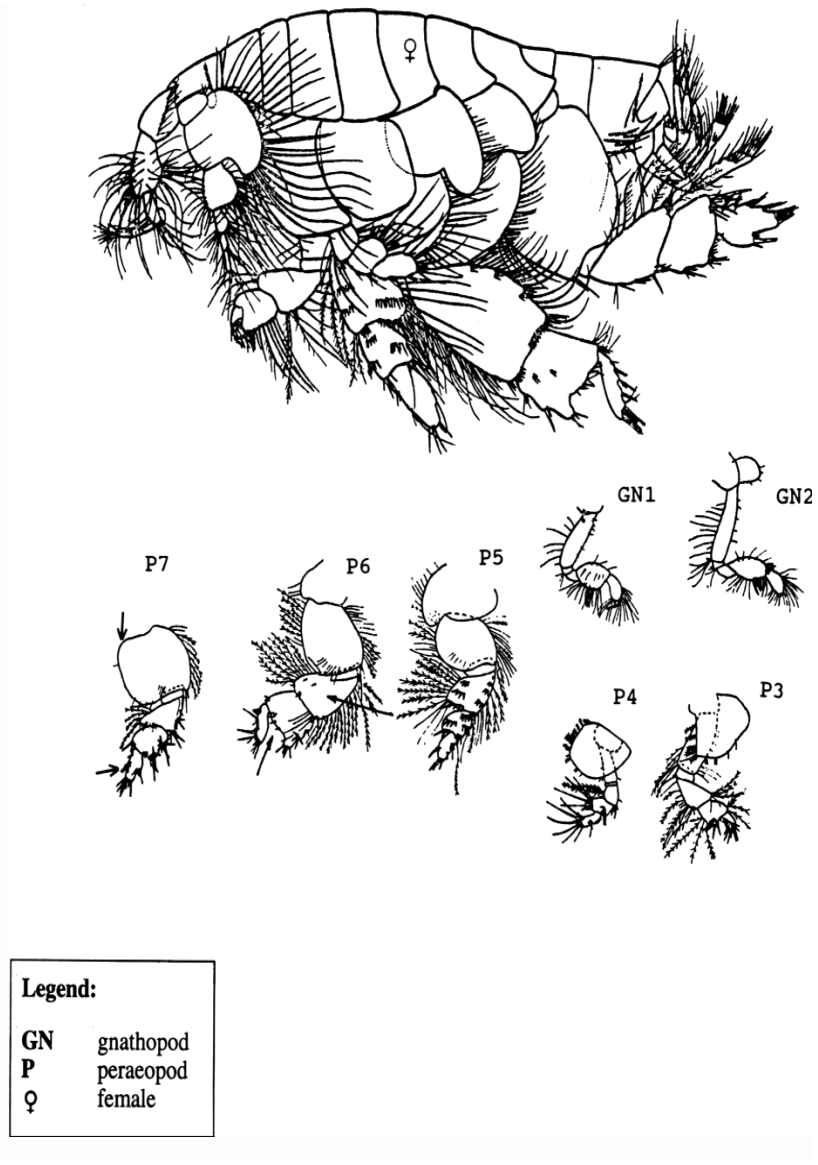


Figure G.1 Taxonomic Illustration of *Eohaustorius estuarius* [a]

[a] Body modified from Barnard (1962); appendages form Bosworth (1973). Reproduced from Bousfield (1990b), with permission. See Appendix E for abbreviations.

(ASTM, 1991a). Very large (i.e., >5 mm) individuals should not be used, because they might be senescent.

Laboratory Testing and Tolerance

This species has a broad salinity tolerance. With no acclimation to salinity, *E. estuarius* has shown good survival rates (≥95%) at salinities from 2 to 28 g/kg (DeWitt *et al*., 1989). The species is tolerant of a wide range of sediment grain sizes, with generally little if any effect on survival whether coarse-grained or very fine-grained (predominantly silt and clay) clean sediments are tested (DeWitt *et al*., 1989; Paine and McPherson, 1991a). However, because this species normally inhabits sandy sediments and some correlation between survival and sediment grain size might exist (DeWitt *et al*., 1989; Paine and McPherson, 1991b), toxicity tests should include clean reference sediments with a range of particle sizes characteristic of those of the test sediment(s).

Ten-day survival rates for *E. estuarius* held in control sediment are generally ≥95% when tested at 15 ± 2 °C across a 2 to 28 g/kg salinity range (DeWitt, 1989) or at 28 g/kg (Paine and McPherson, 1991a,b). Generally, *E. estuarius* is slightly less sensitive than *R. abronius* to contaminants (ASTM, 1991a; Paine and McPherson, 1991a). However, some examples exist where a greater sensitivity to contaminated sediment has been reported for *E. estuarius* (DeWitt *et al*., 1989; Paine and McPherson, 1991b).

Eohaustorius estuarius is greyish-brown or yellowish-brown, with a dark oval spot on the dorsal surface. They are cup-shaped and "ghost-like". Individuals swim slowly, dorsal side down, often in a spiral pattern, and do not float well but are easy to catch with a pipette, when sieved from control to sediment. When returned to sediment, they reburrow rapidly (within 10 min). Because they do not float and blend in easily with the colour of the sediment, specimens of *E. estuarius* are more difficult to remove from test sediments than those of *R. abronius* and certain other species of infaunal amphipods, although changing the water used for sieving will help their recovery at the end of the test. *Eohaustorius estuarius* is a desirable species for testing sediments that have salinities of pore water between 2g/kg and 28 g/kg.

Appendix F of Environment Canada (1998a) should be consulted for additional and more recent information on the grain-size tolerance limits for *E. estuarius*. This appendix also provides information on the tolerance of this species to ammonia in "water only" and "spiked sediment" tests, its known tolerance limits for porewater salinity, its historical control performance, and historical LC50s for "water only" reference toxicity tests with cadmium.

Eohaustorius washingtonianus (Barnard)

Distinctive Taxonomic Features

Eohaustorius washingtonianus is a member of the gammaridean family Haustoriidae. This and other haustoriids have the following characteristics (Bosworth, 1973) (see Figure H.1): body short, very broad, urosome deflected beneath pleosome; basal segments of antennae and peraeopods strongly broadened and spinose, adapted for burrowing in sand; mouthparts of filter-feeding type, with large setose lobes on maxillae and maxillipeds; gnathopods slender, unlike, simple or minutely cheliform; peraeopods 3 to 7 lacking dactyls; pleopods powerfully modified, peduncles short, broad; uropod rami linear (not falcate); coxal gills lacking on P7; brood plates medium broad.

Eohaustorius washingtonianus is distinguished from other haustoriids by the following combination of characteristics (Bosworth, 1973; see Figure H.1): peraeopods 3 and 4 strongly differing in form and size; telson lobes widely separated, attached posterodorsally; peraeopod 5, outer face of segments 4 and 5, and margins, with strong spine clusters; peraeopod 7, basis sparsely setosed behind, with stout proximal cusp; peraeopod 7, segment 6, posterior margin with three spine clusters.

Species Distribution

Eohaustorius washingtonianus is distributed along the west coast of North America from southeastern Alaska to Oregon (Bousfield, 1990a, b). Suitable collection sites exist west of Victoria, B.C. (Witty's Lagoon or the exposed side of Esquimalt Lagoon); at West Beach, Whidbey Island (Washington State); and at the main beach at Point Roberts, Washington State (Bousfield, 1990a, b; Paine and McPherson 1991a, b; Yee *et al*., 1992).

Ecological Requirements

This free-burrowing species of amphipod is found on surf-exposed and semiprotected sand beaches, from mid-water level to at least shallow subtidal (Bosworth, 1976; Bousfield, 1990a, b). There is some indication from collection records that the vertical distribution of this species becomes lower the further south it is found, although the extent of subtidal distribution remains unresolved (Bosworth, 1976; Bousfield, 1990a). *Eohaustorius washingtonianus* is the most common haustoriid species on beaches of British Columbia (Bousfield, 1990a, b).

The species tolerates a wide range of salinities (usually 12 to 33 g/kg) and surface summer temperatures (10 to 22 °C) (Bousfield, 1990b). At a B.C. subtidal collection site adjacent to Esquimalt Lagoon, salinities just above the sediment were 36 g/kg, 35 g/kg, and 35 g/kg, and temperatures were 8 °C, 11 °C, and 7 °C, during the months of January, June, and October, respectively (McPherson and Paine, 1991a, b; Yee *et al*., 1992).

Eohaustorius washingtonianus normally resides in the surficial layer of clean, medium-fine sand with relatively low organic content (Bousfield, 1990b). Sediment particle sizes at a B.C. collection site have ranged from 0.13 to 2.0 mm, with the preponderance (62 to 80%) 0.25 to 0.5 mm (McPherson and Paine, 1991a, b; Yee *et al.*, 1992).

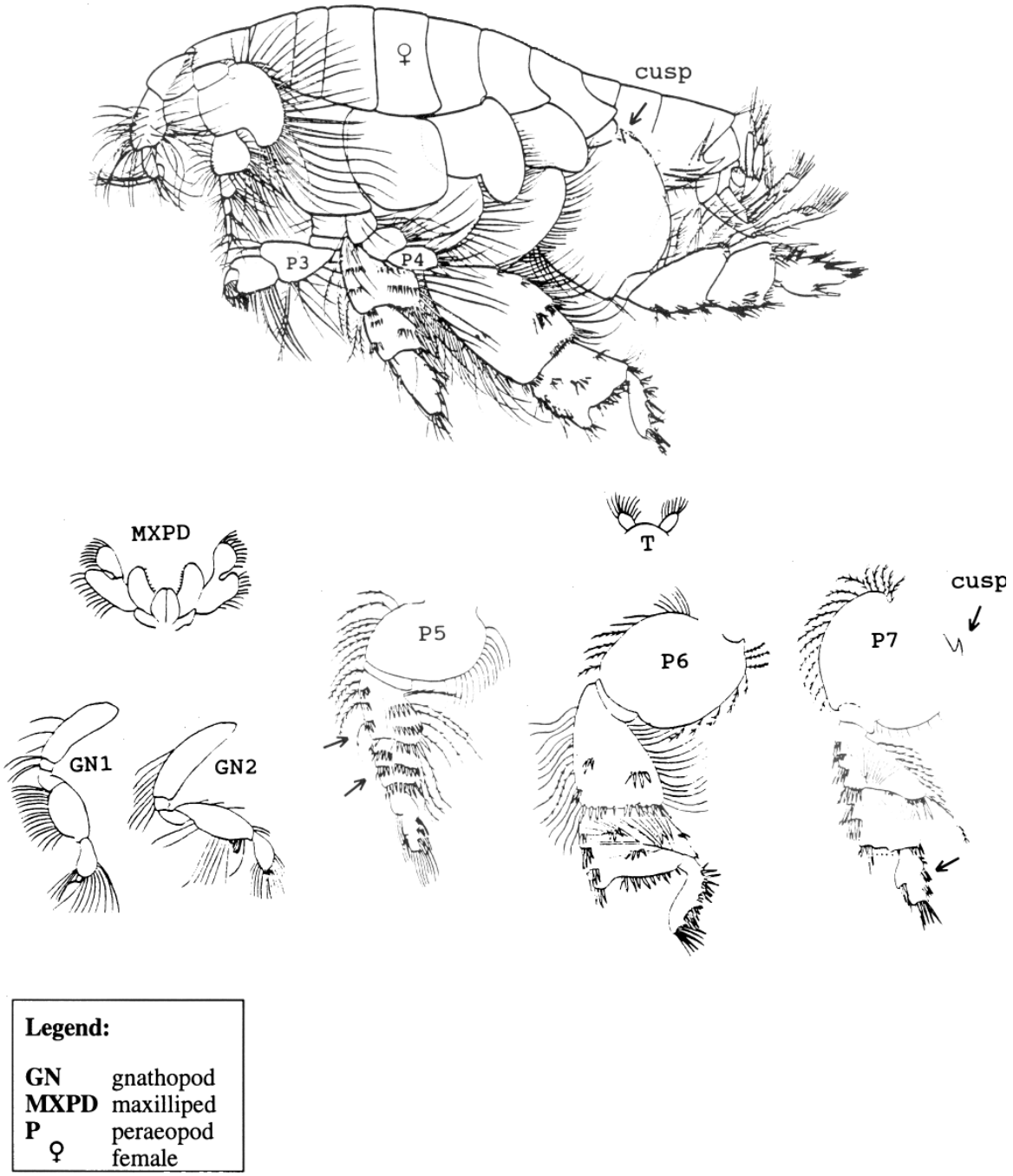


Figure H.1 Taxonomic Illustration of *Eohaustorius washingtonianus* [a]

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[a] From Thorsteinson (1941), as provided by Bousfield (1990b). See Appendix E for abbreviations.

Life Cycle and Age Class for Tests

The life cycle of *E. washingtonianus* is presumably annual, with some ovigerous females present throughout the year (Bosworth, 1976; Bousfield, 1990b). In Oregon, the percentage of gravid females is highest in February (60 to 70%), with 10 to 20% gravid females throughout the remainder of the year (Bosworth, 1976). No consistent trends are evident seasonally with regard to the relative abundance of males and females, although there is a tendency toward more females than males (Bosworth, 1976).

The percentage of smaller individuals (<3 mm) reaches a maximum in June (Bosworth, 1976). Juvenile and adult animals measuring 2.5- to 5-mm total length are available year-round (Bosworth, 1976), and should be used for the toxicity test. Very large (i.e., >5 mm) individuals should not be used because they might be senescent.

Laboratory Testing and Tolerance

Eohaustorius washingtonianus appears to be somewhat less euryhaline than *E. estuarius* (Bosworth, 1976). Laboratory studies with *E. washingtonianus* showed good (95 to 100%) survival during a 2-day exposure to salinities of 14 g/kg to 28 g/kg at temperatures of 5 °C, 11 °C, or 15 °C[b]. However, at salinities ≤6 g/kg, poor (≤25%) survival was evident for this species at each of these test temperatures (Bosworth, 1976). In these studies, acute survival of *E. washingtonianus* was reduced when tests were conducted at 21 °C.

Insufficient data are presently available to determine if this species is sensitive to grain-size effects. An initial study by Environment Canada researchers of laboratory performance of *E. washingtonianus*, demonstrated an overall mean 10-day survival rate of 87% for fine-grained reference sediment (31% of particles, <0.06 mm; 95%, <0.13 mm), and a 10-day survival rate of 91% for coarse-grained reference sediment (97%, 0.13 to 0.5 mm) (Paine and McPherson, 1991a). In a subsequent study, survival rates for *E. washingtonianus* held for 10 days in fine-grained reference sediment were reduced relative to those held in more coarse-grained control sediment (as was also the case for *E. estuarius*; however, the authors questioned whether this difference reflected a grain-size effect (Paine and McPherson, 1991b).

Interlaboratory studies performed with *E. washingtonianus* found that the 10-day survival rate for this species in control sediment was below the 90% criterion for test acceptability; mean values ranged from 79 to 89%, depending on laboratory and test (Paine and McPherson, 1991a, b). Subsequent studies, whereby greater care was taken to reduce handling stress and minimize temperature changes during transport and the initial period of laboratory acclimation, demonstrated acceptably high (95 to 98%) 10-day survival rates in control sediment (Yee *et al*., 1992). Interlaboratory studies with *E. washingtonianus* and other species of amphipods common to Canada's coastal waters indicate that *E. washingtonianus* is among the most sensitive of those species studied (including *R. abronius*) to samples of contaminated sediments (Paine and McPherson, 1991a, b). Based on these findings, *E. washingtonianus* is recommended as a species of amphipod appropriate for this test. Experience to date

[b] When *E. estuarius* were tested using the same salinity and temperature regimes, good (95 to 100%) 48-h survival was found at salinities ≥6 g/kg and temperatures ranging from 5 to 21 ˚C (Bosworth, 1976).

indicates that considerable care must be taken during collection, transport, and acclimation of this species to achieve acceptable (mean, ≥90%) survival rates for controls.

Eohaustorius washingtonianus cannot be distinguished from *E. estuarius* without microscopic observation. *Eohaustorius washingtonianus* is greyish-brown or yellowish-brown, with a dark oval spot on the dorsal surface. Animals are cup-shaped and "ghost-like". Individuals swim slowly, dorsal side down, often in a spiral pattern, and do not float well but are easy to catch with a pipette, when sieved from control sediment. Upon return to sediment, they reburrow rapidly (within 10 min). Because they do not float and blend in easily with the colour of the sediment, specimens of *E. washingtonianus* are more difficult to remove from test sediments than those of *R. abronius* and certain other species of infaunal amphipods, although changing the water used for sieving will help their recovery at the end of the test.

Appendix E of Environment Canada (1998a) should be consulted for additional and more recent information on the grain-size tolerance limits for *E. washingtonianus*. This appendix also provides information on the tolerance of this species to ammonia in "water only" and "spiked sediment" tests, its known tolerance limits for porewater salinity, its historical control performance, and historical LC50s for "water only" reference toxicity tests with cadmium.

Foxiphalus xiximeus (Barnard)

Distinctive Taxonomic Features

Like *R. abronius* this species of marine amphipod is a member of the gammaridean family Phoxocephalidae. *Foxiphalus xiximeus* is distinguished from other phoxocephalids by the following characteristics (Jarrett and Bousfield, 1992) (see Figure I.1): rostrum full, not laterally incised; eyes large in both sexes; dactyls of P3 and P4 distinct, not minute; segments 4 and 5 of P5 strongly broadened, but of P7 only slightly broadened; abdominal side plate 3, hind margin with setae at corner only; right mandible totally lacking lacinia mobilis.

Species Distribution

Foxiphalus xiximeus is found along the west coast of North America, ranging from the Aleutian Islands to southern California (Jarrett and Bousfield, 1992). In British Columbia, this species is common around Vancouver Island (e.g., James Island, near Sidney; Witty's Lagoon and Esquimalt Lagoon, west of Victoria) and the central coast, but rare on the Queen Charlotte Islands. It has been identified in coastal waters of southeastern Alaska and Prince William Sound, and also at Unimak Island, Aleutians (Jarrett and Bousfield, 1992).

Ecological Requirements

Foxiphalus xiximeus inhabits clean, medium-to-fine sand. Sediment particle sizes at collection sites have ranged from 0.13 to 1 mm, with the majority 0.25 to 0.5 mm (Paine and McPherson, 1991a, b). During field collections, measurements of seabed water quality have shown the following: salinity 35 g/kg; pH 7.8; temperature 8 to 11 °C. This species can be found intertidally at the low-water mark, or subtidally to depths of at least 25 m.

Life Cycle and Age Class for Tests

The life cycle of *F. xiximeus* has not been studied, although field collections indicate that it has an annual life cycle (one brood per year). Large juvenile or adult individuals, measuring 3- to 6-mm total length, should be used in the toxicity test. Animals of this size are available year-round at subtidal collection sites, and are easily handled in the toxicity test. Very large, mature individuals (i.e., > 6 mm) should not be used because they might be senescent.

Laboratory Testing and Tolerance

The range of tolerance of *F. xiximeus* to salinity and temperature extremes has not been studied. Collection-site salinities indicate that the species is suitable for testing sediment samples when salinities of pore water are ≥25 g/kg.

Limited information is available regarding the effect of sediment particle size or organic enrichment on the acute (10-day) survival of this species. Comparative tests by three laboratories, using coarse (97% of particles, 0.13 to 0.5 mm) and fine (30% of particles, ≤0.06 mm; 63%, 0.06 to 0.13 mm) reference sediments, showed similarly high survival rates (Paine and McPherson, 1991a). For test sediments with up to 72% mud (silt and clay; ≤0.06 mm), survival rates as high as 95% have been recorded (Paine and McPherson, 1991b). High (≥90%) survival rates were also noted for sediment samples with values of up to 124 g/kg total volatile residue (Paine

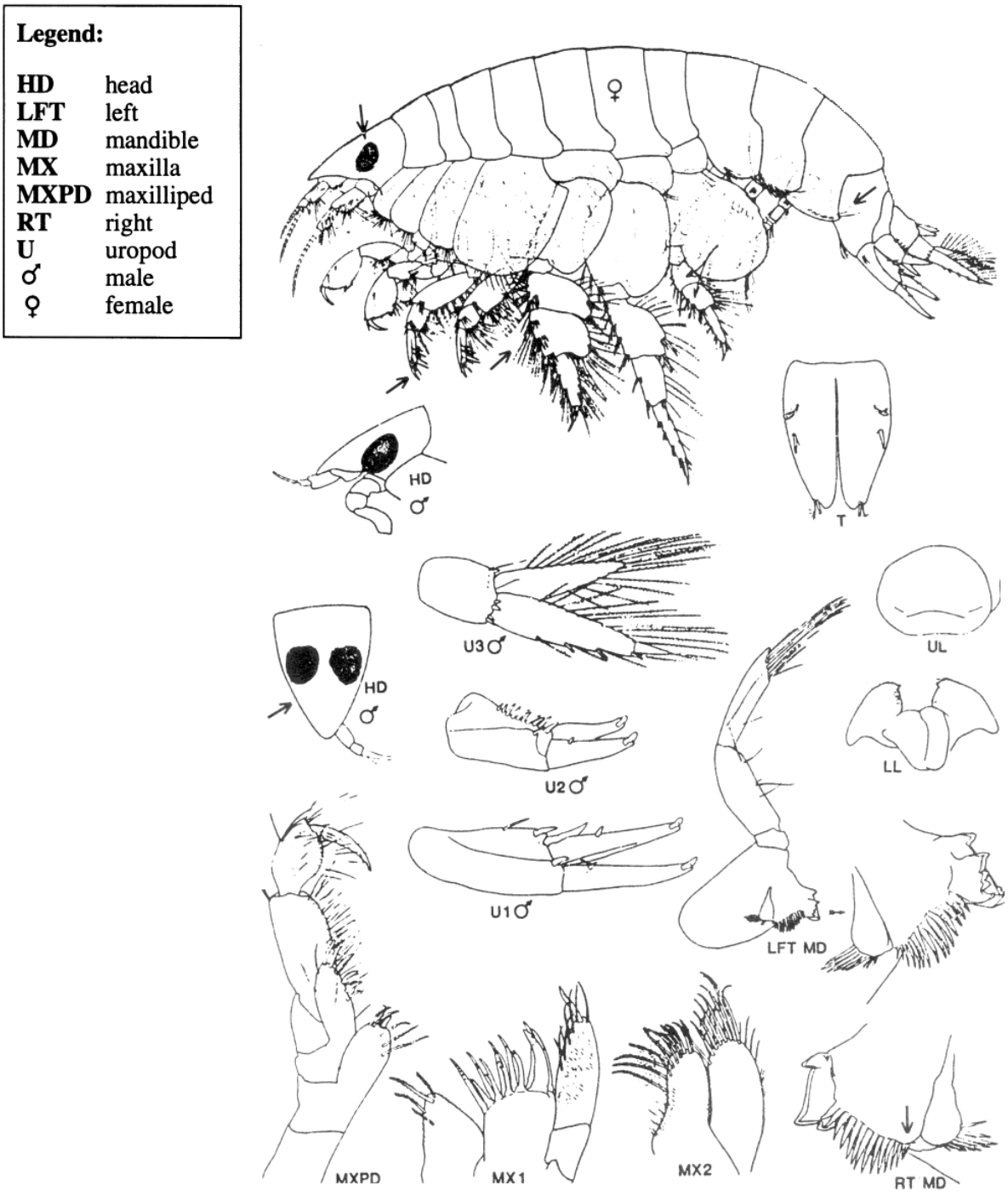


Figure I.1 Taxonomic Illustration of *Foxiphalus xiximeus*[a]

[a] Reproduced from Jarret and Bousfield (1992), with permission. See Appendix E for abbreviations.

and McPherson, 1991b). These data show little influence of sediment particle size or organic content on laboratory survival of *F. xiximeus*.

Ten-day survival rates in control sediment average 98% in tests conducted to date at 15 ± 2 °C. Comparative tests with contaminated sediments indicate that, overall, the sensitivities of *F. xiximeus, R. abronius*, and *E. estuarius* are similar (Paine and McPherson, 1991a, b).

Foxiphalus xiximeus reburies quickly (within 10 min) when transferred from control sediment to this or other clean sediment. Individuals can swim quickly. When sieved, they will float on their side on the side on the surface of the seawater, facilitating their collection. These animals are translucent, brown to grey in colour, often with a black or brown stripe on the dorsal surface. The anterior end of the animal is pointed, and there is frequently a distinctive white patch on the posterior end. *Foxiphalus xiximeus* is shaped similar to *R. abronius*, but is more compressed laterally. Because populations of *F. xiximeus* are frequently found together with *R. abronius* and other species of amphipods, care must be taken while sorting this species. Except for this detractor, ease of handling *F. xiximeus* in the laboratory and the field, and its use in toxicity tests, is similar to that for *R. abronius*.

Leptocheirus pinguis (Stimpson)

Distinctive Taxonomic Features

Leptocheirus pinguis is a member of the gammaridean amphipod family Aoridae, superfamily Corophioidea (open-ended tube dwellers). It is distinguished from other aorids by a combination of characteristics (see Figure J.1): body, stout, heavy; coxal plates large, deep, strongly setose below; gnathopod 1 (male), only moderately (not enormously) larger than in female; gnathopod 2, slender, simple; peraeopods 5 to 7, bases broad; urosome segments 1 and 2 with dorsal clumps of setae and/or cusps; uropod 3 subequally biramous (Bousfield, 1973, 1990b).

Leptocheirus pinguis is distinguished from *L. plumulosus* by its distally narrowed and angled coxa 1, the distally narrowed anterior lobe of coxa 5, and the cuspate and spinose telson (Bousfield, 1990b).

Species Distribution

Leptocheirus pinguis is found along the North American Atlantic coast, from southern Labrador to Virginia. It is common in the cold, outer-coast estuaries of eastern Canada. Along the outer coast of Nova Scotia, it has been collected in cold estuaries of Guysborough Harbour, Bourgeois Inlet, and Lennox Passage at Grandique Point. In southwestern Nova Scotia, this species has been found at Split Point, Clarke's Harbour, and Middle West Pubnico, at extreme low-water levels. Within the Bay of Fundy (Minas Basin and Minas Channel), *L. pinguis* has been collected at Kingsport, Diligent River, and Spencer's Island, all at low-water level (Bousfield, 1958; Bousfield and Leim, 1960; Bousfield and Laubitz, 1972).

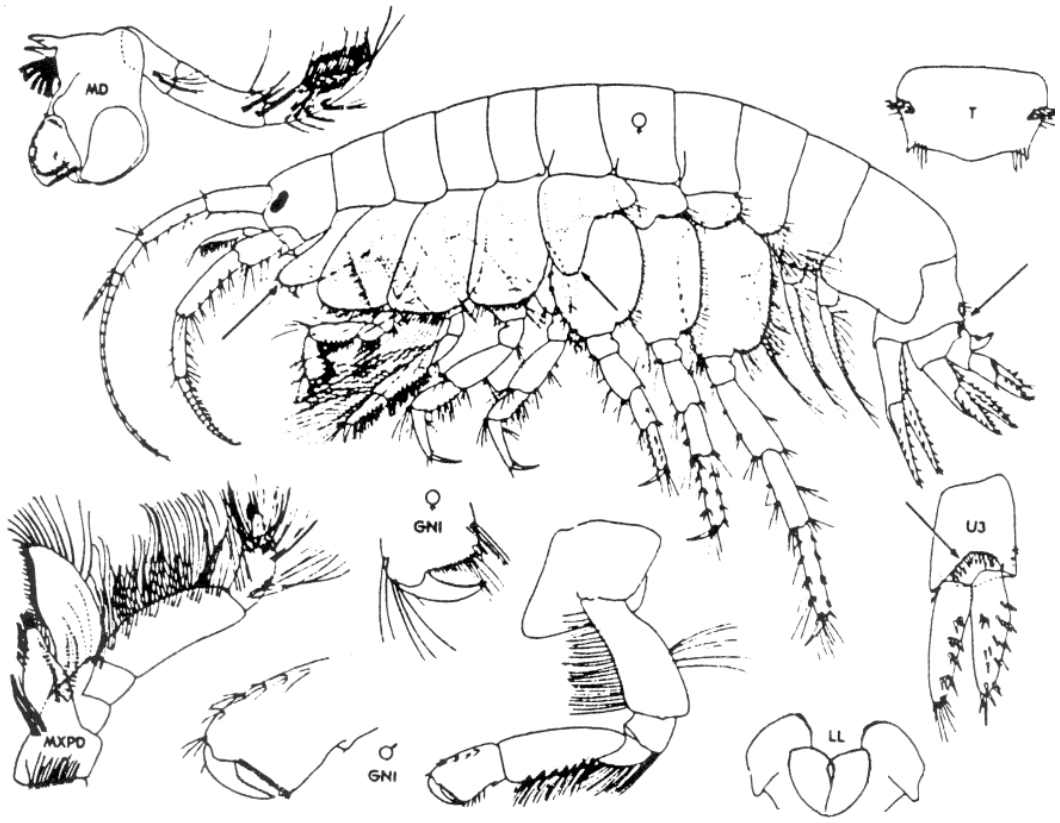
Ecological Requirements

Leptocheirus pinguis occurs from low intertidal, to subtidal depths of more than 250 m, on fine sediments (silty sand or mud), especially in channels of outer-coast estuaries where current flow is appreciable. A boreal species, *L. pinguis* tolerates winter temperatures near freezing as well as a limited range of temperatures in summer (usually 10 to 15 °C, rarely above 20 °C). It is normally found where salinities are $\geq$25 g/kg (Bousfield, 1990b).

The animal constructs flimsy, parchment-like tubes of bottom sediments and debris. Although a tube-dweller, *L. pinguis* is almost certainly a deposit feeder and thus is expected to be reasonably sensitive to contaminated sediment (Bousfield, 1990b).

Life Cycle and Age Class for Tests

The life history and reproductive biology of *L. pinguis* has not been studied. The species probably has a 2-year life cycle, with ovigerous females in April though June (Bousfield, 1973). Field collections in June have identified large individuals (16 to 18 mm; probably second-year animals) together with medimum-sized animals (approximately 5 to 8 mm) and some very small (<1 mm) individuals (Paine and McPherson, 1991b). Juvenile or adult animals, 4- to 10-mm total length, are available during the year except over the winter period (January through March, perhaps April) when ice can cover collection sites. Populations within this size range should be used for the toxicity test. Larger individuals should not be used because they might be senescent and/or create overcrowded conditions in the test.



Legend:

GN	gnathopod	**MXPD**	maxilliped	♂	male
LL	lower lip	**T**	telson	♀	female
MD	mandible	**U**	uropod		

Figure J.1 Taxonomic Illustration of *Leptocheirus pinguis*[a]

[a] Reproduced from Bousfield (1973), with permission. See Appendix E for abbreviation.

Laboratory Testing and Tolerance

Ranges of salinity and temperature tolerance for *L. pinguis* have not been studied in the laboratory, nor have the effects of particle size or organic content on laboratory survival been examined in depth. At a test temperature of 15 ± 2 °C, 10-day survival rates in control or reference sediments have averaged 96% (Paine and McPherson, 1991b). These sediments have comprised 70% (control) or 64% (reference) mud (≤ 0.06 mm), and contained total volatile residues of up to 72 g/kg (Paine and McPherson, 1991b).

Insufficient information is presently available to judge the sensitivity of *L. pinguis* to contaminated sediment, relative to other species of estuarine or marine amphipods. Preliminary data, using three samples of test sediment, indicate that *R. abronius* might be somewhat more sensitive, although this finding was not consistent for each of the test sediments examined (Paine and McPherson, 1991b).

Leptocheirus pinguis is brownish-grey in colour, and is distinguished by numerous dark bands or stripes dorsally across their bodies. Eyes are small but evident. Animals of this species swim similar to *R. abronius*, and burrow quickly (within 10 min) when transferred to clean sediment. *Leptocheirus pinguis* often floats on the water surface after being sieved from sediment. Individuals curl up when resting on the sieve, but do not curl when floating (Paine and McPherson, 1991b).

Rhepoxynius and abronius (Barnard)

Distinctive Taxonomic Features

This species of marine amphipod is a member of the gammaridean family Phoxocephalidae. It is distinguished from other phoxocephalids by the following characteristics (Barnard, 1960; Jarrett and Bousfield, 1992) (see Figure K.1): rostrum slender, sharply incised in front of eyes (dorsal view); epistome acutely produced in front; gnathopods weakly subchelate, wrist (carpus) elongate; basis of P7, hind margin with 8 to 10 shallow cusps; peraeopods 5 to 7 dactyls long, slender; uropods 1 and 2, peduncles strongly spinose behind.

Species Distribution

The genus *Rhepoxynius* is widely found along the east and west coasts of North America (Barnard and Barnard, 1982; Jarrett and Bousfield, 1992). Abundant populations of *R. abronius* occur along the west coast, ranging from central British Columbia to southern California. This species is not found on the east coast of North America. Within British Columbia, *R. abronius* is common from Vancouver Island (e.g., McKenzie Beach, near Tofino; Pachena Bay, near Bamfield) to the northern Queen Charlotte Islands.

Ecological Requirements

Rhepoxynius abronius inhabits clean sands on the continental shelf and along the lower portion of estuaries where salinities are no lower than 20 g/kg. The species is found mainly on outer, high-salinity beaches with full-ocean salinity. This infaunal amphipod can burrow as deep as 6 cm, but normally inhabits the upper 2 cm of sediment (Swartz *et al*., 1985a). Its occurrence is common in uncontaminated sediment in the lower intertidal and nearshore subtidal zones, although it is typically a subtidal species found to depths of 274 m (Barnard and Barnard, 1982).

In nature, this organism survives well in a broad range of sediment grain sizes (e.g., percent composition of silt or clay ranging from 1 to 79%), although it seems to prefer well-sorted fine sand to sandy silt (ASTM, 1991a). This species is more sensitive to contaminated sediment than a variety of other marine benthic species including some bivalves, copepods, cumaceans, oligochaetes, and polychaetes (Swartz *et al*., 1985a; Chapman, 1986). Field observations indicate that *R. abronius*, and phoxocephalid amphipods in general, are not found in contaminated areas (Swartz *et al*., 1982; Swartz *et al*., 1986). *Rhepoxynius abronius* is a meiofaunal predator that also feeds on algae and detritus (ASTM, 1991a).

Life Cycle and Age Class for Tests

Rhepoxynius abronius has an annual life cycle (one brood per year), with recruitment occurring during the late winter through the spring months (Kemp *et al*., 1985; ASTM, 1991a). Large juvenile or adult individuals, measuring 3- to 5-mm total length, should be used in the toxicity test. Animals of this size are available year-round, and are easily handled in the toxicity test. Mature males and females have been found to be equally sensitive to test substances (ASTM, 1991a); thus a mixed population of both sexes can be used in the test. Very large mature individuals (i.e., >5 mm) should not be used because they might be senescent. It is necessary to change year classes sometime

Legend:

EPIST	epistome
GN	gnathopod
HD	head
IR	inner ramus
LFT	left
LL	lower lip
MD	mandible
MX	maxilla
MXPD	maxilliped
OR	outer ramus
P	peraeopod
RT	right
T	telson
U	uropod
UL	upper lip
♂	male
♀	female

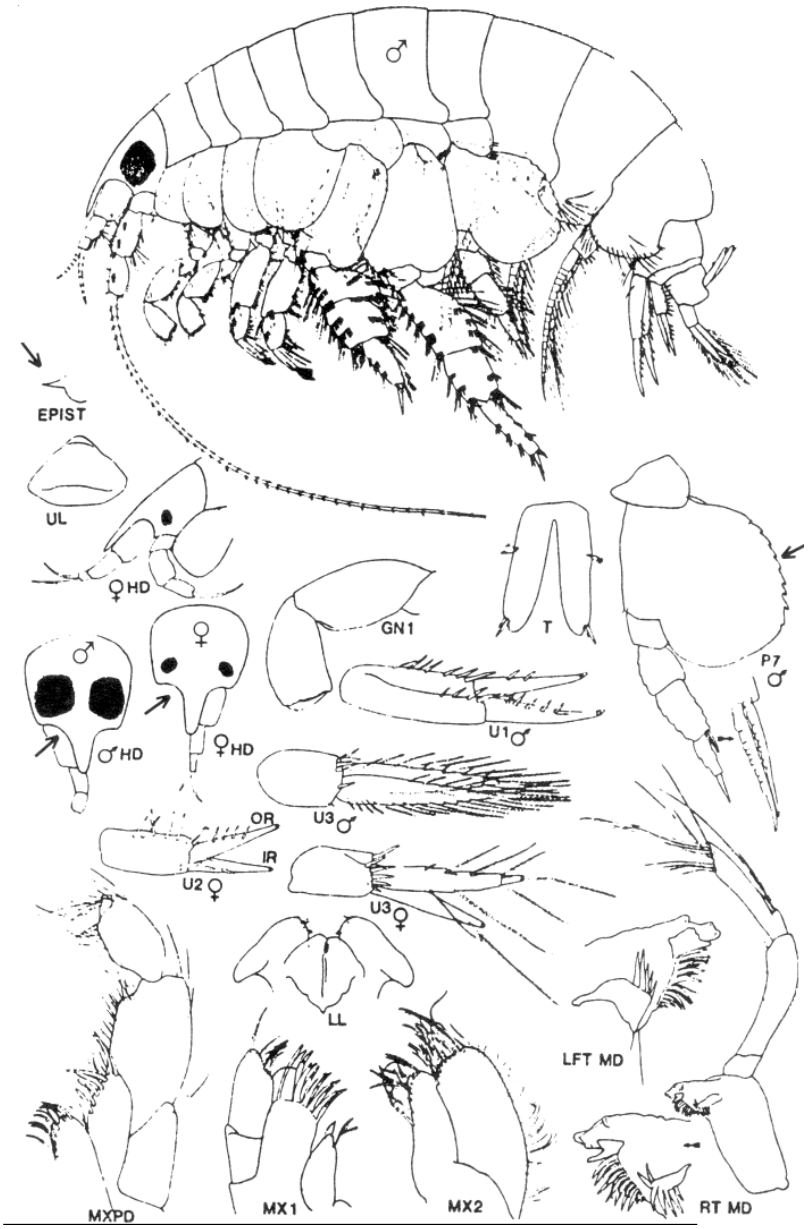


Figure K.1 Taxonomic Illustration of *Rhepoxynius abronius*[a]

[a] Reproduced from Jarret and Bousfield (1992), with permission. See Appendix E for abbreviations.

during the summer, when the old adults die and are replaced by maturing juveniles.

Laboratory Testing and Tolerance

Rhepoxynius abronius is a desirable test species when testing for the acute toxicity of sediment samples with salinities of pore water of 25 g/kg or higher. Reduced survival of this species has been demonstrated in the laboratory at lower salinities (Swartz *et al*., 1985a; ASTM, 1991a). *Rhepoxynius abronius* is tolerant of substantial organic enrichment of sediment, and is able to survive 10-day exposures to sediment with very low oxidation-reduction potential (Eh) values (Swartz *et al*., 1985a).

In the laboratory, *R. abronius* reburies quickly (within 10 min) when transferred from control sediment to other clean sediment. Individuals can swim quickly, although many curl and float on the water surface when sieved, making their collection easy. Their colour has been variously described as salmon pink to yellowish, greyish-brown, or white with a pinkish-brown hue. Eyes and gut are evident; animals are thick-bodied. Larger animals have a noticeable whitish area on their side. This species is very easy to work with in all phases of testing.

The species is tolerant of a wide range of sediment grain sizes, although a number of studies have shown some reduction in survival rate when this species is held for 10 days in very fine-grained (predominantly silt and clay), clean sediment (DeWitt *et al*., 1988; Long *et al*., 1990; McLeay *et al*., 1991). The inclusion of sample(s) of reference sediment with grain sizes similar to those of the test sediment(s) (see Section 5.1) accounts for this effect.

When acclimated to and tested at 15 ± 2 °C, the 10-day survival of *R. abronius* in control sediment is generally 95% or greater (ASTM, 1991a; McLeay *et al.*, 1991; Paine and McPherson, 1991a,b). In comparative tests using this and other species of marine or estuarine amphipods, *R. abronius* was shown to be among those most sensitive to samples of contaminated sediment (Paine and McPherson, 1991a). The 10-day toxicity test using *R. abronius* has been demonstrated to be very useful in detecting sediment toxicity. This test can be used in a variety of regulatory, monitoring, and research applications.

Appendix D of Environment Canada (1998a) should be consulted for additional and more recent information on the grain-size tolerance limits for *R. abronius*. This appendix also provides information on the tolerance of this species to ammonia in “water only” and “spiked sediment” tests, its known tolerance limits for porewater salinity, its historical control performance, and historical LC50s for “water only” reference toxicity tests with cadmium.

Appendix L

Logarithmic Series of Concentrations Suitable for Toxicity Tests [a]

Column (Number of concentrations between 10.0 and 1.00, or between 1.00 and 0.10)[b]						
1	2	3	4	5	6	7
10.0	10.0	10.0	10.0	10.0	10.0	10.0
3.2	4.6	5.6	6.3	6.8	7.2	7.5
1.00	2.2	3.2	4.0	4.6	5.2	5.6
0.32	1.00	1.8	2.5	3.2	3.7	4.2
0.10	0.46	1.00	1.6	2.2	2.7	3.2
	0.22	0.56	1.00	1.5	1.9	2.4
	0.10	0.32	0.63	1.00	1.4	1.8
		0.18	0.40	0.68	1.00	1.3
		0.10	0.25	0.46	0.72	1.00
			0.16	0.32	0.52	0.75
			0.10	0.22	0.37	0.56
				0.15	0.27	0.46
				0.10	0.19	0.32
					0.14	0.24
					0.10	0.18
						0.13
						0.10

[a] Modified from Rochinni *et al.* (1982).

[b] A series of five (or more) successive concentrations should be chosen from a column. Midpoints between concentrations in column (x) are found in column (2x + 1). The values listed can represent concentrations expressed as percentage by weight (e.g., mg/kg) or weight-to-volume (e.g., mg/L). As necessary, values can be multiplied or divided by any power of 10. Column 1 might be used if there was considerable uncertainty about the degree of toxicity. More widely spaced concentrations (differing by a factor <0.3) should not be used.