TEST SPECIFIC CHECKLIST

Revised: June 1999

Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods (GM)

Reference Method for Determining Acute Lethality of Sediment to Marine or Estuarine Amphipods (RM)

Parameter	Specification	Met Specifics Y	N	NA
Sample Preparation				
Homogenization	Sample mixed thoroughly on day preceding test	...	...	...
	Liquid separated from sample during transport and storage remixed within the sample **(Must GM & RM)**	...	...	...
	For each sample included in a test, mixing conditions (duration & T°) are to be as similar as possible **(Must GM & RM)**	...	...	...
	Immediately after mixing, sample placed in labeled test chambers **(Must RM)**	...	...	...
Characterization	Each sample (including control and reference sediment) is analyzed for: whole sediment for particle size and TOC **(Must GM & RM)**; porewater for salinity, pH and ammonia **(Must RM)**	...	...	...
Sieving	Sample not wet sieved **(Must GM & RM)**	...	...	...
Pre-aeration	Overlying water in each vessel (including controls) aerated overnight before introduction of test organisms **(Must GM)**	...	...	...
Temperature	Upon arrival at lab, T° and date of receipt recorded **(Must GM & RM)**	...	...	...
Test Conditions				
Test Facility	Isolated from physical disturbances; well ventilated; dust and fumes minimized; non toxic construction material **(Must RM)**; isolated from acclimation area	...	...	...
Test Type	Static	...	...	...
Test Duration	10 days	...	...	...
Test T°	15 ± 2 °C except for *A. virginiana* which is 10 ± 2 °C **(Must GM & RM)**	...	...	...
Light Quality	Overhead florescent	...	...	...
Light Intensity	500 - 1000 lux at surface of overlying water; uniform	...	...	...
Photoperiod	Continuous light **(Must GM & RM)**	...	...	...
Aeration	Overlying water in each test vessel to be aerated overnight and throughout the test **(Must GM & RM)**	...	...	...
Aeration Rate	Continuous and gentle (2 to 3 bubbles/s) **(Must GM & RM)**	...	...	...
	Not disturb surface of sediment layer	...	...	...
Vessel Size & Type	1L glass beaker or jar, internal diameter ~ 10 cm, covered **(Must RM)**	...	...	...
Sediment Volume	Identical volume of each sample in each replicate vessel **(Must GM)**	...	...	...
	175 mL (~2 cm thick) **(Must RM)**; sediment be smoothed	...	...	...
	Sediment in test chambers not to be stirred with the overlying water or otherwise disturbed at any time before or during the test **(Must RM)**	...	...	...
Water Volume	Overlying water added to the 750 mL mark on vessel one day before test start and made up to 950 mL mark after amphipods are introduced	...	...	...
Renewal of Solution	None	...	...	...
Test Water	Clean seawater, natural or reconstituted; same as that used for acclimation (same salinity and T°); D.O. 90-100% saturation **(Must RM)**	...	...	...
Vessel Labeling	Clearly labeled/coded: test substance, conc. and replicate # **(Must GM & RM)**	...	...	...
	Date and time of test initiation on data sheets **(Must RM)**	...	...	...
Vessel Position	Each set of replicate treatments positioned randomly within the test facility	...	...	...
# Replicates/Conc.	≥ 5 field replicates, each a discrete (different) sample from the same location	...	...	...
	≥ 5 lab replicates for each field replicate **(Must GM)**	...	...	...
	≥ 5 replicates per conc. if multi-conc. test with contaminant-spike sediment performed for regulatory purpose **(Must GM)**	...	...	...
# Organisms/Vessel	20 per vessel	...	...	...
Organisms Selection	⅓ more amphipods than required for test be sieved to allow for selection of healthy organisms	...	...	...
	Distribute amphipods to vessel using randomized block design	...	...	...
	Allow 1 h for amphipods to rebury, replacing amphipods that are not buried (except for *E. washingtonianus, C. volutator & A. virginiana*) **(Must RM)**	...	...	...
Removal of Dead	None	...	...	...
Feeding Regime	None **(Must RM)**	...	...	...
Vessel Cleaning	Wash with lab detergent; 3 distilled water rinses, a rinse in 10% nitric acid, ≥ 2 rinses in distilled water and ≥ 2 rinses with test water	...	...	...
Endpoint	Mean (± SD) % survival at Day 10 **(Must GM & RM)**	...	...	...

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Observations & Measurements				
Overlying Water	T° and D.O. measured at test start and ≥ 3 times/week on non-consecutive days for at least 1 test vessel representing each treatment **(Must GM & RM)**	...	...	...
	Salinity, pH and ammonia **(RM)**measured at start and end of test for at least 1 test vessel representing each treatment **(Must GM & RM)**	...	...	...
	Probe inspected and rinsed between samples **(Must GM & RM)**	...	...	...
Air flow	Each test vessel checked regularly to confirm that the airflow is uninterrupted and not excessive **(Must GM)**	...	...	...
	If at any time during the test, the airflow is stopped, D.O. of overlying water is to be measured and airflow re-established **(Must GM & RM)**	...	...	...
Amphipods	Each test vessel checked regularly to note if amphipods are swimming in the overlying water or floating at the water surface **(Must GM & RM)**	...	...	...
	Floating amphipods gently pushed down into water using glass rod	...	...	...
Porewater	Analyses for pH, salinity and ammonia undertaken within 24h of test start **(Must RM)**; recommend at test initiation	...	...	...
	Salinity measured at least at test start for each test substance (including controls and reference sediments)	...	...	...
	Probe inspected and rinsed between samples **(Must GM & RM)**	...	...	...
Mortality	At test end, sediment from each test vessel sieved (1 mm or smaller) to remove organisms and determine if they are dead or alive **(Must GM & RM)**	...	...	...
	Animals are considered dead if they fail to show any movement (magnifying device) in response to gentle prodding **(Must RM)**	...	...	...
	Missing animals are presumed dead	...	...	...
Test Organisms				
Species				
•Pacific/Arctic	*Eohaustorius estuarius, Eohaustorius washingtonianus, Rhepoxynius abronius*	...	...	...
•Atlantic/Arctic	*Amphiporeia virginiana, Corophium volutator* **(GM)**	...	...	...
Source	Selection of the species take into consideration the known/anticipated physiochemical characteristics of the material together with the known tolerance limits of the species to these characteristics **(Must GM & RM)**	...	...	...
	All organisms used in a test from one population and source **(Must GM & RM)**	...	...	...
	Wild population (preferably) collected subtidally or intertidally from clean sediment; or laboratory reared; taxonomically verified	...	...	...
Age	Large immature or young mature amphipods (3 - 10 mm in length, depending on species); as uniform as possible in age and size	...	...	...
	Mature females (bearing embryos) or large individuals not be used **(Must RM)**	...	...	...
Amphipod Collection				
Collection Site	Previously demonstrated to have an abundance of organisms of correct size and age, and species taxonomically confirmed	...	...	...
Site Measurements	Salinity and T° of surface and bottom water measured	...	...	...
Sieving	A minimum 2 to 4cm layer sieved (0.5 to 1mm mesh screen) sediment be placed in the bottom of the containers	...	...	...
	Water from collection site is then added to form a layer of ≥ 2 cm of water	...	...	...
	Sieved amphipods are added to containers; density in each container not exceeding 1 amphipod/cm^2	...	...	...
	Additional sieved sediment collected for use as control sediment and for physicochemical analyses	...	...	...
Apparatus	All apparatus & containers used for collecting/sieving/transporting organisms and sediment be clean and made of non-toxic materials **(Must GM & RM)**	...	...	...
	Apparatus used only for handling and transport of live animals and control sediment **(Must GM)**	...	...	...
Handling	Handling of amphipods minimized; amphipods dropped, injured or that contact dry surface discarded **(Must GM & RM)**	...	...	...

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Holding/Acclimation				
Apparatus	All containers and accessories that might contact organisms, seawater or control sediment be clean, rinsed and made of non-toxic material **(Must GM)**	...	...	...
	Materials such as copper, zinc, brass, galvanized metal, lead, and natural rubber not come in contact with apparatus, equipment or samples of water or sediment **(Must GM)**	...	...	...
Observations	Upon receipt of filed-collected animals at the lab, T°, salinity, D.O. and pH of the overlying water in 1 or more of the containers holding animals and sediment be determined and recorded	...	...	...
	Any dead organisms observed on the surface of the sediment counted and removed, together with any debris evident	...	...	...
Holding Amphipods	No sieving or sorting of amphipods until the day that the test is started	...	...	...
	Amphipods (density ≤ 1 amphipod/cm^2) be held unfed in a minimum 2 to 4cm layer of sieved sediment from the collection site, with at least 2cm deep of overlying water	...	...	...
Acclimation	Salinity of seawater same as that for overlying seawater in test	...	...	...
	T° of 15 ± 2 °C except for *A. virginiana* which is 10 ± 2 °C	...	...	...
	D.O. of 90 - 100 % of air saturation **(Must RM)**	...	...	...
Acclimation Rate	Salinity ≤ 5 g/(kg.d) **(Must RM)**; measured daily during adjustment period	...	...	...
	T°≤ 3 °C/day **(Must RM)**; measured daily during adjustment period	...	...	...
	Thereafter, T°, salinity, pH, and D.O. measured at least at start and end of remaining acclimation period (ie: 2 to 10d) **(Must RM)**	...	...	...
Lighting	Overhead (fluorescent or equivalent); 500-1000 lux at surface of water	...	...	...
	Constant/continuous throughout holding/acclimation period **(Must GM & RM)**	...	...	...
Duration acclimation	2 - 10 days once T°, lighting, and salinity adjusted to that of test water & test conditions **(Must GM & RM)**	...	...	...
	If holding/acclimation exceeds 2 d, the overlying water is to be replaced continuously or periodically with air-saturated, fresh seawater adjusted to the required temperature and salinity	...	...	...
Selection Amphipods	Remove and discard inactive amphipods that have emerged from sediment or do not bury at any time during holding/acclimation period **(Must RM)**	...	...	...
	On the day of the test, select active and healthy amphipods with typical appearance (size & range) and behaviour of that species **(Must GM & RM)**	...	...	...
QA/QC				
Validity Criteria	Mean 10-d survival in control sediment be ≥ 85% for *E. washingtonianus*; ≥80% for *A. virginiana*; and ≥ 90% for all other species **(Must GM & RM)**	...	...	...
	Results for a test sediment (grain size characteristics & pore water salinity) are within the application limits specified for the species used in test **(Must RM)**	...	...	...
Reference Toxicant	A 4d-LC50 static test, in seawater only, using cadmium **(Must RM)** or a 10d-LC50 test with spiked control sediment using copper, cadmium or fluoranthene **(GM)**	...	...	...
	To be performed on each batch of field-collected amphipods used for testing **(Must GM & RM)**	...	...	...
	Initiated within 1 d of the start of the sediment test **(Must RM)**	...	...	...
	4d-LC50 seawater only is performed in 1L glass beakers with ≥ 800mL of test solution and minimum of 10 amphipods per test chamber; 6 treatments; replicates not required; no sediment; in the dark, without aeration; other conditions are similar to sediment test; valid if control survival at 96h is ≥ 85% for *E. washingtonianus*, and ≥90% for the other species	...	...	...
Warning Chart	Prepared and updated for each species of amphipod used **(Must GM & RM)**	...	...	...
Control Sediment	Obtained from amphipod collection site, sieved through 0.5 mm screen using test water **(Must RM)**	...	...	...
Reference Sediment	One or more samples taken from clean site in the general vicinity of test sediment site, (ideally particle size and organic content within range of test sediment) tested with field collected sediment or similar substance	...	...	...

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Reference Sediment.	The mean 10-d survival rate in the replicate samples of reference sediment is to be at least 70% for *A. virginiana*, 75% for *E. washingtonianus*, and 80% for *R. Abronius* and *E. estuarius*, to be eligible and acceptable for comparison with results for test sediments **(Must RM)**.	...	...	...
Dilution Water.	Before use, adjusted to test T°; to D.O. of 90-100% air saturation; and to salinity of 28 ± 2 g/kg **(Must GM & RM)**.	...	...	...
	D.O., salinity and pH measured in each test chamber at start and end of reference toxicant test **(Must GM & RM)**.	...	...	...
Sample Handling				
Containers.	Non-toxic material for transport and storage **(Must GM & RM)**.	...	...	...
	New or thoroughly rinsed used containers **(Must GM & RM)**.	...	...	...
Holding Time.	Test to be initiated within 6 weeks after sampling **(Must GM & RM)**.	...	...	...
	Recommend test initiation within 2 weeks after sampling.	...	...	...
Holding Conditions.	Store in dark in airtight containers at 4 ± 2 °C **(Must GM & RM)**.	...	...	...
	Samples be kept from freezing, partially freezing or drying out during transport or storage **(Must GM & RM)**.	...	...	...
Labeling.	After collection, containers sealed and labeled or coded **(Must GM & RM)**.	...	...	...
	Include at least a code which identify sample type, source, location, replicate number and date of collection **(Must GM & RM)**.	...	...	...
Transport.	If sample ≥ 7°C, cool to 1 - 7°C (with ice or frozen gel packs); transport in dark at 4 ± 3 °C).	...	...	...
Test Report				
Test Substance.	Sample type, source; sampling location, method and schedule; nature, appearance, properties **(Must GM & RM)**.	...	...	...
	Information on labeling or coding of the test substance **(Must GM & RM)**.	...	...	...
	Collection, transport and storage details **(Must GM)**.	...	...	...
	Person providing/collecting sample **(Must GM)**.	...	...	...
	Dates for sample collection, receipt at test facility and start & end of definitive test **(Must GM & RM)**.	...	...	...
Test Organisms.	Species, source and date of collection **(Must GM & RM)**.	...	...	...
	Procedures used to sort, identify and handle amphipods **(Must GM)**.	...	...	...
	Description of holding and acclimation conditions **(Must GM)**.	...	...	...
	Daily % amphipods that emerged from control sediment during holding/acclimation period **(Must GM)**.	...	...	...
	Any unusual appearance, behaviour, or treatment of the organisms, before their use in the test **(Must RM)**.	...	...	...
Facilities/Apparatus.	Name and address of testing laboratory **(Must GM & RM)**.	...	...	...
	Person(s) performing the test **(Must GM & RM)**.	...	...	...
	Description of systems for lighting and regulating T° in facility **(Must GM)**.	...	...	...
	Description of test vessels and lids, aeration system and apparatus **(Must GM)**.	...	...	...
	Description of procedures used to clean or rinse apparatus **(Must GM)**.	...	...	...
Control Sediment & Test Water.	Type and source of control sediment and test water **(Must GM & RM)**.	...	...	...
	Type and quantity of any chemicals added to test water **(Must GM)**.	...	...	...
	Sampling/storage procedures & conditions for control sediments **(Must GM)**.	...	...	...
	Pretreatment of control sediment and test water **(Must GM)**.	...	...	...
	Measured quality of control sediment & test water before and/or at start of test **(Must GM & RM)**.	...	...	...
Test Method.	Brief mention of method, if standard **(Must GM & RM)**.	...	...	...
	Design and description, if specialized procedure or modification of standard method **(Must GM & RM)**.	...	...	...
	Procedures used for mixing or manipulating sediments before use **(Must GM)**.	...	...	...
	Time interval between sediment preparation and testing (ie: ≤ 24h) **(Must GM)**.	...	...	...
	Procedure used for preparing stock and/or chemical test solutions **(Must GM)**.	...	...	...

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Test Method (con't).	Procedure used for preparing and testing reference toxicants **(Must GM)**.	...	...	...
	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 **(Must GM)**.	...	...	...
	Use of preliminary or range-finding test **(Must GM)**..	...	...	...
	Frequency and type of observations made during test **(Must GM & RM)**.	...	...	...
	Programs and methods used for calculating statistical endpoints **(Must RM)**.. .	...	...	...
Test Conditions.	Number replicate vessels for each treatment **(Must GM & RM)**.	...	...	...
	Test conc. if applicable **(Must GM)**.	...	...	...
	Depth/weight/volume of sediment and water in test vessels **(Must GM & RM)**..	...	...	...
	# of organisms per test vessel **(Must GM & RM)**.	...	...	...
	Light intensity adjacent to surface of overlying water in test vessels **(Must GM)**.	...	...	...
	Aeration rate and manner of application to test vessels, before and during test **(Must GM)**.	...	...	...
	Any measurements showing D.O.< 60% in overlying water **(Must RM)**.	...	...	...
Test Results.	Any physical/chemical measurements made on control and reference sediments, test substances, porewater; and control/dilution water; before and during test **(Must GM & RM)**.	...	...	...
	Appearance of control and reference sediments, test substances and overlaying seawater and changes noted during test **(Must GM)**.	...	...	...
	Results for range-finding test (if conducted) **(Must GM)**.	...	...	...
	Biological endpoints calculated for each test sediment and results of any statistical comparisons **(Must GM & RM)**.	...	...	...
	Any LC50's and EC50's (and 95% CL) determined for test chemicals or reference toxicants, including statistical method used **(Must GM & RM)**.	...	...	...
	Results for tests with any reference toxicant(s) performed during the toxicity test, with mean value (±2 SD) for the same reference toxicant(s) derived in previous tests (ie: in the form of a warning chart) **(Must GM)**.	...	...	...
	Specification as to whether results are based on measured or nominal concentrations of test substance **(Must GM)**..	...	...	...
	Results of any other observations or analyses made on the reference and control sediments and test substance(s) **(Must GM)**..	...	...	...
	Anything unusual about the test, problems encountered, remedial measures taken **(Must GM & RM)**.	...	...	...
Info Kept On-File	Do lab SOPs indicate that the information on Section 7.2 of the EPS 1/RM/35 method must be kept on file for 5 years? **(Must RM)**.	...	...	...
	For details of this information, see EPS 1/RM/35, section 7.2.			

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