

TEST SPECIFIC CHECKLIST

Revised: December 2001

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

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

Note: Shaded text reflects Dec. 2000 method amendments

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

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

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

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

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<u>Info Kept On-File</u>	Do lab SOPs indicate that the information on Section 8.2 of the EPS 1/RM/14 method must be kept on file for 5 years? (Must RM) For details of this information, see EPS 1/RM/14, section 8.2.

Note: In 2016, there was an amendment to the introduction section of “Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna*”. The amendment indicated the salinity limits of the test method, and specified that salinity is measured by refractometry. Specifications for the measurement of salinity have been described in more detail in “Reference Method for Determining Acute Lethality Using Threespine Stickleback”, and “Reference Method for Determining Acute Lethality Using *Acartia tonsa*.” The specifications in these more recent methods supercede the 2016 amendment to the introduction of “Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna*”.