

This checklist is a summary of the requirements and recommendations in the Environment and Climate Change Canada test method. As a summary, it will not contain all supplementary information. If there is a discrepancy between the checklist and the Environment and Climate Change Canada test method, the test method is taken as the definitive source.

Y= Yes, meets requirements; N= No, does not meet requirements; NA= not applicable.

DO = dissolved oxygen; temp = temperature; conc = concentration(s); sal = salinity; min = minute(s); h = hour; # = number (of);

SD = standard deviation; ‰ = parts per thousand, equivalent to g/kg

<b>TEST SPECIFIC CHECKLIST</b>							
<b>Reference Method for Determining Acute Lethality Using Threespine Stickleback</b>							
Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
<b>Sample Handling: Effluent</b>							
Salinity	Salinity of effluent is > 10‰ and is discharging directly to estuarine or marine receiving waters <b>(must)</b>						
Containers	Made of nontoxic material; new or thoroughly cleaned and rinsed with clean water before use <b>(must)</b>						
	Each sample container is filled completely to exclude air						
Volumes	Single-conc Test: ≥ 20 L; Multi-conc Test: ≥ 40 L						
Labelling	Immediately after filling, each sample container is sealed and labeled or coded <b>(must)</b>						
	Label and/or records include a code or sample identifier, sample type, source, sampling method, date and time of collection, and name of sampler(s) <b>(must)</b>						
Holding Time	Test is initiated within 5 days after sampling <b>(must)</b> ; recommend within 3 days after sampling						
	Date and time of receipt of the sample(s) at lab is recorded <b>(must)</b>						
Holding Conditions	Sample temp is measured and recorded upon receipt at lab <b>(must)</b>						
	Samples are kept between 1 and 8 °C if more than 2 days in transit or when ambient temp is extreme (i.e., > 30 °C or < 1 °C), and in darkness throughout transport						
	Samples are kept from freezing during transport or storage <b>(must)</b>						
	Samples are held in the dark at 4 ± 2 °C for a brief period in full, sealed container(s) within a refrigerated facility; or are held in full, sealed container(s) at 15 ± 1 °C overnight if test to be started the next day <b>(must)</b>						
<b>Sample Handling: Chemicals</b>							
Containers	Sealed and coded or labelled upon receipt <b>(must)</b>						
Labelling	Label and/or record(s) includes a code or sample identifier with required information (i.e., chemical name, supplier, date received) <b>(must)</b>						
Properties	Information on the properties of the test chemical is obtained, including: concentration of major ingredients, solubility in seawater (natural or artificial), vapour pressure, chemical						

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		Y	N	NA	Y	N	NA
Properties cont.	stability, dissociation constants, toxicity to humans and aquatic organisms, biodegradability and data-sheets on safety aspects (e.g., Safety Data Sheets)						
	Acceptable procedures for preparing aqueous solutions of the chemical are obtained and reported and/or solubility in control/dilution water is determined experimentally where aqueous solubility is in doubt or problematic						
	Other available information such as structural formulae, degree of purity, nature and percentage of significant impurities, presence and amounts of additives, and n-octanol:water partition coefficient is obtained and recorded.						
	An acceptable analytical method for measuring the chemical in seawater at concentrations intended for the test is known along with the precision and accuracy of the analysis						
Holding Conditions	Storage conditions (e.g., temp, protection from light), as dictated by the nature of the chemical, and standard operating procedures for chemical handling are followed						
<b>Sample Preparation: Effluent</b>							
Mixing and Subsampling	Contents of each sample container are thoroughly agitated before pouring and subsamples are combined prior to use for preparing aliquots <b>(must)</b>						
DO, pH, Salinity	Measured in unadjusted, undiluted effluent before preparation of test solutions <b>(must)</b>						
Temp	Measured in unadjusted, undiluted effluent before preparation of test solutions and adjusted to 15 ± 1 °C if outside that range <b>(must)</b>						
	No use of immersion heaters or microwaves <b>(must)</b>						
Pre-aeration	All test solutions and controls for 30 min at a rate of 6.5 ±1 mL/min·L through an air stone <sup>1</sup> at the end of which DO is measured in at least the highest test conc <b>(must)</b>						
	Second period only if DO in highest test conc is < 70% or > 100%; pre-aeration continued at 6.5 ±1 mL/min·L until DO is 70–100% or 90 additional min, whichever is shorter <b>(must)</b>						
Filtering	Samples are not filtered prior to testing <b>(must)</b>						
pH Adjustment	No pH adjustment of sample or test solution <b>(must)</b>						
Salinity Adjustment	No salinity adjustment of sample <b>(must)</b>						
Solution Preparation	Same water is used for preparing control and all test solutions <b>(must)</b>						

<sup>1</sup> Air stones acceptable for use are: (i) Marina®, 2.5 cm length x 1.5 cm diameter, cylindrical (one use only); (ii) AS1 silica glass, 3.8 cm length x 1.3 cm width, rectangular (re-usable after proper cleaning); or (iii) alternate air stone that has been shown to perform equivalently to the Marina® or AS1 air stone.

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<b>Sample Preparation: Chemicals</b>							
Solution Preparation	Test solutions are typically prepared by adding aliquots of a stock solution in control/dilution water; alternatives include adding quantities of chemical directly to control/dilution water to give nominal strengths for testing; or by salinity adjustment of aqueous samples (i.e., chemical formulations in water) by adding dry ocean salts directly to the sample or test solutions to adjust the salinity to within the desired range						
	If stock solutions are used, conc and stability of test chemical in solution is determined						
	If deionized, distilled, or fresh water is used to prepared the stock solution, dry ocean salts are used to adjust the salinity of each test solution to within the desired range						
	Nominal concentrations are prepared and reported in consideration of any salinity adjustment <b>(must)</b>						
	Unstable stock solutions are newly prepared <b>(must)</b> ; and stock solutions subject to photolysis are shielded from light						
Solvent	Water is the preferred solvent for preparing stock solutions; emulsifiers or dispersants are not used unless formulated with the test chemical; organic solvent is used only if no other method of test solution preparation is available						
	Solubilizing agent is used sparingly and does not exceed the conc that affects the survival of threespine sticklebacks or a maximum of 0.1 mL/L in any test solution; preliminary solvent only test conducted if toxicity of solubilizing agent is unknown						
	If solvent (or equivalent) is used, an additional control solution (i.e., solvent control) is prepared with the conc of solubilizing agent that is present in the most concentrated solution of the test chemical <b>(must)</b>						
<b>Test Conditions</b>							
Facility and Apparatus	Tests are isolated from general disturbances <b>(must)</b>						
	Test area is ventilated and free from physical disturbances or airborne contaminants; dust and fumes are minimized; test area is isolated from areas where test solutions are prepared or equipment is cleaned						
	All apparatus and supplies that contact test/stock solutions or control/dilution water do not contain substances that can be leached or dissolved in amounts that adversely affect the test organism <b>(must)</b> ; and minimize sorption of materials from water						
	Lab has the instruments to monitor basic water quality (e.g., temp, sal, DO, and pH) and is prepared to accurately and promptly analyze other variables (e.g., ammonia) <b>(must)</b>						
	All test vessels, measurement devices, stirring equipment, and fish-transfer pails are clean and rinsed in accordance with standard operating procedures <b>(must)</b>						

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Facility and Apparatus cont.	Facilities are appropriate for degree of hazard associated with samples and risk of sample and apparatus contamination							
	Equipment is cleaned and rinsed in accordance with good laboratory practice <b>(must)</b>							
Test Type	Static (no renewal of test solutions) <b>(must)</b>							
	Samples are not agitated (other than that provided by required aeration) <b>(must)</b>							
Duration	96 hours <b>(must)</b>							
Temperature	15 ± 1 °C; measured in test solutions <b>(must)</b>							
Lighting	Same as that defined for acclimation (i.e., full spectrum; 100 to 500 lux) <b>(must)</b>							
Photoperiod	16 ± 1 h light: 8 ± 1 h dark and coincides with timing used for acclimation <b>(must)</b> ; preferably with 15-30 min transition							
DO range	70 to 100% air saturation							
	Test is initiated after pre-aeration regardless of whether DO range is achieved							
Aeration	Aeration is throughout the test at a rate of 6.5 ±1 mL/min ·L in all solutions <b>(must)</b> ; checked daily							
	Provided by bubbling compressed air through clean air stones <b>(must)</b>							
Test Vessels	Glass, Plexiglas®, acrylic, polypropylene, polyethylene, or polyethylene-lined <b>(must)</b> ; loosely covered							
	Liners, if used, are discarded at the end of the test <b>(must)</b>							
	Test vessels (e.g., type, size, shape) are identical for all test solutions <b>(must)</b>							
	Each test vessel is clearly coded or labeled as to conc and start-date and -time <b>(must)</b>							
# Test Conc	Single-conc Test	1 (100% effluent or test solution) plus control(s) <b>(must)</b>						
	Multi-conc Test	≥ 5 plus control(s) <b>(must)</b>						
		Highest conc is full-strength effluent; each successive conc must have at least 50% of the strength of the next higher one <b>(must)</b>						
# Replicates/ Conc	Single-conc Test	Minimum 1 vessel per conc <b>(must)</b> ; recommend 3						
	Multi-conc Test	Minimum 1 vessel per conc <b>(must)</b> ; more for chemical testing						
# Organisms/ Conc	≥10 fish per test concentration and control(s) <b>(must)</b> ; may be divided between two or more vessels at the same concentration to meet required limit on loading							
Randomization	Concentrations are positioned randomly within the test facility for multi-conc test <b>(must)</b>							
	Healthy, acclimated fish are taken randomly from the acclimation tanks <b>(must)</b>							
	The order of adding fish to each test solution is randomized <b>(must)</b>							

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Test Volume/ Loading Density	Test solution depth is ≥15 cm and identical for all test solutions <b>(must)</b>							
	Test solution volumes are identical in each vessel and well mixed just before use <b>(must)</b>							
	Loading density of fish in each test vessel is ≤ 0.5 g fish/L as determined by the mean wet weight of control fish (in dilution-water control) at the end of the test <b>(must)</b>							
Control/Dilution Water	Same type(s) as described for holding and acclimation water; preferably identical to holding/acclimation water							
	Same water is used for preparing control(s) and all test solutions less than 100% <b>(must)</b>							
	Adjusted to 15 ± 1°C prior to use <b>(must)</b>							
Control/Dilution Water (Chemical Testing)	DO is 90 to 100% air saturation and not supersaturated <b>(must)</b> ; aerated if necessary using vigorous aeration with oil-free compressed air and acceptable air stones							
	As per effluent test; additional option includes receiving water; artificial seawater is recommended if a high degree of standardization is needed and the salinity of all test concentrations should be within 1‰ of the controls.							
	If receiving water used as control/dilution water, a separate control using the lab's normal control/dilution water is included <b>(must)</b>							
# Control/Test	For multiple concurrent tests at various salinities control/dilution water is from a single source with salinities adjusted using dry salts or fresh water							
	One or more dilution-water control solutions are prepared per test <b>(must)</b>							
	Control solution(s) and fish are used for only one toxicity test and/or one effluent sample <b>(must)</b>							
	Salinity Control	A salinity control (with salinity adjusted to within 1‰ of the effluent sample or highest test concentration) is included in the test if the salinity of the sample (or highest test concentration) is > 5‰ higher or lower than the salinity to which the fish have been acclimated <b>(must)</b>						
Prepared as described for control/dilution water <b>(must)</b>								
Salinity is >10‰ and ≤ 35‰ <b>(must)</b>								
Feeding Regime	Fish are not fed 16 h before the start of the test; nor during the test <b>(must)</b>							
Endpoint	Single-conc Test	Percent Mortality at 96 hours						
	Multi-conc Test	Mortality; 96-h LC50 and its 95% confidence limits <b>(must)</b>						
		Dilution-water control is used for calculations in effluent tests <b>(must)</b>						
		Method of calculation is reported <b>(must)</b>						

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Calculations (Chemical Testing)	Mean percent mortality for fish at the end of the test is calculated and reported for each test concentration, if replicates are used <b>(must)</b>							
	If solvent used: only the data from the solvent control is used to calculate the LC50, or for calculating other statistical endpoints							
<b>Observations and Measurements</b>								
Temp	At start and end of test in each test solution including control(s) as a minimum <b>(must)</b> ; daily measurement is recommended							
DO	At start and end of test in each test solution including control(s) as a minimum <b>(must)</b>							
pH	At start and end of test in each test solution including control(s) as a minimum <b>(must)</b>							
Initial/Final Measurements	Initial measurements are done after the pre-aeration period; final measurements are done after biological observations							
Salinity	At the start of the test in each test solution including control(s) as a minimum <b>(must)</b>							
	Measured using conductivity or refractometry <b>(must)</b>							
	Instruments for measuring salinity are properly operated and maintained as required by accreditation programs and are calibrated and verified routinely <b>(must)</b>							
	Further investigation of effluent ion composition is done where high total dissolved solids are suspected							
Salinity Method QA	Performance-based approach used to confirm suitability/acceptability of method <b>(must)</b> :							
	Conductivity	Calibrated daily when in use with certified conductivity standard <b>(must)</b> ;						
		A conductivity standard close to the conductivity of the effluent sample and a conductivity cell with a cell constant appropriate for use in high ionic strength solutions are used						
		Verified to accurately measure seawater salinity using a certified seawater standard; tolerance limit for accuracy is within 1‰ <b>(must)</b>						
	Refractometry	Reported conductivity accounts for temperature <b>(must)</b>						
		Calibrated daily when in use with purified water at 0‰ <b>(must)</b>						
		Verified to accurately measure seawater salinity using a certified seawater standard; tolerance limit for accuracy is within 1‰ <b>(must)</b>						
	Verification for accuracy is carried out after calibration							
Chemical Concentration (Chemical Testing)	Chemical conc is measured in aliquots from high, medium, and low test conc and control at beginning and end of test, as minimum; samples are preserved, stored and analyzed using appropriate methods for analysis in seawater; if conc declined by > 20%, tests are repeated with more frequent renewals							

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Chemical Concentration (Chemical Testing) cont.	If concentrations are measured, results are calculated and expressed in terms of measured concentrations; test solutions are characterized by the geometric mean measured concentrations to which fish were exposed						
	Appearance of sample and test solutions during preparation, at the start of the test and any obvious changes during the test are noted and recorded						
Baskets	Baskets, if used to facilitate monitoring of fish, are placed in all vessels including controls and are big enough to allow fish to move throughout the test vessel <b>(must)</b>						
	Baskets are thoroughly cleaned and rinsed with control/dilution water before use <b>(must)</b>						
Fish Appearance/ Behaviour	At 24, 48, 72, and 96 hours <b>(must)</b> ; more frequent observations are made during the initial day of the test						
Mortality	At 24, 48, 72, and 96 hours <b>(must)</b> ; more frequent observations are made during the initial day of the test						
	Dead fish are recorded and removed during each observation period <b>(must)</b> ; overt sublethal effects and/or differences from control fish are recorded during each observation period						
Control Fish Length and Weight	Mean fork length of fish in dilution-water control is determined and recorded at the end of the test <b>(must)</b> ; data is used to assess if recommended size of test fish was met						
	Mean wet weight of fish in dilution-water control is determined and recorded at the end of the test <b>(must)</b>						
	Mean wet weight is determined after fish are blotted dry and within 30 minutes of removal from the tank <b>(must)</b>						
	Mean wet weight data is used to confirm that the required range of weights for test fish and loading density were met <b>(must)</b>						
Morph	Each fish in each treatment is examined to determine its morphological form						
<b>Test Organism</b>							
Species	<i>Gasterosteus aculeatus</i> <b>(must)</b>						
	Taxonomic identification of species is provided and documented by qualified taxonomist or barcoding at least once for each collection site or supplier <b>(must)</b>						
	Confirmation of test species for each shipment <b>(must)</b>						
Source	Cultured or captured from coastal marine or estuarine waters (i.e., no fish originating from populations inhabiting fresh water) <b>(must)</b>						
	All fish used in a test are derived from the same population and source <b>(must)</b>						
	All fish used in a test are free of overt signs of disease or parasites <b>(must)</b>						

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Source cont.	Records accompanying each batch include: quantity and source of test organisms, supplier's/collector's name(s), date of shipment, date of arrival at lab, and arrival condition (i.e., mortality, temperature, DO, pH, and salinity)( <b>must</b> )						
	For Atlantic populations, batches are examined to remove blackspotted stickleback ( <b>must</b> )						
	All batches are examined to remove morphs lacking a caudal keel (i.e., freshwater morphs)						
Acclimation	Fish are held under specified physicochemical conditions for ≥ 1 week prior to use in the test ( <b>must</b> )						
	Acclimation period immediately precedes fish use in a test ( <b>must</b> )						
	Temperature: 15 ± 2°C ( <b>must</b> ); rate of change ≤ 5°C/day						
	Salinity: within 5‰ of salinity for control/dilution water to be used in the test ( <b>must</b> ); rate of change ≤ 5‰ per day						
	DO: 80 to 100% saturation						
	pH: 7.0 to 8.5						
Age/Size	Photoperiod: 16 ± 1 hours light: 8 ± 1 hours dark ( <b>must</b> ); 15- to 30-min transition Light: full spectrum; 100 to 500 lux						
	Average wet weigh of fish is between 0.20 and 1.2 g, based on control fish at the end of the test ( <b>must</b> )						
	Fork length of largest fish is not more than 1.6 times that of the smallest fish in the same test, based on control fish at the end of the test						
	Males displaying breeding colours and gravid females are not used in the test ( <b>must</b> ) Fish lacking caudal keel are not used in the test						
Health Criteria	Mortality in acclimation tanks is monitored and recorded a minimum of 5 days/week ( <b>must</b> ); recommend daily						
	Cumulative rate of mortality is <2% during 7-day period preceding the day the test is started ( <b>must</b> )						
	If cumulative mortality is 2 to 10%, acclimation is extended for at least an additional 7 days and until cumulative 7-day mortality of <2% is achieved for the 7-day period preceding the day the test is started ( <b>must</b> )						
	Cumulative mortality of >10% per week, during any 7-day period makes the group of fish unacceptable for future use if death is caused by disease or aquatic contaminants ( <b>must</b> ); if deaths are caused by other factors, fish may be used provided mortalities decline to <2% during the 7-days preceding the day the test is started						

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Test Fish Use and Disposal	Individual fish are only used once as test or control organisms <b>(must)</b>						
	Surviving fish used in the test (including controls) are disposed of in a humane manner at the end of the test <b>(must)</b>						
	Fish are euthanized according to CCAC guidelines <b>(must)</b>						
<b>Culture/Holding Conditions</b>							
Facility and Apparatus	Holding and acclimation tanks are made of nontoxic materials <b>(must)</b>						
	Holding and acclimation tanks are located away from physical disturbances and separate from test tanks						
	Holding tanks may be indoors or outdoors; acclimation tanks are indoors or outdoors covered with lids fitted with photoperiod-controlled lighting						
Water Temperature	8 to 17 °C						
DO and Aeration	DO maintained at 80 to 100%; supplemental aeration using filtered, oil-free compressed air is provided if necessary; supersaturation (if any) is remedied						
pH	7.0 to 8.5						
Lighting	Full spectrum; 100 to 500 lux						
Photoperiod	16 ± 1 h light; 8 ± 1 h dark <b>(must)</b> ; 15- to 30-minute transition period						
Water Quality	Uncontaminated natural or reconstituted/artificial seawater						
	Artificial seawater is made up to desired salinity by adding commercially available dry ocean salts to suitable fresh water and mixed thoroughly during addition <b>(must)</b>						
	Sources of fresh water for preparing artificial seawater are deionized or distilled water; uncontaminated ground or surface water; or dechlorinated municipal drinking water						
	Dechlorinated water is free of any harmful concentration of chlorine or chlorinated compounds upon fish exposure <b>(must)</b> <sup>2</sup>						
	Artificial seawater is aerated continuously and vigorously for ≥12 h before use <b>(must)</b> ; longer periods (≥3 days) are recommended						
	Commercially available sea salts used to prepare artificial seawater have previously been shown to support good survival and health of threespine stickleback						

<sup>2</sup> The guideline value for total residual chlorine (TRC) for the protection of marine life is ≤0.5 µg/L (CCME, 1999). Analytical techniques used to measure TRC in the treated supply of dechlorinated water should ideally have detection limits low enough to assure that TRC is below the guideline, however this might be unrealistic for methods used in the laboratory for routine measurements. EPS 1/RM/10, 2<sup>nd</sup> edition indicates that the use of equipment that can measure TRC down to 20 µg/L is acceptable as this level has been shown not to affect stickleback survival.

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Water Quality cont.	Seawater supply is monitored as frequently as required to document quality and variation for: sal, pH, DO, and TRC (if using dechlorinated water), as a minimum; and as appropriate suspended solids, total organic carbon, ammonia, metals, and pesticides						
	Method and duration for storage of batches of seawater (natural and artificial) are appropriate						
Handling/Transfer	Handling and transfer procedures minimize stress						
	Any fish that are dropped or injured are not used for testing <b>(must)</b>						
	Dip nets are rinsed (dilution water) between transfers if contact is made with test solution						
	Seawater in fish-transfer pails is aerated to maintain DO at 80 to 100% during transfer						
Feeding	Fish are fed once or more per day with bloodworms and/or brine shrimp (fresh or frozen) at a daily ration (dry food basis) approximating 1 to 5% of wet body weight, depending on temperature, fish size and consumption rate; alternatively freeze dried bloodworms and/or brine shrimp or recognized commercial fish food						
	Food size is appropriate and methods/duration for food storage follows manufacturer's recommendations						
Water Renewal	Static-replacement, recirculated (with biological filtration), or continuous flow						
	For static-replacement and recirculated systems holding densities are $\leq 0.9$ g fish/L <b>(must)</b> ; recommend $\leq 0.5$ g fish/L; water filtration and/or renewal is adequate for maintaining fish health						
	For flow-through systems holding densities can be higher; flow is adequate for maintaining fish health (e.g., $\geq 2$ L/min)						
	Ammonia levels for holding/acclimating are $\leq 2$ mg/L of total ammonia; ammonia is monitored frequently enough to ensure safe levels						
Cleaning	Tanks are kept clean of excess food and faeces (e.g., siphoned) and are disinfected and rinsed thoroughly before introducing a new batch of fish						
	Chlorinated or iodophore compounds, n-alkyldimethylbenzyl ammonium chloride, or biodegradable detergent are used as disinfectants						
Disease	If chemically treated for disease, fish are not used for a minimum of 2 weeks <b>(must)</b>						
Monitoring	Fish inspected daily for disease or parasites; dead or moribund fish, fish with disease or parasites, and males and females displaying breeding characteristics are removed immediately, if observed						
	Water temperature, DO, sal, pH, water flow (if applicable), ammonia, and mortalities are monitored in tanks at regular intervals <b>(must)</b>						
	Wet weights of $\geq 10$ fish are determined at regular intervals to assess/confirm feeding rates and holding densities, as well as effluent volume and fish size for testing						

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<b>QA/QC</b>								
Validity Criteria	Test is invalid if >10% control fish (combined data, if replicates are used) die and/or exhibit atypical/stressed behaviour <b>(must)</b>							
	Results for each set of controls used in a test are examined to determine if they independently meet the test validity criteria <b>(must)</b>							
	Tests using salinity control: if test results in either salinity control or dilution-water control fail to meet validity criteria, test is invalid <b>(must)</b>							
	Tests using solvent control: if test results in either solvent control or dilution-water control fail to meet validity criteria, test is invalid <b>(must)</b>							
Reference Toxicant	Reagent grade phenol; 96-h LC50 is determined and expressed as mg/L							
	Phenol stock solutions are prepared on day of use or shown to remain stable if stored <b>(must)</b>							
	Frequency is within 14 d of test start of toxicity test and upon acclimation of a new batch of fish <b>(must)</b>							
	Fish are from the same batch held in the lab and used in the effluent test <b>(must)</b>							
	Test is performed using the same conditions, procedures and control/dilution water as that used in the effluent test <b>(must)</b>							
	Concentrations of stock solutions and low, medium, and high test concentrations are measured chemically using appropriate methods, or stored for future analysis							
	If stored, ref. tox. aliquots are held in the dark at 4 ± 2°C <b>(must)</b> ; phenol solutions should be preserved before storage, and stored aliquots analyzed promptly if required							
	LC50 calculations are based on measured concentrations if they differ (i.e., ≥ 20%) from nominal ones and if the accuracy of the analyses is satisfactory							
Warning Chart	Prepared using 96-h LC50 results and continually updated with each new reference toxicity test <b>(must)</b>							
	Log conc used in all calculations of mean and standard deviation <b>(must)</b> ; and in all plotting procedures							
	Each new LC50 for the reference toxicant is compared with previously established limits of the chart							
	LC50 is acceptable if within warning limits (± 2 SD on log scale)							
	Laboratory observes trends and variation (i.e., reasonable spread of warning limits) for warning chart; and appropriate actions are taken if an LC50 falls outside the warning limits and/or outside control limits (± 3 SD on log scale)							

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<b>Test Report (all items here are required, i.e. must be reported)</b>								
Effluent or Chemical	Name and location of operation generating the effluent							
	Date and time of sampling							
	Type of sample (e.g., whole effluent, final mill effluent, etc.) or coding as provided to the laboratory personnel							
	Information on labelling or coding for each sample							
	Brief description of sampling point							
	Sampling method (e.g., grab, batch, 24-h composite etc.)							
	Name of person(s) collecting sample							
Test Facilities and Conditions	Date and time sample received at test facility and temperature of sample upon receipt							
	Test type and method (e.g., single-concentration as specified in 2 <sup>nd</sup> ed EPS 1/RM/10)							
	Name and city of testing laboratory							
	% mortality of fish in holding/acclimation tank(s) from which test fish are taken, as recorded daily (or, as a minimum, for 5 of the 7 days spanning a weekly period) for the 7-day period immediately preceding the test							
	Species of test organism							
	Date and time for start of toxicity test							
	Person(s) performing the test and verifying the results							
	The pH, temperature, DO, and salinity of unadjusted, undiluted effluent, just before preparing test solutions							
	Method used (with citation) for measuring salinity of effluent (or chemical sample), control/dilution water, and test solutions							
	Confirmation that sample or solution was not filtered; indication if any additional tests with filtration or maintaining solids in suspension were performed							
	Confirmation that no adjustment of sample or solution pH occurred; indication of procedure used for any pH adjustment if both pH-adjusted and non-adjusted tests were run (see Section 4.2)							
	Confirmation that no adjustment of sample or solution salinity occurred; indication if any parallel test run using salinity-control water as dilution water (see Section 4.2)							
Indication of aeration of test solutions (rate and time) before introduction of fish; rate of aeration throughout the test								
Concentrations and volumes tested, including control(s), and indication of any replication								

<b>TEST SPECIFIC CHECKLIST</b>							
<b>Reference Method for Determining Acute Lethality Using Threespine Stickleback</b>							
Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Facilities and Conditions cont.	Measurements of DO, pH, and temperature determined for each test solution including control(s) at the beginning and end of the test, as a minimum; as well as salinity of each test solution at the beginning of the test						
	Number of fish added to each test vessel						
	Mean fork length of fish in the dilution-water control at the end of the test, together with the range of the values measured						
	Mean wet weight of individual fish in the dilution-water control at the end of the test						
	Calculated loading density (g/L) of fish in dilution-water control solution(s)						
Results	Number of mortalities of fish in each test solution including the control(s), at 24, 48, 72, and 96 hours; number of control fish showing atypical/stressed behaviour						
	Mean percent mortality of fish in test solutions and control(s), at 96 hours, if a single-conc or multi-conc test is performed using replicate solutions; mean number of control fish showing atypical/stressed behaviour if replicate control solutions						
	Estimate of 96-h LC50 and 95% confidence limits in multi-conc tests, if statistically achievable; methods used for calculating statistical endpoints						
	Most recent 96-h LC50 (with 95% confidence limits) for reference toxicity test(s) performed with fish from the same batch of fish used in the effluent (or chemical) test; reference chemical(s); date test initiated; historic geometric mean LC50 and warning limits ( $\pm 2$ SD)						
	Anything unusual about the test, any problems encountered, and any remedial measures taken						
Deviations	Deviations from any "must" requirements are reported and details provided <b>(must)</b>						
Information Kept On-File	Do lab SOPs indicate that the additional reporting requirements in Section 9.2 of the EPS 1/RM/10 second edition method must be kept on file for 5 years? For details of this information, see EPS 1/RM/10, second edition, section 9.2.						

**Notes:**