Shaded Text reflects February 2011 method amendment changes.

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

TEST SPECIFIC CHECKLIST

Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Sample/Preparation							
Filtering	None; if indigenous organisms, filter through a sieve with 60 µm mesh openings **(Must)**						
D.O. Measurement	In each sample just before its use **(Must)**						
Pre-aeration	Only if D.O. measured in the sample indicate that 1 or more test conc. would be outside 40 - 100 % of air saturation, then pre-aerate the sample						
	Rate of pre-aeration minimal and controlled **(Must)**; and not exceeding 100 bubbles/min						
	Duration of pre-aeration is the lesser of 20 min and attaining 40% of air saturation; test initiated at ≤ 20 min regardless of whether D.O. of 40 - 100% was achieved **(Must)**						
pH adjustment	pH measured in each sample just before its use **(Must)**						
	No adjustment of pH of sample or solution; a second (pH adjusted) test might be required						
T° Adjustment	T° to be measured in each sample on arrival at lab **(Must)**						
	Sample adjusted to ± 1°C of the test T° before starting the test **(Must)**						
	No use of immersion heaters **(Must)**						
Salinity Adjustment	Salinity of each sample measured before starting the test						
	Sample adjusted to 28 - 32 g/kg using natural or artificial HSB, dry ocean salts, reagent grade salts, or deionized water only						
Test Conditions							
Test Facility	Isolated from general laboratory disturbances						
	Instruments available to measure basic water quality variables ((T°, D.O., pH, salinity / conductivity) and lab prepared for other analyses (ie: ammonia) **(Must)**						
Test Type	Static						

TEST SPECIFIC CHECKLIST

Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Duration	Recommend 10-min sperm + 10-min sperm and egg (options include 20-min + 20-min or 60-min + 20-min, both longer durations recommended for Arbacia)						
Test T°	15 ± 1°C for green sea urchins, Pacific purple sea urchins and eccentric sand dollars; and 20 ± 1°C for *Arbacia* and white sea urchins						
Lighting	Normal laboratory lighting or natural sunlight; variable photoperiod						
Salinity	28 - 32 g/kg; preferably 30 g/kg; each test solution within 1 g/kg of the control; adjust using natural or artificial HSB (with a salinity of 90 ± 1g/kg) commercially available dry ocean salts, reagent-grade salts or deionized water						
	Sample not warmed to test temperature before salinity adjustment **(Must)**						
	Any sample to which dry salts added is aged **(Must);** to age sample, salt added while stirring, held for 16-24h at 4 ±2°C in dark within sealed container **(Must)**						
	Nominal test conc. adjusted and reported in consideration of any salinity adjustments **(Must)**						
In-test pH	7.5 - 8.5 minimum range **(Must)**; preferably 8.0 - 8.2						
D.O. Range	40 – 100% air saturation						
Aeration	None						
Vessel Size & Type	Borosilicate glass vials or tubes **(Must)**; 20 mL capacity for 10 mL test solution; with covers						
	Identical for each treatment in a given test **(Must)**						
Test Volume	10 mL recommended (5 mL and 2 mL options)						
Renewal of Test Solution	None						
Dilution/ Control Water	Filtered (60 µm) uncontaminated lab seawater, reconstituted seawater, or filtered (60 µm) upstream receiving water						
	Salinity: 28 - 32 g/kg **(Must)**; recommend 30 g/kg; salinity adjusted using natural or artificial HSB, dry ocean salts, reagent grade salts or deionized water						
	Water used in any given test from same source (if natural seawater) or same batch (if reconstituted seawater) **(Must)**						
	Any HSB, dry ocean salts, or reagent-grade salts used, be from the same source as that used to adjust the salinity of the sample or test solutions **(Must)**						
	pH: 7.5 - 8.5 **(Must)**; recommend 8.0 - 8.2						

TEST SPECIFIC CHECKLIST

Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Dilution/ Control Water cont.	Adjusted to ± 1°C of the test T° before starting the test **(Must)**						
	D.O: 90 - 100 % air saturation **(Must)**; not supersaturated						
	Same water used for preparing control and all test conc. **(Must)**						
	If any HSB is added to sample or test solutions to adjust salinity, the toxicity test include a set of controls prepared using only this HSB and deionized water, adjusted to the test salinity 30 ± 2 g/kg **(Must)**						
	If dry ocean salts or reagent-grade salts added, the toxicity test includes set of controls prepared using same source, batch and conc. as that added to test sample **(Must)**						
	During prolonged storage (>1 day), natural or artificial seawater prepared for use as dilution water should be refrigerated (4±2°C) to minimize microbial growth						
	Artificial seawater should not be used after 14 days following its preparation						
	If uncontaminated receiving water used as control/dilution water, an additional control is run using lab seawater normally used for performing fertilization tests and shown to achieve valid test results **(Must)**						
	Any test using dilution water which differs from HSB or salt control in any respect include a separate set of controls prepared using this same dilution water **(Must)**						
Vessel Identification	Each vessel clearly labelled or positions coded so that conc. and replicates can be identified **(Must)**						
# Test Conc.	≥7 plus control to estimate ICp **(Must)**; ≥10 recommended						
	1 plus control for single conc. test						
# Replicates/Conc.	≥3 for each conc. and control(s) for ICp **(Must)** and ≥4 in single conc. test **(Must)**						
	Test start with equal # of replicates for each conc. and controls **(Must)**						
# Organisms/Vessel	2000 eggs per vessel for 10 mL test volume (1000 eggs for 5 mL test volume and 400 eggs for 2 mL test volume); sperm:egg ratio is ascertained by trial as that which gives 80% fertilization in controls (normally 200:1 to 2500:1)						
Egg Suspension	1 mL per vessel for 10 mL test volume (0.5 mL for 5 mL test volume and 0.2 mL for 2 mL test volume)						
Sperm Suspension	0.1 mL per vessel for 10 mL test volume (0.05 mL for 5 mL test volume and 0.02 mL for 2 mL test volume)						

TEST SPECIFIC CHECKLIST
Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Feeding Regime	None						
Vessel Cleaning	Disposable recommended						
	All test vessels, measurement devices and stirring equipment and pails for transferring organisms thoroughly cleaned/rinsed in accordance with good laboratory practice **(Must)**; Control/dilution water used in final rinse						
Substance Testing	Solubilizing agent control solution be run, if used, and at highest concentration present in test **(Must)**						
	Agent concentration not exceed 0.1mL/L						
Endpoints	Multi conc. test: ICp (and 95% confidence limits) for fertilization success						
	Initial plot of raw data (% fertilization) against log highly recommended; any major disparity between graphic and computer derived ICp resolved **(Must)**						
	Calculation of ICp and it's 95% CL via non-linear regression analysis provided assumptions of normality and homoscedasticity are met **(Must);** data assessed for outliers						
	More than one model fit to data and model with best fit chosen for generation of ICp and 95% CL (**Must)**						
	Endpoints generated by regression analysis are bracketed by test concentrations; endpoint is not extrapolated beyond highest test concentration **(Must)**						
	Lowest test concentration inducing zero (or near-zero) % fertilization kept in data set, subsequent high test concentration(s) removed before regression analyses **(Must)**						
	Hormesis data entered directly for regression (no trimming of data points)						
	Linear interpolation (ex. ICPIN) used to derive ICp if data do not allow regression statistics						
	If data exhibited hormesis and ICPIN is used, control responses entered for those concentrations which demonstrated hormesis **(Must)**						
	For each conc. including control, mean (±SD) % fertilization as determined at end of test is reported **(Must)**						
	Single conc. test: % fertilization and whether significantly lower than control; for each test solution or treatment including control, mean (±SD) % fertilization **(Must)**						

TEST SPECIFIC CHECKLIST
Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Endpoints cont.	Porewater tests: % fertilization and whether significantly lower than control or reference						
EPS/RM/27 Amendments	Has the laboratory incorporated the Feb. 2011 Amendments into lab SOPs?						
Observations and Measurements							
D.O. + pH + T° + salinity	Measured in representative (controls + at least high, medium and low conc.) aliquot of test solutions when they are prepared **(Must)**						
Fertilization	% of fertilized eggs among 100 to 200 inspected microscopically (100X magnification) for each test vessel **(Must)**; consistency of counting checked by trials						
	Clearly abnormal or dead eggs are omitted from the count whether they are fertilized or not						
Test Organisms							
Species	Test carried out with one of the following species: *Strongylocentrotus droebachiensis* (green sea urchin), *Strongylocentrotus purpuratus* (Pacific purple sea urchin); *Dendraster excentricus* (eccentric sand dollar); *Lytechinus pictus* (white sea urchin), or *Arbacia punctulata* (Atlantic purple sea urchin) **(Must)**						
Source	All adults used to provide gametes for a test derived from the same batch and source						
	Wild population; biological supply houses; commercially; or from other laboratories						
Shipping Test Organisms	All information needed to properly identify adult echinoids provided with each shipment **(Must)**						
	Records accompanying each batch of test organisms must include quantity and source, supplier's name, date of shipment and arrival, arrival condition, and species identification **(Must)**						
	During transit appropriate T°, DO and salinity conditions should be maintained, shipping containers should be insulated and T° recorded on departure and arrival (if shipped dry, DO and salinity maintenance do not apply)						
Age	Gametes obtained from mature and gravid echinoids (3-10 cm in diameter, depending on species)						

TEST SPECIFIC CHECKLIST

Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Health Criteria	Holding conditions satisfactory if organisms can deliver viable gametes that meet needs and validity criteria of the test						
Culture/ Holding Conditions for Echinoids Maintained in Lab for Extended Time (>3 days)							
T°	12 ±2°C for green sea urchins; 10 ±2°C for Pacific purple urchins, 13 ±2°C for the eccentric sand dollar; 17 ±2°C for *Arbacia* and 13 ±2°C for white sea urchins						
pH	7.5 - 8.5 **(Must)**; 8.0 ±0.2 preferred						
D.O.	80 – 100% air saturation						
	Remedial measures taken if dissolved gases > 100% saturation **(Must)**						
Salinity	28 - 34 g/kg (preferably 30 - 32 g/kg); not less than 25 g/kg **(Must)** and should not exceed 36 g/kg						
	Rate of salinity change ≤5 g/kg/day **(Must),** should be ≤3 g/kg/day						
Lighting	Low intensity normal laboratory light; 16-h light: 8-h dark; normal daylight, seasonal photoperiod; or complete darkness						
Feeding	Sea urchins: kelp (or other macroalga) or romaine lettuce, spinach and carrots; always available; old or decomposing food removed						
	Sand dollars: provide sediment with detritus and alga, use lighting to encourage growth of alga, if necessary, add shredded eel grass, spinach, flaked fish food, cultured alga or algal paste						
Cleaning	Tanks cleaned and disinfected (if necessary) before introducing new batch of adults						
	Removal of old alga, fecal material and debris daily or as required						
Disease/ Mortality	Monitor daily, mortality should be ≤2%/d averaged over 7 d preceding collection of gametes, and cumulative mortality over the same 7-d period ≤20% **(Must)**; remove dead, diseased or moribund animals; discard groups of diseased animals						
Holding Water	Reconstituted or clean natural seawater						
	Renewed continuously (flow-through: flow of 5-10 L/d for each organism, and equal to the tank volume in 6-12 h) or periodically to prevent buildup of metabolic wastes (target values ≤0.02 mg/L unionized ammonia, ≤0.06 mg/L nitrite)						

TEST SPECIFIC CHECKLIST

Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Holding Water cont.	If reconstituted sea water is used, made up by adding HSB, commercially-available dry ocean salts or reagent-grade salts to fresh water **(Must) ;** if made using dry salts is aerated for ≥24 h **(Must);** aeration for ≥3 days recommended						
	Any artificial HSB prepared using commercial or reagent grade salts is filtered (≤1 μm) and aerated for ≥24h **(Must)** aeration for ≥3 d recommended; HSB from natural seawater should be filtered (≤1 μm)						
	Unused portions of prepared natural or artificial HSB should be capped and stored in the dark at 4±2°C until used						
Water Depth	≥ 20 cm for sea urchins; ≥ 10 cm water with 2 - 3 cm of sediment or sand rich in detritus for sand dollars						
Acclimation	Recommend gradual acclimation and holding adults a minimum of 3 - 4 days at test T° and salinity and in same water as control/dilution water to be used in test prior to gamete collection						
	Temperature rate of change ≤ 5°C /d **(Must)**						
Facility & Apparatus	Aquaria, troughs or tanks made of nontoxic materials (~ 50 - 150 L water)						
	Holding tanks located away from physical disturbances, preferably separate from area used for testing						
	Held in groups of ≤20 to avoid mass spawning						
Specific Conditions for Holding Adults for Immediate Use (≤3d)							
Gamete Collection	Gametes collected ≤3 d after adults arrive at lab						
	Confirmation should be obtained from supplier that adults are mature and eggs are viable						
T°	T° at shipping at/near test T°						
Acclimation	Gradual exposure to testing lab's holding conditions and/or control/dilution water						
	Adults spawned on same day as arrival at lab held for minimum of 3 h to observe health and move to testing conditions **(Must)**						
Lighting	Recommend lighting representing test conditions						
Salinity	Adjustment should be <5 g/kg/day, can be higher if test validity criteria is met and sensitivity of gametes in reference toxicant tests not affected						
Feeding	Not required						
Mortality	Adults cumulative mortality ≤20% for 7-d period prior to shipment **(Must)**						

TEST SPECIFIC CHECKLIST

Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Gamete Handling							
Gamete Collection	Adults are stimulated to spawn using 0.5 to 1 mL of 0.5 *M* KCl through the peristomal membrane into the coelom for sea urchins; and 0.5 mL of the solution through the mouth for sand dollars (alternatively, stimulate the shell for 30 seconds with 12 volts D.C. for Arbacia)	☐	☐	☐	☐	☐	☐
	A second injection used if no spawning in 5 or 10 min	☐	☐	☐	☐	☐	☐
	Collection of spawn terminated within 15 min of start of steady spawning	☐	☐	☐	☐	☐	☐
	Gametes from males pooled and gametes from females pooled before transferring to test vessels, perform gamete check to ensure only good quality gametes are selected for test **(Must)**	☐	☐	☐	☐	☐	☐
	Sperm should represent ≥3 male adults and eggs ≥3 female adults	☐	☐	☐	☐	☐	☐
Gamete Check	3-5 females and ≥3 males selected for microscopic examination of gametes **(Must)**	☐	☐	☐	☐	☐	☐
	"Good quality" eggs from each female fertilized with 5-7 drops of diluted sperm (i.e. 20-50 μL dry sperm in 10 mL filtered seawater) from each of the "good-quality" batches of sperm	☐	☐	☐	☐	☐	☐
	Mixture of sperm and eggs observed microscopically after 10 min; Sperm quality assessed for motility, activity, clumping and fertilization success, and egg quality for shape, colour, size and fertilization success	☐	☐	☐	☐	☐	☐
	If no pre-test run to determine optimal sperm:egg ratio, good quality gametes pooled from ≥3 males and ≥3 females, as determined in gamete check, are used **(Must)**	☐	☐	☐	☐	☐	☐
	If good quality gametes not available from ≥3 males and ≥3 females, fewer adults can be used if pre-test done to determine optimal sperm:egg ratio for a given batch of gametes **(Must)**	☐	☐	☐	☐	☐	☐
Gamete Pre-test	Uses 2 replicates of control/dilution water and 1 replicate of each of 3 conc. of reference toxicant tested with each of several (~5) sperm:egg ratios to determine optimum; ratios should cover wide range (~10-fold difference in sperm conc.)	☐	☐	☐	☐	☐	☐
	Ratio chosen based on % fertilization results in control/dilution water (targeting 80%) and that which maximizes potential for reference toxicant result to fall within warning limits of control chart	☐	☐	☐	☐	☐	☐

TEST SPECIFIC CHECKLIST
Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Gamete Pre-test cont.	Pre-test recommended for porewater tests; and should include addition of 2 replicates of control porewater	☐	☐	☐	☐	☐	☐
Spawning Males	"Dry spawning" recommended; collect sperm from bottom of petri dish or beaker or from surface of animal (sand dollars should be spawned in a minimal amount of seawater)	☐	☐	☐	☐	☐	☐
	Care taken collecting dry sperm to avoid contamination with water or KCl solution **(Must)**	☐	☐	☐	☐	☐	☐
	Semen collected "dry" held on ice for 4 h before "activation" in seawater then used in test in the subsequent 30 - 120 min	☐	☐	☐	☐	☐	☐
	Semen collected "wet" used to start test in a period of ≥ 0.5h to ≤ 2h.; in interim, sperm stored on ice in minimal control/dilution water	☐	☐	☐	☐	☐	☐
Spawning Females	"Wet spawning" recommended; animal placed aboral side down on small beaker (50 - 250 mL) filled with control/dilution water at test T°; water decanted from gametes after spawning complete. Alternatively, animal placed aboral side up in a vessel with control/dilution covering test (shell) by 1 cm; Eggs collected off surface and placed in beaker/appropriate vessel	☐	☐	☐	☐	☐	☐
	Eggs washed 3 X by diluting with 100 mL control/dilution water, mixing, settling for 10 min and decanting	☐	☐	☐	☐	☐	☐
	Eggs can be held for 4 h at test T° before use (recommend gentle aeration)	☐	☐	☐	☐	☐	☐
Sperm Density	Sperm density estimated with hemocytometer (diluted 100x to 10,000x, using 10% glacial acetic acid made up with control/dilution water); count sperm in middle 400 small squares	☐	☐	☐	☐	☐	☐
	# sperm/mL in initial suspension = (dilution factor) x (# sperm counted) x (hemocytometer conversion factor) x (conversion of mm^3 to mL) ÷ (# squares); for standard Neubauer hemocytometer: # sperm/mL = 100 x (# sperm) x 4000 x 1000 ÷ 400	☐	☐	☐	☐	☐	☐
	Sperm density adjusted to desired conc. (determined by sperm:egg ratio selected) using control/dilution water	☐	☐	☐	☐	☐	☐
	Alternative technique is to use turbidity or optical density as indication of # sperm/mL	☐	☐	☐	☐	☐	☐
Egg Density	Eggs counted using a Sedgwick-Rafter cell (add ≤ 1 mL of mixed suspension, as required, diluted 10-, 100-, or 1000-fold for counting)	☐	☐	☐	☐	☐	☐

TEST SPECIFIC CHECKLIST

Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Egg Density cont.	Egg suspension adjusted to 2000 eggs/mL by adding control/dilution water or decanting water, as necessary						
Sperm:Egg Ratio	Optimum sperm:egg ratio determined by pre-test in each lab to give 80% fertilization under control conditions; should be determined just before each test with gametes to be used in that test						
	Alternatively to a pre-test, replicates of 2-3 sperm:egg ratios for each test conc. including controls could be used and results for the ratio yielding a control fertilization rate closest to 80% would be used in calculating the endpoints						
Gamete Exposure	0.1 mL of sperm solution added to each test vessel (containing 10 mL of test solution); at end of sperm exposure, 1 mL of mixed egg exposure is added to each test vessel; at end of sperm and egg exposure, test is terminated by adding either 2 mL or less of 1% glutaraldehyde or 2 mL or less of 10% buffered formalin to each test vessel						
	A timing procedure should be used for adding sperm to vessels in sequence (eg: 1 vessel every 5 sec) and eggs should be added in the same sequence (order of vessels) with same timing to equalize exposure periods						
	All fluid delivered from pipette enter test solution rather than striking the side of the vessel and pipette tip not touch test solution **(Must)**						
	Suspension of female gametes mixed after every second or third addition						
	After each addition, all vessels thoroughly mixed						
Counting Preserved Eggs	Preserved eggs counted within 3 d of test completion; vessels sealed during storage						
QA/QC							
Test Validity Criteria	Test is invalid if the mean fertilization rate in all replicates of the control water is < 60%, or ≥98% **(Must)**						
	A positive and logical dose-effect curve should be obtained						
	If two sets of control solutions are used, results of toxicity test are valid only if each set of controls meets validity criteria						
	No pooling of controls to calculate endpoints if validity criteria not met and/or if controls are statistically different by t-test **(Must)**						
Reference Toxicant	Perform within 14 d of definitive test, or concurrently with definitive test for every new batch of adults if held >3 d **(Must)**; concurrent tests use same batch of gametes as definitive test **(Must)**						

TEST SPECIFIC CHECKLIST

Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Reference Toxicant cont.	For gametes collected from adults ≤3 d of arrival at lab, portion of the batch tested for reference toxicant under same experimental conditions and concurrently with definitive test **(Must)**						
	Standard test with ICp endpoint using the same procedure as the definitive test **(Must)**						
	Copper is recommended						
	Using same water as culture dilution/water **(Must)**						
Warning Chart	Prepared for each reference toxicant and continually updated **(Must)**						
	Within acceptable warning limits (± 2 SD on log scale)						
	If ICp outside control limits (mean ±3 SD), or if endpoints fell between control and warning limits >5% of the time, test should be repeated and aspects of test carefully scrutinized						
Sample Handling							
Containers	Non-toxic materials for sample and transport containers, new containers or thoroughly rinsed used containers **(Must)**						
	Collapsible polyethylene or polypropylene containers recommended						
	Upon collection, sample containers filled, sealed and labeled/coded **(Must)**						
Volume	2 L						
Labeling	Include at least sample type, source, date and time of collection and name of sample collectors						
Holding Time	Test to be initiated within 3 days after sampling (or following preparation of elutriate) **(Must)**						
	Recommend test initiation within 1 day after sampling						
Holding Conditions	Make effort to keep samples cool throughout their period of transport **(Must)** at 1 - 7°C (preferably 4 ± 2°C) using regular ice or frozen gel packs						
	Upon collection, if sample > 7 °C, cool to 1 - 7°C with regular ice or frozen gel packs (not dry ice) **(Must)**						
	Sample be kept from freezing during transport or storage **(Must)**						
	Any remaining portion(s) of sample held for possible additional testing be stored in darkness in sealed containers without air headspace at 4 ± 2°C **(Must)**						
Subsample Mixing	Subsample (ie: a sample divided between 2 or more containers) mixed together to ensure their homogeneity **(Must)**						

TEST SPECIFIC CHECKLIST
Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Sample Aliquots	Each sample in a collection container thoroughly agitated just before pouring **(Must)**						
Test Report							
Sample Data	Brief description of sample type and volume or weight (if dry chemical) if and as provided to the lab **(Must)**						
	Information on labelling or coding, for each sample **(Must)**						
	Date of sample collection; date and time sample received at lab **(Must)**						
	For effluent or leachate, T° of sample upon receipt at lab **(Must)**						
	D.O. and pH of sample of wastewater or receiving water just before its preparation and use **(Must)**						
	Date of sample generation and use and procedure for preparation for elutriate or any liquid extracted from sediments or similar solids **(Must)**						
Test Organism	Species and source **(Must)**						
	Brief description of holding time and conditions for adults **(Must)**						
	% mortalities among adults shipped and held ≤3 d and/or weekly; and/or % mortalities among the adults being acclimated and held for >3 d **(Must)**						
	Any unusual apparearance, behaviour, or treatment of adults or gametes, before the test is started **(Must)**						
Test Facilities	Name and address of test laboratory **(Must)**						
	Name of person(s) performing the test **(Must)**						
	Brief description of test vessels (size, shape, type of material) **(Must)**						
Control/ Dilution Water	Type and source of water used as control and dilution water **(Must)**						
	Type(s), source(s) and collection procedure of control and/or reference pore water used, if any **(Must)**						
	Type & quantity of any chemical(s) added to control or dilution water **(Must)**						
Test Method	Citation of biological test method used **(Must)**						
	Design and description if specialized procedure **(Must)**						
	Brief description of procedure for preparation of hypersaline brine and duration of aging **(Must)**						
	Brief description of procedure(s), products used, and duration of aging for any salinity adjustments for control/dilution water, sample, or test solutions **(Must)**						
Test Method cont.	Brief description of procedure(s) if any sample or test solutions received filtration and/or pH adjustment **(Must)**						

TEST SPECIFIC CHECKLIST

Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
	Brief description of frequency and type of all observations and all measurements made during test **(Must)**	☐	☐	☐	☐	☐	☐
	Program(s) and methods used for calculating statistical endpoints **(Must)**	☐	☐	☐	☐	☐	☐
Test Conditions	Design and description if any deviation from or exclusion of any of the procedures and conditions specified in test method document **(Must)**	☐	☐	☐	☐	☐	☐
	Number, concentration, volume, and depth of solutions in test vessels, including controls **(Must)**	☐	☐	☐	☐	☐	☐
	Number of replicates per treatment **(Must)**	☐	☐	☐	☐	☐	☐
	Brief statement indicating that gamete viability check was performed, and whether a pre-test was performed **(Must)**	☐	☐	☐	☐	☐	☐
	Estimated number of sperm per vessel and sperm:egg ratio **(Must)**	☐	☐	☐	☐	☐	☐
	# males and females used to pool sperm and eggs **(Must)**	☐	☐	☐	☐	☐	☐
	Brief statement concerning aeration (if any, give rate, duration) of sample or test solutions before starting the test **(Must)**	☐	☐	☐	☐	☐	☐
	T°, salinity, pH, D.O. in aliquot of test solutions & controls at test start **(Must)**	☐	☐	☐	☐	☐	☐
	Period of time test vessels are stored prior to enumerating results **(Must)**	☐	☐	☐	☐	☐	☐
	Dates when test was started and statement of test duration **(Must)**	☐	☐	☐	☐	☐	☐
	Date when reference toxicity test was performed and brief statement indicating whether it was performed under the same experimental conditions as test sample; description of any deviation/ exclusion of procedures/ conditions specified in test method document **(Must)**	☐	☐	☐	☐	☐	☐
Test Results	Number of fertilized and unfertilized eggs counted for each vessel (including control groups) at the end of the test **(Must)**	☐	☐	☐	☐	☐	☐
	Mean (±SD) % fertilized eggs or proportion fertilized for each test vessel including controls **(Must)**	☐	☐	☐	☐	☐	☐
	ICp and 95% confidence limits for the percent fertilization or proportion fertilized data; details regarding any weighting techniques and indication of quantitative statistics used **(Must)**	☐	☐	☐	☐	☐	☐
	Any outliers and the justification for their removal **(Must)**	☐	☐	☐	☐	☐	☐
Test Results cont.	Results and duration of any toxicity tests with the reference toxicant(s) performed at the same time or within 14 days of the test, together with the geometric mean value (± 2 SD) for the same reference toxicant(s) as derived at the test facility in previous tests with the same species **(Must)**	☐	☐	☐	☐	☐	☐

TEST SPECIFIC CHECKLIST
Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
	Anything unusual about the test, any problems encountered, any remedial measures taken **(Must)**	☐	☐	☐	☐	☐	☐
Information Kept On-File	Do lab SOPs indicate that the information on Section 9.2 of the EPS 1/RM/27 method must be kept on file for 5 years? **(Must)** *For details on this information, see EPS 1/RM/27, section 9.2	☐	☐	☐	☐	☐	☐

Notes: