

EPA Region 10 Guidance for Conducting Effluent Toxicity Tests Using West Coast Sea Urchins and Sand Dollars

Effluent toxicity tests using west coast sea urchins and sand dollars that are conducted in compliance with NPDES permit requirements should be performed following the sperm cell test protocol described by Dinnel, Link, and Stober, 1987. Additional technical and statistical guidelines for conducting these tests are provided in the EPA "Chronic Marine Toxicity Test Manual" (U.S. EPA, 1988). However, the following summary of recommended conditions for conducting sperm cell tests with west coast echinoderms is to provide interim guidance that is more consistent with the present EPA protocol describing a sperm fertilization test using the east coast sea urchin, Arbacia punctulata (U.S. EPA, 1988).

EPA test development efforts are expected to produce a draft "West Coast Echinoderm Sperm Fertilization Test" protocol later this year that will replace Region 10's guidance.

References:

- Dinnel, P.A., J.M. Link, and Q.J. Stober. 1987. Improved methodology for a sea urchin sperm cell bioassay for marine waters. Arch. Environ. Contam. and Toxicol., 16: 23-32.
- U.S. EPA. 1988: Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio, EPA/600/4-87/028.

Other Sources of Information:

- U.S. Environmental Protection Agency, Environmental Research Laboratory, Hatfield Marine Science Center, Newport, Oregon
- U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, Rhode Island

Summary of Recommended Test Conditions for Conducting Sperm Fertilization Tests Using the Purple Sea Urchin, Strongylocentrotus purpuratus, Green Sea Urchin, Strongylocentrotus droebachiensis, Red Sea Urchin, Strongylocentrotus franciscannus, and Sand Dollar, Dendraster excentricus.

1. Test Type	Static
2. Salinity	30 o/oo \pm 2 o/oo
3. Temperature	12 \pm 1°C
4. Light Quality and Intensity	Ambient laboratory illumination, 10-20 uE/m ² /s (50-100 ft-c).
5. Test Vessel Size	Disposable (Glass) Liquid Scintillation Vials (20-mL); Not Pre-cleaned.
6. Test Solution Volume	10 mL
7. No. of Spawning Adults	Two to four males and females, with sperm and eggs being pooled separately.
8. Sperm Collection and Storage Procedure	"Dry" sperm collection technique ^a , with pooled sperm stored on ice. Sperm must be used within one hour of being collected.
9. Number of Eggs per Test Vessel	2000 eggs
10. Sperm:Egg Ratio	Depends on species. (Note: Each control and effluent treatment should be tested using several sperm:egg ratios, with fertilization being scored only at that ratio yielding 70% - 90% fertilization success in the seawater controls. Tests achieving less than 50% fertilization success in the seawater controls are considered invalid.)
11. Number of Replicates per Treatment	4 (minimum of 3)
12. Dilution Water	Uncontaminated natural seawater; deionized water mixed with hypersaline brine or artificial sea salts.
13. Dilution Factor	0.3 or 0.5
14. Test Duration	1 hr and 20 min (60 min sperm exposure plus 20 min fertilization period).

15. Aeration	None
16. Effect Measured	Fertilization success as indicated by the presence of a fertilization membrane.
17. Number of Treatments per Test	Minimum of five effluent concentrations and a control.
18. Reference Toxicant	The toxicity (EC50) of a reference toxicant <u>must</u> be determined concurrently with each test.
19. Egg Control	An egg control (eggs minus sperm) should be included with each test to reveal any contamination of the eggs with sperm or the presence of false positives, i.e. eggs that appear to be fertilized in the absence of sperm.
20. Egg-Effluent Control	An egg-effluent control (eggs plus effluent, minus sperm) should be included with each test to reveal the presence of false positives, i.e. eggs that appear to be fertilized in the presence of effluent, but in the absence of sperm.

^a "Dry" Sperm Collection Technique:

Sea urchins and sand dollars are induced to spawn by KCl-injection as described by Dinnel, et al., 1987. Eggs are also collected and washed as described in the Dinnel protocol, however, sperm are collected by the "dry" (seawater-free) sperm collection technique outlined below.

Spawning males are removed immediately from the seawater-filled beakers and placed, right-sided-up, in a shallow tray containing seawater. The males should be partially immersed in the seawater, taking care that the water does not cover the gonophores located at the top of their tests (shells). Sperm are then collected from the gonophores using a pipet or syringe. The "dry" sperm are stored on ice until diluted (and activated) with seawater for counting and testing. The sperm must be used in a toxicity test within one hour of collection.