

Members of the Review Panel for Tropical collector urchin fertilization test method:

Document for review:

The Tropical collector urchin, *Tripneustes gratilla*, fertilization test method in its final form will become Chapter 18 in an updated version of the 'Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to West Coast Marine and Estuarine Organisms' (West Coast marine methods manual). This fertilization method estimates the chronic toxicity of effluents and receiving waters to the gametes of the tropical sea urchin (*Tripneustes gratilla*) during a static, non-renewal 60-minute sperm exposure and a subsequent 20-minute exposure period, following the addition of eggs, for measuring the fertilizing capacity of the sperm. The purpose of the test is to determine the concentrations of a test substance that reduce fertilization of exposed gametes, relative to that of the control.

The Tropical collector urchin, *Tripneustes gratilla*, fertilization test method described in the document under review provides a standardized procedure and set of test acceptability criteria based on methodological variations of this test used historically with this species. It is generally consistent with other urchin fertilization test methods, especially the West Coast urchin fertilization test using *Strongylocentrotus purpuratus*. Therefore, these supporting materials are provided:

- West coast marine methods manual, selected sections
 - chapter 16, Pacific urchin fertilization test method
 - Appendices A – L
 - References
- Published Tropical urchin fertilization methods comparison (ET&C 2003 22:2191 – 2194)

Reviewers' charge:

This panel comprised of peer reviewers and technical commenters has been convened to contribute to EPA's review of this method. The peer reviewers should conduct this review similarly to the way they would review other scientific products. Specifically, the entire method should be reviewed for attributes such as 'content and scope', 'organization and presentation', as identified in the Technical Manuscript Review Form (EPA-363 provided to peer reviewers). In addition, both reviewers and technical commenters are asked to comment on the usefulness of specific aspects of the test conduct and acceptability criteria that have been standardized in this version. These aspects appear in red in the method text, and are compared in the table below to the West Coast urchin fertilization test method.

Reviewers and commenters are asked to complete their tasks in one month during which time they should feel free to contact the authors for any additional information. All contributions will be considered in the development of the final method to be included in the updated West Coast methods manual.

With appreciation,
Diane Nacci and Amy Wagner, US EPA

	Pacific urchin fertilization method	Reference	Tropical urchin fertilization method	Reference
Urchin species	<i>Strongylocentrotus purpuratus</i>	West Coast manual, section 16	<i>Tripneustes gratilla</i>	Tropical urchin method
Sperm:egg testing ratio			Spectrophotometric count optional	
	Optional pre-test	p. 416	Pre-trial, 45 minute exposure	p. 2, 26, 29, 33 (Figure 7 to be revised)
	S:E range: 100 - 3000:1		S:E range: 125 - 2500:1	p. 31
	'oversperming control'			
	Lowest S:E w ≤ 90% control fertilization	Table 6, p 424	Lowest S:E w < 90% control fertilization?	Table 6, p 34
Definitive Test	W/out pre-test, if S:E ≤ 500:1		Conducted ≤ 3 hr post sperm collection using newly prepared sperm solution; 60 minute exposure	p. 24, 29, 36
Test Acceptability Criteria	≥ 70% control fertilization; and appropriate sperm counts; %MSD < 25%	Table 7, p 435	S:E ≤ 2500: 1 (± 10%) = < 50,000,000 sperm/mL; ≥ 70% control fertilization	p 37, 42, Table 7