THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**IN SITU SEDIMENT TOXICITY TESTING**
APPLICATION:	**SEDIMENT AND AQUEOUS TOXICITY TESTING**
TECHNOLOGY NAME:	**Sediment Ecotoxicity Assessment Ring**
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The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field and laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The Advanced Monitoring Systems (AMS) Center, one of six verification centers under ETV, is operated by Battelle in cooperation with EPA's National Risk Management Research Laboratory. The AMS Center evaluated the performance of a sediment toxicity testing device for in situ sediment toxicity testing. This verification statement provides a summary of the test results for the Space and Naval Warfare Systems Center Pacific (SPAWAR) Sediment Ecotoxicity Assessment Ring (SEA Ring) for determining the toxicity of contaminated sediment and overlying water on benthic and aqueous organisms.

VERIFICATION TEST DESCRIPTION

This verification testing of the SEA Ring was conducted in two rounds, initially from November 16 through December 7, 2012, while the second round was conducted in March 2013 at the SPAWAR Systems Center (SSC) Pacific Bioassay Laboratory in San Diego, CA. The primary evaluation assessed survival, growth, and bioaccumulation of contaminants in the aquatic and benthic organisms exposed in the SEA Ring compared to

responses achieved in the laboratory using standard ASTM and EPA methods. For the water column toxicity tests, two organisms (Pacific topsmelt [*Atherinops affinis*] and mysid shrimp [*Americamysis bahia*]) were evaluated at four different copper concentrations (0, 100, 200 and 400 µg/L). For the sediment toxicity tests, three different species (bent-nosed clam [*Macoma nasuta*], marine amphipod [*Eohaustorius estuarius*], and marine polychaete [*Neanthes arenaceodentata*]) were tested for sediment toxicity and bioaccumulation. Four sediment types (two control sediments, a metals contaminated sediment [MS] and a polychlorinated biphenyl [PCB] contaminated sediment [PSNS]) were used for the sediment toxicity tests. The PSNS sediment was included for bioaccumulation testing. The clam was not exposed to the MS sediment. Simultaneous reference toxicant tests were conducted with copper sulfate at five different concentrations to assess test precision and the health and sensitivity of each batch of test organisms. Clams were not included in the reference toxicant tests. Table VS-1 provides information on the number of organisms and number of replicates included for each test.

Table VS-1. Toxicity Test Summary

	# of Replicates	# of Organisms per Chamber	Exposure Period (days)
Water Column Toxicity Test			
Mysid Shrimp	5	10	4
Topsmelt	5	5	4
Sediment Toxicity Test			
Amphipod	5	20	10
Clam	5	3	14
Polychaete	5	20	20
Reference Toxicant Test			
Mysid Shrimp	5	10	4
Topsmelt	5	5	4
Amphipod	3	10	4
Polychaete	3	10	4

Test acceptability criteria were used to evaluate each of the parameters measured in the water column and sediment toxicity tests. Survival was considered acceptable when the mean survival among the replicates was ≥ 90%. If the mean percent survival was less than 90%, the test was repeated. For comparison of the mean percent survival among the replicates, a coefficient of variation of less than 25% was targeted. For the reference toxicant tests, the laboratory tests were considered acceptable if the LC50 was within ± two standard deviations of the running mean for the testing laboratory.

The main objective of this verification test was to generate performance data on the SEA Ring for assessing water column toxicity, contaminated sediment toxicity, and bioaccumulation potential using indigenous organisms. The performance of the SEA Ring was evaluated against traditionally used EPA and ASTM methods. The SEA Ring tests were evaluated on the following performance parameters:

- Repeatability - the variability in biological response among the five replicate exposure chambers in a SEA Ring.
- Comparability - comparison between results obtained from tests in the SEA Ring and traditional EPA and ASTM laboratory methods.
- Intra-unit Reproducibility - to determine if different SEA Rings are capable of producing the same results.
- Operational factors (qualitative assessment) - ease of use, training and sustainability (sampling time, waste produced, and the amount of protective equipment required by the individual operating the technology).

The SEA Ring was operated according to the SEA Ring manual and the technology representative's instructions by SPAWAR and Battelle staff. The technology representative was available to answer questions and provide support. QA oversight of verification testing was provided by Battelle, AMEC Environment and Infrastructure,

and EPA. Battelle and AMEC QA staff conducted technical systems audits of all laboratory testing, and Battelle QA staff conducted a data quality audit of at least 10% of the test data. This verification statement, the full report on which it is based, and the test/QA plan for this verification test are available at http://www.epa.gov/nrmrl/std/etv/verifiedtechnologies.html#site.

TECHNOLOGY DESCRIPTION

The following is a description of the technology, based on information provided by the technology representative. The information provided below was not verified in this test.

The SEA Ring (U.S. Patent No. 8,011,239) is an integrated, field tested, toxicity and bioavailability assessment device. This device was developed at SPAWAR in San Diego, California and is commercially available from Zebra-Tech, Ltd. The unit consists of 10 cylindrical chambers fixed to a circular ultra-high molecular weight polyethylene platform. The top end of each chamber is fitted with an integrated, multifunctional cap. The cap includes both overlying water intake and outlet ports, and an organism delivery port (opening for an optional modified plastic 30 cubic centimeter [cc] syringe). The intake port connects to a peristaltic pump that is housed in the center of the device and powered by rechargeable batteries stored in a separate housing underneath the pump. The pump is programmable to provide chamber water volume exchange at a rate (range ~6 to >50 turnovers per day) desired for the site- or project-specific preferences.

The SEA Ring was designed to evaluate toxicity in the water column (WC), sediment water interface (SWI), and/or surficial sediment (SED). The SED chambers are open on the bottom, 10 inches in length, 2.75 inches in diameter, and extend 5 inches below the base of the system. Small sediment dwelling organisms can be introduced into the SED chambers through the organism delivery port built into the cap with a modified 30 cc plastic syringe. The syringe is plugged with a silicone stopper inside the test chamber to retain the organisms until desired release. For larger organisms, a ½ inch stainless steel mesh is integrated into the bottom opening of the exposure chamber, allowing organisms to be preloaded prior to deployment. The WC and SWI chambers are 5 inches in length, 2.75 inches in diameter, and have a closed bottom. The bottom consists of a solid plastic polyethylene cap or mesh insert for SWI testing. Organisms for the WC and SWI tests can be loaded in the laboratory or in the field immediately prior to deployment. The center of the circular platform houses a custom-built peristaltic pump and battery. These components are fully encased and water tight. The intake to the test chambers is located on top of the cap. Each inlet is directly connected to the pump through individual tubes that pass over the pump roller. As the pump rotor turns, compressing and releasing pressure on the tubing, ambient water from the surrounding area is circulated through each chamber. Water then leaves each chamber through an outlet port also located in the cap. The inlet and outlet ports house small screens to prevent the loss of organisms from the chamber. A water quality sensor or passive sampler can also be attached to one of the chambers. Water quality sensors are used to measure a variety of physical parameters including pH, temperature, depth, salinity, conductivity, and dissolved oxygen from inside the exposure chambers.

VERIFICATION RESULTS

The verification of the SEA Ring is summarized below.

Repeatability

This verification parameter tested the variability among five replicates within a SEA Ring using both the sediment and WC toxicity tests. To determine standard deviation and standard error of the sample mean for a given set of treatments, the coefficient of variation (CV) was calculated. A CV of less than 25% was targeted. For the WC toxicity tests, the CV was less than 25% for the control treatments for both the mysid and topsmelt tests. For the mysid toxicity test, the 200 and 400 µg/L treatments had CV values greater than 25%. For the topsmelt toxicity test, all copper concentrations greater than 0 µg/L (control) had CVs greater than 25%. With increasing copper concentrations, organism mortality increased as did replicate variability, which was (and is typically) observed in the parallel standard laboratory tests. For the sediment toxicity tests, the CV was less than 25% for survival (and growth for the polychaete) for all species and all sediment types, indicating low variability across chambers within the SEA Ring for a given treatment. The CV was also less than 25% for growth of polychaete in both the control and contaminated sediments. Bioaccumulation was also determined and there was no detectable bioaccumulation for any species under the control sediment treatment.

Comparability

Comparability was measured as the ability of the SEA Ring to provide similar results to the traditional EPA/ASTM methods under controlled laboratory conditions. Comparability was investigated for the sediment toxicity, WC toxicity and bioaccumulation tests for identical treatments in the SEA Ring to the laboratory tests. In both sediment and WC toxicity tests, there was no statistically significant difference in survival for any of the treatments, indicating that the result obtained from the SEA Ring was no different from the results obtained by EPA and ASTM laboratory methods. Polychaete growth was determined by measuring the wet weight collectively of the organisms in each replicate after the exposure period. A statistical comparison of the growth of polychate between the SEA Ring and laboratory tests showed no statistically significant difference for the control sediment exposures, but there were significant differences for both the MS and PSNS sediment exposures based on the wet weights. There was no significant difference between the SEA Ring and laboratory tests on the growth of the polychaete in the MS sediment exposure based on dry weight. Comparability between the SEA Ring and laboratory tests for the bioaccumulation revealed no significant differences for any of the species tested.

Reproducibility

Reproducibility compared mean percent survival in two SEA Rings where identical tests were conducted. This was measured using the WC toxicity test with mysid and topsmelt for two copper concentrations: the seawater control and 200 µg/L treatments. No statistically significant differences were found in comparisons between the mean percent survival obtained from the two SEA Rings for either species tested.

Operational Factors

The SEA Ring was operated in the laboratory by the staff at SPAWAR, and also by a Battelle staff member. During a 4-hour period, the Battelle staff member was trained on use of the SEA Ring, including loading of organisms and measurement of water quality parameters. The Battelle staff member found the SEA Ring easy to operate, but noted that care must be taken when loading some species due to their small size. It should be noted that this is also the case with standard laboratory test methods. The SEA Ring was found to be easy to transport by one person. The waste obtained when operating the SEA Ring was minimal. No maintenance was required when the Battelle staff was onsite.

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