**Laboratory Testing In Support of *Site Specific Water Quality Criteria Assessment and Hydrographic Data Collection for New Bedford Harbor***

# TASK 2B
# TOXICITY IDENTIFICATION EVALUATION TESTING WITH MYSIDS AND SEA URCHINS

**Data Report**

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***Quality Criteria Assessment and Hydrographic Data Collection for New Bedford Harbor***

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# NEW BEDFORD HARBOR TOXICITY IDENTIFICATION EVALUATION

## Introduction and Background

Task 2B of SAIC's Site Specific Water Quality Assessment Study is a follow-on study conducted to resolve cause(s) of toxicity observed in Suspended Particulate Phase testing (SPP; Task 2A). Task 2A found that only one of six site samples, NBH-202, was found to be toxic to *Americamysis bahia,* the species chosen for SPP testing. Hence, SPP from NBH-202 was further evaluated using a sequential toxicity identification evaluation (TIE) testing approach (SAIC, 2002). TIEs are used to identify cause and affect relationships between toxicity observed in toxicity tests and factors that have contributed to the observed effects. These relationships are revealed through manipulations that remove the toxicity of individual toxicant classes (e.g., metals, organics, or ammonia) from (e.g., SPP and elutriates). Associated reductions in toxicity are used to characterize causative factors. It was expected that the cause of acute toxicity in the NBH-202 sample would be due principally to copper, PCBs, confounding factors, or a combination of factors. Per EPA Marine TIE methodology (EPA, 1994) two species were tested, as differential sensitivity to specific toxicants provide additional evidence regarding the factors causing toxicity. For this study, the mysid *(A. bahia)* survival test and the sea urchin *(Arbacia punctulata)* larval development test were selected because they were previously used in monitoring of potential dredging-related water column impacts in Upper New Bedford Harbor (Nelson, 1991), and because they are relatively sensitive to PCBs and copper, respectively. Results from the TIE tests will contribute to the basis for an approach to derive Water Effect Ratios (Task 2C) and site specific protective exposure limits for New Bedford Harbor aquatic life.

## Methods

### *Sample Collection, Preparation and Transport*

Sediment and water collection for the TIE conducted with NBH-202 were described in the Task 2A report, "Suspended Particulate Phase Acute Toxicity Tests with Myids" (SAIC, 2002). The samples were stored (4 ± 2° C) at the toxicity testing laboratory (SAIC's subcontractor, Aquatec Biological in Williston, VT) from 12 October to 28 October 2002. On 28 October 2002, new SPP was prepared for TIE manipulations and testing. Suspended Particulate Phase samples were prepared as described in the Task 2A report (SAIC, 2002) except that GP-2 artificial sea salts were substituted for the commercial Forty Fathoms® artificial seasalt mixture because GP-2 may be more reliable with the sea urchin larval development test used in the TIE (Aquatec, personal communication). The volume of prepared SPP required for mysid testing was sub-sampled, and the remaining SPP was prepared for the sea urchin larval development tests with *Arbacia punctulata* by centrifuging for approximately10 minutes at 6000 rpm to remove fine particulates that may inhibit larval development. SPP was shipped overnight to SAIC's Newport, RI laboratory for TIE manipulations (see below), and TIE samples were subsequently shipped back to Aquatec for toxicity testing to commence on 30 October 2002.

To serve as a positive control for the TIE tests, SAIC prepared a spiked solution using GP-2 artificial seawater, neat copper chloride (Sigma Chemical) and neat Arochlor 1242 (PP-310) standard from Ultra Scientific, North Kingstown, RI. The copper was spiked from a 10 mg/L stock solution prepared in deionized water manipulated to a pH of 2.0 with nitric acid to result in a test concentration of 120 ug copper/L. Aliquots of 100 mg Aroclor 1242/L in methanol were added to the copper-spiked sample to result in a nominal concentration of 200 µg/L. The copper spike is expected to be largely dissolved and stable (Lussier et al., 1999), while the nominal Aroclor concentration would be expected to be approximately an order of magnitude higher than the actual exposure concentration (Ho et al., 1997). Concentrations were chosen to approximate those that would affect approximately 50% of at least on of the test species (based on known LCs0 or ECs0). While copper and PCBs were the only constituents in the spiked sample for sequential TIE treatments (see TIE Manipulations and Testing, below), ammonia was added from a 1,000 mg/L standard solution (Orlon) to produce a 14 mg/L concentration in the spike prior to the final individual TIE treatments. The ammonia was added immediately prior to the TIE treatments that affect ammonia so that that the effects of treatments to reduce copper and PBC toxicity would not be obscured by ammonia toxicity.

Upon arrival at each laboratory, samples were inspected to determine their temperature and condition (e.g., caps in place or leakage). All samples met transit protocols. Standard chain-of-custody procedures were followed. Chain-of custody (CoC) forms were signed and copied. SAIC retains copies of the CoCs, along with test data in experiment binders and project files.

***Organism Selection and Source***

Mysids for testing were supplied by Aquatic Biosystems in Fort Collins Colorado. They were hatched on 28 October, received at Aquatec on 30 October, and the test was initiated on the same day. Newly hatched Artemia were fed to mysids on each day prior to test initiation, and daily feeding continued during the test.

Mysids were evaluated using a standard reference toxicant water-only test with potassium chloride. In this test, survival is determined in each of two replicate chambers to which ten animals have been added. The reference test uses a six dilution series with concentrations ranging between 0.1 and 1.0 g/L, and is used to determine LCs0 values for comparison with Control Chart values. Aquatec's Control Chart for the mysid *(A. bahia)* includes > 20 tests from mysid tests conducted since 1999. Sea Urchins used in TIE tests were from Aquatec's in-house cultures. Along with the TIE tests, sea urchin larval development was tested in a standard reference toxicant series with copper sulfate as the toxicant.

***Toxicity Identification Evaluation Manipulations and Testing***

In all, four samples, GP-2 control water, spiked water, SPP site sample, and centrifuged SPP site sample were used in TIE testing. The GP-2 control water served as a negative control to monitor for potential ancillary effects associated with the TIE manipulations described below. The spiked water served as a positive control to document the effectiveness of the

manipulations in reducing toxicity as intended, and the two site samples were prepared to resolved contributors to toxicity in mysids and sea urchins respectively. For the spiked sample, in addition to the 100% undiluted samples, the untreated samples and sodium thiosulfate-treated samples were diluted in a series to include 50%, 25% and 10% dilutions. These extra samples served to discriminate the expected reduction in toxicity that would occur with the first TIE treatment, and to characterize the over-all sensitivity of the organisms to the untreated sample (e.g., to demonstrate differences in sensitivity between the two test species). Centrifuged samples were used for the sea urchin test because physical damage to these organisms may occur when exposed to high concentrations of particulate matter.

***Sample Manipulations***

As illustrated in Figure 1, the TIE manipulations involved a series of sequential manipulations followed by two independent treatments. The principle of the sequential approach is that as each sample is treated and tested for toxicity, a potential source of toxicity can be identified or eliminated. The procedure begins with untreated samples, followed by the most specific treatments and ends with the most general. For SPP constituents, STS and EDTA act quite specifically on certain groups of common heavy metal contaminants. By treating the metals first, and then applying filtration and Solid Phase Extraction (SPE) to remove organic contaminants, reductions in toxicity following each individual treatment can be associated with specific toxicant groups.

By applying the independent *Ulva* treatment and associated pH adjustments at the end of the sequential treatments, the role of ammonia as a contributor to toxicity can be more clearly discerned. The *Ulva* addition is best suited as a final treatment because it could also remove metals and organics to varying degrees. Its application as final treatment limits uncertainty in the interpretation of results. Similarly, pH adjustments can affect the toxicity of multiple potential contaminants, including certain metals and potentially toxic organic compounds. The elimination or reduction of toxicity due to these groups prior to pH adjustment facilitates the direct association between pH change and commensurate changes in the relative toxicity of both ammonia and sulfides due to ionic shift.

Untreated SPP is sub-sampled to determine baseline toxicity for the SPP, provide a starting point to assess relative changes in toxicity associated with each subsequent treatment. Likewise, sub-sampling occurs after each treatment for TIE toxicity testing. The objective of each treatment step is described below.

Sequential Treatments

*Establish Baseline Toxicity with Untreated sample:* For this step, sub-samples of untreated SPP are tested to assess toxicity relative to TIE-manipulated sub-samples. Even though SPP tests was performed during toxicity screening (Task 2A) new baseline samples should still be collected and tested to correspond temporally with the manipulated treatments for each sample.

*Reduce Metals Concentrations with STS and EDTA:* Two treatments are conducted in sequence to reduce bioavailability of metals, specifically by rendering them unavailable for direct uptake into cell tissues. First is the addition of sodium thiosulfate (STS; Na2S203) and second is the addition of ethylenediaminetetraacetic acid (EDTA). Reduction in toxicity of the sample after either or both treatments indicates the presence of metals in toxic concentrations.

a. Reduce Cationic Metals and Oxidants with STS: Sodium thiosulfate addition was performed as the first metals reduction step because it is generally effective with a smaller subset of metal contaminants relative to EDTA. It is reported by EPA to be most effective in reducing toxicity due to $Cd^{2+}$, $Cu^{2+}$, $Ag^{1\div}$ and Hg (with lesser affinity for $Ni^{2+}$, $Zn^{2+}$, $Pb^{2+}$ and $Mn^{z\div}$ (U.S. EPA 1994)). Reduction in toxicity of the sample after STS treatment indicates the above metals are present in toxic concentrations. Sodium thiosulfate is added at the rate of 50 mg/L with no apparent effects on test species (U.S. EPA, 1996).

b. Chelate Cationic Metals with EDTA: This reducing agent chelates divalent cationic metals (i.e., $AI^{2+}$, $Ba^{2+}$, $Fe^{2\div}$, $Mn^{2+}$, $Sr^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $Pb^{2+}$, $Cd^{2+}$, 002+, and $Zn^{2+}$) (U.S. EPA., 1996). Reduction in toxicity of the sample after EDTA treatment indicates that members of the above listed group of metals are present in toxic concentrations. If reduction in toxicity does not occur with STS, but does occur with EDTA addition, there are two potential explanations. One possibility is that the metals causing toxicity are amongst the group that is less reactive with STS (NI , Zn , Pb and Mn ) and the other is that the magnitude of toxicity was high enough that the addition of both reducing agents was required to affect toxicity. Generally, a fully or partially toxic response following the sequential EDTA treatment indicates that something other than divalent cationic metallic compounds are a major contributor to sediment toxicity. In other words, either metals are not toxic, or alternatively, if the samples remain fully toxic (i.e., no normal response is observed), other toxic agents may be masking the reductions in toxicity associated with metals. EDTA is added at the rate of 60 mg/L with no apparent effects on test species. According to the marine TIE guide (1996) this could potentially chelate 26 mg of divalent metal per liter.

The absence of reduction in toxicity indicates that metals are not toxic in the sample, and/or that remaining constituents are present at levels that still influence toxicity and/or that the toxic load of metals in the sample exceeded the binding capacity of the TIE agents.

*Extract Particulate-associated Contaminants with Filtration:* Because filtration may remove metals and organics, the placement of the filtration step after the treatments for

metals (STS and EDTA) reduces ambiguity of interpretations associated with filtration effects. Filtration is operationally defined by filter type and the filtration procedure used. To assure the removal of all suspended particles that could clog or compromise the integrity of the SPE column used in the following procedure, samples were filtered with 0.45 mm membrane filter (i.e., polyvinylidene fluoride to minimize sorption of organics). Toxicity tests conducted on the pre- and post-filtered fraction permit elucidation of potential toxicity associated with large colloids or particulates in the SPP. Filtration has not been found to affect the concentrations of sample ammonia. Filters used in this step were retained for any subsequent analyses that would be helpful if reduction in toxicity occurred due to filtration.

*Extract Organics with a Solid-phase Extraction (SPE) Column:* In this step, filtered SPP samples were eluted through a SPE column (Waters C18) to remove organic compounds (Waters, 2001). According to general recommended manufacturer's procedures, the samples were eluted through the column at a rate of 10 ml/min. For each sample, the column was exchanged after 500 ml was eluted. The column was monitored visually to limit the possibility that its capacity would be exhausted prior to elution of 500 ml. Nevertheless, prevention of column break-through cannot be assured for samples with unknown constituents, and removal of toxic organic toxicants may be incomplete.

Independent Treatments

*Remove Ammonia with Ulva:* For saltwater samples, treatment with the green seaweed *(Ulva lactuca)* is generally more effective than zeolite in removing ammonia. However, this treatment may also remove other residual sources of toxicity to varying degrees, including metals and organics. *Ulva* is a cosmopolitan macroalgae, and is generally found in estuarine lagoons, often floating on mudflats. It inhabits the upper to mid-intertidal, and in some locations may be found up to the subtidal zone and is associated with nutrient-enriched conditions. For this study, the algae was collected on the day prior to test treatments and held in aerated seawater at 15°C. Batches of *Ulva* to be added to each sample were prepared by weighing out lg of *Ulva* per 15 ml sample. Whole leaves of *Ulva* were used to treatment each sample. The pre-weighed batches were held together with skewer sticks and stored in seawater until addition. After addition, the samples were incubated for 5 hours at 15°C (Ho et al., 1997; 1999).

*Manipulate Ammonia and Sulfide with Adjusted pH:* As noted above, methods to remove ammonia, while generally effective, may provide inconclusive evidence to deduce ammonia toxicity. Hence, it is useful to conduct pH manipulations to provide additional evidence of ammonia toxicity, as well as discriminate between ammonia and hydrogen sulfide as potential toxicants. To achieve a reduction in pH, dilute hydrochloric acid (e.g. 1N) is added in small increments (μLs), followed by mixing, and measurement, repeating the procedure until the target (pH= 7.0 to 7.5) is achieved. If toxicity decreases with

decreased sample pH, ammonia is suspected, while an increase in toxicity with lower pH would implicate hydrogen sulfide or residual metals.

***TIE Exposures***

Mysids were exposed with ten animals in each of three replicates. In all other respects, the mysid tests with each treatment were conducted as described in the report for Task 2A.

Tests with the sea urchin, *Arbacia punctulata,* were conducted according to methods developed by SAIC, as reported in "Laboratory Testing In Support of Environmental Assessment NAE O&M Projects" (U.S. EPA and U.S. ACE, 2002). The test chambers were 20 mL polyethylene scintillation vials. Ten milliliter aliquots of elutriate were added to each of three replicate chambers per sample. Tests were conducted in a temperature-controlled chamber at 20 ± 1°C. Gametes for the test were collected and mixed as follows:

> Four male urchins were placed in seawater in shallow bowls. Males were stimulated to release sperm by touching the shell for about 30 seconds with the steel electrodes of a 12 V transformer. Sperm were collected using a 1 mL disposable syringe fitted with an 18-gauge, blunt tipped needle. The sperm were diluted with seawater to achieve approximately 1 X $10^8$ sperm/ml, held on ice and used within 1 hr of release.
>
> Four female urchins were placed in seawater in shallow bowls. Females were stimulated to release eggs by touching the shell as described above. Eggs were collected and held at room temperature for up to two hours with aeration. The eggs were washed two times with seawater by gentle centrifugation (500xg) for two minutes in a conical centrifuge tube. The eggs were diluted with seawater to a concentration of 2,000 eggs/mL and were aerated until used. Sperm and egg suspensions were mixed to a final concentration of 1:500 egg: sperm ratio.

After 60 minutes, fertilization was confirmed (100% in this case) and 1 mL of fertilized egg suspension was added to 10 mL of sample in each of three replicates and was incubated for 72 hours at 20 ± 1EC. The test was terminated by adding 2 mL of preservative to each vial.

One mL of suspension from each of the three replicates was transferred to a Sedgwick-Rafter counting chamber. Embryos were examined using a compound microscope (100X). One hundred embryos were examined for normal (i.e., not delayed) development as indicated by the presence of the pluteus larva.

The number of normal pluteii larvae and the number of abnormal pluteii larvae per 100 organisms were counted, as well as the total number of surviving organisms per ml.

For both tests, acceptable dissolved oxygen concentrations were documented to be in the range of 7.8 to 8.2 mg/L at the start of the test, and 5.3 to 6.6 mg/L at the end of the test. Salinity increased by = 3 mg/Kg, from 31 mg/Kg at test initiation, pH ranged between 7.8 and

8.2, across samples, with no apparent temporal trend. All water quality parameters were acceptable (U.S. EPA/U.S. ACE, 1998; U.S. ACE, 1991 ). Ambient laboratory lighting was set for constant light during the test exposure period.

Full strength SPP solutions were analyzed for ammonia on day 0. Samples were diluted 1 to 10 with deionized water. Total ammonia was measured spectrophotometrically.

***Data Analysis***

Mean responses to baseline and TIE treatments were calculated, for mysids and sea urchins. Responses are presented for performance control, the spiked sample and NBH-202 samples. For mysids, results are expressed for both 48 hr and 96 hr responses. For sea urchins, results are expressed as percent normal development and survival relative to controls.

## Results

*Quality Assurance/Quality Control*

Up to 96 hrs, control responses for mysids through all treatments remained > 90%. For sea urchins, control responses, normal development ranged from 98 to 100% and survival counts ranged from 83 to 92 per ml. These results, along with documentation of acceptable water quality, confers validity of test results.

The summary report for reference toxicant testing with mysids and sea urchins using potassium chloride and copper sulfate is presented at the end of the Toxicity Test Data Report provided by Aquatec (Appendix A). The $LC_{50}$ for *A. bahia* was 0.360 g/L (as potassium), well within the Control Chart lower and upper boundaries of 0.11 and 0.83 g/L, established the normal response of these organisms. The $EC_{50}$ calculated for *A punctulata* was 30.9 µL (as copper) is equivalent to the value reported previously reported for this test (SAIC, 1994).

*Chemical Exposure Concentrations*

Results from the toxicity testing component of the TIE study are best interpreted in the context of the chemical exposure levels present in the untreated toxic sample under investigation. This is accomplished by using hazard Quotients (HQ= measured chemical concentrations divided by species-specific $LC_{50}$s or $EC_{50}$s) to represent expected sensitivity of the test species to the chemical exposure. In a single toxicant exposure, HQs less than 1 would result in less than 50% adverse affect while HQs > 1 would generally result in higher percentage of exposed organisms affected; the higher the HQ, the greater and more likely the observation of high percentage effects. For the current study, HQs were derived using chemical concentrations presented in the Task 2A report, Appendix C, and literature values that to represent effect concentrations for each of the toxicants of concern.

Table 1 presents HQs for the spike sample and the site sample (NBH-202), for the two species. Based on the chemical exposure concentrations, the mysid is expected to be more sensitive to PCBs in the TIE testing with NBH-202 (HQ=1.36 vs. 0.02, respectively) given

the lower *(i.e.,* more sensitive) $LC_{50}$ value, while sea urchins would be more sensitive to copper (HQ = 5.43 vs. 0.64, respectively) and ammonia (HQ = 17.7 vs. 0.82, respectively). The comparison of the spike sample and the NBH sample HQs show that the test concentrations in the spike approximated the concentrations of the toxicants of concern in the site sample, except for ammonia, where a reduced potency was chosen to increase the likelihood of demonstrating an effective treatment for the more sensitive sea urchin response.

In summary, the analyses of the chemical exposures suggest that both copper and PCB concentrations are in the exposure range were toxicity could occur, depending on species sensitivity and site-specific water quality conditions. Also, the spike concentrations are in the proper range to adequately assess the effectiveness of the TIE treatments in mitigating the toxic response.

***Toxicity Identification Evaluation Test Results and Interpretation***

Summaries of the TIE toxicity tests with mysids and sea urchins are provided in Tables 1 and 2, respectively, synthesized from the raw data presented in Appendix A (Aquatec data report). Changes in toxicity are highlighted in yellow, and are indicative of reduction/removal of bioavailability of a toxic constituent that was present in the untreated sample.

The most relevant findings from TIE treatments for each of the targeted toxicant classes are reviewed below, particularly with regard to the relationship between expected toxicity based on species-specific HQs, and observed responses. The results from the spike sample are presented first, to establish the interpretive process.

Results for the Spiked Sample

*Metal treatments (STS, EDTA):* Tables 2a and 2b show TIE results from 48 hour and 96 hour tests with mysids. Untreated sample results show complete mortality in both 100% and 50% exposures. STS completely removed toxicity in the 50% dilution, and in the undiluted sample survival reached 90% following STS treatment, and 100% following EDTA treatment. This indicates that copper was causing the majority of the toxicity in the untreated sample, given that the metal treatments alone were successful in improving survival to 100% despite the presence of PCBs in the sample. The mysid results also indicate that toxicity of copper was greater than would be expected for exposures to copper alone (i.e., no survival, but HQ was <1; see Table 1), indicating that copper was more toxic in the presence of Aroclor).

Sea urchin results are presented in Tables 3a (survival) and 3b (larval development). While larval development is generally the more sensitive endpoint, and the one most commonly reported for the embryo-larval test (U.S. EPA, 2002), both endpoints demonstrated responses to TIE treatments of the spiked sample. Unlike mysids, only partial mortality was observed in sea urchins exposed to the spike samples. The survival endpoint was less reliable, as a clear dose-response pattern (survival proportional to

concentration) was not observed. Where survival responses were low in untreated samples (25% and 50% dilutions), the metal treatments appeared to increase survival, indicating that toxic forms of copper were removed (one anomaly occurred, with lower survival in the STS treatment than in the untreated sample, but EDTA restored survival to 91%). Sea urchin larval development was more affected by copper than expected, with high toxicity occurring in all untreated samples, including the 10% dilution (HQ= 0.7). Copper effects on sea urchin normal development in the spike was removed by STS in the 10% dilution, and by the combination of STS and EDTA in the 100% dilution, indicating that, even for this more sensitive endpoint, the TIE treatments were effective in removing copper from the sample.

*Organics Treatment (PCBs):* In mysid 48 and 96 hr exposures (Table 2), PCB in the spike was not toxic. This indicates that after available copper was bound the concentration of PCB was insufficient to cause toxicity. Because the estimated HQ was 1.2 for PCB in the sample, it is possible that the estimated concentration was less toxic to mysids than predicted. However, the actual exposure concentration of Aroclor used to derive the HQ (10% of the nominal concentration; losses expected to result largely from sorption to exposure chambers) is uncertain, such that the expectation of toxicity was equally uncertain. Results from the TIE treatments for particulates and organics were similar to control responses, indicating that the treatments had no adverse affect on survival. Similarly, the sea urchin normal development was not affected by either the particulate or organic treatments of the spiked sample.

*Ulva Treatment:* Ammonia was added to the non-toxic C18 -treated sample to demonstrate efficiency of ammonia removal. For mysids, the concentration of ammonia added (HQ= 0.3) was not be expected to result in toxicity, and the absence of toxicity in the spike sample (90%) indicates that *Ulva* had no adverse affect on survival (Table 2). For the sea urchin, the *Ulva* treatment did not improve larval development (0.3%), indicating that the treatment did not reduce ammonia to a non-toxic level (Table 3b). For the survival endpoint (Table 3a), the 41% survival response at the spike concentration can be used for comparison with results obtained in the site sample (see below), where ammonia is a natural constituent of the sediment matrix.

*Low pH (Independent Post-C18 Treatment):* As with the *Ulva* treatment, ammonia was added to the non-toxic C18 -treated sample to reduce the proportion of the more toxic unionized ammonia form through pH reduction. In the mysid tests, the ammonia-spiked low-pH sample was not toxic, as expected, although the finding is somewhat uncertain due to variability of pH over time. Similarly, the spiked low-pH sample was non-toxic to sea urchin survival and larval development, indicating that the reduction in unionized ammonia was sufficient to remove toxicity.

Site sample NBH-202

*Metal treatments (STS, EDTA):* Table 2a shows that for mysids at 48 hours, the EDTA increased survival from 20 to 37%, indicating that metal(s) have likely contributed to toxicity in the filed sample. The 96 hour results (Table 2b) indicate an increased level of toxicity in the untreated sample could not be mitigated by the metal treatments. It also suggests the possibility that reductions in toxicity due to the metal treatments were masked by other sample constituents that remained at highly toxic levels after the STS and EDTA treatments (discussed below).

Table 3a shows that the elutriate prepared from the Harbor sediment was highly toxic, both in survival and development of sea urchin larvae. Sea urchin survival and larval development did not improve following treatments to bind metals, even though the copper concentration appears to be similar to the spiked sample where reduction in toxicity did occur. This indicates a presence of residual contributors to toxicity, including organics, ammonia and/or copper and other metals that were not completely bound by the TIE treatments.

*Organics treatment (PCBs):* For mysids, the filtration and C18 steps each sequentially removed site sample toxicity at 48 hours (increasing survival to 70 and 93%, respectively; Table 2a), indicating that organics were the principal contributors the toxicity observed at this exposure interval. As with the metal treatment, the 96 hour results (Table 2b) indicate a residual source of toxicity (discussed below) that precluded observed reductions in toxicity due to the metal treatments.

For sea urchins, larval development was not improved by filtration and $C_{18}$ treatments of the site sample (Table 3b), while a slight trend of increasing survival was observed (count per ml increasing from 9% in the untreated sample to 16% in the filtered sample and 21% after the C18 treatment; Table 3b).

*Ulva Treatment: Ulva* treatment of the site sample was performed to remove ammonia as a source of toxicity. In the NBH-202 sample, *Ulva* completely removed toxicity to mysids at 96 hrs (Table 2b). survival remained at <10% prior to the *Ulva* treatment. This indicates that the mortality due to ammonia did likely mask potential chemical toxicity removed by previous sequential TIE treatments. *Ulva* may also reduce residual toxicity associated with metals and organics. This fact will be important in interpreting the results of the Low pH treatment discussed below.

In the sea urchin exposures to the site sample, the *Ulva* treatment had a large impact on sea urchin survival (increased to 65% from 21%; Table 3a). This indicates that survival was affected by ammonia, and possibly other residual toxicants, as noted above. *Ulva* did not increase normal development (the principal, and more sensitive endpoint for this test; Table 3b). The concentration of total ammonia through the $C_{18}$ treatment was 37 mg/L and was reduced by the *Ulva* treatment to 7.8 mg/L (as unionized, 0.06 mg/L). Reported $EC_{50}$s for this

species exposed to ammonia are as low as1.7 mg/L and 0.06 mg/L as total and unionized ammonia respectively, indicating that the treatment may not have removed enough ammonia; hence ammonia most likely remained a factor contributing to toxicity.

*Low pH (independent post-$C_{18}$ treatment):* Mysid survival at 48 hours was lower with the low pH treatment than it was following the $C_{18}$-treatment. Normally, ammonia toxicity would be reduced by this treatment, but in this case, an increased toxicity could be due to residual copper. Copper toxicity may is inversely related to pH in some marine organisms (Ho et al., 1999b) not sequestered by the STS and EDTA treatments. The low pH shift can increase the proportion of the toxic $Cu^{2+}$ ion by an order of magnitude within the pH range evaluated for this study (Leckie and Davis, 1979)

The low pH treatment resulted in 27% sea urchin survival (indicating that unionized ammonia may not have been the principal toxicant for this endpoint. Larval development did not improve with the low pH treatment, most likely due to residual ammonia and other residual toxicants.

**Summary of Findings for Site Specific Water Quality Study**

The TIE conducted in this study addressed the relative roles of metals, organic constituents and ammonia are contributors to toxicity associated with SPP generated from a New Bedford Harbor sediment (NBH 202). The sequential TIE method relies on evaluation of results from multiple treatments and multiple species. Results with spiked samples demonstrated that the sea urchin (particularly larval development) is more sensitive to copper and ammonia relative to the mysid, in fact, too sensitive for the purposes of this study. Accordingly, the 48 hour mysid results were determined to be most useful in identifying sources of toxicity prior to the *Ulva* treatment. For mysids following 48-hour exposures to 100% SPP, survival gradually increased from 20% to 90%, apparently due to treatments for both metals and organics.

The SPP and elutriate for NBH-202 at 100% strength was highly toxic to both species. *Ulva* eliminated and reduced toxicity, respectively in the 96-hour mysid and sea urchin survival results, where prior treatments had been ineffective. This indicates that ammonia toxicity masked the removal of toxicity that would have been occurred in prior sequential steps that target metals and organics.

Specific Hazard Quotients and TIE results generally both support the finding of multiple sources of toxicity. Copper and ammonia toxicity to sea urchins appeared to have exceeded the capacity of the TIE treatments to sufficiently limit observed effects. Mysids were most affected by PCBs and ammonia, but their sensitivity to copper appears to increase with near-toxic levels of PCBs, as seen with the spike sample responses. The role of PCBs is the most uncertain of the three toxicants due to the need to use toxicity values derived for specific PCB mixtures (e.g. Aroclor 1242) that are different from the mixture presented in the NBH sediment sample.

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SAIC. 1993. Toxicity testing to support the New York/New Jersey Harbor Site specific criteria study. Science Applications Intl. Corp. Final Report to EPA. Contract No. 68-C8-0066. Work Assignment C-4-94. Internal Report # 1037.

U.S. EPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA/600/4-90/027.

U. S. Environmental Protection Agency and United States Army Corps of Engineers. 1991. "Evaluation of dredged material proposed for ocean disposal: Testing manual," EPA/503R 91/001 Washington, DC.

US EPA. 1996. Marine Toxicity Identification Evaluation (TIE), Phase I Guidance Document. EPA/600/R-096/054. US EPA Office of Research and Development.

U. S. Environmental Protection Agency and United States Army Corps of Engineers. 1998. Evaluation of dredged material proposed for inland disposal: Testing manual, Washington, DC.

## *Figure 1. Simplified Flow Diagram for Sequential TIE: Fractionation, Testing and Interpretation*

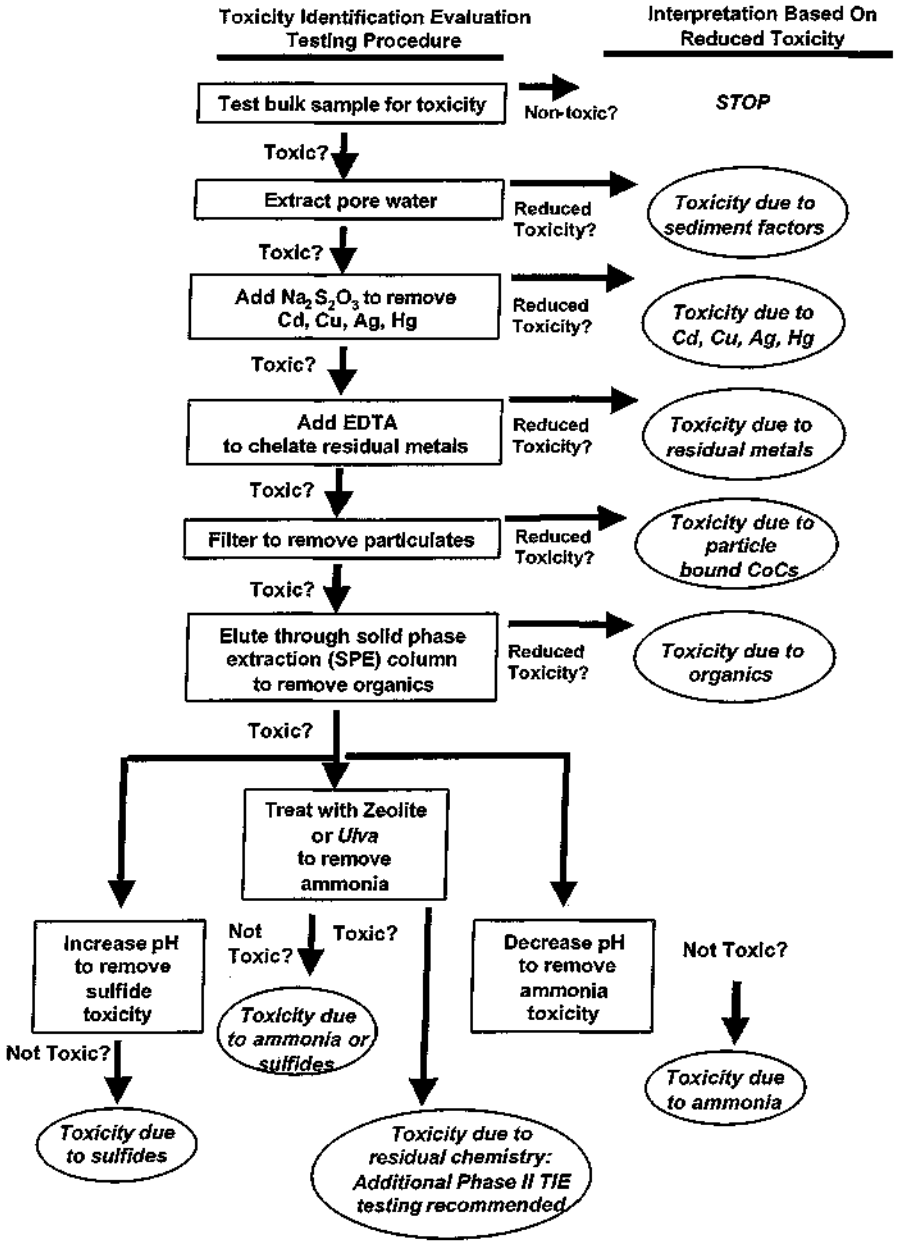


Table 1. Species-specific elutriate Hazard Quotients for chemical exposures to *Americamysis bahia* and *Arbacia punctulata* exposed to New Bedford Harbor Suspended Particulate Phase samples.

| | Mysid (*Americamysis bahia*[1]) | | | |
|---|---|---|---|---|
| Analyte | Acute $LC_{50}$ (ug/L) | Reference for Acute value | HQ for Spike[1,2,3] | HQ for NBH-202-Elutriate |
| Copper | 153 | a,g | 0.78 | 0.64 |
| PCB | 17 | c | 1.18 | 1.36 |
| Unionized ammonia | 1.94 | f | 0.26 | 0.82 |

| | Sea Urchin (*Arbacia punctulata*[1]) | | | |
|---|---|---|---|---|
| Analyte | Acute $EC_{50}$ (ug/L) | Reference for Acute value | HQ for Spike[1,2,3] | HQ for NBH-202-Elutriate |
| Copper | 18 | g | 6.67 | 5.43 |
| PCB | 1000 | d | 0.02 | 0.02 |
| total ammonia | 4.06 | e | 3.45 | 9.33 |
| Unionized ammonia | 0.09 | b, e | 5.56 | 17.71 |

1 - Hazard Quotient = elutriate concentration/species $LC_{50}$ (larval development for sea urchin)

2 - Hazard Quotients for spiked sample based on estimate from nominal concentrations

3 Copper = 100% nominal concentration and PCB =10% nominal concentration[h]

a Nacci, Jackim and Walsh. 1986.

b. Bay, S. R. Burgess and D. Nacci. 1993.

c Ho, K.T., R.A. McKinney, A.Kuhn, M.C. Pelletier, and R.M. Burgess.1997.
Value for Aroclor1242; Aroclor 1254 = 57 ug/L

d Adams and Slaughter-Williams. 1988.

e National Beological Service. 1996. Value used is geometric mean of values from Bay et al. and NBS.

f Miller, D.C., S. Poucher, J.A. Cardin and D. Hansen. 1990.
geo. Mean = 1.94 mg/L unionized ammonia

g. SAIC 1993.

h. Ho et al., 1999b.

Table 2 Survival in the mysid, *Americamysis bahia*, after exposures to Spiked Water and Suspended Particulate Phase sediment in the New Bedford Harbor TIE study.

A. 48 hour results

| | TIE Treatment[1] Result (% Survival) | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Metals | | Particulates | Organics | Ammonia | |
| Sample-dilution % | Untreated | STS | EDTA | Filtered | $C_{18}$ | *Ulva* | Low pH[2] |
| **Spike - 50 %** | 0 | 100 | | | | | |
| **Spike - 100 %** | 0 | 90 | 100 | 100 | 93 | 90 | 100 |
| **STA 202 100%** | 20 | 20 | 37 | 70 | 93 | 90 | 23 |
| | | | | | | | |
| **PC-100 %** | 100 | 100 | 100 | 100 | 93 | 90 | 100 |

B. 96 hour results

| | TIE Treatment[1] Result (% Survival) | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Metals | | Particulates | Organics | Ammonia | |
| Sample-dilution % | Untreated | STS | EDTA | Filtered | $C_{18}$ | *Ulva* | Low pH[2] |
| **Spike - 50 %** | 0 | 100 | | | | | |
| **Spike - 100 %** | 0 | 80 | 97 | 100 | 93 | 90 | 97 |
| **STA 202 100%** | 0 | 0 | 0 | 0 | 3 | 90 | 3 |
| | | | | | | | |
| **PC-100 %** | 100 | 100 | 97 | 97 | 100 | 90 | 100 |

[1] Treatments were sequential, from left to right (except Low pH, which followed $C_{18}$- *Ulva*).
Blank cell indicate that no sample was tested.
Yellow highlighting indicates apparent reduction (> 15%) in toxicity.
Bold outline indicates statistically significant change in toxicitiy (a= 0.05).
No toxicity tests were conducted on Spike dilutions after the STS treatment.

Table 3. Responses of the sea urchin, *Arbacia punctulata,* after exposures to spiked water and sediment elutriate in the New Bedford Harbor TIE study.

A. Survival at 72 hrs.

| | TIE Treatment[1] Result (% Survival)[2] | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Metals | | Particulates | Organics | Ammonia | |
| Sample-dilution % | Untreated | STS | EDTA | Filtered | $C_{18}$ | *Ulva* | Low pH |
| **Spike - 10 %** | 82.0 | 85.0 | | | | | |
| **Spike - 25 %** | 26.0 | 76 | | | | | |
| **Spike - 50 %** | 54.7 | 79 | | | | | |
| **Spike - 100 %** | 81.0 | 35.0 | 91 | 90.0 | 87.7 | 41.3 | 84.0 |
| **STA 202 100%** | 8.7 | 17 | 4.7 | 16 | 21 | 65 | 27.0 |
| | | | | | | | |
| **PC-100 %** | 90 | 88.0 | 82.3 | 87.7 | 92.3 | 83.0 | 93.3 |

B. Normal development at 72 hours.

| | TIE Treatment[1] Result (% Normal Development)[3] | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Metals | | Particulates | Organics | Ammonia | |
| Sample-dilution % | Untreated | STS | EDTA | Filtered | $C_{18}$ | *Ulva* | Low pH |
| **Spike - 10 %** | 0.7 | 99 | | | | | |
| **Spike - 25 %** | 0.0 | 0.0 | | | | | |
| **Spike - 50 %** | 0.0 | 0.0 | | | | | |
| **Spike - 100 %** | 0.0 | 0.0 | 98 | 98.3 | 98.0 | 0.3 | 96.7 |
| **STA 202 100%** | 0.0 | 0.0 | 0.3 | 3.0 | 1.3 | 0 | 0.0 |
| | | | | | | | |
| **PC-100 %** | 100 | 99.7 | 99.7 | 99.7 | 97.7 | 98.7 | 99.3 |

[1] Treatments were sequential, from left to right (except Low pH, which followed $C_{18}$- *Ulva*).

[2] The survival endpoint is defined as number of larvae present in 1 ml.

[3] The normal larval development endpoint is defined as achievement of the pluteus stage

Blank cell indicate that no sample was tested.

Yellow highlighting indicates apparent reduction (> 15%) in toxicity.

Bold outline indicates statistically significant change in toxicitiy (a= 0.05).

# Aquatec Biological Sciences

Ecology | Environmental Toxicology | Natural Resource Assessments | Microbiology

December 2, 2002

Ms. Sherry Poucher
SAIC
221 Third Street
Newport, Rhode Island 02840

Dear Ms. Poucher:

Enclosed please find a report (two copies, one bound, one unbound) of the toxicity test results for TIE preparations with *Americamysis bahia* and *Arbacia puntulata* completed on samples received on October 31, 2002 (New Bedford).

If you have any questions regarding the report, please contact Dr. Philip C. Downey or me.

Sincerely,

John Williams
Manager, Environmental Toxicology

273 Commerce Street, Williston, VT 05495 Tel: 802.860.1638 Fax: 802.658.3189

# Aquatec Biological Sciences

Ecology | Environmental Toxicology | Natural Resource Assessments | Microbiology

## Toxicity Detail Report

Science Applications International Corp
221 Third Street

Newport, RI 02840

Date: 12/2/2002
Project: 02065
SDG 6560
Site: New Bedford

Method Description Sea Urchin, Arbacia punctulata, 72-h embryo development
Method: TIEAP
Species: *Arbacia punctulata*

| Number | Treatment | Conc(%) | Day | Replicate Normal Development (normal/counted) A | B | C | Average Normal (%) |
|---|---|---|---|---|---|---|---|
| 023135 | Control-Filtered | 100 | 3 | 99 / 100 | 99 / 100 | 98 / 100 | 98.7 |
| 023136 | NBH_SPP_Cent-C18 | 100 | 3 | 1 / 50 | 3 / 100 | 0 / 100 | 1.6 |
| 023138 | Spike-C18 | 100 | 3 | 96 / 100 | 99 / 100 | 99 / 100 | 98.0 |
| 023139 | Control-C18 | 100 | 3 | 96 / 100 | 98 / 100 | 99 / 100 | 97.7 |
| 023140 | NBH_SPP_Cent-Ulva | 100 | 3 | 0 / 100 | 0 / 100 | 0 / 100 | 0.0 |
| 023142 | Spike-Ulva | 100 | 3 | 0 / 100 | 0 / 100 | 1 / 100 | 0.3 |
| 023143 | Control-Ulva | 100 | 3 | 99 / 100 | 100 / 100 | 97 / 100 | 98.7 |
| 023144 | NBH_SPP_Cent-LOpH | 100 | 3 | 0 / 100 | 0 / 100 | 0 / 100 | 0.0 |
| 023146 | Spike-LOpH | 100 | 3 | 96 / 100 | 97 / 100 | 97 / 100 | 96.7 |
| 023147 | Control-LOpH | 100 | 3 | 99 / 100 | 100 / 100 | 99 / 100 | 99.3 |
| 023148 | NBH_SPP_Cent-Untreat | 100 | 3 | 0 / 50 | 0 / 50 | 0 / 50 | 0.0 |
| 023150 | Spike-Untreated | 10 | 3 | 1 / 100 | 1 / 100 | 0 / 100 | 0.7 |
| 023150 | Spike-Untreated | 25 | 3 | 0 / 100 | 0 / 100 | 0 / 100 | 0.0 |
| 023150 | Spike-Untreated | 50 | 3 | 0 / 100 | 0 / 100 | 0 / 100 | 0.0 |
| 023150 | Spike-Untreated | 100 | 3 | 0 / 100 | 0 / 100 | 0 / 100 | 0.0 |
| 023151 | Control-Untreated | 100 | 3 | 100 / 100 | 100 / 100 | 99 / 100 | 99.7 |
| 023152 | NBH_SPP_Cent-STS | 100 | 3 | 0 / 50 | 0 / 100 | 0 / 50 | 0.0 |
| 023154 | Spike-STS | 10 | 3 | 99 / 100 | 99 / 100 | 98 / 100 | 98.7 |
| 023154 | Spike-STS | 25 | 3 | 0 / 100 | 0 / 100 | 0 / 100 | 0.0 |
| 023154 | Spike-STS | 50 | 3 | 0 / 100 | 0 / 100 | 0 / 100 | 0.0 |
| 023154 | Spike-STS | 100 | 3 | 0 / 100 | 0 / 100 | 0 / 100 | 0.0 |
| 023155 | Control-STS | 100 | 3 | 100 / 100 | 100 / 100 | 99 / 100 | 99.7 |
| 023156 | NBH_SPP_Cent-EDTA | 100 | 3 | 0 / 50 | 1 / 50 | 0 / 25 | 0 8 |
| 023158 | Spike-EDTA | 100 | 3 | 95 / 100 | 99 / 100 | 100 / 100 | 98.0 |
| 023159 | Control-EDTA | 100 | 3 | 99 / 100 | 100 / 100 | 100 / 100 | 99.7 |
| 023160 | NBH_SPP_Cent-Filtered | 100 | 3 | 3 / 100 | 6 / 100 | 0 / 100 | 3.0 |
| 023162 | Spike-Filtered | 100 | 3 | 100 / 100 | 100 / 100 | 95 / 100 | 98.3 |
| 023163 | Seawater | 0 | 3 | 100 / 100 | 99 / 100 | 100 / 100 | 99.7 |

Submitted By:


273 Commerce Street, Williston, VT 05495 Tel: 802.860.1638 Fax: 802.658.3189

# Aquatec Biological Sciences

Ecology | Environmental Toxicology | Natural Resource Assessments | Microbiology

## Toxicity Detail Report

Science Applications International Corp
221 Third Street

Newport, RI 02840

Date: 12/2/2002
Project: 02065
SDG 6560
Site: New Bedford

Method Description: TIE Using Mysidopsis bahia
Method: TIEMY
Species: *Mysidopsis bahia*

| | | Conc (%) | Test End Day | Start Count | Replicate Survival A | B | C | Average Survival (%) |
|---|---|---|---|---|---|---|---|---|
| 23135 | Control-Filtered | 100 | 4 | 10 | 10 | 10 | 9 | 96.7 |
| 23137 | NBH_SPP_202-C18 | 100 | 4 | 10 | 0 | 1 | 0 | 3.33 |
| 23138 | Spike-C18 | 100 | 4 | 10 | 9 | 9 | 10 | 93.3 |
| 23139 | Control-C18 | 100 | 4 | 10 | 10 | 10 | 10 | 100 |
| 23141 | NBH_SPP_202-Ulva | 100 | 4 | 10 | 10 | 9 | 8 | 90 |
| 23142 | Spike-Ulva | 100 | 4 | 10 | 10 | 9 | 8 | 90 |
| 23143 | Control-Ulva | 100 | 4 | 10 | 10 | 9 | 8 | 90 |
| 23145 | NBH_SPP_202-LOpH | 100 | 4 | 10 | 3 | 0 | 0 | 10 |
| 23146 | Spike-LOpH | 100 | 4 | 10 | 10 | 9 | 10 | 96.7 |
| 23147 | Control-LOpH | 100 | 4 | 10 | 10 | 10 | 10 | 100 |
| 23149 | NBH_SPP_202-Untreated | 100 | 4 | 10 | 0 | 0 | 0 | 0 |
| 23150 | Spike-Untreated | 100 | 4 | 10 | 0 | | | |
| 23151 | Control-Untreated | 100 | 4 | 10 | 10 | 10 | 10 | 100 |
| 23153 | NBH_SPP_202-STS | 100 | 4 | 10 | 3 | 0 | 0 | 10 |
| 23154 | Spike-STS | 50 | 4 | 10 | 10 | | | |
| 23154 | Spike-STS | 100 | 4 | 10 | 8 | | | |
| 23155 | Control-STS | 100 | 4 | 10 | 10 | 10 | 10 | 100 |
| 23157 | NBH_SPP_202-EDTA | 100 | 4 | 10 | 0 | 0 | 0 | 0 |
| 23158 | Spike-EDTA | 100 | 4 | 10 | 10 | 9 | 10 | 96.7 |
| 23159 | Control-EDTA | 100 | 4 | 10 | 10 | 10 | 9 | 96.7 |
| 23161 | NBH_SPP_202-Filtered | 100 | 4 | 10 | 0 | 0 | 0 | 0 |
| 23162 | Spike-Filtered | 100 | 4 | 10 | 10 | 10 | 10 | 100 |
| 23163 | Seawater | 0 | 4 | 10 | 10 | 10 | 10 | 100 |

Submitted By: _____

Aquatec Biological Sciences

Ecology | Environmental Toxicology | Natural Resource Assessments | Microbiology

## Quality Assurance Report

| | | |
|---|---|---|
| Science Applications International Corporation | Date: | 12/2/2002 |
| 221 Third Street | Project: | 02065 |
| | SDG | 6560 |
| Newport, RI 02840 | Site: | New Bedford |

Qualifiers and Special Conditions

For the untreated spike sample (sample 23150) and the STS-treated spike sample (sample 23154) dilutions of 10%, 25%, 50%, and 100% sample were tested with Arbacia. For the mysids there was only enough sample to run the 100% (one replicate for the untreated spike) or the 50% and 100% (one replicate each for the STS-spike).

Dissolved oxygen concentrations were low in two treatments, sample 23156 and sample 23160 and were aerated briefly before starting the toxicity tests.

For the Arbacia punctulata embryo development test, a subsample of 100 embryos was counted and scored for normal/abnormal development. When it was evident that few embryos survived in some test solutions, only 50 embryos were scored. These replicates were sample 23136 replicate A; sample 23148 replicates A,B,C; and sample 23152 replicates A,C.



273 Commerce Street, Williston, VT 05495 Tel: 802.860.1638 Fax: 802.658.3189

# Supportive Documentation

Chain-Of-Custody

Toxicity Test Methods

Sea Urchin, Arbacia Punctulata, 72-h embryo development

TIE Using Mysidopsis bahia

Standard Reference Toxicant Control Charts

Science Applications International Corporation

# Chain-Of-Custody

# Aquatec Biological Sciences
## Chain-of-Custody Record

273 Commerce Street
Williston, VT 05495
TEL: (802) 860-1638
FAX: (802) 658-3189

| COMPANY INFORMATION | COMPANY'S PROJECT INFORMATION | SHIPPING INFORMATION |
|---|---|---|
| Name: Sherry Pelletier | Project Name: New Bedford | Carrier: Fed Ex |
| Address: 221 Third Street | Project Number: | Airbill Number: 8383 0250 4326 |
| City/State/Zip: Newport RI 02840 | Sampler Name(s): John Williams (SPP Prep) | Date Shipped: 10/29/02 |
| Telephone: (401) 847-4210 | Quote #: Client Code: | Hand Delivered: ☐ Yes ☐ No |
| Facsimile: | | |
| Contact Name: Sherry | | |

| SAMPLE IDENTIFICATION (NOTE1) | COLLECTION DATE | COLLECTION TIME | GRAB | COMPOSITE | MATRIX | ANALYSIS/REMARKS (NOTE 2,3) | VOLUME/CONTAINER TYPE/ PRESERVATIVE (NOTE 4) | NUMBER OF CONTAINERS |
|---|---|---|---|---|---|---|---|---|
| NBH-202-SPP | 10/29/02 | 1305 | | | SPP | TIE Manipulations | 1 gal unpres | 1 (cool) |
| NBH-202-SPP-Clarified by centrifuge | 10/29/02 | 14:00 | | | SPP Clear | TIE Manipulations | 500 mL unpres | 2 (1 full 1 partial) |

| Relinquished by: (signature) | DATE | TIME | Received by: (signature) |
|---|---|---|---|
| | 10/29/02 | 13:13 | Picked up for Fed Ex |
| Relinquished by: (signature) | DATE | TIME | Received by: (signature) |
| Relinquished by: (signature) | DATE | TIME | Received by: (signature) |

NOTES TO SAMPLER(S): (1) Limit Sample Identification to 30 characters, if possible; (2) Indicate designated Lab Q.C. sample and type (e.g.:MS/MSD/REP) and provide sufficient sample; (3) Field duplicates are separate sample; (4) e.g.: 40 ml/glass/$H_2SO_4$

Notes to Lab:

Distribution: Original Accompanies Shipment; Copy to Coordinator Field Files

An Employee-Owned Company Science Applications International Corporation

# Chain of Custody Record

Science Applications International Corporation/ 221 Third Street/ Admiral's Gate/ Newport RI 02840 phone (401)847-4210 fax (401)849-9786

Project: NBH - Dredging

Client Name and Contact: Maguire / ACE. : Sherry Poucher

| Sample No. | Containers No. | Containers Type | Collection Date | Collection Time | Sample Description | Requested Parameters |
|---|---|---|---|---|---|---|
| Control | 1 | 185mL | 10/30/02 | 14:00 | Filtered | TIE |
| NBH_SPP_Cont | 1 | 60mL | ↓ | 15:00 | C18 | ↓ |
| NBH_SPP_202 | 1 | 150mL | ↓ | ↓ | C18 | ↓ |
| Spike | 1 | 185mL | ↓ | ↓ | C18 | ↓ |
| Control | 1 | 185mL | ↓ | ↓ | C18 | ↓ |
| NBH_SPP_Cont | 1 | 60mL | ↓ | 1530 | WIVA | ↓ |
| NBH_SPP_202 | 1 | 150mL | ↓ | ↓ | WIVA | ↓ |
| Spike | 1 | 185mL | ↓ | ↓ | WIVA | ↓ |
| Control | 1 | 185mL | ↓ | ↓ | WIVA | ↓ |
| NBH_SPP_Cont | 1 | 60mL | ↓ | ↓ | 10 PH | ↓ |
| NBH_SPP_202 | 1 | 150mL | ↓ | ↓ | 10 PH | ↓ |
| Spike | 1 | 185mL | ↓ | ↓ | 10 PH | ↓ |
| Control | 1 | 185mL | ↓ | ↓ | 10 PH | ↓ |
| | | | | | | |
| Seawater | 1 | 1 Gal | 11/30/02 | | ~~SW~~ EPA AED SeaWater | Use for Dilutions |

Total: 18

| Packed/Released By | Date | Time | Received By | Date | Time | Remarks: |
|---|---|---|---|---|---|---|
| Signature: Kate A. Montgomery | 10/30/02 | 16:00 | Signature: | | | |
| Printed Name: Kate A. Montgomery | | | Printed Name: | | | |
| **Released By** | **Date** | **Time** | **Received By** | **Date** | **Time** | |
| Signature: Kate A. Montgomery | 10/30/02 | 16:00 | Signature: | 10/31/02 | 09:30 | |
| Printed Name: Kate A. Montgomery | | | Printed Name: Ted Williams | | | |
| Final Destination: Aquatec Williston, VT | | | Contact Name and Phone Number: John Williams 802 860 1638 | | | Shipping Method: Fed-Ex |
| | | | | | | Page 2 of (2) |

An Employee-Owned Company *Science Applications International Corporation*

# Chain of Custody Record

Science Applications International Corporation/ 221 Third Street/ Admiral's Gate/ Newport RI 02840 phone (401)847-4210 fax (401)849-9786

Project: NBH - Dredging

Client Name and Contact: Maguire/ACE : Sherry Poucher

| Sample No. | Containers No. | Containers Type | Collection Date | Collection Time | Sample Description | Requested Parameters |
|---|---|---|---|---|---|---|
| NBH_SPP-Cent. | 1 | 60mL | 10/30/02 | 11:00 | Untreated (unt.) | TIE |
| NBH_SPP_202 | 1 | 150mL | | | Untreated (unt.) | |
| SPIKE | 1 | 150mL | | | Untreated (unt.) | |
| Control | 1 | 150mL | | | Untreated (unt.) | |
| NBH_SPP_Cent | 1 | 60mL | | 12:00 | Sodium Thiosulfate (STS) | |
| NBH_SPP_202 | 1 | 150mL | | | Sodium Thiosulfate (STS) | |
| SPIKE | 1 | 150mL | | | Sodium Thiosulfate (STS) | |
| Control | 1 | 150mL | | | Sodium Thiosulfate (STS) | |
| NBH_SPP_Cent | 1 | 60mL | | 13:00 | EDTA | |
| NBH_SPP_202 | 1 | 150mL | | | EDTA | |
| SPIKE | 1 | 150mL | | | EDTA | |
| Control | 1 | 150mL | | | EDTA | |
| NBH_SPP_Cent | 1 | 60mL | | 14:00 | Filtered | |
| NBH_SPP_202 | 1 | 150mL | | | Filtered | |
| SPIKE | 1 | 185mL | | | Filtered | |
| Total: | 15 | | | | | |

| Packed/Released By | Date | Time | Received By | Date | Time | Remarks |
|---|---|---|---|---|---|---|
| Signature: Kate A. Montgomery | 10/30/02 | 16:00 | Signature: | | | Rec. Temp 1.3°C |
| Printed Name: Kate A. Montgomery | | | Printed Name: | | | |
| **Released By** | **Date** | **Time** | **Received By** | **Date** | **Time** | |
| Signature: Kate A. Montgomery | 10/30/02 | 16:00 | Signature: | 10/31/02 | 09:30 | |
| Printed Name: Kate A. Montgomery | | | Printed Name: John Williams | | | |
| Final Destination: Aquatec Williston, VT | | | Contact Name and Phone Number: John Williams 8028601638 | | | Shipping Method: Fed-Ex |
| | | | | | | Page 1 of (2) |

# Toxicity Test Methods

Science Applications International Corporation

# Test Protocol

| Client: SAIC | Project: 02065, New Bedford TIE | SDG: 6560 |
|---|---|---|
| Test Description: *Arbacia punctulata* Embryo development Toxicity Test | | |

ASSOCIATED PROTOCOL: EPA/ACE 1998. *Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Inland Testing Manual* (EPA-823-B-98-004)

| | |
|---|---|
| 1. Test type: | Static, no renewal |
| 2. Test temperature: | 20 ± 1°C |
| 3. Light quality: | Ambient laboratory illumination |
| 4. Photoperiod: | Continuous illumination |
| 5. Test chamber size: | 20-mL HDPE scintillation vials |
| 6. Test solution volume: | 20 ml / replicate |
| 7. Renewal of test concentrations: | None |
| 8. Age of test organisms: | Embryos, approximately 1-h old |
| 9. No. embryos / test chamber: | ~ 2000 |
| 10. No. of replicate chambers / concentration: | 3 |
| 11. No. of embryos / concentration: | ~ 6000 |
| 12. Feeding regime: | None |
| 13. Cleaning: | None during test |
| 14. Aeration: | None |
| 15. Dilution water: | Seawater |
| 16. Test concentrations: | 100% for SPP and spike; 10%, 25%, 50%, %100% for spiked untreated and spiked STS treatment |
| 17. Controls: | Seawater |
| 18. Test duration: | 72 hours |
| 19. Monitoring: | Daily: Temperature<br>Day 0: DO, temperature, pH, salinity. |
| 19. End points: | Embryo development |
| 20. Reference toxicant test: | Copper sulfate 48-h embryo development |
| 21. Test acceptability (control performance): | 70% or greater normal development in control |
| 22. Data interpretation: | Embryo development |

# Test Protocol

| Client: SAIC | Project: 02065, New Bedford TIE | SDG: 6560 |
|---|---|---|
| Test Description: *Americamysis bahia* 96-h acute toxicity | | |

ASSOCIATED PROTOCOL: EPA/ACE 1998. *Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Inland Testing Manual* (EPA-823-B-98-004)

| | |
|---|---|
| 1 Test type: | Static, no renewal |
| 2. Test temperature: | 25 ± 1°C |
| 3. Light quality: | Ambient laboratory illumination |
| 4. Photoperiod: | 16 h light, 8 h dark |
| 5. Test chamber size: | 250-mL disposable polystyrene |
| 6. Test solution volume: | Nominally, 200 ml / replicate |
| 7. Renewal of test concentrations: | None |
| 8. Age of test organisms: | 1 – 5 days |
| 9. No. mysids / test chamber: | 10 |
| 10. No. of replicate chambers / concentration: | 3 |
| 11. No. of mysids / concentration: | 30 |
| 12. Feeding regime: | Daily, 0.2 mL *Artemia* nauplii |
| 13. Cleaning: | None during test |
| 14. Aeration: | None during test |
| 15. Dilution water: | Seawater |
| 16. Test concentrations: | 100% for SPP and spike. Insufficient sample available to test 10% or 25% spiked sample. |
| 17. Controls: | Seawater |
| 18. Test duration: | 96 hours |
| 19. Monitoring: | Daily: Temperature<br>Days 0, 4: DO, temperature, pH, salinity. |
| 19. End points: | Survival |
| 20. Reference toxicant test: | Potassium chloride |
| 21. Test acceptability (control performance): | 90% or greater survival in control |
| 22. Data interpretation: | Survival (%) |

# Sea Urchin, Arbacia Punctulata, 72-h embryo development

Science Applications International Corporation

For the *Arbacia punctulata* embryo development test, percent survival may be estimated by using the number of embryos (including normal and abnormal) from a 1-mL aliquot removed from each test vial (preserved embryos) after the test was ended. Presence of any embryo material, no matter how undeveloped or degraded, was scored as "a live embryo" (Actual survival could not be verified because the embryos were preserved.). Data were recorded on the bench sheet labeled as "# in 1-mL".

Percent surviving may be calculated by:

["# in 1-mL" X 23) / 2000] X 100 = percent survival

23 = the total volume of solution per vial, including preservative

2000 = the nominal number of embryos added per test vial when the test was started.

One exception to this is for Sample 23152 ("Cent SPP-STS") Replicate B. The total volume in this vial was 13 mL after preservation.

Percent surviving = [(29 X 13) / 2000] X 100 = 18.8%

| Client: SAIC | Project: 02065, New Bedford TIE | SDG: 6560 |
|---|---|---|
| Test Description: *Arbacia punctulata* Embryo development Toxicity Test | | |

72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in (2) 1-mL |
|---|---|---|---|
| 23136 A | 1 | 49 | 10 |
| Cent SPP B | 3 | 97 | 29 |
| C-18 C | 0 | 100 | 24 |
| | | | |
| 23138 A | 96 | 3 | 89 |
| SPIKE B | 99 | 1 | 91 |
| C-18 C | 99 | 1 | 83 |
| D | | | |
| 23139 A | 96 | 4 | 101 |
| Control B | 98 | 2 | 85 |
| C-18 C | 99 | 1 | 91 |
| | | | |
| I/D | JT 11/6/02 | | |

WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | 7.9 | | | |
| DO | 6.9 | | | |
| Temp | 19.2 | | | 20.3 |
| Salinity | 30 | | | |
| pH | 7.6 | | | |
| DO | 8.6 | | | |
| Temp | 20.3 | | | 20.4 |
| Salinity | 30 | | | |
| pH | 7.8 | | | |
| DO | 7.7 | | | |
| Temp | 20.9 | 19.9 | | 20.3 |
| Salinity | 80 | | | |
| | 10/31/02 | 11/1 | 11/2 | 11/3 JW |

72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in 1-mL |
|---|---|---|---|
| 23140 A | 0 | 100 | 73 |
| Cent SPP B | 0 | 100 | 70 |
| ULVA C | 0 | 100 | 51 |
| | | | |
| 23142 A | 0 | 100 | 35 |
| SPIKE | 0 | 100 | 53 |
| ULVA C | 1 | 99 | 36 |
| | | | |
| 23143 A | 99 | 1 | 88 |
| Control | 100 | 0 | 79 |
| ULVA C | 97 | 3 | 82 |
| | | | |
| I/D | JT 11/6/02 | | |

WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | 7.3 | | | |
| DO | 4.1 | | | |
| Temp | 21.6 | | | 20.5 |
| Salinity | 80 | | | |
| pH | 7.5 | | | |
| DO | 5.1 | | | |
| Temp | 20.2 | | | 20.4 |
| Salinity | 80 | | | |
| pH | 7.3 | | | |
| DO | 5.2 | | | |
| Temp | 20.4 | | | 20.5 |
| Salinity | 30 | | | |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW |

72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in 1-mL |
|---|---|---|---|
| 23144 A | 0 | 100 | 38 |
| Cent SPP B | 0 | 100 | 24 |
| LO PH C | 0 | 100 | 19 |
| | | | |
| 23146 A | 96 | 4 | 90 |
| SPIKE | 97 | 3 | 76 |
| LO PH C | 97 | 3 | 86 |
| | | | |
| 23147 A | (1) 99 | 1 | 97 |
| Control | 100 | 0 | 98 |
| LO PH C | 99 | 1 | 85 |
| D | | | |
| I/D | JT 11/6/02 | | |

WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | 7.2 | | | |
| DO | 5.7 | | | |
| Temp | 21.0 | | | 20.6 |
| Salinity | 30 | | | |
| pH | 8.6 | | | |
| DO | 8.8 | | | |
| Temp | 19.8 | | | 20.6 |
| Salinity | 30 | | | |
| pH | 7.1 | | | |
| DO | 8.0 | | | |
| Temp | 20.4 | | 20.3 | 20.3 |
| Salinity | 30 | | | |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW |

(2) # in 1-mL = 1 mL aliquot of well mixed vial content. Target loading = 2000 embryos per vial. Vial volume = 23 mL (20 mL test sol. + 1 mL embryos + 2 mL preservative)

Aquatec Biological Sciences Williston, Vermont ApTIEToxForms

Reviewed by: _____ Date: 11/6/02

Abnormals are spherical multicellular/granular masses.

(1) Considered marginally normal. Pluteus large, small, slightly

Embryo count × 23 = estimated # embryos

## BIOLOGICAL AND WATER CHEMISTRY DATA

| Client: SAIC | Project: 02065, New Bedford TIE | SDG: 6560 |
|---|---|---|
| Test Description: *Arbacia punctulata* Embryo development Toxicity Test | | |

### 72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in 1-mL |
|---|---|---|---|
| 23148 A | 0 | 50 | 11 |
| Cent SPP B | 0 | 50 | 8 |
| UNT C | 0 | 50 | 5 |
| | | | |
| I/D | J | 11/8/02 | |

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | 7.9 | | | |
| DO | 8.4 | | | |
| Temp | 20.3 | | | 20.2 |
| Salinity | 30 | | | |
| | 10/31/02 | 11/1 | 11/2 | 11/3 |

J J

### 72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in 1-mL |
|---|---|---|---|
| 23150 A | 1 | 99 | 70 |
| SPIKE B | 1 | 99 | 89 |
| UNT C | 0 | 100 | 87 |
| 10% | | | |
| 23150 A | 0 ① | 100 | 18 ① |
| SPIKE B | 0 | 100 | 30 |
| UNT C | 0 | 100 | 80 |
| 25% | | | |
| 23150 A | 0 ① | 100 | 68 ① |
| SPIKE B | 0 | 100 | 57 |
| UNT C | 0 | 100 | 39 |
| 50% | | | |
| 23150 A | 0 ① | 100 | 69 ① |
| SPIKE B | 0 | 100 | 75 |
| UNT C | 0 | 100 | 99 |
| 100% | | | |
| I/D | J | 11/8/02 | |

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | | | | |
| DO | | | | |
| Temp | | | | 20.3 |
| Salinity | | | | |
| pH | | | | |
| DO | | | | |
| Temp | | | | 20.5 |
| Salinity | | | | |
| pH | | | | |
| DO | | | | |
| Temp | | | | 20.5 |
| Salinity | | | | |
| pH | 7.6 | | | |
| DO | 8.7 | | | |
| Temp | 19.9 | 20.5 | 20.5 | 20.5 |
| Salinity | 30 | | | |
| | 10/31/02 | 11/1 J | 11/2 J | 11/3 |

J J

### 72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # embryos in 1 mL |
|---|---|---|---|
| 23151 A | 100 | 0 | Not counted |
| Control | 100 | 0 | 93 |
| UNT C | 99 | 1 | 87 |
| | | | |
| I/D | J | 11/6/02 | |

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | 8.0 | | | |
| DO | 8.2 | | | |
| Temp | 20.5 | | | 20.5 |
| Salinity | 30 | | | |
| | 10/31/02 | 11/1 | 11/2 | 11/3 |

J

① Abnormal embryos are undeveloped spheres - arrested development at very early stage.

Solution vol = 23 mL, unless otherwise noted
(20 mL original test vol + 1 mL embryos + 2 mL formalin) J

J

## BIOLOGICAL AND WATER CHEMISTRY DATA

| Client: SAIC | Project: 02065, New Bedford TIE | SDG: 6560 |
|---|---|---|
| Test Description: *Arbacia punctulata* Embryo development Toxicity Test | | |

### 72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in 1-mL |
|---|---|---|---|
| 23152 A | 0 | 50 | 11 |
| Cent SPP B ① | 0 | 100 | 29 |
| STS C | 0 | 50 | 11 |
| | | | |
| I/D | 0 | 11/8/02 | |

① Total vol Rep B = 13 mL.

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | 7.7 | | | |
| DO | 8.4 | | | |
| Temp | 20.9 | | | 20.6 |
| Salinity | 31 | | | |
| | 10/31/02 | 11/1 | 11/2 | 11/3 JW |

O

### 72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in 1-mL |
|---|---|---|---|
| 23154 A | 99 | 1 | 87 |
| SPIKE B | 99 | 1 | 79 |
| STS C | 98 | 2 | 89 |
| 10% | | | |
| 23154 A | 0 | 100 | 85 |
| SPIKE B | 0 | 100 | 76 |
| STS C | 0 | 100 | 66 |
| 25% | | | |
| 23154 A | 0 | 100 | 87 |
| SPIKE B | 0 | 100 | 69 |
| STS C | 0 | 100 | 81 |
| 50% | | | |
| 23154 A | 0 | 100 | 39 |
| SPIKE B | 0 | 100 | 46 |
| STS C | 0 | 100 | 24 |
| 100% | | | |
| I/D | J | 11/8/02 | |

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | | | | |
| DO | | | | |
| Temp | | | | 20.6 |
| Salinity | | | | |
| pH | | | | |
| DO | | | | |
| Temp | | | | 20.6 |
| Salinity | | | | |
| pH | | | | |
| DO | | | | |
| Temp | | 20.3 | 20.7 | 20.5 |
| Salinity | | | | |
| pH | 7.8 | | | |
| DO | 8.6 | | | |
| Temp | 20.1 | | | 20.6 |
| Salinity | 30 | | | |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW |

O O J

### 72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in 1-mL |
|---|---|---|---|
| 23155 A | 100 | 0 | 99 |
| Control | 100 | 0 | 81 |
| STS C | 99 | 1 | 84 |
| | | | |
| I/D | J | 11/8/02 | |

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | 8.0 | | | |
| DO | 8.7 | | | |
| Temp | 20.3 | | | 20.5 |
| Salinity | 30 | | | |
| | 10/31/02 | 11/1 | 11/2 | 11/3 JW |

J

## BIOLOGICAL AND WATER CHEMISTRY DATA

| Client: SAIC | Project: 02065, New Bedford TIE | SDG: 6560 |
|---|---|---|
| Test Description: *Arbacia punctulata* Embryo development Toxicity Test | | |

### 72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in 1-mL |
|---|---|---|---|
| 23156 A | 0 | 50 | 8 |
| Cent SPP B | 1 | 49 | 10 |
| EDTA C | 0 | 28 | 5 |
| | | | |
| 23158 A | 95 | 5 | 107 |
| SPIKE B | 99 | 1 | 81 |
| EDTA C | 100 | 0 | 84 |
| | | | |
| 23159 A | 99 | 1 | 76 |
| Control B | 100 | 0 | 90 |
| EDTA C | 100 | 0 | 81 |
| | | | |
| I/D | J | 11/11/02 | |

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | 7.4 | | | |
| DO | 1.7/7.2 ← | | | |
| Temp | 20.6 | | | 20.6 |
| Salinity | 30 | | | |
| pH | 7.8 | | | |
| DO | 8.6 | | | |
| Temp | 20.1 | | | 20.6 |
| Salinity | 30 | | | |
| pH | 7.8 | | | |
| DO | 8.3 | | | |
| Temp | 20.5 | | | 20.6 |
| Salinity | 30 | | | |
| | 10/31/02 | 11/1 | 11/2 | 11/3 JW |

Aerated before reading.

J

### 72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in 1-mL |
|---|---|---|---|
| 23160 A | 3 | 97 | 20 |
| Cent SPP B | 6 | 94 | 17 |
| FILT C | 0 | 100 | 11 |
| | | | |
| 23162 A | 100 | 0 | 92 |
| SPIKE B | 100 | 0 | 90 |
| FILT C | 95 | 5 | 88 |
| | | | |
| 23135 A | 99 | 1 | 88 |
| Control B | 99 | 1 | 97 |
| FILT C | 98 | 2 | 78 |
| | | | |
| I/D | J | 11/11/02 | |

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | 7.9 | | | |
| DO | 3.4/7.0 ← | | | |
| Temp | 20.5 | | | 20.6 |
| Salinity | 30 | | | |
| pH | 7.6 | | | |
| DO | 8.0 | | | |
| Temp | 19.9 | | | 20.6 |
| Salinity | 30 | | | |
| pH | 7.7 | | | |
| DO | 7.4 | | | |
| Temp | 20.5 | | | 20.6 |
| Salinity | 30 | | | |
| | 10/31/02 | 11/1 | 11/2 | 11/3 JW |

Aerated before reading.

J

### 72-h BIOLOGICAL DATA

| Sample | # Normal | # Abnormal | # in 1-mL |
|---|---|---|---|
| 23163 A | 100 | 0 | 83 |
| Seawater B | 99 | 1 | 75 |
| C | 100 | 0 | 85 |
| | | | |
| I/D | J | 11/11/02 | |

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 |
|---|---|---|---|---|
| pH | 8.0 | | | |
| DO | 8.6 | | | |
| Temp | 20.4 | 20.4 | 20.7 | 20.5 |
| Salinity | 33 | | | |
| | 10/31/02 | 11/1 | 11/2 J G | 11/3 JW |

J J

Client: SAIC Project: New Bedford 02065 SDG: 6560

## Egg Collection and Dilution

Egg injection time: 12:45 No. females used: 4

#eggs in 9:1 dilution of egg stock = 327 = 3270 eggs/mL

Egg dilution:

Volume egg stock = 200 mL

Egg count / 200 = DF = 1.635

(DF X vol. Egg stock) – vol. Egg stock = = 127

volume of FSW added to egg stock = 127

Recount = 227 eggs - 2ddnl 40 mL.

Confirmation: #eggs in 9:1 dilution of egg stock. Final egg count =

Final volume of egg stock: 367

Total number of eggs in egg stock: 367 x 2000 = 734,000 eggs

Total number of eggs X 500 = number of sperm required: = 367,000,000 ($3.67 \times 10^8$)

## Sperm Collection and Dilution

Sperm injection time: 12:10 No. males used: 4

Add ~~0.25~~ 0.4mL σ mL sperm to Vial A (containing ~~10~~ 20mL σ mL seawater. Serially dilute to Vials B, C, and D. Add 5 mL 10% acetic acid/seawater to vial C. Transfer 1 mL from Vial C to Vial E (contains 4 mL seawater).

Hemacytometer count:, Vial E X $10^4$ = Side 1: 171 Side 2: 187 Avg. 179.

Avg. X 0.001 = X sperm X $10^7$ = 0.179 X $10^7$ = 0.0179 X $10^8$

Sperm concentration Vial A = 40 X Vial E = 0.716 X $10^8$

Sperm concentration Vial B = 20 X Vial E = X $10^8$

Sperm concentration Vial D = 5 X Vial E = X $10^8$

Vial selected for sperm stock = Vial

Sperm dilution to obtain 500:1 (sperm:egg) $3.67 \times 10^8$

Number of eggs in egg stock X 500 =

Vial selected as sperm stock = A $0.716 \times 10^8$ sperm per mL

Target #sperm / sperm stock per mL = volume of sperm stock to add to egg stock. 3.67 / 0.716 = 5.12 mL

| Date / Time Sperm added to egg stock | Fertilization in 1:9 dilution of embryo stock | Time Embryo Development Test Started |
|---|---|---|
| 13:54 | 100/100 = 100% | 15:00 |

Initials: σ Date: 10/31/02.

Test preserved 11/3/02 JW 15:00

Reviewed by: σ Date 11/1/02

Laboratory: Aquatec Biological Sciences, Inc. Williston, Vermont

ApEmbryoE&SP

Peak Table: ammonia

File name: A:\110502A.RST
Date: November 05, 2002
Operator: JJG

| Peak | Cup | Name | Type | Dil | Wt | Height | Calc. (mg/L) | Flags |
|---|---|---|---|---|---|---|---|---|
| 1 | 6 | Sync | SYNC | 1 | 1 | 174482 | 1.034395 | |
| 2 | 0 | CarryOver | CO | 1 | 1 | 2415 | 0.011859 | |
| 3 | 0 | CarryOver | CO | 1 | 1 | 182 | -0.001407 | LO |
| B | 0 | Baseline | RB | 1 | 1 | 0 | -0.002491 | BL |
| B | 0 | Baseline | RB | 1 | 1 | 0 | -0.002491 | BL |
| 6 | 1 | Cal 0 | C | 1 | 1 | 134 | -0.001694 | LO |
| 7 | 2 | Cal 1 | C | 1 | 1 | 35152 | 0.206406 | |
| 8 | 3 | Cal 2 | C | 1 | 1 | 67995 | 0.401583 | |
| 9 | 4 | Cal 3 | C | 1 | 1 | 167974 | 0.995725 | |
| 10 | 5 | Cal 4 | C | 1 | 1 | 841872 | 5.000472 | |
| 11 | 0 | Blank | U | 1 | 1 | -1383 | -0.010710 | LO |
| B | 0 | Baseline | RB | 1 | 1 | 0 | -0.002491 | BL |
| 13 | 6 | ICV | U | 1 | 1 | 171595 | 1.017242 | |
| 14 | 1 | ICB | U | 1 | 1 | -300 | -0.004271 | LO |
| 15 | 31 | 22621CTEND | U | 1 | 1 | 13549 | 0.078027 | |
| 16 | 32 | 22622CTEND | U | 1 | 1 | 2549 | 0.012652 | |
| 17 | 33 | 22623CTEND | U | 1 | 1 | 1827 | 0.008364 | |
| 18 | 34 | 22624CTEND | U | 1 | 1 | 865 | 0.002650 | |
| 19 | 35 | 22625CTEND | U | 1 | 1 | 1336 | 0.005449 | |
| 20 | 36 | 22626CTEND | U | 1 | 1 | 1742 | 0.007861 | |
| 21 | 37 | 22643CTEND | U | 1 | 1 | 9685 | 0.055061 | |
| 22 | 38 | 22644CTEND | U | 1 | 1 | 3578 | 0.018770 | |
| 23 | 39 | 22645CTEND | U | 1 | 1 | 12628 | 0.072553 | |
| 24 | 40 | 22646CTEND | U | 1 | 1 | 2160 | 0.010347 | |
| 25 | 5 | CCV | U | 1 | 1 | 845598 | 5.022618 | |
| 26 | 1 | CCB | U | 1 | 1 | -1442 | -0.011063 | LO |
| B | 0 | Baseline | RB | 1 | 1 | 0 | -0.002491 | BL |
| 28 | 41 | 22647CTEND | U | 1 | 1 | 420 | 0.000003 | |
| 29 | 42 | 22648CTEND | U | 1 | 1 | 3940 | 0.020925 | |
| 30 | 43 | 22655CTEND | U | 1 | 1 | 3573 | 0.018741 | |
| 31 | 44 | 22656CTEND | U | 1 | 1 | 91378 | 0.540535 | |
| 32 | 45 | 22657CTEND | U | 1 | 1 | 12101 | 0.069422 | |
| 33 | 46 | 22658CTEND | U | 1 | 1 | 3716 | 0.019593 | |
| 34 | 47 | 22659CTEND | U | 1 | 1 | 3217 | 0.016628 | |
| 35 | 48 | 22660CTEND | U | 1 | 1 | 8246 | 0.046514 | |
| 36 | 49 | 22661CTEND | U | 1 | 1 | 9784 | 0.055649 | |
| 37 | 50 | 22662CTEND | U | 1 | 1 | 6265 | 0.034737 | |
| 38 | 5 | CCV | U | 1 | 1 | 841834 | 5.000246 | |
| 39 | 1 | CCB | U | 1 | 1 | -1163 | -0.009403 | LO |
| B | 0 | Baseline | RB | 1 | 1 | 0 | -0.002491 | BL |
| 41 | 51 | 22663CTEND | U | 1 | 1 | 8469 | 0.047840 | |
| 42 | 52 | 22668CTEND | U | 1 | 1 | 25019 | 0.146189 | |
| 43 | 53 | 23035SPP (201) | U | 10 | 1 | 191119 | 11.332678 | SPP Ammonias 10/17/02 |
| 44 | 54 | 23036SPP (202) | U | 10 | 1 | 637488 | 37.857109 | |
| 45 | 55 | 23037SPP (204) | U | 10 | 1 | 21825 | 1.272105 | |
| 46 | 56 | 23038SPP (205) | U | 10 | 1 | 154018 | 9.127696 | |
| 47 | 57 | 23039SPP (206) | U | 10 | 1 | 105337 | 6.234899 | |
| 48 | 58 | 23040SPP (207) | U | 10 | 1 | 232029 | 13.763792 | |
| 49 | 59 | 23059SPP (REF) | U | 10 | 1 | 23615 | 1.378450 | |
| 50 | 60 | 23137TIE – (202) | B | end of mysid test, C-18 | 1 | 628496 | 37.323933 | TIE Ammonia 11/4/02 |
| 51 | 5 | CCV | U | 1 | 1 | 852684 | 5.064609 | |
| 52 | 1 | CCB | U | 1 | 1 | -1146 | -0.009300 | LO |
| B | 0 | Baseline | RB | 1 | 1 | 0 | -0.002491 | BL |
| 54 | 61 | 23141TIE – (202) | U | end of mysid test, ULV A | 1 | 1315797 | 7.816853 | |

| Peak | Cup | Name | Type | Dil | Wt | Height | Calc. (mg/L) | Flags |
|---|---|---|---|---|---|---|---|---|
| 55 | 62 | 23153TIE (202) end of mysid test, STS | U | 1 | 1 | 610643 | 36.263691 | |
| 56 | 5 | CCV | U | 1 | 1 | 850516 | 5.051842 | |
| 57 | 1 | CCB | U | 1 | 1 | -1047 | -0.008715 | LO |
| B | 0 | Baseline | RB | 1 | 1 | 0 | -0.002491 | BL |

# Suspended Particulate Phase Preparation for TIE

| Client: SAIC | Project: 02065, New Bedford FEIR | SDG: 6519 |
|---|---|---|

**SPP / Elutriate Preparation:**
Quantitatively mix Site Sediment with matched Site Water in a 1:4 ratio. Mix this solution 30 minutes with a mixer. At approximately 10 min intervals, manually stir to ensure complete mixing. Allow the solution to settle 1 hour. At 1 hour remove the SPP for the toxicity tests. Ideally, approximately 4.7 L or SPP is needed for the TIE, however, we may be limited by sediment quantity. Approximately 4.2 L of SPP will be shipped to SAIC for the mysid TIE. A sub-sample of approximately 500 mL will be centrifuged (10 min @ 6000 RPM) for the *Arbacia* TIE and shipped to SAIC. The SPP prep water will be the matched site water for Sample 202: Our lab numbers 23024 (sediment) and 23030 (water).

**SPP / Elutriate Preparation (October 29, 2002)**

| Water & Sediment Samples | Volume Sediment: Water (mL) | SPP Mix Time | SPP Settle Time | SPP TOX Vol for Mysid | SPP TSS Vol For *Arbacia* | Spin 1 Time 6000 RPM 10 min 4°C |
|---|---|---|---|---|---|---|
| | | | | | + mysid | |
| 23030<br>202-W-ELUT<br>23024<br>202-ELUT | 4800 mL<br>$H_2O$, 1200mL<br>Sed = 6L | 11:35 –<br>12:05 | 12:05 –<br>13:05 | ~1200 | baseline. | 13:50 –<br>14:00 |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | JW 10/29/02 | | | | | |

# TIE Using Mysidopsis bahia

Science Applications International Corporation

# BIOLOGICAL AND WATER CHEMISTRY DATA

| Client: SAIC | Project: 02065, New Bedford TIE | SDG: 6560 |
|---|---|---|
| Test Description: *Americamysis bahia* Acute Toxicity Test | | |

## NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23137 A | 10 | 6 | 1 | 0 | 0 |
| SPP B | 10 | 8 | 4 | 3 | 1 |
| C-18 C | 10 | 6 | 1 | 0 | 0 |
| | | | | | |
| 23138 A | 10 | 10 | 10 | 9 | 9 |
| SPIKE B | 10 | 10 | 10 | 9 | 9 |
| C-18 C | 10 | 10 | 10 | 10 | 10 |
| D | | | | | |
| 23139 A | 10 | 10 | 10 | 10 | 10 |
| Control B | 10 | 10 | 10 | 10 | 10 |
| C-18 C | 10 | 10 | 10 | 10 | 10 |
| | 10 | T | | | |
| I/D/T | 10/31/02 | 11/1 15:00 | 11/2 JG | 11/3 JW | 11/4 |

## WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | 7.7 | | | 8.1 | 8.4 |
| DO | 8.1 | 6.7 | | 7.4 | 7.2 |
| Temp | 20.1 | | 25.0 | 24.2 | 24.9 |
| Salinity | 30 | | | 33 | 33 |
| pH | 7.6 | | | | 7.1 |
| DO | 8.6 | | | | 5.4 |
| Temp | 20.3 | | | 24.2 | 24.7 |
| Salinity | 30 | | | | 32 |
| pH | 7.8 | | | | 7.2 |
| DO | 7.7 | | | | 4.4 |
| Temp | 20.2 T | 25.5 | | | 24.7 |
| Salinity | 30 | | | | 32 |
| | 10/31/02 | 11/1 T | 11/2 JG | 11/3 JW | 11/4 |

Temp probe T2 P25C 10/31/02 22:22

20:30

Ammonia sample 11/4/02

## NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23141 A | 10 | 10 | 9 10 JG | 10 | 10 |
| SPP B | 10 | 9 | 9 | 9 | 9 |
| ULVA C | 10 (1) 9 | 9 | 9 | 8 | 8 |
| | | | | | |
| 23142 A | 10 | 10 | 10 | 10 | 10 |
| SPIKE B | 10 | 9 | 9 | 9 | 9 |
| ULVA C | 10 | 10 | 9 | 9 | 8 |
| | | | | | |
| 23143 A | 10 | 10 | 10 | 10 | 10 |
| Control B | 10 | 10 9 | 9 | 9 | 9 |
| ULVA C | 10 | 9 | 9 | 9 | 8 |
| D | 10 | ~~10~~ | ~~10~~ | | |
| I/D/T | 10/31/02 | 11/1 15:15 | 11/2 JG | 11/3 JW | 11/4 20:20 |

(1) One snail on petri dish Top

20.40

## WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | 7.3 | | | | 8.4 |
| DO | 6.1 | | | | 7.5 |
| Temp | 20.4 | 24.7 | | 24.1 | 24.6 |
| Salinity | 30 | | | | 32 |
| pH | 7.5 | | | | 7.2 |
| DO | 5.1 | | | | 3.5 |
| Temp | 20.2 | | 24.5 | | 24.6 |
| Salinity | 30 | | | | 30 |
| pH | 7.3 | | | | 7.6 |
| DO | 5.2 | | | | 5.9 |
| Temp | 20.4 | | | | 24.7 |
| Salinity | 30 | | | | 31 |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 |

Ammonia sample 11/4/02

## NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23145 A | 10 | 6 | 5 | 5 | 3 |
| SPP B | 10 | 6 | 1 | 0 | 0 |
| LO PH C | 10 | 7 | 1 | 0 | 0 |
| | | | | | |
| 23146 A | 10 | 10 | 10 | 10 | 10 |
| SPIKE B | 10 | 10 | 9 | 9 | 9 |
| LO PH C | 10 | 10 | 10 | 10 | 10 |
| | | | | | |
| 23147 A | 10 | 10 | 10 | 10 | 10 |
| Control B | 10 | 10 | 10 | 10 | 10 |
| LO PH C | 10 | 10 | 10 | 10 | 10 |
| ~~D~~ | ~~10~~ | | | | |
| I/D/T | 10/31/02 | 11/1 15:30 | 11/2 JG | 11/3 JW | 11/4 20:28 |

20.50

## WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | 7.7 | | | 8.0 | 7.8 |
| DO | Not measured | 6.9 | | 7.3 | 6.7 |
| Temp | 21.0 | 24.9 | 24.4 | 24.1 | 24.9 |
| Salinity | 30 | | | 32 | 34 |
| pH | 8.6 | | | | 7.5 |
| DO | 8.8 | | | | 4.9 |
| Temp | 19.8 | | | 24.2 | 24.8 |
| Salinity | 30 | | | | 32 |
| pH | 7.1 | | | | 7.6 |
| DO | 8.0 | | | | 7.3 |
| Temp | 20.4 | | | | 24.8 |
| Salinity | 30 | | | | 32 |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 |

No ammonia sample

Aquatec Biological Sciences Williston, Vermont Fed ✓ Fed ✓ SPPToxForms

Reviewed by: _____ Date: 11/5/02

10/31/02 22:10 - all replicates

12/6/02 All reps fed during monitoring

0 written in wrong space 11/1 + 11/2

11/4/02 FINAL COUNTS MEASURED WHEN TEST CONTAINERS JUST PRIOR TO FINAL COUNTS

## BIOLOGICAL AND WATER CHEMISTRY DATA

| Client: SAIC | Project: 02065, New Bedford TIE | SDG: 6560 |
|---|---|---|
| Test Description: *Americamysis bahia* Acute Toxicity Test | | |

### NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23149 A | 10 | 9 | 5 | 0 | 0 |
| SPP B | 10 | 7 | 1 | 0 | 0 |
| UNT C | 10 | 6 | 0 | 0 | 0 |
| | | | | | |
| I/D/T | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 |

21:09 15:35

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | 7.9 | | 8.5 | 8.5 | ✓ |
| DO | 9.0 | 6.6 | 6.5 | 7.3 | ✓ |
| Temp | 20.0 | | 24.3 | 24.2 | ✓ |
| Salinity | 30 | | 30 | 31 | ✓ |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 |

### NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23150 A | 10 | ✓ | | | |
| SPIKE B | ~~10~~ | | | | |
| UNT C | 10 | | | | |
| ~~10%~~ | | | | | |
| 23150 A | 10 | ✓ | | | |
| SPIKE B | ~~10~~ | | | | |
| UNT C | 10 | | | | |
| ~~25%~~ | | | | | |
| 23150 A | 10 | ✓ | | | |
| SPIKE B | ~~10~~ | | | | |
| UNT C | 10 | | | | |
| ~~50%~~ | | | | | |
| 23150 A | 10 | 0 | 0 | 0 | 0 |
| SPIKE B | ~~10~~ | | | | |
| UNT C | ~~10~~ | | | | |
| 100% | | | | | |
| I/D/T | 10/31/02 | 11/1 R-79 | 11/2 JG | 11/3 | 11/4 |

Insufficient sample to run dilutions (10%, 25%, 50%)

One rep only at 100%

21:10

SPIKE UNT – only enough solution for one rep – 100%

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | | | | | |
| DO | | | | | |
| Temp | | | | | |
| Salinity | | | | | |
| pH | | | | | |
| DO | | | | | |
| Temp | | | | | |
| Salinity | | | | | |
| pH | | | | | |
| DO | | | | | |
| Temp | | | | | |
| Salinity | | | | | |
| pH | 7.6 | 7.8 | | | ✓ |
| DO | 8.7 | 6.9 | | | ✓ |
| Temp | 19.9 | | | | ✓ |
| Salinity | 30 | 30 | | | ✓ |
| | 10/31/02 | 11/1 | 11/2 | 11/3 JW | 11/4 |

### NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23151 A | 10 | 10 | 10 | 10 | 10 |
| Control B | 10 | 10 | 10 | 10 | 10 |
| UNT C | 10 | 10 | 10 | 10 | 10 |
| | | | | | |
| I/D/T | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 |

21:15 15:50 14:00 Fed ✓ 14:00 Fed ✓ 20:30

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | 8.0 | | | | 8.0 |
| DO | 8.2 | | | | 7.3 |
| Temp | 20.5 | 25.2 | 24.5 | 24.2 | 24.9 |
| Salinity | 30 | | | | 34 |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 |

## BIOLOGICAL AND WATER CHEMISTRY DATA

| Client: SAIC | Project: 02065, New Bedford TIE | SDG: 6560 |
|---|---|---|
| Test Description: *Americamysis bahia* Acute Toxicity Test | | |

### NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23153 A | 10 | 10 | 6 | 5 | 3 |
| SPP B | 10 | 7 | 0 | 0 | 0 |
| STS C | 10 | 6 | 0 | 0 | 0 |
| | | | | | |
| I/D/T | 10/31/02 | 11/1 ✓ | 11/2 JG | 11/3 JW | 11/4 |

✓ 21:20 15:57

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | 7.6 | | 8.4 | | 8.3 |
| DO | 7.1 | | 6.4 | | 7.1 |
| Temp | 19.8 | | 24.5 | 24.2 | 24.9 |
| Salinity | 30 | | 30 | | 32 |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 ✓ |

Ammonia sample ✓ 11/4/02 ✓

### NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23154 A | 10 | | | | |
| SPIKE B | 10 | | | | |
| STS C | 10 | | | | |
| 10% | | | | | |
| 23154 A | 10 | | | | |
| SPIKE B | 10 | | | | |
| STS C | 10 | | | | |
| 25% | | | | | |
| 23154 A | 10 | 10 | 10 | 8 | 10 |
| SPIKE B | 10 | / | / | / | / |
| STS C | 10 | / | / | / | / |
| 50% | | | | | |
| 23154 A | 10 | 10 | 9 | 9 | 8 |
| SPIKE B | 10 | / | / | / | / |
| STS C | 10 | / | / | / | / |
| 100% | | 15:57 | | | |
| I/D/T | 10/31/02 | 11/1 JG | 11/2 JG | 11/3 JW | 11/4 ✓ 20:30 |

Insufficient sample to run 10% or 25%. One rep only at 50% & 100%

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | | | | | |
| DO | | | | | |
| Temp | | | | | |
| Salinity | | | | | |
| pH | | | | | |
| DO | | | | | |
| Temp | | | | | |
| Salinity | | | | | |
| pH | | | | | 7.7 |
| DO | | | | | 5.6 |
| Temp | | | | | 25.0 |
| Salinity | | | | | 32 |
| pH | 7.8 | | | | 7.4 |
| DO | 8.6 | | | | 6.4 |
| Temp | 20.1 | | | 24.2 | 25.3 |
| Salinity | 30 | | | | 31 |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 ✓ |

✓ 21:20 SPIKE STS - only enough for 1 50% - one rep; 100% - one rep.

### NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23155 A | 10 | 10 | 10 | 10 | 10 |
| Control B | 10 | 10 | 10 | 10 | 10 |
| STS C | 10 | 10 | 10 | 10 | 10 |
| | | | | | |
| I/D/T | 10/31/02 | 11/1 ✓ | 11/2 JG | 11/3 JW | 11/4 ✓ |

✓ 21:20 16:06 14:00 Fed ✓ 13:50 Fed ✓ 20:40

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | 8.0 | | | | ~~7.4~~ 7.9 ✓ |
| DO | 8.7 | | | | ~~7.4~~ 7.0 ✓ |
| Temp | 20.3 | | | 24.1 | 25.1 |
| Salinity | 30 | | | | 31 |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 ✓ |

## BIOLOGICAL AND WATER CHEMISTRY DATA

| Client: SAIC | Project: 02065, New Bedford TIE | SDG: 6560 |
|---|---|---|
| Test Description: *Americamysis bahia* Acute Toxicity Test | | |

### NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23157 A | 10 | 10 | 8 | 0 | 0 |
| SPP B | 10 | 7 | 0 | 0 | 0 |
| EDTA C | 10 | 6 | 3 | 0 | 0 |
| | | | | | |
| 23158 A | 10 | 10 | 10 | 10 | 10 |
| SPIKE B | 10 | 10 | 10 | 10 | 9 |
| EDTA C | 10 | 10 | 10 | 10 | 10 |
| | | | | | |
| 23159 A | 10 | 10 | 10 | 10 | 10 |
| Control B | 10 | 10 | 10 | 10 | 10 |
| EDTA C | 10 | 10 | 10 | 10 | 9 |
| | | | | | |
| I/D/T | 10/31/02 | 11/1 16:30 | 11/2 JG | 11/3 JW | 11/4 20:35 |

21:28

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | 7.4 | | 8.3 | 8.2 | |
| DO | 8.0 | 6.4 | 6.8 | 7.2 | |
| Temp | 20.3 | 24.7 | | 24.2 | |
| Salinity | 31 | | 30 | 31 | ~~31~~ |
| pH | 7.8 | | | | 7.5 |
| DO | 8.6 | | | | 4.0 |
| Temp | 20.1 | | 24.3 | | 25.2 |
| Salinity | 30 | | | | 32 |
| pH | 7.8 | | | | 7.8 |
| DO | 8.3 | | | | 6.9 |
| Temp | 20.5 | | | 24.2 | 25.3 |
| Salinity | 30 | | | | ~~31~~ 31 |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 |

### NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23161 A | 10 | 10 | 8 | 0 | 0 |
| SPP B | 10 | 9 | 7 | 0 | 0 |
| FILT C | 10 | 10 | 6 | 0 | 0 |
| | | | | | |
| 23162 A | 10 | 10 | 10 | 10 | 10 |
| SPIKE B | 10 | 10 | 10 | 10 | 10 |
| FILT C | 10 | 10 | 10 | 10 | 10 |
| | | | | | |
| 23135 A | 10 | 10 | 10 | 10 | 10 |
| Control B | 10 | 10 | 10 | 10 | 10 |
| FILT C | 10 | 10 | 10 | 10 | 9 |
| | | | | | |
| I/D/T | 10/31/02 | 11/1 16:27 | 11/2 JG | 11/3 JW | 11/4 20:47 |

21:35

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | 7.6 | | | 8.3 | |
| DO | 6.0 | | | 6.6 | |
| Temp | 20.3 | 25.1 | | 24.2 | |
| Salinity | 31 | | | 34 | 32 |
| pH | 7.6 | | | | 7.2 |
| DO | 8.0 | | | | 4.6 |
| Temp | 19.9 | | 24.2 | | 25.2 |
| Salinity | 30 | | | | ~~31~~ 31 |
| pH | 7.7 | | | | 7.8 |
| DO | 7.4 | | | | 6.8 |
| Temp | 20.5 | | | 24.2 | 25.4 |
| Salinity | 30 | | | | 31 |
| | 10/31/02 | 11/1 | 11/2 JG | 11/3 JW | 11/4 |

### NUMBER SURVIVING

| Sample | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| 23163 A | 10 | 10 | 10 | 10 | 10 |
| Seawater B | 10 | 10 | 10 | 10 | 10 |
| C | 10 | 10 | 10 | 10 | 10 |
| | | | | | |
| I/D/T | 10/31/02 | 11/1 16:30 | 11/2 JG 14:15 Fed | 11/3 JW 13:30 Fed | 11/4 20:50 |

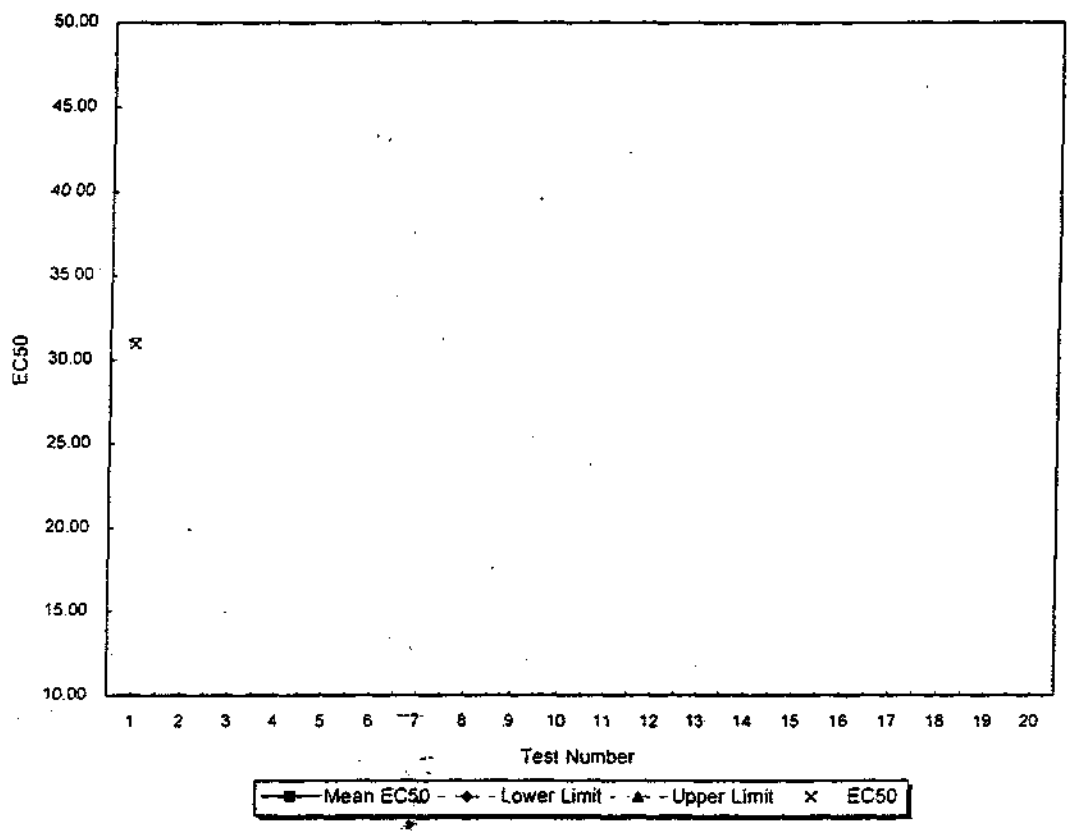
21:38

### WATER CHEMISTRY DATA

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| pH | 8.0 | | | | 8.0 |
| DO | 8.6 | | | | 6.9 |
| Temp | 20.4 | | | 24.1 | 25.0 |
| Salinity | 33 | | | | 31 |
| | 10/31/02 | 11/1 | 11/2 | 11/3 JW | 11/4 |

# Reference Toxicant Control Chart
## *Arbacia punctulata Embryo Development*
## in Copper sulfate (ug/L)

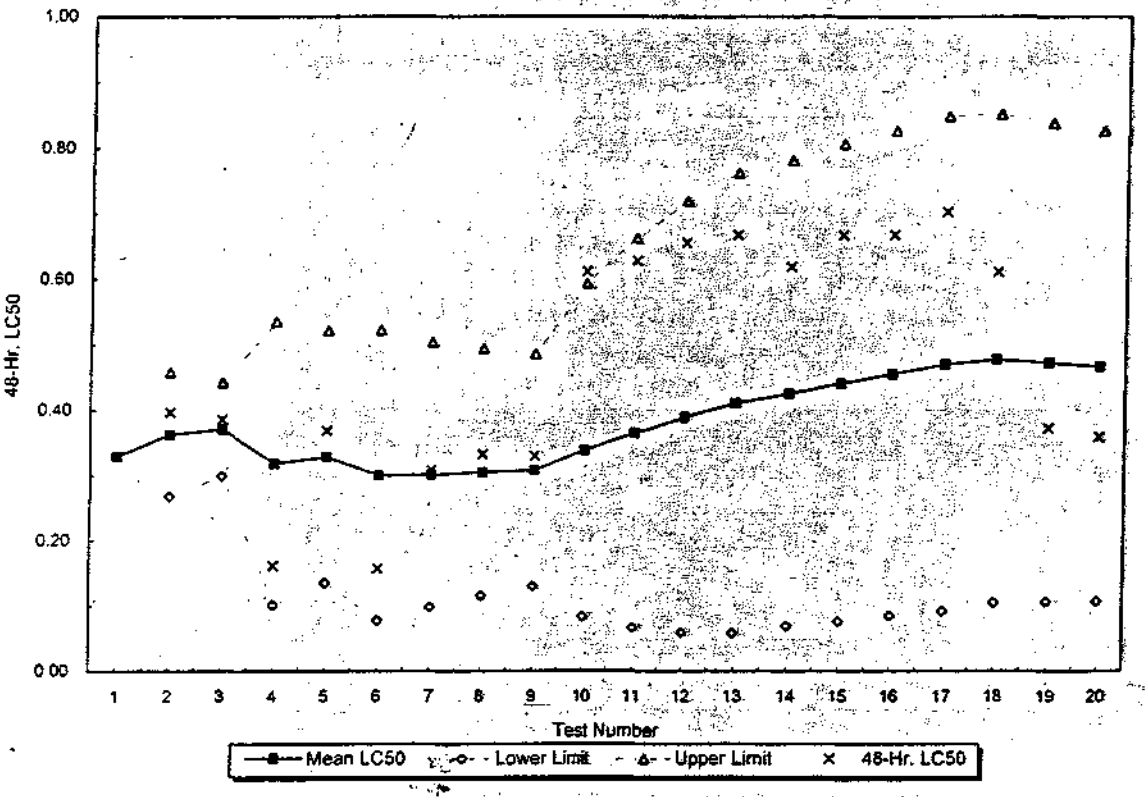
| Test Number | Test Date | 48-h EC50 | Mean EC50 | Lower Limit | Upper Limit | Organism Source |
|---|---|---|---|---|---|---|
| 1 | 10/31/02 | 30.935 | 30.94 | | | Aquatec Biological Sciences |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |
| 6 | | | | | | |
| 7 | | | | | | |
| 8 | | | | | | |
| 9 | | | | | | |
| 10 | | | | | | |
| 11 | | | | | | |
| 12 | | | | | | |
| 13 | | | | | | |
| 14 | | | | | | |
| 15 | | | | | | |
| 16 | | | | | | |
| 17 | | | | | | |
| 18 | | | | | | |
| 19 | | | | | | |
| 20 | | | | | | |




\qaqc\srts\APEmbDevCUSO

# Reference Toxicant Control Chart
## *Americamysis bahia*
## in Potassium chloride (g/L)

| Test Number | Test Date | Organism Age (Days) | 48-Hr. LC50 | Mean LC50 | Lower Limit | Upper Limit | Organism Source |
|---|---|---|---|---|---|---|---|
| 1 | 05/24/01 | 3 | 0.330 | 0.33 | | | Aquatic Research Organisms |
| 2 | 06/06/01 | 3 | 0.397 | 0.36 | 0.27 | 0.46 | Aquatic BioSystems |
| 3 | 07/06/01 | 4 | 0.386 | 0.37 | 0.30 | 0.44 | Aquatic BioSystems |
| 4 | 08/15/01 | 3 | 0.162 | 0.32 | 0.10 | 0.54 | Aquatic Research Organisms |
| 5 | 09/12/01 | 4 | 0.369 | 0.33 | 0.14 | 0.52 | Aquatic BioSystems |
| 6 | 10/05/01 | 3 | 0.157 | 0.30 | 0.08 | 0.52 | Aquatic BioSystems |
| 7 | 12/05/01 | 2 | 0.308 | 0.30 | 0.10 | 0.50 | Aquatic BioSystems |
| 8 | 01/04/02 | 2 | 0.333 | 0.31 | 0.12 | 0.49 | Aquatic Research Organisms |
| 9 | 01/04/01 | 3 | 0.330 | 0.31 | 0.13 | 0.49 | Aquatic BioSystems |
| 10 | 03/07/02 | 3 | 0.612 | 0.34 | 0.08 | 0.59 | Aquatic BioSystems |
| 11 | 03/19/02 | 2 | 0.628 | 0.36 | 0.07 | 0.66 | Aquatic BioSystems |
| 12 | 04/08/02 | 5 | 0.656 | 0.39 | 0.06 | 0.72 | Aquatic BioSystems |
| 13 | 04/10/02 | 4 | 0.668 | 0.41 | 0.06 | 0.76 | Aquatic BioSystems |
| 14 | 06/03/02 | 4 | 0.619 | 0.43 | 0.07 | 0.78 | Aquatic BioSystems |
| 15 | 08/15/02 | 5 | 0.668 | 0.44 | 0.08 | 0.81 | Aquatic BioSystems |
| 16 | 09/11/02 | 4 | 0.668 | 0.46 | 0.08 | 0.83 | Aquatic BioSystems |
| 17 | 09/21/02 | 5 | 0.703 | 0.47 | 0.09 | 0.85 | Aquatic BioSystems |
| 18 | 09/30/02 | 5 | 0.612 | 0.48 | 0.10 | 0.85 | Aquatic BioSystems |
| 19 | 10/18/02 | 4 | 0.373 | 0.47 | 0.11 | 0.84 | Aquatic BioSystems |
| 20 | 11/01/02 | 3 | 0.360 | 0.47 | 0.11 | 0.83 | Aquatic BioSystems |



# Appendix A

# Toxicity Testing Data Report and Statistical Analyses

## Mysid Survival, Growth and Fecundity Test-48 Hr

| | | | |
|---|---|---|---|
| Start Date: | Test ID: NBHMYS48 | Sample ID: | NBH MYS 48 |
| End Date: | Lab ID: | Sample Type: | AMB1-Ambient water |
| Sample Date: | Protocol: EPAA 91-EPA Acute | Test Species: | AB-Americamysis bahia |
| Comments: | New Bedford Harbor, 48hr Americamysis bahia | | |

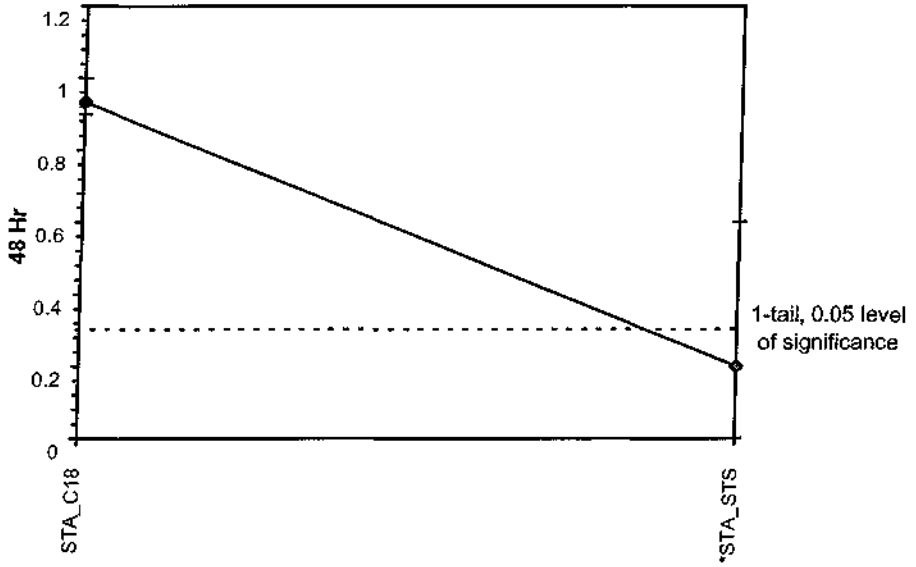
| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| STA_C18 | 1.0000 | 0.9000 | 0.9000 |
| STA_STS | 0.6000 | 0.0000 | 0.0000 |

| | | | Transform: Untransformed | | | | | 1-Tailed | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| STA_C18 | 0.9333 | 1.0000 | 0.9333 | 0.9000 | 1.0000 | 6.186 | 3 | | | |
| *STA_STS | 0.2000 | 0.2143 | 0.2000 | 0.0000 | 0.6000 | 173.205 | 3 | 3.617 | 2.920 | 0.5921 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.860401 | 0.713 | 1.320255 | 2.03981 |
| F-Test indicates equal variances (p = 0.05) | 36 | 199.012 | | |

| Hypothesis Test (1-tail, 0.05) | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|
| Heteroscedastic t Test indicates significant differences | 0.592053 | 0.634342 | 0.806667 | 0.061667 | 0.022421 | 1, 4 |

**Dose-Response Plot**



## Mysid Survival, Growth and Fecundity Test-48 Hr

| | | | |
|---|---|---|---|
| Start Date: | Test ID: NBHURC72 | Sample ID: | NBH URC 72hr |
| End Date: | Lab ID: | Sample Type: | AMB1-Ambient water |
| Sample Date: | Protocol: EPAA 91-EPA Acute | Test Species: | AP-Arbacia punctulata |
| Comments: | New Bedford Harbor, 72hr Urchin Survival | | |

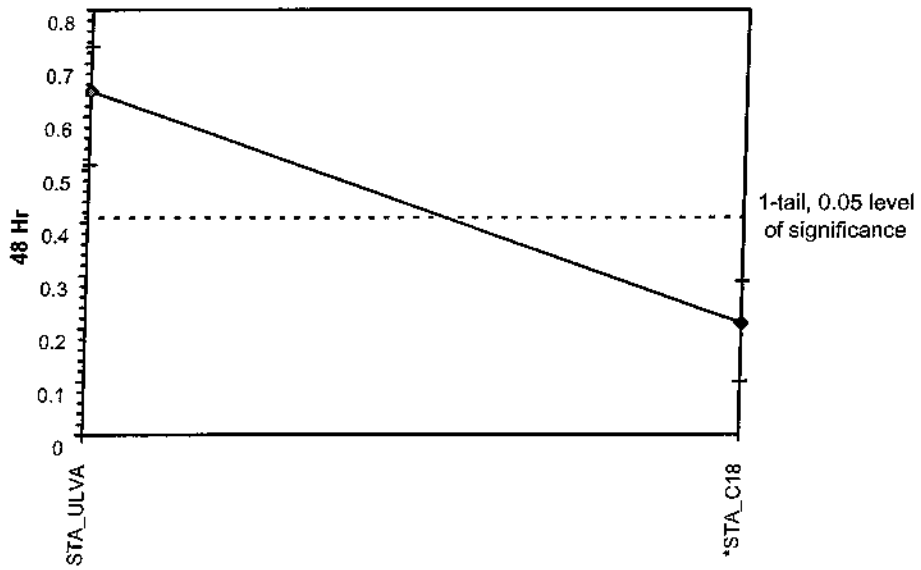
| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| STA_ULVA | 0.7300 | 0.7000 | 0.5100 |
| STA_C18 | 0.1000 | 0.2900 | 0.2400 |

| | | | Transform: Untransformed | | | | | 1-Tailed | |
|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| STA_ULVA | 0.6467 | 1.0000 | 0.6467 | 0.5100 | 0.7300 | 18.449 | 3 | | | |
| *STA_C18 | 0.2100 | 0.3247 | 0.2100 | 0.1000 | 0.2900 | 46.899 | 3 | 4.889 | 2.353 | 0.2102 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.809437 | 0.713 | -0.83728 | -1.71803 |
| F-Test indicates equal variances (p = 0.81) | 1.467354 | 199.012 | | |

| Hypothesis Test (1-tail, 0.05) | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|
| Heteroscedastic t Test indicates significant differences | 0.210199 | 0.325049 | 0.286017 | 0.011967 | 0.008109 | 1, 4 |

**Dose-Response Plot**








Reviewed by: SP

## Mysid Survival, Growth and Fecundity Test-48 Hr

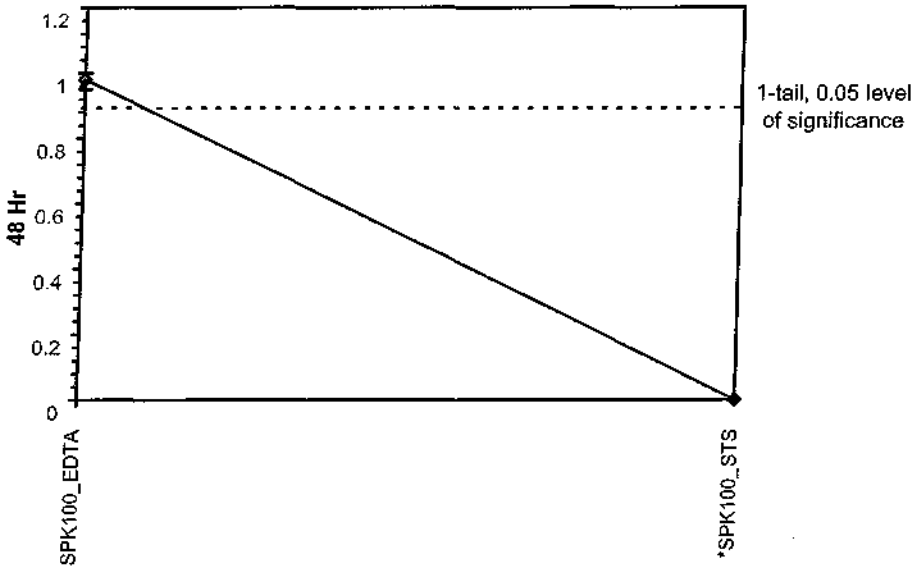
| | | | |
|---|---|---|---|
| Start Date: | Test ID: NBHURC | Sample ID: | NBH URC |
| End Date: | Lab ID: | Sample Type: | AMB1-Ambient water |
| Sample Date: | Protocol: EPAA 91-EPA Acute | Test Species: | AP-Arbacia punctulata |
| Comments: | New Bedford Harbor, Arbacia punctulata, Normal Development | | |

| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| SPK100_EDTA | 0.9500 | 0.9900 | 1.0000 |
| SPK100_STS | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Untransformed | | | | | 1-Tailed | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| SPK100_EDTA | 0.9800 | 1.0000 | 0.9800 | 0.9500 | 1.0000 | 2.700 | 3 | | | |
| *SPK100_STS | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.000 | 3 | 64.156 | 2.920 | 0.0446 |

| Auxiliary Tests | Statistic | | Critical | | Skew | Kurt |
|---|---|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.873051 | | 0.713 | | -1.15254 | 2.5 |
| Equality of variance cannot be confirmed | | | | | | |
| **Hypothesis Test (1-tail, 0.05)** | **MSDu** | **MSDp** | **MSB** | **MSE** | **F-Prob** | **df** |
| Heteroscedastic t Test indicates significant differences | 0.044604 | 0.045514 | 1.4406 | 0.00035 | 3.5E-07 | 1, 4 |

**Dose-Response Plot**






ToxCalc v5.0.23

Reviewed by: SP

## Mysid Survival, Growth and Fecundity Test-48 Hr

| | | | |
|---|---|---|---|
| Start Date: | Test ID: NBHURCS48 | Sample ID: | NBH URC 48 |
| End Date: | Lab ID: | Sample Type: | AMB1-Ambient water |
| Sample Date: | Protocol: EPAA 91-EPA Acute | Test Species: | AP-Arbacia punctulata |
| Comments: | New Bedford Harbor, Arbacia punctulata, Normal Development | | |

| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| SPK10_STS | 0.9900 | 0.9900 | 0.9800 |
| SPK10_UNT | 0.0100 | 0.0100 | 0.0000 |

| | | | Transform: Untransformed | | | | | 1-Tailed | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| SPK10_STS | 0.9867 | 1.0000 | 0.9867 | 0.9800 | 0.9900 | 0.585 | 3 | | | |
| *SPK10_UNT | 0.0067 | 0.0068 | 0.0067 | 0.0000 | 0.0100 | 86.603 | 3 | 207.889 | 2.132 | 0.0100 |

| Auxiliary Tests | Statistic | | Critical | | Skew | Kurt |
|---|---|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.639916 | | 0.713 | | -0.96825 | -1.875 |
| F-Test indicates equal variances (p = 1.00) | 1 | | 199.012 | | | |
| **Hypothesis Test (1-tail, 0.05)** | **MSDu** | **MSDp** | **MSB** | **MSE** | **F-Prob** | **df** |
| Heteroscedastic t Test indicates significant differences | 0.01005 | 0.010185 | 1.4406 | 3.33E-05 | 3.2E-09 | 1, 4 |

**Dose-Response Plot**