

### 3. TOXICITY TESTING PROGRAM

#### 3.1 INTRODUCTION

At the request of SCSPA and URS Greiner, EA performed toxicity testing on 11 project area sediment samples and one reference sediment sample collected by EA personnel. The toxicity testing program consisted of water column acute toxicity testing with sediment elutriate samples, acute toxicity testing of solid phase whole sediment samples, and 28-day survival and bioaccumulation testing of the whole sediment samples. The water column (elutriate) acute toxicity tests were conducted with one vertebrate species: inland silverside (*Menidia beryllina*); and two invertebrate species: opossum shrimp (*Mysidopsis bahia*), and the purple sea urchin (*Arbacia punctulata*). The solid phase sediment acute toxicity test were performed on the opossum shrimp (*Mysidopsis bahia*), and the estuarine amphipod (*Leptocheirus plumulosus*). The 28-day survival and bioaccumulation tests were performed with the marine polychaete *Neanthes virens* and the blunt-nose clam *Macoma nasuta*.

#### 3.2 MATERIALS AND METHODS

##### 3.2.1 Sample Receipt and Homogenization

Collection of the specific samples is previously described in Section 2 of this report. Following collection of the samples, the sediment samples were transported to EA's ecotoxicology laboratory in Sparks, Maryland via refrigerated truck (cooled to 4°C, and arrived at EA on 15 July 1998. Upon receipt, the cores were visually inspected, and the labeled core sleeves were compared against the COC record. The COC records were completed at receipt. Each of the 11 project area samples and the reference sample were logged into the Ecotoxicology Sample Log, and each was assigned a unique accession number. Additionally, the 25 gallons of site water were logged and assigned accession number (AT8-416). When not actively being processed, the samples were stored in a secured walk-in cooler, in the dark, at 4°C.

The sediments from each station were extracted from the core sleeves and were composited and homogenized in pre-cleaned 55-gallon fiberglass containers. For each station, the core section containing the surficial sediments was composited and homogenized first, after an initial examination for indigenous organisms. If any organisms were found in the sample, they were excluded by sieving through a coarse mesh sieve (1 mm). Following the surficial sediments, all of the successive core sections from a given station were composited in the 55-gallon container. Each sample was homogenized until the sediment was thoroughly mixed (including dense clay portions) and was of uniform consistency. Upon completion of compositing and homogenization for each station, subsamples of sediment were removed for bulk chemistry analyses, and the remainder was placed in pre-cleaned buckets for subsequent toxicity testing or was placed directly into pre-cleaned aquaria for the bioaccumulation studies to be initiated with addition of organisms the following day. This allowed the sediments to settle after addition of dilution waters in the aquaria.

### 3.2.2 Water Column Testing

For the water column testing portions of the sediment characterization program, elutriates were prepared in accordance with U.S. EPA (1991) and U.S. EPA/USACE (1994) as well as the Region IV RIM guidance. In preparing the elutriates, a subsample of each homogenized sediment was combined with site water in a 1:4 sediment to water ratio, on a volume/volume basis. The sediment/water combination was thoroughly mixed (stirring and vigorous aeration) for 30 minutes at 20°C, and was then allowed to settle for 1 hour. After settling, the supernatant was siphoned off and used immediately for the water column acute toxicity testing.

#### 3.2.2.1 Acute Toxicity Testing with *Mysidopsis bahia*

Static, non-renewal 96-hour *Mysidopsis bahia* bioassays were initiated 23 July 1998, immediately following the elutriate preparation, and were in accordance with the Green Book (U.S. EPA 1991) and the Region IV Regional Implementation Manual (USACE and U.S. EPA 1993) testing guidelines. The testing was conducted at 20°C ( $\pm 1^\circ\text{C}$ ) and 30 ppt salinity ( $\pm 2$  ppt) with a 16 hour light: 8 hour dark photoperiod. The lot of juvenile *Mysidopsis bahia* used for the testing were obtained from Aquatic BioSystems (ABS) Fort Collins Colorado on 22 July and were acclimated in the EA's laboratory to the proposed test conditions of 20°C and 30 ppt salinity. The *Mysidopsis bahia* lot Mb 389 were fed newly hatched brine shrimp (*Artemia salina*) nauplii daily.

The toxicity tests were performed in accordance with the EA protocol (EA 1996) for *Mysidopsis bahia*: ATS-SAI-OS-06 which was included in the Daniel Island Work Plan (EA 1998). Artificial seawater (30 ppt) was used for holding the *Mysidopsis bahia* prior to testing, and as the dilution water for the *Mysidopsis bahia* static acute toxicity tests. In preparation of artificial seawater, dry sea salts (Forty Fathoms Marine Enterprises, Towson, Maryland) were added to City of Baltimore tap water, which had been passed through a high capacity activated carbon filtration system. The prepared seawater was allowed to age a minimum of 24 hours before use. This synthetic sea water formulation has proven acceptable for aquatic toxicological studies, and Forty Fathoms water has been used successfully at EA for maintaining multigeneration cultures of *Mysidopsis bahia*, and for holding healthy populations of inland silverside (*Menidia beryllina*), larval grass shrimp (*Palaemonetes pugio*) and numerous other marine species.

The elutriate samples were evaluated at a test concentration series of 100, 50, 10 and 0 percent elutriate (0 percent elutriate was a laboratory dilution water control). A site water control treatment was also tested. Prior to preparation of test solutions, subsamples of elutriate and laboratory dilution water were brought to the desired test temperature using a water bath. The test concentrations were prepared on a volume/volume basis. The *Mysidopsis bahia* test was conducted in 1-L beakers containing 200 ml of test solution. For each test concentration and control, there were five replicate test chambers. After preparation of the dilution series, the test solutions were transferred into the test chamber, water quality analyses (dissolved oxygen, pH, temperature, ammonia and salinity) were performed and the juvenile *Mysidopsis bahia* were randomly introduced into the chamber until there were 10 organisms per replicate. The *Mysidopsis bahia* were 3 days old at test initiation. The tests were maintained in an environmentally controlled room at  $20^\circ \pm 1^\circ\text{C}$ . Water quality measurements of temperature, pH

and dissolved oxygen were recorded daily in at least one replicate of each test concentration and control. In addition, daily headcounts were performed, and the number of surviving organisms in each replicate was documented. Due to the size of the probe and the danger of injuring the test organisms, salinity was only measured prior to organism introduction and at the end of the test. Due to their cannibalistic nature, the *Mysidopsis bahia* were fed newly hatched brine shrimp (*Artemia salina*) nauplii at test initiation and at 48 hours. The *Mysidopsis bahia* tests were terminated after 96 hours, at which time water quality analyses were performed, and final headcounts were recorded. Copies of the original data sheets, which include all water quality measurements and test organism survivorship are included in Attachment 3-1.

### **3.2.2.2 Acute Toxicity Testing with *Menidia beryllina***

Similar to the *Mysidopsis bahia* water column testing, static non-renewal 96-hour *Menidia beryllina* (inland silverside) bioassays were also initiated on 23 July 1998 immediately following elutriate prep. The *Menidia beryllina* tests were identical to the *Mysidopsis bahia* with respect to temperature ( $20^{\circ} \pm 1^{\circ} \text{C}$ ), photoperiod (16L:8D), salinity (30 ppt), test concentration series (100, 50, 10, and 0 percent elutriates), number of replicates (5) per concentration, number of organisms per replicate (10) and water quality parameters measured. Initially, 3 lots of *Menidia beryllina* were received on 21-22 July 1998 from Aquatic BioSystems (ABS) Fort Collins, Colorado. The fish in each lot were less than 14 days old and within a 24-hour age range at the time of test initiation. Several of the *Menidia beryllina* elutriate tests had to be re-run due to unacceptable control survival. Two separate lots of *Menidia beryllina* were received from ABS on 04 and 11 August 1998 and the retests with freshly prepared elutriates were initiated on 5 and 13 August 1998. Water quality measurements and headcounts followed that described for the *Mysidopsis bahia* above. Copies of the *Menidia beryllina* raw data sheets are included in Attachment 3-1.

### **3.2.2.3 Acute Toxicity Testing with *Arbacia punctulata***

The third test that was utilized to evaluate the acute toxicity of the sediment elutriate samples was the purple sea urchin *Arbacia punctulata* 48-hour larval development test. Adult sea urchins were acquired from Duke Marine Laboratory, Beaufort, North Carolina, and arrived at EA on 23 July 1998. Upon receipt at EA, the adult sea urchins were inspected and were moved into a walk-in environmental chamber at  $12^{\circ} \text{C}$  to acclimate to the temperature at which the developing embryos would be eventually be tested. Adults which spawned in transit were immediately removed from the population and were discarded.

The collection of sea urchin eggs and sperm, and preparation of gamete dilution were performed according to EA's protocol (EA 1996) which follows guidelines in U.S. EPA (1994). Just prior to test initiation, the sea urchins were injected with 0.5 ml of 0.5 M KCl, to induce spawning. Gametes were collected during the first 15 minutes of spawning and were microscopically inspected to determine normality of eggs and sperm motility. Gametes which were determined to be acceptable for testing were pooled from a minimum of three males and three females, and were used to prepare the sperm and egg suspension for the fertilization procedures. Sperm and eggs were used within 4 hours of spawning. Care was taken to keep male and female urchins, gametes, and the equipment used in the collection and preparation of male and female gametes completely separate to avoid accidental fertilization during gamete preparation. Only combined

gamete preparations which had achieved a minimum of 90 percent fertilization were used in testing. Tests were initiated within 1 hour of egg fertilization.

The *Arbacia punctulata* 72-hour larval development tests were performed in accordance with EA's protocol (EA 1996), U.S. EPA (1994). EA's protocols can be found in the Daniel Island Work Plan (EA 1998). The concentration series used in these tests was 100, 50 and 10 percent elutriate and 30 ppt laboratory dilution water control. Prior to preparation of test solutions, subsamples of each elutriate and the dilution water were brought to the test temperature of  $15 \pm 1$  °C. Test concentrations were prepared on a volume/volume basis.

Test chambers were 30-ml scintillation vials with polypropylene caps. Each test concentration and control had four replicate test chambers containing a minimum of 10 ml of test solution, and a separate chamber for measuring water quality of test solutions during the tests. Extra replicates of controls were prepared to monitor embryo counts at test initiation. The test chambers for each test were randomly loaded into a 12 °C water bath. At test initiation, a minimum of 250  $\mu$ l of fertilized gamete solution was delivered into each test chamber. The tests were maintained at the target temperature of  $12 \pm 1$  °C with a 16-hour light/8-hour dark photoperiod. Water quality parameters (temperature, pH, dissolved oxygen and salinity) were measured in the water quality chambers at test initiation and a minimum of once daily on each test concentration and control. Copies of the original data sheets, including water quality measurements, are provided in Attachment 3-1. At 48 hours, testing was terminated by the addition of a minimum of 1.0 ml of 37 percent buffered formalin to each test chamber.

Observations of embryo development were performed within one week of sample preservation. A minimum of 100 embryos from each replicate test chamber were observed microscopically to determine the proportion of normally to abnormally developed embryos at the pluteus larvae stage.

### **3.2.3 Solid Phase Testing**

The solid phase sediment testing was conducted with opossum shrimp (*Mysidopsis bahia*) and the estuarine amphipod *Leptocheirus plumulosus*. The day prior to test initiation, the sediments and overlying water were added to the test chambers, and the suspended sediments were allowed to settle overnight prior to initiation of the acute toxicity tests the following day. Prior to test initiation, water quality analyses were performed (temperature, pH, dissolved oxygen, ammonia and salinity) to assure target test conditions were met.

#### **3.2.3.1 Solid Phase Testing with *Mysidopsis bahia***

The static non-renewal 96-hour *Mysidopsis bahia* solid phase sediment acute toxicity tests were initiated on 21 July 1998. Test chambers were 1-L beakers containing 200 ml of each site sediment, the reference sediment, and a Wye River (Maryland) control sediment with 800 ml of overlying water in each chamber. The overlying water was 30 ppt salinity Forty Fathoms artificial seawater. The artificial seawater was prepared at least 24 hours in advance of the test and was allowed to age. The test temperature was  $20 \pm 1$  °C and the photoperiod was 16 hours light: 8 hours dark. For each sediment, there were five replicate test chambers, with 10

*Mysidopsis bahia* per replicate. The *Mysidopsis bahia* lot Mb 388 was received from Cosper Environmental on 21 July. The *Mysidopsis bahia* juveniles used for the solid phase sediment testing were 3 days old (all within a 24 hour window) at test initiation. The water quality parameters measured daily were: temperature, pH, dissolved oxygen, and salinity; and measured values were recorded daily in at least one replicate of each sediment treatment and control. The test chambers were visually inspected daily for dead organisms were removed and/or abnormal behavior were recorded when observed.

The test was terminated at 96 hours, at which time final water quality measurements were taken and recorded, and the contents of each test chamber was sieved to recover live/dead *Mysidopsis bahia*. Copies of the raw data sheets with all water quality measurements, headcounts and test observations are included in Attachment 3-1.

### **3.2.3.2 Solid Phase Testing with *Leptocheirus plumulosus***

The static 10-day *Leptocheirus plumulosus* solid phase sediment testing was also initiated on 21 July 1998. The test organisms were obtained from stocks maintained in EA's Ecotoxicology Culture Facility. Organisms were retrieved from the culture sediment (Wye River Control) the day before the test was to be initiated. The culture sediment was passed through graded sieves to obtain uniform age (3-5 mm) *Leptocheirus plumulosus* for testing.

Test treatments included the 11 site sediments, the reference sediment, and a Wye River control sediment. Test chambers were 1-L beakers containing 200 ml of sediment plus 800 ml of overlying synthetic seawater. The overlying water was 20 ppt Forty Fathoms artificial seawater. There were five replicate chambers for each sediment, with 20 *Leptocheirus plumulosus* randomly loaded into each replicate for a total of 100 test organisms per sediment. The test was maintained at  $20 \pm 1$  °C with a 16 hour light: 8 hour dark photoperiod. Water quality measurements of temperature, pH, dissolved oxygen and salinity were performed daily on at least one replicate of each sediment, and values were recorded on the raw data sheets. Test chambers were visually inspected daily for abnormal behavior/lack of burrowing. Each test chamber was fed 5.0 ml of freshly prepared 6 mg/ml of a slurry of finely ground Tetramin flakefood three times per week at a maximum. The overlying water was brought back up to volume (800 ml) on Day 7 to offset evaporative the minimal (<50 ml) loss during the 10-day static acute test. On Day 10, final water quality measurements were recorded, and the individual test chamber contents were emptied into sieves to recover and enumerate the surviving organisms. The *Leptocheirus plumulosus* data sheets are included in Attachment 3-1.

### **3.2.4 Bioaccumulation Testing**

To evaluate the bioavailability of chemical constituents in the site sediments, 28-day bioaccumulation testing was performed with two species: the marine polychaete *Neanthes virens*, and the blunt-nose clam *Macoma nasuta*. Testing was performed on the 11 project area sediments, the reference sediment, and a native control sediment for each species collected at the site of test organism collection. The *Macoma nasuta* were collected from Santa Cruz Bay in California and the *Neanthes virens* were collected from Boothbay Harbor, Maine. Due to the abundance of indigenous organisms, the native control sediments were sieved through 1 mm

sieves prior to testing. Immediately following the receipt and compositing and homogenization of the test sediments on 15 July 1998, aliquots of the sediments were loaded directly into the bioaccumulation test chambers, and overlying water was introduced into each chamber. The test chambers were 10-gallon all glass aquaria, and 3.9 L of sediment and 26 L of overlying water were added to each aquaria. The overlying water was aged 30 ppt salinity Forty Fathoms artificial seawater described earlier in this report.

#### **3.2.4.1 *Neanthes virens* Bioaccumulation Testing**

The adult polychaete worms, *Neanthes virens* for the bioaccumulation study were obtained from Aquatic Research Organisms (ARO) and arrived at EA on 14 July 1998. Upon receipt, the *Neanthes virens* were unpacked, visually inspected, and gradually acclimated to the test conditions of 20°C and 30 ppt salinity. In addition, two 5-gallon buckets of sediment were collected at the *Neanthes virens* collection site and were shipped to EA to serve as a control sediment for the polychaetes.

The whole sediment bioaccumulation studies were performed in accordance with the Region IV RIM and EA's protocol (EA 1996) which was included in the Daniel Island Work Plan (EA 1998). Prior to performance of the bioaccumulation studies, the sediments and overlying water were slowly brought to a testing temperature of 20±1°C. Glass aquaria (37 L) were used as the test vessels, with five replicates for each test, reference and control sediment. A layer of approximately 3–5 cm of sediment (3.9 L of sample) was added to each of the test chambers, followed by approximately 26 L of 30 ppt artificial sea water, to bring the total volume in each test chamber to 30 L. Test organisms were introduced approximately 24 hours after the addition of sediment and overlying water to the test chambers, to allow for settling of suspended sediment. *Neanthes virens* were randomly introduced into each replicate chamber to achieve a final sample size of 20 organisms. The transfer of organisms marked the initiation of the whole sediment bioaccumulation test. A sediment sample was collected from one replicate of each sediment treatment at test initiation to document the initial ammonia concentration that would be transferred to the overlying water during the test.

During the 28-day testing period, the test chambers were maintained at 20±1°C with a 16-hour light/8-hour dark photoperiod with dusk and dawn timers. Gentle aeration (~100 bubbles/min) was provided to each aquarium throughout the test period. Each test chamber was renewed daily by siphoning approximately 80 percent of the laboratory synthetic seawater from the aquaria and replacing with synthetic sea water, taking care to avoid disturbing the sediment surface. Observations of mortality or abnormal behavior when observed on the surface of the sediment in the test were recorded daily, and dead organisms were removed from the test chambers. Measurements of temperature, pH, dissolved oxygen, and salinity of the overlying water were performed daily, before and after renewal, on one replicate of each sample. Copies of the original data sheets, including all water quality measurements, are included in Attachment 3-1.

#### **3.2.4.2 *Macoma nasuta* Bioaccumulation Testing**

The adult blunt-nose clams *Macoma nasuta* were received from K.W. Siewers, Santa Cruz, California on 15 July 1998. Upon receipt, the organisms were removed from the shipping

containers and were visually inspected for any indication of shipping stress. The *Macoma nasuta* were gradually acclimated to the test conditions of 15°C and 30 ppt salinity. Two 5-gallon buckets of *Macoma nasuta* collection site sediments were also received on 17 July 1998 to serve as control sediment for the blunt-nose clam bioaccumulation test. The purpose of the control sediment was to provide an indication of whether acceptable control survival is achieved at the end of the 28-day exposure period and was not intended for bioaccumulation analysis. The *Macoma nasuta* bioaccumulation test procedures followed those described above for *Neanthes virens* with the exception of the test temperature of 15°C, and the food provided to the *Macoma nasuta* was finely ground Tetramin flake food. Gentle aeration was also provided from test initiation in order to maintain dissolved oxygen levels at >60 percent saturation. Copies of the original data sheets, which include all headcount and water quality measurements are included in Attachment 3-1.

### 3.2.5 Statistics

Statistical analyses were performed on the data from the water column, solid phase and bioaccumulation testing, using appropriate procedures which were consistent with the guidance provided in the Green Book (EPA 1991) and the Region IV RIM (USACE and U.S. EPA 1993). Survival (or larval development) of the organisms exposed to the test material for the prescribed time period, was statistically ( $P = .05$ ) compared to either the laboratory control, or the reference sediment as appropriate for each test, using the student t-test. The t-test is based on the assumptions that the observations are independent and normally distributed, and that the variances of the observations are equal between the two groups. The F-Test was used to test for homogeneity of variance. The test for normality was the Shapiro-Wilk's Test. When the data did not meet the normality assumption, the nonparametric test, Wilcoxon's Rank-Sum Test was used to analyze the data. Transformations, such as the Arc Sine (Square Root [Y]) transformation for percentages, were performed when appropriate.

In the water column bioassays, the median lethal concentration (LC50) was calculated for each elutriate sample. Depending on the nature of the specific data sets, calculations were performed using the binomial, moving average or probit methods, based on goodness of fit, as described by Stephan (1977), or the trimmed Spearman-Kärber method (Hamilton 1977). The LC50 is an estimate of the elutriate concentration which is lethal to 50 percent of the test organisms, or which creates a sublethal effect on the development of 50 percent (EC50) of the test organisms, in the time period prescribed by the test. Calculation of the LC50 was performed if there was at least 50 percent adverse effect in the 100 percent elutriate concentration at test termination.

### 3.2.6 Reference Toxicant Testing

In conformance with EA's QA/QC program requirements, reference toxicant testing was performed on all lots of organisms utilized in this testing program. The reference toxicant tests consisted of a graded concentration series of a specific toxicant in water only tests, with no sediment present in the test chambers. The reference toxicant for *Menidia beryllina*, *Neanthes virens* and *Macoma nasuta* was sodium dodecyl sulfate (SDS). The reference toxicant for *Mysidopsis bahia* was potassium chloride (KCl), while the toxicant used for *Arbacia punctulata* was copper chloride ( $\text{CuCl}_2$ ). The *Leptocheirus plumulosus* utilized cadmium chloride ( $\text{CdCl}_2$ ) as a reference toxicant. Test procedures for the reference toxicant tests followed the test

procedures outlined in the EA Protocols for each test species previously presented in the EA Daniel Island Work Plan (EA 1998).

### 3.3 RESULTS

#### 3.3.1 Water Column Testing with *Mysidopsis bahia*

The results of the *Mysidopsis bahia* 96-hour static acute toxicity tests on elutriate samples prepared with the Daniel Island sediment are presented in Table 3-2. For the total of 12 elutriate samples, four separate acute toxicity tests comprised of three elutriate samples and a control treatment each were conducted. There was acceptable control survival (>90 percent) in each of the four *Mysidopsis bahia* tests, with 100 percent control survival in three of the tests and 96 percent control survival in the fourth test. There also was very high survival in the sitewater control (96 percent), indicating that the site water used to prepare the elutriate samples did not contribute toxicity to the elutriates.

For the 12 elutriate samples there was a minimum of 84 percent *Mysidopsis bahia* survival at full strength, 100 percent elutriate. As a result all 12 elutriates had 96-hour LC50 values of >100 percent, indicating a low degree of acute toxicity. The elutriate prepared from the reference sediment displayed a complete lack of toxicity as there was 100 percent survival in all concentrations tested. When the percent survival at 100 percent concentration for each elutriate was statistically compared to the survival in the corresponding control, two elutriate samples (CNB-02 and CNB-03) were determined to be significantly different ( $P=0.05$ ) from the control survival of 100 percent. Though statistically different from the control, the 89 and 90 percent survival in those two 100 percent elutriates respectively were at or very near acceptable control survival ( $\geq 90$  percent) indicating very low levels of toxicity.

The results of the water quality analyses of the test solutions are presented in Table 3-3, where the mean temperature, pH, dissolved oxygen (DO) and salinity are reported for each elutriate and control. All values of the measured parameters were within acceptable ranges, and do not indicate any adverse contributions to effects on the toxicity test results from the testing procedures.

#### 3.3.2 Water Column Testing with *Menidia beryllina*

The water column tests with *Menidia beryllina* were initiated on 23 July 1998 immediately following the sediment elutriate preparation. The number of organisms that were required for this testing program necessitated acquiring three lots of *Menidia beryllina*; all were less than 14 days old, and each lot with an age range of  $\pm 12$  hours. Table 3-4 presents the results of the *Menidia beryllina* 96-hour static acute toxicity testing with the Daniel Island sediment elutriates. Due to unacceptable control survival in certain tests, fresh elutriates were prepared and tests were rerun on 5 and 13 August 1998.

Exposure to 100 percent concentrations of elutriates from CNB-01 and CNB-02 for 96 hours resulted in 100 percent *Menidia beryllina* mortality. For CNB- the 96-hour LC50 was 30.0 percent elutriate. The CNB-02 LC50 was 58.9 percent. The elutriate prepared from the CNB-03

sediment produced an LC50 of 75.4 percent elutriate. None of the remaining elutriates elicited sufficient toxicity (mortality  $\geq$  50 percent) to calculate an LC50 and are presented as >100 percent elutriate. The least toxic elutriate samples based upon percent survival in 100 percent sample were (least toxic first): WDB-01 (96 percent), CPB-01 (49 percent), CPB-03 and Reference Site (92 percent). Similar to the *Mysidopsis bahia* testing, the sitewater was not toxic, and resulted in 94 percent survival; indicating that the elutriates were prepared with a water that was not contributing toxicity to the mixture.

The results of the water quality analyses on the test solutions are presented in Table 3-5. Included with the means calculated for temperature, pH, dissolved oxygen and salinity, are the results of the ammonia analyses performed on the elutriate samples. Comparing the results of the *Menidia beryllina* static acute toxicity tests (Table 3-3) to the water quality summary (Table 3-5) it appears that ammonia may have been a potential contributor to the toxicity pressures observed in the several of the elutriates. The elutriate CNB-01 had the highest total ammonia concentration (17.6 mg/L) and the elutriates CNB-02 and CNB-03 continued that trend closely (17.3 and 12.3 mg/L total ammonia).

### 3.3.3 Water Column Testing with *Arbacia punctulata*

The results of the *Arbacia punctulata* tests, which were initiated on 23 July 1998 are presented in Table 3-6. The test endpoint is quantification of percent normal embryo development to the pluteus stage at 48 hours. All of the site sediment elutriates were toxic to the *Arbacia punctulata* embryos, and the resulting 48-hour EC50s ranged from <10 percent elutriate (CNB-01, CNB-02, CNB-03) to 23.1 percent elutriate (WDB-01). The only non-toxic elutriate to *Arbacia punctulata* was the sample prepared with the reference sediment.

With respect to the elutriates prepared with the sediment samples, the *Arbacia punctulata* results showed a pattern similar to the *Menidia beryllina* findings. All elutriates samples, with the exception of the reference had total ammonia concentration which ranged from a low 3.9 to 17.6 mg/L. The reference elutriate had an ammonia concentration of only 0.7 mg/L total ammonia, and was the only nontoxic elutriate. The results of the water quality analyses (temperature, pH, DO and salinity) on the *Arbacia punctulata* test solutions are presented in Table 3-7.

### 3.3.4 Solid Phase Testing with *Mysidopsis bahia*

The results of the Daniel Island whole sediment toxicity testing with *Mysidopsis bahia* are presented in Table 3-8. The highest survival occurred in the control (culture sediment used for the amphipod testing) and in the reference station sediment, with 96-hour survival in each treatment at 94 percent. None of the sediments were highly or even moderately toxic to *Mysidopsis bahia* since the lowest percent survival at 96 hours was 74 percent (WDR-02). Sediments in which *Mysidopsis bahia* survival was statistically different from the reference site were: CNB-01, CNB-02, CPB-01 and WDR-02. In testing with other species, CNB-01 and CNB-02 have elicited the greatest toxicity, but CPB-01 and WDR-02 have not been as toxic to the other species. None of the other site samples elicited substantial mortality to *Mysidopsis bahia*.

The results of the water quality analyses of temperature, pH, DO and salinity performed on the overlying water in the *Mysidopsis bahia* solid phase tests are presented in Table 3-9. All of the values observed were in normal ranges for dredge material testing.

### 3.3.5 Solid Phase Testing with *Leptocheirus plumulosus*

The results of the *Leptocheirus plumulosus* 10-day static acute toxicity tests on Daniel Island solid phase sediment samples are presented in Table 3-10 and the results of the water quality analyses are reported in Table 3-11. At the end of the 10-day test period there was 100 percent survival in the laboratory control (Wye River sediment□culture sediment), which survival in the site sediments ranged from 72 (CPB-02) to 100 percent (CNB-02) and survival in the reference sediments was only 7 percent. In comparing the survival for each station individually to the control, because the control had no mortality, a large number of stations (8) fell out as statistically different from the control, even though survival was generally high ( $\geq 80$  percent). It is EA best professional opinion that sediments with 80 percent or greater survival did not exert a substantial lethal effect on *Leptocheirus plumulosus*. Besides the reference sediment, which had 7 percent survival, station CPB-03 had the greatest adverse effect, with a 10-day survival of 7 percent. One possible explanation to the *Leptocheirus plumulosus* test results revolves around the fact that as a test species, they prefer the very fine grained sediments with high percentage of clay and silt observed in the culture controls and the sediments from the individual stations; and do not do as well in sediments with a large proportion of sand such as the reference sample. For the purpose of this discussion, the results of the grain size analyses on the Daniel Island sediments are reported in Table 3-12 rather than in the analytical section of this report. From the table, it is possible to add the percent clay and percent silt for each individual sediment, to provide a measure of the “fines” in the sample, which is the physical composition *Leptocheirus plumulosus* prefers. If one compares the “percent fines” from Table 3-12 to the percent *Leptocheirus plumulosus* survival in Table 3-10 there is a good relationship between the two parameters. The highest percent fines (84.6, 76.9, and 73.9 percent) occurred in CNB-03, -02 and -01, and the respective survival rates: 98, 100, and 96 percent survival were the highest in the test, other than the Wye River control. The site sediments with the lowest survival: CPB-03 (72 percent) and WDR-01 (80 percent) had the lowest percent fines (27.6 and 20 percent respectively) of any of the site sediments. The reference sediment by far had the lowest percent fines (3 percent) and likewise the lowest survival (7 percent). Communication with the USACE Waterways Experimental Station (WES) indicate that in the past, they have had acceptable *Leptocheirus plumulosus* survival in sandy sediments as long as the organisms are fed during the test. The *Leptocheirus plumulosus* were fed three times a week during the Daniel Island testing. The USACE WES past experience suggests that another toxicant or unknown factor may have been responsible for the majority of the mortality observed in the reference site treatment which is not accounted for based solely on grain size effects.

Water quality parameters monitored during the tests are presented in Table 3-11. The ranges in water quality observed in these tests are what would be normally expected in the evaluation of dredged material samples.

### 3.3.6 Bioaccumulation Testing with *Neanthes virens*

The results of the *Neanthes virens* bioaccumulation testing (exposure phase only, not analytical results) are presented in Table 3-13. The table lists the number of organisms originally loaded into each replicate chamber, the number recovered out of each replicate after 28 days, the resulting percent survival for each replicate, and the mean survival rate for each sediment treatment (average of 5 replicates). Survival rates for the 28-day test were good to very good, with all sediment treatment mean survival rates being >80 percent. The lowest mean survivals were in stations WDR-03 (84 percent) and CNB-01 (85 percent). The highest mean survival rates were: control (100 percent), reference (97 percent), CNB-03 (96 percent) and WRD-01 (96 percent). The tissue analysis results are discussed in Chapter 5 of this report.

Table 3-14 reports the means for the water quality data gathered during the *Neanthes virens* bioaccumulation testing and represent ranges of values that would be expected in dredged material samples.

### 3.3.7 Bioaccumulation Testing with *Macoma nasuta*

Table 3-15 presents the results of the exposure phase of the *Macoma nasuta* bioaccumulation testing with the Daniel Island sediment samples. The table presents the survival data that was collected at the end of the 28-day sediment exposure period. The water quality parameters of temperature, pH, dissolved oxygen and salinity were monitored daily during the test, and the means for the measured water quality parameters are reported in Table 3-16. Very little *Macoma nasuta* mortality occurred during the bioaccumulation exposure. For the 11 site sediments, the reference sediment, and the control sediment, the lowest mean survival rate for the five replicate sediment treatments was 93 percent survival in station CNB-02. One replicate of that treatment had 88 percent survival. The survival rates in all other sediments ranged from 95-99 percent indicating that the sediment constituents were not present in lethal concentrations. The results of the tissue analyses are discussed in Chapter 5 of this report.

### 3.3.8 Reference Toxicant Results

In conformance with EA's QA/QC program, reference toxicant testing was conducted on all lots of acquired test species, and EA cultured species that were utilized in the Daniel Island testing program. Reference toxicant tests provide an internal quality control check on technical test performance, and also provide an indication of the condition of the lots of test organisms at the time they are used in testing. The results of each reference toxicant test are compared to historical ecotoxicological data to determine if the test results are within the prescribed acceptability limits. Table 3-17 presents the results of the reference toxicity testing that was performed for the Daniel Island program. The table includes test organism lot number, reference toxicant used, the 48-hour LC50 value, and the acceptable control chart limit for the LC50 value. The reference toxicant LC50 value for each of the acquired test organism lots and the EA cultured stock LC50 all fell within the acceptability range based upon historic EA data thus indicating that the organisms were of expected and acceptable quality.

### 3.4 DISCUSSION

Comparisons among species in Table 3-18 indicate that for the water column testing, the *Arbacia punctulata* was most sensitive to the individual sediment sites followed by *Menidia beryllina* and *Mysidopsis bahia*. It is important to note that with the exception of the CNB-01, CNB-02, and CNB-03 samples, a 4.3 to 5.7 fold dilution at the disposal site would produce an LC50 value of >100 percent elutriate. A similar statement can not be made for the above mentioned stations because the lowest elutriate samples produced an effect for the *Arbacia punctulata* tests.

An evaluation of benthic-effects for whole sediment bioassays is based on the Limiting Permissible Concentration (LPC). The LPC is defined as "...that concentration which will not cause unreasonable acute or chronic toxicity or sublethal adverse effects based on bioassay results using...appropriate sensitive marine organisms..." (U.S. EPA/USACE 1991). The proposed dredged material does not meet the LPC if the mortality of the test organism (1) is statistically greater than mortality in the reference sediment, and (2) exceeds reference sediment mortality by at least 10 percent. A of 20 percent reference exceedence is typically used for amphipod tests.

The whole sediment testing presented in Table 3-18 indicated that stations CNB-01, CNB-02, CPB-01, and WDR-02 were significantly different (and 10 percent different) from the reference site. However, the survival of the test sediments ranged from 74 to 88 percent for the sediment samples and 94 percent survival for the reference and laboratory sediment controls. Sediment from Station CPB-03 was only sample that was significantly different (and 20 percent different) from the laboratory sediment controls. The CPB-03 station yielded a 72 percent survival of the estuarine amphipod versus 100 percent survival in the laboratory control. This reduced survival in the CPB-03 sample may be reflective in the less fine grain size characteristics of this sample. It is also important to note that the survival in the reference site was only 7 percent. This poor reference station performance consistent among all of the treatment replicates is thought to be possibly due to the sandy characteristics of the sediment as compared to the laboratory control sediment sample and possibly from another unknown toxicant or factor.

The results of the bioaccumulation testing including the tissue concentrations observed following the 28-day exposure period are presented and further discussed in Chapter 5.