

5. BIOACCUMULATION TESTING PROGRAM

5.1 MATERIALS AND METHODS

5.1.1 Tissue Preparation and Homogenization

At the end of the 28-day exposure period, the test organisms were retrieved from the test chambers, and numbers of surviving organisms were recorded. These procedures and data are presented in the toxicology results in Chapter 3.

To prepare the animals for tissue analyses, surviving organisms were placed in 37-L holding tanks containing 20 ppt artificial sea water and no sediment for 24 hours to purge their digestive tracts. The organisms were not fed during this period. At the end of the 24-hour purging period, the shells of the clams were rinsed with de-ionized (DI) water, the clams were shucked, and the soft tissues and liquids inside the shell were placed into analytically cleaned glass jars. The worms were rinsed with DI water to remove external salts (originating from the purge chambers) and were placed directly into analytically cleaned glass jars. Tissues for each replicate were placed in separate jars, labeled, and stored in a walk-in freezer. The tissues were processed and frozen by the ecotoxicology lab on 15 August 1998 and were held until the appropriate bioaccumulation analyses were determined by URS Greiner and SCSPA and approved by U.S. EPA Region IV and USACE–Charleston. The tissue samples were delivered to EA's analytical laboratory on 09 September 1998. The Chain-of-Custody form is provided in Attachment 5-1.

In the analytical laboratory, the tissues were thawed, and tissue for each individual replicate was homogenized separately. After homogenization, equal aliquots from each of the five replicates for each station were combined to attain sufficient volume for analytical testing. Remaining homogenized tissue from each replicate was re-frozen and stored separately for later verification of individual replicates, if necessary. Tissue homogenization was conducted following EA's analytical laboratory standard procedure for tissue preparation and homogenization. The tissue homogenization SOP is provided as Attachment 5-2.

5.1.2 Analytical Methods and Detection Limits

Tissue samples were analyzed for the same suite of analytes as the sediments, with the exception of TOC, grain size, percent solids, fluoride, and phosphorus. The project-specific analytical methods for tissue analyses are provided in Table 4-1. Detection limits for tissue analyses are provided in Table 5-1. EA's analytical laboratory conducted all tests with the exception of tributyltin and dioxins/furans. DAT, located in Dublin, Ohio, conducted the tributyltin analyses, and Ionics International, Inc., located in Houston, Texas, conducted the dioxin and furan testing.

5.2 BIOACCUMULATION RESULTS

5.2.1 Theoretical Bioaccumulation Potential (TBP)

Prior to conducting the tissue analysis, Theoretical Bioaccumulation Potential (TBP) was calculated to provide a partial basis for selecting appropriate tissue analyses for quantification of

bioaccumulation. The TBP represents the approximate equilibrium tissue concentration that would be expected if the sediment or dredged material were the only source of contaminants to the test organisms. TBP is only determined for nonpolar organic compounds (pesticides, PAHs, PCBs, dioxins and furans) and is not calculated for metals, organic acids or salts, organotins, or methyl mercury. The TBP calculation requires the concentration of the contaminant found in the sediment, the percent TOC in each sediment sample, and the organisms' percent lipid content. TBP was calculated using the methods described in the *Inland Testing Manual (ITM)* (U.S. EPA and USACE 1994). The equation for determining the TBP is as follows:

$$\text{TBP} = \text{BSAF} (C_s / \% \text{TOC}) \% \text{L}$$

Where TBP is expressed in the same concentration units as the C_s and

C_s = Concentration found in the sediment (expressed in any unit); these data are provided in Tables 4-8 (pesticides), 4-10 (PCBs), 4-12 (PAHs), and 4-14 (dioxins);

BSAF = Biota Sediment Accumulation Factor = 4 (Ankley et al. 1992);

% TOC = Total organic carbon in the sediment (expressed as a decimal fraction); these data are provided in Table 4-6;

% L = Lipid content of the organism (expressed as a decimal fraction of whole body weight) (ITM 1994).

If a compound was not detected in the sediment, the detection limit was used as the compound concentration in the calculation. Lipid values for soft-bodied invertebrates vary depending upon the test organisms, but can range up to 1-2 percent of total body wet weight (U.S. EPA/USACE 1994). A 2 percent lipid value was used for the TBP calculations, assuming a worse case bioaccumulation potential.

TBP calculations revealed low bioaccumulation potential for pesticides and PCB congeners (Tables 5-2 and 5-3), with none of the 11 test sediments exceeding reference values for any pesticide or PCB analyte. Of the 18 PAHs analyzed in 11 test sediments, TBP exceeded reference values in 8 of 198 cases, with five of the exceedences for sediments from station CPB-03 (Table 5-4). Of the 7 dioxin congeners analyzed in the 11 test sediments, TBP exceeded reference values 11 of 77 cases (Table 5-5). Six of the 11 occurrences were congener OCDD, the least toxic of all dioxin congeners. No test samples exceeded reference values for any furan congener.

Since TBP values were low and infrequent, protocols for the analytical testing were modified through joint discussions with URS Greiner, U.S. EPA Region IV, and USACE-Charleston. Rather than conducting analytical testing on each of the five replicate tissue samples for each station, it was determined that one pooled sample (composited from the five replicates) for each station would be analyzed for the contaminants of concern.

5.2.2 Tissue Analyses

Results of the tissue chemistry analysis for *Neanthes virens* are presented in Section 5.2.2.1 and results for *Macoma nasuta* are presented Section 5.2.2.2. Analytical results for tissues are reported on a wet weight basis. Lipid content is reported as a percent of total body wet weight. Data qualifiers for the organic data are provided in Table 4-4. Inorganic data qualifiers are provided in Table 4-5. Copies of the final raw data sheets are provided in Attachment 5-1. Analytical narratives, which include a synopsis of laboratory QA/QC results for Laboratory Control Samples and Matrix Spike/Matrix Spike Duplicate Recoveries, are provided in Attachment 5-1. Certificates of Analysis for glassware are provided in Attachment 5-3. EA's analytical laboratory will retain and archive the results of these analyses for seven years from the date of issuance of the final results.

Data Analysis

Because tissues from individual replicates were pooled and tested as one analytical sample, statistical analyses could not be conducted to identify differences in tissue concentrations among stations for each species or to statistically compare the tissue concentrations at each station against tissue concentrations at the reference site. For non-statistical comparisons, percent difference was calculated for cases where tissue concentrations at individual stations exceeded reference site tissue concentrations. If the contaminant was not detected in the reference tissue, but was detected in tissue exposed to the project sediments, the detection limit was used as the reference concentration for the percent difference calculations. In addition to calculating percent difference, tissue concentrations that exceeded the reference concentration were non-statistically compared the U.S. Food and Drug Administration (FDA) action limits (Table 5-6).

5.2.2.1 Tissue Concentrations of *Neanthes virens*

Percent Lipids

Lipid results are provided in Table 5-7. Percent lipids for *Neanthes virens* ranged from <0.084 percent to 0.14 percent of total tissue weight.

Metals

Results of the trace metals analyses are provided in Table 5-8. Of the 12 metals analyzed in 11 test tissues, metals in tissue exceeded the reference value in 12 of 132 cases. Percent difference values for tissues exceeding the reference concentration are provided in Table 5-9. Four of the twelve trace metals were detected in tissue samples at concentrations higher than the concentrations in tissues from the reference site. Tissue concentrations of lead exceeded the reference value at seven of the 11 stations, with the maximum concentration of 0.32 mg/kg at CNB-01 exceeding the reference value by 146 percent. Mercury exceeded the reference value at three stations: CNB-02, WDR-01, and WDR-02. The exceedence values for mercury, however, were lower than or equivalent to the project required detection limit. In addition, the detected mercury levels were lower than the FDA Action Level (Table 5-6). Selenium was detected at a concentration approximately 40 percent higher than the reference value at station WDR-01, and

zinc exceeded the reference value by 184.6 percent at station CPB-03. The only tissues that exceeded the reference concentration by >100 percent were lead at CNB-01 and zinc at CPB-03.

Pesticides

Results of the pesticide analyses are provided in Table 5-10. None of the target pesticides were detected in *Neanthes virens* tissue samples.

PCBs

Results of the PCB analysis are provided in Table 5-11. Six of the twenty-one congeners tested were detected in the tissue samples. The Charleston Naval Base and Cooper River Berthing Area reaches exhibited the highest incidence of concentrations exceeding the reference value. Percent difference values for tissues exceeding the reference concentration are provided in Table 5-12. Congener IUPAC-153 was the most commonly detected congener in the tissue samples, with concentrations exceeding the reference value at eight of 11 stations. Concentrations of the congener IUPAC-8 were substantially higher than the reference value at CNB-01 (124 percent), CPB-02 (158 percent), and CPB-03 (107 percent). All other congeners exceeded the reference by <100 percent. Based upon laboratory narratives, all detected values for congener IUPAC-209 (Table 5-11) originated from a spiked surrogate solution that was added to the samples. Therefore, all detected concentrations of IUPAC-209 originated from the surrogate solution and was not initially present in the samples. Total PCB concentrations did not exceed the FDA Tolerance Limit for PCBs (Table 5-6).

Polynuclear Aromatic Hydrocarbons (PAHs)

Results of the PAHs analyses are provided in Table 5-13. Of the 18 PAHs analyzed in 11 test samples, PAHs numerically exceeded the reference value in 6 of 198 possible cases. Percent difference values for tissues exceeding the reference concentration are provided in Table 5-14. Only three of the eighteen target PAHs were detected in *Neanthes virens* tissue samples: acenaphylene, fluoranthene, and pyrene. Acenaphylene was detected above reference concentrations in tissue exposed to two of the Wando Reach Realignment stations (WDR-02 and WDR-03). Pyrene was detected above the reference concentration in tissue exposed to the three Charleston Naval Base samples. Fluoranthene and pyrene at CNB-01 were detected at concentrations higher than concentrations in the reference tissue (190.6 percent and 463.6 percent higher for fluoranthene and pyrene, respectively). Corresponding concentrations for fluoranthene and pyrene were 9.3 ug/kg and 6.2 ug/kg, respectively.

Dioxins and Furans

Results of the dioxin and furan analyses and associated TEF and TEQ values are provided in Table 5-15. In the 11 test tissues, concentrations exceeded the reference value in 8 of 176 cases. Percent difference values for tissues exceeding the reference concentration are provided in Table 5-16. Two dioxin congeners (1,2,3,4,6,7,8-HpCDD and OCDD) and two furan congeners (1,2,3,4,6,7,8,-HpCDF and OCDF) were detected in the tissue samples. OCDD concentrations exceeded the reference value at four stations (CNB-01, CNB-02, CPB-03, and WDB-01). Concentrations of OCDD detected in the Charleston Naval Base stations ranged from 90 percent

to 96.7 percent above the reference value. Maximum OCDD and OCDF concentrations (62.5 ng/kg and 5.5 ng/kg, respectively) were reported for tissue exposed to sediment from CPB-03; these concentrations exceeded the reference value by 1983 percent and 1000 percent, respectively. Overall, the dioxin and furan congeners detected in the *Neanthes* tissue have the low TEF potency. The combined potency of the dioxin and furan congeners detected at station CPB-03 was only equivalent to 0.1 ng/kg of total 2,3,7,8-TCDD. The dioxin concentrations did not exceed the FDA Action Level (Table 5-6).

5.2.2.2 Tissue Concentrations of *Macoma nasuta*

Percent Lipids

Lipid results are provided in Table 5-17. Percent lipids for *Macoma nasuta* ranged from <0.083 percent to 0.12 percent of total tissue weight. These values are similar to values reported for *Neanthes virens* in this study (Section 5.2.2.1.1).

Metals

Results of the trace metals analyses are provided in Table 5-18. Of the twelve metals analyzed in 11 test tissues, nine trace metals exceeded the reference value in 65 of 132 cases. Percent difference values for *Macoma nasuta* tissues exceeding the reference concentration are provided in Table 5-19. Mercury and tributyltin were the only metals not detected in reference or test tissue samples. Tissue concentrations of copper, lead, and zinc exceeded the reference value in all 11 samples. Concentrations of copper ranged from 9.8 mg/kg to 18.7 mg/kg, with the maximum concentration at station WDB-02 exceeding the reference value by 101 percent. Lead concentrations in tissue ranged from 0.53 mg/kg to 0.84 mg/kg, with the maximum concentration at station WDB-02 exceeding the reference value by 90.9 percent. Zinc concentrations in test tissue ranged from 29.5 mg/kg to 37.9 mg/kg, with the maximum concentration exceeding the reference value by 32.1 percent. The analyte with highest single exceedence value (114 percent) was chromium with a tissue concentration of 4.5 mg/kg at station CPB-02. The only tissues that exceeded the reference by >100 percent were chromium at WPB-02 and copper at WDB-02.

Pesticides

Results of the pesticide analyses are provided in Table 5-20. Of the 11 analytes tested in 11 tissue samples, pesticide concentrations exceeded the reference value in 5 of 121 cases. Percent difference values for tissues exceeding the reference concentration are provided in Table 5-21. Only one target pesticide, DDE, was detected in *Macoma nasuta* tissue. DDE was detected in tissue from five of the 11 stations, including all three Cooper River Berthing Area stations. Concentrations of DDE ranged from 187.9 percent to 354.4 percent above the reference site concentration. The maximum DDE concentration (3 ug/kg) did not exceed the FDA Action Level (Table 5-6).

PCBs

Results of the PCB analysis are provided in Table 5-22. Of the 21 congeners tested in the 11 tissue samples, tissue concentrations exceeded the reference value in 2 of 231 cases. Percent

difference values for tissues exceeding the reference concentration are provided in Table 5-23. Congeners IUPAC-8 and IUPAC-153 were the only detected congeners and were only detected in the *Macoma* tissue exposed to sediment from CPB-03. IUPAC-8 and IUPAC-153 exceeded the reference value by 2.8 percent and 35.9 percent, respectively. Based on laboratory narratives, all detected values for congener IUPAC-209 (Table 5-22) originated from a spiked surrogate solution that was added to the sample. Therefore, IUPAC-209 was not initially present in the samples. Total PCB concentrations did not exceed the FDA Tolerance Limit for PCBs (Table 5-6).

Polynuclear Aromatic Hydrocarbons (PAHs)

Results of the PAHs analyses are provided in Table 5-24. Of the 18 PAHs analyzed in 11 test samples, PAHs numerically exceeded the reference value in 44 of 198 cases. Percent difference values for tissues exceeding the reference concentration are provided in Table 5-25. A total of sixteen of the eighteen target PAHs were detected in *Macoma nasuta* tissue samples. Tissues exposed to the Charleston Naval Base sediment exhibited the highest incidence of detected PAHs, with 14 of the 18 target PAHs detected above the reference value. Thirty-two of the 44 exceedences occurred in tissues exposed to the Charleston Naval Base sediments. Overall, in 30 cases, tissue concentrations exceeded the reference by >100 percent, and 7 of the 30 cases exceeded the reference by >1,000 percent. Acenaphylene exceeded the reference concentration at nine of the 11 stations, with the maximum value of 930 ug/kg at WDB-02, exceeding the reference by 1,560 percent. Pyrene, chrysene, and fluoranthene exceeded the reference values by the highest percentages and were only detected in tissues exposed to the Charleston Naval Base sediments. Maximum concentrations of pyrene, chrysene, and fluoranthene exceeded the reference value by 5809 percent, 2900 percent, and 2681 percent, respectively at CNB-01. Corresponding concentrations of pyrene, chrysene, and fluoranthene were 65 ug/kg, 60 ug/kg, and 89 ug/kg, respectively.

Dioxins and Furans

Results of the dioxin and furan analyses and associated TEF and TEQ values are provided in Table 5-26. In the 11 test tissues, concentrations exceeded the reference value in 6 of 176 cases. Percent difference values for tissues exceeding the reference concentration are provided in Table 5-27. Two dioxin congeners (1,2,3,4,6,7,8-HpCDD and OCDD) and one furan congener (OCDF) were detected in the *Macoma nasuta* tissue samples. All three congeners exceeded reference values by >100 percent in tissues exposed to sediment from WDR-03. OCDD concentrations exceeded the reference value at four additional locations, with concentrations ranging from 200 percent (WDB-01) to 1008 percent (CNB-01) above the reference value. Overall, the dioxin and furans congeners detected in the *Macoma* tissue have low TEF potency. The combined potency of the dioxin and furan congeners at WDR-03 was only equivalent to 0.04 ng/kg of total 2,3,7,8-TCDD. The dioxin concentrations did not exceed the FDA Action Level (Table 5-6).

5.3 DISCUSSION

A summary of the analytes exceeding the reference tissue values for *Neanthes virens* and *Macoma nasuta* is provided in Tables 5-28 and 5-29, respectively. Fourteen percent and 5.9 percent of the sample/contaminant possibilities exceeded the reference values for *Macoma nasuta* and *Neanthes virens*, respectively. Generally, total exceedences were at least twice as prevalent in *Macoma nasuta* tissue compared to *Neanthes virens*, tissue in all four project sampling reaches. Lipid values were similar for both species, eliminating lipids as a potential source of the difference in total number of exceedences.

Macoma nasuta tissue exposed to Charleston Naval Base sediments exhibited the greatest number of total exceedences (50 of 237 cases). Charleston Naval Base tissues exceeded the Cooper River Berthing area, the Wando River Berthing area, and Wando River Realignment area tissues by 60 percent, 95 percent, and 100 percent, respectively (see Table 5-29). The majority of *Macoma nasuta* tissue exceedences were for metals and PAHs, collectively comprising more than 80 percent of the total exceedences.

Total exceedences for *Neanthes virens* tissue were similar for the Charleston Naval Base and the Cooper River Berthing area (8.0 percent and 7.2 percent, respectively) and for the Wando River Berthing and area and Wando River Realignment area (3.2 percent and 4.2 percent, respectively). *Neanthes virens* tissue exceedences were primarily for PCBs (49 percent), metals (23.5 percent) and dioxins/furans (15.5 percent).

The differences in bioaccumulation results for *Neanthes virens* and *Macoma nasuta* could partially be an artifact of differences in morphology and feeding modes. *Macoma* are sessile filter-feeders, and their feeding radius is limited to the length of their siphons. They tend to be selective for smaller particle sizes when foraging. *Neanthes virens* are soft-bodied invertebrates, and they are omnivorous and less selective feeders (Fauchald and Jumar 1979). It is possible that *Macoma nasuta* are being more selective for particle sizes that would be associated with organic contaminants such as PAHs.

Overall, more exceedences occurred in tissues exposed to the Charleston Naval Base sediments than in tissues exposed to sediments from the other three project reaches. TOC concentrations were highest in the Charleston Naval Base sediments, potentially making organic contaminants more available to organisms than stations with lower TOC values and higher concentrations of sand particles. Although several dioxin and furan congeners were detected above the reference value for both *Neanthes virens* and *Macoma nasuta*, the detected congeners have low TEF potency and are the least environmentally active and of the least environmental concern. None of the detected analytes exceeded the FDA Action Levels for fish or shellfish tissue, indicating no potential risk to human health.