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**TOXICOLOGICAL EVALUATION OF
SEDIMENT SAMPLES FROM BURNS
HARBOR, PORTER COUNTY, INDIANA**

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SUMMARY

The United States Army Corps of Engineers (USACE) Chicago District is authorized to maintain the water depths in Burns Harbor at navigable levels. In order to maintain these levels, sediments must be dredged and disposed of at approved disposal sites. To make a 404 (b)1 open-water disposal evaluation, the dredged sediment may be evaluated through a series of toxicological tests to assess its potential for causing an adverse environmental effect. Battelle/Marine Sciences Laboratory (MSL) was contracted by USACE to perform these freshwater toxicity tests. The tests were designed to simulate conditions that organisms living within an aquatic dredged material disposal site might experience during disposal operations, and included both bedded-sediment (solid-phase) and suspended-sediment (elutriate) tests. Test samples were collected by USACE personnel and composited into three test treatments representing potential dredging areas (Management Units #1, #2, and #3). USACE personnel also collected sediment from four dredged material disposal site reference areas for use in the solid-phase tests. MSL personnel were responsible for collecting an appropriate control sediment for the solid-phase tests.

Four toxicological tests were conducted in support of this program. The solid-phase tests included the amphipod, *Hyalella azteca*, and the midge, *Chironomus tentans*. The elutriate tests included the fathead minnow, *Pimephales promelas*, and the daphnid, *Daphnia magna*. Testing was conducted following standard procedures provided by USACE which are consistent with ASTM protocols and the *Evaluation of Dredged Material Proposed for Discharge in Inland and Near Coastal Waters - Testing Manual (Draft) Inland Testing Manual* (EPA/USACE 1993), known as the "Draft Inland Testing Manual." The suitability of sediment representing the management units for open-water disposal was evaluated following the guidelines contained in the Draft Inland Testing Manual.

Sediment representing Management Unit #1 produced statistically significant acute toxicity and reduced growth in the solid-phase *C. tentans* test and the *P. promelas* elutriate test. An LC₅₀ of 76.16% elutriate was calculable for *P. promelas* test. High ammonia levels associated with the 100% elutriate concentrations in Management Unit #1 appeared to contribute to test organism mortality. Sediment representing Management Unit #2 produced acute toxicity in the 25% and 100% concentrations of the elutriate relative to the other concentrations in the *P. promelas* test, and reduced growth in the *C. tentans* solid-phase test. An LC₅₀ was not calculable for the *P. promelas* test, as greater than 80% survival was observed in all elutriate concentrations.

The results of these toxicological tests suggest that sediment from Management Units #2 and #3 are acceptable for open-water disposal. Sediments from Management Unit #1 produced statistically significant biological effects and may not be suitable for open-water disposal. Solid-

phase test results suggest that material from Management Unit #1 may be unacceptable for open-water disposal if test results are compared to the references BH-Ref-93-01 and BH-Ref-93-03. Elutriate tests suggest that this material may be unacceptable for open-water disposal if the concentration of dissolved plus suspended contaminants, after allowance for mixing, exceeds 0.01 of the toxic (LC_{50}) concentration beyond the boundaries of the mixing zone. Using these guidelines, numerical modeling may be necessary to determine compliance for this sediment. If high ammonia concentrations in the elutriate were the primary reason for test organism mortality, sediment from Management Unit #1 may not be excluded based on elutriate test results, as ammonia is considered a non-persistent chemical.

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1.0 INTRODUCTION

1.1 BACKGROUND

The United States Army Corps of Engineers (USACE) Chicago District is responsible for maintaining the water depths in Burns Harbor at authorized navigable levels. In order to maintain these levels, sediments must be dredged and disposed at approved disposal sites. As described in Section 404 of the federal Clean Water Act, the sediments may be evaluated through a series of toxicological tests to evaluate the potential for the dredged material to cause an adverse environmental effect. Battelle/Marine Sciences Laboratory (MSL) was contracted by USACE to perform the freshwater toxicity tests as described in the *Great Lakes Materials Testing and Evaluation Manual*. These tests are designed to simulate conditions that organisms living within an aquatic dredged material disposal site might experience during disposal operations and included both solid phase and elutriate tests.

The solid-phase tests are designed to examine the effects of the dredged sediment on sensitive benthic invertebrates. The amphipod, *Hyalella azteca*, and the midge, *Chironomus tentans*, were used in this study. The elutriate tests are designed to examine the effects of dissolved and suspended particulate phase of the sediment. The fathead minnow, *Pimephales promelas*, and the cladoceran, *Daphnia magna*, were used for the elutriate tests.

1.2 EXPERIMENTAL DESIGN

The USACE Chicago District was responsible for the collection of sediment from 12 test stations, and 4 reference stations. Field sampling of the 12 test stations and 1 reference site (BH-Ref-93-01) occurred on June 15 and 16, 1993. Three composite samples (management units) representing the 12 test stations were prepared in the field and shipped with the reference sample to the MSL. The compositing strategy and approximate location of the test stations is depicted in Figure 1. Management Unit #1 was created from four stations taken from the East Harbor Arm (project depth -27 ft). Management Unit #2 was created from five stations in the Outer Harbor (project depth -28 ft). Management Unit #3 was created from three stations taken in the West Harbor Arm (project depth -27 ft). After examining the material from reference BH-Ref-93-01, it was decided by USACE and MSL personnel that the coarse grain size present at this nearshore site was not comparable to the grain size exhibited in the management units.

As a result, three deep lake reference stations were sampled on July 16, 1993: BH-Ref-93-02, BH-Ref-93-03, and BH-Ref-93-04 (Figure 2). Toxicological testing occurred after all samples were received at the MSL. In addition to the management units and the reference

FIGURE 1. Study Area Map for the Compositing Strategy and Test Stations, Burns Harbor Program

FIGURE 2. Study Area Map for the Reference Site Locations, Burns Harbor Program

treatments, a control sediment was collected from Lake Leland in Washington State on July 19, 1993. This sediment served as a clean control for the solid-phase tests and was used to assess test organism health and the appropriateness of the test system.

The experimental design for this study is summarized in Tables 1 and 2. The solid-phase tests evaluated eight sediment treatments: three management unit treatments, four reference treatments, and one control treatment. Five replicate containers were used for each treatment with ten organisms per replicate. Reference toxicant evaluations for both species included six concentrations with three replicates per concentration. Ten organisms were exposed per replicate. The elutriate tests were performed on the three management unit treatments. The experimental design for the elutriate tests included six concentrations of elutriate per treatment. For the *P. promelas* test, four replicates were tested per elutriate concentration, with ten organisms per replicate. The reference toxicant evaluation for *P. promelas* included six concentrations with three replicates per concentration. Ten organisms were exposed per replicate. For the *D. magna* test, ten replicates were tested per elutriate concentration with one neonate per replicate. The reference toxicant evaluation for *D. magna* included six concentrations with three replicates per concentration. Five organisms were exposed per replicate. All tests were performed following standard protocols developed jointly by the Lake Superior Research Institute, University of Wisconsin-Superior, Superior, Wisconsin; the Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency; Science Applications International, Hackensack, New Jersey; and ASci Corporation, Duluth, Minnesota. Specific test methods are discussed in Chapter 2.

TABLE 1. Experimental Design for *H. azteca* and *C. tentans* Solid-Phase Tests

Parameter	Specification
Test Material:	Test and Reference sediment samples were collected from Lake Michigan; control sediment was collected from Lake Leland in Washington state.
Test Organisms:	<i>H. azteca</i> and <i>C. tentans</i>
Test Type:	Solid-phase static renewal
Test Endpoint:	Acute toxicity and biomass
Test Duration:	10 days
Reference Toxicant:	Potassium Chloride (KCl)
Replicates Per Treatment:	5
Organisms Per Replicate:	10
Test Conditions:	Temperature: 22 to 24°C (<i>H. azteca</i>) Temperature: 21 to 24°C (<i>C. tentans</i>) D.O.: ≥3.4 mg/L (>40% saturation @23°C), both species pH: 6.5 to 9.0 pH units (both species) Hardness: 25 to 300 mg/L CaCO ₃ (<i>H. azteca</i>) Hardness: 30 to 195 mg/L CaCO ₃ (<i>C. tentans</i>) Alkalinity: 25 to 300 mg/L CaCO ₃ (<i>H. azteca</i>) Alkalinity: 30 to 195 mg/L CaCO ₃ (<i>C. tentans</i>) Specific Conductivity: 50 to 500 µmhos (both species)
Testing Protocols:	Brooke, L.T., D.J. Call, G.T. Ankley, D.A. Benoit, C.W. West, and R. A. Hoke. "A Short-Term Method for Estimating the Toxicity of Solid Phase Sediment to the Amphipod <i>Hyalella azteca</i> ." Call, D. J., L.T. Brooke, G.T. Ankley, D.A. Benoit, C.W. West, and R.A. Hoke. "A Short-Term Method for Estimating the Toxicity of Solid Phase Sediment to <i>Chironomus Tentans</i> [sic]." Sediment Collection Dates: June 15 and 16, 1993: Management Units 1-3, BH-Ref-93-01 July 16, 1993: BH-Ref-93-02, BH-Ref-93-03, BH-Ref-93-04 July 19, 1993: Lake Leland Control
Test Dates:	<i>H. azteca</i> : July 30 to August 9, 1993 <i>C. tentans</i> : July 29 to August 8, 1993

TABLE 2. Experimental Design for *P. promelas* and *D. magna* Elutriate Tests

Parameters	Specification
Test Material:	Test sediment samples collected from Lake Michigan
Test Organisms:	<i>P. promelas</i> and <i>D. magna</i>
Test Type:	Elutriate test with static renewal
Test Endpoint:	Acute toxicity (both species); biomass (<i>P. promelas</i>)
Test Duration:	7 days (<i>P. promelas</i>); 21 days (<i>D. magna</i>)
Reference Toxicant:	Sodium chloride (NaCl)
Replicates Per Treatment:	4 (<i>P. promelas</i>); 10 (<i>D. magna</i>)
Organisms Per Replicate:	10 (<i>P. promelas</i>); 1 (<i>D. magna</i>)
Test Conditions:	Temperature: 24 to 26°C (<i>P. promelas</i>) Temperature: 19 to 21°C (<i>D. magna</i>) D.O.: ≥3.4 mg/L (>40% saturation @25°C) (<i>P. promelas</i>) D.O.: ≥3.3 mg/L (>40% saturation @20°C) (<i>D. magna</i>) pH: 6.5 to 9.0 (<i>P. promelas</i>) pH: 6.8 to 8.5 (<i>D. magna</i>) Hardness: 25 to 300 mg/L CaCO ₃ (<i>P. promelas</i>) Hardness: 160 to 180 CaCO ₃ (<i>D. magna</i>) Alkalinity: 25 to 300 mg/L CaCO ₃ (<i>P. promelas</i>) Alkalinity: 10 to 120 CaCO ₃ (<i>D. magna</i>) Specific Conductance: 50 to 500 µmhos (both species)
Testing Protocols:	Brooke, L.T., D.J. Call, G.T. Ankley, T.N. King, D.A. Benoit, J.S. Denny, and R.A. Hoke. "A Short-Term Method for Estimating the Chronic Toxicity of Dredged Material Elutriates to the Fish <i>Pimephales promelas</i> ." Call, D. J., L.T. Brooke, G.T. Ankley, D.A. Benoit, and R.A. Hoke. "A Method for Estimating the Chronic Toxicity of Sediment Elutriates to <i>Daphnia magna</i> ."
Sediment Collection Dates:	June 15 and 16, 1993: Management Units 1-3
Test Dates:	<i>P. promelas</i> : July 21 to July 28, 1993 <i>D. magna</i> : July 29 to August 19, 1993

2.0 METHODS

2.1 LABWARE PREPARATION

All glassware, stainless-steel or titanium utensils, Nalgene, Teflon, and other laboratory containers and equipment underwent cleaning procedures to avoid contamination of samples. Glassware, including test containers, aquaria, and sediment transfer dishes, was washed with warm, soapy water, rinsed five times with deionized water, then soaked in a 10% reagent grade nitric acid bath for a minimum of 4 h. After soaking, glassware was rinsed with deionized water five times and allowed to dry. Polyvinyl chloride (PVC), Nalgene, and Teflon tools were also washed and soaked in acid baths in the same manner as for glassware. Stainless-steel bowls, spoons, spatulas, and other utensils were washed with warm, soapy water, rinsed five times with deionized water, and allowed to air dry. They were then rinsed with methylene chloride under a fume hood, and the residual methylene chloride was allowed to evaporate under the hood. Neoprene stoppers and polyethylene sheets or other porous materials were washed with warm, soapy water and rinsed five times with deionized water. These items were then "seasoned" by continuous soaking in 0.45- μ m-filtered seawater for at least 2 days prior to use.

Equipment used to determine water quality, such as pH, dissolved oxygen (D.O.), temperature, and salinity, was calibrated according to the manufacturers' specifications and internal MSL procedures.

2.2 SEDIMENT COLLECTION

Sediment samples were collected from 12 test stations and 1 reference station in Lake Michigan by the USACE Chicago District on June 15 and 16, 1993. Sediment from three additional reference stations, located in deeper waters of Lake Michigan and more representative of the test sediments, was sampled on July 16, 1993. USACE personnel composited the test sediments into three management units in the field. Samples were placed in precleaned plastic containers and packed in coolers containing Blue Ice to maintain a temperature of $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$. All sediment samples were shipped to the MSL via overnight delivery and arrived at the appropriate temperature. On July 19, 1993, a control sediment was collected from Lake Leland, Washington, by MSL personnel using a ponar grab. The sediment was placed in a precleaned, epoxy-coated bucket and stored in a cooler containing Blue Ice to maintain a temperature of $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The control sediment sample was delivered to the MSL within 4 h of collection. All sediment samples were stored in the MSL's walk-in cold room and maintained at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ until used in the toxicological tests.

2.3 SEDIMENT SAMPLE PREPARATION

Sediment sample preparation consisted of all steps in the laboratory between delivery of the samples to MSL and the preparation of sediments for biological testing. Sediment used for biological testing was prepared within the 6-week holding period, as specified by the USACE. During this holding time, the samples were received at the MSL, inventoried against chain-of-custody forms, processed for testing, and used in the biological tests.

2.3.1 Solid-Phase Preparation

"Solid phase" refers to the whole sediment, as opposed to suspended or dissolved elutriate phases. In biological tests, the solid phase of sediments represents 1) dredged material once it has settled at an aquatic disposal site, 2) the existing environment of a disposal site without dredged material (reference sediment), or 3) the environment of a benthic test organism (control sediment).

Prior to use in testing, reference and control sediments were press sieved through a 1.0-mm screen to remove predators. After sieving, each sediment was mixed in a large stainless steel bowl using stainless steel utensils. Test sediments were mixed in an identical fashion. Once a sediment sample or composite was thoroughly homogenized, it was referred to as a "sediment treatment," indicating that all necessary processing was completed and the sediment was ready for use in biological testing. All sediment treatments (test, reference, and control) were then placed in precleaned epoxy-coated containers that were sealed, labeled, and maintained at approximately 4°C until needed for biological testing.

2.3.2 Elutriate Preparation

The elutriate of sediment composites was used to evaluate water column effects of open-water disposal of dredged material. The elutriate is the liquid supernatant that remains after intermixing sediment with water and allowing heavier particles to settle to the bottom. Because this preparation does not involve filtration, this phase contains suspended particles as well as "dissolved" constituents. The elutriate tests simulate effects that might be expected by both the physical presence of the suspended particles and the chemical toxicity of contaminants associated with the particles or dissolved fractions. The process is intended to approximate exposure conditions created as a result of materials being discharged through the water column during dredge-disposal operations.

The first step of preparation was creating a 4:1 (volume:volume) water to sediment slurry in 1-L glass jars with Teflon-lined lids. The jars were marked at 200 mL and 400 mL, filled with fresh water to the 200-mL mark, and homogenized sediment was added until the water was displaced to the 400-mL mark. The jar was then filled to 1 L with fresh water. A set of 12 jars of sediment and water was placed on a shaker table and agitated for 30 min at a shaking rate of 120 to 150 cycles/min. After shaking, the slurry was poured into 500-mL Teflon containers with tightly

fitting lids. These containers were placed in a centrifuge and spun for 15 min at approximately 740 x g. The 15-min centrifugation was necessary to ensure that test organisms would be visible at the first observation after exposure to elutriate test treatments. After centrifugation, the elutriate was stored in precleaned glass carboys at 4°C ±2°C until it was used in toxicological tests.

2.4 TEST ORGANISM PROCUREMENT AND CARE

Four species of test organisms were used for this study: the amphipod, *H. azteca*, the midge, *C. tentans*, the fathead minnow, *P. promelas*, and the cladoceran, *D. magna*. None of the organisms for this program were cultured at Battelle MSL and were obtained from Environmental Consulting and Testing in Superior Wisconsin, a commercial supplier.

Test organisms were shipped via overnight delivery to the MSL along with specification sheets stating the water chemistry records for the laboratory of the commercial supplier. Upon receipt, water quality analysis was performed on the shipping containers and included temperature, pH, D.O., conductivity, hardness, and alkalinity. If the water quality parameters in the shipping containers differed from the MSL acclimation tanks (i.e., temperature difference of greater than 2°C), then the water was allowed to equilibrate to acclimation conditions by incremental dilution of the shipping containers, using a slow drip system.

Test organisms were maintained according to the procedures described in Section 2.5 and water quality parameters were measured daily in the acclimation/holding tanks until the organisms were used in the toxicity tests. *H. azteca* were held for two days prior to testing. *C. tentans* and *P. promelas* were held for 24 h prior to testing. Since <24-h-old *D. magna* were required for testing, adults were held for nine days prior to testing to ensure a sufficient number of young would be available.

2.5 TOXICOLOGICAL TESTING PROCEDURES

2.5.1 Solid-Phase Static Renewal Test Using *H. azteca*

This 10-day solid-phase test was conducted on three test sediment samples (management units), four reference sediment treatments, and a control sediment treatment following the procedures described in Brooke et al., "A Short-Term Method for Estimating the Toxicity of Solid Phase Sediment to the Amphipod *Hyalella azteca*." Five replicates were tested for each sediment sample, with 10 organisms in each test chamber. One replicate of each sediment exposure was monitored daily for D.O. and temperature. Hardness, alkalinity, specific conductance, pH, and total ammonia were measured at the beginning and end of the test from one replicate of each sediment exposure. The test endpoint was survival after 10-day exposure to sediment. A 4-day reference toxicant exposure using potassium chloride (KCl) was used to assess test organism sensitivity. A test summary is presented in Table 3.

TABLE 3. Summary of the Test Design for the *H. azteca* Static-Renewal Test

Parameter	Specification
Test Type:	Solid-phase sediment toxicity test with renewal of overlying water (4 volume additions per day)
Temperature (°C):	23 ±1°C
Light quality:	Ambient laboratory levels
Light intensity:	8 to 16 µE M ⁻² s ⁻¹ ; 800 to 1600 lux or 75 to 150 ft-c
Photoperiod:	16 h light, 8 h dark
Test chamber:	1-L glass jar
Test sediment volume:	200 mL
Overlying water volume:	150 to 175 mL; variable due to water renewal siphoning cycle
Renewal of test solutions:	4 volume additions per day done intermittently
Age of test organisms:	7 to 14 days old
Organisms per chamber:	10
Replicate test chambers per concentration:	5
Reference toxicant:	KCl, 5 concentrations plus control
Feeding regime:	YCT food, fed 0.5 mL daily to each test chamber
Aeration:	None
Dilution water:	Culture water
Test chamber cleaning:	If test chamber screens become clogged, gently brush outside of screen only
Dissolved oxygen:	≥40% of saturation; if this saturation cannot be maintained, increase the rate of renewal of overlying water
Test duration:	10 days
Endpoints:	Survival
Test acceptability:	80% or greater survival in the control sediments; dissolved oxygen ≥40% saturation; mean test temperature 23 ±1°C
Sample requirements:	Storage of dredged material at 4°C ±2°C; sediment should be homogenized and tests initiated within 6 weeks of collection
Dredged material required:	A minimum of 600 mL from each test and reference site

2.5.2 Solid-Phase Static Renewal Test Using *C. tentans*

This 10-day solid-phase test was conducted on three test sediment treatments (management units), four reference sediment treatments, and a control sediment treatment following the procedures described in Call et al., "A Short-Term Method for Estimating the Toxicity of Solid Phase Sediment to *Chironomus Tentans* [sic]." Five replicates were tested for each sediment sample, with 10 organisms in each test chamber. One replicate of each sediment exposure was monitored daily for D.O. and temperature. Hardness, alkalinity, specific conductance, pH, and total ammonia were measured at the beginning and end of the test from one replicate of each sediment exposure. The test endpoint was survival after 10-day exposure to sediment. A 4-day reference toxicant exposure using KCl was performed to assess test organism sensitivity. A test summary is presented in Table 4.

2.5.3 Elutriate Test Using *P. promelas*

This 7-day static-renewal test was conducted on elutriates of the three management units following the procedures described in Brooke et al., "A Short-Term Method for Estimating the Chronic Toxicity of Dredged Material Elutriates to the Fish *Pimephales promelas*." Six elutriate concentrations were tested using a dilution factor of 0.5, resulting in elutriate concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 0% (dilution water). Four replicates were tested for each concentration with 10 organisms per replicate. Test solutions were renewed daily by warming the renewal to test temperature, gently siphoning off approximately 80% of the old test solution, and slowly adding the new solution. This process minimized test organism disturbance. Water temperature was monitored daily in at least five locations in the test chamber array. Dissolved oxygen and pH were measured daily in old and new solutions in a control chamber and in one replicate of each concentration. Hardness, alkalinity, and specific conductivity were measured in the initial control water and in one replicate of the 6.25%, 50%, and 100% concentrations at the start and end of the test. Test endpoints were survival and growth (biomass). A 4-day reference toxicant exposure using analytical grade NaCl was also performed to assess test organism sensitivity. A test summary is presented in Table 5.

2.5.4 Elutriate Test Using *D. magna*

This 21-day static-renewal elutriate test was conducted on elutriates of the three management units following the procedures described in Call et al., "A Method for Estimating the Chronic Toxicity of Sediment Elutriates to *Daphnia magna*." Six elutriate concentrations were tested using a dilution factor of 0.5, resulting in elutriate concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 0% (dilution water). Ten replicates were tested for each concentration with one daphnid per test chamber. Test solutions were renewed on days 2, 4, 7, 9, 11, 14, 16, and 18. To renew test solutions, a second set of test containers was prepared. On test solution renewal day, the elutriates were brought to test temperature via a water bath, mixed with dilution

TABLE 4. Summary of the Test Design for the *C. tentans* Static-Renewal Test

Parameters	Specification
Test Type:	Solid-phase sediment toxicity test with renewal of overlying water
Temperature (°C):	22 to 23 ±1 °C
Light quality:	Ambient laboratory levels
Light intensity:	10-20 µE M ⁻² s ⁻¹ ; 50-100 ft-c
Photoperiod:	16 h light, 8 h dark
Test chamber:	1-L flow-through glass jar
Test sediment volume:	200 mL
Overlying water volume:	150 to 175 mL; variable due to water renewal siphoning cycle
Renewal of test solutions:	4 volume additions per day done intermittently
Age of test organisms:	Second instar larvae 11 to 13 days of age (post-oviposit)
Organisms per chamber:	10
Replicate test chambers per concentration:	5
Reference toxicant:	KCl, 5 concentrations plus control
Feeding regime:	Feed 1.0 mL daily to each chamber of blended Tetrafin goldfish food containing 4.0 mg of dry solids
Aeration:	No aeration is allowed. If D.O. is less than 40% of saturation, the renewal rate of the overlying water should be increased
Dilution water:	Culture water
Water quality monitoring:	Daily measurements of water temperature, D.O., and pH; hardness, alkalinity, specific conductance, pH, and total ammonia measured twice during the test
Duration:	10 days
Endpoints:	Larval survival and biomass (dry weight)
Test acceptability:	70% or greater survival in the control sediments; dissolved oxygen ≥40% saturation; mean test temperature 23 ±1°C
Sample requirements:	Storage of dredged material at 4°C ±2°C; sediment should be homogenized and tests initiated within 6 weeks of collection
Dredged material required:	A minimum of 600 mL from each test and reference site

TABLE 5. Summary of the Test Design for the *P. promelas* Elutriate Test

Parameter	Specification
Test Type:	Static with renewal
Temperature (°C):	25 ±1°C
Illumination quality:	Ambient laboratory illumination
Illumination:	540 to 1080 lux or 50 to 100 ft-c (ambient laboratory levels)
Photoperiod:	16 h light, 8 h dark
Test chamber size:	300 to 400 mL
Test solution volume:	200 mL
Renewal of test solutions:	Daily
Age of test organisms:	Less than 48 h old
Larvae per chamber	10
Replicate test chambers per concentration:	4
Reference toxicant:	NaCl, 5 concentrations plus control
Feeding regime:	Feed 0.1 mL brine shrimp nauplii suspension per test chamber three times daily, or 0.15 mL twice daily
Aeration:	None
Dilution water:	Culture water
Elutriate concentrations:	Minimum of 5 test site elutriate concentrations with a performance control (water-only)
Dilution factor:	0.5
Test duration:	7 days
Endpoints:	Survival and biomass (dry weight)
Test acceptability:	80% or greater survival in the control solutions; mean weight per control fish ≥0.25 mg at end of test
Sample storage:	Store sediment and elutriate for renewals at 4°C ±2°C. Elutriate (in sufficient volume for entire test, i.e. ≥13.95 L) should be prepared for testing within 1 month of sample collection and used to initiate the test within 24 hr of preparation
Sediment volume required:	A minimum of 1,500 mL for each test treatment

water to achieve the appropriate test solution concentrations, and added to the second set of test containers. Each *D. magna* was then transferred to the new solution, and the old containers were examined for the presence of young. Water temperature was monitored daily in at least five locations in the test chamber array; D.O. and pH were measured daily in old and new solutions in a control chamber and in one replicate of each concentration. Hardness, alkalinity, and specific conductance were measured in the initial control water and one replicate of each concentration at the start and end of the test and on days 8, 15, and 21. Test endpoints were survival and reproduction. A 2-day reference toxicant exposure using analytical grade NaCl was performed to assess test organism sensitivity. A test summary is presented in Table 6.

2.6 LABORATORY INSTRUMENTATION

Temperature control was established using the MSL's boiler/heat exchanger coupled to a YSI Temperature Controller. This controller moderated the mixing of the warmed water with cold to produce the appropriate temperature for each test. Temperatures were recorded with Fluke Model 51 digital thermometers, calibrated monthly to an ErTco L-68397 certified thermometer. pH was measured with Orion SA-250 pH meters calibrated daily to standard pH-7 and pH-10 buffers manufactured by RICCA, Arlington, Texas. Dissolved oxygen was measured with YSI Model 57 Dissolved Oxygen Meters calibrated daily to 100% oxygenated deionized water. Specific conductivity was measured with a YSI Model 33 meter which was calibrated with 0.01M KCL. Hardness and alkalinity were measured with HACH Test Kits Model 5B, and Model AL-AP respectively.

2.7 STATISTICAL ANALYSIS AND RANDOMIZATION

All solid-phase and elutriate toxicity tests were designed as completely random tests using the discrete uniform random-number generator available in the computer program Excel. Organisms were randomly allocated to treatments, and treatments were randomly positioned on water tables. Special care was taken with *P. promelas* individuals in order to eliminate bias caused by variable mobility of the fish (otherwise, easily caught fish would be used earlier than more mobile fish).

2.7.1 Statistical Analysis of Solid-Phase Tests

The survival and biomass data from the solid-phase tests were first transformed using the arcsine square root of the proportion of organisms surviving and on the average weight per organism. The arcsine square-root transformation stabilizes the within-class variances and allows the data to be interpreted using analysis of variance (ANOVA). The treatments were tested for normality and homogeneity of variance using a program called Toxstat. To determine statistically significant differences of test treatments relative to the four reference treatments and the pooled

TABLE 6. Summary of the Test Design for the *D. magna* Elutriate Test

Parameters	Specification
Test Type:	Static with renewal
Temperature (°C):	20°C ± 1°C
Light quality:	Fluorescent bulbs (e.g. Grow-lux or vita-life)
Light intensity:	30 to 100 ft-c
Photoperiod:	16 h light, 8 h dark
Test chamber size:	100 mL
Test solution volume:	80 mL
Renewal of test solutions:	Days 2, 4, 7, 9, 11, 14, 16, and 18
Age of test organisms:	Less than 24 h
Neonates per chamber:	1
Replicate test chambers per concentration:	10
Reference toxicant:	NaCl, 5 concentrations plus control
Feeding regime:	Feed three times weekly when solutions are renewed to result in final concentrations in each test chamber of 5 mg/L of trout chow suspension and 10 ⁶ cells/L of <i>Selenastrum capricornutum</i>
Aeration:	None
Dilution water:	Hard reconstituted water of 150 to 180 mg/L CaCO ₃ hardness and a pH range of 6.8 to 8.5
Elutriate concentrations:	Minimum of 5 test site elutriate concentrations with a performance control (water-only)
Dilution factor:	0.5
Test duration:	21 days
Endpoints:	Survival and reproduction
Test acceptability:	80% or greater survival and an average of 40 or more young/surviving females in the water-only control solutions
Sample storage:	Store sediment and elutriate for renewals at 4°C ± 2°C. Elutriate (in sufficient volume for entire test, i.e. ≥ 13.95 L) should be prepared for testing within 1 month of sample collection and used to initiate the test within 24 h of preparation
Sediment volume required:	4.0 L from each test site

response of references BH-Ref-93-02, BH-Ref-93-03, and BH-Ref-93-04 (BH-Ref-Comp), survival and biomass data were compared using the conservative Fisher's Protected Least Significant Difference (LSD) test. This test is a multiple-range comparison that provides information about how each sediment treatment compares to all other treatments. An α level of 0.05 was used to compare the toxicity data.

2.7.2 Statistical Analysis of Elutriate Tests

The elutriate tests were analyzed using a variety of statistical methods based on the endpoint, normality, and variance. In all cases, the statistical analyses followed the methods suggested in the protocols listed in Section 2.5. The *P. promelas* survival test was first transformed using the arcsine square root of the proportion of organisms surviving the test. The biomass tests required no transformations prior to statistical analysis. The results from both the survival and biomass tests were tested for normality and homogeneity using Toxstat. If the data were either not normally distributed or had heterogeneous variance, the non-parametric, Steel's Many-One Rank Test was used to analyze the data.

The survival data for *D. magna* were analyzed by the Toxstat program using the Fisher's Exact Test. This test investigates the hypothesis that the proportion of surviving organisms in the control (0% elutriate) is equal to the proportion of surviving organisms in each of the elutriate concentrations (i.e., 6.25% through 100% elutriate). The reproduction data for the *D. magna* test were analyzed for normality and homogeneity of variance using Toxstat. If the data were normally distributed and there was homogeneous variance, then Dunnett's Tests was used to determine whether statistically significant differences occurred for any of the elutriate treatment means. If the data were either not normally distributed or had heterogeneous variance the non-parametric, Steel's Many-One Rank Test was used to analyze the data.

2.8 QUALITY ASSURANCE/QUALITY CONTROL

Quality assurance/quality control (QA/QC) procedures consisted of verification of all test chamber labels and verification of all data points by a person not involved in either task. In addition, at least 10% of the mortality observations were verified by another person. Before testing began, detailed testing procedures were written, reviewed, and read by the laboratory staff. During testing, water quality data were collected daily. Organism care information and water quality instrument calibration were recorded on standard forms. These data were included in Battelle Standard Data Accumulation Notebooks which become a permanent auditable part of the program.

3.0 TOXICOLOGICAL TESTING RESULTS

3.1 *H. azteca* SOLID-PHASE TEST

Summary toxicity, water quality, and reference toxicant data related to the test are presented in Appendix A. The water quality parameters for the acute and reference toxicant tests (temperature, pH, D.O., and specific conductivity) were measured daily in at least one replicate, and were within the accepted ranges shown in Appendix A. Hardness, alkalinity, and ammonia were measured on preselected days in one replicate of the test treatments. All water quality parameters were within range for this test, the acceptable control survival of 94% validates it. Reference toxicant results are presented in Table A.3. The concentration of the reference toxicant potassium chloride (KCL), which was lethal to 50% of the test organisms (LC_{50}), was calculated at 0.33 g/L of KCL, with a 95% confidence interval of 0.31 g/L to 0.35 g/L.

The results of the *H. azteca* test are summarized in Table 7. Test organism survival ranged from 90% in Management Unit #2 and Reference BH-Ref-93-04 to 100% in Reference BH-Ref-93-03. Analysis of these data using Fishers LSD showed no statistically significant differences among any of the treatment groups, as all groups are included in a common statistical group (a). For example, all treatments in Table 7 had the common letter (a) under statistical group; therefore, none of these sediment treatments was statistically different from another.

3.2 *C. tentans* SOLID-PHASE TEST

Summary toxicity, water quality, and reference toxicant data related to the test are presented in Appendix B. The water quality parameters for the acute and reference toxicant tests (temperature, pH, D.O., and specific conductivity) were measured daily in at least one replicate, and were within the accepted ranges shown in Appendix B. Hardness, alkalinity, and ammonia were measured in one replicate of the acute test only, on pre selected days. All water quality parameters were within range, with the exception of alkalinity and D.O. Upper alkalinity limits were exceeded for nearly every treatment; D.O. levels fell below acceptable minimums on Test Day 2. Neither of these exceedences appeared to affect the test results, as evidenced by the acceptable control survival of 85%. Reference toxicant results are presented in Table B. 4. The calculated LC_{50} for KCL to *C. tentans* was 4.5 g/L of KCL, with a 95% confidence interval of 4.2 g/L to 4.9 g/L.

Table 8 presents the acute toxicity test results for *C. tentans*. Test organism survival ranged from 26% in Reference BH-Ref-93-02 to 88% in Reference BH-Ref-93-03. Survival in management unit treatments ranged from 54% to 80%. The lowest survival (26%) was observed in BH-Ref-93-02. The reason for this low survival is unknown, as water quality

TABLE 7. Results of Statistical Analysis of *H. azteca* Survival Data

<u>Sediment Treatment</u>	<u>Percent Survival</u>	<u>Statistical Grouping^(a)</u>
Management Unit #2	90.0	a
Reference BH-Ref-93-04	90.0	a
Reference BH-Ref-93-02	92.0	a
Management Unit #3	94.0	a
Management Unit #1	94.0	a
Reference BH-Ref-93-01	96.0	a
Reference BH-Ref-93-03	100.0	a
Control	94.0	NA ^(b)

- (a) Sediment treatments with the same statistical grouping (a,b,c) show no statistically significant differences.
(b) NA Not included in statistical analysis.

TABLE 8. Results of Statistical Analysis of *C. tentans* Survival Data

<u>Sediment Treatment</u>	<u>Percent Survival</u>	<u>Statistical Grouping^(a)</u>
Reference BH-Ref-93-02	26.0	a
Management Unit #1	54.0	a b
Management Unit #3	72.0	b c
Reference BH-Ref-93-04	76.0	b c
Management Unit #2	80.0	c
Reference BH-Ref-93-01	84.0	c
Reference BH-Ref-93-03	88.0	c
Control	85.0	NA ^(b)

- (a) Sediment treatments with the same statistical grouping (a,b,c) show no statistically significant differences.
(b) NA Not included in statistical analysis.

parameters associated with this sediment were not appreciably different from those of other treatments. The Fisher's LSD test was used to statistically analyze the survival data. This test compares all treatments to every other treatment. However, since the objective of this report is to determine the suitability of each management unit relative to a given reference treatment, we will focus on the comparisons of each management unit to the four reference treatments. Management Unit #1 produced a statistically significant reduction in test organism survival relative to Reference Treatments BH-Ref-93-01 and BH-Ref-93-03. Test organism survival in Management Unit #1 was 30% to 34% lower, respectively. Management Units #2 and #3 did not produced statistically significant reductions in survival relative to the four references.

Since the reference stations BH-Ref-93-02, BH-Ref-93-03, and BH-Ref-93-04 are very close together (Figure 2), the pooled survival data for these references (designated BH-Ref-Comp) were also statistically compared to those of the management units. The results of these comparisons are presented in Table 9. This table shows that there was no significant difference in survival between the management units and BH-Ref-Comp.

The results of the biomass test are shown in Table 10. The average weight of *C. tentans* ranged from 2.18 mg in Management Unit #1 to 3.50 mg in the reference BH-Ref-93-03. Statistical analyses was conducted using Fisher's LSD. The average weight per test organism exposed to Management Unit #1 was significantly lower relative to BH-Ref-93-03 (2.18 mg versus 3.50 mg). Test organism weight in Management Unit #2 was also significantly lower relative to this reference (2.60 mg versus 3.50 mg). Significant decreases in test organism weight were not observed in Management Unit #3 relative to the four references, nor were significant decreases in weight observed in any treatment relative to references BH-Ref-93-01, BH-Ref-93-02, and BH-Ref-93-04. Comparison of the Management Units to BH-Ref-Comp produced no statistically significant difference in biomass.

3.3 *P. promelas* ELUTRIATE TEST

Summary toxicity, water quality, and reference toxicant data related to the test are presented in Appendix C. The water quality parameters for the acute and reference toxicant tests (temperature, pH, DO, and specific conductivity) were measured daily in at least one replicate. Temperature, pH, and D.O. were within the acceptable ranges established in Appendix C. Specific conductivity was within the acceptable ranges only for Management Unit #3. The conductivity for Management Unit #1 and #2 exceeded the acceptable limit by 150 μ mhos. Hardness, alkalinity, and ammonia were measured on pre selected days, in one replicate of the test treatments. The values for alkalinity were all within acceptable ranges

TABLE 9. Results of Statistical Analysis of *C. tentans* Survival Data in Management Units Versus BH-Ref-Comp

<u>Sediment Treatment</u>	<u>Percent Survival</u>	<u>Statistical Grouping^(a)</u>
Management Unit #1	54.0	a
BH-Ref-Comp ^(b)	63.3	a
Management Unit #3	72.0	a
Management Unit #2	80.0	a

(a) Sediment treatments with the same statistical grouping (a) show no statistically significant differences.

(b) Pooled survival data for BH-Ref-93-02, BH-Ref-93-03, and BH-Ref-93-04.

TABLE 10. Results of Statistical Analysis of *C. tentans* Biomass Data

<u>Sediment Treatment</u>	<u>Average Weight Per Animal (mg)</u>	<u>Standard Deviation</u>	<u>Statistical Grouping^(a)</u>
Management Unit #1	2.18	0.33	a
Reference BH-Ref-93-02	2.18	0.79	a
Management Unit #2	2.60	0.19	a b
Reference BH-Ref-93-01	2.74	0.66	a b
Reference BH-Ref-93-04	2.90	0.52	a b c
Management Unit #3	3.16	0.73	b c
Reference BH-Ref-93-03	3.50	0.57	c
Control	2.42	0.58	NA ^(b)

(a) Sediment treatments with the same statistical grouping (a,b,c) show no statistically significant differences.

(b) NA Not included in statistical analysis.

with the exception of Management Unit #1. This exceedence did not appear to affect test organism survival. This test is validated by acceptable survival in the control (0% elutriate) for each management unit. Reference toxicant results are presented in Table C.4. The concentration of the reference toxicant NaCl, which was lethal to 50% of the test organisms (LC₅₀), was calculated at 7.5 g/L of NaCL, with a 95% confidence interval of 6.9 g/L to 8.1 g/L.

The results of the acute toxicity test and statistical analyses for *P. promelas* are shown in Table 11. Management Unit #1 was analyzed using Steel's Many-One Rank Test because of the non-normal distribution and heterogeneity of variance. Management Unit #2 and #3 were analyzed by Dunnett's Test due to normal distribution and homogeneity of variance. For Management Unit #1, the 100% elutriate produced only 22.5% survival and was statistically significantly different from the other percent elutriate concentrations. This appears to be due in part to high levels of ammonia associated with this treatment. The maximum ammonia value of 16.0 mg/L observed in the 100% elutriate is approximately twice the observed 96-h LC₅₀ observed for this species (Verschuere 1983). The 25% and 100% elutriate concentrations for Management Unit #2 were statistically significantly different from the other elutriate concentrations, though survival was over 80%. The maximum ammonia level associated with Management Unit #2 was 7.5 mg/L (see Table C.5). None of the elutriate concentrations for Management Unit #3 differed significantly from each other.

Table 12 is a summary of the biomass data and statistical analysis for *P. promelas*. All of the data used in the biomass test were normally distributed and had homogeneous variance. Subsequently, the data were analyzed using Dunnett's Test of the arcsine square root of the average organism weight (in milligrams) per management unit. The 100% elutriate for Management Unit #1 was statistically significantly different from the other percent elutriates for that unit. There were no statistically significant differences between percent elutriates for Management Unit #2 or #3.

TABLE 11. Statistical Summary Information for Survival in the *P. promelas* Test. Bold Indicates Statistical Significance Compared to the Other Concentrations of Elutriate for a Given Management Unit.

<u>Sediment Treatment</u>	<u>Percent Elutriate</u>	<u>Mean Percent Survival</u>
Management Unit #1 ^(a)	0.0	100.0
Management Unit #1	6.25	90.0
Management Unit #1	12.5	95.0
Management Unit #1	25.0	95.0
Management Unit #1	50.0	92.5
Management Unit #1	100.0	22.5
Management Unit #2 ^(b)	0.0	97.5
Management Unit #2	6.25	95.0
Management Unit #2	12.5	97.5
Management Unit #2	25.0	85.0
Management Unit #2	50.0	97.5
Management Unit #2	100.0	82.5
Management Unit #3 ^(b)	0.0	97.5
Management Unit #3	6.25	92.5
Management Unit #3	12.5	85.0
Management Unit #3	25.0	97.5
Management Unit #3	50.0	87.5
Management Unit #3	100.0	90.0

(a) Analyzed using Steel's Many-One Rank Test, because of non-normal distribution and heterogeneous variance.

(b) Analyzed using Dunnett's Test, because of normal distribution and homogeneous variance.

TABLE 12. Statistical Summary Information for Biomass in the *P. promelas* Test. Bold Indicates Statistical Significance Compared to the Other Concentrations of Elutriate for a Given Management Unit

<u>Sediment Treatment</u>	<u>Percent Elutriate</u>	<u>Average Weight Per Animal (mg)</u>	<u>Standard Deviation</u>
Management Unit #1	0.0	0.53	0.02
Management Unit #1	6.25	0.55	0.06
Management Unit #1	12.5	0.57	0.07
Management Unit #1	25.0	0.57	0.03
Management Unit #1	50.0	0.57	0.05
Management Unit #1	100.0	0.41	0.05
Management Unit #2	0.0	0.55	0.05
Management Unit #2	6.25	0.58	0.08
Management Unit #2	12.5	0.55	0.02
Management Unit #2	25.0	0.59	0.08
Management Unit #2	50.0	0.59	0.02
Management Unit #2	100.0	0.49	0.02
Management Unit #3	0.0	0.58	0.06
Management Unit #3	6.25	0.60	0.03
Management Unit #3	12.5	0.64	0.03
Management Unit #3	25.0	0.58	0.02
Management Unit #3	50.0	0.60	0.09
Management Unit #3	100.0	0.68	0.02

3.4 *D. magna* ELUTRIATE TEST

Summary toxicity, water quality, and reference toxicant data related to the test are presented in Appendix D, Tables D.1 through D.5. The water quality parameters for the acute and reference toxicant tests (temperature, pH, D.O., and specific conductivity) were measured daily in at least one replicate. D.O. and specific conductivity were within the acceptable ranges established in Appendix D; temperature was low during one day of the test. Hardness, alkalinity, and ammonia were measured on preselected days in one replicate of the test treatments. The upper ranges for hardness and alkalinity were exceeded in all management units. No acceptable ranges have been established for ammonia, though ammonia was highest in the 100% elutriate concentrations of Management Unit #1. Upper pH levels were exceeded slightly in Management Units #1 and #3. The above exceedances occurred across all concentrations, but did not appear to affect test organism survival, as evidenced by high control survival. *D. magna* survival in the 0% elutriates was 90%, 90% and 80% for Management Units #1, #2, and #3 respectively, and validate the test. Reference toxicant results are presented in Table D.4. The concentration of the reference toxicant potassium chloride NaCl, which was lethal to 50% of the test organisms (LC₅₀), was calculated at 2.0 g/L of NaCl, with a 95% confidence interval of 1.6 g/L to 2.5 g/L.

The results of *D. magna* survival and neonate production are summarized in Table 13. Survival for any of the management units for any given elutriate concentration was never lower than 80%. Statistical significance for the survival data was analyzed using Fisher's Exact Test. This test compares the 0% elutriate to each of the dilution concentration results. None of the management units was statistically significantly different for any elutriate concentration. The reproduction data as measured by neonate production was analyzed using the Toxstat software package, which was developed to analyze chronic test data in support of "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (Weber et al. 1989). The reproduction data was first subjected to tests for determining normality and homogeneity of variance. The data failed to meet at least one of these assumptions; subsequently, the non-parametric test, Steel's Many-One Rank Test was used for statistical analysis. This test compares each elutriate concentration to the other concentrations to determine whether a significant decrease in neonate production occurred. None of the management units produced statistically significant differences among any of the elutriate concentrations.

TABLE 13. Summary of *D. magna* Survival and Neonate Production

<u>Sediment Treatment</u>	<u>Percent Flutriate</u>	<u>Percent Survival</u>	<u>Neonate Production</u>		
			<u>Total</u>	<u>Average</u>	<u>S.D.^(a)</u>
Management Unit #1	0.0	90.0	757	84.1	11.0
Management Unit #1	6.25	100.0	821	82.1	6.6
Management Unit #1	12.5	90.0	740	82.2	7.0
Management Unit #1	25.0	100.0	839	83.9	13.6
Management Unit #1	50.0	100.0	937	93.7	17.5
Management Unit #1	100.0	90.0	691	76.8	25.3
Management Unit #2	0.0	90.0	753	83.7	7.9
Management Unit #2	6.25	100.0	821	82.1	8.9
Management Unit #2	12.5	100.0	863	86.3	11.6
Management Unit #2	25.0	100.0	912	91.2	7.5
Management Unit #2	50.0	100.0	948	94.8	13.1
Management Unit #2	100.0	90.0	838	93.1	12.3
Management Unit #3	0.0	80.0	603	75.4	17.7
Management Unit #3	6.25	100.0	835	83.5	6.8
Management Unit #3	12.5	100.0	925	92.5	10.7
Management Unit #3	25.0	100.0	1011	101.1	11.8
Management Unit #3	50.0	90.0	1082	120.2	7.8
Management Unit #3	100.0	100.0	982	98.2	19.9

(a) Standard deviation.

4.0 DISCUSSION

According to the guidance presented in the *Draft Inland Testing Manual* (EPA/USACE 1993), potential dredged material may be unacceptable for open-water disposal if acute toxicity is observed in solid-phase and elutriate tests using sensitive freshwater species. For solid-phase tests, the *Draft Inland Testing Manual* states that dredged material is predicted to be acutely toxic to benthic organisms when test organism mortality 1) is statistically greater than in the reference sediment, and 2) exceeds mortality or other appropriate endpoint in the reference sediment by at least 10%. For elutriate tests, the *Draft Inland Testing Manual* states that dredged material is predicted to be acutely toxic to water column organisms if the concentration of dissolved plus suspended contaminants, after allowance for mixing, exceeds 0.01 of the toxic concentration (LC₅₀) beyond the boundaries of the mixing zone. At this time, there are no guidelines for evaluating chronic/sublethal effects with respect to dredged material disposal, though these tests were performed using *C. tentans*, *P. promelas*, and *D. magna*. What follows is a summary of solid-phase and elutriate test results for the Burns Harbor Program. These summaries compare the toxicological effect observed in each management unit to the four reference areas.

4.1 SOLID-PHASE TEST RESULTS

Solid-phase test results are presented in Table 14. This table shows that acute toxicity was only observed in *C. tentans* for Management Unit #1 relative to the references BH-Ref-93-01 and BH-Ref-93-03. Acute toxicity was not observed when the data for the management units were compared to the pooled survival data for BH-Ref-93-02, BH-Ref-93-03, and BH-Ref-93-04 (BH-Ref-Comp). Reduced *C. tentans* biomass was observed in Management Units #1 and #2 relative to the reference BH-Ref-93-03. All biomass was greater than that observed in the control. No other acute toxicity or reduction in biomass was observed. Under the guidelines presented in the *Draft Inland Testing Manual*, dredged material associated with Management Unit #1 may not be acceptable for open-water disposal if toxicological testing results for this management unit are compared to those of the references BH-Ref-93-01 or BH-Ref-93-03. Based on these tests, Management Units #2 and #3 are acceptable for open-water disposal.

4.2 ELUTRIATE TEST RESULTS

Elutriate test results are summarized in Table 15. This table shows that acute toxicity to *P. promelas* was observed in the 100% elutriate of sediment from Management Unit #1 and the 100% and 25% elutriates of Management Unit #2 relative to the other concentrations. It is believed that elevated ammonia concentrations associated with the 100% elutriate of Management Unit #1 may have contributed to test organism mortality, as the 16.0 mg/L

TABLE 14. Summary of Solid-Phase Test Results

<u>Toxicological Test</u>	<u>Management Unit #1</u>	<u>Management Unit #2</u>	<u>Management Unit #3</u>
<u><i>H. azteca</i> Survival</u>			
BH-Ref-93-01	No acute toxicity	No acute toxicity	No acute toxicity
BH-Ref-93-02	No acute toxicity	No acute toxicity	No acute toxicity
BH-Ref-93-03	No acute toxicity	No acute toxicity	No acute toxicity
BH-Ref-93-04	No acute toxicity	No acute toxicity	No acute toxicity
<u><i>C. tentans</i> Survival</u>			
BH-Ref-93-01	Acute toxicity	No acute toxicity	No acute toxicity
BH-Ref-93-02	No acute toxicity	No acute toxicity	No acute toxicity
BH-Ref-93-03	Acute toxicity	No acute toxicity	No acute toxicity
BH-Ref-93-04	No acute toxicity	No acute toxicity	No acute toxicity
BH-Ref-Comp	No acute toxicity	No acute toxicity	No acute toxicity
<u><i>C. tentans</i> Biomass</u>			
BH-Ref-93-01	No effect	No effect	No effect
BH-Ref-93-02	No effect	No effect	No effect
BH-Ref-93-03	Reduced biomass	Reduced biomass	No effect
BH-Ref-93-04	No effect	No effect	No effect
BH-Ref-Comp	No effect	No effect	No effect

TABLE 15. Summary of Elutriate Test Results

<u>Toxicological Test</u>	<u>Management Unit #1</u>	<u>Management Unit #2</u>	<u>Management Unit #3</u>
<i>P. promelas</i> Survival	Acute toxicity in 100% elutriate relative to other concentrations	Acute toxicity in 100% and 25% elutriates relative to other concentrations	No effect
<i>P. promelas</i> Biomass	Reduced biomass in 100% elutriate relative to other concentrations	No effect	No effect
<i>D. magna</i> Survival	No effect	No effect	No effect
<i>D. magna</i> Reproduction	No effect	No effect	No effect

concentration observed in the 100% elutriate is approximately twice the reported LC₅₀ for this species (Verschuere 1983). An LC₅₀ of 76.16% elutriate was observed in Management Unit #1; an LC₅₀ could not be calculated for Management Unit #2 or #3, as survival was over 85% for all elutriate concentrations. Reduced biomass was also observed in the 100% elutriate of Management Unit #1 relative to the other concentrations. The influence of ammonia on *P. promelas* biomass is not known. There was no observed toxicity or reduced reproduction in the *D. magna* test. These toxicological test results suggest that sediment from Management Units #2 and #3 are acceptable for open-water disposal. Sediments from Management Unit #1 produced statistically significant biological effects and may not be suitable for open-water disposal.

According to the *Draft Inland Testing Manual*, if 0.01 of the acutely toxic concentration exists beyond the boundaries of the mixing zone after allowance for initial mixing, dredged material may not be acceptable for open-water disposal. This means that numerical modeling of these data may be necessary to determine compliance for Management Unit #1. This modeling is beyond the scope of this document.

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*All are Standard Operating Procedures, which have no dates.

APPENDIX A

H. azteca TEST INFORMATION

TABLE A.1. Summary Information for *H. azteca* Test

<u>Sediment Treatment</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Missing</u>	<u>Mean Percent Survival</u>
Management Unit #1	1	10	0	0	
Management Unit #1	2	10	0	0	
Management Unit #1	3	10	0	0	
Management Unit #1	4	10	0	0	
Management Unit #1	5	7	0	3	94.0
Management Unit #2	1	9	0	1	
Management Unit #2	2	10	0	0	
Management Unit #2	3	6	0	4	
Management Unit #2	4	10	0	0	
Management Unit #2	5	10	0	0	90.0
Management Unit #3	1	8	0	2	
Management Unit #3	2	10	0	0	
Management Unit #3	3	10	0	0	
Management Unit #3	4	9	1	0	
Management Unit #3	5	10	0	0	94.0
Reference BH-Ref-93-01	1	9	0	1	
Reference BH-Ref-93-01	2	9	0	1	
Reference BH-Ref-93-01	3	10	0	0	
Reference BH-Ref-93-01	4	10	0	0	
Reference BH-Ref-93-01	5	10	0	0	96.0
Reference BH-Ref-93-02	1	9	1	0	
Reference BH-Ref-93-02	2	10	0	0	
Reference BH-Ref-93-02	3	8	0	2	
Reference BH-Ref-93-02	4	9	1	0	
Reference BH-Ref-93-02	5	10	0	0	92.0
Reference BH-Ref-93-03	1	10	0	0	
Reference BH-Ref-93-03	2	10	0	0	
Reference BH-Ref-93-03	3	10	0	0	
Reference BH-Ref-93-03	4	10	0	0	
Reference BH-Ref-93-03	5	10	0	0	100.0
Reference BH-Ref-93-04	1	10	0	0	
Reference BH-Ref-93-04	2	10	0	0	
Reference BH-Ref-93-04	3	6	0	4	
Reference BH-Ref-93-04	4	9	0	1	
Reference BH-Ref-93-04	5	10	0	0	90.0

TABLE A.1. (contd)

<u>Sediment Treatment</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Missing</u>	<u>Mean Percent Survival</u>
Control Sediment	1	9	0	1	
Control Sediment	2	8	0	2	
Control Sediment	3	10	0	0	
Control Sediment	4	10	0	0	
Control Sediment	5	10	0	0	94.0

TABLE A.2. Summary of Water Quality Data for *H. azteca*

Sediment Treatment	Temperature (°C)		pH		D.O. (mg/L)		Hardness (mg/L CaCO ₃)		Alkalinity (mg/L CaCO ₃)		Ammonia (mg/L)		Specific Conductivity (µmhos)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Acceptable Ranges	22.0	24.0	6.5	9.0	>3.4	NA ^(a)	25	300	25	300	NE ^(b)	NE	50	500
Management Unit #1	21.4	23.8	7.9	8.3	4.3	7.7	170	170	187	187	1.6	0.5	340	340
Management Unit #2	21.5	23.9	7.9	8.2	4.8	7.8	170	170	204	204	0.1	0.3	310	340
Management Unit #3	21.9	23.8	8.0	8.3	4.9	7.9	153	170	187	204	0.2	0.3	320	340
BH-Ref-93-01	21.5	23.7	8.0	8.4	4.9	8.0	153	170	187	204	0.02	0.15	310	330
BH-Ref-93-02	21.6	23.7	7.9	8.3	5.0	7.9	153	170	187	187	0.23	0.48	320	340
BH-Ref-93-03	21.3	23.8	7.9	8.3	4.4	7.8	170	170	187	204	0.1	0.2	320	350
BH-Ref-93-04	21.8	23.8	8.0	8.3	5.0	8.0	170	170	187	221	0.01	0.07	320	340
Control	21.5	23.7	7.7	8.1	4.7	7.7	153	153	187	187	0.11	0.16	260	330

(a) NA Not Applicable.

(b) NE Acceptable ranges not established for ammonia.

TABLE A.3. Summary of Survival Data for *H. azteca* Reference Toxicant Test

<u>Concentration</u> <u>(g KCl/L)</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Missing</u>	<u>Mean</u> <u>Percent Survival</u>
0.00	1	8	2	0	
0.00	2	10	0	0	
0.00	3	9	1	0	90.0
0.24	1	10	0	0	
0.24	2	9	1	0	
0.24	3	10	0	0	96.7
0.34	1	4	6	0	
0.34	2	2	8	0	
0.34	3	2	8	0	26.7
0.49	1	1	9	0	
0.49	2	0	10	0	
0.49	3	2	8	0	10.0
0.70	1	0	10	0	
0.70	2	0	10	0	
0.70	3	0	10	0	0.0
1.00	1	0	10	0	
1.00	2	0	10	0	
1.00	3	0	10	0	0.0

The calculated LC₅₀ is 0.33 g/L KCl with a 95% confidence interval of 0.31 to 0.35 g/L KCl.

TABLE A.4 Summary of Water Quality Data for the *H. azteca* Reference Toxicant Test.

Concentration (g KCl/L)	Temperature (°C)		pH		D.O.		Specific Conductivity (µmhos)	
	Min	Max	Min	Max	Min	Max	Min	Max
Acceptable Ranges	22.0	24.0	6.5	9.0	>3.4	NA(a)	50	500
0.0	22.5	23.2	8.3	8.7	7.1	8.2	330	330
0.24	22.5	23.3	8.3	8.7	7.2	8.2	800	800
0.34	22.6	23.3	8.4	8.7	7.2	8.8	1000	1000
0.49	22.6	23.3	8.3	8.6	7.0	8.6	1200	1200
0.70	22.6	23.3	8.3	8.6	7.0	8.5	1500	1500
1.00	22.6	23.3	8.3	8.6	7.2	8.4	2300	2300

(a) NA Not applicable.

APPENDIX B

C. tentans TEST INFORMATION

TABLE B.1. Summary Information for *C. tentans* Test

<u>Sediment Treatment</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Missing(a)</u>	<u>Mean Percent Survival</u>
Management Unit #1	1	7	0	3	
Management Unit #1	2	7	0	3	
Management Unit #1	3	7	0	3	
Management Unit #1	4	2	0	8	
Management Unit #1	5	4	0	6	54.0
Management Unit #2	1	8	0	2	
Management Unit #2	2	8	0	2	
Management Unit #2	3	10	0	0	
Management Unit #2	4	6	0	4	
Management Unit #2	5	8	0	2	80.0
Management Unit #3	1	9	0	1	
Management Unit #3	2	6	0	4	
Management Unit #3	3	7	0	3	
Management Unit #3	4	6	0	4	
Management Unit #3	5	8	1	1	72.0
Reference BH-Ref-93-01	1	10	0	0	
Reference BH-Ref-93-01	2	9	0	1	
Reference BH-Ref-93-01	3	8	1	1	
Reference BH-Ref-93-01	4	8	0	2	
Reference BH-Ref-93-01	5	7	2	1	84.0
Reference BH-Ref-93-02	1	4	0	6	
Reference BH-Ref-93-02	2	0	1	9	
Reference BH-Ref-93-02	3	6	0	4	
Reference BH-Ref-93-02	4	2	0	8	
Reference BH-Ref-93-02	5	1	0	9	26.0
Reference BH-Ref-93-03	1	9	1	0	
Reference BH-Ref-93-03	2	9	0	1	
Reference BH-Ref-93-03	3	7	0	3	
Reference BH-Ref-93-03	4	8	0	2	
Reference BH-Ref-93-03	5	11(b)	0	0	88.0
Reference BH-Ref-93-04	1	5	0	5	
Reference BH-Ref-93-04	2	8	0	2	
Reference BH-Ref-93-04	3	8	0	2	
Reference BH-Ref-93-04	4	9	0	1	
Reference BH-Ref-93-04	5	8	1	1	76.0

TABLE B.1. (contd)

<u>Sediment Treatment</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Missing</u>	<u>Mean Percent Survival</u>
Control Sediment	1	7	1	2	
Control Sediment	2	9	0	1	
Control Sediment	3	10	0	0	
Control Sediment	4	15 ^(c)	2	3	
Control Sediment	5	10	0	0	85.0

- (a) *C. tentans* adults may have flown away during water quality and/or termination.
 (b) 11 animals placed in test container instead of 10 (one flew away at end of test).
 (c) 20 animals placed in test container instead of 10.

TABLE B.2. Growth Data for *C. tentans* Test

<u>Sediment Treatment</u>	<u>Replicate</u>	<u>Number of Animals Weighed^(a)</u>	<u>Average Weight Per Animal (mg)</u>	<u>Average Weight Per Treatment (mg)</u>	<u>Standard Deviation</u>
Management Unit #1	1	7	1.90		
Management Unit #1	2	7	2.10		
Management Unit #1	3	7	2.70		
Management Unit #1	4	2	2.30		
Management Unit #1	5	4	1.90	2.18	0.33
Management Unit #2	1	7	2.40		
Management Unit #2	2	8	2.80		
Management Unit #2	3	10	2.50		
Management Unit #2	4	6	2.50		
Management Unit #2	5	8	2.80	2.60	0.19
Management Unit #3	1	9	3.00		
Management Unit #3	2	6	2.00		
Management Unit #3	3	7	3.60		
Management Unit #3	4	6	3.90		
Management Unit #3	5	8	3.30	3.16	0.73
Reference BH-Ref-93-01	1	10	2.90		
Reference BH-Ref-93-01	2	9	3.60		
Reference BH-Ref-93-01	3	8	2.30		
Reference BH-Ref-93-01	4	8	3.00		
Reference BH-Ref-93-01	5	5	1.90	2.74	0.66
Reference BH-Ref-93-02	1	4	2.70		
Reference BH-Ref-93-02	2	0	NA ^(b)		
Reference BH-Ref-93-02	3	6	3.00		
Reference BH-Ref-93-02	4	2	1.50		
Reference BH-Ref-93-02	5	1	1.50	2.18	0.79
Reference BH-Ref-93-03	1	9	2.80		
Reference BH-Ref-93-03	2	9	3.00		
Reference BH-Ref-93-03	3	7	3.80		
Reference BH-Ref-93-03	4	8	3.80		
Reference BH-Ref-93-03	5	10	4.10	3.50	0.57
Reference BH-Ref-93-04	1	5	2.90		
Reference BH-Ref-93-04	2	8	3.20		
Reference BH-Ref-93-04	3	8	3.20		
Reference BH-Ref-93-04	4	8	2.00		
Reference BH-Ref-93-04	5	8	3.20	2.90	0.52

TABLE B.2. (contd)

<u>Sediment Treatment</u>	<u>Replicate</u>	<u>Number of Animals Weighed</u>	<u>Average Weight Per Animal (mg)</u>	<u>Average Weight Per Treatment (mg)</u>	<u>Standard Deviation</u>
Control Sediment	1	7	2.10		
Control Sediment	2	9	3.10		
Control Sediment	3	10	2.90		
Control Sediment	4	15(a)	1.70		
Control Sediment	5	10	2.30	2.42	0.58

- (a) Number of animals weighed may differ from number surviving due to adults, flying away during water quality and/or termination.
- (b) 20 animals placed in test container instead of 10.

TABLE B.3. Summary of Water Quality Data for *C. tentans*

Sediment Treatment	Temperature (°C)		pH		D.O. (mg/L)		Hardness (mg/L CaCO ₃)		Alkalinity (mg/L CaCO ₃)		Ammonia (mg/L)		Specific Conductivity (µmhos)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Acceptable Ranges	22.0	24.0	6.5	9.0	>3.4	NA ^(a)	30	195	30	195	NE ^(b)	NE	50	500
Management Unit #1	21.2	23.4	7.6	8.5	2.0 ^(c)	8.1	170	170	204	204	1.2	4.5	310	360
Management Unit #2	21.4	23.4	7.5	8.3	1.8 ^(c)	8.1	153	187	170	187	0.6	4.3	320	340
Management Unit #3	21.9	23.4	7.6	8.5	2.1 ^(c)	8.1	153	170	204	204	0.4	4.3	320	340
BH-Ref-93-01	21.7	23.6	7.6	8.5	2.0 ^(c)	8.3	153	170	187	204	0.3	4.4	290	340
BH-Ref-93-02	21.5	23.5	7.7	8.5	3.1 ^(c)	8.1	153	170	187	204	0.8	4.4	320	340
BH-Ref-93-03	21.8	23.4	7.5	8.4	2.5 ^(c)	8.0	170	170	170	238	0.6	3.3	320	350
BH-Ref-93-04	21.2	23.5	7.6	8.4	2.8 ^(c)	8.2	170	170	204	210	0.2	4.3	320	350
Control	21.4	23.4	7.4	8.3	1.9 ^(c)	8.1	153	170	187	187	0.2	4.4	270	330

(a) NA Not Applicable.

(b) NE Acceptable ranges not established for ammonia.

(c) Test Day 2 only; other days within range (aeration applied at night only).

TABLE B.4. Summary of Survival Data for the *C. tentans* Reference Toxicant Test

<u>Concentration</u> <u>(g KCl/L)</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Missing</u>	<u>Mean</u> <u>Percent Survival</u>
0.0	1	8	1	1	
0.0	2	8	2	0	
0.0	3	8	2	0	80.0
3.2	1	8	2	0	
3.2	2	8	1	1	
3.2	3	6	4	0	66.7
4.2	1	4	6	0	
4.2	2	5	5	0	
4.2	3	4	6	0	43.3
5.6	1	2	8	0	
5.6	2	4	6	0	
5.6	3	1	9	0	23.3
7.5	1	0	10	0	
7.5	2	0	10	0	
7.5	3	0	10	0	0.0
10.0	1	0	10	0	
10.0	2	0	10	0	
10.0	3	0	10	0	0.0

The calculated LC₅₀ is 4.5 g/L KCl with a 95% confidence interval of 4.2 to 4.9 g/L KCl.

TABLE B.5 Summary of Water Quality Data for the *C. tentans* Reference Toxicant Test.

Concentration (g KCl/L)	Temperature (°C)		pH		D.O.		Specific Conductivity (µmhos)	
	Min	Max	Min	Max	Min	Max	Min	Max
Acceptable Ranges	22.0	24.0	6.5	9.0	>3.4	NA(a)	50	500
0.0	22.2	23.3	8.1	8.5	6.0	8.4	NM(b)	NM
3.2	22.4	23.2	8.1	8.5	6.0	8.3	NM	NM
4.2	22.3	23.3	8.1	8.5	5.8	8.2	NM	NM
5.6	22.3	23.3	7.9	8.5	4.8	8.1	NM	NM
7.5	22.3	23.2	8.0	8.5	5.9	8.1	NM	NM
10.0	22.3	23.2	8.0	8.4	6.2	8.2	NM	NM

(a) NA Not Applicable.

(b) NM Not Measured.

APPENDIX C

P. promelas TEST INFORMATION

TABLE C.1. Summary Information for *P. promelas* Test

<u>Sediment Treatment</u>	<u>Percent Elutriate</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Mean Percent Survival</u>
Management Unit #1	0.0	1	10	0	100.0
Management Unit #1	0.0	2	10	0	
Management Unit #1	0.0	3	10	0	
Management Unit #1	0.0	4	10	0	
Management Unit #1	6.25	1	9	1	90.0
Management Unit #1	6.25	2	9	1	
Management Unit #1	6.25	3	10	0	
Management Unit #1	6.25	4	8	2	
Management Unit #1	12.5	1	10	0	95.0
Management Unit #1	12.5	2	9	1	
Management Unit #1	12.5	3	10	0	
Management Unit #1	12.5	4	9	1	
Management Unit #1	25.0	1	10	0	95.0
Management Unit #1	25.0	2	9	1	
Management Unit #1	25.0	3	10	0	
Management Unit #1	25.0	4	9	1	
Management Unit #1	50.0	1	9	1	92.5
Management Unit #1	50.0	2	10	0	
Management Unit #1	50.0	3	9	1	
Management Unit #1	50.0	4	9	1	
Management Unit #1	100.0	1	2	8	22.5
Management Unit #1	100.0	2	2	8	
Management Unit #1	100.0	3	3	7	
Management Unit #1	100.0	4	2	8	

TABLE C.1. (contd)

<u>Sediment Treatment</u>	<u>Percent Elutriate</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Mean Percent Survival</u>
Management Unit #2	0.0	1	10	0	
Management Unit #2	0.0	2	9	1	
Management Unit #2	0.0	3	10	0	
Management Unit #2	0.0	4	10	0	97.5
Management Unit #2	6.25	1	10	0	
Management Unit #2	6.25	2	9	1	
Management Unit #2	6.25	3	10	0	
Management Unit #2	6.25	4	9	1	95.0
Management Unit #2	12.5	1	10	0	
Management Unit #2	12.5	2	9	1	
Management Unit #2	12.5	3	10	0	
Management Unit #2	12.5	4	10	0	97.5
Management Unit #2	25.0	1	8	2	
Management Unit #2	25.0	2	8	2	
Management Unit #2	25.0	3	9	1	
Management Unit #2	25.0	4	9	1	85.0
Management Unit #2	50.0	1	9	1	
Management Unit #2	50.0	2	10	0	
Management Unit #2	50.0	3	10	0	
Management Unit #2	50.0	4	10	0	97.5
Management Unit #2	100.0	1	9	1	
Management Unit #2	100.0	2	7	3	
Management Unit #2	100.0	3	8	2	
Management Unit #2	100.0	4	9	1	82.5

TABLE C.1. (contd)

<u>Sediment Treatment</u>	<u>Percent Elutriate</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Mean Percent Survival</u>
Management Unit #3	0.0	1	9	1	97.5
Management Unit #3	0.0	2	10	0	
Management Unit #3	0.0	3	10	0	
Management Unit #3	0.0	4	10	0	
Management Unit #3	6.25	1	9	1	92.5
Management Unit #3	6.25	2	10	0	
Management Unit #3	6.25	3	9	1	
Management Unit #3	6.25	4	9	1	
Management Unit #3	12.5	1	8	2	85.0
Management Unit #3	12.5	2	9	1	
Management Unit #3	12.5	3	9	1	
Management Unit #3	12.5	4	8	2	
Management Unit #3	25.0	1	9	1	97.5
Management Unit #3	25.0	2	10	0	
Management Unit #3	25.0	3	10	0	
Management Unit #3	25.0	4	10	0	
Management Unit #3	50.0	1	10	0	87.5
Management Unit #3	50.0	2	9	1	
Management Unit #3	50.0	3	9	1	
Management Unit #3	50.0	4	7	3	
Management Unit #3	100.0	1	8	2	90.0
Management Unit #3	100.0	2	10	0	
Management Unit #3	100.0	3	8	2	
Management Unit #3	100.0	4	10	0	

TABLE C.2. Biomass Data for the *P. promelas* Test

<u>Sediment Treatment</u>	<u>Percent Elutriate</u>	<u>Animals Weighed</u>	<u>Average Weight Per Animal (mg)</u>	<u>Average Weight Per Treatment (mg)</u>	<u>Standard Deviation</u>
Management Unit #1	0.0	10	0.55		
Management Unit #1	0.0	10	0.51		
Management Unit #1	0.0	10	0.54		
Management Unit #1	0.0	10	0.53	0.53	0.02
Management Unit #1	6.25	9	0.56		
Management Unit #1	6.25	9	0.54		
Management Unit #1	6.25	10	0.48		
Management Unit #1	6.25	8	0.63	0.55	0.06
Management Unit #1	12.5	10	0.55		
Management Unit #1	12.5	9	0.52		
Management Unit #1	12.5	10	0.54		
Management Unit #1	12.5	9	0.67	0.57	0.07
Management Unit #1	25.0	10	0.60		
Management Unit #1	25.0	9	0.60		
Management Unit #1	25.0	10	0.53		
Management Unit #1	25.0	9	0.56	0.57	0.03
Management Unit #1	50.0	9	0.50		
Management Unit #1	50.0	10	0.57		
Management Unit #1	50.0	9	0.61		
Management Unit #1	50.0	9	0.59	0.57	0.05
Management Unit #1	100.0	2	0.45		
Management Unit #1	100.0	2	0.45		
Management Unit #1	100.0	3	0.37		
Management Unit #1	100.0	2	0.35	0.41	0.05

TABLE C.2. (contd)

<u>Sediment Treatment</u>	<u>Percent Elutriate</u>	<u>Animals Weighed</u>	<u>Average Weight Per Animal (mg)</u>	<u>Average Weight Per Treatment (mg)</u>	<u>Standard Deviation</u>
Management Unit #2	0.0	10	0.60	0.55	0.05
Management Unit #2	0.0	9	0.49		
Management Unit #2	0.0	10	0.53		
Management Unit #2	0.0	10	0.57		
Management Unit #2	6.25	10	0.66	0.58	0.08
Management Unit #2	6.25	9	0.60		
Management Unit #2	6.25	10	0.58		
Management Unit #2	6.25	9	0.47		
Management Unit #2	12.5	10	0.54	0.55	0.02
Management Unit #2	12.5	9	0.53		
Management Unit #2	12.5	10	0.58		
Management Unit #2	12.5	10	0.53		
Management Unit #2	25.0	8	0.71	0.59	0.08
Management Unit #2	25.0	8	0.55		
Management Unit #2	25.0	9	0.54		
Management Unit #2	25.0	9	0.54		
Management Unit #2	50.0	9	0.59	0.59	0.02
Management Unit #2	50.0	10	0.60		
Management Unit #2	50.0	10	0.56		
Management Unit #2	50.0	10	0.60		
Management Unit #2	100.0	9	0.50	0.49	0.02
Management Unit #2	100.0	7	0.47		
Management Unit #2	100.0	8	0.50		
Management Unit #2	100.0	9	0.48		

TABLE C.2. (contd)

<u>Sediment Treatment</u>	<u>Percent Elutriate</u>	<u>Animals Weighed</u>	<u>Average Weight Per Animal (mg)</u>	<u>Average Weight Per Treatment (mg)</u>	<u>Standard Deviation</u>
Management Unit #3	0.0	9	0.64		
Management Unit #3	0.0	10	0.60		
Management Unit #3	0.0	10	0.50		
Management Unit #3	0.0	10	0.58	0.58	0.06
Management Unit #3	6.25	9	0.58		
Management Unit #3	6.25	10	0.57		
Management Unit #3	6.25	9	0.61		
Management Unit #3	6.25	9	0.64	0.60	0.03
Management Unit #3	12.5	8	0.69		
Management Unit #3	12.5	9	0.63		
Management Unit #3	12.5	9	0.62		
Management Unit #3	12.5	8	0.62	0.64	0.03
Management Unit #3	25.0	9	0.59		
Management Unit #3	25.0	10	0.59		
Management Unit #3	25.0	10	0.59		
Management Unit #3	25.0	10	0.55	0.58	0.02
Management Unit #3	50.0	10	0.52		
Management Unit #3	50.0	9	0.51		
Management Unit #3	50.0	9	0.66		
Management Unit #3	50.0	7	0.69	0.60	0.09
Management Unit #3	100.0	8	0.68		
Management Unit #3	100.0	10	0.65		
Management Unit #3	100.0	8	0.70		
Management Unit #3	100.0	10	0.67	0.68	0.02

TABLE C.3. Summary of Water Quality Data for *P. promelas*

Sediment Treatment	Temperature (°C)		pH		D.O. (mg/L)		Hardness (mg/L CaCO₃)		Alkalinity (mg/L CaCO₃)		Ammonia (mg/L)		Specific Conductivity (µmhos)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Acceptable Ranges	24.0	26.0	6.5	9.0	>3.4	NA ^(a)	25	300	25	300	NE ^(b)	NE	50	500
Management Unit #1	24.4	25.2	7.7	8.5	5.4	8.9	170	255	204	323	0.9	16.0	340	650
Management Unit #2	24.4	25.2	7.5	8.5	5.2	9.2	170	187	187	221	1.2	7.5	340	650
Management Unit #3	24.5	25.2	8.1	8.5	5.5	8.9	170	187	204	221	1.2	3.0	340	470

(a) NA Not Applicable.

(b) NE Acceptable ranges not established for ammonia.

TABLE C.4. Summary of Survival Data for *P. promelas* Reference Toxicant Test

<u>Concentration (g/L NaCl)</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Missing</u>	<u>Percent Survival</u>
0.0	1	10	0	0	100.0
0.0	2	10	0	0	
0.0	3	10	0	0	
1.0	1	10	0	0	100.0
1.0	2	10	0	0	
1.0	3	10	0	0	
4.0	1	10	0	0	100.0
4.0	2	10	0	0	
4.0	3	10	0	0	
7.0	1	8	2	0	73.3
7.0	2	7	3	0	
7.0	3	7	3	0	
10.0	1	1	9	0	3.3
10.0	2	0	10	0	
10.0	3	0	10	0	
14.0	1	0	10	0	0.0
14.0	2	0	10	0	
14.0	3	0	10	0	

The calculated LC₅₀ is 7.5 g/L NaCl with a 95% confidence interval of 6.9 to 8.1 g/L NaCl.

TABLE C.5 Summary of Water Quality Data for the *P. promelas* Reference Toxicant Test.

NaCl Concentration (g/L)	Temperature (°C)		pH		D.O. (mg/L)		Specific Conductivity (µmhos)		Salinity (ppt)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Acceptable Ranges	22.0	24.0	6.5	9.0	>3.4	NA(a)	50	500	NA	NA
0.0	24.4	24.9	8.1	8.4	5.5	8.1	330	330	0.0	0.0
1.0	24.3	24.8	8.2	8.4	6.1	8.1	2400	2400	0.0	1.0
4.0	24.5	24.9	8.2	8.5	6.2	8.1	8000	8000	4.0	4.0
7.0	24.6	24.9	8.1	8.4	6.1	7.9	12500	12500	6.0	7.0
10.0	24.6	24.8	8.2	8.4	6.0	7.9	17000	17000	10.0	10.0
14.0	24.6	24.7	8.4	8.4	7.9	7.9	23000	23000	14.0	14.0

(a) NA Not applicable.

APPENDIX D

D. magna TEST INFORMATION

TABLE D.1. Summary Information for *D. magna* Test

<u>Management Unit</u>	<u>Percent Elutriate</u>	<u>Percent Survival</u>	<u>Neonate Production</u>		
			<u>Total</u>	<u>Average</u>	<u>S.D.^(a)</u>
1	0.0	90	757	84.1	11.0
1	6.25	100	821	82.1	6.6
1	12.5	90	740	82.2	7.0
1	25.0	100	839	83.9	13.6
1	50.0	100	937	93.7	17.5
1	100.0	90	691	76.8	25.3
2	0.0	90	753	83.7	7.9
2	6.25	100	821	82.1	8.9
2	12.5	100	863	86.3	11.6
2	25.0	100	912	91.2	7.5
2	50.0	100	948	94.8	13.1
2	100.0	90	838	93.1	12.3
3	0.0	80	603	75.4	17.7
3	6.25	100	835	83.5	6.8
3	12.5	100	925	92.5	10.7
3	25.0	100	1011	101.1	11.8
3	50.0	90	1082	120.2	7.8
3	100.0	100	982	98.2	19.9

(a) S.D. Standard deviation.

TABLE D.2. Neonate Production in *Daphnia magna* Test

<u>Management Unit</u>	<u>Percent Elutriate</u>	<u>Replicate</u>	<u>Total Neonate Production</u>
1	0.0	1	66
1	0.0	2	80
1	0.0	3	103
1	0.0	4	73
1	0.0	5	92
1	0.0	6	0(a)
1	0.0	7	89
1	0.0	8	79
1	0.0	9	87
1	0.0	10	88
1	6.25	1	80
1	6.25	2	85
1	6.25	3	70
1	6.25	4	82
1	6.25	5	87
1	6.25	6	82
1	6.25	7	94
1	6.25	8	80
1	6.25	9	86
1	6.25	10	75
1	12.5	1	93
1	12.5	2	84
1	12.5	3	89
1	12.5	4	80
1	12.5	5	81
1	12.5	6	81
1	12.5	7	0(a)
1	12.5	8	74
1	12.5	9	71
1	12.5	10	87
1	25.0	1	84
1	25.0	2	86
1	25.0	3	55
1	25.0	4	91
1	25.0	5	73
1	25.0	6	92
1	25.0	7	76
1	25.0	8	84
1	25.0	9	93
1	25.0	10	105
1	50.0	1	61
1	50.0	2	108
1	50.0	3	89
1	50.0	4	76
1	50.0	5	120
1	50.0	6	107
1	50.0	7	107

TABLE D.2. (contd)

<u>Management Unit</u>	<u>Percent Elutriate</u>	<u>Replicate</u>	<u>Total Neonate Production</u>
1	50.0	8	86
1	50.0	9	88
1	50.0	10	95
1	100.0	1	78
1	100.0	2	59
1	100.0	3	61
1	100.0	4	66
1	100.0	5	29
1	100.0	6	104
1	100.0	7	96
1	100.0	8	94
1	100.0	9	0(a)
1	100.0	10	104
2	0.0	1	87
2	0.0	2	0(a)
2	0.0	3	70
2	0.0	4	82
2	0.0	5	93
2	0.0	6	79
2	0.0	7	79
2	0.0	8	79
2	0.0	9	93
2	0.0	10	91
2	6.25	1	69
2	6.25	2	75
2	6.25	3	83
2	6.25	4	79
2	6.25	5	101
2	6.25	6	78
2	6.25	7	88
2	6.25	8	85
2	6.25	9	87
2	6.25	10	76
2	12.5	1	98
2	12.5	2	110
2	12.5	3	85
2	12.5	4	92
2	12.5	5	77
2	12.5	6	82
2	12.5	7	79
2	12.5	8	77
2	12.5	9	91
2	12.5	10	72

TABLE D.2. (cont'd)

Management Unit	Percent Elutriate	Replicate	Total Neonate Production
2	25.0	1	94
2	25.0	2	76
2	25.0	3	92
2	25.0	4	91
2	25.0	5	90
2	25.0	6	88
2	25.0	7	84
2	25.0	8	103
2	25.0	9	98
2	25.0	10	96
2	50.0	1	104
2	50.0	2	90
2	50.0	3	94
2	50.0	4	92
2	50.0	5	83
2	50.0	6	99
2	50.0	7	97
2	50.0	8	72
2	50.0	9	122
2	50.0	10	95
2	100.0	1	94
2	100.0	2	108
2	100.0	3	94
2	100.0	4	100
2	100.0	5	111
2	100.0	6	82
2	100.0	7	79
2	100.0	8	0(a)
2	100.0	9	76
2	100.0	10	94
3	0.0	1	103
3	0.0	2	78
3	0.0	3	77
3	0.0	4	0(a)
3	0.0	5	60
3	0.0	6	97
3	0.0	7	61
3	0.0	8	53
3	0.0	9	74
3	0.0	10	0(a)
3	6.25	1	80
3	6.25	2	90
3	6.25	3	82
3	6.25	4	87
3	6.25	5	76
3	6.25	6	82

D.4

TABLE D.2. (contd)

<u>Management Unit</u>	<u>Percent Elutriate</u>	<u>Replicate</u>	<u>Total Neonate Production</u>
3	6.25	7	98
3	6.25	8	82
3	6.25	9	75
3	6.25	10	83
3	12.5	1	96
3	12.5	2	83
3	12.5	3	72
3	12.5	4	92
3	12.5	5	104
3	12.5	6	100
3	12.5	7	96
3	12.5	8	108
3	12.5	9	89
3	12.5	10	85
3	25.0	1	85
3	25.0	2	104
3	25.0	3	113
3	25.0	4	100
3	25.0	5	101
3	25.0	6	122
3	25.0	7	82
3	25.0	8	100
3	25.0	9	106
3	25.0	10	98
3	50.0	1	107
3	50.0	2	117
3	50.0	3	125
3	50.0	4	124
3	50.0	5	132
3	50.0	6	121
3	50.0	7	0(a)
3	50.0	8	117
3	50.0	9	112
3	50.0	10	127
3	100.0	1	81
3	100.0	2	104
3	100.0	3	109
3	100.0	4	102
3	100.0	5	84
3	100.0	6	122
3	100.0	7	130
3	100.0	8	66
3	100.0	9	102
3	100.0	10	82

(a) Test organism died, no neonate production.

TABLE D.3. Summary of Water Quality Data for *D. magna*

Sediment Treatment	Temperature (°C)		pH		D.O. (mg/L)		Hardness (mg/L CaCO ₃)		Alkalinity (mg/L CaCO ₃)		Ammonia (mg/L)		Specific Conductivity (µmhos)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Acceptable Ranges	19.0	21.0	6.8	8.5	>3.4	NA ^(a)	160	180	110	120	NE ^(b)	NE	50	500
Mangagement Unit #1	16.3	20.8	8.3	8.6	7.5	9.2	153	238	187	400	0	22.0	290	495
Mangagement Unit #2	16.2	20.8	8.2	8.5	7.1	9.3	170	187	187	312	0	8.0	290	380
Mangagement Unit #3	16.5	20.7	8.2	8.6	7.3	9.2	153	187	187	320	0	6.0	270	400

(a) NA Not Applicable.

(b) NE Acceptable ranges not established for ammonia.

TABLE D.4. Summary of Survival Data for *D. magna* Reference Toxicant Test

<u>Concentration</u> <u>(g KCl/L)</u>	<u>Replicate</u>	<u>Alive</u>	<u>Dead</u>	<u>Missing</u>	<u>Mean</u> <u>Percent Survival</u>
0.0 g/L	1	4	1	0	80.0
0.0 g/L	2	3	2	0	
0.0 g/L	3	4	1	0	
0.0 g/L	4	5	0	0	
0.6 g/L	1	2	3	0	75.0
0.6 g/L	2	5	0	0	
0.6 g/L	3	3	2	0	
0.6 g/L	4	5	0	0	
1.3 g/L	1	1	4	0	30.0
1.3 g/L	2	0	5	0	
1.3 g/L	3	3	2	0	
1.3 g/L	4	2	3	0	
2.5 g/L	1	1	4	0	45.0
2.5 g/L	2	2	3	0	
2.5 g/L	3	4	1	0	
2.5 g/L	4	2	3	0	
5.0 g/L	1	0	5	0	0.0
5.0 g/L	2	0	5	0	
5.0 g/L	3	0	5	0	
5.0 g/L	4	0	5	0	
10.0 g/L	1	0	5	0	0.0
10.0 g/L	2	0	5	0	
10.0 g/L	3	0	5	0	
10.0 g/L	4	0	5	0	

The calculated LC₅₀ is 2.0 g/L KCl with a 95% confidence interval of 1.6 to 2.5 g/L KCl.

TABLE D.5 Summary of Water Quality Data for the *D. magna* Reference Toxicant Test.

NaCL Concentration (g/L)	Temperature (°C)		pH		D.O. (mg/L)		Specific Conductivity (µmhos)		Salinity (ppt)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Acceptable Ranges	19.0	21.0	6.8	8.5	>3.4	NA(a)	50	500	NA	NA
0.0	19.9	20.5	8.2	8.5	6.5	9.0	NM(b)	NM	0.0	0.0
0.6	19.8	20.6	8.2	8.5	6.4	8.9	NM	NM	0.0	1.0
1.3	19.7	20.5	8.3	8.4	7.4	8.9	NM	NM	0.0	0.5
2.5	19.7	20.6	8.2	8.4	7.1	8.7	NM	NM	1.0	2.7
5.0	19.5	20.8	8.1	8.4	6.3	8.7	NM	NM	3.5	4.0
10.0	19.8	19.9	8.3	8.5	7.8	8.7	NM	NM	8.0	9.0

(a) NA Not applicable.

(b) NM Not measured.

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