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Notice

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ABSTRACT

Sediment contamination has resulted in the need to develop an appropriate suite of toxicity tests to assess ecotoxicological impacts on estuarine ecosystems. Existing Environmental Protection Agency (EPA) protocols recommend a number of test organisms, including amphipods, polychaetes, molluscs, crustaceans and fish for use in sediment toxicity tests. While this suite of test animals represents a diverse group of fauna, many of the species recommended by the EPA are not indigenous to all geographic regions of the United States, particularly the Gulf of Mexico and South Atlantic. As a result, environmental risk assessment based on these organisms may not adequately protect ecosystem health in the Gulf of Mexico. Ideally, appropriate test organisms to evaluate sediment toxicity should include species indigenous to the Gulf of Mexico that are representative of a variety of faunal classes and feeding types. Additionally, the toxicity test endpoints should include both lethal (mortality) and sublethal (reproduction, growth, physiological impairment) effects and they should be sensitive to either porewater and/or whole sediment exposures for all major classes of chemical contaminants (trace metals, polycyclic aromatic hydrocarbons (PAHs), pesticides). Finally, test species should be easy to collect and maintain in the laboratory. This study examined the relative sensitivity of a variety of test organisms, broadly distributed throughout the southeastern United States and the Gulf of Mexico to several classes of chemical contaminants in both whole sediment and aqueous/ porewater exposures. Additionally, several rapid screening assays were compared with these more traditional toxicity evaluations. The three model contaminants selected for study were cadmium (an inorganic toxicant), DDT (a persistent organochlorine pesticide) and fluoranthene (a polycyclic aromatic hydrocarbon [PAH]). These compounds represent contaminants frequently measured in sediments throughout the Gulf of Mexico.

Overall, the juvenile clam was the most sensitive species tested in this study from an acute toxicity standpoint. The grass shrimp and the two amphipod species were generally similar in sensitivity to each of the three compounds. The copepod assay, although relatively insensitive in terms of adult mortality, was capable of detecting sublethal effects at contaminant concentrations below those which caused mortality in the other more sensitive species. Both the juvenile clam assay and the copepod partial life cycle test have the potential to serve as sensitive indicators of potential sediment-associated toxicity which might not be detected using standard acute toxicity bioassays.

The differing species sensitivities observed with the different classes of chemical contaminants in this study suggest that a multiple species approach may be more appropriate for a holistic ecological risk assessment of sediment contamination. The "Crustacean Triad" (copepods, amphipods and grass shrimp) provide a battery of tests which predict toxicity to epibenthic and benthic crustaceans with known sensitivity to a variety of chemical contaminants and represent the base of the food chain for most recreationally and commercially important finfish species that utilize estuarine nursery grounds. The addition of the juvenile clam assay provides a herbivorous filter feeder with the ability to bioconcentrate pollutants and which is extremely sensitive in the size range tested (>212<350 μm). Field studies in South Carolina have indicated that sites with high sediment contaminant levels have degraded benthos, with significant effects observed in crustaceans and molluscs. These findings support our laboratory results and suggest that an integrated battery of assays may be most appropriate for estimating field effects.

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Chapter 1

Introduction

Anthropogenically-induced chemical contamination of sediments has resulted in the need to develop an appropriate suite of toxicity tests to holistically assess ecotoxicological impacts on estuarine ecosystems. Existing Environmental Protection Agency (EPA) protocols recommend a number of test organisms, including amphipods, polychaetes, molluscs, crustaceans and fish for use in sediment toxicity tests (EPA, 1991). While this suite of test animals represents a diverse group of fauna, many of the species recommended by the EPA are not indigenous to all geographic regions of the United States, particularly the Gulf of Mexico and South Atlantic. As a result, environmental risk assessment based on these organisms may not adequately protect ecosystem health in the Gulf of Mexico. Ideally, appropriate test organisms to evaluate sediment toxicity should include species indigenous to the Gulf of Mexico that are representative of a variety of faunal classes and feeding types. Additionally, the toxicity test endpoints should include both lethal (mortality) and sublethal (reproduction, growth, physiological impairment) effects and they should be sensitive to either porewater and/or whole sediment exposures for all major classes of chemical contaminants (trace metals, polycyclic aromatic hydrocarbons (PAHs), pesticides). Finally, test species should be easy to collect and maintain in the laboratory.

The purpose of this study was to evaluate the relative sensitivity of a variety of test organisms, broadly distributed throughout the southeastern United States and the Gulf of Mexico to several classes of chemical contaminants in both whole sediment and aqueous/porewater exposures. Additionally, several rapid screening assays were compared with these more traditional toxicity evaluations. The three model contaminants selected for study were cadmium (an inorganic toxicant), DDT (a persistent organochlorine pesticide) and fluoranthene (a polycyclic aromatic hydrocarbon [PAH]). These compounds represent contaminants frequently measured in sediments throughout the Gulf of Mexico. MacDonald (1994) reported that the percentage of stations on the Atlantic Coast of Florida where sediment concentrations exceeded the Threshold Effects Level (TEL) was 15.2 % for Cd, 42.2 % for fluoranthene and 1.2-1.8 % for DDT and related metabolites. On the Gulf Coast the % of TEL exceedances was 12.6 % for Cd, 20.9 % for fluoranthene and 2.5 % for DDT and related metabolites.

Chapter 2

Methods

Description of Species Tested

Palaemonetes pugio

The grass shrimp, *Palaemonetes pugio*, is a common shrimp species found in tidal marsh systems along the east coast of the U.S. and Gulf of Mexico. These shrimp are a major force in accelerating the breakdown of detritus in the estuary and are important dietary components for many fish species (Wood, 1967). *P. pugio* can be found in salinities ranging from 2 to 36 ‰ and are most abundant in vegetated habitats (Anderson, 1985). In South Carolina estuaries, *P. pugio* occur year round at densities ranging from <1000/50m of stream in winter to 28,000/50m of stream in summer. *P. pugio* comprise 56 % of the total stream density on an annual basis, therefore any significant reductions in grass shrimp populations would greatly affect the entire estuarine ecosystem (Scott et al., 1994). These factors have led to *P. pugio* being used as a representative nontarget species in many insecticide toxicity tests (e.g., Mayer, 1987).

Ampelisca abdita

Ampelisca abdita is a tube-dwelling, infaunal amphipod (Bousfield, 1973). This species has a cosmopolitan distribution in coastal waters of the United States ranging from Maine to Florida on the eastern seaboard, throughout the Gulf of Mexico, and in San Francisco Bay (Mills, 1967; Bousfield, 1973; Nichols and Thompson, 1985; Summers, unpublished). It is generally found in sediments consisting of fine sand to mud and it has been reported to range in depth from the intertidal zone to 60 m. Although *A. abdita* has been found during all seasons in several studies conducted in South Carolina, it is generally most abundant during the fall, winter and spring months and is usually only found in areas with salinities greater than 20 ppt (Van Dolah et al., 1990, 1994; Wendt et al., 1990). *A. abdita* is both a deposit and filter feeder and generally constructs a well-developed tube.

Ampelisca verrilli

Ampelisca verrilli is also an infaunal, tube dwelling amphipod that has a cosmopolitan distribution throughout the East coast of the U.S. Extensive surveys of benthic fauna in subtidal portions of the Gulf of Mexico estuaries have not collected *A. verrilli*, but a very closely related species, *A. holmesi* (Mills, 1967), is widely distributed in that region (Summers, unpublished EMAP Louisianian Province data). Additionally, *A. verrilli* has been collected from the west coast of Florida. In South Carolina, *A. verrilli* appears to be most abundant in muddy sand flats near high-salinity inlets at the mouth of estuaries. We have observed dense populations in fine to medium sands from high in the intertidal zone to shallow subtidal bottoms. Bousfield (1973) reports its distribution to extend from the low intertidal zone to 50 m in depth. Although *A. verrilli* can be collected during all seasons, it appears to be more abundant during the warmer months (Wendt et al., 1990). Assays conducted in our laboratory to evaluate the salinity tolerance of *A. verrilli* indicate that it survives well above 20 ppt. It also survives well in all non-toxic sediments that we have tested, ranging from unconsolidated muds with very little sand to very sandy sediments with little or no mud. This species is both a deposit and filter feeder and the tube it constructs is generally not very well developed compared with *A. abdita* tubes, which may increase its exposure to contaminants. In some sediments, it does not appear to construct a tube.

Amphiascus tenuiremis

Amphiascus tenuiremis is an easily-cultured, diosaccid harpacticoid copepod, collected originally in 1988 from North Inlet, SC (Chandler, 1986). This species is more abundant at higher latitudes than SC but is amphi-Atlantic in distribution ranging from the North Sea/Baltic intertidal to the southern Gulf of Mexico. Diosaccid copepods are the most abundant, diverse and widely-distributed family of sediment-dwelling copepods. *Amphiascus tenuiremis* has a generation time of 21 days at 20°C, and is capable of multiple clutches in short periods of time (i.e., 10-14 d post-insemination). This species has been shown to be sensitive to pesticides (Chandler, 1990; Chandler and Scott, 1991), PAHs (Fulton et al., 1997) and trace metals (Green et al., 1993) in acute, chronic and multi-generational bioassays.

Mercenaria mercenaria

Mercenaria mercenaria is a marine filter-feeding, infaunal mollusk in the family Veneridae (Pechenik,1991). These bivalves are commonly known as "northern quahogs," "cherrystones," "littlenecks" or "hard-shell clams." Among bivalves, this clam is second only to oysters in commercial importance in the United States, partly due to its ability to remain tightly closed and live for weeks out of water if refrigerated. *Mercenaria mercenaria* occurs along the East coast of the United States from the Gulf of St. Lawrence to the central Florida coast, with a subspecies, *M. mercenaria texana*, occurring in the northern Gulf of Mexico (Menzel,1988; Dillon and Manzi,1989). Throughout its range, *M. mercenaria* primarily inhabits intertidal to shallow subtidal estuarine areas and filter feeds on detritus and phytoplankton, such as *Isochrysis galbana*.

Mercenaria mercenaria have demonstrated sensitivity to anthropogenic contaminants. Calabrese et al. (1977) determined that some clams exposed to heavy metals exhibited retardation of growth. Such growth retardation may prolong the pelagic life stage of the larvae which may ultimately lead to increased predation, thus decreasing the larval survival rate. Ultimately this results in a lower recruitment into the population and reduced commercial harvest for human consumption.

Brachionus plicatilis

Brachionus plicatilis is a rotifer species in the family Brachionidae, which has a global distribution. This species has been collected from estuarine habitats in both the southeastern U.S. and the Gulf of Mexico. It has also been found on six continents, and several strains are being cultured worldwide (Snell and Persoone, 1989). As with other rotifer species, *B. plicatilis* filter feeds on phytoplankton and bacteria, has a rapid reproductive cycle and short generation time, and can be grown from dormant eggs (cysts) that can be stored for long periods of time. Snell and Persoone (1989) developed an acute toxicity bioassay using this rotifer species for brackish and marine environments, and have shown that it is sensitive to several contaminants. This assay has been further developed by Creasel, Ltd. (Deinze, Belgium) as the Rotoxkit M® toxicity test kit. Since the test can be done rapidly (24 hr exposure), requires relatively little aqueous solution, and is inexpensive to conduct, it was considered to be a worthwhile test protocol for comparison with the other bioassay protocols and species.

Mysidopsis bahia

The mysid, *Mysidopsis bahia*, is a crustacean found in the estuarine waters of the northern Gulf of Mexico from southwestern Florida to Mexico. These crustaceans are ecologically important as food for many fish species and are also important in detritus breakdown. Mysids inhabit shallow water grass flats with salinities ranging from 9 to 29 ‰. Of the mysid species, *M. bahia* has been the most extensively studied. The USEPA, other US government agencies and private laboratories have selected this crustacean as a toxicity model for many of their assessment programs (EPA, 1980, 1994; Nimmo et al., 1977). Aqua Survey, Inc. has developed a rapid toxicity screening test (Mysid IQ Test™) that utilizes a sublethal toxic response in *M. bahia*. The IQ test is based on the ability of a healthy, unstressed organism to ingest and metabolize a fluorigenic substrate, 4-methyllumbelliferyl-β-D-galactoside (MUG). Non-affected organisms are able to cleave galactose from MUG, forming 4-umbelliferone which is strongly fluorescent under longwave UV light. Impacted organisms display reduced fluorescence

which can be quantified relative to control organisms. Since this test can be done rapidly (1 h exposure) and requires little test solution, it was included among the suite of bioassay protocols evaluated.

Microtox and Mutatox

The Microtox and Mutatox screening assays were evaluated to determine their relative sensitivity to the three model toxicants. The Microtox assay utilizes the photoluminescent bacterium, *Vibrio fischeri*, to provide a sublethal toxicity measure which is based on the attenuation of light production by the bacterial cells due to toxicant exposure. The Mutatox assay utilizes a dark strain of *Vibrio fischeri* which reverts to the bioluminescent strain when exposed to mutagenic substances.

Collection and Holding of Test Organisms

Palaemonetes pugio

Adult grass shrimp, *P. pugio*, were collected from Leadenwah Creek, a tidal tributary of North Edisto River estuary, located on Wadmalaw Island, SC. Seawater used for holding and exposures was collected from Bohicket Creek, another tributary of North Edisto River. Adult shrimp (20-35 mm) were acclimated in 76-L tanks at 20°C, 30 ‰ salinity and 12-h light:12-h dark cycle. Shrimp were fed daily with Tetramin Fish Flakes and newly hatched *Artemia*. Shrimp were collected five to seven days prior to testing.

Ampelisca abdita

Since local populations of *A. abdita* were not available for use in the bioassays, all *A. abdita* were obtained either from Science Applications International in Narragansett, Rhode Island, or from East Coast Amphipods, Inc. in Kingston, Rhode Island. Both facilities collected their amphipods from tidal flats in the Pettaquamscutt River which flows into Narragansett Bay, Rhode Island. The majority of specimens tested in our assays ranged in size from 3 to 5 mm. Bousfield (1973) reported this size to be in the juvenile to early adult size range. Prior to testing, *A. abdita* were acclimated in the laboratory to the testing temperature (20°C) for a period of 2-6 days, with daily feeding using *Phaeodactylum tricornutum* or *Chaetocerus* sp.

Ampelisca verrilli

The *A. verrilli* used in this study were collected from intertidal flats in a relatively pristine, undeveloped section of the Folly River near Charleston, South Carolina. The majority of organisms tested in our assays ranged in size from 5 to10 mm, which is considered to be in the juvenile to adult size range (Mills, 1967). Prior to testing, all *A. verrilli* were acclimated in the laboratory and fed using the same protocols described for *A. abdita.*

Amphiascus tenuiremis

The *A. tenuiremis* used in this study were obtained from laboratory stock cultures established in 1988 from North Inlet, SC brood lines. Stock populations were continuously cultured under flow in clean sediments at 30 ‰ salinity, 21°C and 12:12 L:D photoperiod. Populations were fed twice weekly with concentrated (>10^6 cells/ml) phytoplankton. In preparation for each experiment, sediment was removed from the copepod culture system and rinsed with clean seawater on a 125 μm sieve to collect the copepods. Copepods were gently washed into plastic petri dishes where they were sorted by sex. Copepods were kept in clean seawater in petri dishes at 21°C up to 48 h prior to the initiation of an experiment.

Mercenaria mercenaria

Juvenile clams, *M. mercenaria*, were acquired from Atlantic Littleneck Clam Farm located on James Island, SC. Seawater used for holding and exposures was collected from Bohicket Creek, a tidal tributary of North Edisto River estuary. Juvenile clams (>212<350 μm) were acclimated for 24 to 48h in 16 oz precleaned glass jars at 20°C, 30 ‰ salinity and 12-h light:12-h dark cycle. Clams were fed daily *Isochrysis galbana* obtained from the stock culture at the clam farm.

Brachionus plicatilis

The *B. plicatilis* used in this study were supplied as dried cysts in the Rotoxkit M® test kits. The cysts were hatched by incubating them in 10 ml of 20 ‰ artificial seawater under continuous lighting at 25 °C for a period of approximately 28-30 hrs just prior to testing.

Mysidopsis bahia

The *M. bahia* used in the tests were shipped overnight from Flemington, New Jersey to the National Ocean Service laboratory at Charleston, South Carolina. One-day-old mysids placed in

artificial seawater (Forty Fathoms™) containing food were shipped overnight in insulated containers, along with a container of dilution water. The dilution water was the same solution of Forty Fathoms™ seawater mix but without added food. Upon arrival at the laboratory, mysids and their dilution water (28 ± 2 ‰) were transferred to small aquaria (2.5 gallons). An airstone was placed in the aquaria to maintain adequate dissolved oxygen levels. Mysids were fed *Artemia nauplii* larvae. Prior to dosing, the test organisms were held overnight in order to acclimate them to the laboratory conditions, as well as to clear their intestinal tracts prior to toxicity testing.

Reference Toxicant Tests

Sodium dodecyl sulfate (SDS) was used as reference toxicant for most test species to ensure that each batch of organisms used in the contaminant bioassays were of comparable sensitivity. The grass shrimp, amphipods, copepods and clams were tested by exposing 10-20 organisms to various concentrations of SDS for a 24 h period at 20 °C and 30 ‰ salinity (20 μm filtered seawater). Exposure media volumes were 2 L for grass shrimp, 0.8 L for amphipods, 0.050 L for copepods and 0.5 L for the clams. Dose ranges varied for each species, depending on their sensitivity to the toxicant. To develop baseline data, at least five reference toxicant tests were completed prior to conducting any of the model toxicant bioassays. Subsequent SDS bioassays conducted for each definitive test were added to the database to create a running mean. The acceptance criteria for a given batch of animals was defined as the mean LC_{50} for all previous SDS tests ± two standard deviations. Reference toxicant tests for *B. plicatilis* were conducted using serial dilutions of the toxicant, potassium dichromate, which was provided with the test kits. The exposure protocol was the same as described above, except that 30 rotifers were exposed at each potassium dichromate concentration. Percent survival was assessed after 24 hours and compared with the acceptable limits (95% CI) provided by the manufacturer for that batch of animals. A phenol standard was used as a positive control in all microtox assays. Acceptance criteria were provided by the manufacturer. No reference toxicant tests were conducted with the mysid tests.

Analytical Chemistry

Contaminant Stock Solutions

Spiking solutions were prepared by dissolving the toxicant in deionized water (cadmium) or acetone (DDT and fluoranthene). Stock solutions were quantified using either inductively coupled plasma spectroscopy (ICP) (cadmium), gas chromatography with electron capture detection (GC-ECD) (DDT) or high performance liquid chromatography (HPLC) with fluorescence detection (fluoranthene). Stocks were then distributed to each laboratory for use in the aqueous assays which required various dose levels dependent upon the species and/or protocol being tested. All contaminant-spiked sediments were also prepared using these stocks. Specific sediment spiking protocols are described in the sections describing bioassay protocols.

Quantification of Cadmium in Spiked Sediments

Twenty gram aliquots were taken from each batch of cadmium-spiked sediment for analysis. Each aliquot was transferred to a 30-ml acid-washed plastic sample cup. The sample was then covered and dried at 70°C for 24 hours. After drying, the sample was reweighed to determine moisture content. The dried sediment was then ground with a mortar and pestle and transferred to a 20-ml plastic screw-top container.

Ground samples were extracted using a closed-vessel, concentrated acid microwave digestion technique. A 0.5-g subsample of the ground sediment was weighed (0.0001 g) into a Teflon-lined digestion vessel, and 10 ml of concentrated HNO_3 (Instra-analyzed) plus 0.5-ml deionized water was added. The sample was then microwaved using a well ventilated, 600 watt corrosion-resistant digestion microwave (CEM Model MDS-2000) for 2 hours at full power and 120 psi. The sample was allowed to cool, then 2.0 ml of 30% H_2O_2 was added. The vessel was then microwaved for an additional 10 minutes at full power and 80 psi. After cooling, the digestate was filtered (#41 filter paper) into a 50-ml volumetric flask and brought to volume with deionized water. The sample was then transferred by pouring into a 50-ml polypropylene conical centrifuge tube for analysis.

Cadmium-spiked samples were analyzed by inductively coupled plasma spectroscopy (ICP). The instrument (Perkin Elmer Plasma 1000 with autosampler) was calibrated by developing a standard curve. The response factor was determined as the slope of the standard curve line (absorbance/mg cadmium). Sample extracts were analyzed in duplicate and the results averaged and reported as mg/Kg dry weight (dw)

Quantification of Acid Volatile Sulfides (AVS) and Simultaneously Extractable Metals (SEM) in Cadmium-Spiked Sediments

The general procedure for measuring AVS and SEMs was based on Allen et al. (1991) with modifications as described below. Sulfide and metals were released from sediment using a N_2 gas supply system and a reaction/trap system by placing about 5 g of wet homogenized sediment into each of six 500-ml round-bottom flasks. Deionized water (80 ml) was then added to each flask together with a small Teflon-coated stir bar, and the injection ports were sealed with rubber septums. The sediment-deionized water mixtures were then purged with nitrogen for 10 minutes to remove residual oxygen. After the nitrogen flow was stopped, 20 ml of 6 M HCl was added to each flask. The HCl was added through the rubber septum using a syringe to volatilize the sulfides and metals in the sediment sample. The samples were stirred with the magnetic stirrers and the volatilization reaction allowed to proceed for 1.5 hours. Each boiling flask was then filtered through a 0.45-μm membrane filter. The flask was rinsed several times with deionized water, with the rinses added to the filtrate. The volume of the filtrate was measured, and a 50.0-ml aliquot removed for SEM analysis.

Impingers contained 0.5 M NaOH to capture H_2S retained from the boiling flask. The NaOH traps were developed by adding 10 ml of a mixed diamine reagent (Allen et al., 1991) and allowing the mixture to react for 30 minutes. The solution was quantitatively transferred to a 100-ml volumetric flask and brought to volume. Approximately 2 ml of solution were transferred to a cuvette, and the absorbance at 670 nm read using a spectrophotometer (Milton Roy Spectronic Model 601).

A standard sulfite solution was prepared by weighing 12 g of $Na_2S.9H_2O$ into 1.0 L of deionized water. The solution was standardized by the sodium thiosulfate titration procedure described in Allen et al. (1991) using a starch indicator. From the standardized solution, 0 (blank) to 10 ml were pipetted in 1-ml increments, transferred to 100-ml volumetric flasks and developed using the mixed diamine reagent. Absorbance was measured at 670 nm and used with solutions of known concentration to construct a standard curve.

Simultaneously extracted metals (SEMs) were measured in the 50.0-ml aliquot removed from the sediment extract. The acid treatment removes metals which are weakly associated with the sediments and not incorporated in crystalline matrices. Samples were analyzed by ICP for cadmium using the methods previously described.

Quantification of DDT in Spiked Sediments

The methods for extraction and sample preparation for organic contaminants in sediments were similar to those of Krahn et al. (1985) with a few modifications. In preparation for analysis, sediment samples were thawed and allowed to reach room temperature. Visible detritus was removed from the sample, and the sediment thoroughly stirred with a stainless steel spatula. A portion of the sediment was transferred to a beaker and placed on a top-loading balance, where about 8.5 g of sediment was accurately weighed by difference (0.01 g) and placed in a Pyrex mortar. The sediment was then dried by mixing with 100 g of Na_2SO_4 which had been ashed for 16 h at 700°C. The dried sample was transferred to a Pyrex Soxhlet thimble. The sample was then extracted in a Soxhlet apparatus with 250 ml of CH_2Cl_2 for 18 hrs. Sample extracts were reduced in volume by a stream of purified nitrogen using a nitrogen blow-down concentrator (Turbo Vap, Zymark Instruments) to about 0.5 ml. The CH_2Cl_2 was replaced with isooctane and concentrated to a final volume of about 1.0 ml and transferred to an autosampler vial for analysis by gas chromatography (GC) with electron capture detection (ECD).

The instrument (GC-ECD; Hewett-Packard 5890 series II) was configured with one column, a 30-m x 0.25-mm i.d. (0.25-mm film thickness) DB-5 (5% phenyl; J&W Scientific). The initial carrier gas constant average linear velocity was 33 cm/sec. The carrier and detector makeup gasses were helium and nitrogen (95%:5%), respectively. The injector

and detector temperatures were 250°C and 320°C, respectively. The sample was injected (1 μl) using a splitless Grob technique (1 min split time). The initial oven temperature was 50°C with a one-minute hold, followed by an increase to 170°C at 4°C/min, then to 210°C at 1°C/minute, and finally to 310°C at 4°C/min with a 10 min hold. The detector signal was digitized and processed using the Windows-based EZChrom software (Scientific Software Inc.).

The instrument was calibrated using a mixed standard of the target analytes (chlorinated pesticides, NIST SRM 2261). The slope of the response curve with respect to the internal standards was used to quantify the concentrations of the analytes in the unknown (i.e., test) sample. The calibration curve was verified at the beginning of each sample set by injecting the mid-level, continuing calibration, which is a check standard which was required to be with ± 20% of the known value for each analyte; otherwise, the instrument was recalibrated.

Quantification of Fluoranthene in Spiked Sediments

Fluoranthene-spiked sediments were prepared and dried as described above. The dried sample was then transferred to a Pyrex Soxhlet thimble, and the internal standards D_8-naphthalene (200 ng), D_{10}-acenapthalene (200 ng), D_{10}-phenanthrene (502 ng), D_{10}-fluoranthene (497 ng), D_{12}-perylene (102 ng), dibromooctafluorobiphenyl (PCB-103; 20 ng), and 2,2',3,3',4,5,5'6-octachlorobiphenyl (PCB-198; 20ng) were added. The sample was then extracted in a Soxhlet apparatus with 250 ml of CH_2Cl_2 for 18 hours. Sample extracts were reduced in volume by a stream of purified nitrogen using a nitrogen blow-down concentrator (Turbo Vap, Zymark Instruments) to about 0.5 ml. Lipids and other high molecular weight compounds were removed from the sample by gel permeation chromatography. The liquid chromatograph consisted of an autosampler (Gilson Model 231), a Waters HPLC pump (Model 501), two 22.5-mm x 500-mm gel permeation columns in series (Phenomenex Phenogel, 100 A pore size), a UV detector (Linear Model U-106), and a fraction collector (Gilson Model 201). The mobile phase was CH_2Cl_2 at a flow rate of 7 ml/min. A 400-ml sample was injected into the system. Lipids and other high molecular weight compounds were eluted in the first 14 minutes. The fraction of interest was collected beginning 1 minute before the retention time of DBOFBP and ending 2 minutes after perylene. The resulting fraction was reduced in volume as above. The CH_2Cl_2 was replaced with hexane and concentrated to a final volume of about 0.5 ml. At this point, elemental sulfur was removed from the sample by treatment with activated copper. To remove remaining polar interferences, the sample was transferred to a 6-g cyanopropyl solid-phase extraction cartridge (Varian, pre-rinsed with 6 ml of hexane) and eluted with 12 ml of hexane. The eluent was reduced in volume to about 0.5 ml, and 200 μl were transferred to an autosampler vial for analysis by gas chromatography (GC) with electron capture detection (ECD) and GC with ion trap mass spectrometry (GC-ITMS) detection analysis (see below). The hexane in the remainder of the sample (about 200 μl) was replaced with acetonitrile for analysis by high performance liquid chromatography with fluorescence detection (HPLC-fluorescence).

PAHs were additionally quantified using HPLC with fluorescence detection utilizing a method similar to Wise et al. (1988) and Schantz et al. (1990). The instrument consisted of two HPLC pumps (Waters 6000A), a 680 gradient controller (Waters Model 680), and an autosampler (Waters WISP). The column dimensions were 6 mm x 25 cm, with a 5-μm particle size (Supelco LC-PAH). The column was heated to 30° (Fiaton TC-50 column heater controller and a CH-30 column heater). The solvent was pumped at a constant flow rate of 1.5 ml/min with a gradient program that started with a two-minute hold at 60% water: 40% acetonitrile followed by a linear increase to 55% water: 45% acetonitrile in 15 minutes and a final increase to 0% water: 100% acetonitrile in 35 minutes with a 10-minute hold. Fluorescence was monitored with two fluorescence detectors (Perkin Elmer LC-240 and LS-4) connected in series at wavelengths specific to individual PAHs (Appendix B - Table B-1). The separation between deuterated and nondeuterated PAHs was 0.44, 0.40, and 0.41 minutes for phenanthrene, fluoranthene, and perylene, respectively.

Data collection was accomplished using Perkin Elmer Omega II personal computer-based software. A NIST certified PAH standard solution and the deuterated PAH internal standards were used to calibrate the instrument. Sample peaks were identified by retention times and fluorescence at

specific wavelengths. TOC was measured using a Perkin Elmer Model 2400 Series II CHNS/O Analyzer on three replicate 15 g sediment samples.

Aqueous Contaminant Bioassay Protocol

All 24 h aqueous contaminant bioassays were conducted at 20 °C and 30±2‰ salinity. The rationale for the aqueous bioassays was to determine the inherent sensitivity of the test species in seawater exposures where organisms were directly exposed to a given toxicant. Often in sediment exposures, the interaction of the contaminant with the sediment may reduce or prevent exposure of the organism. Thus, the aqueous exposures were used to assess the overall sensitivity of each species which could then be compared with the sediment toxicity test results. Deionized water was the carrier for the cadmium bioassays while acetone was used for the DDT and fluoranthene tests. All test concentrations and the controls received the same carrier concentration. For all tests, the trimmed Spearman-Karber method was used to calculate the LC_{50} for each replicate based on nominal doses, whenever at least one dose produced mortality > 50%. Comparisons of LC50 estimates among species were performed using ANOVA on log (x+1) transformed data. Bonferroni's test was used to identify specific group differences.

Aqueous bioassays with grass shrimp were conducted in 4-L wide-mouth glass jars with five replicate chambers for each test concentration and the control. Two liters of test solution were added to each jar. Six serial dilutions (60%) were used for cadmium and DDT and five were used for fluoranthene. Ten grass shrimp were added to each jar and all jars were then placed in a Revco Environmental Chamber. Tests were run under a 12-h light:12-h dark cycle. Shrimp were not fed during the test. Temperature, dissolved oxygen, salinity and pH were recorded from each control replicate at the beginning and end of the exposure period. Shrimp mortality at the end of the test was recorded from each jar based on lack of movement and the failure to respond to tactile stimulation.

Aqueous bioassays using the two amphipod species involved exposure of 10 amphipods/ replicate 1-liter beaker in 700 ml of toxicant solution under constant aeration and a 12L:12D light cycle. Five replicate groups of amphipods were used for each treatment. Each contaminant test consisted of six treatments containing seawater spiked with the varying doses of the toxicant (60% dilution series) and a control containing seawater with an equivalent carrier dose. Usually, both species were tested at the same time with the same stock solution and all treatment groups randomized with respect to location on the water table. Amphipod mortality at the end of the test was recorded from each jar based on lack of movement and the failure to respond to tactile stimulation.

Copepod bioassays were run in five replicate, 50-ml glass crystallizing dishes containing 45 ml of the appropriate test solution. There were five replicates for each of five toxicant concentrations and the control. Twenty copepods were used per replicate. Bioassays were run in total darkness. After 24 hours, each dish was sieved on a 63 μm sieve and the number of live and dead copepods determined. Temperature, salinity, dissolved oxygen and pH were measured at beginning and end of each bioassay.

Clam bioassays were run under constant aeration and a 12L:12D light cycle in 600 ml Pyrex glass beakers containing 500 ml of test solution, with five replicates for each test concentration and the control. Ten clams were added to each beaker and the beakers were placed in a Revco Environmental Chamber. Clams were not fed during the 24-h test periods. Temperature, dissolved oxygen, salinity and pH were recorded from each control replicate at the beginning and end of the test. At the end of the bioassay, clam mortality from each beaker was recorded. Clam mortality was determined using an Olympus SZH10 Microscope under 7.0 x magnification and Mocha Image Analysis (Jandel Scientific) to capture images of the clams. Clams were determined to be alive if locomotion was exhibited following placement in the petri dish. Some clams which remained closed for several minutes were gently moved by tapping the petri dish or moving the clam onto its umbo to ensure they were alive. Dead organisms were assessed based on a gaping shell and/or no response to tactile stimulation.

Both the aqueous and sediment pore-water assays using *B. plicatilis* were performed using procedures similar to those described by Snell and Persoone (1989), with some modifications. Toxicant doses for the rotifers were mixed from natural seawater (30 ‰) and the same contaminant stocks used for the

amphipod assays. Sediment porewater was obtained from each dose of the spiked sediments used for the amphipod assays by centrifuging approximately 50 ml of sediment at 10,000 RPM for 10 minutes. Rotifers were exposed in multi-well test plates which had one large well for hatching the cysts and six series of additional wells that included one 0.7 ml rinsing well and six 0.3 ml exposure wells/series. Approximately 50 neonates were transferred from the hatching well into each rinsing well which contained one of the toxicant doses or control water. From there, 30 neonates were transferred to six test wells (5 neonates/well) which contained the same dose of toxicant or control water. The plates were then covered and incubated in a 12L:12D light cycle at 25 °C. The number of alive versus dead rotifers were counted after 24 h to determine percent survival for each toxicant dose, and an LC_{50} was computed using the trimmed Spearman Karber method whenever at least one dose resulted in >50% mortality. A spiked-sediment porewater concentration was considered to be toxic if its survival was statistically lower ($p<0.05$) and less than 80% of control survival.

Mysid IQ™ assays were performed following protocols described by the manufacturer (Aqua Survey, 1994). Test organisms were exposed in ultraviolet transmissible acrylic plates (12cm x 8.5cm x 1cm) containing six shallow cylindrical chambers (1cm deep, 3.5cm diameter). Three of the chambers (exposure chambers) were filled with five ml of test solution, and the other three chambers (response chambers) were filled with five ml of Aqua Survey Dilution Water (ASDW). Using a wide bore pipette, six mysids were added to each of the exposure chambers where they were kept for one hour. Next, 0.25 ml of the fluorigenic substrate, 4-methyl-umbelliferyl-B-D-galactoside (MUG), was added to the exposure chambers. After 20 min, mysids were transferred to the response chambers using great care to minimize the amount of substrate entering the chambers. After the mysids were placed in the response chambers, the lights in the room were turned off, and the mysids were placed over a longwave UV lamp. The number of fluorescing mysids were recorded. The proportion of mysids not fluorescing in each treatment was calculated. An EC50 was then calculated using the trimmed Spearman-Karber method.

Sediment Bioassay Protocol

Sediment was collected from a "relatively pristine" site located on the tidally influenced Folly River, SC. Sediment was press-sieved through a 1 mm mesh screen into 5-gallon plastic buckets at the collection site, then transported to the laboratory and stored at 3°C until used in the bioassays (≤ seven days).

Sediment for the grass shrimp, amphipod , Microtox, Mutatox and rotifer assays was spiked in acid washed 4-L wide-mouth plastic jars 24 h before the start of the bioassay, then rolled on a jar mill until thorough mixing had occurred (~2 h). Contaminant spikes were based on the estimated dry weight of the sediment. Deionized water was the carrier for cadmium while DDT and fluoranthene were dissolved in acetone. All test concentrations and the controls received the same carrier concentration.

For the copepod bioassays, sediment was wet-sieved on a 212 μm sieve and collected in a clean 4 L beaker. The sediment slurry was allowed to settle for 24 h at 4°C. Supernatant was removed from the slurry by aspiration. The sediment slurry was transferred to a clean 1 L beaker and homogenized for 30 min. by continuous stirring with a 3" stirring bar. Aliquots of 75-200 ml of homogenized sediment slurry were distributed to clean 250 ml glass beakers and stirred. Appropriate amounts of contaminant were added to stirring sediment slurry. The slurry was homogenized for 2 h before placing 10 ml of each concentration in each of five replicate test chambers containing 20 ml of filtered artificial seawater.

Sediment for the clam bioassays was press-sieved through a 212μm mesh screen for *M. mercenaria* bioassays. Sediment was spiked in acid-cleaned 1000 ml Pyrex beakers with the appropriate amount of testing compound 24 h before the start of the bioassay, then stirred vigorously by hand (~5 mins) until thorough mixing had occurred.

All bioassays were run at 30 ‰ salinity and 20°C. Each day, temperature, dissolved oxygen, salinity and pH were recorded from each control replicate. On days 0, 2 and 8, ammonia was measured in three randomly selected control replicates and one randomly selected treatment replicate. When

possible the trimmed Spearman-Karber method was used to calculate LC50 values as previously described. In those cases when LC50s could not be calculated, mortality in treatment groups was compared with that in the controls using either a t-test or ANOVA on the transformed percentage (arcsine sq. rt. of P).

Grass shrimp bioassays were run in 4-L wide-mouth glass jars. There were five replicates for each concentration and the control. The sieved sediments were allowed to warm to room temperature and then rolled on a jar mill for approximately 30 minutes, since separation into liquid and solid phases may have occurred. Approximately 300 ml of sieved sediment was placed into each 4-L jar, then 1700 ml of 20 μm filtered seawater was added. The jars were capped with Teflon-lined plastic caps through which a 1 mℓ pipette was inserted into a pre-drilled hole. The jars were placed in a Revco Environmental Chamber with airlines attached to the 1 mℓ pipettes. The sediment was allowed to settle under aeration in the bioassay jars for 24-h before the addition of the grass shrimp. After 24-h, ten grass shrimp were added to each jar and the jars returned to the environmental chambers. Bioassays were conducted using 12-h light:12-h dark cycle. Shrimp in each jar were fed Tetramin fish flakes every 48 h in order to reduce mortality from cannibalism. Mortality was determined on day 10.

The sediment bioassays for both amphipod species involved a 10-day static whole sediment assay using procedures similar to those described by Swartz et al., (1985) and ASTM (1993). Test chambers were 1-liter pyrex beakers filled with 200 ml of sediment and 800 ml of seawater and covered with an inverted glass dish. The sediment and seawater were added to the beakers approximately 24 hr prior to inoculating the sediments with amphipods using the procedures described above.

Five replicate series of beakers were tested, with each series consisting of four doses containing sediment spiked with varying concentrations of contaminants as described above and one control containing sediment with an equivalent concentration of either distilled water or acetone only. All tests were conducted under constant lighting to inhibit amphipod emergence from the sediment. Air was provided using oil-free pumps and glass pipettes inserted into the test chambers. For most tests, both species were tested at the same time and all treatment groups were randomly located on the water table.

At the end of each assay, the test chambers were sieved through a 0.5 mm mesh screen and the number of animals alive, dead, or missing was recorded. Sediment test results were considered valid if the overall survival was ≥85% in the control group and no replicate fell below 80% survival. All beakers were also inspected daily to record the number of animals that were found either dead or alive on the surface of the sediments. The size (total length) of the amphipods used in each assay was measured by selecting 10 amphipods from one randomly-selected beaker representing each treatment dose.

Test chambers for the copepod bioassays consisted of 50 ml Teflon® Erlenmeyer flasks fitted with outflow ports covered with 63 μm Nitex mesh. Fifteen *A. tenuiremis* adult males and 15 non-gravid females were added to each test chamber without disturbing the sediment. Chambers were placed in an incubator under flow at 20°C, 30 ‰ salinity and 12:12 L:D cycle for 10 days. Physical parameters were monitored at the beginning and end of an experiment.

Upon completion of an experiment, test chambers were removed from the incubator. Contents of the chamber were washed onto a 63 μm sieve with filtered artificial seawater and rinsed into a plastic petri dish. The contents of each dish were stained with Rose Bengal and preserved with formalin to a final concentration of 5%. The dishes were refrigerated until their contents could be counted under stereo dissection microscope.

Female and male mortality was assessed. In addition, the reproductive endpoints of copepodite, nauplii and clutch size were also examined. In cases where mortality was significant, an LC_{50} value was computed as previously described. In cases where mortality was minimal, reproductive endpoints were compared in a general linear model (GLM) procedure using SAS® statistical software. Significant differences among treatments were detected using

Tukey's Studentized T-Test and differences between test concentrations and the control were assessed using Dunnett's test .

The clam bioassays were run in 600 ml Pyrex glass beakers, with five replicates for each contaminant concentration and the control. The sieved sediments were allowed to warm to room temperature and then stirred vigorously by hand, since separation of liquid and solid phases may have occurred. Approximately 100 ml of spiked sediment was placed into each 600 ml beaker, followed by 300 ml of 20 μm filtered seawater. The beakers were covered with solvent-rinsed aluminum foil through which a 1 mℓ pipette was inserted and placed in a Revco Environmental Chamber with airlines attached to the 1 mℓ pipettes. The sediment was allowed to settle under aeration for 24-h before the addition of the clams. After 24-h, 50 clams were added to each beaker. Bioassays were run at 30 ‰ salinity (20 μm filtered seawater), 20°C, and a 12-h light :12-h dark cycle. Clams in each beaker were fed 5 ml of *Isochrysis galbana* every 48 hours. At the end of ten days, clam mortality was determined from each jar and recorded.

Comparisons of LC50 estimates among species were performed using ANOVA on log (x+1) transformed data. Bonferroni's test was used to identify specific group differences.

Microtox Assay Protocols

Microtox™ assays were performed generally following protocols from Microbics Corporations' Microtox™ Manual (Microbics Corporation, 1992). A phenol standard solution was used as positive control with each microtox bioassay.

For aqueous bioassays, serial dilutions of each contaminant were prepared in a 2% saline diluent. A reagent solution which contained the bacteria was then added to each dilution. Light emission readings were taken after 5 and 15 minutes. The percent decrease in bioluminescence relative to the reagent blank was used to calculate an EC_{50} for each dilution series at both time points using a log-linear regression model. Five replicate assays were performed for each contaminant.

For sediment Microtox™ assays, sediments were collected and spiked with the model contaminants (cadmium, DDT and fluoranthene) as previously described. Spiked sediments were analyzed using established protocols (Microbics Corporation, 1992; Long and Markel, 1992). Samples were evaluated using both the organic extract and solid phase protocols.

The percent decrease in bioluminescence relative to the reagent blank was used to calculate an EC_{50} for each spiked sediment sample. A total of three replicate assays were performed for each of the sediment spikes.

Mutatox Bioassay

The Mutatox™ genotoxicity bioassay was performed as described in Microbics Corporations' Mutatox™ manual using the same DMSO solvent extracts that were prepared for the Microtox organic extract assay (Microbics Corporation, 1992). The Mutatox™ test uses a dark strain of the bioluminescent bacteria, *Vibrio fischeri*, which will revert back to bioluminescent strain if exposed to mutagenic substance. Two assay protocols were utilized. The first, the S-9 assay, utilizes media which contain mammalian hepatic enzymes which can metabolize promutagenic compounds and thus can be used to screen sediments for mutagens which require metabolic activation. The second assay, the direct assay, uses media which contains no mammalian enzymes and thus can be used to screen for mutagens which do not require activation. The mutagenic potential of samples was evaluated using the criteria described in the Microbics Corporations' Mutatox™ Manual. A total of three replicate assays were performed for each spiked sediment. A spiked sediment was considered to be mutagenic if all three replicates met the criteria for mutagenicity.

Chapter 3

Results and Discussion

Analytical Chemistry

Measured concentrations of the contaminant stock solutions were generally similar to nominal values. Measured concentrations were 91.6 ± 3.7% of nominal for cadmium, 107.8 ± 7.2% for DDT and 102.1 ± 2.8% for fluoranthene. Cadmium concentrations measured in spiked sediments used in definitive bioassays were generally quite similar to nominal values, with recoveries ranging from 91-116% of nominal estimates (Table 1). Acid Volatile Sulfide (AVS) levels in these cadmium spiked sediments were low and ranged from 0.019-0.028 μmol/g.

Measured DDT concentrations ranged from 52-96 % of the nominal estimates (Table 2). The mean TOC concentration in these spiked sediments was 0.70%.

Measured fluoranthene concentrations ranged from 78-91% of the nominal values (Table 3). The mean TOC in these spiked sediments was 0.48%. Except where noted, all subsequent discussions of contaminant concentrations will be based on nominal concentrations.

Reference Toxicant Tests

Results obtained from all the reference toxicant tests conducted in conjunction with the Gulf of Mexico Project contaminant bioassays are provided in Appendix A. Only two batches of *P. pugio* failed to meet acceptance criteria. Neither of these tests was associated with a definitive contaminant bioassay. Only one assay using the amphipod, *A. verrilli,* failed to pass acceptance criteria. This assay was repeated with a new batch of animals which passed the reference toxicant criteria limits. All the reference toxicant tests for the definitive assays using the other test species (*A. abdita, B. plicatilis*, *M. mercenaria* and *A. tenuiremis*) provided LC_{50} estimates that met acceptance criteria.

Cadmium

Results obtained for the aqueous cadmium assays for each species are provided in Appendix B and summarized in Table 4. Although a minimum of five replicate cadmium exposures were conducted for each species, some of the test series resulted in either insufficient or excessive responses which precluded computation of an LC_{50} or EC_{50} estimate for that replicate series. Therefore, both the mean LC_{50} (based on only the replicate series which provided an LC_{50} estimate) and a pooled LC_{50} estimate (all replicates combined) are presented. The results using each approach were quite similar in all cases.

Comparison of the results obtained from these aqueous bioassays (Table 4) indicated significant differences among the eight organisms tested ($p < 0.0001$, ANOVA). Based on pairwise multiple comparisons among the species using the Bonferroni test, the juvenile clam, *M. mercenaria*, was the most sensitive species and the copepod, *A. tenuiremis,* was the next most sensitive species. The two amphipod species (*A. abdita* and *A. verrilli*) showed comparable sensitivity to this toxicant and both species were significantly more sensitive to cadmium than the *P. pugio*, Mysid IQ™ or Microtox™ assays. The rotifer, *B. plicatilis*, was the least sensitive species tested.

Table 5 provides a comparison of the 24 h aqueous LC50 values obtained in this study with literature

Table 1. Measured cadmium concentrations and AVS in spiked sediments.

Nominal Concentration (mg/kg dw)	Measured Concentration (mg/kg dw)	SD	% of Nominal	SEM (μmol/g)	AVS (μmol/g)	SEM/AVS
2.5	2.6	0.5	104	0.021	0.02	1.1
10.0	9.5	0.3	95	0.077	0.019	4.1
40.0	37.4	4.6	91	0.315	0.024	13.1
160.0	185.7	28	116	1.247	0.028	44.5

Table 2. Measured DDT and TOC in spiked sediments.

Nominal Concentration (mg/kg dw)	Measured Concentration (mg/kg dw)	SD	% of Nominal	% TOC	DDT Concentration (mg/g OC)
0.64	0.33	0.20	52	0.7	0.09
1.60	1.53	1.34	96	0.7	0.23
4.00	2.58	0.61	65	0.7	0.57
10.00	5.93	0.69	59	0.7	1.43

Table 3. Measured fluoranthene concentrations and TOC in spiked sediments.

Nominal Concentration (mg/kg dw)	Measured Concentration (mg/kg dw)	SD	% of Nominal	% TOC	Fluoranthene Concentration (mg/g OC)
0.78	0.67	0.10	85	0.48	0.16
3.12	2.84	0.39	91	0.48	0.65
12.50	9.79	1.67	78	0.48	2.60
50.00	42.01	4.55	84	0.48	10.42

Table 4. Summary results from aqueous assays with cadmium.

Species	Exposure Period	Mean LC50/EC50 (mg/L)	SD	Statistical Comparisons[1]	Pooled LC50 (mg/L)	Sensitivity Ranking	95% CI (mg/L)
M. mercenaria	24 h	0.4	0.1	A	0.4	1	0.4 - 0.5
A. tenuiremis	24 h	1.5	0.3	B	1.6	2	1.4 - 1.7
A. abdita	24 h	5.7	1.0	C	5.8	3	5.2 - 6.5
A. verrilli	24 h	6.0	1.5	C	5.6	3	5.3 - 6.0
M. bahia	1 h	34.8	10.7	D	34.2	4	26.6 - 43.9
P. pugio	24 h	29.9	8.1	D	31.7	4	23.6 - 42.6
V. fisheri (microtox)	15 min	24.9	3.5	D	NC	4	NC
B. plicatilis	24 h	75.0	3.6	E	74.8	5	71.3 - 78.5

[1] Mean LC50s/EC50s for species sharing the same letter are not significantly different at $\alpha = 0.05$.

Table 5. Sensitivity of selected invertebrate species to cadmium in water column exposures.

Species	Duration	Life Stage[1]	LC50 (μg/L)	Reference
Mysidopsis bahia	96 h	J	12	Cripe, 1994
Palaemonetes pugio	96 h	A	40	Sunda et al., 1978
Homarus americanus	96 h	L	80	Johnson, 1979
Rhepoxynius abronius	96 h	U	147	Hong and Reish, 1987
Penaeus duorarum	96 h	J	312	Cripe, 1994
Callinectes sapidus	96 h	A	320	Frank and Robertson, 1979
Crangon septemspinosa	96 h	A	320	Eisler, 1971
Crassostrea gigas	48 h	E	375	Martin et al., 1981
Palaemonetes vulgaris	96 h	A	420	Eisler, 1971
Mercenaria mercenaria	24 h	J	420	This study
Corophium insidiosum	96 h	U	779	Hong and Reish, 1987
Argopecten irradians	96 h	J	908	Nelson et al., 1976
Amphiascus tenuiremis	24 h	A	1,500	This study
Mya arenaria	96 h	A	2,200	Eisler, 1971
Ampelisca abdita	24 h	J	5,700	This study
Ampelisca verrilli	24 h	A/J	6,000	This study
Palaemonetes pugio	48 h		13,000	Burton and Fisher, 1990
Palaemonetes pugio	24 h	A	29,900	This study
Mysidopsis bahia	1 h	J	34,800 [2]	This study
Brachionus plicatilis	24 h	J	75000	This study

[1] J = juvenile, L = larvae, U = unknown, A = adult, E = embryo
[2] EC50 for fluorescence reduction in Mysid IQ® test

values for other invertebrate species. Little comparable data was available to assess the relative assay and this species was not retested. The value for this species shown in Table 6 is from the muddy sediment bioassay. M. mercenaria was the most sensitive species to cadmium-spiked sediments with sensitivity of the species used in this study with others cited in the literature, since most of the published values were for longer duration exposures. As stated previously, the main purpose of the short-term aqueous bioassays was to provide a basis for comparing the inherent sensitivity of each species used in this study and to relate this inherent sensitivity to the results obtained from the sediment bioassays. Results obtained from the definitive 10-day sediment bioassays (*P. pugio*, *A. verrilli*, *A. abdita, M. mercenaria and A. tenuiremis*), the 24 h *B. plicatilis* sediment porewater assay and the Microtox™ and Mutatox™ bioassays are provided in Appendix B and the results for all species are summarized in Table 6. Preliminary 10-day spiked-sediment bioassays with *P. pugio*, *A. abdita* , *A. verrilli* and *A. tenuiremis* were conducted with a muddy sediment collected from North Inlet, South Carolina. No significant contaminant-related mortality was observed in any of the test species at concentrations as high as 36 mg/Kg dw (*P. pugio*, *A. abdita* , *A. verrilli*) and 45 mg/Kg dw (*A. tenuiremis*). Subsequent AVS analysis revealed extremely high AVS levels (>7 μmol/g) in these sediments which explained the lack of cadmium toxicity. All subsequent spiked-sediment bioassays were conducted with a much sandier sediment collected from Folly Beach, South Carolina which was autoclaved prior to spiking. The AVS levels in this autoclaved sediment were much lower (<0.03

Table 6. Summary of results from sediment assays with cadmium.

Species	Exposure Period	Mean LC50/EC50 (mg/kg)	SD	Statistical Comparisons[1]	Pooled LC50 (mg/kg)	Sensitivity Ranking	95% CI (mg/kg)
M. mercenaria	10 d	< 2.5	----	----	----	1	----
A. verrilli	10 d	4.8	0.4	A	4.5	2	3.9 - 5.1
A. abdita	10 d	12	4.3	A, B	11.8	2	9.9 - 14.2
P. pugio	10 d	18.2	0.2	A, B	17.9	2	16.2 - 19.9
B. plicatilis	24 h	41.5	11.7	B	41.9	3	35.8 - 49.1
A. tenuiremis	10 d	> 45	----	----	----	4	----
V. fisheri (microtox)	5 min	16021603	----	----	----	44	----
V. fisheri (mutatox)			----	----	----		----

[1] Mean LC50s/EC50s for species sharing the same letter are not significantly different at $\alpha = 0.05$.
[2] Lowest sediment cadmium concentration which caused a significant reduction in bioluminescence relative to control in the microtox solid phase bioassay. This was the most sensitive microtox endpoint evaluated.
[3] Lowest sediment cadmium concentration which gave a positive response in the mutatox screening assay.

μmol/g). Unfortunately, the sandy nature of this sediment made it unsuitable for the *A. tenuiremis* assay and this species was not retested. The value for this species shown in Table 6 is from the muddy sediment bioassay.

M. mercenaria was the most sensitive species to cadmium-spiked sediments with 100% mortality at 2.5 mg/kg dw, the lowest concentration tested. *A. verrilli*, *A. abdita* and *P. pugio* were comparable in sensitivity and were the next most sensitive group. The *B. plicatilis* porewater assay was less sensitive than *A. verrilli*, but was not different from the *A. abdita* and *P. pugio* assays. The Microtox™ and Mutatox™ bioassays were the least sensitive of the assays conducted with comparable sediments. Comparisons of aqueous versus sediment toxicity testing indicated generally similar relative sensitivities to cadmium. The copepod, *A. tenuiremis*, was an exception since its relative sensitivity was greater in the aqueous exposure (Tables 4 and 6).

Table 7 provides a comparison of the sensitivity of the species used in this study with other measures of cadmium toxicity from the literature. *M. mercenaria* was the only species tested which showed significant toxicity near the reported TEL and ER-L levels of 0.7 mg/Kg dw and 1.2 mg/Kg dw, respectively. Both amphipod species and the grass shrimp were sensitive to cadmium-spiked sediments at concentrations near the ER-M of 9.6 mg/Kg dw (Long et al., 1995). The remainder of the species tested in this study were only sensitive to cadmium-spiked sediments at concentrations which exceeded the ER-M. As was noted earlier, AVS levels were quite low in the cadmium-spiked sediments used in this study. The SEM/AVS ratio was >1.0 at all cadmium spike levels (Table 1). DiToro et al. (1990) has reported that an SEM/AVS ratio > 1.0 is necessary for the manifestation of cadmium-induced toxicity from sediments. The lowest cadmium spike level (2.5 mg/Kg dw) which caused toxicity in *M. mercenaria* had an SEM/AVS ratio (1.1) which was very near this reported minimum threshold for toxic effects. This suggests that this species is one of the most sensitive organisms tested.

DDT

Results obtained for the 24 h aqueous DDT assays for each of the test species are provided in Appendix C and summarized in Table 8. Results indicated that the *P. pugio* and the Mysid IQ™ tests were the most sensitive endpoints evaluated. *A. verrilli* was the next most sensitive species, with *M. mercenaria* being the third most sensitive species to DDT. The remaining four species were insensitive to DDT at the highest concentration tested (10,000 μg/l). The large differences in apparent sensitivity may have resulted, in part, due to the limited solubility of DDT in water. The reported solubility of DDT in water is~35 μg/l, thus much of the DDT may have been unavailable at the higher exposure concentrations. Table 9 provides a comparison of the 24 h aqueous LC50 values obtained in this study with literature

Table 7. Comparison of the relative toxicity of test species to cadmium in sediment exposures versus other measures of cadmium toxicity.

Species	Significant Effects Concentration mg/kg dry wt.	Effects Code	Source
TEL	0.7		MacDonald, 1994
ER-L	1.2		Long et al., 1995
Mercenaria mercenaria	<2.5	A	This Study
PEL	4.2		MacDonald, 1994
Ampelisca verrilli	4.8	A	This Study
ER-M	9.6		Long et al., 1995
Rhepoxynius abronius	9.8	A	Mearns et al, 1986
Ampelisca abdita	12.0	A	This Study
Palaemonetes pugio	18.2	A	This Study
Brachionus plicatilis	41.5	B	This Study
Brachionus plicatilis	56.8	C	Snell and Persoone,1989
Vibrio fischeri (Microtox)	160	D	This Study
Amphiascus tenuiremis	>45.0	A	This Study
Ampelisca abdita	2600	A	DiToro et al., 1990

A = LC50 in 10 day sediment exposure
B = LC50 in 24 hr exposure to sediment porewater extract
C = LC50 in 24 hr exposure to 30ppt seawater
D = Lowest concentration to elicit significant reduction in fluorescence compared with controls.

Table 8. Summary of results from aqueous assays with DDT.

Species	Exposure Period	Mean LC50/EC50 (μg/L)	SD	Statistical Comparisons[1]	Pooled LC50 (μg/L)	Sensitivity Ranking	95% CI (μg/L)
M. bahia	1 h	5.9	1.7	A	5.1	1	3.9 - 6.6
P. pugio	24 h	9.5	2.3	A	8.9	1	7.5 - 10.5
A.verrilli	24 h	39.8	27.6	B	38.3	2	32.4 - 45.3
M. mercenaria	24 h	612	135	C	615	3	358 - 1057
A. abdita	24 h	>10,000	----	----	----	4	----
A.tenuiremis	24 h	> 10,000	----	----	----	4	----
B. plicatilis	24 h	> 10,000	----	----	----	4	----
V. fisheri (microtox)	15 min	> 10,000	----	----	----	4	----

[1] Mean LC50s/EC50s for species sharing the same letter are not significantly different at $\alpha = 0.05$.

Table 9. Sensitivity of selected invertebrate species to DDT in water column exposures.

Species	Duration	Lifestage[1]	LC_{50} (μg/L)	Reference
Daphnia magna	48 h	U	0.4	Frear and Boyd, 1967
Gammarus fasciatus	96 h	U	0.8	Sanders, 1972
Gammarus lacustris	96 h	U	1	Sanders, 1969
Palaemonetes vulgaris	96 h	U	2	Eisler, 1969
Asselus brevicaudus	96 h	U	4	Sanders, 1972
Daphnia magna	24 h	U	4.4	Sanders and Cope, 1966
Mysidopsis bahia	1 h	J	5.9 [2]	This study
Palaemonetes pugio	24 h	A	9.5	This study
Callinectes sapidus	96 h	U	19	Mahood et al., 1970
Ampelisca verrilli	24 h	J	39.8	This study
Mercenaria mercenaria	24 h	J	612	This study
Ampelisca abdita	24 h	J	>10,000	This study
Brachionus plicatus	24 h	J	>10,000	This study
Amphiascus tenuiremis	24 h	A	>10,000	This study

[1] J = juvenile, L = larvae, U = unknown, A = adult, E = embryo
[2] EC_{50} for fluorescence reduction

Table 10. Summary of results from sediment assays with DDT.

Species	Exposure Period	Mean LC_{50}/EC_{50} (mg/kg)	SD	Statistical Comparisons[1]	Pooled LC50 (mg/kg)	Sensitivity Ranking	95% CI (mg/kg)
A. tenuiremis	10 d	1.0 [2]	----		----	1	----
P. pugio	10 d	4.5	0.5	A	4.5	2	3.6 - 5.6
M. mercenaria	10 d	5.8	1.1	----	6.3	2	4.8 - 8.3
A. abdita	10 d	8.2	0.8	B	8.5	3	7.2 - 10.0
A. verrilli	10 d	8.3	0.9	B	8.5	3	7.2 - 10.0
A. tenuiremis	10 d	> 10.0	----	----	> 10.0	4	----
B. plicatilis	24 h	> 10.0	----	----	> 10.0	4	----
V. fisheri (microtox)	5 min	> 10.0	----	----	> 10.0	4	----
V. fisheri (microtox)	----	> 10.0	----	----	----	----	----

[1] Mean LC50s/EC50s for species sharing the same letter are not significantly different at $\alpha = 0.05$.
[2] Concentration which caused significant reduction in clutch size.

Table 11. *Comparison of the relative toxicity of test species to DDT in sediment exposures versus other measures of DDT toxicity.*

Species	Significant Effects Concentration mg/kg dry wt.	mg/g OC	Effects Code	Source
ER-L	*0.0016*			*Long et al., 1995*
TEL	*0.0028*			*MacDonald, 1994*
Crangon septemspinosa	*0.0310*		*A*	*McLeese and Metcalfe, 1980*
ER-M	*0.0461*			*Long et al., 1995*
PEL	*0.0517*			*MacDonald, 1994*
Amphiiascus tenuiremis	*1*		*B*	*This Study*
Palaemonetes pugio	*4.5*	*0.6*	*C*	*This Study*
Mercenaria mercenaria	*5.8*	*0.8*	*C*	*This Study*
Ampelisca verrilli	*8.3*	*1.2*	*C*	*This Study*
Ampelisca abdita	*8.2*	*1.2*	*C*	*This Study*
Rhepoxynius abronius		*1.0*	*C*	*Swartz et al., 1994*
Eohaustorius estuarius		*2.5*	*C*	*Swartz et al., 1994*
Hyalella azteca		*2.6*	*C*	*Swartz et al, 1994*
Brachionus plicatilis	*>10.0*	*>1.4*	*D*	*This Study*
Vibrio fischeri (Microtox)	*>10.0*	*>1.4*	*E*	*This Study*
Amphiascus tenuiremis	*>10.0*	*>1.4*	*C*	*This Study*
Nereis virens	*>16.5*		*F*	*McLeese et al, 1982*

A = LC50 in 96 hr sediment exposure
B = Significant decrease in clutch size from control after 10 d exposure
C = LC50 in 10 d sediment exposure
D = LC50 in 24 hr exposure to sediment porewater extract
E = Lowest concentration to elicit significant reduction in fluorescence compared with controls.
F = LC50 in 12 d sediment exposure

values for other invertebrate species. Although little comparable data was available for this exposure period, the 24 h LC50 (4.4 μg/l) reported for *D. magna* was similar t the values obtained for *P. pugio* (9.5 μg/l) and *M. bahia* (5.9 μg/l) in this study.

The results from the sediment DDT assays (Appendix C, Table 10) indicated that *M. mercenaria* and *P. pugio* were the two most sensitive species tested based on mortality endpoints and both were more sensitive than the two amphipod species. *A. tenuiremis* was insensitive to DDT-induced mortality at the highest concentration tested (10 mg/kg dw);however, clutch size was reduced in this species at concentrations as low as 1 mg/kg dw (Table 10). Both the *B. plicatilis* porewater assay and the Microtox™ bioassay were insensitive to DDT at the highest concentration tested. In general, all of the LC50 and EC50 values obtained for the species used in this study were higher than the reported TEL, ER-L, ER-M and PEL values (Table 11, MacDonald, 1994; Long et al., 1995).

Fluoranthene

Results obtained for the individual aqueous fluoranthene assays are provided in Appendix D and mortality was observed in most species after 24 h of exposure at the highest concentration tested (800 summarized in Table 12. Due to the fact that <50% μg/l) which was at the limit of solubility, the duration of these tests was extended to 96 h. Both 24 h and 96 h data are presented for *M. mercenaria*. *A. abdita* and *M. mercenaria* were the two most sensitive species tested and both were slightly more sensitive

than *A. verrilli*. *P. pugio* was the next most sensitive organism. The remaining four organisms were insensitive to fluoranthene at the highest concentrations tested. In the Mysid IQ™ test, the organisms exposed to higher fluoranthene concentrations exhibited greater fluorescence than the control organisms. This may have been due to an artifact of the assay protocol since fluoranthene also fluoresces under UV light and uptake of this contaminant by the mysids may have masked any stress-induced reduction in fluorescence. In general, the aqueous LC50s determined for the species used in this study were similar to those reported for other invertebrates (Table 13).

Individual sediment fluoranthene assay results are shown in Appendix D and the results for all species are summarized in Table 14. *M. mercenaria* was by far the most sensitive species to fluoranthene, with > 50% mortality at the lowest concentration tested (0.78 mg/Kg dw). Comparison of aqueous and sediment bioassays with fluoranthene indicated generally similar relative sensitivities for the test species in both exposure matrices (Table 12 and 14). The estimated LC50 (<0.11 mg/g OC/<0.8 mg/Kg dw) for juvenile *M. mercenaria* was lower than the EPA SQC (0.3 mg/g OC) and was similar to the ER-L (0.6 mg/Kg dw) reported by Long et al. (1995). This suggests t hat this is one of the most sensitive species tested and that these criteria may not adequately protect this organism. The next most sensitive species was A. abdita which experienced significant mortality (45% at 50 mg/Kg dw). The remaining species were insensitive to fluoranthene at the highest concentration tested (50 mg/Kg dw) which exceeded the ER-M of 5.1 mg/Kg dw reported by Long et al. (1995) (Table 15).

Table 12. Summary of results from aqueous assays with fluoranthene.

Species	Exposure Period	Mean LC50/EC50 (μg/L)	SD	Statistical Comparisons[1]	Pooled LC50 (μg/L)	Sensitivity Ranking	95% CI (μg/L)
A. abdita	96 h	60.5	12	A	59	1	55 - 63
M. mercenaria	96 h	< 104	----	----	----	1	----
A.verrilli	96 h	113	30	B	108	2	97 - 119
P. pugio	96 h	595	175	C	565	3	411 - 776
A.tenuiremis	96 h	>1600	----	----	----	4	----
B. plicatilis	48 h	> 500	----	----	----	4	----
M. bahia	1 h	> 800	----	----	----	4	----
V. fisheri (microtox)	5 min	> 1100	----	----	----	4	----
M. mercenaria	24 h	652	120	----	735		441 - 1225

[1]Mean LC50s/EC50s for species sharing the same letter are not significantly different at $\alpha = 0.05$.

Table 13. Sensitivity of selected invertebrate species to fluoranthene in water column exposures.

Species	Duration	Life Stage[A]	LC50 (μg/L)	Reference
Mysidopsis bahia	96 h	J	40	EPA, 1978
Ampelisca abdita	96 h	J	60.5	This study
Ampelisca abdita	96 h	J	66.9	Champlin and Poucher, 1991
Mercenaria mercenaria	96 h	J	< 104	This study
Ampelisca verrili	96 h	J	112.7	This study
Palaemonetes pugio	96 h	L	122	Frasca, 1995
Palaemonetes pugio	96 h	J	142.5	Champlin and Poucher, 1991
Neanthes arenaceodentata	96 h		500	Rossi and Neff, 1978
Brachionus plicatus	48 h	L	>500	This study
Palaemonetes pugio	96 h	A	594.6	This study
Mercenaria mercenaria	24 h	J	652	This study
Amphiascus tenuiremis	96 h	A	>1600	This study
Mulinia lateralis	96 h	J	10710	Champlin and Poucher, 1991

[1]J = juvenile, L = larvae, U = unknown, A = adult, E = embryo

Table 14. Summary of results from sediment assays with fluoranthene.

Species	Exposure Period	Mean LC50/EC50 (mg/kg)	SD	Statistical Comparisons	Pooled LC50 (mg/kg)	Sensitivity Ranking	95% CI (mg/kg)
M. mercenaria	10 d	< 0.8	----	----	< 0.8	1	----
V. fisheri (mutatox)	----	3-50[1]	----	----	----	2	----
A. abdita	10 d	> 50[2]	----	----	> 50	2	----
A. tenuiremis	10 d	> 50	----	----	> 50	3	----
A. verrilli	10 d	> 50	----	----	> 50	3	----
B. plicatilis	24 h	> 50	----	----	> 50	3	----
P. pugio	10 d	> 50	----	----	> 50	3	----
V. fisheri (microtox)	5 min	> 50	----	----	> 50	3	----

[1]Assay screened positive for mutagenicity in sediment samples spiked with fluoranthene at 3.12 and 50 ppm but not at 12.5 ppm.
[2]Significant mortality (45%)

Table 15. Comparison of the relative toxicity of test species to fluoranthene in sediment exposures versus other measures of fluoranthene toxicity

Species	Significant Effects Concentration mg/kg dry wt.	mg/g OC	Effects Code	Source
TEL	0.1			MacDonald, 1994
ER-L	0.6			Long et al., 1995
Mercenaria mercenaria	<0.8	<0.11	A	This Study
EPA SQC		0.3		EPA, 1993
PEL	1.5			MacDonald, 1994
Hyalella azteca	2.3 - 7.4	0.5 - 1.5	A	Suedel et al., 1993
Rhepoxynius abronius	3.4 - 10.7	1.9 - 2.2	A	Swartz et al., 1990
ER-M	5.1			Long et al., 1995
Rhepoxinius abronius	8.7 - 19.1	1.4 - 4.4	A	DeWitt et al., 1992
Ampelisca abdita	50	10.4	B	This Study
Ampelisca verrilli	>50.0	>10.4	B	This Study
Palaemonetes pugio	>50	>10.4	B	This Study
Brachionus plicatilis	>50.0	>10.4	B	This Study
***Vibrio fischeri* (Microtox)**	>50	>10.4	C	This Study
Amphiascus tenuiremis	>50.0	>10.4	B	This Study

A = LC50 in 10 day sediment exposure
B = Significant mortality < 80% of control survival
C = LC50 in 24 hr exposure to sediment porewater extract
D = Lowest concentration to elicit significant reduction in fluorescence compared with controls

Chapter 4

Summary and Conclusions

The juvenile clam was the most sensitive species to cadmium in both aqueous and sediment exposures. The sensitivities of the two amphipod species and the grass shrimp to cadmium were similar in both water and sediment exposures, while the rotifer assay was generally less sensitive. The Microtox™ assay was relatively sensitive to cadmium in the aqueous assay, but insensitive to sediment-associated cadmium. The copepod assay was sensitive to cadmium in the aqueous assay; however, its sensitivity to sediment-associated cadmium could not be compared with the other test species. For the most part, the relative sensitivity of the test organisms to sediment-associated cadmium paralleled their sensitivity in the aqueous tests. Only the clam assay was sensitive to sediment-associated cadmium at concentrations near the ER-L (1.2 mg/Kg dw) and TEL (0.7 mg/Kg dw) values. The remaining species were only sensitive at concentrations ≥ ERM (9.6 mg/Kg dw) and PEL (4.2 mg/Kg dw) levels.

The grass shrimp and Mysid IQ™ assays were most sensitive to DDT in aqueous exposures, with *A. verrilli* being the next most sensitive species. *M. mercenaria* was ~10x less sensitive than *A. verrilli*. The remaining assays were ≥ 10x less sensitive than *M. mercenaria*. These apparent large differences may have been due, in part, to the limited solubility of DDT in water (~35 μg/L). DDT concentrations which exceeded the solubility would be mostly unavailable for uptake. The differences of the test species to sediment-associated DDT were less dramatic than those observed in the aqueous tests. The most sensitive species (*P. pugio and M. mercenaria*) were only slightly more sensitive than the two amphipods. The remaining species were insensitive to DDT at the highest concentration tested (10 mg/kg dw). Survival of adult copepods was not affected at 10 mg/Kg dw; however, reproductive output was depressed at DDT concentrations as low as 1mg/kg dw. These findings suggest that DDT may cause sublethal effects in many species at concentrations well below those producing acute toxicity. None of the species tested in this study were sensitive to DDT at concentrations near the ER-L (0.0016 mg/Kg dw) or ER-M (0.0461 mg/Kg dw).

The juvenile clam was the most sensitive species to fluoranthene in both aqueous and sediment exposures and was sensitive to sediment-associated fluoranthene at concentrations at or below the ER-L of 0.6 mg/Kg dw and the EPA sediment quality criterion of 0.3 mg/g OC. The remaining species tested were generally only sensitive to fluoranthene at concentrations ≥ 50 mg/Kg dw.

Overall, the juvenile clam was the most sensitive species tested in this study from an acute toxicity standpoint. The grass shrimp and the two amphipod species were generally similar in sensitivity to each of the three compounds. The copepod assay, although relatively insensitive in terms of adult mortality, was capable of detecting sublethal effects at contaminant concentrations below those which caused mortality in the other more sensitive species. Both the juvenile clam assay and the copepod partial life cycle test have the potential to serve as sensitive indicators of potential sediment-associated toxicity which might not be detected using standard acute toxicity bioassays.

Comparisons of ERL/TEL and ERM/PEL sediment quality guidelines generally indicated that the most sensitive species tested (e.g., Cd-clam, DDT-copepod reproduction and fluoranthene-clam) were sensitive at concentrations at or just above the ERL

values for Cd and fluoranthene. The remaining test species were sensitive to these compounds at concentrations just below or above the ERM. The lack of sensitivity in our suite of bioassays to DDT suggests that existing ERL/ERM and TEL/PEL guidelines may be overly protective. Our most sensitive species value based on copepod reproduction is nearly two orders of magnitude higher than the ERM/PEL guidelines. In sediments where DDT is the only contaminant, our findings suggest that these guidelines may overestimate potential toxicity.

The differing species sensitivities observed with the different classes of chemical contaminants in this study suggest that a multiple species approach may be more appropriate for a holistic ecological risk assessment of sediment contamination. The "Crustacean Triad" (copepods, amphipods and grass shrimp) provide a battery of tests which predict toxicity to epibenthic and benthic crustaceans with known sensitivity to a variety of chemical contaminants and represent the base of the food chain for most recreationally and commercially important finfish species which utilize estuarine nursery grounds. The addition of the juvenile clam assay provides a herbivorous filter feeder with the ability to bioconcentrate pollutants and which is extremely sensitive in the size range tested (>212<350 μm). Field studies in South Carolina have indicated that sites with high sediment contaminant levels have degraded benthos, with significant effects observed in crustaceans and molluscs (F. Holland, South Carolina Department of Natural Resources, personal communication). These findings support our laboratory results and suggest that an integrated battery of assays may be most appropriate for estimating field effects.

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Appendix A

Summary of results obtained from the SDS reference toxicant tests using *P. pugio*, *A. verrilli*, *A. abdita*, *M. mercenaria*, *A. tenuiremis* and from the potassium dichromate reference toxicant tests using *B. plicatilis*

Table A-1. SDS reference toxicant bioassay results for the Gulf of Mexico Project using *P. pugio.* All concentrations are in mg/L.

			Acceptable Test Criteria			
Test Date	Comments	LC50	Average LC50	(-) 2 Stand. Dev	(+) 2 Stand. Dev	SDS Pass/Fail
10:22 AM	Baseline	140.20	140.20	NC	NC	
10:22 AM	Baseline	108.30	124.25	76.60	171.90	
10:22 AM	Baseline	154.90	134.47	91.01	177.92	
10:22 AM	Baseline	115.10	129.63	91.51	167.74	
10:22 AM	Baseline	136.40	130.98	95.10	166.86	
10:22 AM	Baseline	117.30	128.70	90.58	166.82	
10:22 AM	Cd Aqueous (defin.)	102.90	125.01	71.29	178.74	Pass
10:22 AM	Cd Trial Sediment	182.30	132.18	81.61	182.74	Fail
10:22 AM	Cd Trial Sediment	140.50	133.10	69.36	196.84	Pass
10:22 AM	DDT Aq. Rangefinder	200.00	139.79	77.72	201.86	Fail
10:22 AM	DDT Aq. Rangefinder	163.00	141.90	82.25	201.55	Pass
10:22 AM	DDT Aq. Rangefinder	154.90	142.98	85.86	200.11	Pass
10:22 AM	DDT Aqueous (defin.)	145.30	143.16	86.47	199.85	Pass
10:22 AM	Cd Sediment (defin.)	116.60	141.26	86.55	195.98	Pass
10:22 AM	DDT Sediment (defin.)	147.20	141.66	88.39	194.93	Pass
10:22 AM	Fluor Aqueous (defin.)	154.90	142.49	90.51	194.46	Pass
10:22 AM	Fluor Sediment (defin.)	129.30	141.71	89.74	193.69	Pass
	Final Average		**141.71**			

Table A-2. SDS reference toxicant bioassay results for the Gulf of Mexico Project using the amphipod, *Ampelisca abdita*. All concentrations are in mg/l.

			Acceptable Test Criteria			
Test Date	Comments	LC_{50}	Mean LC_{50}	(-) 2 Stand Dev	(+) 2 Stand Dev	SDS Pass/Fail
10:22 AM	baseline	16.5	16.5	NC	NC	
3/16/95	baseline	22.8	19.6	10.7	28.5	
3/16/95	baseline	22.6	20.6	13.4	27.8	
3/16/95	baseline	18.8	20.1	14.0	26.3	
3/16/95	baseline	17.4	19.6	13.8	25.4	
3/28/95	Cd aqueous	20.5	19.8	14.5	25.0	Pass
3/28/95	Cd aqueous	21.6	20.0	15.0	25.0	Pass
5/2/95	Cd sediment	21.6	20.2	15.4	25.0	Pass
5/2/95	Cd sediment	19.5	20.1	15.6	24.6	Pass
6/26/95	DDT aqueous	25.2	20.7	15.3	26.0	Pass
6/26/95	DDT aqueous	20.5	20.6	15.6	25.7	Pass
10/2/95	Cd sediment	19.5	20.5	15.7	25.4	Pass
12/11/95	DDT range finder	21.6	20.6	15.9	25.3	Pass
12/18/95	DDT aqueous	25.2	21.0	15.8	26.1	Pass
12/18/95	DDT aqueous	24.0	21.2	16.0	26.3	Pass
1/23/96	DDT sediment	17.6	20.9	15.6	26.2	Pass
1/23/96	DDT sediment	21.6	21.0	15.8	26.1	Pass
2/26/96	Fluoranthene aqueous	17.6	20.8	15.6	26.0	Pass
2/26/96	Fluoranthene aqueous	19.4	20.7	15.6	25.8	Pass
3/26/96	Fluoranthene sediment	20.6	20.7	15.7	25.7	Pass
3/26/96	Fluoranthene sediment	20.6	20.7	15.8	25.6	Pass
	Final Average		**20.7**			

Table A-3. SDS reference toxicant bioassay results for the Gulf of Mexico Project using the amphipod, *Ampelisca verrilli*. All concentrations are in mg/l.

				Acceptable Test Criteria		
Test Date	Comments	LC_{50}	Mean LC_{50}	(-)2 Stand Dev	(+)2 Stand Dev	SDS Pass/Fail
1/18/95	range finder	66.5	66.5	NC	NC	
1/19/95	baseline data	65.0	65.8	63.6	67.9	
1/19/95	baseline data	61.7	64.4	59.5	69.3	
10:22 AM	baseline data	58.5	62.9	55.8	70.1	
1/19/95	baseline data	54.5	61.2	51.5	71.0	
1/19/95	baseline data	58.2	60.7	51.6	69.8	
2/2/95	baseline data	58.2	60.4	51.8	68.9	
2/2/95	baseline data	66.0	61.1	52.2	69.9	
10:22 AM	baseline data	65.0	61.5	52.8	70.2	
10:22 AM	baseline data	42.3	59.6	44.9	74.2	
10:22 AM	baseline data	58.7	59.5	45.6	73.4	
4/17/95	Cd aqueous	53.0	59.0	45.2	72.7	Pass
10:22 AM	Cd sediment	47.9	58.1	43.5	72.7	Pass
10:22 AM	Cd sediment	54.3	57.8	43.7	72.0	Pass
10:22 AM	DDT aqueous	33.5	NC			Fail
10:22 AM	DDT aqueous	39.7	NC			Fail
10:22 AM	DDT aqueous	42.1	56.8	40.9	72.6	Pass
10:22 AM	DDT aqueous	45.5	56.1	39.7	72.4	Pass
10:22 AM	baseline data	47.9	55.6	39.3	71.9	
10:22 AM	baseline data	45.5	55.0	38.5	71.6	
10:22 AM	baseline data	45.1	54.5	37.8	71.2	
10:22 AM	baseline data	41.1	53.8	36.5	71.2	
10/2/95	Cd sediment	47.9	53.5	36.4	70.6	Pass
10:22 AM	DDT aqueous	58.7	53.8	37.0	70.6	Pass
10:22 AM	DDT aqueous	45.5	53.4	36.6	70.2	Pass
10:22 AM	DDT sediment	40.5	52.9	35.6	70.1	Pass
10:22 AM	DDT sediment	45.5	52.6	35.4	69.7	Pass
10:22 AM	Fluoranthene aqueous	45.5	52.3	35.3	69.3	Pass
10:22 AM	Fluoranthene aqueous	45.5	52.1	35.1	69.0	Pass
10:22 AM	Fluoranthene sediment	55.8	52.2	35.5	68.8	Pass
10:22 AM	Fluoranthene sediment	55.1	52.3	35.9	68.7	Pass
	Final Average		**52.3**			

Table A-4. SDS reference toxicant bioassays for the Gulf of Mexico Project using *Amphiascus* tenuiremis.

Test Date	Comments	SDS LC50	Average LC50	Acceptable Test Criteria (-) 2 Stand Dev	Acceptable Test Criteria (+) 2 Stand Dev	SDS Pass/Fail
10:22 AM		12.13	12.13	NC	NC	
10:22 AM	Baseline	10.52	11.33	9.05	13.60	
10:22 AM	Baseline	12.36	11.67	9.66	13.68	
10:22 AM	Baseline	12.99	12.00	9.90	14.10	
10:22 AM	Baseline	16.86	12.97	8.26	17.69	
10:22 AM	Cd Aqueous	14.76	13.27	8.81	17.73	Pass
10:22 AM	Cd Sediment	14.24	13.41	9.27	17.55	Pass
10:22 AM	DDT (aqueous and sediment)	13.43	13.41	9.58	17.24	Pass
10:22 AM	Fluor (aqueous and sediment)	13.97	13.47	9.87	17.08	Pass
	Final Average		**13.47**			

Table A-5. SDS reference toxicant bioassay results for the Gulf of Mexico Project using M. mercenaria. All concentrations are in mg/L.

Test Date	Comments	LC50	Average LC50	Acceptable Test Criteria (-) 2 Stand. Dev	Acceptable Test Criteria (+) 2 Stand. Dev	SDS Pass/Fail
6/22/95	Baseline	6.29	6.29	NC	NC	
6/22/95(B)	Baseline	6.04	6.17	5.81	6.52	
6/28/95(A)	Baseline	8.27	6.87	4.43	9.30	
6/28/95(B)	Baseline	6.13	6.68	4.56	8.80	
6/28/95(C)	Baseline	7.87	6.92	4.80	9.04	
8/31/95	DDT Aq. Rangefinder	8.26	7.14	4.95	9.33	Pass
10/4/95	Cd Aqueous (defin.)	5.98	6.98	4.79	9.16	Pass
2/21/96	DDT Sediment (defin.)	7.92	7.09	4.97	9.22	Pass
3/7/96	DDT Aqueous (defin.)	6.13	6.99	4.90	9.08	Pass
3/19/96	Fluor Aqueous (defin.)	7.74	7.06	5.03	9.09	Pass
3/30/96	Fluor Sediment (defin.)	7.80	7.13	5.15	9.11	Pass
5/20/96	Cd Sediment (defin.)	7.43	7.16	5.26	9.05	Pass
	Final Average		**7.16**			

Table A-6. Potassium dichromate reference toxicant bioassay results for the Gulf of Mexico Project using the rotifer, *B. plicatilis*. All concentrations are in mg/l.

			Acceptable Test Criteria		
Test Date	Comments	LC_{50}	Batch LC50	lower CI	upper CI
1/18/95	baseline	304.2	323.0	226.0	420.0
10:22 AM	baseline	261.8	323.0	226.0	420.0
10:22 AM	Cd aqueous	303.6	323.0	226.0	420.0
10:22 AM	Cd porewater (lower)	324.7	323.0	226.0	420.0
10:22 AM	Cd porewater (higher)	339.1	323.0	226.0	420.0
10:22 AM	DDT aqueous	278.4	323.0	226.0	420.0
10:22 AM	DDT porewater	301.2	323.0	226.0	420.0
10:22 AM	Fluoranthene aqueous	314.6	323.0	226.0	420.0
10:22 AM	Fluoranthene sediment	315.4	323.0	226.0	420.0

Appendix B.

Results obtained from Cadmium aqueous and sediment bioassays

Toxicant: Cadmium (mg/L)
Matrix: Aqueous

Species: *Palaemonetes pugio*

Duration	Replicate	% Mortality							
		Control	4.8	8	13	22	36.8	61.3	LC_{50}
24 h	A	0	0	20	40	20	60	50	33.2
	B	0	20	10	10	50	60	40	36.8
	C	0	10	20	40	30	30	80	28.5
	D	10	30	40	40	80	40	90	16.4
	E	10	30	40	10	30	50	80	34.5
	Mean	4	18	26	28	42	52	68	29.9
	SD								8.1
	Pooled								31.67
	95% CI								(23.57, 42.56)

Species: *Ampelisca abdita*

Duration	Replicate	% Mortality							
		Control	1.4	2.2	3.7	6.2	10.3	17.2	LC_{50}
24 h	A	0	30	10	20	60	90	100	5.5
	B	0	20	20	60	50	80	90	4.4
	C	10	0	0	40	40	80	100	5.8
	D	0	0	10	50	50	60	80	5.8
	E	0	0	10	20	50	40	100	7.2
	Mean	2	10	10	38	50	70	94	5.7
	SD								1
	Pooled								5.8
	95% CI								(5.2, 6.5)

Species: *Ampelisca verrilli*

Duration	Replicate	% Mortality							
		Control	1.9	3.2	5.4	9	15	25	LC_{50}
24 h	A	10	20	20	40	50	50	20	NC
	B	0	0	50	0	80	50	70	8.1
	C	0	30	20	70	70	80	80	4.6
	D	0	0	30	70	60	50	70	6.1
	E	0	10	10	60	40	60	60	7.7
	F	0	0	0	40	80	100	100	6.3
	G	10	10	10	90	90	100	100	4.2
	H	10	0	10	80	80	100	100	4.8
	Mean	4	9	20	56	69	74	78	6
	SD								1.5
	Pooled								5.6
	95% CI								(5.3, 6.0)

Species: *Amphiascus tenuiremis*

Duration	Replicate	% Mortality						
		Control	0.6	1.1	1.8	3	5	LC_{50}
24 h	A	0	55	45	45	80	90	1.1
	B	0	10	10	55	85	95	1.8
	C	5	10	10	50	80	80	1.9
	D	0	5	30	55	95	95	1.6
	E	5	5	25	95	100	100	1.3
	Mean	2	17	24	60	88	92	1.5
	SD							0.3
	Pooled							1.6
	95% CI							(1.4, 1.7)

Toxicant: Cadmium (mg/L)
Matrix: Aqueous

Species: *Mercenaria mercenaria*

Duration	Replicate	% Mortality Control	0.2	0.3	0.5	0.9	1.5	2.45	LC_{50}
24 h	A	0	0	20	100	100	100	100	0.37
	B	10	20	10	30	80	90	100	0.63
	C	10	10	20	50	100	100	100	0.48
	D	0	10	60	80	100	90	100	0.32
	E	0	10	50	100	100	80	100	0.32
	Mean	4	10	32	72	96	92	100	0.42
	SD								0.14
	Pooled								0.4
	95% CI								(0.35, 0.45)

Species: *Brachionus plicatilis*

Duration	Replicate	% Mortality Control	12	20	33	55	92	LC_{50}
24 h	A	0	0	0	3	13	73	75.6
	B	3	0	0	3	13	70	76.9
	C	0	0	0	3	13	70	76.9
	D	0	0	0	3	7	73	76.9
	E	3	0	3	7	20	87	68.6
	Mean	1	0	1	4	13	75	75
	SD							3.6
	Pooled							74.8
	95% CI							(71.3, 78.5)

Species: *Mysidopsis bahia*

Duration	Replicate	% Not Fluorescing Control	8	13.2	16	22.1	26.5	36.8	44.2	61.3	73.6	123	EC50
1 hr	A	33	33	40	–	33	–	33	–	60	–	–	49.8
	B	0	33	0	–	17	–	67	–	50	–	–	33.2
	C	33	17	0	–	0	–	40	–	67	–	–	44.6
	D	0	–	–	17	–	67	–	83	–	50	83	30.2
	E	0	–	–	33	–	67	–	67	–	100	83	20.6
	F	17	–	–	50	–	33	–	83	–	67	83	30.1
	Mean	14	28	13	33	17	56	47	78	59	72	83	34.8
	SD												10.7
	Pooled												34.2
	95% CI												(26.6,43.9)

Species: *Vibrio fischeri* (Microtox)

Replicate	5 min EC50	15 min EC50
A	374.5	27.7
B	304.0	26.5
C	333.3	26.8
D	257.5	24.4
E	154.4	19.0
MEAN	284.7	24.9
SD	84.4	3.5

Toxicant: Cadmium (mg/kg)
Matrix: Sediment

Species: *Palaemonetes pugio*

Duration	Replicate	% Mortality Control	2.5	10	40	160	LC_{50}
10 Day	A	0	20	20	90	100	18.1
	B	10	10	10	100	100	18.5
	C	0	20	10	100	100	17.7
	D	20	20	10	100	100	17.7
	E	0	20	10	100	100	17.7
	Mean	6	18	12	98	100	18.2
	SD						0.2
	Pooled						17.9
	95% CI						(16.2,19.9)

Species: *Ampelisca abdita*

Duration	Replicate	% Mortality Control	2.5	10	40	160	LC_{50}
10 Day	A	0	20	55	100	100	7.8
	B	0	10	5	100	100	18.9
	C	5	5	30	100	100	13.1
	D	0	20	45	100	100	9.7
	E	0	5	45	100	100	10.4
	Mean	1	12	36	100	100	12
	SD						4.3
	Pooled						11.8
	95% CI						(9.85, 14.2)

Species: *Ampelisca verrilli*

Duration	Replicate	% Mortality Control	2.5	10	40	160	LC_{50}
10 Day	A	50	40	90	100	100	NC
	B	5	15	85	100	100	5
	C	5	10	90	100	100	5
	D	0	25	75	100	100	5
	E	5	25	90	100	100	4.3
	Mean	13	23	86	100	100	4.8
	SD						0.37
	Pooled						4.5
	95% CI						(3.9, 5.1)

Species: *Amphiascus tenuiremis*

Duration	Replicate	% Mortality Control	9	18	36	45	LC_{50}
10 Day	A	23	10	17	33	17	NC
	B	30	0	7	10	37	NC
	C	17	3	23	20	20	NC
	D	33	50	3	20	17	NC
	E	23	40	3	17	23	NC
	Mean	25	21	11	20	23	NC
	SD						NC
	Pooled						NC
	95% CI						NC

Toxicant: Cadmium (mg/kg)
Matrix: Sediment

Species: *Mercenaria mercenaria*

Duration	Replicate	% Mortality					LC_{50}
		Control	2.5	10	40	160	
10 Day	A	0	100	100	100	100	< 2.5
	B	0	100	100	100	98	< 2.5
	C	0	100	98	100	100	< 2.5
	D	0	100	100	100	100	< 2.5
	E	0	100	98	100	100	< 2.5
	Mean	0	100	99	100	99	< 2.5
	SD						
	Pooled						< 2.5

Species: *Brachionus plicatilis*

Duration	Replicate	% Mortality					LC_{50}
		Control	2.5	10	40	160	
10 Day	A	0	0	0	77	77	27.6
	B	0	0	0	50	100	40
	C	0	0	0	52	100	39.1
	D	0	3	0	21	100	60.2
	E	0	3	0	48	100	40.7
	Mean	0	1	0	49	95	41.5
	SD						11.7
	Pooled						41.9
	95% CI						(35.8, 49.1)

Toxicant: Cadmium (mg/L)
Species: *Vibrio fischeri*

Microtox Solvent Extract EC_{50}s for Spiked Sediments

Cadmium Concentration [mg/kg dw]	5 min EC_{50} [mg dw/ml]	15 min EC_{50} [mg dw/ml]
0.0	> 5.8	> 5.8
2.50	>5.7	> 5.7
10.00	> 5.7	> 5.7
40.00	> 5.7	> 5.7
160.00	> 5.7	> 5.7

Microtox Solid Phase EC_{50}s for Spiked Sediment

Cadmium Concentration [mg/kg dw]	5 min EC_{50} [mg dw/ml]
0.0	66.6 (11.2)
2.5	75.6 (11.6)
10.0	64.4 (10.5)
40.0	52.3 (4.9)
160.0	44.4 (7.1)*

*Significantly different from control at $\alpha = 0.05$

Mutatox Results for Spiked Sediment Extracts

Concentration [mg\kg dw]	Direct Assay Time				S-9 Assay Time			
	14	16	20	24	14	16	20	24
0.0	–	–	–	–	–	–	–	–
2.5	–	–	–	–	–	–	–	–
10.0	–	–	–	–	–	–	–	–
40.0	–	–	–	–	–	–	–	–
160.00	–	–	–	–	+	+	–	–

Appendix C.

Results obtained from DDT aqueous and sediment bioassays.

Toxicant: DDT (μg/L)
Matrix: Aqueous

Species: *Palaemonetes pugio*

Duration	Replicate	% Mortality Control	3.90	6.50	10.80	18.00	30	50	LC_{50}
24 h	A	0	20	30	50	70	100	100	10.5
	B	0	0	50	100	100	80	90	6.6
	C	0	0	20	50	70	80	100	12.6
	D	0	40	40	40	90	100	100	8.4
	E	0	30	40	50	70	90	100	9.5
	Mean	0	18	36	58	8 0	90	98	9.5
	SD								2.3
	Pooled								8.9
	95% CI								(7.5, 10.5)

Species: *Ampelisca abdita*

Duration	Replicate	% Mortality Control	800	1300	2200	3600	6000	10000	LC_{50}
24 h	A	0	0	0	0	0	0	0	NC
	B	0	0	0	0	0	0	0	NC
	C	0	0	0	0	0	0	0	NC
	D	0	0	0	0	0	0	0	NC
	E	0	0	0	0	0	0	0	NC
	Mean	0	0	0	0	0	0	0	NC
	SD								NC
	Pooled								NC

Species: *Ampelisca verrilli*

Duration	Replicate	% Mortality Control	2.3	3.9	6.5	10.8	18.0	30.0	50.0	83.3	LC_{50}
24 h	A	10	0	10	20	30	40	80	80	80	18
	B	10	10	10	20	20	40	0	30	20	NC
	C	0	0	0	10	50	10	70	70	80	23.7
	D	0	10	0	10	10	60	60	70	50	16.8
	E	0	0	20	20	10	50	60	20	80	36
	F	0	–	–	10	0	20	60	30	40	NC
	G	0	–	–	10	20	30	70	40	90	30
	H	0	–	–	0	20	30	30	40	50	83.3
	I	0	–	–	0	10	70	30	40	50	83.3
	J	0	–	–	0	10	20	60	80	30	27.3
	Mean	0	4	8	10	18	37	52	50	57	39.8
	SD										27.6
	Pooled										38.3
	95% CI										(32.4, 45.3)

Species: *Amphiascus tenuiremis*

Duration	% Mortality Control	0.6	1.25	2.5	5	10	LC_{50}
24 h	5	0	0	5	15	10	NC
48 h	0	0	0	20	10	25	NC
96 h	0	0	10	25	15	20	NC

Toxicant: DDT (μg/L)
Matrix: Aqueous

Species: *Mercenaria mercenaria*

Duration	Replicate	% Mortality							LC_{50}
		Control	313	625	1250	2500	5000	10000	
24 h	A	0	60	30	80	100	100	100	690
	B	0	50	40	80	100	100	100	690
	C	0	40	30	100	90	100	100	743
	D	0	30	60	100	100	100	100	493
	E	0	30	70	90	100	100	80	442
	Mean	0	42	46	90	98	100	96	612
	SD								135
	Pooled								615
	95% CI								(358, 1057)

Species: *Brachionus plicatilis*

Duration	Replicate	% Mortality						LC_{50}
		Control	1300	2200	3600	6000	10000	
24 h	A	0	0	0	0	3	40	NC
	B	0	0	0	0	0	13	NC
	C	0	0	0	0	0	3	NC
	D	0	0	0	0	3	7	NC
	E	0	0	0	0	0	20	NC
	Mean	0	0	0	0	1	17	NC
	SD							NC
	Pooled							NC

Species: *Mysidopsis bahia*

Duration	Replicate	% Not Fluorescing						LC_{50}
		Control	2.6	4	7.2	12	20	
1 h	A	17	29	33	67	50	83	6.9
	B	0	29	33	33	67	83	8.6
	C	0	43	33	50	100	100	6.5
	D	0	17	50	50	100	100	5.3
	E	33	17	67	67	83	100	4.3
	F	17	33	50	100	100	100	4
	Mean	11	28	44	61	83	94	5.9
	SD							1.7
	Pooled							5.1
	95% CI							(3.9, 6.6)

Species: *Vibrio fischeri* (microtox)

Replicate	5 min EC50	15 min EC50
A	> 10,000	> 10,000
B	> 20,000	> 20,000
C	> 27,000	> 27,000
D	> 50,000	> 50,000
MEAN	NC	NC
SD	NC	NC

Toxicant: DDT (mg/kg)
Matrix: Sediment

Species: *Palaemonetes pugio*

Duration	Replicate	% Mortality					LC_{50}
		Control	0.64	1.60	4.00	10.00	
10 Day	A	10	20	0	30	90	5.0
	B	0	10	10	40	90	4.5
	C	0	10	30	40	80	4.2
	D	0	20	20	50	90	3.8
	E	10	30	30	40	70	5.0
	Mean	4	18	18	40	84	4.5
	SD						0.5
	Pooled						4.5
	95% CI						(3.6, 5.6)

Species: *Ampelisca abdita*

Duration	Replicate	% Mortality					LC_{50}
		Control	0.64	1.60	4.00	10.00	
10 Day	A	10	0	0	15	65	7.6
	B	5	0	5	10	55	9.0
	C	10	5	5	20	55	8.8
	D	5	5	10	15	45	NC
	E	5	5	10	5	70	7.5
	Mean	7	2	6	13	58	8.2
	SD						0.8
	Pooled						8.5
	95% CI						(7.2, 10.0)

Species: *Ampelisca verrilli*

Duration	Replicate	% Mortality					LC_{50}
		Control	0.64	1.60	4.00	10.00	
10 Day	A	10	5	5	10	45	NC
	B	10	5	0	10	70	7.4
	C	10	5	5	10	55	9.0
	D	2	15	30	10	45	NC
	E	0	10	15	20	75	8.5
	Mean	10	8	11	12	58	8.3
	SD						0.88
	Pooled						8.5
	95% CI						(7.2, 10.0)

Species: *Mercenaria mercenaria*

Duration	Replicate	% Mortality					LC_{50}
		Control	0.64	1.60	4.00	10.00	
10 Day	A	0	6	0	50	80	4.3
	B	0	20	10	46	40	> 10
	C	0	10	20	32	70	6.2
	D	0	0	6	26	66	6.9
	E	0	4	20	30	66	5.8
	Mean	0	8	11	37	64	5.8
	SD						1.1
	Pooled						6.3
	95% CI						(4.8, 8.3)

Toxicant: DDT (mg/kg)
Matrix: Sediment

Species: *Brachionus plicatilis*

Duration	Replicate	% Mortality Control	0.64	1.60	4.00	10.00	LC_{50}
24 h	A	0	0	0	0	3	NC
	B	0	0	0	0	0	NC
	C	0	0	0	0	0	NC
	D	0	0	0	0	0	NC
	E	0	0	0	0	0	NC
	Mean	0	0	0	0	1	NC
	SD						NC
	Pooled						NC

Species: *Amphiascus tenuiremis*

Duration	Replicate	% Mortality Control	0.1	1	10	100	LC_{50}
10 Day	A	33	43	33	7	10	NC
	B	33	40	33	17	43	NC
	C	13	10	0	17	27	NC
	D	30	0	3	10	23	NC
	E	10	10	27	17	7	NC
	Mean	24	21	19	13	22	NC
	SD						NC
	Pooled						NC
	95% CI						NC

Clutch size in *Amphiascus tenuriemis* exposed to DDT in sediments for 10 days

DDT	Clutch Size ± SD (eggs/female)
0	8.83 ± 3.02
0.1	7.45 ± 2.87
1.0	7.34* ± 3.09
10.0	6.65* ± 2.93
100.0	6.89* ± 2.98

*significantly different at alpha = 0.05

Toxicant: DDT
Species: *Vibrio fischeri*

Microtox Solvent Extract EC_{50}s for Spiked Sediments

DDT Concentration [mg/kg dw]	5 min EC_{50} [mg dw/ml] (SD)		15 min EC_{50} [mg dw/ml]	
0.00	0.86	(0.36)	0.93	(0.32)
0.64	0.98	(0.15)	1.00	(0.15
1.60	0.79	(0.23)	0.73	(0.20)
4.00	0.72	(0.19)	0.63	(0.15)
10.00	0.81	(0.15)	0.78	(0.15)

Microtox Solid Phase EC_{50}S for Spiked Sediments

DDT Concentration [mg/kg dw]	5 min EC_{50} [mg dw/ml](SD)	
0	9.9	(1.7)
0.64	13.0	(2.0)
1.60	10.1	(3.8)
4.00	7.4	(0.1)
10.00	8.7	(2.1)

Mutatox Results for Spiked Sediment Extracts

Concentration [mg/kg dw]	Direct Assay Time				S-9 Assay Time			
	14	16	20	24	14	16	20	24
0	–	–	–	–	–	–	–	–
0.64	–	–	–	–	–	–	–	–
1.60	–	–	–	–	–	–	–	–
4.00	–	–	–	–	–	–	–	–
10.00	–	–	–	–	–	–	–	--

Appendix D.

Results obtained from Fluoranthene aqueous and sediment bioassays.

Toxicant: Fluoranthene(μg/L)
Matrix: Aqueous

Species: *Palaemonetes pugio*

		% Mortality						
Duration	Replicate	Control	104	173	288	480	800	LC_{50}
96 h	A	0	10	20	50	70	80	313.7
	B	0	10	10	30	30	50	800
	C	0	10	20	40	40	60	619.7
	D	10	10	30	20	30	70	619.7
	E	10	0	10	50	30	60	619.7
	Mean	4	8	18	38	40	64	594.6
	SD							175.4
	Pooled							564.6
	95% CI							(410.6, 776.4)

Species: *Ampelisca abdita*

		% Mortality							
Duration	Replicate	Control	38.4	64.8	108	180	300	500	LC_{50}
96 h	A	0	0	60	100	100	100	100	61.6
	B	10	10	20	90	100	100	100	78.5
	C	0	0	60	100	100	100	100	61.6
	D	0	20	70	90	100	100	100	53.5
	E	20	20	100	100	100	100	100	47.1
	Mean	6	10	62	96	100	100	100	60.5
	SD								11.8
	Pooled								59.1
	95% CI								(55.4, 62.0)

Species: *Ampelisca verrilli*

		% Mortality							
Duration	Replicate	Control	38.9	64.8	108	180	300	500	LC_{50}
96 h	A	10	20	40	40	50	90	90	124
	B	0	0	40	80	90	100	80	75.4
	C	0	10	20	30	60	90	100	139.4
	D	0	0	40	70	80	100	90	87.2
	E	0	10	10	30	70	100	90	137.7
	Mean	2	8	30	50	70	96	90	112.7
	SD								29.6
	Pooled								107.7
	95% CI								(97.3, 119.2)

Species: *Amphiascus tenuiremis*

		% Mortality							
Duration	Replicate	Control	100	170	290	480	800	1600	LC_{50}
24 h	A	15	25	10	20	5	0	20	NC
	B	0	0	5	5	20	15	10	NC
	C	0	10	10	10	10	15	20	NC
	D	5	0	5	0	10	15	20	NC
	E	0	5	5	10	20	5	20	NC
	Mean	4	8	7	9	13	10	18	NC
	SD								NC
	Pooled								NC

Toxicant: Fluoranthene (μg/L)
Matrix: Aqueous

Species: *Mercenaria mercenaria*

Duration	Replicate	% Mortality						
		Control	104	173	288	480	800	LC_{50}
24 h	A	0	10	10	30	50	60	512
	B	0	20	30	40	40	50	800
	C	0	20	30	30	50	50	620
	D	0	20	20	10	30	60	675
	E	10	10	20	40	30	40	>800
	Mean							652
	SD							–
	Pooled							735
	95% CI							(441, 1225)

Species: *Mercenaria mercenaria*

Duration	Replicate	% Mortality						
		Control	104	173	288	480	800	LC_{50}
96 h	A	10	70	70	100	100	100	< 104
	B	10	80	90	100	100	100	< 104
	C	0	80	100	100	100	100	< 104
	D	0	60	100	100	100	100	< 104
	E	10	60	100	100	100	100	< 104
	Mean	6	70	92	100	100	100	< 104
	SD							-
	Pooled							< 104

Species: *Brachionus plicatilis*

Duration	Replicate	% Mortality							
		Control	38.4	64.8	108	180	300	500	LC_{50}
24 h	A	0	0	0	0	4	0	0	NC
	B	0	0	0	0	0	0	0	NC
	C	3	0	0	0	4	0	0	NC
	D	0	0	3	0	3	0	0	NC
	E	3	8	0	0	0	0	0	NC
	Mean	1	2	1	0	2	0	0	NC
	SD								NC
	Pooled								NC

Species: *Mysidopsis bahia*

Duration	Replicate	% Not Fluorescing						
		Control	104	173	288	480	800	EC50
1 h	A	17	17	33	50	33	33	NC
	B	17	0	40	40	33	33	NC
	C	17	0	0	33	33	0	NC
	D	0	0	0	0	0	0	NC
	E	0	0	0	0	0	0	NC
	F	0	0	0	0	0	0	NC
	Mean		3	12	20	17	11	NC
	SD							NC
	Pooled							NC

Species: *Vibrio fischeri* (Microtox)

Replicate	5 min EC50	15 min EC50
A	> 1100	> 1100
B	1057	> 1100
C	> 1100	> 1100
D	> 1100	> 1100
MEAN	NC	NC
SD	NC	NC

Toxicant: Fluoranthene (mg/kg)
Matrix: Sediment

Species: *Palaemonetes pugio*

		% Mortality					
Duration	Replicate	Control	0.78	3.12	12.50	50.00	LC_{50}
10 Day	A	20	10	10	10	10	> 50
	B	0	20	20	20	0	> 50
	C	10	0	0	10	10	> 50
	D	0	10	10	10	10	> 50
	E	30	0	0	0	10	> 50
	Mean	12	8	8	10	8	> 50
	SD						-
	Pooled						> 50

Species: *Ampelisca abdita*

		% Mortality					
Duration	Replicate	Control	0.78	3.13	12.50	50.00	LC_{50}
10 Day	A	0	55	5	0	10	NC
	B	10	0	25	0	25	NC
	C	0	50	5	0	50	NC
	D	0	0	45	5	75	NC
	E	5	0	0	0	65	NC
	Mean	3	21	16	1	45	NC
	SD						NC
	Pooled						NC

Species: *Ampelisca verrilli*

		% Mortality					
Duration	Replicate	Control	0.78	3.12	12.50	50.00	LC_{50}
10 Day	A	15	0	5	0	5	NC
	B	5	5	0	10	25	NC
	C	10	5	0	5	35	NC
	D	10	5	0	0	5	NC
	E	0	10	0	0	10	NC
	Mean	8	5	1	3	16	NC
	SD						NC
	Pooled						NC

Toxicant: Fluoranthene (mg/kg)
Matrix: Sediment

Species: *Amphiascus tenuiremis*

Duration	Replicate	% Mortality					
		Control	0.8	3.1	12.5	50	LC_{50}
10 Day	A	0	17	17	7	0	NC
	B	13	3	17	10	13	NC
	C	17	0	27	7	7	NC
	D	0	17	20	13	0	NC
	E	-	20	13	3	3	NC
	Mean	7	11	19	8	5	NC
	SD						NC
	Pooled						NC
	95% CI						NC

Species: *Mercenaria mercenaria*

Duration	Replicate	% Mortality					
		Control	0.78	3.12	12.50	50.00	LC_{50}
24 h	A	0	76	54	80	86	< 0.78
	B	0	56	78	76	94	< 0.78
	C	0	70	68	86	86	< 0.78
	D	0	36	76	76	80	1.27
	E	4	44	78	90	94	< 0.78
	Mean	1	56	71	82	88	-
	SD						-
	Pooled						< 0.78
	95% CI						

Species: *Brachionus plicatilis*

Duration	Replicate	% Mortality					
		Control	0.78	3.13	12.50	50.00	LC_{50}
24 h	A	0	0	0	0	3	NC
	B	0	0	0	0	0	NC
	C	0	0	0	0	0	NC
	D	0	0	0	0	0	NC
	E	0	0	0	0	0	NC
	Mean	0	0	0	0	1	NC
	SD						NC
	Pooled						NC
	95% CI						NC

Toxicant: Fluoranthene
Species: *Vibrio fischeri*

Microtox Solvent Extract EC_{50}s for Spiked Sediments

Fluoranthene Concentration [mg/kg dw]	5 min EC_{50} [mg dw/ml]	(SD)	15 min EC_{50} [mg dw/ml]	(SD)
0	0.83	(0.23)	0.81	(0.33)
0.78	1.41	(0.31)	2.11	(1.82)
3.12	4.49	(0.82)*	3.38	(1.93)
12.50	0.87	(0.18)	1.41	(1.28)
50.0	1.83	(1.35)	1.31	(0.75)

*Significantly different from control $\alpha = 0.05$

Microtox Solid Phase EC_{50}s for Spiked Sediments

Fluoranthene Concentration [mg/kg dw]	5 min EC_{50} [mg dw/ml]	(SD)
0.0	7.2	(1.2)
0.78	7.1	(1.0)
3.12	8.8	(1.6)
12.5	7.8	(0.2)
50.0	9.0	(2.6)

Mutatox Results for Spiked Sediments

Concentration [mg/kg dw]	Direct Assay Time 14	16	20	24	15 min EC_{50} [mg dw/ml] (SD) 14	16	20	24
0	—	—	—	—	—	—	—	—
0.78	—	—	—	—	—	—	—	—
3.12	—	—	—	—	—	—	+	+
12.5	—	—	—	—	—	—	—	—
50.0	—	—	—	—	—	—	+	—

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