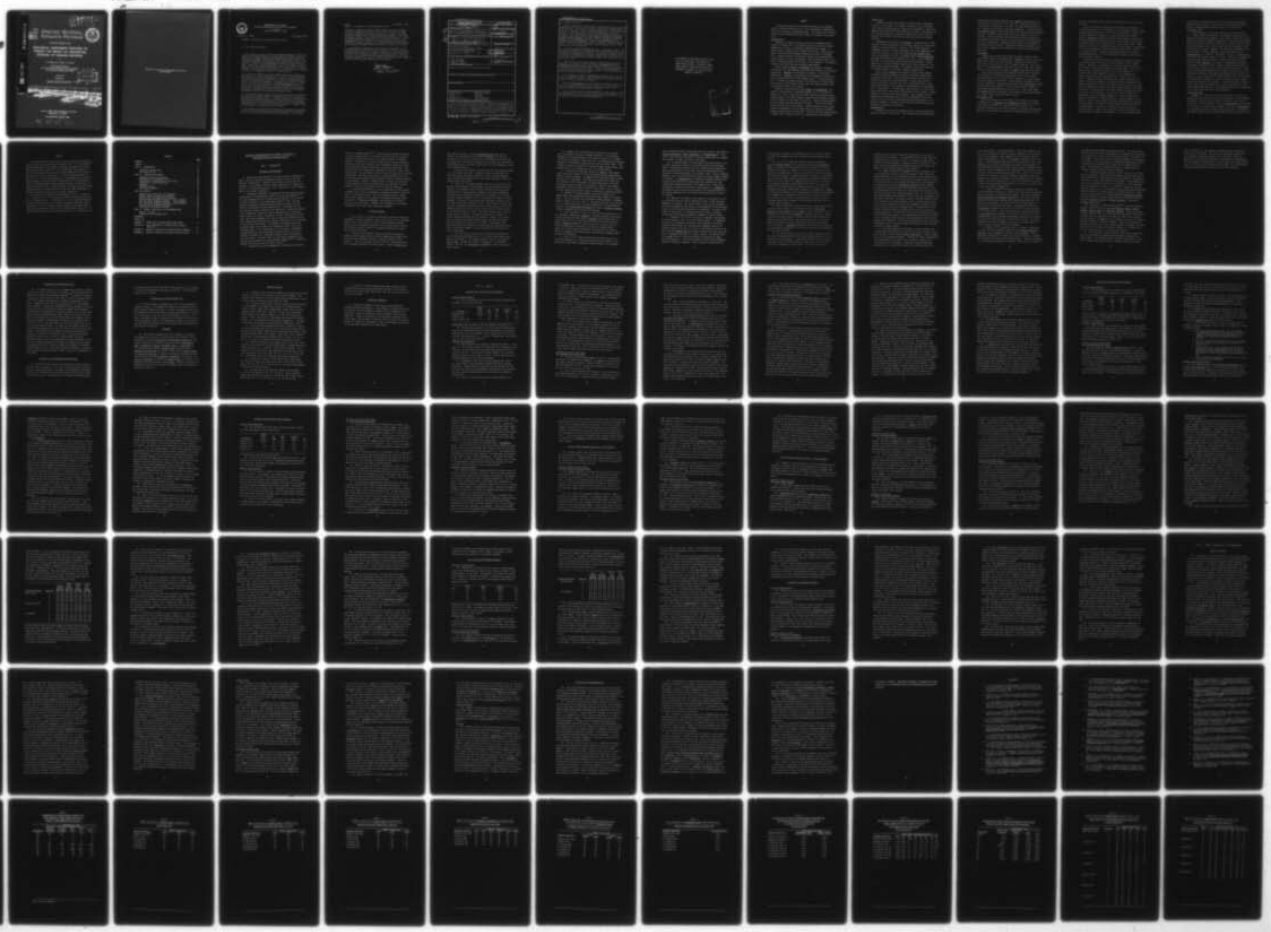
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DREDGED MATERIAL RESEARCH PROGRAM

TECHNICAL REPORT D-78-50

BIOLOGICAL ASSESSMENT METHODS TO PREDICT THE IMPACT OF OPEN-WATER DISPOSAL OF DREDGED MATERIAL

by

P. J. Shuba, H. E. Tatem, J. H. Carroll

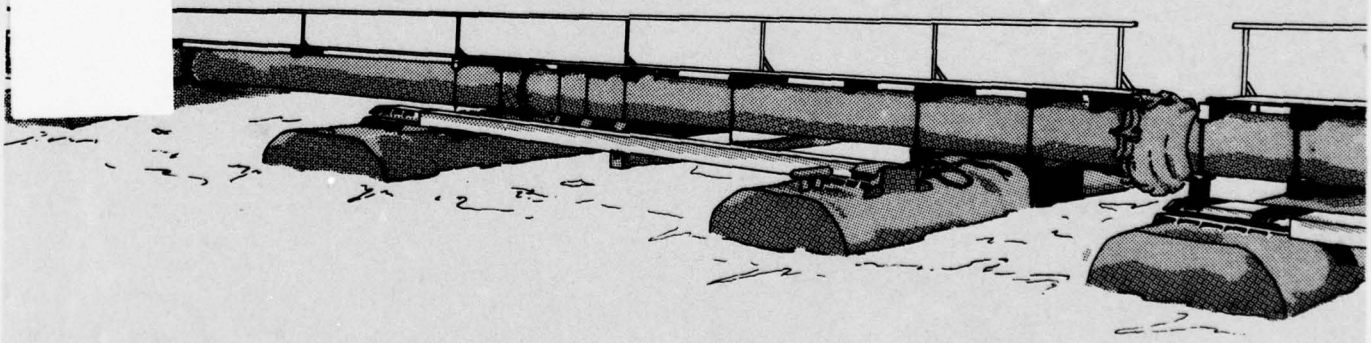
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Prepared for Office, Chief of Engineers, U. S. Army
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Under DMRP Work Unit No. 1E08

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30 September 1978

SUBJECT: Transmittal of Technical Report D-78-50

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1. This technical report, Biological Assessment Methods to Predict the Impact of Open-Water Disposal of Dredged Material, presents data and discusses the results of laboratory experiments conducted with heavily contaminated sediments and selected aquatic invertebrate animals. The work was accomplished at the Waterways Experiment Station during 1976 and 1977 as a part of the Dredged Material Research Program's Environmental Impact and Criteria Development Project. This study was one of several work units included under Task 1E, Pollution Status of Dredged Material. The objective of the study was to establish and test standard procedures for conducting bioassays of dredged material.

2. Section 103 of Public Law 92-532 (Marine Protection, Research and Sanctuaries Act of 1973) and Section 404 of Public Law 92-500 (Federal Water Pollution Control Act Amendments of 1972) require that certain ecological evaluations be made prior to disposal of dredged material in marine or freshwater environments. Guidelines and criteria for these two sections have been published in the Federal Register; Vol. 42(7) 11 Jan 77 for Section 103 and Vol. 40(173) 5 Sep 75 for Section 404. These guidelines generally require bioassays, among other evaluation procedures. Data developed from this study were used to help prepare implementation manuals for the required bioassay tests.

3. Test sediments were obtained from areas where chemical contaminants were known to be present, such as the Duwamish River at Seattle and New York Harbor shipping channels. Chemical analyses of these materials revealed the presence of metals, chlorinated hydrocarbons, petroleum hydrocarbons, and other contaminants. Animals used in the experiments included marine, estuarine, and freshwater forms such as shrimp, clams, small crustaceans, polychaete worms, and others.

4. The results of numerous acute bioassays with contaminated sediments indicate that sediment elutriates and suspended particulate phases may be toxic to small crustaceans under some circumstances. Also it was

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found that exposure of sensitive benthic animals to these sediments resulted in significant toxicity in some cases. However, the above findings were generally the exception and not the rule, even though tests were conducted with heavily contaminated sediments. Also these findings are subject to many qualifications since there are, at present, no standard methods or animals for use in sediment bioassays. One recommendation in this report is that additional work be conducted to determine the normal variability in bioassays conducted with sediments and to establish standard laboratory species for tests of this type.

5. The information published in this report will contribute to the understanding of the complex nature of sediment, water, and chemical/biological interactions in the aquatic environment. Also the data will help establish a baseline from which to develop meaningful regulatory criteria. It is expected that the methodology employed in this study and interpretation of the results will be of significant value to persons concerned with CE dredged material permit programs.

JOHN L. CANNON
Colonel, Corps of Engineers
Commander and Director

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This report describes numerous bioassay experiments where representative aquatic invertebrates were exposed to heavily contaminated sediments and standard liquid (elutriate) and suspended particulate phases of the sediments. The purpose of the work was to develop biological methods for assessing the effects of open-water disposal of dredged material on water column and benthic animals, prior to actual disposal. Sediments and liquid phases were analyzed for selected chemical constituents in conjunction with the bioassays.

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20. ABSTRACT (Continued).

Sediment samples were collected from the Vicksburg area as well as from the Duwamish River at Seattle, the James River in Virginia, and shipping channels in New York Harbor. These materials contained a wide variety of environmental contaminants. Animals and control or reference sediments were obtained from relatively clean Gulf Coast areas or from the Mississippi River system near Vicksburg. Some organisms were obtained from other workers and cultured in the laboratory. Marine test animals included Acartia, Mysidopsis, Palaemonetes, Neanthes, Rangia, Mercenaria, and benthic amphipods and isopods. Freshwater animals included Palaemonetes (freshwater species), Daphnia, Corbicula, Musculium, and the isopod Lirceus. Survival of exposed animals was compared to control survival using statistical methods to determine a significant adverse effect. Preliminary sublethal bioassays using larval growth as the critical parameter were also accomplished.

The results varied depending upon the level of contaminants present in the material, the sensitivity of the organisms being tested, and the length of time the sediments were held in the laboratory. Test sediments contained high concentrations of contaminants such as metals, polychlorinated biphenyls, chlorinated and petroleum hydrocarbons, and kepone. However, none of the sediments tested, with one exception, were found to be clearly toxic to a majority of the test animals used.

There were cases where animals exposed to sediment mixtures survived at rates equal to or greater than control animals. Mysidopsis and juvenile Palaemonetes were exposed to high concentrations (90 percent) of a sediment liquid and suspended particulate phases. Survival of test animals was equal to or greater than control survival. However, another group of Mysidopsis was exposed to the same sediment (solid phase) for 96 hr. Survival of controls was significantly greater than test animals.

In a separate test, freshwater Musculium were exposed to four concentrations of a contaminated sediment. Control survival was 95 percent, survival of animals exposed to 1 percent sediment was 92 percent, and survival of those exposed to 2 percent sediment was 25 percent.

Four-day-old Palaemonetes larvae were exposed to Perth Amboy sediment for 33 days. The sediment proved to be toxic but only after 7 days exposure. It was also determined that exposure to the contaminated sediment, under static conditions, significantly slowed growth of the larvae.

It is now required that all material scheduled for ocean disposal, including dredged material, be tested for biological effects prior to disposal. The work discussed in this report should be helpful to those responsible for planning appropriate bioassays for contaminated sediments.

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SUMMARY

Contaminated sediments were obtained from marine and freshwater areas of the United States for use in the development of standard procedures for bioassays of dredged material. Test animals were collected from the field, obtained from other workers or cultured in the laboratory.

Duwamish River

Sediment was collected from two sites in the Duwamish River at Seattle in February 1976. The primary site was in the river channel immediately in front of a mechanical clamshell bucket dredge. A smaller amount of material was obtained from Slip No. 1 where a major spill of polychlorinated biphenyls (PCBs) had occurred in September 1974. Chemical analyses revealed PCBs, metals, and petroleum hydrocarbons present in these two sediments. PCB concentrations were 0.38 ppm for Duwamish sediment and 0.66 ppm for Slip sediment. The sediments contained over 300.0 ppm total petroleum hydrocarbons.

Marine copepods Acartia and Tigriopus were exposed to Duwamish liquid phase (LP) and suspended particulate phase (SPP) in four separate experiments. Tigriopus was unaffected by exposure to 100 percent Duwamish SPP. Acartia, a very sensitive test organism, was exposed to four concentrations of LP and SPP ranging from 0.1 to 100.0 percent for 24 hr. Survival of animals exposed to 100 percent LP or SPP averaged over 60 percent while control survival was only slightly higher, indicating little potential for toxicity.

Estuarine clams and grass shrimp, Rangia and Palaemonetes, were exposed in glass aquaria to Duwamish sediment (0.1 to 5.0 percent by volume) for 14 days. Clams were relatively unaffected; however, grass shrimp exposed to the higher sediment concentrations averaged 55 percent survival compared to 80 percent for controls. Clams and shrimp exposed to Slip sediment suffered negligible mortality. Tissue from exposed animals was analyzed for PCB content. Animals exposed to Slip sediment contained approximately 0.68 ppm PCBs while those exposed to Duwamish sediment averaged about 0.35 ppm PCBs. Tissue of control animals exposed to washed sand or disposal site water contained about 0.06 ppm PCBs.

Bailey Creek

Freshwater sediment was obtained from Bailey Creek at Hopewell, Virginia, in April 1976. The sediment contained 2.3-2.5 ppm kepone, 158.0 copper, and 880.0 ppm zinc among other contaminants. Analyses of sediment LP and SPP showed both contained less than 0.5 ppm kepone; the SPP contained almost three times more kepone than the LP. Thus the majority of the kepone in the SPP was associated with fine sediment particles.

Experiments were conducted with Daphnia. Animals exposed to 100 percent LP and SPP revealed 88 and 50 percent mortality, respectively, compared to zero mortality for controls. This clearly indicated significant toxicity. However, additional tests with Daphnia and the LP and SPP conducted 2-3 weeks later with the same sediment batch failed to demonstrate toxicity. Freshwater grass shrimp Palaemonetes were exposed to Bailey Creek sediment for 6 days. Control mortality was high, yet deaths among exposed shrimp were much higher. Shrimp exposed to 2.5 percent sediment showed only 26 percent survival. Exposed shrimp accumulated kepone. The highest tissue levels found were for animals exposed to the higher sediment concentrations.

Ninety clams, Corbicula, were exposed to Bailey Creek sediment along with 90 control animals in natural Yazoo River sediment which contained little kepone. Clams accumulated kepone but there were no mortalities during the 3-week experiment. Control clams held in natural fresh water contained less than 1.0 ppb kepone after 3 weeks while clams exposed to test sediment contained about 140.0 ppb kepone after 7 days exposure. Interestingly, clams exposed to 1 percent sediment contained over three times as much kepone, after 3 weeks, as those exposed to 5 percent Bailey Creek sediment. This suggests that the sediment was readsorbing kepone during the experiment and also that clams are able to depurate accumulated kepone during exposure to contaminated sediments. Analyses of clam tissue for heavy metals did not demonstrate significant accumulation.

Windmill Point

Sediment was obtained from Windmill Point in the James River near Hopewell, Virginia, in June 1976. This material was also contaminated

with kepone but at levels below 0.2 ppm. Daphnia bioassays were conducted with LP and SPP at 20°, 25°, and 30°C. Significant mortality occurred only at 100 percent LP at 30°C. The data indicated that control animals were stressed at this high temperature. In another experiment Daphnia exposed to 100 percent SPP at 30°C survived much better than controls. Freshwater grass shrimp larvae were exposed to SPP at 20° and 30°C. Control survival at 92 hr averaged 60 percent compared to approximately 25 percent survival of larvae exposed to 100 percent SPP at the two temperatures. Animals exposed to lower SPP concentrations survived as well as controls. Adult grass shrimp were exposed to 1, 2, and 5 percent Windmill Point sediment for 14 days without significant mortality.

Perth Amboy

Sediment was received from two New York Harbor channels, Perth Amboy and Bay Ridge, in September 1976. Acartia was used in two experiments with LP and SPP of both sediments. Significant toxicity was noted after 21 hr exposure to the Perth Amboy LP and SPP. The LP and SPP of the Bay Ridge sediment were also found toxic after 24 hr exposure; however, the SPP was not as harmful to Acartia as the Perth Amboy SPP.

Larval grass shrimp were also exposed to Perth Amboy LP and SPP. The 100 percent LP and SPP were significantly toxic to the larvae. Control survival at 120 hr was 90 percent compared to 5 and 50 percent for larvae exposed to LP and SPP. Larvae exposed to Bay Ridge LP and SPP were unaffected. Small estuarine opossum shrimp (Mysidopsis) exposed to Perth Amboy and Bay Ridge LP for 72 hr were affected. Again the Perth Amboy sediment produced greater mortality. Adult grass shrimp and Mercenaria clams were exposed to Perth Amboy and Bay Ridge sediments for 14 days without significant adverse effect.

Perth Amboy - 2nd

Additional sediment from Perth Amboy and Bay Ridge was obtained in December 1976. Mysidopsis and Palaemonetes juveniles were exposed to Bay Ridge LP and SPP for 3 days without adverse effect, i.e., survival at 90 percent LP and SPP was equal to or greater than controls. Mysids were also exposed to 10 percent Bay Ridge sediment for 14 days.

Survival of sediment-exposed mysids was significantly less than control survival.

Mysids and juvenile grass shrimp were exposed to Perth Amboy LP. The sediment had been in the laboratory for approximately 2 weeks. Grass shrimp exposed to 90 percent LP for 96 hr were affected. Control survival was 100 percent compared to 75 percent survival for exposed animals. Mysidopsis exposed to 100 percent Perth Amboy LP and SPP were also affected but the difference between control survival and survival of exposed animals was not significant at the 95 percent level. Mysids exposed to 10 percent Perth Amboy sediment, a high sediment concentration, for 2 days were harmed, but again statistical tests did not indicate a significant effect. Juvenile grass shrimp exposed to 10 percent Perth Amboy sediment for 45 days showed about 95 percent survival which did not indicate toxicity.

Initial work with the Perth Amboy sediment had suggested substantial toxicity while tests with the second shipment of this sediment had not. Therefore, an experiment was planned to show the effects of this sediment on survival and growth of a larval shrimp. Forty grass shrimp larvae, divided into groups of five, were exposed to 1 or 3 percent Perth Amboy sediment for 33 days. The experiment was a static bioassay with water replacement every 2 days after day 6. A slurry of sediment and water was poured into beakers containing the larvae. Some larvae exposed to the higher sediment concentration were adversely affected by the method of exposure, which resulted in a high level of sediment particles in the water column during the initial hours of the test. Larvae exposed to the lower sediment concentration began to die after 7 days exposure. After 33 days, control survival was 88 percent; survival of animals exposed to 1 percent sediment was 65 percent; and survival of animals exposed to 3 percent sediment was 55 percent. Growth of these animals was slowed significantly by exposure to the sediment. At 18 days the average weight of control larvae was 2.13 mg compared to 1.81 mg for 1 percent larvae and 1.70 mg for 3 percent larvae. Control larvae continued to grow more rapidly than exposed animals throughout the experiment. After the exposure period

the survivors were placed in clean, filtered seawater and sediment-exposed animals recovered. Twenty days after the exposure period the weight of sediment-exposed larvae was equal to control values.

Perth Amboy - 3rd

A final shipment of Perth Amboy sediment was received in April 1977. Chemical analyses showed that this sediment was much more contaminated than reference sediments. Total volatile solids were 0.3-0.5 percent for reference sediments compared to 4.5 percent for Perth Amboy. Total PCBs were between 0.04 and 0.05 ppm for reference material and 5.4 ppm for Perth Amboy. Since the previous work with Perth Amboy LP and SPP had been inconclusive, emphasis was placed upon experiments with benthic animals. Benthic amphipods, tentatively identified as Parahaustorius, were exposed to clean sand, kaolinite, or approximately 3.2 percent Perth Amboy sediment for 9 days. Mortality was highest for sediment-exposed animals; however, the levels were not significant. Benthic isopods, Sphaeroma, were exposed to approximately 5 percent Perth Amboy sediment for 4 days. Mortality among exposed animals was over 80 percent compared to less than 20 percent for controls. Thus, the same contaminated sediment produced highly different results when tested with two benthic crustaceans from different taxa. Mysids were exposed to about 5.3 percent Perth Amboy sediment with controls exposed to a clean natural sediment for 10 days in static aquaria. Exposed mysids survived better than controls. In another experiment, mysids and larval grass shrimp were exposed to 2.1 percent sediment for 7 days without significant mortality. The sediment had been in the laboratory for 3 weeks at the time of this final experiment.

Long Island Sound

Sediment was collected in April 1977 from four locations at a dredged material disposal site in Long Island Sound. The four samples were mixed. Chemical analyses showed 4.29 percent total volatile solids and 1.37 ppm total PCBs. Mysids and Parahaustorius were exposed to 3.2 percent Long Island Sound sediment for 11 days. Parahaustorius was established in sandy reference sediment before the test sediment was added. Survival was not significantly affected by exposure to the

test sediment. In another experiment, mysids and larval grass shrimp were exposed to 2.0 percent sediment for 7 days. Again, there was no significant mortality. Eighty Sphaeroma were exposed to this sediment 5 percent concentration) for 4 days without significant mortality. Thus, three experiments utilizing four different organisms all indicated little potential for toxicity.

Vicksburg area

Sediment was obtained from a small stream in the Vicksburg area which receives runoff from a nearby chemical plant. Chemical analyses of the material, which appeared to be very clean, revealed high levels of volatile solids (4.5 percent) and PCBs (12.8 ppm). Total aromatic petroleum hydrocarbons were also much higher than control sediment values. Freshwater grass shrimp were exposed in static aquaria and under flow-through conditions. Shrimp were first placed in the exposure chambers containing reference sediment and observed for 4 days with no deaths noted. Control tanks then received 2 percent reference sediment while experimental tanks received 2 percent test sediment. Control survival was excellent. The effect of the test sediment was rapid and dramatic. At 24 hr exposure test animals suffered 100 percent mortality in the static system and over 50 percent mortality in the flow-through system. This sediment demonstrated stronger toxicity than any previous sediment tested. These results were complicated, however, by the fact that addition of the test sediment to the exposure tanks caused pH to rise from 7.5 to between 9.0 and 9.7. This change in water pH was rapid and resulted in values which are above the commonly accepted range (7.0-9.0) for culturing freshwater animals. Natural freshwater areas usually have pH values between 6.5 and 8.5.

Adult fingernail clams, Musculium, were exposed to this sediment at two concentrations, 0.2 and 5.0 percent, for 8 days. Mortality was significant at the 5 percent concentration but pH values were 8.6 in test chambers compared to 7.8 for control chambers. Another experiment with the same animals showed that 2 percent Vicksburg sediment was harmful to these clams. The freshwater isopod Lirceus was also utilized. Sediment concentrations were 0.2 and 2.0 percent. Mortality was higher

among animals exposed to 2.0 percent sediment but not significantly higher.

These preliminary experiments have shown that contaminated sediments can cause significant toxicity to aquatic invertebrates in laboratory experiments. However, it should be emphasized that these data are the result of developmental bioassays where animals were exposed, generally under static conditions, to a range of sediment concentrations. Since a bioassay by definition attempts to determine toxic concentrations, it should not be surprising that there were cases where high sediment or LP concentrations caused significant mortality. Considering the sediments used for these tests, it is surprising that these contaminated sediments proved to be relatively nontoxic in many cases. There were cases where initial experiments would indicate toxicity while additional work would fail to confirm the initial results.

Most of these experiments were acute or short-term exposures. One test with grass shrimp larvae illustrated the potential of contaminated sediments for causing chronic adverse effects such as effects on growth or reproductive capacity.

PREFACE

The study reported herein presents data and discusses the results of work conducted at the Environmental Laboratory (EL), U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Mississippi, during the period February 1976 to May 1977. The investigation was conducted as part of the Dredged Material Research Program (DMRP) which was sponsored by the Office, Chief of Engineers, U. S. Army, and was authorized by Congress in the 1970 River and Harbor Act. The program was a nationwide study designed to provide definitive information on the environmental impact of dredged material disposal operations and to develop new or improved disposal practices. The research was conducted as part of Task 1E, "Pollution Status of Dredged Material," Work Unit 1E08, "Development of Bioassay Methodologies Using Selected Benthic Organisms."

The research was planned and accomplished by Drs. P. J. Shuba and H. E. Tatem and Mr. J. C. Carroll with the expert technical assistance of Ms. D. D. Hardin and Mr. J. P. McKinney, all of the Ecosystems Research and Simulation Division, EL. The work was performed under the general supervision of Dr. R. M. Engler, Project Manager, Environmental Impact and Criteria Development Project, and Dr. John Harrison, Chief, EL. The study benefited from the advice and direction of Dr. R. D. Peddicord, EL.

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BIOLOGICAL ASSESSMENT METHODS TO PREDICT THE IMPACT OF OPEN-WATER DISPOSAL OF DREDGED MATERIAL

PART I: INTRODUCTION

Objectives and Rationale

1. Bioassay has been defined as "any test in which organisms are used to detect or measure the presence or effect of one or more substances or conditions."[1] Alderdice stated there are three parts to a bioassay: (a) a stimulus, such as a drug, insecticide, or industrial waste; (b) a subject which may be a cell, a tissue, or a total organism; and (c) the subject's response.[2]

2. Biological assessments ("bioassays") have been used by a regulatory agency to establish water quality criteria and standards.[3,4,5] The basic approach has been to expose selected species to the toxicant or waste of interest for a predetermined period of time. The LC_{50} (lethal concentration causing 50 percent mortality during the test period) is determined from the resulting data. Bioassays have also been conducted using an endpoint other than death. Sublethal parameters such as immobility, respiratory rate, swimming behavior, feeding behavior, blood cell counts, reproductive success, and growth have been used. When these parameters were used, an EC_{50} was determined (effective concentration producing the response in 50 percent of the organisms in the selected time period). The majority of bioassays have been performed using a waste dissolved in appropriate water, such as the receiving water, or the liquid phase of a solid waste. Recommended standard procedures for liquid phase bioassays exist,[6,7] but until recently, procedures for conducting bioassays using solid materials have been absent. Presently there are four reports available which discuss various methods for testing sediment toxicity.[8-11] The EPA/CE method[8] relied heavily upon the work of Swartz.[9]

3. Bioassays have been recommended for evaluating the potential

effects of dredged sediment.[12,13] The tests and procedures described in this report were part of an effort to develop tests for assessing potential environmental impacts of open-water disposal of dredged material. Sediments were collected from various locations throughout the United States and returned to the Environmental Laboratory (EL) of the Waterways Experiment Station (WES), Vicksburg, Mississippi. Sediments were used in bioassays and subsamples of sediment, animal tissue, soluble phase, and suspended particulate phase were analyzed for selected contaminants. The major objective was to develop methods that would aid in evaluating potential adverse chemical effects of aquatic disposal of the sediment. Therefore, sediments were collected from areas that received industrial and/or municipal wastes and would have a strong potential for toxic or sublethal effects to the test organisms.

4. Different approaches were used in attempting to find standard procedures that could be recommended to regulatory personnel during the course of the work described. The basic methodology was to expose water column animals to the soluble and suspended particulate phases (see PART II: MATERIALS AND METHODS), and epifaunal and infaunal species to the solid phase. Sediments from freshwater and saltwater environments were tested using appropriate organisms.

Literature Review

5. The scientific literature contains many reports of biological assessment of various substances using a wide variety of test organisms. However, few of the reports are concerned with chemically complex wastes such as contaminated sediments. This review discusses some of the literature directly related to biological assessment of complex wastes in the laboratory.

6. Decoursey and Vernberg[14] conducted biological assessment studies on water samples collected during dredging and disposal operations from three dredging locations in Charleston Harbor, South Carolina. The three locations varied in salinity, necessitating the use of different organisms for each location. Juvenile Daphnia pulex

were used for Location I, larval Palaemonetes pugio were used for Location II, and larvae of the polychaete Polydora were used for Location III. For each location, water was collected and tested from the dredge site, from 200 yd downstream of the dredge site, and from the diked disposal area. Survival, respiration rate, and swimming rate were the parameters measured.

7. Mortality was greatest in runoff water from the upland disposal site, also termed weir water. Dredge site water was generally less toxic than water from 200 yd downstream. Respiratory and swimming rates were adversely affected by weir water when compared to the other sampling sites and controls.

8. Hoss et al.[15] prepared sediment extracts using sea water from Beaufort, North Carolina, and sediments from Charleston Harbor, South Carolina. The sediments were collected from seven stations and were representative of areas regularly dredged by the Corps of Engineers. The extracts were prepared by adding 500 g of sediment to 1 litre of filtered seawater, shaking for 2 hr, and allowing the mixture to settle overnight. Dilutions of the extracts were then made at 100, 75, 20, 2 and 1 percent and used in toxicity tests. Seven species of larval fish collected from nearby estuaries were used in the testing. Most of the sediment extracts were toxic to one or more species of fish, but only at the 100 and 50 percent levels. The larvae survived well at concentrations below 50 percent. The authors attribute the toxicity to soluble compounds released from the sediments. However, the extracts were not filtered and some particulate matter may have been present which could have had an effect on the larvae. The sediment from Charleston Harbor was disposed of in diked disposal areas and the overflow is potentially harmful to larval fish based on the results presented in the paper.

9. The toxic effect of heavy metals released from sediment collected from proposed dredging sites in Los Angeles Harbor was investigated by McConaugha.[16] The organisms used for toxicity testing were Acartia tonsa, a planktonic copepod, and Tisbe sp., an epibenthic copepod. The rock crab Pachygrapsus crassipes was used in heavy metal uptake studies.

10. Sediment was collected from 13 stations for the toxicity tests and separate filtrates ("standard elutriates") were prepared. Sediment and unfiltered seawater were mixed in a ratio of 1:4, shaken for 30 min, and allowed to settle for 1 hr. The supernatant was filtered through 0.45 μ Millipore filters and then used in toxicity tests. Five sediment stations were sampled for the metal uptake study; however, this supernatant was not filtered after the 1 hr settling period.

11. After 96 hr of exposure, survival of Tisbe sp. was significantly lower than controls in only one elutriate and was significantly higher in three elutriates. A. tonsa survival was significantly reduced in seven of the elutriates. Rock crabs were exposed for 7 days, but the variation in the concentration of heavy metals found in the gill tissue was too great to make any conclusions. However, the sediment preparation from one station killed all of the crabs in 48 hr, indicating toxic material was present either as dissolved or particulate matter.

12. Emerson[17] collected sediment from four stations in Los Angeles Harbor and prepared extracts using various sediment to seawater ratios. The test solutions were prepared by mixing seawater to sediment ratios of 100:1, 10:1, 4:1, and 2:1. The author calls his test solutions of 4:1 a standard elutriate, but the solutions were not centrifuged or filtered. Adult Ophryotrocha labronica and larvae of Capitella capitata, benthic polychaetes, were used as test organisms.

13. After exposure for 96 hr, no mortality had occurred in any of the treatments containing O. labronica. Solution prepared from the four stations produced mortality among C. capitata larvae during 96-hr exposure tests, but in all cases the mortality was less than 50 percent. Adult O. labronica exposed to extracts from one of the stations for 28 days produced fewer offspring in all water to sediment ratios except for the 100:1 which produced more offspring than the controls. The author suggested that some disposal operations may stimulate reproduction of polychaetes.

14. Grice et al.[18] conducted short-term mortality tests (24 to 48 hr) and long-term (up to 18 days) reproduction and survival of offspring tests using marine copepods and acid-iron waste. The copepods

were collected from Block Island Sound, New York, and were identified as Calanus finmarchicus, Temora longicornus, and Pseudocalanus sp. Dilutions of the acid waste were made in filtered seawater and the copepods were exposed to various dilutions of the waste.

15. At concentrations of the acid waste that had pH values of 6.5 or lower, significant mortality of the three species occurred among adults. The authors pointed out that the concentrations producing the toxic pH values exist for less than 3 min and the mortality was not indicative of what might happen in the disposal area. Further, copepods exposed to the toxic concentrations of waste in buffered seawater showed no mortality. C. finmarchicus was transferred through acid-waste dilutions with pH values and for time periods that simulated the time-concentration exposures expected at the disposal area. Under those conditions, no mortalities occurred. Reproduction of T. longicornis was inhibited in the test solutions used for the long-term studies, but the authors emphasize that the concentrations used would not persist for 18 days.

16. Among the conclusions of the authors it was pointed out that the pH may have been the factor producing toxicity, rather than some component of the waste. The authors did not point out that changing the pH could change the form of one or more components of the acid waste as well as the biological availability of components. The paper illustrated the importance of considering dilution as well as chemical species when evaluating the response of animals to toxicity studies.

17. Buikema et al.[19] used 15 species of freshwater invertebrates and three species of fish to test oil refinery wastes. In attempting to develop a standard test and method, they formulated an arbitrary reference mixture (ARM) that contained many compounds found in oil refinery effluents and known to be toxic to many forms of aquatic life. They found that Daphnia pulex was the most sensitive species tested and gave a recommended procedure for toxicity testing. The ARM was toxic to D. pulex within 48 hr and the procedure was reproducible among various laboratories. Their work pointed out some of the requirements needed for regulatory bioassays. These included a procedure that is

relatively easy to conduct, uses exposure times of as short a duration as technically possible, and can be reproduced among separate laboratories.

18. Lee et al.[20] conducted bioassays using the freshwater cladoceran Daphnia magna and the saltwater grass shrimp Palaemonetes pugio. Preparation of the standard elutriate was modified by using different percentages of sediment (5, 10, 15, and 20 percent) and by sparging with compressed air rather than shaking. In some cases growth media were used instead of dredge site water to prepare the elutriates. The 10 percent sediment elutriate prepared from Bridgeport Harbor sediments had a toxic effect on P. pugio in 96-hr tests, while elutriates from Ashtabula and Corpus Christi Harbors had little or no toxicity. Manganese was released from all sediments tested and its effect was determined using acute lethal 96-hr bioassays on D. magna and P. pugio. No effects were observed at the concentrations used with either organism. Lee recommended the abandonment of bulk chemical analysis in favor of the standard elutriate test for determining water column effects and bioassays using benthic organisms for determining long-term effects of dredged material disposal.

19. Gannon and Beeton[21] conducted bioassays using dredged material from Great Lakes harbors. They used benthic fauna (Pontoporeia, Gammarus, and Chironomous larvae), Daphnia, native zooplankton, native phytoplankton, and the alga Cladophora. Sediment selectivity, benthos viability, and algal uptake of carbon-14-labeled carbon dioxide were among the assay methods used. Algal assays using direct counts and light scattering were unsuccessful because the algal cells clumped with the sediment. Cladophora experiments failed because the algae did not grow without the addition of soil extract which made interpretation of the results difficult.

20. For the bioassays using carbon-14 uptake, sediment "extracts" were used by Gannon and Beeton rather than suspended sediments. Cell numbers were not determined. An increased incorporation of carbon-14 into algal cells was observed during a 4-day period. This was interpreted as a stimulation of algal growth. If this were true, the

population would increase, resulting in an increase in the rate of incorporation of carbon-14. Lee and Plumb[22] point out that when the carbon-14 data are corrected for time of contact, the algal photosynthetic activity decreased. However, the data did indicate in many cases that as the percentage of sediment extract was increased, the amount of total carbon-14 taken up also increased.

21. Gannon and Beeton[23] recommended the use of sediment selectivity and benthos viability tests they devised. While their data demonstrated that the test organisms did prefer certain sediments over others, there were no clear-cut correlations between chemical or physical characteristics of the sediment and selection by the organisms. It is interesting to note that Pontoporeia affinis selected sediments collected from open-water areas containing high proportions of sand. The organisms used in the study were collected from an open-water area where the sediments had a high percentage of sand. It is possible that the organisms simply chose sediments to which they were accustomed, and under other conditions, could easily adapt to different sediment types. It is possible that certain sediments were not selected because they did not contain suitable nutrients for P. affinis, including a native bacterial flora which these organisms prefer. Interpretation of the benthos viability studies was complicated by the lack of dissolved oxygen measurements. As stated by the authors, the possibility existed that the high oxygen demand of some sediments may have caused the death of test organisms, rather than any toxic materials that may have been present in the sediments. Their work demonstrated the need for physical measurements, such as dissolved oxygen concentrations, when conducting bioassays on complex wastes.

22. Bryan and Hummerstone[24] compared estuarine sediments that contained high concentrations of heavy metals with the levels of heavy metals present in the polychaete Nereis diversicolor, a benthic inhabitant of the contaminated sediments. The data for copper indicated that the concentrations in the worms were, in general, related to the concentrations in the sediments. Sediments containing high mean concentrations of copper had polychaetes that also contained high

mean concentrations. The concentrations of zinc, lead, manganese, and iron in the worms were relatively constant regardless of the concentrations in the sediments. They suggested that the organisms may have regulatory mechanisms for zinc, lead, manganese, and iron, but not for copper. These mechanisms would exclude the accumulation of zinc, lead, manganese, and iron by the organisms, but copper could accumulate in some equilibrium concentration that would be related to sediment concentrations. Bryan and Hummerstone also suggested that where there were no obvious correlations between concentration (e.g., zinc) in the sediment and worms, in addition to possible regulatory mechanisms in the polychaetes, the concentration available to the organisms may not be directly related to the total concentration. Their work illustrated the need to consider the bioavailable concentration of a toxicant in a complex waste as well as the total concentration.

23. Renfro[25] added ^{65}Zn to marine sediments to determine what effect polychaetes would have on release of the metal from the sediment and how much would be taken up by the animals. The marine polychaetes Nereis diversicolor and Hermione hystrix were used as test organisms. Experiments with flowing seawater and sediments indicated that in the absence of polychaetes, 1 to 3 percent of the ^{65}Zn was desorbed in 18 days and 3 to 9 percent in 30 days. The burrowing activity of N. diversicolor increased the loss of ^{65}Zn to three to seven times that lost in their absence. After 5 days exposure to radioactive sediment, N. diversicolor had accumulated 0.2 percent of the total ^{65}Zn in the sediment-water system, and when transferred to nonradioactive sediment, the worms lost about 30 percent of their ^{65}Zn in 3 days. The author estimated that at least 60 days are required for the polychaetes to reach a steady-state with ^{65}Zn in the sediment.

24. Nimmo et al[26] studied the effect of sediments contaminated with polychlorinated biphenyls (PCB) on shrimp (Penaeus duorarum, P. setiferus, and P. aztecus) and fiddler crabs (Uca minax). PCB contaminated sediments were collected from various locations in Escambia Bay, Florida, and placed in flow-through aquaria. Animals were exposed to the contaminated sediments for 30 days. Whole tissues of crabs and

hepatopancreas from shrimp were analyzed for PCB. The authors found that PCB as "Aroclor 1254" accumulated in both animals and in most cases the concentration in the tissue was directly related to the amount in the sediment. The authors could not determine whether the PCB entered the animals' tissue by ingestion of contaminated sediment particles or by absorption of chemicals that leached from the sediment into the water. This failure to determine a source is a common problem in studies of chemical uptake by benthic organisms.

25. The marine sediment toxicity tests of Schwartz et al.[9] were major contributors to the solid phase bioassay procedure described in the EPA CE manual.[8] Using benthic marine invertebrates, they measured the effect of burial under different thicknesses of clean sediment and the effect of sediment particle size on mortality. Their organisms were not sensitive to burial of up to 15 mm of sediment and were not affected by grain size. A flow-through bioassay apparatus was described using five species of benthic animals for sediment bioassays. Their results indicated that the burrowing amphipods (Paraphoxus epistomus) were very sensitive and that cumaceans were more sensitive than the molluscs (Protothaca stamina and Macoma inquinata) or polychaetes (Glycinde picta) used.

26. The reports of Prater and Anderson[10,11] discussed freshwater sediment bioassays using the organisms Hexagenia limbata, Asellus communis, Daphnia magna, and Pimephales promelas. They used a unique bioassay apparatus with a recirculating water system that enabled them to expose the four organisms to the same test water after it passed over the sediment. D. magna was very sensitive, but all of the animals exhibited mortality in the test sediments. While they discussed the problems of trying to use total chemical analysis of sediment to predict biological effects, Prater and Anderson attempted to correlate their data of percent mortality to total chemical analysis.

27. This literature review illustrates some of the problems that need to be considered in designing and conducting bioassays using complex wastes. The problems are both technical and practical. Technical considerations include physical variables caused by the waste,

such as turbidity, pH, and changes in dissolved oxygen concentration, that need to be monitored. The selection of appropriate animals and test conditions, realistic dilution of the test waste, length of exposure, and total versus bioavailable concentrations of contaminants are also technical considerations. Practical aspects of design include cost of the assay, ease of conducting the bioassay, and reproducibility among separate laboratories as well as in the same laboratory.

PART II: MATERIALS AND METHODS

Sampling Locations and Methods

Duwamish River and Elliott Bay samples

28. Sediment and water samples were collected on 20 February 1976. Sediments were collected from two sites within the Duwamish River, Seattle, Washington. River sediment was collected about 300 m east of the 14th Street bridge in the center of the river from a depth of 10 m. Dredge site water was also collected from this site 1 m above the sediment surface. Slip sediment was collected at the entrance to Slip No. 1 at a depth of 10 m. Disposal site water was collected at Buoy D in disposal site No. 2 of Elliott Bay. Water depth at the disposal site was 66 m, but water was collected only from the surface and from 15 m. The disposal site was being used for Duwamish River sediment disposal and a dump had occurred approximately 2 hr before water samples were collected. Sediment samples were collected with an Ekman dredge and water samples were collected using a Van Dorn sampler.

Bailey Creek and James River samples

29. Sediment was collected from Bailey Creek on 5 April 1976 approximately 457 m upstream from the point where the creek empties into the James River at Hopewell, Virginia, and midway between the shores. A Peterson dredge was used to collect the sediment at a water depth of 1 m. James River water was collected in the middle of the ship channel approximately 0.8 km downstream from Bailey Creek. A "Little Guzzler" pump was used to collect the water from a depth of 5 m.

Windmill Point and James River samples

30. Sediment was collected on 9 June 1976 from a proposed dredge site in the James River at Windmill Point. A Ponar dredge was used and the water depth was 7 m. Dredge site water was collected 1 m above

the sediment surface using a "Little Guzzler" pump. The disposal site was adjacent to the dredge site and water was collected from three depths: surface, 2 m, and 3.5 m. These samples were mixed in equal proportions to obtain a composite water column sample for use as the dilutent in the bioassays.

Perth Amboy Channel and Bay Ridge Channel sediments of New York Harbor

31. Sediment samples from the Perth Amboy (PA) and Bay Ridge Channels were collected by personnel of the U. S. Army Engineer District, New York, on 9 September 1976. The samples were shipped by air freight in 50-litre polyethylene ice chests to EL, WES, and were received on 14 September 1976. The depth of both ship channels was 10 to 12 m at the sediment collection sites.

32. Additional sediment was collected at later dates by the same personnel from the same areas of New York Harbor. Sediment from the Bay Ridge Channel was collected on 9 December 1976 and arrived at WES on 13 December 1976 and was stored at $4^{\circ}C$ until used. A Smith-MacIntyre grab sampler was used. Sediment from PA Channel of New York Harbor was collected on 20 December 1976 and arrived at WES on 23 December 1976. The sediment was stored at $21 \pm 2^{\circ}C$ until used.

33. A third collection of sediment was obtained from the PA Channel on 12 April 1977 and arrived at WES on 15 April 1977. The PA sediment was a subsample of a larger collection and was used as part of a joint EPA/CE series of experiments to evaluate methods proposed as indicators of the pollution potential of dredged material designated for ocean disposal.

Vicksburg, Mississippi, area sediments

34. Sediment was collected several times for use in bioassays from a small stream in the Vicksburg, Mississippi, area. The stream flows into the Mississippi River and receives large amounts of industrial and domestic waste products because of its proximity to a municipal waste treatment plant and a chemical manufacturing facility. Water depth at the sediment collection site was approximately 0.5 m. The sediment was collected with shovels and transported to the laboratory in polyethylene buckets.

Preparation of Liquid Phase (LP)

35. The liquid phase (LP) of the sediments was prepared by mixing four parts of water with one part of sediment. The LP was analyzed for chemical constituents by established methods.[6,27] Methods for preparation of the LP were similar to those used to produce a standard elutriate.[28] The sediment was measured by volumetric displacement of the water. Dredge site water was used to prepare the LP of the Duwamish River sediments. Reconstituted Freshwater[29] (RCF) (Appendix A) was used to prepare the LP for the Bailey Creek and Windmill Point sediments. Instant Ocean (IO) (Appendix B) was used to prepare LP for the New York Harbor samples and dechlorinated, aged (30 days or more) tap water was used for the preparation of LP of the Vicksburg area sediment. The 4:1 mixture was shaken at approximately 100 rpm for 0.5 hr and allowed to settle for 1-2 hr. The supernatant was poured or siphoned into 0.5 or 1.0 litre centrifuge bottles. The amount of centrifugation varied for each location depending on the size and volume of the particulate matter present. The objective was to remove as much particulate matter as possible to facilitate the filtration step. Filtration was through Millipore filters (0.20 μ to 0.45 μ) using pressure filtration. Bioassay testing of the LP and suspended particulate phase (SPP) varied with each sediment depending on the amount of sediment and the number of organisms available. Generally animals were exposed to LP and SPP concentrations of 1, 10, 50, and 100 percent when possible. Methods for each individual bioassay are given in PART III prior to presentation of the results.

Preparation of Suspended Particulate Phase

36. The SPP for each location was prepared in a manner similar to the LP, except that after the 1- to 2-hr settling phase the supernatant was only lightly centrifuged. The amount of centrifugation was dependent on the size and amount of particulate matter present and the animal that was to be tested. The objective was to remove the minimum amount

of particulate matter that would still allow visibility of the animals so they could be observed at appropriate intervals. The centrifuged liquid was not filtered.

Preparation of the Solid Phase (SP)

37. Various amounts of sediment were used in the development of sediment bioassays. The sediment was added as a concentrated slurry made by adding sediment and water to a flask, swirling the flask until the sediment was distributed evenly, pouring the sediment over the surface of the water in the test unit, and allowing it to settle. The waters used were the same as described in the preparation of the LP for the corresponding location. The developmental nature of the testing required different approaches and the variations will be discussed for each location in PART III.

Organisms

38. Invertebrate organisms from both freshwater or saltwater environments were used for the locations that were studied. Marine zooplankton used were Acartia tonsa and Tigriopus californicus. Epibenthic crustaceans for saltwater locations included Palaemonetes pugio, Palaemonetes vulgaris, Mysidopsis bahia, and Mysidopsis sp. The burrowing marine amphipod Parahaustorius and marine isopod Sphaeroma quadridentatum were tested. Clams included Rangia cuneata and Mercenaria mercenaria. Animals for freshwater sampling locations included the grass shrimp Palaemonetes kadiakensis, the Asiatic clam Corbicula manilensis, the water flea Daphnia pulex, the fingernail clam Musculium, and the freshwater isopod Lirceus. Methods, locations, and holding procedures varied and will be discussed under the appropriate experiment in PART III.

Chemical Analyses

39. Sediment, tissue, and water samples were analyzed for selected chemical constituents in conjunction with various bioassays. The analyses were conducted by contract using private laboratories, as well as the Analytical Laboratory Group (ALG), EL, WES.

40. Sediment, animal tissue, and water samples from the exposures for the Duwamish River samples were analyzed for polychlorinated biphenyls (PCB), arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), Lead (Pb), and zinc (Zn) by American Bacteriological and chemical Company, P. O. Box 1557, Gainesville, Florida 32602. The method for PCB analyses was provided by Region X of the U. S. Environmental Protection Agency (Appendix C). The other constituents were analyzed by methods given in References 6 and 27.

41. The ALG analyzed Duwamish River water, disposal site water, LP, SPP, and sediment interstitial water for nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), ammonium plus ammonia-nitrogen (NH_3-N), total Kjeldahl nitrogen (TKN), total organic carbon (TOC), total inorganic carbon (TIC), orthophosphate-phosphorus (PO_4-P), and As, Cd, Cu, Fe, Mn, Ni, Pb, and Zn using methods described in References 6 and 27.

42. Sediment and animal tissue samples used in the Bailey Creek exposure were analyzed for the pesticide kepone by Jennings Laboratory, 1118 Cyprus Avenue, P. O. Box 851, Virginia Beach, Virginia 23451. Sediment was also analyzed by that laboratory for NO_2-N, NO_3-N, PO_4-P, total phosphorus (TP), NH_3-N, TKN, TOC, TIC, Cu, Fe, Mn, Pb, Zn, and Hg. The methods used by that laboratory are given in Appendix D.

43. Disposal site water, LP, SPP, and RCF used in the Bailey Creek toxicity studies were analyzed by ALG for NO_2-N, NO_3-N, PO_4-P, NH_3-N, TKN, TOC, TIC, Cd, Ni, Zn, Mn, Pb, Cu, Fe, and As using methods described in References 6 and 27.

44. Jennings Laboratories analyzed Windmill Point sediment, LP, and SPP for kepone, Zn, Mn, Pb, Cu, and Fe. The ALG analyzed dredge and disposal site waters, LP, and SPP for NO_2-N, NO_3-N, NH_3-N, TKN, PO_4-P, TOC, TIC, Ni, Cd, Pb, Fe, Cu, Zn, Mn, As, and Hg.

45. The LP of the Perth Amboy and Bay Ridge sediments and the Instant Ocean used in the New York Harbor bioassays were analyzed by ALG for NO_2-N, NO_3-N, NH_3-N, TKN, PO_4-P, TOC, TIC, Cd, Ni, Zn, Mn, Pb, Cu, Fe, As, and Hg.

Statistical Analysis

46. Statistical analyses of the bioassay data followed the methods described in Reference 8. These analyses included calculating the variance for each treatment, Cochran's test for homogeneity of variances, a t-test or analysis of variance (ANOVA), and a multiple-range test. Results are reported at the 95 percent confidence level in all cases. Data on chemical analyses were not treated statistically because of insufficient replication. Some of the bioassay results were not statistically analyzed for the same reason.

PART III: RESULTS

Duwamish River and Elliott Bay Bioassays

Physical characteristics

47. The following table lists the water quality characteristics measured at the collection sites:

Location	Water Depth m	DO ppm	Temp °C	Salinity ppt	pH
Disposal site	surface	9.5	8.5	13	7.0
Disposal site	15	9.5	8.0	26	6.9
River sediment site	9	9.0	7.5	15	6.9

Measurements were not taken at the slip sediment collection site.

48. Particle-size analyses of the river and slip sediments were done with a Coulter Electronic Particle Counter Model TA II using an aperture tube with 100-μ opening. River sediment had a size range of 2 to 100 μ with a mean of 15 μ. Slip No. 1 sediment had a size range of from 2 to 80 μ with a mean of 10 μ.

Chemical characteristics

49. The LP and SPP were prepared as described in PART II. After the 1- to 2-hr settling period, the supernatant was centrifuged 3 min at 7000 rpm. The resultant liquid had some particulate matter and was tested as the SPP. A portion was filtered through 0.45 μ pore-size Millipore filters and was tested as the LP. The two phases were also analyzed chemically, as was the bulk sediment.

50. Subsamples of the river and slip sediments were sent to Dr. Louis DiSalvo, Naval Biomedical Research Laboratory, Oakland, California. Chemical analyses indicated 2301 and 1224 ppm oil and grease in the river and slip sediments, respectively, and 413 and 338 ppm total petroleum hydrocarbons in the river and slip sediments, respectively.

51. Slip No. 1 was the location of a 260-gal PCB spill on

13 September 1974. A large amount of the PCB settled into the sediment of Slip No. 1 and other parts of the Duwamish River.[30] The contamination of the sediment was a cause of great concern to various agencies when the sediment was scheduled for dredging. The contaminated sediment was, therefore, an ideal candidate for biological assessment methodology development.

52. Table 1 lists the concentrations of PCB and trace elements found in Slip No. 1 and Duwamish River sediments. Although the spill at Slip No. 1 was primarily Aroclor 1242, the gas-liquid chromatographic scans of the extracts matched more closely with Aroclor 1254. The slip sediment contained almost twice as much PCB as the river sediment. The trace elements are not in unusually high concentrations when compared to chemical analyses of other harbor sediments.

53. Table 2 lists PCB and trace element concentrations of water samples used in the bioassays. Of the elements analyzed, Mn, Ni, Cu, and As were released in the soluble form when dredge site water was mixed with river sediment to prepare the LP. Manganese levels in the LP were increased by a factor of 25 compared to the levels in the dredge site water. The other three metals were increased by a factor of two. The sediment-mixing procedure reduced the concentrations of Cd and Zn in the LP. The concentrations given for the river and slip SPP are those of the soluble plus the particulate-associated components. The data demonstrate that most of the Cd and much of the Fe and Pb present in the SPP are closely associated with the particulate matter.

Bioassays using the liquid phase
and suspended particulate phase

54. The marine copepod Acartia tonsa was used as a test organism with the LP of Duwamish River sediments. A. tonsa was collected from Big Lagoon, Pensacola, Florida, in February 1976, at a salinity of 20 ppt and temperature of 13°C. Animals were maintained in IO at 20 ppt and 22 to 25°C until used in the tests.

55. Static, acute bioassays were conducted. The LP was diluted with unfiltered disposal site water to obtain LP concentrations of 0.1, 1.0, 10.0, and 100 percent. Salinity was 20 ppt and the pH ranged

from 7.0 to 8.0 in the test units. Test water volume was 100 ml per culture dish (40 mm x 80 mm Pyrex crystallizing dishes). Temperature was maintained at 20°C in a Psychrotherm Incubator (New Brunswick Scientific Co.) with a 12-hr-Light:12-hr-Dark cycle. Four replicates of each exposure condition were used with approximately five copepods per dish.

56. Table 3 lists the number of survivors in a 24-hr test. Mortality was high in the disposal site water as well as the IO controls. Survival was best at 0.1 and 1.0 percent LP (87 and 90 percent, respectively). There was no observable difference between survival in the disposal site water, IO, and the 10 and 100 percent LP.

57. The marine copepod Acartia tonsa and the tidal pool copepod Tigriopus californicus were used as test organisms with the SPP of Duwamish River sediments. A. tonsa was from the same collection used in the liquid phase test. T. californicus was provided by Dr. Louis DiSalvo, Naval Biomedical Research Laboratory, Oakland, California, in February 1976. Both organisms were maintained in IO at 20 ppt and a temperature of 22 to 25°C until used in the tests. Table 4 lists the results of a 24-hr test using A. tonsa. This test was run concurrently with the LP A. tonsa test and all experimental conditions were the same as discussed in that survival was excellent at 1.0, 10, and 100 percent SPP concentrations and higher than in the disposal site water or IO controls. The presence of suspended particulates seemed to enhance the survival of the animal.

58. The Acartia test was repeated with the exception that six replicates were used for each exposure condition and a 50 percent SPP concentration was used instead of the 0.1 percent concentration. The disposal site water and 10 percent SPP had the highest number of survivors (Table 5). Mortality was high in all other treatments. The 100 percent SPP concentration produced greater mortality in the second Acartia test compared to the first. The discrepancy between the two tests using I. tonsa with the SPP could be the result of the organisms being in the lab for an additional 2 weeks in the second test. The sediment and disposal site water used were also 2 weeks older at the start of the second test.

59. A test using the SPP and T. californicus was conducted. The experimental conditions were the same as those used in the A. tonsa tests. Results of a 96-hr exposure are reported in Table 6. Significant mortality was not observed under any of the experimental conditions.

Bioassay of the sediment solid phase

60. The estuarine grass shrimp Palaemonetes pugio and the marsh clam Rangia cuneata were exposed to Slip No. 1 and Duwamish River sediment. The majority of testing was conducted using river sediment because the river was actively being dredged and disposal was in the open-water of Elliott Bay. Animals were collected at Pensacola, Florida, in February 1976. Grass shrimp were collected in Big Lagoon at a salinity of 20 ppt and temperature of 13°C. Clams were collected from a marsh in Escambia Bay, Florida, at a salinity of 5 ppt and temperature of 15°C. Upon arrival at the laboratory, the clams were slowly acclimated to a salinity of 20 ppt and the shrimp were kept at the salinity of the collection site.

61. Fourteen 37.8-litre all-glass aquaria were used as exposure chambers for the test. Sixteen litres of disposal site water was added to each of 12 aquaria, and two aquaria received 16 litres of IO. Salinity for all aquaria was 20 ppt. Forty shrimp and 15 clams were placed in each aquarium and observed for 16 hr. No mortalities occurred. After the acclimation period, various amount of river sediment and disposal site water were mixed into a slurry and added to the test aquaria. The concentration of sediment and water used in the slurry was such that when added to the aquaria, the final volumes of sediment (vol/vol) were 0.1, 0.5, 1.0, and 5.0 percent. Aquaria that did not receive sediment had either additional disposal site water, a 5 percent slurry of clean sand, or additional IO poured into them. The final volume of each aquarium was 18 litres and there were two replicates of each exposure condition. Animals were observed each day for 14 days. Temperature, dissolved oxygen (DO) and pH were measured each day. The aquaria were not aerated initially; however, after 3 to 4 hr of exposure, slight aeration was supplied to each aquarium because the DO concentration was less than 2 ppm in the tanks with a 5 percent sediment concentration.

62. Addition of the sediment produced substantial turbidity, particularly at the higher sediment concentrations. The animals were not visible for about 6 hr. The loose sediment settled evenly over the bottom of the aquaria. The gills of the grass shrimp were coated with particulate matter, but they swam in a normal manner. All clams were closed at the 6-hr observation. The sediment additions did not change the salinity or temperature of the disposal site water. The pH did decrease slightly from 7.5 to 7.2 in the 5 percent exposure tank and decreased to 7.3 in the other sediment tanks. Dissolved oxygen concentrations were reduced in all sediment exposure tanks from 6.0 ppm to 1.8 ppm in the 5.0 percent aquaria, 4.0 ppm in the 1.0 percent aquaria, 4.5 ppm in the 0.5 percent aquaria, and 5.0 ppm in the 0.1 percent aquaria 2 hr after the addition of sediment. After aerating for 48 hr, the 0.5 and 1.0 percent aquaria returned to a DO concentration of approximately 6.0 ppm, while the 1.0 and 5.0 percent aquaria increased to about 5.5 ppm. Concentrations of DO generally remained at these levels for the balance of the experiment.

63. Twenty-four hours after the addition of sediment, a few clams at all sediment concentrations were siphoning and moving through the sediment. The gill areas of the shrimp had cleared. On day three, all shrimp were fed Tetramin fish food and responded in a normal manner. Shrimp were fed daily after day three. Clams were not fed during the experiment. Table 7 lists the number of shrimp and clams surviving after 336 hr (14 days) of exposure to Duwamish River sediment. Very little mortality occurred among the clams under any of the exposure conditions. There were mortalities of shrimp among all treatment levels and controls. Table 8 shows the percent mortality for the shrimp in the same experiment. There was no obvious relationship between the sediment concentration and shrimp mortality. However, the greatest mortality occurred among shrimp exposed to 0.5 and 1.0 percent sediment. Shrimp exposed to 0.1 percent sediment revealed higher mortality than animals exposed to 5.0 percent sediment. These data indicate that the sediment did release contaminants to the water which adversely affected the shrimp. It is possible that mortality was lower at the 5.0 percent

sediment concentration because of the greater surface area available for readsorption of the contaminants after the initial release. Control mortality in the IO aquaria may be explained by accumulation of metabolites in the static test system, the length of the test, and cannibalism.

64. For exposure to Slip No. 1 sediment, two 65.6-litre all-glass aquaria were used. Thirty-six litres of IO at 20 ppt salinity was added to each aquarium. One hundred grass shrimp and 40 clams were placed in each aquarium and acclimated for 24 hours. A slurry of slip sediment was added to one aquarium to produce a concentration of 5 percent. The other aquarium received additional IO. Mortality and behavior were observed for 14 days. Rangia had 100 percent survival in both tanks. Five shrimp died in the sediment exposure aquarium and five animals died in the control aquarium.

65. Surviving animals from all sediment exposures were collected on day 14 and frozen for chemical analyses. Table 9 lists the concentrations of PCBs found in surviving animals. The results represent whole tissue analyses for the grass shrimp and all tissue excluding the shell for the clams. The concentrations were lower than background for grass shrimp exposed to disposal site water and the 5 percent sand control, but higher in grass shrimp exposed to the river and slip sediment with the exception of the 0.1 percent river sediment. The clams had higher than background PCB concentrations at all sediment exposures.

66. R. cuneata were also analyzed for trace element concentrations in survivors of the 14-day exposure. Table 10 lists the final concentrations found in whole tissue samples excluding the shells. Nickel, Cd, As, and Cu concentrations were approximately the same as background levels at all exposure conditions, and there was no relationship between sediment concentration and final total trace element concentration. Lead was higher than background at all exposure conditions, and high concentrations of Fe were found in the 5 percent slip and 5 percent river sediment-exposed clams. Zinc was higher than background only in the 5 percent slip sediment exposure, and Mn was higher in the 1.0 and 5.0 percent river and 5.0 percent slip sediment exposures.

Bailey Creek and James River Bioassays

Physical characteristics

67. The following data were collected on 5 April 1976 when sediment was collected from Bailey Creek near the James River ship channel:

Location	Water Depth m	DO ppm	Temp. °C	Salinity ppt	pH
Bailey Creek	Surface	1.5	18	0.5	5.5
Bailey Creek	1	0.5	18	0.5	5.5
Ship Channel	5	7.6	14	0.5	5.0

Bailey Creek is a freshwater area and receives sewage from the city of Hopewell, Virginia. The sediment had the appearance of sewage sludge, and gas was bubbling from all areas in the creek.

Chemical characteristics

68. Table 11 lists the concentration of kepone and trace elements in the sediment and water samples used in the bioassays. The sediments contained relatively high concentrations of kepone, Cu, Pb, and Zn. Kepone was released from the sediment into the James River water during the preparation of the soluble phase. Copper, Mn, As, and Pb were also released from the sediment in soluble form.

Bioassays using the liquid phase and suspended particulate phase

69. The freshwater cladoceran Daphnia pulex was used as a test organism with the LP and SPP of Bailey Creek sediments. D. pulex was obtained from Dr. Art Buikema, Center for Environmental Studies, Virginia Polytechnic Institute, Blacksburg, Virginia. The animals were maintained in 75.6 litres all-glass aquaria and fed a diet of yeast suspension and mixed algae. Test chambers were 250-ml Pyrex beakers with a final liquid volume of 200 ml.

70. Table 12 shows the results of a range-finding bioassay using LP and SPP prepared with sediment from Bailey Creek and water from the James River ship channel. One hundred percent LP exhibited significant

toxicity at 4 hr. One hundred percent SPP was also toxic, but mortalities began later than in the LP. Mortalities in 50 percent and 10 percent LP and SPP were slightly higher than in the ship channel water controls.

71. Tables 13 and 14 list the results of a follow-up bioassay with the Bailey Creek samples. There was no disposal site water remaining, and the LP and SPP were diluted with Rocky Springs water. Rocky Springs is a clear, unpolluted stream approximately 30 miles south of Vicksburg, Mississippi.

72. The range-finding bioassay indicated that the toxic levels for the LP and SPP were above 50 percent. Therefore, 60, 70, 80, 90, and 100 percent LP and SPP were used as test concentrations. From the data in Tables 13 and 14, it can be seen that none of the concentrations were toxic to Daphnia pulex in the 91.5-hr test period.

73. The difference in toxicity of the two tests may have been caused by a number of variables, either alone or in combination. These variables included:

a. The range-finding bioassay (Table 12) was conducted in the laboratory where the room temperature fluctuated between 22 and 26°C, while the second experiment was conducted in a controlled environmental chamber at 20 ± 1°C.

b. The first experiment was conducted with a natural light-dark cycle, while the second had a controlled 12-hr-L: 12-hr-D cycle.

c. Diluent water in the first experiment was James River disposal site water. Rocky Springs water was used as the diluent in the second experiment, but this should not affect the results with 100 percent LP and 100 percent SPP.

d. The LP and SPP were refrigerated at 4°C, but were 3 weeks older in the second experiment.

Bioassay using the solid phase

74. The freshwater grass shrimp Palaemonetes kadiakensis and the clam Corbicula manilensis were collected from the Mississippi River system in April 1976 and returned to the laboratory in aerated collection site (natural) water. Shrimp were held in Reconstituted Freshwater (RCF) mixed with water from the animal collection site (50 percent

of each). Charcoal filters were used to filter the water in the shrimp holding tanks. Clams were maintained in ice chests in natural sediment beneath aerated natural water. Temperature in the holding tanks varied from 19 to 25°C.

75. For the P. kadiakensis exposure, twenty-four 18.9-litre aquaria were prepared containing 8 litres of RCF and 55 grass shrimp each. The aquaria were placed in water baths at 20 ± 1°C during the experiment. RCF was used instead of natural James River water because the pH of the natural water was below 6.0. Six exposure conditions including controls were used with four replicates of each treatment. Shrimp were introduced to the aquaria for 24 hr and were dosed with sediment from Bailey Creek on days one and two. Half of the sediment was mixed with 1 litre of water and added each day. Final volume in all tanks was 10 litres. Before addition of the sediment, pH was 7.4 to 7.5 in all aquaria and DO was >9.0 ppm. After the sediment was added, DO decreased at the higher sediment concentrations. Therefore, these tanks were aerated periodically during the first 2 days of the 6-day experiment. Final sediment concentrations were 0.5, 1.25, 2.5, and 5.0 percent by volume, and controls included RCF and RCF with 5 percent clean sand added. The exposure tanks were checked daily with small nets and dead shrimp removed and frozen for chemical analyses.

76. Results of the exposures are presented in Table 15. Observations made on day one revealed an unexpected result. The RCF control animals were dying at a faster rate than animals exposed to the sediment. It was later determined that there were two problems with the RCF. First the reverse osmosis system, which was used to make the RCF, was flushed out the day before the RCF was prepared. Additionally, the RCF was used in the experiment less than 1 week after it had been prepared instead of being held for 30 days, as is recommended. Thus, the RCF used in the experiment may have been contaminated.

77. In spite of the problems with the RCF, the Bailey Creek sediments were shown to be toxic to freshwater grass shrimp at the higher sediment concentrations. An average of over 50 shrimp died in each aquarium after 6 days exposure to 5 percent sediment, while water and

sand controls lost an average of less than 20 shrimp each (Table 15). There is a trend revealed also in that more shrimp survived at the lower sediment concentrations than did at the higher concentrations. A complicating factor is the DO levels in the various aquaria. DO was consistently lower at the higher sediment concentrations. The daily check for dead organisms plus the normal movements of the shrimp kept a large percentage of the sediments continuously suspended. On day six, DO levels in the control tanks were 7.5 to 8.5 ppm as compared to 3.5 to 4.5 ppm in the 5 percent sediment tanks. Approximately four or five shrimp were unaccounted for at the end of the experiment and this was attributed to cannibalism during the test.

78. Statistical analysis of the data in Table 15 included analysis of variance (ANOVA) and a multiple range test. The results demonstrated than in spite of the high mortalities in the controls, there was a significant difference (0.05 level) in survival between the water and sand controls and the 2.5 and 5.0 percent sediment concentrations.

79. P. kadiakensis tissue from various sediment exposures was analyzed for kepone and trace element concentrations. In some cases, animals were pooled to obtain enough tissue for kepone analysis. Chemical analyses of the shrimp tissue demonstrated that the shrimp exposed to sediments containing 2 to 3 ppm kepone accumulated this chlorinated hydrocarbon in a relatively short time (Table 16). The highest levels found in the pooled samples of dead animals exposed to the different sediment concentrations were 0.33 ppm and 3.8 ppm. Control organisms that died showed negative levels of kepone. Animals that survived had much lower levels than the dead animals at the same sediment concentration. To summarize the P. kadiakensis experiment, mortality increased as the concentration of Bailey Creek sediment was increased. Animals that died had higher tissue concentrations of kepone than those that survived. Animals, both dead and living, accumulated kepone in relation to the amount of sediment they were exposed to; the higher the sediment concentrations, the more kepone the animals had in their tissues, with the exception of the 2.5 and 5.0 percent sediment exposures.

80. Trace element accumulation was also determined for P.

kadiakensis exposed to Bailey Creek sediment. Table 17 lists the results of trace element analyses for whole tissue samples. Nickel, As, and Cd were not accumulated. Zinc, Mn, and Cu were higher than background in most cases and lower in a few cases. Lead accumulated in day six animals exposed to sediment and was high in dead day four and day six animals. Iron was accumulated in most cases. However, in attempting to analyze trace element uptake, there was no obvious trend for P. kadiakensis.

81. Corbicula manilensis (except for Rocky Springs water controls) were allowed to establish themselves in 1.5 litres of Yazoo River sediment and 16.5 litres of water (one-half RCF and one-half disposal site) for 24 hr. Eighteen aquaria were prepared with 30 clams in each. The aquaria were in water baths with the temperature regulated to 20 ± 0.5°C. Three aquaria contained only water and clams while 15 also contained 1.5 litres (by volume) of Yazoo River sediment that was collected with the clams. All aquaria were gently aerated throughout the experiment. Clams in their natural sediment were exposed to 1.0, 2.0, and 5.0 percent Bailey Creek sediment concentrations, which were poured into the aquaria in 1 litre of James River water on days one and two. Final volume in all aquaria was 20 litres. The experiment was continued for 3 weeks and there were three replicates of each treatment. There were no mortalities during the experiment. One replicate aquarium of each treatment and one control were harvested each week. Clams were removed from their shells, the tissue frozen, and sent off for analysis of kepone and trace elements. A subjective observation was made with day 17 animals exposed to Bailey Creek sediment: their mantels were softer and more fragile than had been observed with the previously harvested experimental animals or control animals harvested at the same time.

82. Table 18 lists the initial kepone concentration in the sediment and water samples used in the kepone uptake experiment. Yazoo River sediment and Rocky Springs water did not contain measurable concentrations of kepone. The initial concentration of kepone in the water would have been about 40 ppb since the James River water was mixed with Rocky Springs water in a ratio of 1:1.

83. Table 19 lists the concentration of kepone for the various exposure conditions on days 7, 12, and 17. Yazoo River sediments-A are the tanks containing 1.5 litres of Yazoo River sediment with the clams, but only water was added to those tanks. Yazoo River-B had 1 litre (5 percent final volume) of Yazoo River sediment poured over the animals.

84. Although the Yazoo River sediments and Rocky Springs water did not contain measurable concentrations of kepone, the animals exposed to them accumulated low levels of kepone. Either the animals accumulated kepone, which was present at very low concentrations, or a natural product in the clam tissues, which interferes with the kepone analyses, was present. The data do not allow differentiation of the possibilities.

85. At day 7 the animals exposed to Bailey Creek sediment contained approximately the same concentration of kepone, regardless of the sediment concentration. By day 12, there was a slight decrease except for the 1.0 percent sediment, and the Rocky Springs control contained detectable levels of kepone (<0.3 ppb). At day 17, kepone in the Rocky Springs control and Yazoo River-A animals had increased slightly. Animals in Bailey Creek sediment had further reductions, except the 1.0 percent sediment-exposed animals that had the highest concentration. Apparently, the animals started to regulate the uptake of kepone, or began to metabolize it since they were kept in contact with the sediment for the entire 17 days. It is also possible that less kepone was available in the water column at the higher sediment concentrations because of readsorption to sediment particles.

86. Trace element uptake was also studied in the Corbicula exposure. Table 20 lists the initial concentrations of Cu, Fe, Mn, Pb, Zn, and Hg found in Yazoo River and Bailey Creek sediment and James River and Rocky Springs water.

87. Table 21 lists the concentrations of Cu, Fe, Mn, Pb, Zn, and Hg found in the animals for the various exposures on days 7, 12, and 17. In general, Corbicula did not accumulate any of the trace elements from the sediment. The concentrations of Cu and Zn were very high in the Bailey Creek sediment compared to the animals' natural (Yazoo River) sediment, which shows that bioaccumulation bears little relationship to sediment concentrations of these metals.

Windmill Point and James River Bioassays

Physical characteristics

88. The following table lists some of the water quality characteristics measured at the collection sites:

Location	Water Depth m	DO ppm	Temp. °C	Salinity ppt	pH
Disposal site	Surface	7.8	25.0	1.0	7.3
Disposal site	2	7.7	23.8	0.5	7.4
Disposal site	3.5	7.6	24.8	1.0	7.4
Dredge site	7	7.6	24.7	0.5	7.3

All samples were collected during low tide.

89. Upon return to the lab, dredge site water had a pH of 7.7 and disposal site water had a pH of 8.2. Suspended particulate phase and the liquid phase prepared with dredge site sediment and water had a pH of 7.2 and 8.0, respectively.

Chemical characteristics

90. Table 22 lists the results of kepone analyses for the sediment and waters used in the bioassays. Two preparations of SPP and LP were used in testing, and their concentrations are listed separately along with the sediment and water used for each preparation. The concentration listed for Windmill Point sediment on 16 June 1976 is an average of three replicates subsampled from a single container. The values reported were 170, 230, and 260 ppb for the samples and indicated the variability of the analytical method used. Both LP preparations contained more kepone than the dredge site water used to prepare them, indicating kepone was released from the sediment.

91. Table 23 lists the results of selected trace element analyses on samples used in the bioassays. Manganese was the only heavy metal released in large quantities in the liquid phase.

Bioassays using the liquid phase
and suspended particulate phase

92. The SPP and LP prepared from Windmill Point sediment were tested at 20, 25, and 30°C in bioassays using D. pulex. Test chambers were 250-ml Pyrex beakers with a final liquid volume of 200 ml each. The organisms were kept in environmental chambers on a 12-hr-D:12-hr-L cycle at the appropriate temperature. Table 24 lists the results of the bioassay conducted at 20°C. After 95 hr of incubation, very few mortalities had occurred; the highest mortality was in 100 percent disposal site water (four of the 30 animals). After 115 hr, four animals were dead in 100 percent LP; no further mortalities occurred in the disposal site water.

93. Table 25 shows the results of incubation at 25°C. After 94 hr, six animals were dead in 100 percent disposal site water and six were also dead in 100 percent SPP. No mortalities had occurred in 100 percent LP. At 114 hr, one mortality had occurred in 100 percent LP, six in disposal site water, and eight in 100 percent SPP.

94. Table 26 lists the results of incubation at 30°C. Very few mortalities occurred until the 71-hr observation. At that time all 30 animals in 100 percent LP were dead and 21 were dead in 50 percent LP. However, only five animals were dead in 100 percent SPP, and 14 were dead in 100 percent disposal site water. By 93 hr, only one animal remained alive and that was in 100 percent SPP.

95. An additional D. pulex bioassay was conducted at 30°C with a freshly prepared SPP and LP and included additional controls. Table 27 lists the results. After 23 hr of incubation, high mortality occurred in all test units except 100 and 50 percent SPP. The RCF controls also had a large number of survivors at 23 hr. At 49 hr, very few survivors were found in the test units with the exception of 100 percent SPP where 23 animals were still alive. The data of Table 27 show that at 30°C the mortalities are cuased primarily by temperature rather than the test solutions. It is interesting that the particulate matter in the SPP afforded some protection to D. pulex.

96. Seventy-five P. kadiankensis larvae (5 days old) were used in an experiment with SPP prepared with dredge site water from the James

River and Windmill Point sediments. Fifteen individuals (three replicates) were exposed at each concentration. Temperature was 20 ± 1°C and animals were maintained under a 12-hr-L:12-hr-D cycle. Controls included 15 animals in filtered natural water (Mississippi River) mixed with distilled water (1:1) and 15 animals in dredge site water. Data are presented in Table 28. The results indicate toxicity to the larvae at the highest concentration of SPP. After 117 hr of exposure, one animal of 15 was alive at 100 percent SPP, while 11 and 7 survived in dredge site water and control water, respectively.

97. A second experiment was conducted with P. kadiankensis larvae. Forty-five larvae, 16 hr old, were placed in 200 ml of either control water or 100 or 50 percent SPP. Control water for the larvae was natural water mixed with distilled water (1:1). The natural water contained some algae and small copepods. The experiment was conducted at 30°C under a 12-hr-L:12-hr-D photoperiod. The results are listed in Table 29. Survival was excellent for 24 hr. However, at 90 hr most of the experimental animals were dead (80 percent mortality in each treatment), while 40 percent of the controls had died.

Bioassays using solid phase

98. Fifteen 37.8-litre all-glass aquaria were prepared, each containing 18 litres of disposal site water. There were three replicates of each exposure condition with 20 animals per replicate. The aquaria were placed in water baths with a controlled temperature of 20 ± 1°C. After a 24-hr acclimation period, the sediment was introduced as a slurry in dredge site water. Treatments consisted of 1, 2, or 5 percent Windmill Point sediment in disposal site water. One-half the volume of sediment was poured on day one and the remainder was poured on day two. The final volume in all aquaria was 20 litres.

99. Animals were counted daily with the aid of fine-mesh nets. Use of the nets stirred the sediment at each sampling, causing turbidity. Before the initial sediment dose, DO was between 8.0 and 8.8 ppm, temperature was 20 ± 1°C, and pH was lowered to 7.5-7.6 in the experimental aquaria after addition of the sediment. Aquaria were aerated with the result that DO remained above 8.0 after the sediment additions. The experiment was continued for 14 days.

100. Table 30 shows the number of survivors after 14 days exposure. Highest mortalities occurred in the disposal site water (33 percent) and the 2 percent sediment exposures (32 percent). The 1 and 5 percent sediment treatments had 15 and 18 percent mortalities, respectively. However, control mortality was greater than or equal to mortality in the experimental aquaira, making interpretation of the results difficult. It does appear that the Windmill Point sediments showed virtually no toxicity to P. kadiakaneis, especially when compared to the Bailey Creek sediment.

New York Harbor Sediment Bioassays - First Shipment

101. Sediment samples were collected from the Perth Amboy (PA) and Bay Ridge (BR) Channels on 9 September 1976 by personnel from the New York District. A Smith-MacIntyre grab sampler was used to collect the sediment at water depths of 10 to 12 m. Water quality parameters were not measured at the collection site.

Bioassays using the liquid phase and the suspended particulate phase

102. Acartia tonsa was an organism used for toxicity tests with LP and SPP for PA and BR sediments. The LP and SPP were prepared using IO at 28 ppt salinity, and IO was also used as the dilution water. Salinity of all solutions was 29 ppt and the temperature was 24 ± 2°C. Crystallizing dishes (150 mm x 50 mm) were used as test chambers with a final volume of 100 ml in each dish. The pH of the test solutions ranged from 7.5 to 7.8 and the IO control had a pH of 8.3

103. The results are presented in Tables 31 and 32. Control mortality was a definite problem. The copepods seemed to be extremely sensitive to handling. They survived and reproduced in a mass culture in the laboratory in IO, but transfer by pipet to the test chambers resulted in much less vigorous animals. Table 31 (PA) shows that 100 percent LP, the 50 percent SPP, and the 10 percent SPP were very toxic. They produced 100 percent mortality to the test organisms in 21 hr,

while controls exhibited an average of 23 percent mortality after the same exposure period. The 10 percent LP produced more mortalities than were observed in controls.

104. The 100 percent BR LP produced 100 percent mortality in 24 hr and the 10 percent LP was also toxic (76.6 mortality in 24 hr) (Table 32). The 50 percent SPP was toxic with 100 percent mortality in 48 hr. However, control survival was only 40 percent after 48 hr, making the results difficult to interpret.

105. Larvae of the grass shrimp Palaemonetes vulgaris were exposed to the LP and SPP of the sediment from both sampling locations. The larvae were eight to 12 days old. Dilution water was IO at 28 ppt and the test temperature was 23 ± 1°C. The results are shown in Tables 33 and 34. Control survival over the 7-day test period was 85 percent. The LP and SPP of PA sediment were more toxic than the BR LP and SPP, but substantial toxicity was observed only at 100 percent LP and SPP of the PA sediment.

106. Mysidopsis bahia were obtained from a U. S. EPA laboratory at Gulf Breeze, Florida, and were used in preliminary tests with the LP of PA and BR channel sediments. Test salinity was 29 ppt and the temperature was 21 ± 2°C. There were some control mortalities, but shrimp exposed to the PA and BR SP had a greater number of mortalities than the controls (Table 35).

Bioassays using the solid phase

107. The estuarine grass shrimp Palaemonetes vulgaris and the hardshell clam Mercenaria mercenaria were used as test organisms with the SP of the sediments collected at PA and BR ship channels. The animals were placed in 37.8-litre aquaria containing 15 litres of IO. The aquaria were in water baths that were temperature controlled at 24 ± 1°C. Test salinity was 28 ppt. After a 24-hr acclimation period, the sediment was mixed with IO, and the slurries were poured into the aquaria. Final concentrations of sediment were 1 and 5 percent by volume for the shrimp exposure and 5 percent by volume for the clam exposure. Sediment was not added to the IO control tanks, and final volume in all tanks was 18 litres.

108. Introduction of the sediment slurry resulted in an average drop in the pH of the water from 8.2 to 7.8 or 7.9 and an immediate decrease in the DO from 6.5 ppm to 3.0 to 4.0 ppm. DO levels remained low in the experimental tanks for 6 to 8 hr after introduction of the slurry. The shrimp were fed Tetramin and freshly hatched Artemia, and the clams were fed algal suspensions every other day. After 1 week, 10 litres of water was removed from each tank and replaced with clean IO. The results for the shrimp tests are shown in Tables 36 and 37. A total of four shrimp died in the controls compared to 10 at the 5 percent PA concentrations. However, ANOVA of the data presented in Table 36 showed no statistical difference between survival in the controls and sediment-exposure aquaria (Table 37). No mortalities occurred among the clam exposures either in the controls or experimental aquaria (Table 38).

New York Harbor Sediment Bioassays - Second Shipment

109. Members of the New York District collected sediment from BR Channel on 9 December 1976 and from PA Channel on 20 December 1976. The samples arrived at WES on 13 December 1976 and 23 December 1976. Bay Ridge sediment was stored at 4°C and PA sediment at 21°C until used. Water quality measurements were not taken at the sediment collection sites.

Bioassays using the LP and SPP of Bay Ridge Sediment

110. The Bay Ridge sediment was used 2 days after arrival to prepare the SPP and LP. Instant Ocean was used to prepare the phases and as dilution water in the bioassays.

111. Mysidopsis bahia and juvenile Palaemonetes vulgaris were tested simultaneously but in separate chambers. Salinity was 26 ppt and the temperature was 21 ± 1°C. Initial pH of the solutions were IO 8.1, LP 7.6, and SPP 7.7. Dissolved oxygen levels were 5.5 ppm in all treatments initially and aeration was not supplied to the test chambers. One-litre beakers were used as test chambers with a final liquid volume of 900 ml each.

112. The results are given in Tables 39 and 40. Mysidopsis bahia (Table 39) survived better at all concentrations of LP and SPP than in the controls which had only 45 percent survival. Statistical analysis (t-test) of the data showed juvenile P. vulgaris survived the 72-hr exposure to 90 percent LP approximately the same as the controls (Table 40).

Bioassays using the solid phase of Bay Ridge sediment

113. A group of mixed species of mysids (primarily M. bahia, but possibly including M. bigelowi and M. almyra) were collected in November 1976 from the Pensacola, Florida, area. Salinity at the collection site was 18 ppt and temperature was approximately 13°C. The opossum shrimp were transported to the laboratory in aerated collection site water and slowly acclimated to 26 ppt salinity and 21 ± 3°C in IO.

114. Ten 1-litre beakers were prepared, each containing 500 ml of 26 ppt IO. A slurry containing 100 ml of BR sediment and 400 ml of IO was prepared and poured into five of the beakers. The remaining beakers received an additional 500 ml of IO. After allowing 30 min settling time, five mysids were introduced to each beaker. All beakers were supplied with aeration. Animals were exposed to the sediment for 96 hr. Many of the test organisms were observed to be partially buried in the sediment. At the end of the test period, 76 percent of the controls were alive and 60 percent of the test animals had survived. Data are shown in Table 41.

115. The 4-day exposure to the BR sediment resulted in greater mortality among test organisms than among controls. The difference was found to be statistically significant at the 95 percent confidence level using a one-tailed t-test analysis.

Bioassays with the LP and SPP of Perth Amboy Sediment

116. Mysids, primarily M. bahia, and juvenile grass shrimp, P. vulgaris, were tested with the PA liquid phase. Tests were conducted in 1-litre glass beakers, temperature was 20 ± 2°C, salinity was 26 ppt, and the photoperiod was 14-hr-L:10-hr-D. Aeration was provided for

the mysids. The LP was prepared using 0.20 μ pore-size Millipore filters. The sediment had been in the laboratory for approximately 2 weeks. Results are shown in Tables 42 and 43. Five mysids and three grass shrimp were used in each replicate. Survival of mysids was similar to controls; however, young grass shrimp exposed to 90 percent LP for 4 days exhibited greater mortality than control animals. The difference was found to be significant at the 95 percent level.

117. Mysids from the November 1976, Pensacola, Florida, collection were used in additional tests with the PA sediment. Animals were exposed to 100 percent LP or 100 percent SPP. Five mysids were placed in litre beakers containing 800 ml of the test solution. All chambers were aerated during the test. Table 44 presents the results at 49 hr. Control mortality was 20 percent while mortality of animals exposed to 100 percent SPP was 40 percent. Survival of exposed animals was not significantly different from control survival at the 95 percent level.

Bioassays using the solid phase of Perth Amboy sediment

118. Mysids were exposed to 10 percent SP (vol/vol) of Perth Amboy sediment. Each group of test organisms was separately acclimated in 1 litre of IO at 28 ppt salinity and a temperature of 22 ± 1°C for 38 hr before being transferred to the exposure aquaria. A 12-hr-L: 12-hr-D photoperiod was used during the experiment. Two hundred and fifty ml of sediment was added to the 2250 ml of IO at 28 ppt salinity and shaken for 30 min. The mixture was then transferred into aquaria that were in a temperature-controlled water bath. When the sediment had settled, the acclimated animals were transferred to appropriate exposure and control aquaria.

119. The results are shown in Table 45. After 48 hr exposure, survival in the controls was 80 percent (16 animals) and 50 percent (10 animals) in the 10 percent SP exposure. A t-test, however, showed no statistical difference at the 95 percent confidence level between control and experimental animals.

120. Juvenile grass shrimp, P. vulgaris, were exposed to Perth Amboy SP for 45 days under controlled conditions (data not shown).

Eight 18.9-litre aquaria were prepared, four containing 16 litres of IO and four with 14.4 litres of IO and 1.6 litres by volume of PA sediment. The sediment was mixed with 2.4 litres of IO and poured into the test aquaria before grass shrimp were introduced. Approximately 30 min after the sediment was added, the DO levels of the test aquaria averaged 5.6 ppm and the pH of the water was 7.7. Oxygen levels in the control aquaria averaged 7.2 ppm while the pH was 8.0. Initially there were 80 control shrimp and 85 experimental shrimp. All aquaria were in a temperature-regulated water bath with temperature maintained at 23 ± 1°C. The aquaria were aerated for 5 days, after which charcoal filters were placed in both control and experimental chambers. Shrimp were fed either brine shrimp or Tetramin fish food daily.

121. Throughout the 45-day experiment, DO levels remained above 6.0 ppm for both control and experimental tanks. The pH values for the controls were 7.9 to 8.0. The pH values for experimentals increased from 7.7 to 8.1 to 8.2 for 6 days and then remained at those levels for the remainder of the test. Mortality for both groups of shrimp was negligible with five control shrimp and four experimental shrimp dying during the long-term experiment. Thus, no apparent harmful effects of this material on juvenile grass shrimp were demonstrated. A few female shrimp in both treatments became gravid during the experiment.

122. Two groups of P. vulgaris larvae, each group from a single female, were exposed to the LP and SP of PA sediment. Larvae were initially either 2 or 4 days old. They were divided into groups of five and placed in 1-litre beakers containing 500 ml of IO at 25 ppt salinity. Beakers were held in an incubator at 20 ± 1°C and 12-hr-L: 12-hr-D photoperiod during the test. Eight beakers were controls and received an additional 500 ml of IO, eight received a slurry of PA sediment and IO which produced a final volume of 3 percent sediment, and eight received 500 ml of LP resulting in a concentration of 50 percent LP. Larvae from one of the two hatches were also exposed to a 1 percent (10 ml sediment:990 ml IO) mixture. The PA slurry was prepared with a blender and poured into the beakers containing the larvae. The

experiment was continued for 33 days with larvae counted daily and fed brine shrimp nauplii.

123. Dissolved oxygen and pH were checked before and after addition of sediment or LP. The DO levels were lowest in the 3 percent sediment beakers. Values as low as 3.0 ppm were recorded immediately after addition of the sediment. Control DO values were 7.2 to 7.6 ppm. Two hr after the addition of the sediment, the DO values were 4.3 to 5.0 in the 3 percent sediment beakers. Average pH values for the various treatments at 4 hr were 7.9 for controls, 7.9 for 50 percent LP, 7.8 for 1 percent sediment, and 7.7 for 3 percent sediment. Six days after the experiment began, 600 ml of test solution was replaced with clean IO for all test conditions. This substantially reduced the LP concentration; however, the sediment concentration in the beakers remained essentially unchanged and was partially suspended by the addition of IO. Half of the liquid in the beakers was replaced every 2 to 3 days after day 6. Data showing survival of the larvae are presented in Table 46.

124. The data show that exposure of grass shrimp larvae to 50 percent PA LP for 6 days (with lower LP levels thereafter) was not harmful. Larvae exposed to 1 and 3 percent PA sediment were harmful when compared to control animals. Survival of all larvae exposed to the PA sediment was sngnificantly lower at either the 99 or 95 percent confidence level from control survival. The 3 percent sediment mixture revealed substantial toxicity during the test period. There was evidence that the initial pouring of the 3 percent sediment slurry was detrimental to the young larvae. There were many dead larvae after 48 hr exposure to the 3 percent sediment. It is likely that this effect was due to the high level of suspended particulate sediment present in the exposure beakers during the first few hours of the test, combined with the overall decrease in water quality such as low DO levels. However, chemical toxicity of the sediment was indicated since exposed larvae continued to die as the experiment progresses. Also larvae exposed to the 1 percent sediment survived the first 7 days of the test but began to die thereafter.

125. Growth of the larvae discussed above was monitored during the

experiment and for 20 days afterwards. Twenty individual larvae from each treatment were weighed each week to the nearest 0.1 mg using a Mettler H64 balance. Larvae were chosen at random, blotted lightly with an absorbent tissue, and weighed. Wet weight was determined. Handling of the larvae was not a problem except during the first weighing when a few larvae appeared stressed by the procedure. After the 33-day exposure period, larvae were moved to small glass aquaria containing clean, charcoal-filtered IO. Growth was checked for 20 additional days. Data for one of the hatches are shown in Table 47.

126. These data show the effect of the sediment quite clearly. The weight of larvae exposed to sediment LP and SP was greater than controls at 6 days. However, 5 days later larvae exposed to the SP weighed significantly less than controls while larvae exposed to LP were equal to controls. This trend generally continued during and for a few days after the exposure period. After 20 days in clean, filtered IO the mean weight of sediment-exposed larvae was not significantly different from controls. This experiment has demonstrated methods for determining toxic and sublethal effects of a contaminated sediment.

New York Harbor Sediment Bioassays - Third Shipment

Physical characteristics

127. Members of the New York District collected sediment from the PA Channel on 12 April 1977. The sediment arrived at WES on 15 April 1977 and was stored at 4°C until used. Subsamples of the sediment were sent to other laboratories, including some EPA laboratories, for use in bioassays with benthic animals. Water quality measurements were not taken at the sediment collection site.

Chemical characteristics

128. Table 48 shows the results of chemical analyses for total concentration of selected constituents in the control and experimental sediments. The PA test sediment contained high concentrations of all trace elements determined (As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn)

and far exceeded concentrations found in control sediments. Total sulfides, chemical oxygen demand (COD), and immediate oxygen demand (IOD) were also higher in the test sediment. The pesticide DDT and its degradation products were generally not in higher concentrations than in the control sediments. Dieldrin was high in the Pensacola control sediment. Polychlorinated biphenyls were higher in the test sediment than the control sediments and total alkanes were slightly higher in the two test sediments. Total aromatic hydrocarbons and the other petroleum hydrocarbons were either not detectable or at very low ppb levels.

Bioassays using the solid phase of Perth Amboy sediment

129. An animal collecting trip was conducted during March 1977 to Horn Island which is approximately 8 miles southeast of Biloxi, Mississippi, in the Gulf of Mexico. Benthic burrowing amphipods and marine isopods were collected near the island in 1 m of water from sediments that were primarily sand and shell. Salinity was 22 ppt and water temperature was 18°C. The animals were returned to the laboratory in aerated collecting site water. In the laboratory, the amphipods were placed in flow-through chambers containing a 5- to 6-cm layer of sand and IO at a salinity of 22 ppt and temperature of 24 ± 1°C. The amphipods were held for 2 weeks under those conditions and appeared very healthy; when removed from the sand and then placed back on the sand surface, they immediately burrowed. The salinity was then raised slowly over a period of 1 week to 30 ppt. Water changeover in the holding tanks was approximately one-half volume (26 litres) per day. The amphipods used for the bioassays were tentatively identified as members of the genus Parahaustorius. The isopods were identified as Sphaeroma quadridentatum. S. quadridentatum were held and acclimated under static conditions at the same temperature and salinity used for the amphipods.

130. Parahaustorius was used in a 9-day static test with PA sediment. Test containers were 5.68-litre all-glass aquaria and were established in the following manner. Four hundred ml of clean sand was placed in each aquarium with 3 litres of IO at a salinity of 30 ppt.

Twenty animals 3 to 6 mm in length, were transfrred to each test unit, and the aquaria were placed in a water bath at 25 ± 1°C for 24 hours. The test was started by adding various slurries to the aquaria; five each received 200 ml IO, five each received 100 ml of kaolinite in 100 ml IO, and five each received 100 ml PA sediment in 100 ml IO. Aeration was supplied to all aquaria. Temperature, DO, salinity, and pH were monitored. Temperature was 25 ± 1°C and salinity remained at 30 ppt throughout the experiment. Water was renewed at 4, 49, 102, 154, and 202 hr. Seventy-five percent of the water was changed at 4 and 49 hr, and 66.6 percent at each subsequent change. Below are listed some of the changes in DO and pH that occurred during the test.

		Before Sediment Addition		Immed. After Sediment Addition		Before 49-hr Water Change		After 49-hr Water Change	
Exposure Condition	Replicate	DO	pH	DO	pH	DO	pH	DO	pH
Sand control	1	6.3	8.0	6.3	8.0	6.2	8.1	5.9	8.0
	2	6.3	8.0	6.3	8.0	6.2	8.1	5.9	8.0
	3	6.2	8.0	6.3	8.1	6.4	8.1	6.1	8.1
	4	6.3	8.0	6.2	8.0	6.5	8.1	6.1	8.0
	5	6.2	8.0	6.3	8.0	6.5	8.1	5.9	8.0
Kaolinite control	1	6.3	8.0	6.3	7.7	6.6	8.0	5.8	7.9
	2	6.3	8.0	6.3	7.5	6.6	8.0	5.9	7.9
	3	6.2	8.0	6.2	7.5	6.6	8.0	5.8	7.9
	4	6.2	8.0	6.2	7.5	6.6	8.0	6.0	7.9
	5	6.4	8.0	6.3	7.6	6.6	8.0	5.9	7.9
PA sediment	1	6.2	8.0	3.2	7.9	6.2	7.9	5.0	7.8
	2	6.2	8.0	3.2	7.9	6.4	8.0	5.3	7.9
	3	6.3	8.0	3.3	7.9	6.5	7.9	5.2	7.9
	4	6.2	8.0	3.2	7.9	6.4	7.9	4.3	7.8
	5	6.3	8.0	3.5	7.9	6.4	7.9	5.6	7.9

The above data for the 49-hr water change are typical values obtained before and after changing water. The only major decrease in DO occurred immediately after the initial sediment addition. The animals were fed a small amount of Tetramin and Cerophyll on day 1 and every other day thereafter. Animals remained in the sediment during the light period (14 hr L:10 hr D) but holes and tracks were visible in the kaolinite and PA sediment after the dark period.

131. After 9 days of exposure, the sediment was washed through a 1-mm mesh sieve and the number of surviving animals was recorded. Table 49 gives the results of the test using Parahaustorius. Three animals died in the sand controls, five died in the kaolinite, and nine died in the PA sediment. Cochran's test for homogeneity of variances showed that the variances were homogeneous, and analysis of variance (ANOVA) and the Student-Newman-Keuls multiple range test demonstrated that there was no statistical difference between survival of the control and experimental animals at the 95 percent confidence level.

132. The tests using the isopods were carried out under static conditions using IO in round glass aquaria, 20.0 cm in diameter by 7.5 cm high. Five aquaria were used for controls and five as experimentals. The tests were run at a salinity of 28 ppt and the temperature was 21 ± 2°C. The initial pH of the PA bioassay control and treatment units was 8.0 and 7.9, respectively.

133. The control and test units were established by adding 1000 ml of IO and 100 ml of washed sand into each aquarium. Twenty adult isopods, 4 to 6 mm in length, were placed in each aquarium. After the isopods were introduced, 50 ml of test sediment was added to each of the five treatment aquaria, producing a 2- to 3-mm layer of test sediment over the reference sediment. The control units contained only reference sediment and IO.

134. The animals were exposed to the PA sediment for 4 days, the sediment from each test unit washed through a 1.0-mm sieve, and the test animals recovered and counted. The isopods were considered alive if they showed any movement or response to gentle probing. Specimens not recovered were considered dead.

135. The mortality data for the PA sediment bioassay are presented in Table 50. The mean isopod mortality was 81 percent after 96 hr of exposure to the PA sediment and only 18 percent in the controls. The treatment mortality was significantly higher than the control mortality at the 95 percent confidence level as determined with a one-tailed t-test for unpaired data. The data indicated a significant toxic effect of the PA sediment to S. quadridentatum.

136. Opossum shrimp, Mysidopsis bahia, were reared in the laboratory in IO at 28 ppt salinity from an original group of animals collected in January 1977 at Pensacola, Florida. Temperature was 24 ± 2°C in the holding tanks. Reference sediment was obtained from Weeks Bay, Alabama, a small estuary east of Mobile Bay.

137. Ten 18.9-litre all-glass aquaria were prepared containing 7.2 litres of IO at 28 ppt salinity and 0.8 litre of Weeks Bay reference sediment. The aquaria were placed in a temperature-controlled water bath at 25 ± 2°C for the duration of the experiment (10 days). Reference sediment was sieved using a 1.4-mm sieve before being added to the exposure tanks. Very few infaunal organisms were found in the sediment.

138. Twenty-four hours after establishing the exposure tanks in the water bath, 200 adult mysids were separated from the laboratory population and divided into groups of 20. Twenty animals were added to each of the tanks and fed freshly hatched Artemia. The shrimp were allowed to acclimate for 48 hr. Dissolved oxygen was greater than 6.0 ppm in all tanks, while pH varied from 7.9 to 8.0 standard units. All aquaria were provided with aeration throughout the exposure period.

139. After the acclimation period, approximately 0.4 litre of PA sediment was mixed with 0.4 litre of IO and slowly poured into each of the five experimental aquaria for a final test sediment concentration of approximately 5.3 percent. Additional sediment was not added to the control aquaria. It was impossible to count the mysids in either the control or test aquaria because of their small size and the turbidity of the water in the static test chambers. Two hours after addition of the PA sediment, 75 percent of the water in control and test aquaria were replaced. Changing the water resulted in increased turbidity; however, DO levels remained above 6.0 ppm. Thereafter, water was changed every 48 hr for 6 days. After day 6, the water changes were discontinued because it was thought that they were more harmful to the mysids than the static water. Throughout the experiment the DO levels remained above 6.0 ppm; pH values were generally 7.8 to 7.9 except immediately after the water changes when they were 7.9 to 8.1, usually slightly higher in the PA aquaria.

140. After 10 days exposure to the test and reference sediments the aquaria were emptied by siphoning the water through a small aquarium net and pouring the last few millilitres of water through a similar net. Mysids caught in the net were placed in culture bowls and separated into adults and young. Since the mysid life cycle is 18 to 24 days at the test temperature used, it would be expected that some natural mortalities would occur and that young would be produced during the exposure.

141. Table 15 shows the results. No dead adult mysids were found. Some of the young mysids found were dead but not decomposed, which indicated they were killed by handling at the end of the experiment. Control mortality was greater than 40 percent. Control animals produced a greater number of surviving offspring but the number of control offspring was not statistically different from the number of sediment-exposed offspring that survived (data not shown).

142. Ten exposure dishes were prepared with 1840 ml of IO; 40 ml of Pensacola reference sediment was mixed with 40 ml of IO and poured into the dishes. Ten adult mysids and ten Palaemonetes intermedius larvae (8 days old) were then added to each dish. After a 24-hr acclimation period with aeration the water temperature was 25 ± 1°C, DO was 5.5 to 5.9 ppm, and pH was 8.1 to 8.2.

143. The 10 dishes were divided into two groups of five each. The first group received an additional 40 ml of reference sediment while the second group received 40 ml of PA sediment. The PA sediment had been in the laboratory 3 weeks. The sediments were mixed with IO before being poured. The final sediment concentration was 2 percent and the experiment was run for 7 days. One-half of the water was changed in all dishes on day 5. Test organisms were fed Artemia daily. The temperature was 24 ± 1°C; DO was 6.4 ppm in the reference sediment dishes, and 5.8 to 6.2 ppm in the PA treatments. The final pH readings were 7.6 for reference sediment and 7.4 to 7.5 for PA sediment. The data are presented in Table 52.

144. Ninety-six percent of the control and experimental mysids survived the test. There was no statistical significance at the

95 percent confidence level between larval survival under the two conditions; the PA sediment was not harmful to the mysids or grass shrimp larvae when compared to the reference sediment.

Long Island Sound Sediment Bioassays

Physical characteristics

145. Sediment was collected from four sites on a 90-m disposal mound in the dredged material disposal site of New York Bight of Long Island Sound (LIS), New York. The sediment was collected on 28 April 1977 by members of the New York District. A Plessey grab sampler was used to collect the sediment and the following water quality measurements were taken:

Site	Depth m	Temp. °C	Salinity ppt	pH
1	20	7.8	32.9	8.3
2	33	7.7	33.1	8.3
3	38	6.7	32.8	8.2
4	38	7.8	33.4	8.2

Approximately 4 litres of sediment were collected from each site and shipped on ice to WES. The sediment arrived at WES on 3 May 1977. Sediment from the four sites was thoroughly mixed together and used as a composite sample in the bioassays.

Chemical characteristics

146. Table 48 shows the results of chemical analyses for total concentrations of selected constituents of LIS sediment. The sediment contained high concentrations of As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn. Total DDT and total PCB were also high, as were total alkanes.

Bioassays using the solid phase of Long Island Sound sediment

147. The sediment collected from the LIS disposal site was used in a test with Parahaustorius sp. and Mysidopsis sp. The method of exposure for Parahaustorius was the same as the PA tests and the animals

used were from the same collection. The mysids were reared in the laboratory from animals collected in Pensacola, Florida, about 5 months prior to the test. The major differences between the LIS Parahaustorius test and the PA test were that (a) sand was not poured on the sand controls; (b) kaolinite was not used; and (c) mysids were added to the test aquaria after the 4-hr water change. Below are some typical changes in DO and pH that occurred during the test.

Exposure Condition	Replicate	Before Sediment Addition		After Sediment Addition		Before 4-hr Water Change		After 4-hr Water Change	
		DO	pH	DO	pH	DO	pH	DO	pH
Sand Control	1	6.3	8.2	6.3	8.2	6.6	8.1	6.2	8.1
	2	6.2	8.2	6.2	8.2	6.6	8.1	6.2	8.2
	3	6.4	8.2	6.3	8.2	6.7	8.1	6.5	8.2
	4	6.4	8.2	6.4	8.2	6.7	8.1	6.6	8.2
	5	6.3	8.2	6.2	8.2	6.7	8.1	6.6	8.2
LIS Sediment	1	6.3	8.2	4.2	7.9	5.8	8.0	5.1	8.0
	2	6.3	8.2	4.3	7.9	5.6	8.1	5.3	8.0
	3	6.3	8.2	4.0	7.9	6.2	8.1	5.4	8.0
	4	6.4	8.2	4.5	8.0	6.1	8.1	5.4	8.0
	5	6.4	8.2	4.3	7.9	6.3	8.1	5.6	8.0

148. The most dramatic drop in DO occurred immediately after the addition of the sediment, but none of the aquaria went below 4.0 ppm and the DO was above 5.0 ppm within 2 hr after sediment addition.

149. Ten mysids were added to each aquarium about 2 hr after the 4-hr water change. Freshly hatched Artemia were fed to the mysids every other day during the test. After 11 days of exposure (10.5 days for the mysids), the number of surviving animals was recorded. Table 53 gives the results for each species in the LIS sediment test and Table 54 gives the number of survivors. A t-test using the data of Table 54 showed there was no statistical difference between total survival in the controls and experimental aquaria at the 95 percent confidence level.

150. Ten exposure dishes were prepared with 1840 ml of IO; 40 ml of Pensacola reference sediment was mixed with 40 ml of IO and poured in the dishes. Ten adult mysids and ten P. intermedius larvae (8 days

old) were added to each dish. After a 24-hr acclimation period with aeration, the water temperature was 24 ± 1°C, DO was 5.5 to 5.9 ppm, and pH was 8.1 to 8.2.

151. The 10 dishes were divided into two groups of five each. The first group each received an additional 40 ml of reference sediment while the second group each received an additional 40 ml of LIS sediment. The LIS sediment had been in the laboratory 8 days and final test sediment concentration was 2 percent. One-half of the water was changed in all dishes on day 5. Test organisms were fed Artemia daily. The final temperature was 24 ± 1°C; DO was 6.4 ppm in the reference sediment dishes and 5.8-6.2 ppm in the LIS treatments. The final pH readings were 7.6 for reference sediment and 7.4-7.5 for the LIS sediment. The data are presented in Tables 55 and 56.

152. Survival of grass shrimp larvae exposed to the control sediment was 64 percent while larvae exposed to LIS sediment had 52 percent survival. The mysids and 96 percent survival in the control sediment, and a 90 percent survival in the LIS sediment.

153. Statistical analysis of the data in Table 56 indicated no statistically significant difference between mortalities in the controls and experimental test units at the 95 percent confidence limit.

154. Tests using S. quadridentatum were carried out under static conditions in round glass aquaria, 20.0 cm in diameter by 7.5 cm high. Five aquaria were used for the controls and five for the treatments. Instant Ocean dissolved in aged, dechlorinated tap water was used in holding the test animals and as test water. The tests were run at a salinity of 28 ppt and temperature was 21 ± 2°C. The initial pH of the LIS bioassay control and treatment units was 8.1 and 7.8, respectively.

155. The control and treatment test units were established by adding 1000 ml of IO and 100 ml of reference sediment to each aquarium. The reference sediment was washed sand. For the LIS bioassay, 16 isopods were put into each test chamber. After the isopods were introduced, 50 ml of test sediment was added to each of the five replicate treatment aquaria. This produced a 2- to 3-mm layer of test sediment over the reference sediment. The control units contained only the initial dose of reference sediment.

156. The LIS tests were conducted for 4 days after which the sediments of each test unit were washed through a 1.0-mm sieve and test animals were recovered and counted. The isopods were considered alive if they showed any movement or response to gentle probing. Specimens not recovered were considered dead.

157. Table 57 shows the 96-hr mortality data for the LIS sediment bioassay. There was little difference in the control and treatment mortalities. Seventy-seven and one-half percent of the controls survived while 65 percent of the experimental survived. However, statistical analyses of the data demonstrated no difference between control and experimental mortalities at the 95 percent confidence level.

Vicksburg Area Sediment Bioassays

Physical characteristics

158. Sediment was collected from a small stream in the Vicksburg, Mississippi, area on several occasions and used in bioassays within 48 hr after collection. The stream receives wastes from a nearby chemical plant as well as from the Vicksburg sewage treatment plant.

Chemical characteristics

159. Table 58 lists the results of chemical analyses of selected constituents for the control and experimental sediments. Control sediment was collected from the same location that Palaemonetes kadiakensis used in the bioassays were collected. The experimental sediment had higher concentrations of total sulfides and and oil and grease than the other sediment. Arsenic, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were high in the experimental sediment, but were also high in the control sediment. Total concentrations of DDT, PCB, alkanes, and aromatic hydrocarbons were much higher in the experimental sediment than in the control sediment.

Bioassays using the solid phase of Vicksburg area sediments

160. Palaemonetes kadiakensis, freshwater grass shrimp, were collected from the backwater pools of the Mississippi River system

near Vicksburg. The animals were returned to the laboratory in aerated collection site water. When the site water reached the temperature of laboratory-aged tap water, all animals were transferred to flow-through bioassay units and observed for 24 hr. No mortalities occurred. The animals were then transferred to polyethylene baskets (20 per basket) and distributed to the appropriate test unit. Six flow-through troughs and six 27.8-litre aquaria were used in the experiment. Three units of each system were controls and three were experimentals. Each unit received two baskets with 20 animals per basket. Two percent control sediment (taken from the animal collection site) was then added to all units. The animals were observed for an additional 4 days. No mortalities occurred. The control tanks received an additional 2 percent control sediment and the experimental tanks received 2 percent of contaminated test sediment from a stream in the Vicksburg area. Total final volume was 52 litres in each flow-through unit and 20 litres in each static unit; 4 percent of the final volume in each test unit was sediment. The flow-through system had one-half volume change of water every 24 hr. Water used in all units was aged (30 days or more), dechlorinated tap water.

161. Control survival was excellent, with almost 100 percent of the animals alive at the end of the test (Tables 59 and 60). One hundred percent mortality occurred in the static experimental within 24 hr. Mortality was also high in the flow-through experimentals with approximately 50 percent of the animals dying within 24 hr.

162. The results of the flow-through bioassay were encouraging. Control survival was good and indicated the observed effects were caused primarily by the sediment. While the sediment used in the experiments exhibited toxic effects, interpretation of the mortality data is complicated by the changes in pH that occurred. The test sediment caused a rise in pH from 7.5 to between 9.0 and 9.7. The rise in pH would have caused other chemical changes which stressed the animals, and it is difficult to estimate how these parameters affected the sensitivity of the grass shrimp toward the contaminants present in the sediment.

163. Adult Musculium were collected from Brown's Lake at WES from shallow sediments by sieving sand and silt through a 2-mm sieve. The clams were placed in small glass aquaria containing fresh water and about 2 cm of clean sand and held at laboratory temperature and light levels until they were used for bioassays. Testing was usually done within 1 week of collecting organisms.

164. Adult Musculium were used in two clam bioassays. In the first test, Clam Test I, two concentrations of sediment were tested, 0.2 and 5 percent. The test containers used were 190 x 100 mm crystallizing dishes. Each unit contained 50 ml of sand and 1000 ml of aged tap water. The test units were established by first adding the water and sand, then 10 clams to each, followed by the addition of the test sediment. No additions were made to the controls. Four replicate units were run for both treatment levels and for the sand control.

165. Clam Test II was established in the same manner as the first test but 15 clams were placed in each test unit and the treatment levels tested were 5 percent, 3 percent, 2 percent, and 1 percent sediment in addition to washed sand controls.

166. Clam Test I ran for a period of 8 days and Clam Test II was terminated on day 4. Observations for clam mortality were made at 24-hr intervals and dead clams were removed throughout both tests.

167. The bioassays were run at laboratory ambient light (8 hr L: 16 hr D) and temperature levels. The temperature remained at about 22 ± 2°C. All test containers were covered with plexiglass plates to reduce evaporation. The DO of the test waters remained at about saturation (8.0 ppm) throughout both experiments. The initial pH was 7.8 for the control and 0.2 percent treatment and 8.6 for the 5 percent treatment in Clam Test I. The pH was 8.6 for all test treatments in Clam Test II and 8.0 in the control.

168. For Clam Test I mean mortality values of the treatments were compared with the control values using ANOVA and the Student-Newman-Keuls multiple range test (Table 61). The 5 percent sediment treatment mortality was significantly higher than the control mortality at the

95 percent confidence level. No difference was found between mortalities in the 0.2 percent treatment and control.

169. Statistical analyses for the data on day 4 of Clam Test II included Cochran's Test for homogeneity of variances, a one-way analysis of variance, and the Student-Newman-Keuls multiple range test for mean comparison (Table 62). The mean comparison indicated no statistical difference in the control and the 1 percent sediment treatment, but all other treatment mortalities were significantly higher than the control mortalities at the 95 percent confidence level.

170. The isopods Lirceus sp. were collected from below a spillway of Brown's Lake by handpicking the animals from the bottom of submerged rocks and debris. Isopods were maintained in small glass aquaria in aged tap water under laboratory temperature of 22 ± 4°C and an 8-hr-L: 16-hr-D cycle until used in the bioassay.

171. A 96-hr static sediment bioassay was run on the Vicksburg area freshwater sediment using adult Lirceus sp. as the test organism. The procedure was the same as that used in the clam tests with 10 isopods per container and four replicate containers per treatment. The total volume of water and test sediment was 1000 ml with 50 ml of washed sand in each treatment and control container. The treatment levels tested were 0.2 and 2 percent sediment and washed sand controls. Dead isopods were counted and removed at 24-hr intervals.

172. The pH of the test waters immediately after addition of test sediment was 7.8 for the control and 0.2 percent treatment and 8.4 for the 2 percent treatment. Dissolved oxygen was at saturation during the test. Temperature was 22 ± 2°C and an 8-hr-L:16-hr-D light cycle was used.

173. The isopod response to the test sediment is summarized in Table 63. The 2 percent sediment level produced a mean mortality of 22.5 percent after 4 days. Control mortality was 7.5 percent during the same period. The 2 percent sediment treatment produced six mortalities above the control and 0.2 percent sediment treatments, but the difference was not statistically significant at the 95 percent confidence level.

PART IV: SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary of Results

174. The purpose of the work described in the previous section was to develop biological methods as an aid in evaluating the potential ecolological impact of contaminated dredged material scheduled for open-water disposal. To this end, sediments contaminated with trace elements, pesticides, PCB, kepone, petroleum hydrocarbons, and other environmental contaminants were obtained and analyzed for selected chemical and physical parameters. They were subsequently used in bioassay experiments with invertebrate animals captured in the field or obtained from other researchers. Some of the animals were successfully cultured in the laboratory and offspring were used in later tests.

175. Test sediments were collected from the Duwamish River, Seattle, Washington; Bailey Creek and Windmill Point, James River, Virginia; Perth Amboy and Bay Ridge Channels, New York Harbor; a Long Island Sound disposal site, New York; and a contaminated stream in the Vicksburg, Mississippi, area. Reference sediments were collected along with organisms from visually clean areas and were generally found to be much less contaminated than test materials. Test organisms used ranged from the small marine copepod Acartia tonsa to the estuarine grass shrimp Palaemonetes pugio to the large, hardy marine clam Mercenaria mercenaria. Freshwater animals included the small cladoceran crustacean Daphnia pulex and the freshwater grass shrimp P. kadiakensis. Some of the organisms used in these developmental experiments represent animals which have not been previously used in experiments of this type. The opossum shrimp Mysidopsis, the amphipod Parahaustorius, and the freshwater clam Musculium, among others, fall into this category.

176. Approximately five separate experiments were conducted with the Duwamish River sediment, five with the Bailey Creek sediment, six with the Windmill Point material, sixteen with the Perth Amboy and Bay Ridge sediments, four with Long Island Sound sediments, and five with a Vicksburg area sediment. Additional preliminary experiments

with these materials and with the reference toxicant $HgCl_2$ were also completed. Individual tests are discussed in the following paragraphs.

Duwamish River

177. The concentrations of PCB found in the Duwamish River and Slip No. 1 sediments were not high compared to previously reported values. A recent report has indicated that most of the PCB spilled in 1974 at Slip No. 1 was recovered with sediment and placed in an upland disposal pond.[30] Based on PCB concentrations reported by Fulk et al.,[31] for some U. S. harbors, the values reported here were average. However, the values are low compared to those reported by Young and McDermott-Ehrlich[32] for sediments collected near the coast of California and extremely low when compared to concentrations reported by Nadeau and Davis,[33] and Ahmed[34] for various areas of the Hudson River. The concentrations of total trace elements in the sediments are high for As and Pb, based on a comparison of chemical analyses of other sediments given in this report and those of Brannon et al,[35] Katz et al,[36] and Slowey et al.[37] The other trace elements reported are in the concentration ranges found by those workers.

178. The Duwamish River LP did not produce greater mortality of A. tonsa than the disposal site water (Table 3). Survival was greater in 0.1 and 1.0 percent LP than in controls and in the higher concentrations of LP. When exposed to the SPP, the first test (Table 4) indicated that particulate matter was providing one or more positive survival factors (such as microorganisms for food or adsorption of contaminants) because survival of A. tonsa was better in the three highest concentrations than in the controls or in the lowest SPP concentration. However, a second test (Table 5) using the SPP and the same collection of A. tonsa showed great variability in mortality among the treatments. Survival in the second test was best in the disposal site water and in the 10 percent SPP treatments and lowest in the 100 percent SPP.

179. Specific conclusions about the toxicity of the LP or SPP of Duwamish River sediments toward A. tonsa could not be made at this point because of the variability in the results. However, it was decided

that A. tonsa would be a good test organism if the problems of transporting and handling the animals could be solved.

180. T. californicus was exposed to the SPP of Duwamish River sediments for 96 hr (Table 6). The organisms survived exposure to all SPP concentrations, and it was decided to concentrate on A. tonsa as a marine zooplankton test organism. The decision was based on observations in handling T. californicus and other bioassays (not reported) that demonstrated they were not very sensitive to toxicants. Also, discussion with EPA personnel revealed that T. californicus was much less sensitive than A. tonsa to a number of heavy metals.

181. The results of the mortality study using P. pugio exposed to Duwamish sediments were difficult to interpret because control mortality was high, and in some cases, similar to the percent mortality of experimental animals (Tables 7 and 8). Observed mortality in the artificial seawater controls was higher than would normally be expected. This was probably due to the static nature of the experiment where the shrimp were held for 14 days in unfiltered water. Also these animals exhibit cannibalistic tendencies under crowded conditions. Thus, even though the test conditions were poor, more control shrimp survived the experiment than those exposed to the Duwamish sediment. The percent mortality of animals exposed to 0.5 and 1.0 percent SP was more than twice as high as the IO controls. Rangia cuneata survived well at all exposure levels and in the controls. Chemical analyses of whole tissue samples showed that both organisms accumulated PCB from the sediment (Table 9). The data does not allow the differentiation of the immediate source of the PCB, directly from the sediment or by way of the water. However, the important consideration is that sediments contaminated with PCB were a source of contamination for the animals. The average level of tissue contamination of animals prior to the experiment and those exposed to disposal site water and clean sand was 0.09 ppm PCB for shrimp and 0.05 ppm PCB for clams. These levels may be compared to those of animals exposed to the Duwamish and slip sediments, which were much higher.

Bailey Creek

182. The concentration of the insecticide kepone found in the

Bailey Creek sediment was high and satisfactory for toxicity and uptake studies (Table 11). Chemical analyses indicated that kepone was released in a soluble form when Bailey Creek sediment was mixed with James River water. The concentrations of Cu, Pb, and Zn were high in the sediment when compared to some reported values.[29,30,31] Arsenic, Cu, Mn, and Pb were released in the LP (Table 11).

183. Bioassays using D. pulex and the LP and SPP of Bailey Creek sediment indicated that 100 percent LP and 100 percent SPP were extremely toxic to the organisms in the 96-hr exposure period. The 10 and 50 percent concentrations were not toxic (Table 12). Repeating the experiment with the same preparations of LP and SPP 3 weeks later demonstrated no toxicity of the 100 percent solutions toward D. pulex (Tables 13 and 14). Possible reasons for the differences have been discussed in PART III. The reasons are speculative, but strongly suggest the need for standard conditions, such as light cycle and temperature, and the important requirement of using freshly prepared test solutions made from recently collected sediment and water.

184. The mortality experiment using P. kadiakensis and Bailey Creek sediment demonstrated that the sediment exposure was toxic toward adult grass shrimp after 6 days of exposure (Table 15). The exact source of the toxicity cannot be determined because of the complex chemical nature of the sediments. The experiment does suggest that animals exposed to sediment from Bailey Creek through dredging and disposal operations could suffer mortalities. The large number of mortalities in the RCF water controls hinder interpretation of the data, but the mortalities in the 5.0, 2.5, and 1.25 percent sediment exposure tanks were statistically significant (95 percent confidence level) from mortalities in the sand controls. Chemical analyses of whole tissue samples on the grass shrimp (Table 16) demonstrated that kepone was taken up by sediment-exposed animals. Unfortunately, many of the analyses were performed on pooled tissue samples because there was not enough tissue for individual determinations. However, the trend seemed to be an increase of kepone in the tissue of dead animals up to day 4. The dead animals had higher concentrations of kepone than did live

animals, with the exception of days 5 and 6 pooled samples from the 2.5 and 5.0 percent sediment exposures. Trace element analyses on portions of the grass shrimp tissue did not reveal any obvious trends in uptake (Table 17). Some exposed animals did contain concentrations of Zn, Mn, Pb, and Fe that were higher than initial tissue levels.

185. Corbicula manilensis, the Asiatic clam, was also exposed to Bailey Creek sediment. The concentrations of kepone and trace elements found in the sediment and water samples are given in Tables 18 and 20. Kepone was not detected in the clams at the beginning of the experiment. After 7 days of exposure to the SP, the animals had accumulated approximately 150 ppb kepone at all exposure concentrations (Table 19). After 12 days of exposure, the concentrations in the 1.0 and 2.5 percent SP exposures were unchanged, but the 5.0 percent exposure showed a slight decrease. At 17 days exposure the 2.5 and 5.0 percent SP exposures showed large decreases in the whole tissue concentrations of kepone. The clams may be able to regulate the uptake and excretion of kepone, or they may have the ability to metabolize kepone. It is interesting that animals exposed to the least amount of Bailey Creek sediment retained the highest levels of kepone at the end of the experiment. A similar situation was noted in general with PCBs and the Duwamish River sediments. The reason for this is probably related to the capacity of the sediments to incorporate environmental contaminants, thus removing them from the water column. The experiment did demonstrate that if C. manilensis are exposed to kepone-contaminated sediment, their tissue will concentrate the pesticide. In their natural environment, they could serve as a source of kepone into the aquatic food web. Trace element analyses of the clam tissue (Table 21) indicated that the concentrations of Cu, Fe, Mn, Pb, Zn, and Hg did not increase or decrease to any large extent from initial tissue concentrations. The concentration of Cu in the Bailey Creek sediment was 163.5 ppm, but animals exposed to this material for up to 17 days in static aquaria contained an average of 4.69 ppm Cu in their tissues (Table 21).

Windmill Point

186. Sediments from Windmill Point were also known to contain kepone; samples collected contained an order of magnitude less kepone than Bailey Creek samples (Table 22). None of the trace elements analyzed (Table 23) were in high concentrations when compared to references 35, 36, and 37 and the other sediments discussed in this report. Manganese was the only trace element released from the sediment in a soluble form (Table 23).

187. Bioassays using D. pulex as the test organism and the LP and SPP of Windmill Point sediment indicated no toxicity at 20°C and 25°C (Tables 24 and 25). Tests conducted at 30°C (Tables 26 and 27) showed that the temperature was the cause of toxicity for the organisms because of the high mortality at all experimental and control levels, except for the second test (Table 27) where survival was 76.6 percent in the 100 percent SPP and 26.6 percent in the 50 percent SPP. The SPP offered some positive survival factors to D. pulex during the early portions of the exposure. On the other hand, P. kadiakensis larvae exposed to the SPP of the Windmill Point sediment had higher mortalities in the SPP at 30°C than the controls. Control mortalities at 90 hr were 40 percent compared to 80 percent mortality at the 100 percent SPP concentration. The effect of exposure to 100 percent SPP at 20°C for 92 hr was essentially the same as at 30°C. Windmill Point sediment was not harmful to freshwater grass shrimp based on data shown in Table 30. The data indicated that these animals prefer turbid water over clearer disposal site water.

Perth Amboy and Bay Ridge

188. Bioassays using the LP and SPP of Perth Amboy and Bay Ridge sediment (first shipment) indicated that the higher concentrations (100 and 50 percent) were significantly toxic toward A. tonsa (Tables 31 and 32). The Perth Amboy LP and SPP were also toxic toward larvae of P. vulgaris at the 100 percent levels (Table 33); however, these phases of the Bay Ridge sediment (Table 34) were not toxic toward t' larvae. The LP from both locations was toxic to adult M. bahia durin the 72-hr exposure period (Table 35). The SP from both locations was

not toxic toward adult P. vulgaris at concentrations of 1 and 5 percent (Tables 37 and 38), nor were there any mortalities among adult M. mercenaria exposed to 5 percent SP from each location (Table 38).

189. Results of bioassays using the first shipment of Perth Amboy and Bay Ridge sediments indicated that one or more phases of the sediments were toxic toward A. tonsa, M. bahia, and P. vulgaris larvae and were not toxic toward adult P. vulgaris and M. mercenaria.

190. Bioassays using a second shipment of Bay Ridge sediment demonstrated that adult M. bahia were not toxiologically sensitive to the LP or SPP (Table 39) and that the LP was not toxic to juvenile P. vulgaris (Table 40). The 10 percent SP of Bay Ridge sediment was significantly toxic toward adult M. bahia (Table 41), probably due to the fairly high sediment concentration combined with the static test conditions. Mysidopsis exposed to Perth Amboy LP were unaffected (Table 42), but young grass shrimp exposed to the same LP were adversely affected (Table 43).

191. The second shipment of sediment from Perth Amboy was toxic to Mysidopsis sp. at the 100 percent LP and SPP and the 10 percent SP (Tables 44 and 45), but the difference between survival of controls and exposed animals was not significant. Since short-term bioassays with Mysidopsis indicated little toxicity (Tables 42, 44, 45) yet one test with grass shrimp juveniles (Table 43) had indicated toxicity, a long-term bioassay with grass shrimp larvae was prepared. The toxicity data are shown in Table 46. They reveal that both 1 and 3 percent Perth Amboy sediment concentrations were toxic to the larvae. The 50 percent LP was not harmful to the shrimp. Larvae exposed to the contaminated sediment continued to die during the exposure period. Data in Table 47 show the sublethal effects of the sediment on growth of the larvae during the exposure period and for 20 days afterwards. Growth of larvae exposed to the sediment was significantly less than controls. Animals that survived seemed to recover from the exposure when placed in filtered seawater without the Perth Amboy sediment as shown by the growth data (Table 47).

192. Chemical analyses of the third shipment of sediment from

the Perth Amboy channel revealed it was contaminated with oil and grease, trace elements, organochlorine pesticides, and PCB (Table 48). Bioassays using the benthic burrowing amphipod Parahaustorius, the opossum shrimp M. bahia and Mysidopsis sp., and larvae of Palaemonetes intermedius indicated the sediment was not significantly toxic to any of these animals (Tables 49, 51, 52). The Perth Amboy SP was, however, significantly toxic toward the isopod Sphaeroma quadridentatum after 4 days of exposure (Table 50).

Long Island Sound

193. Sediment collected from a dredged material disposal site in Long Island Sound was also found to contain many chemical contaminants (Table 48). However, upon bioassay of 10 percent SP, no toxicity was observed toward Parahaustorius sp., Mysidopsis sp. (two tests), P. intermedius larvae, and S. quadridentatum (Tables 53, 54, 55, 56, and 57).

Vicksburg

194. Sediment collected from Vicksburg had higher concentrations of many of the constituents analyzed when compared to the control sediment used in the bioassays (Table 55). The SP of the Vicksburg sediment exhibited high toxicity toward P. kadiakensis in bioassays utilizing both static and flow-through chambers (Tables 59 and 60). The interpretation of the data is complicated by the fact that the experimental sediment caused an increase in pH of the test water. From a regulatory aspect, however, this may not be a concern because a toxic effect would indicate a problem with open-water disposal of the sediment, regardless of the exact source of the toxicity of the sediment. The impact, rather than whether the impact was caused by physical or chemical means, should be the prime concern. The toxicity exhibited in the P. kadiakensis exposure strongly indicated a potential problem. A further indication of the problem is seen from the results of two bioassays using the freshwater clam Musculium (Tables 61 and 62). Significant toxicity was observed at the 2, 3, and 5 percent SP. However, toxicity was not observed using the isopod Lireceus and the SP of Vicksburg sediment (Table 63).

Conclusions and Recommendations

195. Much research needs to be done to develop bioassay methods suitable for determining potential environmental impacts of the disposal of contaminated dredged material. The conclusions and recommendations discussed here are specifically aimed at dredged material bioassays, but may have general application to the testing of other solid wastes.

196. The physical water quality changes that may occur, such as pH, DO, salinity, and temperature, should be monitored. The pH changed in many cases when the solid phase was added to the various test waters. The DO concentration usually decreases upon the addition of the solid phase to the test water. In many cases the decreases are quite drastic, and it is recommended that gentle aeration be supplied to all experimental and control aquaria. Investigators should become familiar with field measurements of changes in pH and DO measured during actual dredged material disposal operations. Relating field measurements to the changes occurring in the test aquaria may contribute toward meaningful interpretation of the bioassay results. Temperature and salinity were not affected by addition of sediment. The parameters mentioned are considered the minimal number of monitored parameters. Any additional parameter that may be changed by a particular sediment should also be measured at regular intervals.

197. The pH of the test units is also of concern when comparing the liquid and suspended particulate phases of a sediment to control results. In some cases the control water will have a pH slightly higher or lower than these phases. The pH is also a matter of concern in many cases because the pH of the dredge site water may be quite different from that of the disposal site water. When this is the case, the pH of the experimental chambers will be a function of the percent of disposal site water used to make the dilution, and pH becomes a variable in the bioassay. To avoid this complication, it is recommended that disposal site water be used to prepare the LP and SPP as well as being used as the diluent. When a sufficient volume of disposal site water cannot be collected, IO is an acceptable substitute.

198. The importance of using recently collected sediment and water samples was indicated by the results of several bioassays described in this report. It is recommended that sediment and water be collected as discussed in Reference 8, and be immediately placed at 4°C until used. The sediment and water samples should not be used for biological testing after they are 14 days old. The LP and SPP should be used within 24 hr after preparation and should be stored at 4°C during the interval between their preparation and use. The conflicting results observed when certain of the bioassays were repeated could have been caused by the differences in the age of the test solutions. In situations that necessitate the use of synthetic fresh water or salt water, the waters should be aged for 30 days prior to being used.

199. A spectrum of chemical analyses are recommended when time and funding will allow. The chemical constituents listed in Table 55 are prime candidates for analyses, and if every sediment used in the bioassays were examined for those parameters, the results could aid in the interpretation of toxicity data. However, it must be remembered that because of the very complex nature of sediments, it will not be possible in most cases to pinpoint the cause of toxicity.

200. A great deal of research must be conducted to find suitable animals for bioassays. Ideally, the most sensitive species found at or near the disposal site should be used. That is not always practical, and usually organisms that can be easily collected and maintained in the laboratory are used instead. Of the organisms used for the bioassays discussed in this report, the following saltwater organisms show much promise and it is recommended that additional work be conducted with them: Acartia tonsa, Sphaeroma quadridentatum, Mysidopsis bahia and other Mysidopsis species, Parahaustorius, and larvae of Palaemonetes (P. pugio, P. vulgaris, and P. intermedius). Adults of saltwater species of Palaemonetes seem to be very tolerant to the various phases of sediment and are not generally recommended as bioassay organisms except for bioaccumulation tests. Tigriopus californicus seemed to be very tolerant toward the LP and SPP, and the clams Rangia cuneata and Mercenaria mercenaria were not sensitive to the SP of sediments and are

not recommended as test organisms for bioassays. However, the clams were good indicators of chemical contamination of tissues.

201. The freshwater organisms Palaemonetes kadiakensis, Daphnia pulex, and Musculium are recommended as test species. Lirceus and Corbicula manilensis did not show promise as test organisms for toxicity studies, but C. manilensis is recommended for uptake studies.

202. These recommendations are based on bioassays conducted at WES, general handling and maintenance in in the laboratory, review of the scientific literature, and discussion with personnel from bioassay laboratories throughout the United States. The successful use of any organism depends on the ability of the scientific personnel to properly collect, culture, and handle the test organisms. Research is needed in all of these areas to determine the suitability and to aid in the selection of sensitive bioassay organisms from freshwater and ocean environments.

203. Current legislation[13] requires the testing of each sediment phase. Emphasis should be placed on SP testing. The basic recommended procedure[8] is to add animals to aquaria containing disposal site water and allow the animals 48 hr to acclimate to the test conditions. The sediment is then added as a slurry. This approach worked well with most of the animals discussed in this report. It is suggested that Mysidopsis sp. be added approximately 4 hr after the addition of the sediment. When using Mysidopsis, it must also be remembered that at 20 to 25°C the life cycle of the animal is 18 to 22 days. If the test is conducted for about 14 days, natural mortalities and reproduction must be considered.

204. Flow-through bioassays are recommended over static procedures. Sediment can be added as a slurry and allowed to settle, after which the water can be turned on to simulate disposal site conditions.

205. Finally, a great deal of experimentation must be conducted to determine the consistency or variability of the SP bioassay procedure currently suggested[8] and any future procedures that are published. The inconsistency of certain bioassays in this report have been discussed and possible causes suggested, but the "natural" variability of the

SP bioassay is unknown. Experiments designed to determine the reproducibility of the experiments and the contributing factors should be conducted.

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Table 1

Concentration of Polychlorinated Biphenyls and Trace Elements in Sediment Samples Used in the Slip No. 1 and and Duwamish River Bioassays

Constituent	Concentration, ppm	
	Slip No. 1 Sediment	Duwamish River Sediment
PCB	0.66	0.38
As	20	50
Cd	3	3
Cu	64	51
Fe	43,400	43,700
Ni	40	42
Mn	80	83
Pb	80	42
Zn	168	106

Note: PCB concentrations are reported as Aroclor 1254 and on a wet weight basis. Heavy metal concentrations are reported on a a dry weight basis.

Table 2

Concentration of Polychlorinated Biphenyls and Trace Elements in Water Samples Used in the Slip No. 1 and Duwamish River Bioassays

Constituent	Concentration, ppb				
	Unfiltered Disposal Site Water	Unfiltered Dredge Site Water	Duwamish River SPP	Duwanish River LP	Slip No. 1 SPP
PCB	ND	ND	3	ND	3
As	3	2	7	4	33
Cd	2	5	31	1	2
Cu	7	3	6	6	13
Fe	725	620	2700	500	4100
Ni	25	6	25	15	15
Mn	35	55	1800	1400	4100
Pb	55	15	35	13	40
Zn	85	170	115	100	110

Note: ND = not detected.

Table 3

Number of Survivors of *Acartia tonsa* in Duwamish River Liquid Phase (LP)

Exposure Condition	Number of Survivors		
	0 hr	3 hr	24 hr
Disposal site water	20	18	12
Instant Ocean	21	19	15
0.1 percent LP	23	23	20
1.0 percent LP	21	21	20
10 percent LP	22	20	14
100 percent LP	22	22	12

Table 4

Number of Survivors of *Acartia tonsa* In Duwamish River Suspended Particulate Phase (SPP), Test One

Exposure Condition	Number of Survivors		
	0 hr	3 hr	24 hr
Disposal site water	20	18	12
Instant Ocean	21	19	15
0.1 percent SPP	20	20	16
1.0 percent SPP	28	28	28
10.0 percent SPP	23	23	23
100 percent SPP	24	24	23

Table 5

Number of Survivors of *Acartia tonsa* in Duwamish River Suspended Particulate Phase (SPP), Test Two

Exposure Condition	Number of Survivors		
	0 hr	2 hr	24 hr
Disposal site water	30	30	21
Instant Ocean	21	21	7
1 percent SPP	30	30	17
10 percent SPP	30	30	22
50 percent SPP	30	30	17
100 percent SPP	30	30	12

Table 6

Number of Survivors of Tigriopus californicus in Duwamish River Suspended Particulate Phase (SPP)

Exposure Condition	Number of Survivors					
	0 hr	2 hr	24 hr	48 hr	72 hr	96 hr
Disposal site water	30	30	30	30	30	30
Instant Ocean	30	30	30	30	30	30
10 percent SPP	30	30	30	30	29	29
50 percent SPP	30	30	30	30	30	30
100 percent SPP	30	30	30	30	28	28

Table 7

Number of Survivors in the Bioassay Using Duwamish River Solid Phase (SP) and the Animals *Palaemonetes pugio* and *Rangia cuneata* after Fourteen Days Exposure

Exposure Condition	Number of Survivors			
	0 hr		366 hr	
	Shrimp	Clams	Shrimp	Clams
Disposal site water	80	30	57	29
Instant Ocean	80	30	64	27
5.0 percent sand	80	30	50	26
0.1 percent SP	80	30	50	29
0.5 percent SP	80	30	38	29
1.0 percent SP	80	30	40	30
5.0 percent SP	80	30	53	28

Table 8

Percent Mortality of *Palaemonetes pugio* after Fourteen Days

Exposure to Duwamish River Solid Phase (SP)

Exposure Condition	Percent Mortality
Disposal site water	28.8
Instant Ocean	20.0
5.0 percent sand	37.5
0.1 percent SP	37.5
0.5 percent SP	52.5
1.0 percent SP	50.0
5.0 percent SP	33.8

Table 9

Concentration of Polychlorinated Biphenyls Accumulated by Surviving Animals Exposed to Duwamish River and Slip Number One Solid Phase (SP) for Fourteen Days

Exposure Condition	PCB Concentration, ppm	
	Palaemonetes pugio	Rangia cuneata
Starting tissue level	0.12	0.06
Disposal site water	0.07	0.07
5.0 percent sand	0.09	<0.02
0.1 percent river SP	0.09	0.29
0.5 percent river SP	0.37	0.15
1.0 percent river SP	0.68	0.42
5.0 percent river SP	0.45	0.32
5.0 percent slip SP	0.69	0.67

Table 10

Concentration of Trace Elements Accumulated by Surviving *Rangia cuneata* Exposed to Duwamish River and Slip Number One Solid Phase (SP) for Fourteen Days

Exposure Condition	Heavy Metal Concentration, ppm							
	Ni	Cd	Pb	As	Fe	Cu	Zn	Mn
Starting tissue level	0.71	0.24	0.68	1.3	57	2.6	14	2.2
Disposal site water	0.56	0.38	1.1	1.3	39	3.0	14	0.54
5.0 percent sand	0.73	0.39	1.5	1.8	43	3.0	14	0.59
0.1 percent river SP	0.61	0.30	2.0	1.5	49	2.3	14	0.84
0.5 percent river SP	0.80	0.27	1.2	1.4	64	2.7	14	2.6
1.0 percent river SP	0.46	0.25	1.3	2.5	63	2.4	13	5.0
5.0 percent river SP	0.53	0.26	1.2	1.7	140	2.5	13	5.6
5.0 percent slip SP	0.55	0.26	1.2	1.5	107	1.8	29	7.6

Table 11

Concentration of Kepone and Trace Elements in Bailey Creek Sediment and Water Samples Used in the Bioassays

Constituent	Concentration, ppm			
	Bailey Creek Sediment	James River Water	SPP	LP
Kepone	2.3	0.08	0.32	0.13
As	0.08	0.002	0.024	0.022
Cd	0.50	0.001	0.001	0.003
Cu	158.0	0.002	0.250	0.072
Fe	12,000.0	0.800	3.200	0.820
Ni	4.0	0.150	0.150	0.130
Mn	146.0	0.015	0.240	0.160
Pb	135.0	<0.002	0.200	0.040
Zn	880.0	0.300	1.150	0.300
Hg	0.25	<0.002	<0.002	<0.002

Table 12

Number of Survivors of Daphnia pulex Exposed to Bailey Creek Liquid Phase (LP) and Suspended Particulate Phase (SPP) at Room Temperature

Exposure Condition	Replicate	Number of Survivors				
		0 hr	4 hr	21 hr	45 hr	93 hr
Ship Channel Water	1	10	10	10	10	8
	2	10	10	10	10	9
	3	10	10	10	10	10
	4	10	10	10	10	10
100 percent LP	1	10	4	3	1	1
	2	10	8	5	1	0
	3	10	6	4	3	3
	4	10	7	3	0	0
50 percent LP	1	10	9	9	9	3
	2	10	10	10	10	10
	3	10	10	10	10	10
	4	10	10	10	10	10
10 percent LP	1	10	10	10	10	9
	2	10	10	10	10	7
	3	10	10	10	10	7
	4	10	10	10	10	6
100 percent SPP	1	10	10	9	6	2
	2	10	10	8	5	1
	3	10	9	4	1	0
	4	10	10	8	8	7
50 percent SPP	1	10	10	7	7	7
	2	10	10	9	8	8
	3	10	8	8	8	8
	4	10	8	8	8	8
10 percent SPP	1	10	10	10	10	10
	2	10	10	10	10	9
	3	10	10	10	9	9
	4	10	9	9	9	9

Table 13

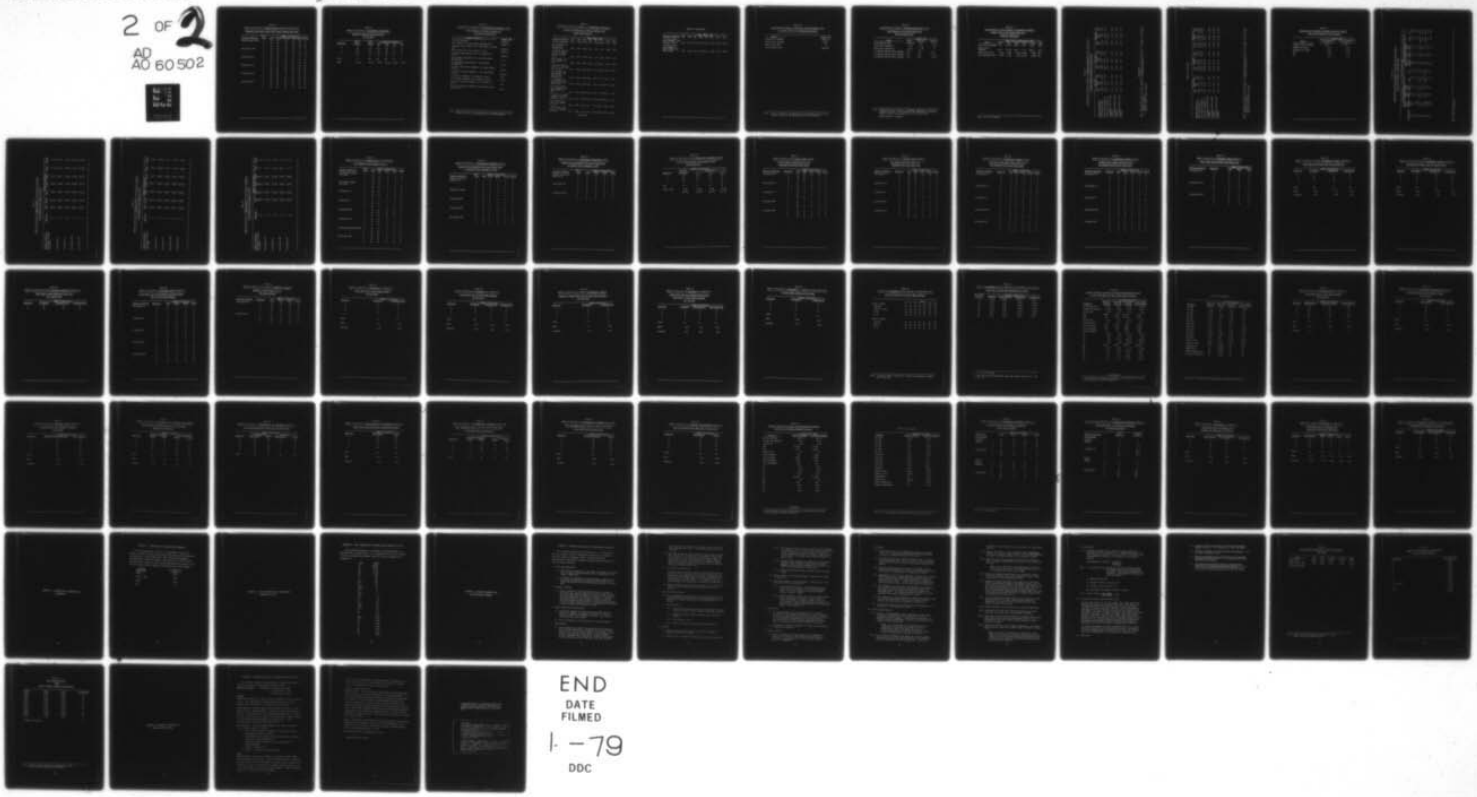
Number of Survivors of *Daphnia pulex* Exposed to Bailey Creek Liquid Phase (LP) under Standard Conditions

Exposure Condition	Replicate	Number of Survivors					
		0 hr	3.5 hr	27.5 hr	44 hr	68 hr	91.5 hr
Rocky Springs water	1	10	10	10	10	10	9
	2	10	10	10	10	10	9
	3	10	10	10	10	10	10
100 percent LP	1	10	10	9	9	9	9
	2	10	10	10	10	10	10
	3	10	10	10	10	10	10
90 percent LP	1	10	10	10	10	10	10
	2	10	10	10	10	10	9
	3	10	10	8	8	8	8
80 percent LP	1	10	10	10	10	9	9
	2	10	10	10	10	9	9
	3	10	10	10	10	10	9
70 percent LP	1	10	10	10	10	10	10
	2	10	10	10	10	10	10
	3	10	10	10	9	9	9
60 percent LP	1	10	10	10	10	10	10
	2	10	10	10	10	10	10
	3	10	10	10	10	8	8

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Table 14

Number of Survivors of *Daphnia pulex* Exposed to Bailey Creek Suspended Particulate Phase (SPP) under Standard Conditions

Exposure Condition	Replicate	Number of Survivors					
		0 hr	2.5 hr	27.5 hr	44 hr	68 hr	91.5 hr
Rocky Springs water	1	10	10	10	10	10	9
	2	10	10	10	10	10	9
	3	10	10	10	10	10	10
100 percent SPP	1	10	10	10	10	9	9
	2	10	10	10	10	9	9
	3	10	10	10	10	10	10
90 percent SPP	1	10	10	9	9	8	8
	2	10	10	10	10	10	9
	3	10	10	10	10	10	10
80 percent SPP	1	10	10	10	10	10	10
	2	10	10	10	10	10	10
	3	10	10	10	10	10	10
70 percent SPP	1	10	10	9	9	9	9
	2	10	10	10	10	10	10
	3	10	10	10	10	10	10
60 percent SPP	1	10	10	10	10	10	10
	2	10	10	10	10	9	9
	3	10	10	10	10	10	10

Table 15

Number of Survivors of *Palaemonetes kadiakensis*

Exposed to Bailey Creek Solid Phase

for Six Days

Replicate	Water Control	Sand Control	Percent Solid Phase			
			0.5	1.25	2.5	5.0
1	29	39	32	26	17	2
2	19	35	33	20	6	0
3	20	34	36	29	23	3
4	29	31	44	30	11	2
Total	97	139	145	105	57	7
Mean	24.25	34.75	36.25	26.25	14.25	1.75

Table 16

Concentration of Kepone in *Palaemonetes kadiakensis* Tissue after Exposure to Various Sediment and and Water Concentrations

Exposure Condition	Kepone, ppb
Tissue Background	Negative
RCF controls; all dead animals through day 4	Negative
5 percent sand controls; all dead animals through day 4	Negative
RCF controls; all live animals on day 6	Negative
5 percent sand controls; all live animals on day 6	Negative
All sediment concentrations; all dead animals through day 2	11.13
All sediment concentrations; dead animals through day 3	45.24
0.5 and 1.25 percent sediment; all dead animals on day 4	326.67
2.5 and 5.0 percent sediment; all dead animals on day 4	3800.00
0.5 percent sediment; live animals on day 6	5.25
1.25 percent sediment; live animals on day 6	<1.00
2.5 and 5.0 percent sediment; live animals on day 6	7.46
2.5 and 5.0 percent sediment; dead animals from days 5 and 6	20.14

Note: Negative indicates concentration of the compound was below the detection limit of the method used or was not present.

Table 17

Concentration of Trace Elements in Palaemonetes kadiakensis Tissue after Exposure to Various Sediment and Water Concentrations

Exposure Condition	Heavy Metal, ppm							
	Cd	Ni	Zn	Mn	Pb	Cu	Fe	As
Tissue background	0.50	0.09	14.63	1.51	3.94	12.26	8.07	<0.01
RCF controls; all dead animals through day 4	0.38	0.09	9.45	0.76	1.87	7.87	9.89	<0.01
5 percent sand controls; all dead animals through day 4	0.48	0.08	12.56	1.26	1.11	9.66	12.85	<0.01
RCF controls; all live animals on day 6	0.08	0.06	17.96	1.80	1.55	18.56	9.68	<0.01
5 percent sand controls; all live animals on day 6	0.07	0.09	16.90	2.98	4.27	12.96	10.34	<0.01
All sediment concentrations; all dead animals through day 2	0.08	0.08	12.96	3.09	0.17	9.37	67.81	<0.01
All sediment concentrations; dead animals day 3	0.10	0.08	22.97	6.29	1.35	8.19	207.74	<0.01
0.5 and 1.25 percent sediment; all dead animals on day 4	0.21	0.12	20.20	4.51	22.57	7.95	137.50	0.01
2.5 and 5.0 percent sediment; all dead animals on day 4	0.40	0.11	19.91	4.43	0.97	12.89	130.43	0.02
0.5 percent sediment live animals on day 6	0.10	0.08	17.93	2.29	7.17	18.23	47.41	<0.01
1.25 percent sediment; live animals on day 6	0.10	0.08	12.66	2.53	5.36	18.60	15.19	<0.01

(Continued)

Table 17 (Concluded)

Exposure Condition	Heavy Metal, ppm							
	Cd	Ni	Zn	Mn	Pb	Cu	Fe	As
2.5 and 5.0 percent sediment; live animals on day 6	0.26	0.07	16.10	1.66	3.64	12.47	21.61	<0.01
2.5 and 5.0 percent sediment; dead animals from days 5 and 6	0.16	0.09	17.51	5.73	10.74	6.68	71.62	<0.01

Table 18

Concentration of Kepone in Sediment and Water Samples Used in an Uptake Study with *Corbicula manilensis*

Sample	Kepone, ppb
Yazoo River sediment	Negative
Bailey Creek sediment	2500
James River water	75
Rocky Springs water	Negative

Note: Negative indicates concentration of the compound was below the detection limit of the method used or was not present.

Table 19

Concentration of Kepone in Corbicula manilensis Tissue after Exposure to Various Sediment and Water Concentrations

Sample	Kepone, ppb		
	Day 7	Day 12	Day 17
Rocky Springs water	Negative	<0.3	0.53
Yazoo River sediment A	3.65	0.63	2.04
Yazoo River sediment B	15.12	1.08	*
1.0 percent Bailey Creek sediment	135	140	110
2.5 percent Bailey Creek sediment	145	120	23.07
5.0 percent Bailey Creek sediment	150	100	34.64

Note: Background level of kepone in Corbicula manilensis was negative. Negative indicates concentration of the compound was below the detection limit of the method used or was not present.

* Sample broken in shipping

Table 20

Concentration of Trace Elements in Sediments and Water Samples Used in the Uptake Study with *Corbicula manilensis*

Sample	Heavy Metal Concentration, ppm					
	Cu	Fe	Mn	Pb	Zn	Hg
Yazoo River sediment	10.5	12,125	745.0	4.8	32.5	0.26
Bailey Creek sediment	163.5	12,300	149.0	1.3	850.0	0.39
James River water	0.002	0.800	0.015	<0.002	0.300	NM
Rocky Springs water	<0.001	0.200	0.030	<0.001	0.058	NM

Note: NM = not measured.

Table 21

Concentration of Trace Elements in Corbicula manilensis after Exposure to Various Sediment and Water Concentrations

Exposure Condition	Cu, ppm			Fe, ppm			Mn, ppm		
	Day 7	Day 12	Day 17	Day 7	Day 12	Day 17	Day 7	Day 12	Day 17
Rocky Springs water	2.18	0.94	8.63	30.45	33.3	25.2	2.73	3.68	2.01
Yazoo River sediment A	4.22	2.19	4.50	65.67	35.9	42.2	7.42	6.52	5.16
Yazoo River sediment B	4.57	5.22	*	40.2	90.3	*	5.05	10.18	*
1.0 percent Bailey Creek sediment	3.11	4.30	3.45	43.0	53.4	81.0	8.53	8.61	8.95
2.5 percent Bailey Creek sediment	2.92	7.75	6.41	59.5	88.9	61.8	7.83	11.63	5.26
5.0 percent Bailey Creek sediment	2.63	6.99	4.21	47.0	78.7	55.2	4.54	8.84	7.07

(Continued)

Note: Background levels of trace elements in Corbicula manilensis were Cu, 7.97 ppm; Fe, 78.06 ppm; and Mn, 6.98 ppm.

* Samples broken during shipping

Table 21 (Concluded

Exposure Condition	Pb, ppm			Zn, ppm			Hg, ppm		
	Day 7	Day 12	Day 17	Day 7	Day 12	Day 17	Day 7	Day 12	Day 17
Rocky Springs water	0.18	0.21	0.20	13.92	19.00	16.16	<0.002	<0.002	<0.002
Yazoo River sediment A	0.23	0.25	0.24	16.82	15.02	19.43	<0.002	<0.002	<0.002
Yazoo River sediment B	0.23	0.03	*	19.98	19.31	*	<0.002	0.021	*
1.0 percent Bailey Creek sediment	0.24	0.27	0.20	18.43	24.11	17.49	0.005	<0.002	<0.002
2.5 percent Bailey Creek sediment	0.27	0.24	0.23	17.64	20.41	22.02	0.004	<0.002	<0.002
5.0 percent Bailey Creek sediment	0.33	0.28	0.30	20.06	19.28	16.80	0.004	<0.002	<0.002

Note: Background levels of trace elements in Corbicula manilensis were Pb, 0.20 ppm; Zn, 20.46 ppm; and Hg, <0.002 ppm.

* Samples broken during shipping

Table 22

Concentration of Kepone in Sediment and Water Samples Used in the Windmill Point Bioassays

Sample	Kepone, ppb	
	First Preparation 16 Jun 76	Second Preparation 23 Jun 76
Windmill Point sediment	220	110
Dredge site water	0.10	0.07
Disposal site water	0.20	0.08
SPP	0.44	0.45
LP	0.34	0.15

Table 23

Concentration of Trace Elements in Sediment and Water Samples

Used in the Windmill Point Bioassays

	Trace Element, ppb									
	First Preparation, 16 Jun 76					Second Preparation, 23 Jun 76				
Constituent	Windmill Point Sediment	Disposal Site Water	Dredge Site Water	SPP	LP	Windmill Point Sediment	Disposal Site Water	Dredge Site Water	SPP	LP
As	NM	1.0	1.0	1.0	2.0	NM	1.0	1.0	2.0	3.0
Cd	NM	0.7	1.0	1.0	0.5	NM	0.1	0.3	0.2	NM
Cu	160	3.0	3.0	3.0	4.0	NM	2.0	3.0	6.0	3.0
Fe	19,052	365.0	122.0	1,470.0	73.0	NM	70.0	98.0	3,900.0	119.0
Ni	NM	<4.0	<4.0	<4.0	NM	NM	<0.3	<0.3	0.3	<0.3
Mn	5,680	18.0	5.0	1,320.0	850.0	NM	4.0	3.0	1,240.0	850.0
Pb	5,380	<1.0	3.0	2.0	1.0	NM	<1.0	<1.0	4.0	<1.0
Zn	1,510	5.0	5.0	6.0	5.0	NM	5.0	5.0	9.0	NM

Note: NM = not measured.

Table 24

Number of Survivors of Daphnia pulex in the Bioassay Conducted with Windmill Point Samples at 20°C

Exposure Condition	Replicate	Number of Survivors						
		0 hr	18 hr	23 hr	47 hr	72 hr	95 hr	115 hr
100 percent disposal site water	1	10	10	10	9	9	9	9
	2	10	10	10	10	8	7	7
	3	10	10	10	10	10	10	10
100 percent LP	1	10	10	10	10	10	10	9
	2	10	10	10	10	9	9	9
	3	10	10	10	10	9	9	8
50 percent LP	1	10	9	9	9	9	9	8
	2	10	10	10	10	10	10	9
	3	10	10	10	10	10	10	10
100 percent SPP	1	10	10	10	10	10	10	10
	2	10	10	10	10	9	9	9
	3	10	10	10	10	10	10	10
50 percent SPP	1	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	10
	3	10	10	10	10	10	10	10

Table 25

Number of Survivors of *Daphnia pulex* in the Bioassay Conducted with Windmill Point Samples at 25°C

Exposure Condition	Replicate	Number of Survivors						
		0 hr	19 hr	24 hr	47 hr	72 hr	94 hr	114 hr
100 percent disposal site water	1	10	10	10	10	9	9	9
	2	10	10	10	10	10	10	10
	3	10	10	10	5	5	5	5
100 percent LP	1	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	9
	3	10	10	10	10	10	10	10
50 percent LP	1	10	10	10	10	9	9	9
	2	10	10	10	10	10	9	9
	3	10	10	10	10	10	8	7
100 percent SPP	1	10	10	10	8	8	8	8
	2	10	10	10	10	10	8	6
	3	10	10	10	10	9	8	8
50 percent SPP	1	10	10	10	10	9	9	9
	2	10	10	10	10	10	10	9
	3	10	10	10	10	10	10	10

Table 26

Number of Survivors of *Daphnia pulex* in the Bioassay Conducted with Windmill Point Samples at 30°C

Exposure Condition	Replicate	Number of Survivors					
		0 hr	17 hr	22 hr	46 hr	71 hr	93 hr
100 percent disposal site water	1	10	10	10	10	7	0
	2	10	10	10	10	8	0
	3	10	10	10	8	1	0
100 percent LP	1	10	10	10	10	0	0
	2	10	10	10	10	0	0
	3	10	9	8	7	0	0
50 percent LP	1	10	10	10	10	1	0
	2	10	10	9	8	0	0
	3	10	10	10	10	8	0
100 percent SPP	1	10	10	10	10	10	0
	2	10	10	10	8	8	0
	3	10	10	10	10	7	1
50 percent SPP	1	10	10	10	10	7	0
	2	10	10	10	10	7	0
	3	10	10	10	10	3	0

Table 27

Number of Survivors of *Daphnia pulex* in the Bioassay with Windmill Point Samples at 30 °C

Exposure Condition	Replicate	Number of Survivors				
		0 hr	4 hr	23 hr	29 hr	49 hr
100 percent disposal site water	1	10	10	4	4	2
	2	10	6	0	0	0
	3	10	10	0	0	0
100 percent dredge site water	1	10	10	3	3	1
	2	10	8	0	0	0
	3	10	10	4	3	0
100 percent LP	1	10	10	0	0	0
	2	10	10	0	0	0
	3	10	9	0	0	0
50 percent LP	1	10	9	0	0	0
	2	10	10	7	7	5
	3	10	7	4	0	0
100 percent SPP	1	10	10	10	10	8
	2	10	10	10	10	8
	3	10	10	8	8	7
50 percent SPP	1	10	10	6	5	4
	2	10	10	6	2	2
	3	10	10	8	8	2
100 percent growth medium	1	10	9	0	0	0
	2	10	10	7	4	0
	3	10	10	0	0	0
100 percent RCF	1	10	10	9	5	0
	2	10	10	2	1	0
	3	10	10	8	4	0

Table 28

Number of Survivors of Palaemonetes kadiakensis Larvae Exposed to the Suspended Particulate Phase (SPP) of Windmill Point Sediments at 20°C

Exposure Condition	Replicate	Number of Survivors				
		0 hr	42 hr	92 hr	117 hr	165 hr
Natural:distilled water (1:1)	1	5	3	3	3	3
	2	5	5	2	1	1
	3	5	5	3	3	2
Dredge site water	1	5	5	5	5	5
	2	5	5	2	2	1
	3	5	5	4	4	3
10 percent SPP	1	5	3	2	2	1
	2	5	5	5	5	5
	3	5	5	4	4	4
50 percent SPP	1	5	4	3	3	1
	2	5	5	4	3	2
	3	5	4	4	3	3
100 percent SPP	1	5	3	1	0	0
	2	5	3	2	1	0
	3	5	4	1	0	0

Table 29

Number of Survivors of Palaemonetes kadiakensis Larvae Exposed to the Suspended Particulate Phase (SPP) of Windmill Point Sediment at 30°C

Exposure Condition	Replicate	Number of Survivors			
		0 hr	24 hr	48 hr	90 hr
Natural: distilled water (1:1)	1	5	5	5	3
	2	5	5	4	3
	3	5	5	5	3
50 percent SPP	1	5	5	2	0
	2	5	5	2	2
	3	5	5	4	1
100 percent SPP	1	5	5	3	1
	2	5	5	4	1
	3	5	5	4	1

Table 30

Number of Survivors of the Palaemonetes kadiakensis Adults in the 14-Day-Bioassay Using Windmill Point Solid Phase (SP)

Replicate	Number of Survivors			
	Disposal Site Water	Percent SP		
		1	2	5
1	10	16	14	15
2	14	18	14	17
3	16	17	13	17
Sum	40	51	41	49
Mean (± SD)	13.33 (±3.06)	17.00 (±1.00)	13.66 (±0.58)	16.33 (±1.16)

Table 31

Number of Survivors of *Acartia tonsa* Exposed to Perth Amboy Liquid Phase (LP) and Suspended Particulate Phase (SPP)

Exposure Condition	Replicate	Number of Survivors				
		0 hr	4 hr	21 hr	45 hr	93 hr
100 percent IO	1	10	10	10	10	5
	2	10	10	7	7	4
	3	10	10	6	6	3
100 percent LP	1	10	3	0	0	0
	2	10	2	0	0	0
	3	10	5	0	0	0
10 percent LP	1	10	8	5	5	2
	2	10	7	4	2	2
	3	10	10	7	7	3
50 percent SPP	1	10	7	0	0	0
	2	10	7	0	0	0
	3	10	9	0	0	0
10 percent SPP	1	10	8	0	0	0
	2	10	8	0	0	0
	3	10	10	0	0	0

Table 32

Number of Survivors of Acartia tonsa Exposed to

Bay Ridge Liquid Phase (LP) and

Suspended Particulate Phase (SPP)

Exposure Condition	Replicate	Number of Survivors				
		0 hr	4 hr	24 hr	28 hr	48 hr
100 percent IO	1	10	10	10	10	5
	2	10	10	7	7	4
	3	10	10	6	6	3
100 percent LP	1	10	6	0	0	0
	2	10	5	0	0	0
	3	10	5	0	0	0
10 percent LP	1	10	8	1	1	1
	2	10	8	1	1	0
	3	10	10	5	4	2
50 percent SPP	1	10	7	2	2	0
	2	10	10	3	2	0
	3	10	6	1	1	0
10 percent SPP	1	10	7	5	5	5
	2	10	8	3	3	1
	3	10	10	6	5	5

Table 33

Number of Survivors of *Palaemonetes vulgaris* Larvae Exposed to Perth Amboy Liquid Phase (LP) and Suspended Particulate Phase (SPP)

Exposure Condition	Replicate	Number of Survivors				
		0 hr	48 hr	96 hr	120 hr	168 hr
100 percent IO	1	5	5	3	3	2
	2	5	5	5	5	5
	3	5	5	5	5	5
	4	5	5	5	5	5
100 percent LP	1	5	4	2	1	0
	2	5	3	2	0	0
	3	5	4	2	0	0
	4	5	4	3	0	0
50 percent LP	1	5	5	5	5	5
	2	5	5	5	5	5
	3	5	5	4	4	4
	4	5	5	5	5	5
100 percent SPP	1	5	2	1	1	0
	2	5	5	3	1	1
	3	5	5	4	3	0
	4	5	5	5	5	1
50 percent SPP	1	5	5	5	5	5
	2	5	5	5	5	5
	3	5	5	5	5	4
	4	5	4	4	4	4

Table 34

Number of Survivors of Palaemonetes vulgaris Larvae

Exposed to Bay Ridge Liquid Phase (LP)
and Suspended Particulate Phase (SPP)

Exposure Condition	Replicate	Number of Survivors				
		0 hr	48 hr	96 hr	120 hr	168 hr
100 percent IO	1	5	5	3	3	2
	2	5	5	5	5	5
	3	5	5	5	5	5
	4	5	5	5	5	5
100 percent LP	1	5	5	5	5	5
	2	5	5	5	5	5
	3	5	4	3	3	3
	4	5	5	5	5	5
50 percent LP	1	5	5	5	5	5
	2	5	5	5	5	5
	3	5	5	5	5	5
	4	5	5	5	5	5
100 percent SPP	1	5	5	5	5	5
	2	5	5	5	5	5
	3	5	4	4	4	4
	4	5	4	4	4	4
50 percent SPP	1	5	5	5	5	4
	2	5	5	5	5	5
	3	5	5	5	5	5
	4	5	5	5	5	5

Table 35

Number of Survivors of *Mysidopsis bahia* Exposed to Perth Amboy and Bay Ridge Liquid Phase (LP)

Exposure Condition	Replicate	Number of Survivors		
		0 hr	48 hr	72 hr
100 percent IO	1	3	3	3
	2	3	3	2
	3	3	3	2
	4	3	3	2
75 percent PA LP	1	3	2	1
	2	3	2	1
	3	3	0	0
	4	3	1	1
75 percent BR LP	1	3	2	1
	2	3	2	0
	3	3	3	3
	4	3	3	2

Table 36

Number of Survivors of Adult Palaemonetes vulgaris Exposed to Perth Amboy Solid Phase (SP) for Fourteen Days

	Number of Survivors		
Replicate	IO Control	1 percent SP	5 percent SP
1	9	6	9
2	10	8	5
3	9	9	9
4	8	8	7
Total	36	31	30
Mean	9.00	7.75	7.50
Variance	0.67	1.58	3.67

Table 37

Number of Survivors of Adult Palaemonetes vulgaris Exposed to Bay Ridge Solid Phase (SP) for Fourteen Days

Replicate	Number of Survivors		
	IO Control	1 percent SP	5 percent SP
1	9	10	9
2	10	9	10
3	9	8	8
4	8	9	10
Total	36	36	37
Mean	9.00	9.00	9.25
Variance	0.67	0.67	0.92

Table 38

Number of Survivors of Adult Mercenaria mercenaria Exposed to Perth Amboy or Bay Ridge Solid Phase (SP) for Fourteen Days

Replicate	Number of Survivors		
	IO Control	5 percent PA SP	5 percent BR SP
1	15	15	15
2	15	15	15

Table 39

Number of Survivors of *Mysidopsis bahia* Exposed to Liquid Phase (LP) and Suspended Particulate Phase (SPP) of Bay Ridge Sediment

Exposure Condition	Replicate	Number of Survivors			
		0 hr	24 hr	48 hr	72 hr
100 percent IO	1	5	5	5	3
	2	5	4	3	3
	3	5	3	2	1
	4	5	5	2	2
90 percent LP	1	5	4	4	4
	2	5	5	4	2
	3	5	5	5	5
	4	5	4	4	4
70 percent LP	1	5	5	5	5
	2	5	4	4	3
	3	5	4	4	3
	4	5	5	4	3
50 percent LP	1	5	5	5	5
	2	5	4	2	2
	3	5	5	3	3
	4	5	4	4	4
90 percent SPP	1	5	5	5	5
	2	5	5	5	5
	3	5	5	4	4
	4	5	4	4	4

Table 40

Number of Survivors of Juvenile Palaemonetes vulgaris

Exposed to Liquid Phase (LP) of

Bay Ridge Sediment

Exposure Condition	Replicate	Number of Survivors			
		0 hr	24 hr	48 hr	72 hr
100 percent IO	1	10	10	10	9
	2	10	9	9	8
	3	10	9	9	9
	4	10	10	10	10
90 percent LP	1	10	10	10	8
	2	10	9	9	8
	3	10	10	10	10
	4	10	8	8	7

Table 41

Number of Survivors of Mysidopsis sp. Exposed to Solid Phase (SP) of Bay Ridge Sediment for Four Days

Replicate	Number of Survivors	
	IO Control	10 percent BR SP
1	4	3
2	4	2
3	3	3
4	4	3
5	4	4
Total	19	15
Mean	3.8	3.0
Variance	0.56	0.50

Table 42

Number of Survivors of *Mysidopsis* sp. Exposed to Liquid Phase (LP) of Perth Amboy Sediment for Four Days

Replicate	Number of Survivors		
	IO Control	50 percent LP	90 percent LP
1	4	5	5
2	5	5	4
3	5	3	5
4	5	4	5
Total	19	17	19
Mean	4.75	4.25	4.75
Variance	0.25	0.91	0.25

Table 43

Number of Survivors of Juvenile Palaemonetes vulgaris Exposed to Liquid Phase (LP) of Perth Amboy Sediment for Four Days

Replicate	Number of Survivors	
	IO Control	90 percent LP
1	3	2
2	3	1
3	3	2
4	3	3
Total	12	9
Mean	3	2.25
Variance	0.0	0.75

Table 44

Number of Survivors of *Mysidopsis* sp. Exposed to Liquid Phase (LP) and Suspended Particulate Phase (SPP) of Perth Amboy Sediment for Two Days

Replicate	Number of Survivors		
	IO Control	100 percent LP	100 percent SPP
1	5	3	2
2	3	4	3
3	4	2	3
4	4	4	4
Total	16	13	12
Mean	4.00	3.25	3.00
Variance	0.67	0.91	0.67

Table 45

Number of Survivors of *Mysidopsis* sp. Exposed to Solid Phase (SP) of Perth Amboy Sediment for Two Days

Replicate	Number of Survivors	
	IO Control	10 percent SP
1	5	3
2	3	0
3	4	4
4	4	3
Total	16	10
Mean	4.00	2.25
Variance	0.67	3.08

Table 46

Survival of *Palaemonetes* Larvae Exposed to Liquid Phase (LP) and Solid Phase (SP) of Perth Amboy Sediment

	Days						
Group I Larvae	0	2	4	7	11	19	33
Controls	40	40	40	39	38	35	35
50% LP (6 days)	40	40	40	39	36	35	33
1% SP	40	40	40	40	36	31	26
3% SP	40	31	31	31	31	26	22
Group II Larvae							
Controls	40	39	38	36	34	32	28
50% LP	40	38	37	33	33	28	27
3% SP	40	23	22	21	21	15	14

Note: Initially, Group I larvae were 4 days old and Group II larvae were 2 days old.

Table 47

Growth of *Palaemonetes* Larvae* Exposed to Liquid Phase (LP) and Solid Phase (SP) of Perth Amboy Sediment for 33 Days

Days After Exposure	Mean Wet Weight, mg			
	Controls	50 Percent LP	1 Percent SP	3 Percent SP
6	0.49	0.61	0.56	0.52
11	1.12	1.13	1.01**	0.91**
18	2.13	2.12	1.81**	1.70**
25	2.70	2.53	2.16**	2.21**
32	3.01	2.76	2.36**	2.42**
39	3.84	3.14	3.15**	2.89**
52	5.47	6.22	5.21	5.41

* N = 15 to 20 larvae

** These values are significantly less than control values at $p > .05$ or $p > .01$

Table 48

Chemical Analyses of Control and Experimental Sediments Used in the Perth Amboy and Long Island Sound Bioassays

Parameter	Concentration, ppm*			
	Pensacola Sediment	Weeks Bay Sediment	Perth Amboy Sediment	Long Island Sound Sediment
Percent moisture	21.02	21.17	58.93	50.03
Percent dry material	78.98	78.83	41.07	49.97
Percent TOC	0.08	0.08	2.24	2.05
COD	3,860	8,420	119,300	74,670
IOD	213	280	4,120	3,870
Percent TVS	0.32	0.50	4.81	4.29
Total sulfides	41	68	589	478
Oil and grease	1,260	5,040	6,801	4,680
Total nitrogen	103	189	2,930	1,430
Total phosphorus	35	46	2,070	1,050
As	0.86	1.03	24.80	33.20
Cd	0.04	0.12	1.58	1.33
Cr	1.29	31.70	196.00	139.00
Cu	2.58	3.96	304.00	143.00
Fe	3,010	1,830	41,200	28,000
Hg	0.07	0.32	2.79	2.58
Mn	4.3	19.8	393.0	286.0
Ni	3.0	2.8	85.0	52.5
Pb	1.3	3.2	88.3	57.3
Zn	11.0	10.7	422.0	256.0

(Continued)

* Concentrations are parts per million on a dry weight basis except where specified as percent composition

Table 48 (Concluded)

Parameter	Concentration, ppm*			
	Pensacola Sediment	Weeks Bay Sediment	Perth Amboy Sediment	Long Island Sound Sediment
op' DDE	0.42	0.006	0.03	0.005
op' DDD	0.004	ND	0.13	ND
op' DDT	0.008	ND	0.03	ND
pp' DDE	0.85	0.014	0.08	0.01
pp' DDD	0.013	ND	0.04	ND
pp' DDT	0.03	ND	0.05	ND
Total DDT	1.32	0.02	0.72	0.015
PCB 1242	0.02	0.02	3.7	0.514
PCB 1254	0.02	0.03	1.53	0.78
PCB 1260	0.002	0.003	0.153	0.078
PCB Total	0.042	0.053	5.38	1.37
Dieldrin	7.05	ND	0.002	0.001
Alkanes Total	0.003	0.001	0.006	0.005
Aromatic Total	<0.001	0.0014	<0.001	<0.001
Phenenthrene	ND	0.00001	ND	ND
Naphthalene	ND	0.0002	ND	ND
Methyl naphthalene	ND	0.0004	ND	ND
Dimethyl naphthalene	ND	0.0008	ND	ND

Note: ND = not detected (concentrations below detection limits).

Table 49

Number of Survivors of Parahaustorius sp. Exposed to Solid Phase (SP) of Perth Amboy Sediment for Nine Days

	Number of Survivors		
Replicate	Sand Control	Kaolinite Control	Perth Amboy SP
1	19	17	19
2	20	19	18
3	19	20	17
4	20	19	18
5	19	20	19
Total	97	95	91
Mean	19.4	19.0	18.2
Variance	0.3	1.5	0.7

Table 50

Number of Survivors of Sphaeroma quadridentatum Exposed to Solid Phase (SP) of Perth Amboy Sediment for Four Days

Replicate	Number of Survivors	
	Sand Control	Perth Amboy SP
1	13	4
2	20	3
3	19	6
4	16	3
5	14	3
Total	82	19
Mean	16.40	3.80
Variance	9.30	1.70

Table 51

Number of Survivors of Mysidopsis bahia Exposed to Solid Phase (SP) of Perth Amboy Sediment for Ten Days

Replicate	Number of Survivors	
	Weeks Bay Control Sediment	Perth Amboy SP
1	6	12
2	13	10
3	17	12
4	14	14
5	9	15
Total	59	63
Mean	11.8	12.6
Variance	18.70	3.80

Table 52

Number of Survivors of *Mysidopsis* and *Palaemonetes intermedius* Larvae Exposed to Solid Phase (SP) of Perth Amboy Sediment for Seven Days

	Number of Survivors			
	Pensacola Sediment		Perth Amboy SP	
Replicate	Mysids	Larvae	Mysids	Larvae
1	9	6	9	9
2	10	9	9	5
3	10	6	10	10
4	10	5	10	10
5	9	6	10	6
Total	48	32	48	38
Mean	9.6	6.4	9.6	7.6
Variance	0.3	2.3	0.3	4.3

Table 53

Number of Survivors of Parahaustorius and Mysidopsis Exposed to Long Island Sound Solid Phase (SP) for Eleven Days

	Number of Survivors			
	Parahaustorius		Mysidopsis	
Replicate	Sand Control	LIS SP	Sand Control	LIS SP
1	17	18	8	8
2	19	17	9	9
3	16	11	7	8
4	16	16	7	6
5	16	13	7	5
Total	84	75	38	36

Table 54

Number of Survivors of *Parahaustorius* and *Mysidopsis* Exposed to Long Island Sound Solid Phase (SP) for Eleven Days

Replicate	Number of Survivors	
	Sand Control	LIS SP
1	25	26
2	28	26
3	23	19
4	23	22
5	23	18
Total	122	111
Mean	24.4	22.2
Variance	4.8	14.2

Table 55

Number of Survivors of *Palaemonetes intermedius* Larvae and Adult *Mysidopsis bahia* Exposed to Long Island Sound Solid Phase (SP) for Seven Days

	Number of Survivors			
	Pensacola Sediment		LIS SP	
Replicate	Larvae	Mysids	Larvae	Mysids
1	6	9	6	10
2	9	10	3	9
3	6	10	4	10
4	5	10	6	9
5	6	9	7	7
Total	32	48	26	45

Table 56

Number of Survivors of *Palaemonetes intermedius* Larvae and Adult *Mysidopsis bahia* Exposed to Long Island Sound Solid Phase (SP) for Seven Days

Replicate	Number of Survivors	
	Pensacola Sediment	LIS SP
1	15	16
2	19	12
3	16	14
4	15	15
5	15	14
Total	80	71
Mean	16.00	14.20
Variance	3.00	2.20

Table 57

Number of Survivors of Sphaeroma quadridentatum Exposed to Long Island Sound Solid Phase (SP) for Four Days

Replicate	Number of Survivors	
	Sand Control	LIS SP
1	12	9
2	14	9
3	10	12
4	12	13
5	14	9
Total	62	52
Mean	12.40	10.40
Variance	2.80	3.80

Table 58

Chemical Analyses of Control and Experimental Sediments Used in the Vicksburg Sediment Bioassays

Parameter	Concentration, ppm*	
	Control Sediment	Vicksburg Sediment
Percent moisture	38.06	51.07
Percent dry material	61.94	48.93
Percent TOC	1.02	2.49
COD	48,060	48,700
IOD	1,130	10,060
Percent TVS	3.7	4.5
Total sulfides	176	1,530
Oil and grease	5,290	13,000
Total nitrogen	365	495
Total phosphorus	498	555
As	17.5	12.5
Cd	0.23	0.25
Cr	69.9	90.3
Cu	27.9	41.6
Fe	30,500	23,300
Hg	0.21	0.13
Mn	384	762
Ni	32.6	63.2
Pb	9.3	13.5
Zn	97.8	110.0

(Continued)

* Concentrations are parts per million on a dry weight basis except where specified as percent composition

Table 58 (Concluded)

Parameter	Concentration, ppm*	
	Control Sediment	Vicksburg Sediment
op' DDE	0.005	0.30
op' DDD	ND	0.11
op' DDT	ND	0.07
pp' DDE	0.01	0.015
pp' DDD	ND	0.35
pp' DDT	ND	0.09
Total DDT	0.015	0.80
PCB 1242	0.514	8.2
PCB 1254	0.78	4.2
PCB 1260	0.078	0.42
PCB total	1.37	12.8
Dieldrin	0.001	0.005
Alkanes total	<0.001	0.010
Aromatic total	0.00012	0.025
Phenanthrene	ND	0.001
Naphthalene	0.00012	0.003
Methyl naphthalene	ND	0.007
Dimethyl naphthalene	ND	0.014

Note: ND = not detected (concentrations below detection limits).

Table 59

Number of Survivors of Palaemonetes kadiakensis Exposed to Solid Phase (SP) of Vicksburg Area Sediment for Seventy-two Hours

Condition		Number of Survivors			
		0 hr	24 hr	48 hr	72 hr
Flow-through					
Control Sediment	1	40	40	40	40
	2	40	40	40	40
	3	40	40	40	39
Vicksburg SP	1	40	23	17	17
	2	40	22	6	6
	3	40	16	9	9
Static					
Control Sediment	1	40	40	40	D
	2	40	40	40	D
	3	40	40	40	D
Vicksburg SP	1	40	0	0	D
	2	40	0	0	D
	3	40	0	0	D

Note: D = discontinued.

Table 60

Total and Percent Mortality of Palaemonetes kadiakensis Exposed to Solid Phase (SP) of Vicksburg Area Sediment for Seventy-two Hours

Exposure Condition		Total Mortality	Percent Mortality
Flow-through			
Control Sediment	1	0	0
	2	0	0
	3	1	2.5
Vicksburg SP	1	23	57.5
	2	34	85.0
	3	31	77.5
Static			
Control	1	0	0
	2	0	0
	3	0	0
Vicksburg SP	1	40	100
	2	40	100
	3	40	100

Table 61

Number of Survivors of *Musculium* sp. Exposed to Solid Phase (SP) of Vicksburg Area Sediment for Eight Days, Clam Test I

Replicate	Number of Survivors		
	Sand Control	0.2 Percent SP	5 percent SP
1	7	9	0
2	9	10	0
3	10	5	0
4	9	8	1
Total	35	32	1
Mean	8.75	8.00	0.25
Variance	1.58	4.66	0.25

Table 62

Number of Survivors of *Musculium* sp. Exposed to Solid Phase (SP) of Vicksburg Area Sediment for Four Days, Clam Test II

	Number of Survivors				
Replicate	Sand Control	1% SP	2% SP	3% SP	5% SP
1	14	12	2	7	1
2	15	15	9	2	2
3	15	13	3	7	1
4	13	15	1	3	2
Total	57	55	15	19	6
Mean	14.25	13.75	3.75	4.75	1.50
Variance	0.92	2.25	12.92	6.92	0.33

Table 63

Number of Survivors of Lirceus sp. Exposed to Solid Phase (SP) of Vicksburg Area Sediment for Four Days

Replicate	Number of Survivors		
	Sand Control	0.2 Percent SP	2 Percent SP
1	9	9	8
2	9	10	6
3	10	9	9
4	9	9	8
Total	37	37	31
Mean	9.25	9.25	7.75
Variance	0.25	0.25	4.75

APPENDIX A: COMPOSITION OF RECONSTITUTED FRESHWATER

APPENDIX A: COMPOSITION OF RECONSTITUTED FRESHWATER

The following formula is from U. S. Environmental Protection Agency, "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians," 660/3-75-009, April 1975, National Environmental Research Center, Corvallis, Oregon. The formula is for "soft" water and formulae for waters of other softness and hardness are given in the reference along with pH, hardness, and alkalinity for each formulae.

Chemical Constituent	Concentration mg/l
$NAHCO_3$	48.0
$CaSO_4 \cdot 2H_2O$	30.0
$MgSO_4$	30.0
KCl	2.0

APPENDIX B: IONIC COMPOSITION OF INSTANT OCEAN SYNTHETIC SEA SALT

APPENDIX B: IONIC COMPOSITION OF INSTANT OCEAN SYNTHETIC SEA SALT

The following information is provided by Aquarium Systems, Inc., manufacturers of Instant Ocean. At a temperature of 15°C and a salinity of 34 ppt, Instant Ocean Synthetic Sea Salt has the following ionic composition:

Ion	ppm
Cl	18,400
Na	10,220
SO_4	2,518
Mg	1,238
Ca	390
K	370
HCO_3	142
Br	60
H_3BO_3	25
Sn	6
SiO_3	3
PO_4	1.3
Mn	1.2
Fe	1.0
MoO_4	0.6
S_2O_3	0.3
Li	0.2
Rb	0.1
I	0.07
EDTA	0.06
Al	0.04
Zn	0.02
V	0.02
Co	0.01
Fe	0.01
Cu	0.003

APPENDIX C: METHOD OF ANALYSIS FOR POLYCHLORINATED BIPHENYLS

APPENDIX C: METHOD OF ANALYSIS FOR POLYCHLORINATED BIPHENYLS

The following information was provided by U. S. Environmental Protection Agency, Region X Laboratory, Surveillance And Analysis Division, Seattle, Washington, 98101, and used by American Bacteriological and Chemical Company to perform chemical analysis for polychlorinated biphenyls.

1. Scope and Application

   1.1 This method is applicable to all types of sediments from fresh water or marine origin varying widely in mineralogical and organic composition.

   1.2 The method is applicable for most non-ionic organochlorine pesticides and commercially available PCB mixtures in concentration greater than 0.010 microgram per gram of wet sediment.

2. Summary of Method

   2.1 The wet sediment is extracted with acetone in a Soxhlet extractor, the acetone evaporated to a low volume, diluted with petroleum ether and dried over anhydrous sodium sulfate. After petroleum ether is reduced in volume, it is passed through a florisil column to separate the pesticides and PCBs from background interferences. Analysis of the extracts from the column is conducted with a gas chromatograph equipped with an electron capture detector (GC/EC).

3. Sample Handling and Preservation

   3.1 Collect the samples in 8-ounce glass jars which have been previously cleaned with solvents and heated in a muffle furnace at 320^{o}C for a minimum of 24 hours. All jar tops are fitted with teflon liners.

   3.2 Store the samples at 4^{o}C and analyze as soon as possible.

4. Interferences

   4.1 Trace quantities of sulfur and related materials interfere with the GC/EC measurement. Treatment of the extract before injection into the gas chromatograph by rapid mixing of the extract with elemental mercury by use of a Vortex-Genie stirrer eliminates this interference. However, some organochlorine pesticides such as heptachlor, BHC, and dieldrin

react with mercury during this treatment and low values for these compounds may result. PCBs remain unaffected by this treatment.

4.2 Care must be taken to remove materials from glassware, glass wool, and sodium sulfate used in the analysis, which affect the EC detector. All glassware is scrupulously washed and then heated at 400°C for 30 minutes in a muffle furnace. Before use, rinse with petroleum ether. Anhydrous sodium sulfate is similarly heated and the glass wool is extracted with petroleum ether before use.

4.3 Dicarboxylic Acid Esters

Electron capture detectors respond to these materials which are widely used as plasticizers and interfere with the determination of the organochlorine pesticides. Application of two different GC columns helps to confirm pesticide and PCB in the presence of these esters. If available, apply gas chromatographic/mass spectrometric technique for confirmation.

4.4 Soxhlet thimbles must be pre-extracted for 6 to 8 hours with acetone to remove impurities that interfere with the EC detector.

5. Apparatus and Materials

5.1 Gas chromatograph equipped with an electron capture detector, a strip chart recorder, and glass gas chromatographic columns packed with suitable materials. See reference 13.3 for details.

5.2 Other Materials:

5.2.1 Kuderna-Danish glassware (K-D) including Snyder columns, evaporative glass receiver ampules, and ampule stoppers.

5.2.2 Soxhlet extractors, 300-ml capacity with 33 x 80 mm thimbles.

5.2.3 Microsyringes, 10 µl.

5.2.4 Florisil PR grade (60-100 mesh) stored at 130°C.

6. Reagents

6.1 Sodium sulfate, anhydrous granular, analytical reagent, muffled at 400°C for 24 hours.

6.2 Diethyl ether - nanograde, redistilled in glass, if necessary.

6.2.1 Must contain 2 percent ethanol and be free of peroxides by following test: to 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add 1 ml of freshly prepared 10 percent KI solution. Shake and let stand 1 minute. No yellow color should be observed in either layer.

6.2.2 Decompose ether peroxides by adding 40 g of 30 percent ferrous sulfate solution to each litre of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

6.2.3 Distill deperoxidized ether in glass and add 2 percent ethanol.

6.3 Benzene, Hexane, and Acetone-Nanograde, redistilled in glass, if necessary.

6.4 Pesticide Standards - Reference grade. Aroclors 1221, 1232, 1242, 1248, 1254, 1260, and 1016.

6.4.1 Stock standard solutions - Dissolve 100 mg of each pesticide to 60 ml benzene in a 100-ml volumetric flask. Dilute to volume with redistilled hexane. Solution contains 1 mg/ml.

6.4.2 Working standard - Pipet 1.0 ml of each stock solution into a single 100-ml volumetric flask. Dilute to volume with hexane. Solution contains 10 µg/l of each standard. Repeat operation until appropriate concentrations are obtained for preparation of calibration graphs. See reference 13.1.

7. Calibration

7.1 Gas chromatographic operating conditions are considered acceptable if the response to aldrin is at least 50 percent of full scale when 0.10 ng is injected for electron detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2 percent of full scale.

7.2 Standards are injected frequently as a check on the stability of operating conditions.

8. Quality Control

8.1 Duplicate, blanks, and spiked samples are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended.

9. Procedure

9.1 Approximately 20 g of wet homogeneous sediment is weighed into a 33- x 80-mm preextracted thimble and extracted with acetone in a Soxhlet extractor for 6 to 8 hr.

9.2 A second portion of the sediment is weighed into a previously dried and weighed dish. Dry at 105°C for 1 day and reweigh. The results are used to calculate the percent solids of the sample.

9.3 After the extraction period is over, the solvent flask is removed from the Soxhlet and fitted with a Snyder column, and the solvent is concentrated on a steam bath to 30 ml.

9.4 Approximately 150 ml of petroleum ether is added to the cooled flask with swirling. Enough anhydrous sodium sulfate, previously heated at 400°C to remove impurities, is added to remove any water present. Usually 20 to 30 g of drying agent is necessary, but quantities vary with sample type and size.

9.5 After standing with occasional swirling for 15 to 20 minutes, the content is decanted into a prerinsed K-D flask. The remaining material is washed with four 50-ml portions of petroleum ether. The combined washings are added to the K-D flask and the contents concentrated to 2-3 ml.

9.6 The concentrate is chromatographed on Florisil with 200 ml each of solutions containing 6 percent and 15 percent diethyl ether in petroleum ether as described in Section 10.

9.7 The separate fractions are collected and concentrated in K-D flasks to 5 ml and analyzed by GC/EC.

10. Florisil Fractionation

10.1 Prepare a chromatographic column containing 4 inches (after settling) of activated Florisil topped with 0.5 inch of anhydrous, granular NA_2SO_4. A small wad of glass wool, preextracted with petroleum ether, is placed at the bottom of the column to retain the Florisil.

NOTES: (1) If the oven is of sufficient size, the columns may be prepacked and stored in the oven, withdrawing columns a few minutes before use.
(2) The amount of Florisil needed for proper elution should be determined for each lot of Florisil.

10.2 Place a 500-ml Erlenmeyer flask under the column and prewet the packing with petroleum ether (40-50 ml, or a sufficient volume to completely cover the Na_2SO_4 layer. If air is

introduced, channeling may occur, resulting in an inefficient column.

10.3 Using a 5-ml Mohr or a long, disposable pipet, immediately transfer the sediment extract (about 5 ml) from the evaporator tube onto the column and permit it to percolate through.

10.4 Rinse tube with two successive 5-ml portions of petroleum ether, carefully transferring each portion to the column with the pipet.

NOTE: Use of the Mohr or disposable pipet to deliver the extract directly onto the column precludes the need to rinse down the sides of the column.

10.5 Prepare two Kuderna-Danish evaporative assemblies complete with 10-ml graduated evaporative concentrator tubes. Place one glass bead in each concentrator tube.

10.6 Replace the 500-ml Erlenmeyer flask under the column with a 500-ml Kuderna-Danish assembly and commence elution with 200 ml of 6 percent diethyl ether in petroleum ether (Fraction I). The elution rate should be 5 ml per minute. When the last of the eluting solvent reaches the top of the Na_2SO_4 layer, place a second 500-ml Kuderna-Danish assembly under the column and continue elution with 200 ml of 15 percent diethyl ether in petroleum ether (Fraction II).

10.7 To the second fraction only, add 1.0 ml of hexane containing 200 ng of aldrin, place both Kuderna-Danish evaporator assemblies in a water bath and concentrate extract until about 5 ml remain in the tube.

10.8 Remove assemblies from bath and cool to ambient temperature.

10.9 Disconnect collection tube from Kuderna-Danish flask and carefully rinse joint with a small amount of hexane.

10.10 Attach modified micro-Snyder column to collection tubes, place tubes back in water bath, and concentrate extract to 1 ml. If preferred, this may be done at room temperature under a stream of nitrogen.

10.11 Remove from bath and cool to ambient temperature. Disconnect tubes and rinse joints with a small amount of hexane. Proceed to 9.7

NOTE: The extent of dilution or concentration of the extract at this point is dependent on the pesticide concentration in the substrate being analyzed and the sensitivity and linear range of the electron capture detector being used in the analysis.

11. Calculations

11.1 Using the absolute method, pesticide concentrations are determined by direct comparison to a standard when the injection volume and response are close to that of the sample. The concentration of pesticide in the sample is calculated as follows:

$$\text{micrograms/g (wet weight)} = \frac{(A)(B)(V_t)}{(V_i)(W_{sed})}$$

where A = µg Std./Std. area (Concentrations for calibration graphs are normally expressed in nanograms or picograms. These concentrations must be converted to micrograms for substitution into the formula)

B = Sample aliquot area

V_i = Volume of extract injected (µl)

V_t = Volume of total extract (µl)

W_s = Wet weight of sediment extracted in grams

11.2 $\text{Percent solids} = \frac{\text{(dry weight)}}{\text{(wet weight}} \times 100$

12. Precision, Accuracy, and Detection Limits

The detection limit for p,p'-DDT, using a 20-g sample and a 5-ml final extract volume, is less than 0.010 µg/g. This method consistently gives 85 to 105 percent yields for aldrin p,p'-DDE, p,p'-DDT, p,p'=DDD, and Aroclor 1242 PCB. Three interlaboratory experiments have been conducted to determine the reliability of the method. The first, a split sample with Oregon State University, was used to determine p,p'-DDT, its isomers, and metabolites in sediments (Table C1). The second, a sample split with several groups, was for determination of PCBs in sediments (Table C2) and the third, a set of split sediments with the Idaho Department of Environmental and Community Services Laboratory, was taken for analysis of p,p'-DDT, its isomers, and metabolites (Table C3).

The results of analysis for these experiments show 2.6, 15, and 15 percent differences with known and mean values. Considering the type of samples (split in field) and the low level of pollutants (near 0.010 µg/g), the differences are reasonably good.

13. References

13.1 Analysis of Pesticide Residue in Human and Environmental Samples, Edited by J. F. Thompson, U.S. EPA, RTP (1973).

13.2 Methods for Organic Pesticides in Water and Wastewater, (1971) EPA, NERC, Cincinnati, Ohio.

13.3 Methods Recommended for the Determination of Chlorinated Hydrocarbons and Pesticides, J. J. Lichtenberg, EPA, AQCL, Cincinnati, Ohio (1971).

13.4 Interlaboratory Calibration Study for Assessing Data Reliability in Chlorinated Hydrocarbon Analysis, U.S. EPA Coastal Pollution Research Branch (Feb 1975) Edited by W. Hom and S. Pavlou (Special report 59 Ref. No. M7537).

Table C1

Interlaboratory Comparison for DDT in Sediments,

OSU - EPA

Sample	DDE	DDD	o,p'-DDT	p,p'-DDT	ΣDDT*
38028 (EPA)	0.010	0.010	0.013	0.022	0.055
Known values (OSU)	0.008	0.009	0.012	0.024	0.053
Percent difference					3.6

Note: Concentrations expressed in micrograms per gram.
* ΣDDT = sum of DDT and metabolites

Table C2

Summary of Interlaboratory Comparison of PCBs in Duwamish Sediments

Laboratory	Total PCB, µg/g
A	0.613
B	0.551
C	0.765
D	0.529
E	0.683
F	0.633
G	0.486
H	0.532
I (EPA Lab)	0.695
J	0.720
Mean	0.621
	±0.079

Table C3

Split Sample Analysis

ΣDDT*

State of Idaho - Region X Laboratories

Sample	Idaho	EPA	% Difference
06605	0.053	0.048	9
06601	0.019	0.016	16
06575	0.010	0.010	0
06573	0.010	0.010	0
06565	0.033	0.060	40
06563	0.022	0.016	27
06564	0.112	0.112	0
06604	0.899	0.908	10
06689	1.050	0.865	17
Average % difference			15

Note: Concentrations expressed in micrograms per gram.

* ΣDDT = p,p'-DDT isomers and metabolites

APPENDIX D: METHODS OF ANALYSIS FOR KEPONE AND HEAVY METALS

APPENDIX D: METHODS OF ANALYSIS FOR KEPONE AND HEAVY METALS

The following information was provided by Jennings Laboratories, 1118 Cypress Avenue, Virginia Beach, Virginia, 23451.

METHODS OF ANALYSIS LAB REPORTS # 14714 (May 29, 1976)
14758 (June 9, 1976)
14864 (July 13, 1976)

Sediment

Preliminary Preparation: Sample spread on aluminum foil and allowed to air dry. Dry sample broken up and screened to remove particles greater than 2 mm diameter. Dry samples stored in glass.

Kepone Analysis: 10-gram sample above extracted in Soxhlet 16 hours using 150 ml 1:1 benzene-methanol. Extract filtered and evaporated to dryness. Extract made up to 25 ml using Hexane, and extract cleaned up prior to GC using either florisil or oleum methods. Sample diluted to 25 ml using benzene containing 1 percent methanol. Inject 3 microlitres on 3 percent OV-1 Column @ 200 C.

Metal Analysis: 1 gram dry sample digested with nitric-perchloric acid, filtered, made up to 100 ml.

a. Cd, Cu, Fe, Mn, and Zn determined on AA using both flame and graphite furnace as a check.
b. Lead determined by graphite furnace and checked by Dithizone extraction - spectrophotometric.
c. Nickel determined by graphite furnace and checked by Dimethylglyoxime.
d. Mercury - flameless AA.
e. Arsenic - flameless AA using EDL lamp.

Water

Kepone Analysis: 500 ml water (1000, or preferably 2000 ml sample used when available) extracted 3 times using 50 ml benzene. Combined benzene extracts dried through anhydrous sodium sulfate, cleaned up if necessary on florisil, concentrated to almost dryness, and made up to 2.0 ml using benzene containing 1 percent methanol. Three microlitres injected on 3 percent OV-1 column at 200 C.

Metals: Mercury determined on original sample by flameless AA. On other metals the water is concentrated to 10 percent of original volume and determinations made as listed above.

Tissues - Shrimp and Clams

Kepone Analysis: Samples drained on paper towels, and 10-gram sample ground in mortar and pestle, transferred to blender using 200 ml 35 percent solution Acetonitrile in water, and blended at high speed for 2 minutes. Material filtered through Whatman #40 paper, and filtrate transferred to separate funnel, washed with 75 ml 50-50 petroleum ether-ethyl ether followed by 10 ml saturated NaCl in 100 ml water. Water phase discarded and ether phase dried through anhydrous sodium sulfate and evaporated; residue made up to 5 ml with benzene containing 1 percent methanol. Three microlitres injected on 3 percent OV-1 Column at 200 C.

Metals: Shrimp and clams digested in nitric-hydrochloric acid mixture, evaporated to near dryness, diluted with 10 ml hydrochloric acid, heated, diluted with water, and filtered through #42 Whatman paper; filtrate and washings made up to 50 ml.

Metals determined as in sediments and water.

US GOVERNMENT PRINTING OFFICE: 1978-740-235/7

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

Shuba, Peter J
Biological assessment methods to predict the impact of open-water disposal of dredged material / by P. J. Shuba, H. E. Tatem, J. H. Carroll. Vicksburg, Miss. : U. S. Waterways Experiment Station ; Springfield, Va. : available from National Technical Information Service, 1978.
77, [86] p. ; 27 cm. (Technical report - U. S. Army Engineer Waterways Experiment Station ; D-78-50)
Prepared for Office, Chief of Engineers, U. S. Army, Washington, D. C., under DMRP Work Unit No. 1E08.
References: p. 74-77.

1. Aquatic anamals. 2. Benthic fauna. 3. Bioassay. 4. Dredged material. 5. Dredged material disposal. 6. Open-water disposal. 7. Pollutants. 8. Sediment. 9. Soil contamination. 10. Water pollution. I. Carroll, Joe H., joint author. II. Tatem, Henry E., joint author. III. United States. Army. Corps of Engineers. IV. Series: United States. Waterways Experiment Station, Vicksburg, Miss. Technical report ; D-78-50.
TA7.W34 no.D-78-50