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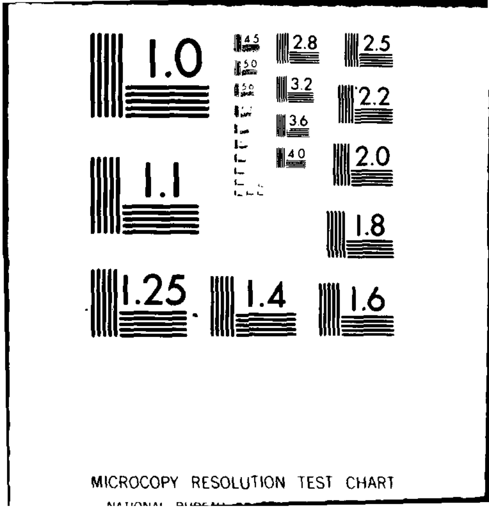
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MH Salazar
SC U'ren
SA Steinert

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April 1980
Final Report: March 1978 — January 1980

Prepared for
Naval Facilities Engineering Command

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SAN DIEGO, CALIFORNIA 92152

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Technical Director

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The authors also wish to thank Tom Peeling for initially suggesting that we get involved in this work and then making the contacts necessary for us to begin. Since his farsightedness was not truly acknowledged while he was at NOSC, we wish to give it recognition now.

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Bioassays were conducted on sediments from 13 sites associated with Navy activities in San Diego Bay using liquid, particulate, and solid phase test solutions. Sediments were pre-sampled to determine the most contaminated area at each site based on the relative concentration of cadmium, chromium, copper, and mercury. Five different marine species representing a variety of phylogenetic levels and feeding types were used in the toxicity tests with the most contaminated sediments including copepods, mysids, fish, clams, and worms. The results of the bioassays suggest that dredged material from Navy Piers 1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 13, JK, and Chollas Creek Channel should not have a significantly adverse effect on the marine environment during disposal operations.

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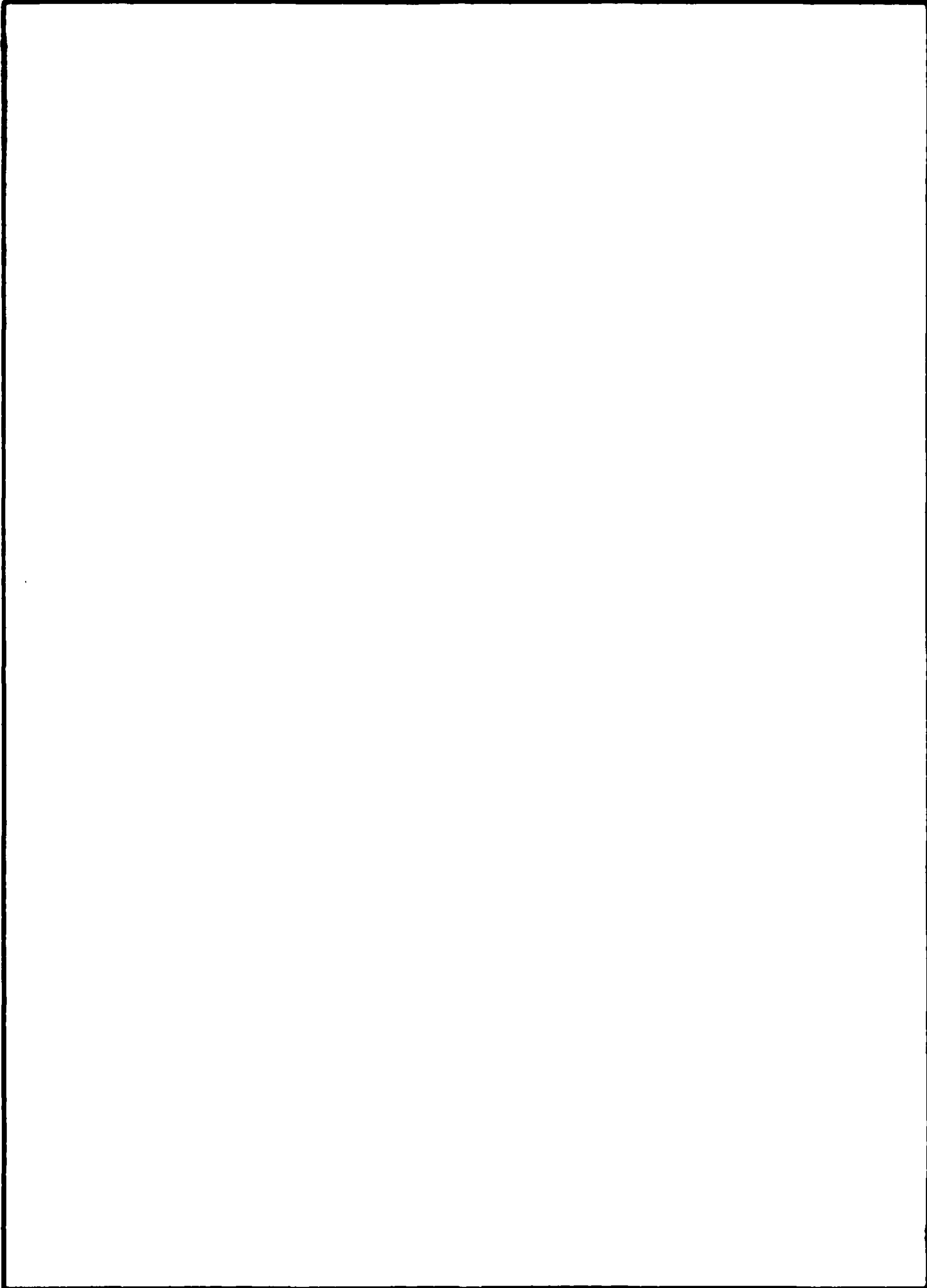

CONTENTS

INTRODUCTION . . . page 3

MATERIALS AND METHODS . . . 5

- Selection of Sampling Site . . . 5
- Collection of Sediment . . . 6
- Preparation of Liquid and Particulate Phases . . . 10
- Test Animals for Liquid and Particulate Phases . . . 11
- Test Procedures for Liquid and Particulate Phases . . . 11
- Test Animals for the Solid Phase . . . 12
- Collection of Test Animals . . . 12
- Test Procedures for the Solid Phase . . . 12
- Bioaccumulation Procedures . . . 13
- Analysis of Tissue and Sediment Samples . . . 13
- Data Analysis Procedures . . . 14

RESULTS . . . 16

- Pier 2 . . . 16
- Piers 1, 3, 4 . . . 19
  - Pier 1 . . . 19
  - Pier 3 . . . 19
  - Pier 4 . . . 22
- Piers 5, 6, 8 . . . 21
  - Pier 5 . . . 24
  - Pier 6 . . . 24
  - Pier 8 . . . 24
- Piers 10, 11, 12 . . . 24
  - Pier 10 . . . 25
  - Pier 11 . . . 25
  - Pier 12 . . . 27
- Piers 13, JK, Chollas Creek . . . 27
  - Pier 13 . . . 27
  - Pier JK . . . 28
  - Chollas Creek Channel . . . 28
- Sediment Analysis . . . 30
- Bioaccumulation . . . 31
- Statistical Analyses . . . 31

DISCUSSION . . . 37

- Pier 2 . . . 37
- Piers 1, 3, 4 . . . 39
- Piers 5, 6, 8 . . . 39
- Piers 10, 11, 12 . . . 39
- Piers 13, JK, Chollas Creek . . . 41

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SUMMARY . . . 42

CONCLUSIONS . . . 45

REFERENCES . . . 46

INTRODUCTION

As part of maintenance dredging and pier construction, the Navy must dredge in the immediate vicinity of 12 different piers in San Diego Bay to accommodate ships assigned to NAVSTA San Diego. The existing water depth varies between 15 and 30 feet below mean lower low water (MLLW). The proposed dredging project will increase the depth to a minimum of 20 feet at Chollas Creek and a maximum of 35 feet at Pier 2 (Fig. 1). A 2-foot overdredge will ensure that the project depth is attained. Dredging will be done by commercial contract and should be completed in 268 working days. About 1 million cubic yards will be dredged from an area of approximately 1 million square yards, which includes 300,000 cubic yards of construction dredging for Pier 2. The existing Pier 2 is being demolished and the new pier is being constructed simultaneously. Although Pier 2 will be dredged after construction, there is a great urgency to dredge around the other piers because some ships are scraping the bottom now.

Since no acceptable land disposal sites are available on Navy property and no other land sites are feasible, ocean dumping of the dredged material is necessary. The dredged material will be barged and dumped at an interim disposal site (LA-5) designated by the Environmental Protection Agency (EPA) and located 7.7 nautical miles from Pt. Loma. Disposal will be at a depth of 600 feet and within a 1000-yard radius of coordinates 32° 36′ 50″ N and 117° 20′ 40″ W.

According to Section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972, sediments to be dumped into ocean waters must be evaluated to determine the potential environmental impact. Evaluations must be in accordance with criteria in the *Federal Register* Vol. 42, No. 7, Tuesday 11 January 1977. Previously, the criteria were based only on chemical analyses of dredged material. Bioassays, which provide more direct estimates of the potential for adverse effects, are now emphasized. The intent of the law is to prevent an adverse environmental impact from the ocean dumping of dredged material.

Under certain conditions, depending on sediment history, grain size, and disposal area, dredged material may be considered environmentally acceptable for ocean dumping without conducting bioassays. Dredged material that does not meet the criteria for exclusion must receive a full technical evaluation. This includes tests on the liquid, particulate, and solid phases with appropriate sensitive marine organisms. In these tests, liquid phase elutriates and suspended particulate phase slurries are used to simulate water column conditions during ocean dumping. The solid phase sediments are used to simulate benthic conditions after disposal. Biological evaluations must also include an assessment of contaminant accumulation in the tissues of marine organisms.

Since disposal is part of the proposed project and the dredged material did not meet the criteria for exclusion, the Navy conducted an ecological evaluation of the sediments. This will supplement the Environmental Impact Assessment for construction and maintenance dredging. To assist applicants and to standardize test procedures, the EPA and the Corps of Engineers (COE) have published a manual for the "Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters." According to this manual, dredge permit applicants must demonstrate that the dredged material does not adversely affect the environment. The following are the results of the ecological evaluation of sediments from Piers 1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 13, JK, and the Chollas Creek Channel to demonstrate that they qualify for ocean dumping under the new law. This was one of the first attempts to comply with the new manual, and a number of technical and

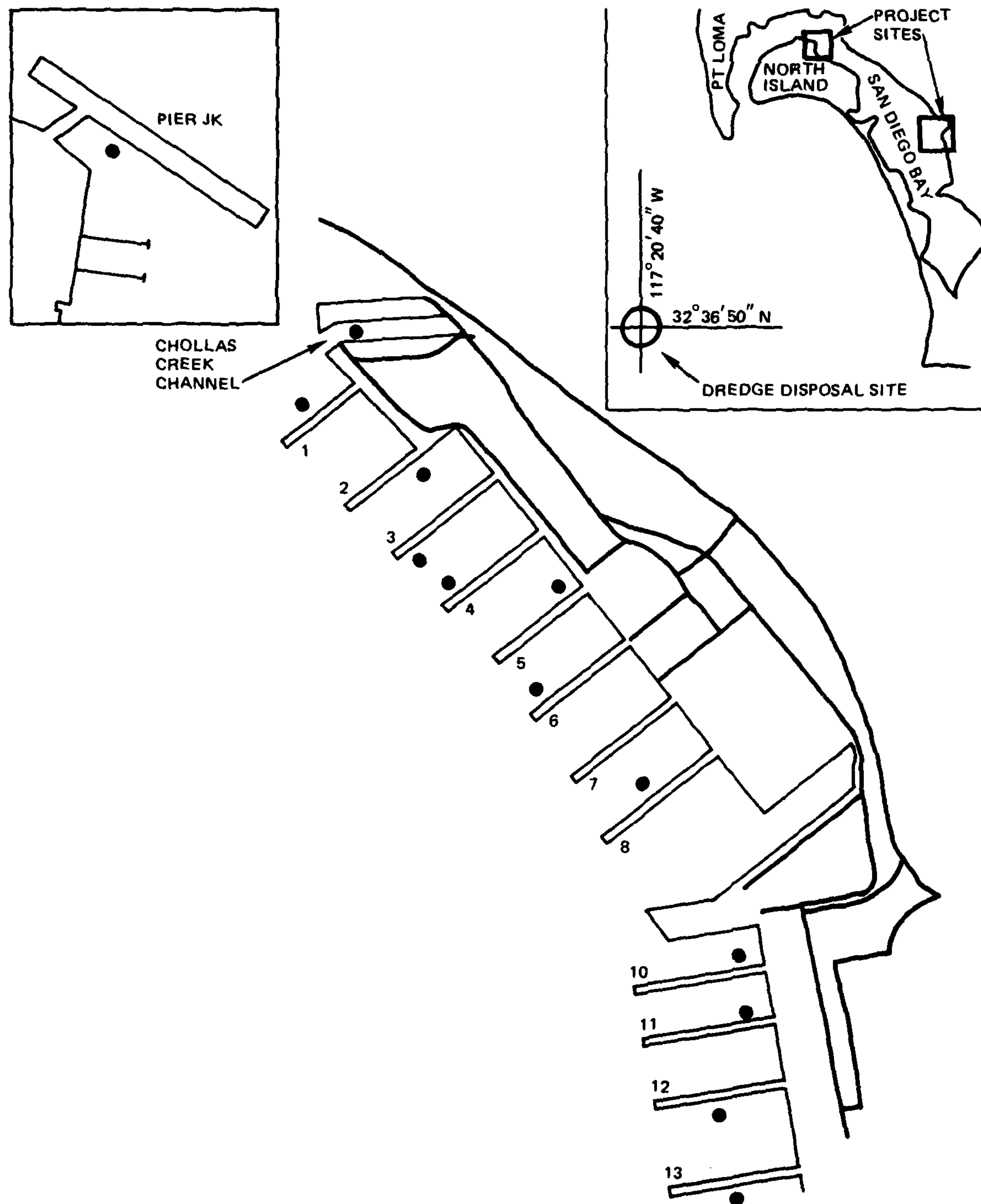

Figure 1. Dredge sites, disposal site, and sediment collection sites (●). (See Fig. 2 for preliminary core sample sites used to determine sediment collection sites.)

practical problems were encountered. These problems are documented herein to assist the COE and future applicants.

MATERIALS & METHODS

SELECTION OF SAMPLING SITE

The manual suggests that sediment be collected from a minimum of three sampling stations within each dredging area. Since there were 13 dredge areas to be assessed, this would have required 39 separate bioassays. The cost and time requirements for 39 bioassays rendered this approach impractical. To reduce the number of bioassays, we did a preliminary survey to determine the most contaminated site at each pier. We used sediment from the most contaminated site to conduct one bioassay per dredge area. The estimated volume of dredged material represented by one bioassay ranged from 20,000 to 318,600 cubic yards (Table 1). It was reasoned that if the dredged material from the "worst site" at each pier was not significantly toxic to the five test species, it would be superfluous to conduct two more bioassays at each pier.

The pollutants used as indices of greatest site contamination are the most toxic heavy metals commonly associated with shipboard operations: cadmium (Cd), chromium (Cr), copper (Cu), and mercury (Hg). They were selected by the Navy with guidance from EPA, COE, and the Regional Water Quality Control Board. The Pier 2 bioassay is a special case because it was the first one conducted (March, 1978); it was conducted separately; and the tests had to be repeated. For Pier 2 we used cadmium, lead, mercury and zinc since these

Table 1. The estimated volume of dredged material to be removed from each site.

Dredge Site	Sediment Volume (yd^3)
Pier 1	75,100
Pier 2	318,600
Pier 3	23,100
Pier 4	46,000
Pier 5	34,000
Pier 6	47,600
Pier 8	90,200
Mole Pier	25,500
Pier 10	47,300
Pier 11	56,000
Pier 12	90,600
Pier 13	90,800
Pier JK	20,000
Chollas Creek	99,700
Total	1,064,500

data had been acquired just prior to the first bioassay. Since significant contamination by other pollutants was not detected in previous sampling by the Naval Ocean Systems Center (NOSC, unpublished data) and the Southern California Coastal Water Research Project (SCCWRP, 1975) "worst sites" within each dredge area were selected by determining the concentration of these four metals. These bioassays were based on the concept that the most important criterion for determining environmental impact is animal mortality. Assessing the absolute concentration of any pollutant in the sediment is of secondary importance. Bioassays were used to determine if contaminants in test sediment were present in toxic amounts.

Seven core samples were taken at each pier to locate the worst station (Fig. 2). The sediment samples were collected with plastic coring devices and placed in Zip-Loc plastic bags. They were freeze-dried, ground, and pelletized for analysis with an ORTEC X-ray fluorescence spectrophotometer, which is sensitive to concentrations as low as 1 ppm for the metals we used. These data are presented in Table 2. Sites were ranked by relative units, on a percentage basis of the maximum allowable concentration for each metal. According to the old regulations, the maximum allowable concentrations in dredged material were 1.2 ppm for mercury and 2.3 ppm for cadmium (75 ppm for lead and 190 ppm for zinc in the Pier 2 cores). The allowable limits for chromium and copper are not listed in the old regulations, so theoretical values were extrapolated from water quality data with assistance from the Regional Water Quality Control Board. Theoretical sediment limits of 20 and 50 ppm were determined for chromium and copper, respectively.

COLLECTION OF SEDIMENT

Test sediments were collected by SCUBA divers using plastic buckets with O-ring-sealed lids. Plastic buckets were used instead of sediment samplers as the manual suggests because of the large volume required. Divers would swim to the bottom and fill the buckets with sediment scooped from the upper 20–30 cm of bottom material. Immediately after scooping, the buckets were sealed to prevent any sediment from washing out on the way to the surface (lids were not used in the first two Pier 2 sediment collections). The sediment-filled buckets were brought to the surface and dumped into 56-liter Coleman ice chests lined with large plastic bags to reduce contamination. The chests were used to maintain the temperature of sediments between collection and storage. The sediments were stored at 4±1°C and used as soon as possible after collection. Sediment was never stored for more than 2 weeks. Approximately 100 liters of treatment sediment (two chests) were required to conduct one bioassay. Three bioassays were conducted concurrently (the first Pier 2 bioassays were done separately), and 200 liters of reference sediment were required. Reference sediment was collected in an uncontaminated area off North Island between Pier JK and the mouth of the bay.

Every effort was made to use reference sediment that was characteristic of the disposal site. However, the disposal site sediment has not been fully characterized. Available information indicated that the average grain size at the LA-5 dump site is about 0.03 mm (Emery & Butcher, 1952). Unfortunately, the "cleanest" sediment in San Diego Bay had a grain size significantly larger than that at the disposal site. It was felt that using uncontaminated sediment was more important than matching the grain size. The average grain size of our reference sediment was 0.0782 mm. Grain sizes for reference and treatment sediments were measured by the tube drop method and are listed in Table 3. The heavy

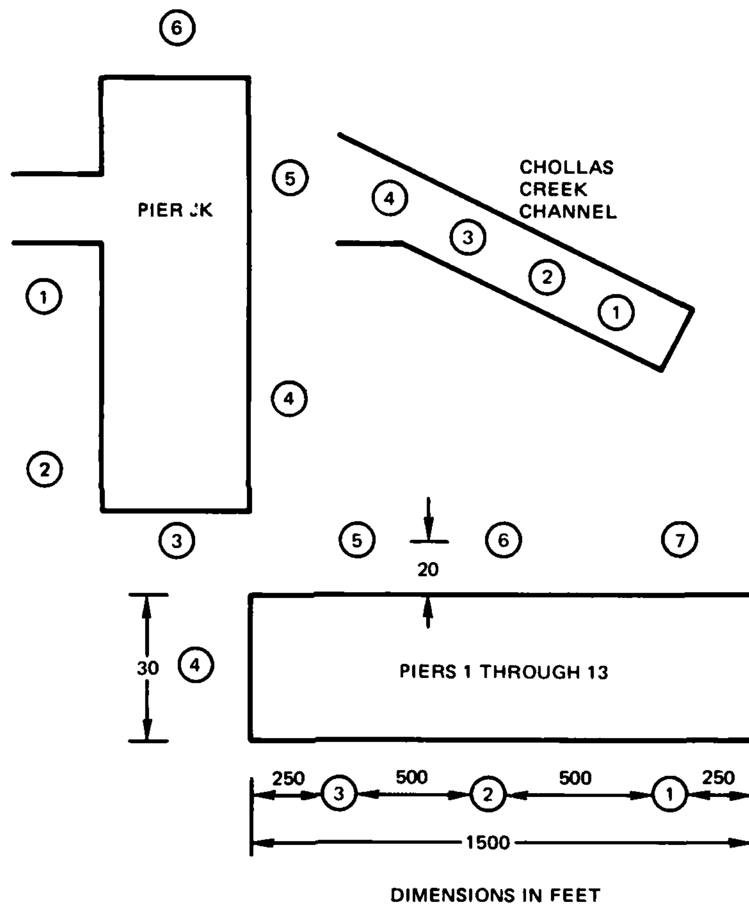

Figure 2. Core sample sites. (Subsequent analyses used to select most contaminated site around each pier.)

Table 2. Concentration of cadmium, chromium, copper and mercury (lead and zinc for Pier 2) in preliminary core samples (the most contaminated site around each pier is indicated by bracketed values, concentration in ppm).

Dredge Site		Core 1	Core 2	Core 3	Core 4	Core 5	Core 6	Core 7
Pier 1	Cd	15.8	14.7	0.0	10.4	11.3	**8.2**	0.0
	Cr	185.3	225.4	207.3	131.6	166.9	**217.9**	157.1
	Cu	256.9	260.8	317.4	318.8	283.5	**410.0**	284.3
	Hg	0.1	6.5	0.1	27.3	12.4	**34.0**	1.6
Pier 2	Cd	**6.6**	6.2	6.6	2.0	1.0	5.0	6.6
	Hg	**5.7**	8.7	9.2	4.2	0.7	5.9	2.5
	Pb	**592.0**	240.0	178.0	140.0	40.0	100.0	240.0
	Zn	**1,024.0**	680.0	760.0	280.0	164.0	392.0	952.0
Pier 3	Cd	15.3	14.3	**17.4**	10.4	13.8	13.0	9.4
	Cr	239.3	277.0	**255.8**	111.3	198.8	215.3	247.0
	Cu	773.3	422.1	**613.5**	218.6	570.7	484.7	592.5
	Hg	0.1	10.2	**9.1**	26.0	8.2	8.3	0.8
Pier 4	Cd	11.8	6.1	5.6	6.5	**11.5**	20.3	30.3
	Cr	284.3	356.4	309.9	270.8	**698.7**	181.0	343.4
	Cu	583.4	396.6	517.0	349.9	**463.6**	345.3	661.9
	Hg	0.5	0.1	0.1	21.6	**6.1**	11.7	0.1
Pier 5	Cd	8.7	19.4	4.3	7.7	0.0	19.1	**7.5**
	Cr	275.5	208.9	258.6	167.6	192.9	273.6	**285.3**
	Cu	1,714.6	432.7	492.8	257.3	646.4	765.7	**1,109.0**
	Hg	114.2	142.4	124.4	80.4	162.7	125.5	**155.3**
Pier 6	Cd	18.9	7.7	13.6	12.0	**6.4**	16.3	17.0
	Cr	242.7	221.8	189.2	178.5	**198.3**	243.5	148.3
	Cu	525.2	368.7	383.7	236.4	**296.2**	371.9	283.2
	Hg	109.1	61.2	78.8	47.0	**141.8**	85.2	64.9
Pier 8	Cd	7.3	7.2	4.7	12.7	11.0	**12.0**	4.0
	Cr	185.8	178.4	147.4	186.6	159.7	**163.4**	215.2
	Cu	370.9	141.8	693.7	231.9	302.1	**372.1**	394.6
	Hg	95.0	56.8	135.3	58.6	121.2	**188.2**	140.0
Pier 10	Cd	3.4	13.7	0.0	17.9	7.3	24.1	**19.0**
	Cr	236.9	213.5	235.3	188.6	187.9	210.5	**225.8**
	Cu	397.8	303.2	312.1	220.0	281.8	329.0	**371.9**
	Hg	63.1	47.3	40.9	37.3	49.6	70.8	**90.1**
Pier 11	Cd	9.0	8.6	13.3	3.3	0.0	16.7	**14.0**
	Cr	231.0	206.1	209.8	164.9	209.5	206.7	**236.4**
	Cu	374.8	360.0	312.6	197.6	287.3	308.4	**379.9**
	Hg	89.3	67.8	32.1	47.2	43.6	134.1	**153.3**
Pier 12	Cd	14.1	**12.1**	8.7	3.0	25.4	16.1	12.0
	Cr	228.3	**195.0**	187.4	170.5	211.3	224.7	112.1
	Cu	354.0	**389.5**	285.2	143.4	284.7	332.0	150.2
	Hg	68.0	**81.3**	57.3	46.3	46.5	67.2	56.0
Pier 13	Cd	3.0	**3.0**	2.0	–	4.0	1.0	2.0
	Cr	111.0	**151.0**	89.0	–	0.0	54.0	90.0
	Cu	130.9	**161.0**	91.0	–	152.0	27.0	134.0
	Hg	1.2	**1.0**	1.0	–	1.0	2.0	3.0
Pier JK	Cd	**13.9**	0.9	0.0	8.9	9.9	22.1	0.0
	Cr	**33.4**	58.6	49.5	132.5	148.1	175.4	176.2
	Cu	**143.7**	132.6	108.7	240.8	169.2	172.3	205.9
	Hg	**77.4**	72.5	71.4	48.7	31.7	34.3	32.6
Chollas	Cd	15.3	14.7	**5.5**	5.1			
Creek	Cr	97.8	124.5	**96.7**	88.3			
Channel	Cu	60.0	83.0	**112.6**	79.6			
	Hg	15.5	16.8	**43.0**	30.3			

Table 3. Mean sediment grain size at each dredge site.

Pier	Mean Grain Diameter (mm)	Sediment Type
1	0.0027	Silty clay
3	0.0026	Silty clay
4	0.0051	Clayey silt
Control	0.0954	Very fine sand
5	0.0069	Clayey silt
6	0.0134	Sand-silt-clay
8	0.0081	Sand-silt-clay
Control	0.0819	Very fine sand
10	0.0031	Silty clay
11	0.0035	Silty clay
12	0.0082	Sand-silt-clay
Control	0.0878	Very fine sand
13	0.0024	Silty clay
JK	0.0412	Very fine sand
Chollas Creek	0.0268	Silty sand
Control	0.0461	Very fine sand
2 (Mar 78)	0.0100	Sand-silt-clay
Control	0.1100	Very fine sand
2 (Apr 78)	0.0200	Sand-silt-clay
Control	0.0800	Very fine sand
2 (Dec 79)	0.2624	Gravely sand
Control	0.0461	Very fine sand

metal concentrations for reference and treatment sediments were determined with a Perkin-Elmer atomic absorption spectrophotometer equipped with a graphite furnace.

We did not acid wash plastic samplers and storage containers as the manual suggests. Acid washing plastic containers is a procedure used to reduce heavy metal contamination. If trace quantities are being examined (ppb range), contamination from the plastic could add significantly to the amount detected in the sample. If the amount present is in the ppm range, as in our samples, the addition of such a small amount is insignificant. Similarly, clean plastic buckets, plastic core samplers, and storage bags contribute very little contamination. The ratio of sediment volume to container surface area was large in all our collections and the contact time was very short. For these reasons, acid washing the plastic containers was unnecessary.

The manual also suggests that disposal site water be used if possible, but this was impractical because of the large volume required and the distance to the site. Additionally, since the seawater from our flow-through system is very similar to that off the coast, it seemed reasonable to use this natural seawater for the bioassays. The seawater system at the NOSC Marine Mammal Facility has been used to maintain a wide variety of marine organisms during its 7 years of operation. The seawater inlet for this system is on the ocean side of Pt. Loma. Periodic water analyses have shown that the four heavy metals used in our bioassays are present in only trace amounts of 1 ppb or less. These concentrations are similar to those found in open-ocean water (Berhard and Zattera, 1975). Analyses of tissues from filter-feeding invertebrates we have maintained in flow-through laboratory aquariums have shown no significant accumulation of heavy metals, hydrocarbons, or pesticides. "Control water" was this natural seawater passed through large sand filters and 0.45-μ filters.

PREPARATION OF LIQUID AND PARTICULATE PHASES

Slurries for the liquid and particulate phase tests were prepared in 50-gallon plastic trash cans. These "new" plastic trash cans were "aged" by holding seawater in them for 2 weeks to leach out the plasticizers. It was not practical to prepare the slurries as the manual suggests because of the large volume required. In most cases 120 liters of control water was added to 30 liters of treatment sediment, but the sediment-to-water ratio was always 1:4. The slurries were mixed by vigorous bubbling with compressed air at a rate of 50 liters per minute. Every 10 minutes we stirred the slurries with a large plastic paddle. After 30 minutes of bubbling, the air stones were removed and the slurries were allowed to settle for at least 1 hour or until the supernatant was clear enough to reveal the sediment on the bottom of the container. If the supernatant did not settle long enough, the resulting particulate phase solutions would be too turbid to permit viewing and counting the animals. The liquid phase was prepared by pumping the supernatant through filters, the smallest having a pore size of 0.45μ. The pump and filter system consisted of a ¾-horsepower magnetic pump (Serfilco) and four CPVC filter chambers holding 20-inch cartridges. No metallic parts came in contact with the test solutions. The chambers, in series, held 100-, 20-, 10-, and 0.45-μ pleated filters. In the particulate phase, the supernatant was pumped directly into the test tanks without filtration. Control water was used for diluting treatment solutions as well as in the control tanks.

TEST ANIMALS FOR LIQUID AND PARTICULATE PHASES

For liquid and particulate phases the manual suggests using phytoplankton or zooplankton, a crustacean or mollusk, and a fish. We used a copepod (planktonic crustacean), a mysid (hypoplanktonic crustacean), and a fish for the liquid and particulate phases. The manual further suggests that organisms from the disposal site be used where possible. It was not practical to use species from the disposal site because they are not well known and not easily collected.

Acartia tonsa is a ubiquitous copepod in southern California coastal waters. It was used in all tests. *Metamysidopsis elongata* was selected as a representative mysid species since it is also common in this area and can be easily maintained in the laboratory. Taxonomically, *Metamysidopsis elongata* is very similar to *Mysidopsis*, the genus suggested in the manual. *Metamysidopsis elongata* was used in all tests except the second bioassay, when they could not be collected in sufficient numbers, and we used *Acanthomysis macropsis* instead. The speckled sanddab (*Citharichthys stigmaeus*) was the third species selected for the liquid and particulate phase tests. It is one of the most common fish in southern California. The fish we used ranged in size from 40 to 110 mm in length, with a mean of 75.3 mm.

TEST PROCEDURES FOR LIQUID AND PARTICULATE PHASES

Fish were held in ten-gallon tanks, mysids in 6-liter battery jars, and copepods in 400-ml beakers. All liquid and particulate phase tests were run under static conditions for 96 hours. Three replicates were used for each experimental and control condition and ten organisms were exposed in each replicate. The liquid and particulate phases consisted of 10, 50, and 100% solutions of dredged material from each pier. Control water was used to prepare controls and make dilutions for the liquid and particulate phase solutions.

During liquid and particulate phase tests, the temperature was maintained at 13-14°C, salinity between 33 and 34 ppt, dissolved oxygen between 7.8 and 8.0 ppm, and pH between 7.8 and 8.1. Measurements were made daily in each container with a Horiba U-7 water checker. To comply with the manual, the fish, copepods, and mysids were not fed during the initial bioassay with Pier 2 sediments. Procedures were modified in subsequent tests where necessary to increase survival. Copepods were fed algae, mysids were maintained on brine shrimp nauplii, and fish were not fed in the second Pier 2 bioassay. During the bioassays on Piers 1, 3, and 4 the copepods were aerated and fed algae. Survival was high, but since there was no difference in dissolved oxygen, aeration was not necessary. During the bioassays on Piers 5, 6, and 8 the copepods were fed algae and not aerated, and algal blooms may have contributed to the high mortality observed. In all subsequent tests the copepods were not fed or aerated. Mysids were fed 20 to 30 brine shrimp nauplii per animal per day in all tests after Pier 2. The copepods and mysids received moderate aeration of about 3-7 ml/min. During all tests, the fish tanks were vigorously aerated at a rate between 500 and 1300 ml/min.

Live animals were counted at the end of each test. It was impractical to count live copepods and mysids every day as suggested in the manual since the only reliable method involved removing the animals with medicine droppers. This procedure would severely stress the delicate test organisms and could adversely affect the results. Fish and clams were counted daily and dead animals removed. Worms burrowing in the sediment could not be counted each day because it would disrupt the sediment regime.

TEST ANIMALS FOR THE SOLID PHASE

We used a mysid, a polychaete worm, and a clam for the solid phase bioassays. *M. elongata* was used in the first Pier 2 bioassay, *Acanthomysis macropsis* in the second Pier 2 bioassay, and *M. elongata* in all subsequent bioassays. A polychaete worm (*Neanthes arenaceodentata*) was used in every solid phase test. In the first Pier 2 bioassay *Macoma nasuta* was selected as the most sensitive and representative clam available. After the donor population began to decline, we attempted to obtain *Parvilucina tenuisculpta*, but the supplier could only find them adjacent to a sewage outfall. Finally, as a third choice, we used *Protothaca staminea*. This species might be less sensitive, but is much more abundant and was used in all subsequent bioassays. They ranged in size from 28 to 58 mm, with a mean of 42.1 mm.

COLLECTION OF TEST ANIMALS

The five species of marine organisms we used were purchased from four commercial suppliers. Copepods, clams, and fish were collected by Pacific Bio Marine, Venice, California. Clams and fish were collected several days prior to each set of bioassays, and copepods were collected on the first day of the test. Clams (*P. staminea*) were collected by hand in Los Angeles Harbor, and fish (*C. stigmaeus*) were taken in an otter trawl in Santa Monica Bay. Copepods (*A. tonsa*) were collected by slowly towing a net (250-μ mesh) just beneath the ocean surface and off the entrance to King Harbor, Redondo Beach, California. Copepods for the March and April 1978 bioassays for Pier 2 were collected by Marine Biological Consultants, Costa Mesa, California. However, control copepod mortality was high, so we changed suppliers and survival increased. Mysids (*M. elongata*) were collected on the first day of the test by Marine Ecological Consultants, Solana Beach, California, using a modified epibenthic sled towed offshore near San Diego. The worms (*N. arenaceodentata*) were cultured by Dr. Donald Reish at California State University, Long Beach, and gathered on the first day of the test.

TEST PROCEDURES FOR THE SOLID PHASE

The solid phase tests were conducted for 10 days. The temperature, salinity, dissolved oxygen, and pH were the same as in liquid and particulate phase tests. They were also measured daily in each container. Water temperature was maintained between 13 and 14°C for all bioassays even though temperature at the disposal site in 600 feet of water is probably 8-10°C. We used a higher temperature because it was closer to the optimum for maintaining these particular test animals. It is not necessary for physical parameters of the tests to duplicate conditions at the disposal site, as the manual suggests, if the organisms used to represent the site perceive other conditions as optimum. As another example, an irradiance of 1200 microwatts/cm^2 suggested by the manual is neither representative of the disposal site nor necessary for maintaining these animals. Cool white fluorescent bulbs were used to approximate the spectral output of the sun, but irradiance in the test containers was significantly less than 1200 microwatts/cm^2. The irradiances were measured with a photometer. For each test species the values obtained (microwatts/cm^2) are as follows: *C. stigmaeus* (180-900); *N. arenaceodentata* (1-500); *M. elongata* (1-500); *P. staminea* (200-500); and *A. tonsa* (7-85). The light regime consisted of a 14L:10D cycle for all bioassays (12L:12D for the first Pier 2 bioassay).

In the first Pier 2 bioassay all three species were included in each 10-gallon tank. The tanks, like the fish tanks, were vigorously aerated between 500 and 1300 ml/min. In the second Pier 2 bioassay, clams and worms were held in the 10-gallon tanks. Mysids were held in 6-liter battery jars with an aeration between 3 and 7 ml/min to minimize injury. In subsequent tests, mysids and worms were kept in the battery jars and clams were maintained separately in the 10-gallon aquariums. The mysids were separated to eliminate injuries caused by turbulent aeration and to facilitate counting. The worms were put in the small containers with mysids to minimize the sediment volume to be sieved for counting. Five replicate tanks were prepared for treatments and controls. Twenty individuals of each species were placed in each tank.

The treatment tanks consisted of a 30-mm layer of reference sediment covered with 15 mm of treatment sediment. After sediments were placed in these tanks, they were filled with control water and test organisms added. We did not add the treatment layer after the clams and worms had been added, as the manual recommends, because it was too difficult to coordinate collection of all the animals and sediment with the start of the test unless the tanks were prepared on the same day just prior to adding the animals. The manual also suggests replacing 75% of the water in the 10-gallon tanks 24 hours after the test organisms are added. To avoid mysid injuries, seawater was not replaced in the first Pier 2 bioassay. In subsequent tests, where mysids were held in the battery jars, the seawater in the 10-gallon tanks was replaced in accordance with the procedures suggested in the manual. To avoid injury or stress, the seawater in the 6-liter battery jars containing mysids was not changed.

BIOACCUMULATION PROCEDURES

At the end of the solid phase tests, clams from treatment and control tanks were transferred to freshly prepared tanks containing only control water. This enabled the clams to purge ingested sediment. After 24 hours, clams were removed and their tissues frozen for subsequent bioaccumulation estimates. Although the manual does not require heavy metal analyses of sediments, they were analyzed to show that the sediments actually used were high in heavy metals. This supported our "worst site" selection.

ANALYSIS OF TISSUE AND SEDIMENT SAMPLES

Tissue and sediment samples were analyzed by atomic absorption spectrophotometry to determine the concentration of cadmium, chromium, and copper. Samples were collected and kept frozen until they were analyzed. Tissue and sediment samples were freeze-dried, weighed, and acid-digested at a column temperature of 8°C (23°C for Pier 2 sediments). Tissue samples were digested for 6 hours and sediment samples for 12–18 hours. They were diluted to a volume of 100 ml before being analyzed. Since sediment would not dissolve completely, samples were ensonified after the final dilution. The ensonification broke up and suspended the residual particles.

The mercury concentration in tissue and sediment samples was determined with cold-vapor spectrophotometry (Laboratory Data Control U.V. Monitor). We did the mercury analyses of sediments and tissues from Piers 1, 3, and 4; but when our instrument became inoperative, the analyses were performed by contract (Environmental Engineering Laboratory). All samples were digested as outlined above. The low (8°C) digestion temperature was required to keep mercury in solution and eliminate losses due to vaporization.

DATA ANALYSIS PROCEDURES

The statistical methods outlined in the manual were followed wherever possible. However, in some cases the recommended procedures violated assumptions of the statistical tests, so we modified them to accommodate our data. The statistical procedures for assessing bioaccumulation potential were followed exactly with Cochran's test for homogeneity of variances, an Analysis of Variance (ANOVA), and the Newman-Keuls multiple range test if a significant difference was detected. However, if the variances were not homogeneous, we used the Kruskal-Wallis analysis of variance by ranks and Dunnett's test. For comparing survival of control and treatment organisms in liquid, particulate, and solid phase tests, the manual suggests Student's t-test. This test requires homogeneous variances between test groups and samples from normally distributed populations. Our discrete-count survival data (unlike measurement data for bioaccumulation assessments) do not meet these requirements. Discrete data are not represented by a normal distribution when counts are low (Dixon & Massey, 1969). Additionally, the variances between groups tested were frequently not homogeneous. Therefore, instead of the t-test we used the nonparametric equivalent, the Mann-Whitney U-test (Zar, 1974), to compare treatment and control survival in all the Pier 2 tests, where only one comparison was made. In all other bioassays where tests were conducted in sets of three and there were several comparisons to be made, we used the non-parametric equivalent of the ANOVA, the Kruskal-Wallis analysis of variance by ranks. If a significant difference was detected among groups, we used Dunnett's test to identify which particular survival was significantly different from the others.

In liquid, particulate, and solid phase bioassays, the survival of treatment organisms for each pier tested was individually compared to the survival of control organisms using the Mann-Whitney U-Test or the Kruskal-Wallis analysis of variance by ranks and Dunnett's test. In liquid and particulate phase tests, survival in 100% test solutions was compared to control survival. If the survival was significantly different ($p \leq 0.05$) in liquid and particulate phase tests, the LC 50 (concentration lethal to 50% of the test organisms) was calculated. The manual suggests that the concentration of dredged material at the dump site after 4 hours of initial mixing should be less than 0.01 of the lower 95% confidence limit of the LC 50. The limiting permissible concentration (LPC) is the concentration of dredged material after 4 hours. It was calculated from information supplied by the manual, the Navy, and dredging contractors. These calculations are presented in Table 4. Taking into consideration the hopper volume, barge speed, disposal site depth, and other parameters, we calculated the LPC to be approximately 0.10% of the original liquid phase concentration and 0.04% of the original suspended particulate phase concentration. If survival was significantly lower in treatment tanks than in control tanks, the application limit was calculated. This is 0.01 of the lower 95% confidence limit of the LC 50. If the application limit is less than the LPC, then the dredged material will probably have an adverse effect on the environment. An acceptable application limit is one that exceeds the LPC, since a large number indicates a high concentration of dredge material is necessary for significant mortality.

Hypothetical "worst case" data were derived to find out how high mortality could be without exceeding the LPC. The application factor of 0.01 introduces an environmentally conservative approach in extrapolating from bioassay to natural conditions. Using this factor, it was determined that the lower 95% confidence limit of the LC 50 could not be less than 4% of the original particulate phase concentration or 10% of the original liquid phase concentration after a dilution of 4 hours. A hypothetical line was drawn (dose vs mortality) by extrapolating from the methods suggested by Litchfield and Wilcoxon (1949). The line was plotted so the lower 95% confidence limit of the liquid phase LC 50 was not less than 10% of the

Table 4. Calculations used to determine the concentration of liquid and suspended particulate phases after four hours of initial mixing.

A. Initial mixing zone volume (V_m):

$$V_m = \pi(100)^2 d + 200\,wd + (200 + w)(ut + \ell)d$$

$$V_m = 1{,}469{,}301\ m^3$$

where

d = appropriate depth value (20 m)
w = width of disposal vessel (13.4 m)
ℓ = length of disposal vessel (67.1 m)
u = speed of vessel (2.5 m/sec)
t = time to empty vessel (100 sec)

B. Volume of liquid phase material (V_w):

$$V_w = \frac{P_b - P_d}{P_w - P_d}(V_T) = 1506\ m^3$$

where

P_b = bulk density (1.5)
P_d = particle density (2.6)
P_w = density of liquid phase (1.0)
V_T = volume of disposal vessel (2190 m^3)

C. Percent of original liquid phase concentration at the disposal site after initial mixing (C_w):

$$C_w = \frac{V_w}{V_m}(100) = 0.10\%\ \text{(Dilution factor of 976)}$$

D. Volume of suspended particulate phase material (V_{sp}):

$$V_{sp} = (V_T - V_w)\,\frac{(P_c + P_s)}{100} = 616\ m^3$$

where

P_c = percent clay in dredge sediment (40%)
P_s = percent silt in dredge sediment (50%)

E. Percent of the original suspended phase concentration at the disposal site after initial mixing (C_{sp}):

$$C_{sp} = \frac{V_{sp}}{V_m}(100) = 0.04\%\ \text{(Dilution factor of 2500)}$$

original concentration of dredged material. This line was used to determine that mortality after only 4 hours in liquid phase tests would have to be greater than 33, 83, and 93% in 10, 50, and 100% concentrations of the liquid phase slurries, respectively, to exceed the LPC. For the particulate phase, a hypothetical line was drawn so that the lower 95% confidence limit of the LC 50 was not less than 4% of the original concentration of dredged material. In this case, 4-hour mortality would have to be greater than 60, 93, and 97% in 10, 50, and 100% concentrations of particulate phase slurries to exceed the LPC. If mortality does not exceed these percentages, then the sediment should not have an adverse effect on the environment. Further, since our data were 96-hour values instead of 4-hour values, a safety margin is introduced because the dilution period is 24 times longer. These hypothetical data represent the maximum mortality possible without exceeding the LPC

When hypothetical lines are drawn at the maximum limits without exceeding the LPC, small changes in mortality (i.e., the death of one animal increases mortality by 3%) in any of the test solutions could alter the line and exceed the LPC. For example, if our hypothetical liquid phase mortality for 10, 50, and 100% test concentrations was 33, 87, and 93% (instead of 33, 83, and 93%), respectively, the LPC would be exceeded. Similarly, small changes in hypothetical mortality for the particulate phase tests could alter the line and exceed the LPC.

There are a large number of hypothetical results that can be derived by drawing lines used to calculate application limits that do not exceed the LPC. Factors which affect the lines used to determine application limits include the number of organisms tested and the slope function of the line (Litchfield and Wilcoxon 1949). The chi square statistical test which determines the "goodness of fit" of the line to the three plotted points allows enormous variability. Within these bounds, the vast majority of results from liquid and particulate phase solutions will be environmentally acceptable.

RESULTS

PIER 2

Our first bioassay was conducted in 1978 on sediments from Pier 2. At this time animal maintenance techniques were being developed and there were several procedural problems. This bioassay was repeated several times. These results are presented in Tables 5 and 6. After two complete bioassays (March and April 1978) only fish and mysid results in liquid and particulate phases were considered reliable and clam and worm results in solid phase tests. In the March bioassay the survival of copepods and mysids in liquid and particulate phase tests was very low, and the data were not analyzed. Mortality could not be attributed to the sediments, and the results were inconclusive. Fish survival in 100% liquid and particulate phase solutions was only 33 and 47%, respectively, but not significantly different from control survival (50%). Mysid survival in March solid phase tests was low and not analyzed. The survival of clams and worms in solid phase tests was 74 and 88%, respectively, and not significantly different from survival in controls.

In the April bioassay, there was no significant difference between survival in treatments and controls with any species. Copepod survival in liquid and particulate phases and mysid survival in the solid phase were very low, and the data were not analyzed. In liquid phase tests, mysid survival varied between 87 and 93%. Fish survival was 100% in all concentrations tested. In particulate phase tests, mysid survival ranged between 70 and 90%.

Table 5. The number of mysid shrimp (*Metamysidopsis elongata*), clams (*Macoma nasuta*), and polychaete worms (*Neanthes arenaceodentata*) surviving after 10 days of exposure to sediment from Naval Pier 2 (solid phase, 20 organisms started in each tank).

		Run 1 (3/78)		Run 2 (4/78)		Run 3 (12/79)	
	Replicate	Control	Treatment	Control	Treatment	Control	Treatment
M. elongata	1	13	0	10	2	20	16
	2	0	0	8	0	20	16
	3	6	0	10	2	20	13
	4	0	0	6	2	20	18
	5	0	0	7	5	20	10
		19%	0%	41%	11%	100%	73%
M. nasuta	1	13	15	20	20		
	2	16	18	19	19		
	3	10	11	20	20		
	4	10	17	20	20		
	5	13	13	19	19		
		62%	74%	98%	98%		
N. arenaceodentata	1	20	13				
	2	20	20				
	3	20	15				
	4	19	20				
	5	20	20				
		99%	88%				

Table 6. The number of mysid shrimp (*Metamysidopsis elongata*), fish (*Citharichthys stigmaeus*), and copepods (*Acartia tonsa*) surviving after 96 hours of exposure to liquid and particulate solutions of sediments from Naval Pier 2 (liquid and particulate phases, 10 organisms started in each tank). The mysid *Acanthomysis macropsis* was used during Run 2 in place of *M. elongata*.

			Liquid				Particulate			
			Concentration of test medium				Concentration of test medium			
		Replicate	10%	50%	100%	Control	10%	50%	100%	Control
M. elongata	Run 1 (3/78)	1	0	0	0	0	0	7	8	0
		2	1	0	0	1	2	4	6	0
		3	2	0	1	2	0	5	4	0
			10%	0%	3%	10%	7%	53%	60%	0%
	Run 2 (4/78)	1	10	9	7	10	9	8	8	10
		2	10	9	9	10	6	10	8	10
		3	8	10	10	9	6	9	8	7
			93%	93%	87%	97%	70%	90%	80%	90%
	Run 3 (12/79)	1			9	10			10	9
		2			9	9			10	9
		3			10	9			10	10
					93%	93%			100%	93%
C. stigmaeus	Run 1 (3/78)	1	7	7	4	8	8	3	3	8
		2	1	5	5	3	2	6	5	3
		3	7	4	1	4	4	4	6	4
			50%	53%	33%	50%	47%	43%	47%	50%
	Run 2 (4/78)	1		10	10	10		10	9	10
		2		10	10	9		10	10	9
		3		10	10	10		9	10	10
				100%	100%	97%		97%	97%	97%
A. tonsa	Run 1 (3/78)	1	5	5	0	5	6	8	5	1
		2	7	3	3	6	7	5	1	1
		3	0	3	1	6	5	3	1	4
			40%	37%	13%	57%	60%	53%	23%	20%
	Run 2 (4/78)	1	3	4	0	3	6	3	1	3
		2	3	4	5	1	1	0	1	3
		3	5	3	0	3	8	5	5	5
			37%	37%	17%	23%	50%	27%	23%	37%
	Run 3 (12/79)	1			0	10			8	10
		2			4	8			8	8
		3			3	8			8	8
					23%	87%			80%	87%

Fish survival was 97% in all concentrations tested. There was no 10% solution of liquid and particulate phases with fish because too few were collected. Clam survival increased to 98% in both treatment and control tanks.

Mysid and copepod tests were repeated in December 1979, when techniques were perfected. The liquid and particulate phase tests with mysids were conducted with 100% solutions even though previous results indicated no significant toxic effects. They were repeated in order to provide corroboration for similar tests with copepods conducted at the same time. Mysid survival in liquid (93%) and particulate phases (100%) was not significantly different from the controls. Liquid and particulate phase tests with copepods were also conducted with only 100% solutions. In liquid phase tests, copepod survival was 23% and significantly different than the controls (87%). This 77% mortality in 100% solutions is probably within acceptable limits, because it is much less than the mortality required to exceed the LPC (93% – derived from hypothetical "worst-case" conditions). This supposition is further supported by particulate phase results of 80% treatment survival and 87% control survival, which show no significant effect from slurries prepared from the same sediment. In the majority of bioassays copepod survival was higher in particulate phase tests than in liquid phase tests. We believe this difference is significant and it will be discussed in more detail later. In solid phase tests, mysid survival was 73% and significantly different from the controls. The statistically significant difference between treatment and control was caused, in part, because there was no mortality in control tanks.

PIERS 1, 3, 4

The Pier 1, 3, and 4 bioassays were done simultaneously to expedite the tests and minimize costs. One control was used for all three bioassays. These results are presented in Tables 7 and 8. In liquid and particulate phase tests, the survival of control copepods was 83%, control fish 97%, and control mysids 89%. In the solid phase tests survival of control mysids was 85%, control clams 96% and control worms 100%. There was no significant difference between survival of treatment and control groups in any of the tests with sediment from Piers 1, 3, and 4.

PIER 1

In liquid phase tests, treatment survival varied from 87 to 93% for mysids, 83 to 93% for fish, and 83 to 90% for copepods. In particulate phase tests, treatment survival varied between 93 and 100% for mysids, and 83 to 90% for copepods. Fish survival in particulate phase tests was 100% in all three concentrations tested. In Pier 1 solid phase tests, treatment survival was 93% for mysids, 99% for clams, and 100% for worms.

PIER 3

In liquid phase tests, treatment survival ranged between 82 and 96% for mysids, and 80 to 87% for copepods. Fish survival was 97% in all three concentrations. In particulate phase tests, survival ranged from 96 to 100% for mysids, 97 to 100% for fish, and 83 to 100% for copepods. In solid phase tests survival was 84% for mysids, 97% for clams, and 90% for worms.

Table 7. The number of mysid shrimp (*Metamysidopsis elongata*), littleneck clams (*Protothaca staminea*), and polychaete worms (*Neanthes arenaceodentata*) surviving after 10 days of exposure to sediments from Naval Piers 1, 3, and 4 (solid phase, 20 organisms started in each tank). August, 1979.

	Replicate	Control	Pier 1	Pier 3	Pier 4
M. elongata	1	17	19	19%	20
	2	16	20	15	19
	3	16	18	20	18
	4	16	20	20	20
	5	20	16	20	17
		85%	93%	94%	94%
P. staminea	1	20	20	19	20
	2	19	20	20	20
	3	20	20	18	20
	4	17	20	20	20
	5	20	19	20	20
		96%	99%	97%	100%
N. arenaceodentata	1	20	20	20	20
	2	20	20	11	20
	3	20	20	19	20
	4	20	20	20	20
	5	20	20	20	20
		100%	100%	90%	100%

Table 8. The number of mysid shrimp (*Metamysidopsis elongata*), fish (*Citharichthys stigmaeus*), and copepods (*Arcartia tonsa*) surviving after 96 hours of exposure to liquid and particulate solutions of sediments from Naval Piers 1, 3, and 4 (liquid and particulate phases, 15 shrimp, 10 fish, and 10 copepods started in each tank). August, 1979.

Species	Concentration of Test Medium	Replicate	Liquid Pier 1	Liquid Pier 3	Liquid Pier 4	Particulate Pier 1	Particulate Pier 3	Particulate Pier 4	Control
M. elongata	10%	1	14	10	15	14	15	14	14
		2	13	12	14	15	15	15	13
		3	15	15	14	13	14	13	13
			93%	82%	96%	93%	98%	93%	89%
	50%	1	14	14	15	14	14	13	
		2	15	15	13	15	15	13	
		3	10	14	15	15	14	14	
			87%	96%	96%	98%	96%	89%	
	100%	1	14	15	11	15	15	15	
		2	14	9	13	15	15	14	
		3	14	14	13	15	15	13	
			93%	84%	82%	100%	100%	93%	
C. stigmaeus	10%	1	10	10	10	10	10	10	9
		2	6	9	10	10	9	10	10
		3	9	10	10	10	10	10	10
			83%	97%	100%	100%	97%	100%	97%
	50%	1	9	10	9	10	10	10	
		2	9	10	10	10	10	10	
		3	10	9	10	10	10	10	
			93%	97%	97%	100%	100%	100%	
	100%	1	9	10	10	10	10	10	
		2	10	9	10	10	10	10	
		3	9	10	10	10	10	10	
			93%	97%	100%	100%	100%	100%	
A. tonsa	10%	1	10	9	9	10	8	8	9
		2	8	6	10	8	9	9	8
		3	9	9	9	9	8	8	8
			90%	80%	93%	90%	83%	83%	83%
	50%	1	9	9	6	8	9	10	
		2	9	6	7	9	9	10	
		3	8	9	10	9	9	10	
			87%	80%	77%	87%	90%	100%	
	100%	1	8	9	9	8	10	10	
		2	8	9	5	8	6	9	
		3	9	8	9	9	9	10	
			83%	87%	77%	83%	83%	97%	

PIER 4

In liquid phase tests, survival in treatment tanks ranged from 82 to 96% for mysids, 97 to 100% for fish, and 77 to 93% for copepods. In particulate phase tests, survival ranged from 89 to 93% for mysids and 83 to 100% for copepods. Fish survival was 100% at all three concentrations of Pier 4 particulate phase water. In solid phase tests, survival in the treatment tanks was 94% for mysids, 100% for clams, and 100% for worms.

PIERS 5, 6, 8

The bioassays for Piers 5, 6, and 8 were also conducted simultaneously. One set of controls was used for the liquid, particulate, and solid phase tests. These results are presented in Tables 9 and 10. In liquid and particulate phase tests, survival of control mysids was 97%, control fish 100%, and control copepods 80%. In solid phase tests, the survival of control mysids was 88%, control clams 99% and control worms 97%.

Table 9. The number of mysid shrimp (*Metamysidopsis elongata*), littleneck clams (*Protothaca staminea*), and polychaete worms (*Neanthes arenaceodentata*) surviving after 10 days of exposure to sediments from Naval Piers 5, 6 and 8 (solid phase, 20 organisms started in each tank). September, 1979.

	Replicate	Control	Pier 5	Pier 6	Pier 8
M. elongata	1	18	17	20	14
	2	16	20	20	20
	3	19	19	15	19
	4	19	19	20	20
	5	16	20	20	20
		88%	95%	95%	93%
P. staminea	1	20	19	20	20
	2	20	19	20	20
	3	20	20	19	18
	4	19	20	20	18
	5	20	20	19	18
		99%	98%	98%	94%
N. arenaceodentata	1	19	20	18	20
	2	19	19	19	19
	3	19	20	20	20
	4	20	20	19	20
	5	20	20	20	20
		97%	99%	96%	99%

Table 10. The number of mysid shrimp (*Metamysidopsis elongata*), fish (*Citharichthys stigmaeus*), and copepods (*Acartia tonsa*) surviving after 96 hours of exposure to liquid and particulate solutions of sediments from Naval Piers 5, 6, and 8 (liquid and particulate phases, 10 organisms started in each tank). September, 1979.

	Concentration of Test Medium	Replicate	Liquid Pier 5	Liquid Pier 6	Liquid Pier 8	Particulate Pier 5	Particulate Pier 6	Particulate Pier 8	Control
M. elongata	10%	1	9	10	7	10	10	10	9
		2	10	10	10	9	10	10	10
		3	10	9	10	10	9	10	10
			97%	97%	90%	97%	97%	100%	97%
	50%	1	10	8	10	10	10	10	
		2	10	10	9	10	10	10	
		3	10	9	8	10	8	10	
			100%	90%	90%	100%	93%	100%	
	100%	1	8	9	10	10	10	10	
		2	9	10	10	10	10	9	
		3	10	9	10	10	10	10	
			90%	93%	100%	100%	100%	97%	
C. stigmaeus	10%	1	10	9	10	10	10	9	10
		2	10	10	10	10	10	10	10
		3	10	10	10	10	10	10	10
			100%	97%	100%	100%	100%	97%	100%
	50%	1	10	10	10	10	10	10	
		2	10	10	9	10	10	10	
		3	9	10	10	10	10	10	
			97%	100%	97%	100%	100%	100%	
	100%	1	10	10	10	10	7	10	
		2	10	10	10	10	10	10	
		3	10	10	10	9	10	10	
			100%	100%	100%	97%	90%	100%	
A. tonsa	10%	1	8	8	5	10	5	6	8
		2	8	8	7	9	6	7	8
		3	9	7	7	9	8	5	8
			83%	77%	63%	93%	63%	60%	80%
	50%	1	7	7	5	7	3	6	
		2	8	7	7	9	4	5	
		3	4	10	5	7	7	9	
			63%	80%	57%	77%	47%	67%	
	100%	1	3	4	4	5	5	4	
		2	8	6	5	8	5	5	
		3	2	7	7	9	8	6	
			43%	57%	53%	73%	70%	50%	

PIER 5

There was no significant difference between survival of treatment and control groups in any of the tests. In liquid phase tests, treatment survival ranged from 90 to 100% for mysids and 97 to 100% for fish. Copepod survival varied from 43 to 83%. Survival was low but not significantly different from that in control tanks due to the high variance among the replicates. Since survival was low, we calculated the copepod LC 50 for Pier 5 to be 73%. The corresponding application limit (0.41%) is greater than the liquid phase LPC (0.1%). In particulate phase tests survival varied from 97 to 100% for mysids, 97 to 100% for fish, and 73 to 93% for copepods. Again, copepod survival was much higher in the particulate phase tests than in the liquid phase tests. In solid phase tests the survival in treatment tank was 95% for mysids, 98% for clams, and 99% for worms.

PIER 6

There was no significant difference between survival in treatment and control tanks with any species except copepods in the liquid phase. In liquid phase tests, survival varied from 90 to 97% for mysids and 97 to 100% for fish. Copepod survival varied from 57 to 90%. Copepod survival at 100% liquid phase was significantly different from control survival. These mortalities are within acceptable ranges when the LPC is compared to the application limit. The Pier 6 LC 50 for copepods in the liquid phase is 360%. The corresponding application limit is 1.16%, which is greater than the liquid phase LPC of 0.1%. In particulate phase tests, survival varied from 93 to 100% for mysids, 90 to 100% for fish, and 47 to 70% for copepods. Copepod survival was lowest in the 50% treatment and highest in the 100% treatment. This indicates survival of copepods was not related to dose of treatment solutions.

PIER 8

There was no significant difference between survival in treatment and control tanks with any species except copepods in the liquid and particulate phases. In liquid phase tests, survival varied from 90 to 100% for mysids, and 97 to 100% for fish. Copepod survival varied from 53 to 63% and was significantly different from control survival. Again, these mortalities are within acceptable limits when the application limit is compared to the LPC. The Pier 8 LC 50 for copepods in the liquid phase is 180%. The corresponding application limit of 0.15% is greater than the liquid phase LPC of 0.1%. In particulate phase tests, survival varied from 97 to 100% for mysids and 97 to 100% for fish. Copepod survival varied from 50 to 67%, and the highest concentration tested had significantly greater mortalities than the control. As in the liquid phase test, these copepod mortalities are not considered significant. The Pier 8 LC 50 for copepods is 215% in the particulate phase. The corresponding application limit is 0.28%, which is much greater than the particulate phase LPC of 0.04%. In solid phase tests, survival was 93% for mysids, 94% for clams, and 99% for worms.

PIERS 10, 11, 12

The bioassays for Piers 10, 11, and 12 were also done simultaneously. One set of controls was used for the liquid, particulate, and solid phase tests. These results are presented in Tables 11 and 12. In liquid and particulate phase tests, the survival of control mysids was

Table 11. The number of mysid shrimp (*Metamysidopsis elongata*), littleneck clams (*Protothaca staminea*), and polychaete worms (*Neanthes arenaceodentata*) surviving after 10 days of exposure to sediments from Naval Piers 10, 11 and 12 (solid phase, 20 organisms started in each tank). November, 1979.

	Replicate	Control	Pier 10	Pier 11	Pier 12
M. elongata	1	17	18	12	20
	2	20	14	13	20
	3	13	15	15	20
	4	20	14	12	16
	5	18	11	18	19
		88%	72%	70%	95%
P. staminea	1	19	19	19	20
	2	19	20	19	19
	3	19	19	20	20
	4	19	20	20	20
	5	20	20	19	20
		96%	98%	97%	99%
N. arenaceodentata	1	19	19	18	20
	2	20	15	15	17
	3	20	19	19	20
	4	20	20	20	20
	5	19	15	20	15
		98%	88%	92%	92%

77%, control fish 90% and control copepods 67%. In solid phase tests, survival of control mysids was 88%, control clams 96% and control worms 98%.

PIER 10

There was no significant difference between survival in treatment and control tanks with any species except mysids in the solid phase. In liquid phase tests, survival varied from 70 to 77% for mysids, 90 to 97% for fish, and 50 to 60% for copepods. In particulate phase tests, survival varied from 83 to 97% for mysids, 89 to 100% for fish, and 63 to 77% for copepods. In solid phase tests, mysid survival was 72%, and the difference between treatment and control was statistically significant. Treatment survival was 98% for clams and 88% for worms.

PIER 11

There was no significant difference between survival in treatment and control tanks with any species except mysids in the solid phase. In liquid phase tests, survival varied from 47 to 70% for mysids, 93 to 97% for fish, and 50 to 70% for copepods. In particulate phase

Table 12. The number of mysid shrimp (*Metamysidopsis elongata*), fish (*Citharichthys stigmaeus*), and copepods (*Acartia tonsa*) surviving after 96 hours of exposure to liquid and particulate solutions of sediments from Naval Piers 10, 11, and 12 (liquid and particulate phases, 10 organisms started in each tank). November, 1979.

Species	Concentration of Test Medium	Replicate	Liquid Pier 10	Liquid Pier 11	Liquid Pier 12	Particulate Pier 10	Particulate Pier 11	Particulate Pier 12	Control
M. elongata	10%	1	9	6	6	7	9	6	7
		2	8	6	6	9	10	9	8
		3	4	2	7	9	8	10	8
			70%	47%	63%	83%	90%	83%	77%
	50%	1	7	7	5	10	9	9	
		2	9	8	7	9	10	8	
		3	7	9	8	10	10	9	
			77%	80%	67%	97%	97%	87%	
	100%	1	7	4	6	9	10	9	
		2	8	7	8	10	9	10	
		3	8	10	8	8	10	10	
			77%	70%	73%	90%	97%	97%	
C. stigmaeus	10%	1	10	9	8	9	10	10	8
		2	10	9	10	10	10	9	9
		3	9	10	8	10	10	10	10
			97%	93%	87%	97%	100%	97%	90%
	50%	1	10	9	10	10	10	9	
		2	9	9	10	9	10	8	
		3	8	10	10	9	9	9	
			90%	93%	100%	93%	97%	87%	
	100%	1	10	10	10	7	10	7	
		2	10	9	10	10	10	10	
		3	8	10	10	9	9	8	
			93%	97%	100%	87%	97%	83%	
A. tonsa	10%	1	4	8	6	5	5	7	6
		2	6	5	6	7	5	8	7
		3	8	8	4	7	6	8	7
			60%	70%	53%	63%	53%	77%	67%
	50%	1	7	7	4	6	5	6	
		2	5	3	5	6	7	7	
		3	6	5	6	8	6	4	
			60%	50%	50%	67%	60%	57%	
	100%	1	2	4	1	8	3	6	
		2	6	6	1	7	6	7	
		3	7	7	3	8	6	4	
			50%	57%	17%	77%	50%	57%	

tests, survival ranged between 90 and 97% for mysids, 97 and 100% for fish, and 50 to 60% for copepods. In solid phase tests, mysid survival was 70% and significantly different from control survival. Treatment survival was 97% for clams and 92% for worms.

PIER 12

There was no significant difference between survival in treatment and control tanks with any species except copepods in the liquid phase. In liquid phase tests, survival varied from 23 to 73% for mysids and 87 to 100% for fish. Copepod survival varied from 17 to 53%. There was a statistically significant difference between control and treatment survival at the highest concentration tested. The Pier 12 LC 50 for copepods in the liquid phase could not be calculated because the data were too heterogeneous to plot a straight line (dose vs. mortality). However, copepod mortality was well below the hypothetical "worst case" mortality required to exceed the liquid phase LPC (see Discussion). In particulate phase tests, survival varied from 33 to 97% for mysids, 82 to 97% for fish, and 57 to 77% for copepods. Again, copepod survival was much higher in the particulate phase tests than in liquid phase tests. In solid phase tests, survival was 95% for mysids, 99% for clams, and 92% for worms.

PIERS 13, JK, CHOLLAS CREEK

The bioassays for Piers 13, JK, and the Chollas Creek Channel were done simultaneously. One set of controls was used for liquid, particulate, and solid phase tests. These results are presented in Tables 13 and 14. In liquid and particulate phases the survival of control mysids was 93%, control fish 100%, and control copepods 87%. In solid phase tests, the survival of control mysids was 100%, control clams 98%, and control worms 92%.

PIER 13

There was no significant difference between survival in treatment and control tanks with any species except copepods in liquid and particulate phase tests. In liquid phase tests survival varied from 93 to 97% for mysids and 97 to 100% for fish. Copepod survival varied from 33 to 73% and the highest concentration tested was significantly different from control survival. These mortalities are within acceptable ranges when the LPC is compared to the application limit. The Pier 13 LC 50 for copepods in the liquid phase is 34.5%. The corresponding application limit is 0.23%, which is greater than the liquid phase LPC of 0.1%. In particulate phase tests, survival varied from 93 to 100% for mysids and 97 to 100% for fish.

Copepod survival ranged between 20 and 73% and was significantly different at the highest concentration tested. The LC 50 for Pier 13 particulate phase tests with copepods could not be calculated because the data were too heterogeneous. Since a statistically valid line could not be drawn between the three points, we constructed three lines using two points per line to bracket all possible combinations of variability. The resulting LC 50's for copepods ranged between 16.5 and 66% in the particulate phase. The corresponding application limits varied between 0.08 and 0.55% and were greater than the particulate phase LPC of 0.04%. These results suggest that the sediment would not be significantly toxic to copepods during disposal. In Pier 13 solid phase tests, survival was 100% for mysids, 98% for clams, and 95% for worms.

Table 13. The number of mysid shrimp (*Metamysidopsis elongata*), littleneck clams (*Protothaca staminea*), and polychaete worms (*Neanthes arenaceodentata*) surviving after 10 days of exposure to sediments from Naval Piers 13, JK and Chollas Creek Channel (solid phase, 20 organisms started in each tank). December, 1979.

	Replicate	Control	Pier 13	Pier JK	CC Channel
M. elongata	1	20	20	18	20
	2	20	20	20	20
	3	20	20	20	16
	4	20	20	20	20
	5	20	20	18	20
		100%	100%	96%	96%
P. staminea	1	20	18	20	20
	2	20	20	20	20
	3	20	20	20	20
	4	19	20	19	20
	5	19	20	20	19
		98%	98%	99%	99%
N. arenaceodentata	1	15	16	17	20
	2	17	20	16	18
	3	20	20	18	20
	4	20	19	18	20
	5	20	20	17	20
		92%	95%	86%	98%

PIER JK

There was no significant difference between survival of treatment and control groups in any of these tests. In liquid phase tests, survival varied from 97 to 100% for mysids, 97 to 100% for fish, and 60 to 77% for copepods. In particulate phase tests, survival ranged from 97 to 100% for mysids and 53 to 80% for copepods. Fish survival in particulate phase tests was 100% at all three concentrations tested. In solid phase tests, treatment survival was 96% for mysids, 99% for clams, and 86% for worms.

CHOLLAS CREEK CHANNEL

There was no significant difference between survival in treatment and control tanks with any species except copepods in the liquid phase. In liquid phase tests survival varied from 90 to 100% for mysids. Fish survival was 100% in all concentrations tested. Copepod survival ranged between 17 and 43%. The LC 50 for Chollas Creek liquid phase tests with copepods is 9.9%. The corresponding application limit is 0.04%. In this case, the 4-hour LPC (0.1%) was greater than the 96-hour application limit. However the application limit

Table 14. The number of mysid shrimp (*Metamysidopsis elongata*), fish (*Citharichthys stigmaeus*), and copepods (*Acartia tonsa*) surviving after 96 hours of exposure to liquid and particulate solutions of sediments from Naval Piers 13, JK, and Chollas Creek Channel (liquid and particulate phases, 10 organisms started in each tank). December, 1979.

	Concentration of Test Medium	Replicate	Liquid Pier 13	Liquid Pier JK	Liquid CC Channel	Particulate Pier 13	Particulate Pier JK	Particulate CC Channel	Control
M. elongata	10%	1	10	10	10	10	9	10	9
		2	10	9	10	10	10	10	9
		3	9	10	10	10	10	10	10
		Total	97%	97%	100%	100%	97%	100%	93%
	50%	1	10	10	10	10	10	10	
		2	9	10	8	9	10	10	
		3	9	10	9	10	10	10	
		Total	93%	100%	90%	97%	100%	100%	
	100%	1	10	10	10	9	10	9	
		2	9	10	9	9	10	10	
		3	10	9	9	10	10	10	
		Total	97%	97%	93%	93%	100%	97%	
C. stigmaeus	10%	1	10	10	10	10	10	9	10
		2	10	9	10	10	10	10	10
		3	10	10	10	10	10	10	10
		Total	100%	97%	100%	100%	100%	97%	100%
	50%	1	10	10	10	10	10	10	
		2	10	10	10	9	10	10	
		3	9	10	10	10	10	10	
		Total	97%	100%	100%	97%	100%	100%	
	100%	1	10	10	10	10	10	10	
		2	10	10	10	10	10	10	
		3	10	10	10	10	10	10	
		Total	100%	100%	100%	100%	100%	100%	
A. tonsa	10%	1	6	8	5	8	6	8	10
		2	8	7	3	7	2	8	8
		3	8	8	5	3	8	8	8
		Total	73%	77%	43%	60%	53%	80%	87%
	50%	1	5	5	1	8	8	5	
		2	3	7	6	8	8	6	
		3	5	6	4	6	8	6	
		Total	43%	60%	37%	73%	80%	57%	
	100%	1	6	3	3	3	5	6	
		2	3	8	1	1	8	9	
		3	1	7	1	2	9	5	
		Total	33%	60%	17%	20%	73%	66%	

was calculated for a dilution period 24 times longer than the dilution period for the LPC. If the extended dilution period is taken into account, the LPC would decrease tremendously, and the LPC would be less than the application limit (see Discussion). In Chollas Creek particulate phase tests, survival varied between 97 and 100% for mysids, 97 and 100% for fish and 57 and 80% for copepods. In solid phase tests, survival was 96% for mysids, 99% for clams, and 98% for worms.

SEDIMENT ANALYSIS

The concentrations of cadmium, chromium, copper and mercury in sediments used for these bioassays are presented in Table 15, and the values used to determine the "worst site" are presented in Table 2. Comparing these two tables reveals some disparities between preliminary core sample concentrations and concentrations in sediment used in the bioassays. There are several reasons for this difference. Among these are differences in analysis by X ray fluorescence versus atomic absorption and the inability to return to precisely the same sampling location. Also there was variability in sediment heavy metal concentrations caused by small spatial variations in sampling. However, there is no particular pattern to this disparity between core sample concentrations and test sediment concentrations. It is clear that treatment sediments were very high and reference sediments very low in all metals used as indices. But some of the values seem unrealistically high, particularly the mercury concentrations determined by Environmental Engineering Laboratory. Even some of our own values for the other metals were much higher than expected. This justifies our selection procedure to locate the most contaminated sites.

Table 15. The concentration (ppm) of cadmium, chromium, copper and mercury in sediments. Controls are included for comparison.

Pier	Cadmium	Chromium	Copper	Mercury
1	11.8	131.2	243.1	0.7
3	14.5	147.1	261.5	18.5
4	22.7	175.0	638.5	0.8
Control	2.0	26.9	15.5	0.0
5	9.6	299.5	995.0	254.4
6	5.0	127.7	464.6	44.0
8	8.0	205.2	715.7	165.1
Control	2.4	52.8	8.2	9.5
10	22.9	124.2	282.4	66.5
11	32.5	143.6	373.0	48.0
12	11.2	84.1	282.1	44.6
Control	8.8	27.7	17.8	5.4
13	28.0	254.8	312.3	58.2
JK	7.5	119.9	178.8	13.1
Chollas Creek	5.0	68.9	80.4	17.8
2	5.2	42.4	123.8	18.5
Control	2.1	79.7	24.7	10.7

BIOACCUMULATION

Tissue concentrations of cadmium, chromium, copper, and mercury from clams used in solid phase tests (10 days) are presented in Table 16. In only 3 of 52 comparisons was there a significant difference between treatment and control tissue concentrations. The concentration of chromium in clams exposed to sediments from Piers 11 and 12 was 4.3 and 5.4 ppm; this was significantly higher than the concentration of chromium in clams exposed to reference sediment (3.5 ppm). The tissue concentration of mercury from clams in Pier 8 sediment was 0.6 ppm and significantly greater than the tissue concentration of mercury from clams in reference sediment (0.4 ppm). The *Macoma nasuta* we used in the Pier 2 bioassays did not provide sufficient tissues, therefore no statistical comparison could be made because the tissues were pooled for analysis.

Table 16. The mean concentration (ppm) of cadmium, chromium, copper and mercury in the tissues of test clams (*Protothaca staminea*). Each value represents five samples. For Pier 2 each value represents one pooled sample of *Macoma nasuta* tissue.

Pier	Cadmium	Chromium	Copper	Mercury
1	4.6	1.1	7.1	0.2
3	3.9	2.5	8.8	0.2
4	4.3	4.9	8.9	0.1
Control	4.4	4.7	7.5	0.2
5	7.5	5.5	18.3	0.4
6	7.1	5.8	21.1	0.5
8	7.9	4.5	17.0	0.6
Control	6.7	10.7	15.7	0.4
10	4.2	4.3	9.0	0.4
11	4.8	4.3	9.4	0.3
12	6.0	5.4	13.7	0.2
Control	7.9	3.5	9.1	0.2
13	14.2	5.1	17.3	0.5
JK	14.0	5.9	24.6	0.7
Chollas Creek	15.7	4.1	18.8	0.6
Control	13.9	4.0	16.7	0.4
2	0.6	1.1	30.5	0.2
Control	0.5	1.8	26.5	0.3

STATISTICAL ANALYSES

The following tables summarize the results of statistical analyses and other procedural calculations outlined in the manual for assessing environmental impact. Table 17 gives the statistical values used to assess the potential for bioaccumulation of pollutants. Tables 18, 19, and 20 give the statistical values used to identify significant mortality in solid, liquid, and particulate phase tests, respectively. If a significant difference was detected, an LC 50 was calculated and 0.01 of its lower confidence limit compared to the limiting permissible concentration (LPC). These values are presented in Tables 21 and 22.

Table 17. Statistical values for determining bioaccumulation potential.

COMPARISON	COCHRAN's TEST		ANOVA (F) or KRUSKAL-WALLIS (H)		NEWMAN-KEULS or DUNNETT's TEST	
	C	C (.05)	F or H	F or H (.05)	q′	q (.05)
Chromium						
C vs P 1,3,4			Not significant			
C vs P 5,6,8			Not significant			
C vs P 10,11,12	0.82*	0.63	H=12.49*	7.82		
C vs P 10					1.45	1.92
C vs P 11					3.03*	1.64
C vs P 12					2.65*	2.06
C vs P 13, JK,CC	0.52	0.63	F=1.32	3.24		
Cadmium						
C vs P 1,3,4			Not significant			
C vs P 5,6,8			Not significant			
C vs P 10,11,12			Not significant			
C vs P 13, JK,CC			Not significant			
Copper						
C vs P 1,3,4	0.509	0.63	F=2.75	3.24		
C vs P 5,6,8	0.42	0.63	F=2.19	3.24		
C vs P 10,11,12	0.35	0.63	F=1.26	3.24		
C vs P 13, JK,CC	0.46	0.63	F=2.91	3.24		
Mercury						
C vs P 1,3,4			Not significant			
C vs P 5,6,8	0.35	0.63	F=4.71*	3.24		
C vs P 5					0.82	2.95
C vs P 6					2.04	2.95
C vs P 8					4.08*	3.58
C vs P 10,11,12	0.642*	0.63	Not significant			
C vs P 13, JK,CC	0.642*	0.63	H=7.43	7.81		

C: Control

P: Pier

*: Statistically significant difference

Not significant: determined by inspection, no calculations made

Table 18. Statistical values for determining significant mortality in solid phase tests.

COMPARISON	KRUSKAL-WALLIS H_c	$H_{(.05)}$	DUNNETT's TEST		$q'_{(.05)(1),\infty,P}$
Metamysidopsis elongata					
C vs P 1,3,4	3.01	7.82			
C vs P 5,6,8	4.34	7.82			
C vs P 10,11,12	8.90*	7.82	P 10	3.14*	1.64
C vs P 10			P 11	2.37*	1.92
C vs P 13, JK,CC	1.47	7.82			
Protothaca staminea					
C vs All Piers	Survival was not significantly different				
Neanthes arenaceodentata					
C vs P 1,3,4,5,6,8	Survival was not significantly different				
C vs P 10,11,12	1.01	7.82			
C vs P 13, JK,CC	6.07	7.82			

Pier 2

COMPARISON	DATE	MANN-WHITNEY U, U'	$U_{.05}$
C vs *M. elongata*	4/78	0,25*	21
	12/79	0,25*	21
C vs *N. arenaceodentata*		9,16	21
C vs *M. nasuta*		Not significant	

C: Control

P: Pier

*: Statistically significant difference

Not significant: determined by inspection, no calculations made

Table 19. Statistical Values for determining significant mortality in liquid phase tests.

COMPARISON	KRUSKAL-WALLIS H_c	KRUSKAL-WALLIS $H_{(.05)}$	DUNNETT's TEST q'	DUNNETT's TEST $q_{(.05)(1),\infty,P}$
Metamysidopsis elongata				
C vs All Piers (100%)	< 3.91	7.82		
Citharichthys stigmaeus				
C vs All Piers (100%)	< 3.33	7.82		
Acartia tonsa				
C vs P 1,3,4 (100%)	0.65	7.82		
C vs P 5,6,8 (100%)	5.31	7.82		
C vs P 10,11,12 (100%)	6.42	7.82		
C vs P 13, JK,CC (100%)	8.27*	7.82		
C vs P 13			2.68*	1.92
C vs P JK			1.01	1.64
C vs CC			5.02*	2.06

Pier 2

COMPARISON	DATE	MANN-WHITNEY U, U′	$U_{(.05)}$
C vs *M. elongata*	4/78	Not significant	
C vs *C. stigmaeus*	4/78	Not significant	
C vs *A. tonsa*	4/78	3,3	9
C vs *M. elongata*	12/79	Not significant	
C vs *A. tonsa*	12/79	Not significant	

C: Control

P: Pier

*: Statistically significant difference

Not significant: determined by inspection, no calculations made

Table 20. Statistical values for determining significant mortality in particulate phase tests.

COMPARISON	KRUSKALL-WALLIS H_c	$H_{(.05)}$
Metamysidopsis elongata		
C vs All Piers (100%)	<6.85	7.82
Citarichthys stigmaeus		
C vs All Piers (100%)	<1.87	7.82
Acartia tonsa		
C vs P 1,3,4 (100%)	6.15	7.82
C vs P 5,6,8 (100%)	4.82	7.82
C vs P 10,11,12 (100%)	7.12	7.82
C vs P 13, JK,CC (100%)	7.01	7.82

Pier 2

COMPARISON	DATE	MANN-WHITNEY U, U'
C vs *M. elongata*	4/78	Not significant
C vs *C. stigmaeus*	4/78	Not significant
C vs *A. tonsa*	4/78	Not significant
C vs *M. elongata*	12/79	Not significant
C vs *A. tonsa*	12/79	Not significant

C: Control

P: Pier

Not significant: determined by inspection, no calculations made

TABLE 21. Liquid phase LC 50's and LPC's for liquid phase tests with significant differences in mortality.

PIER	ORGANISM	LC 50	CONTRIBUTION TO χ^2	UPPER CONFIDENCE LIMIT	LOWER CONFIDENCE LIMIT	0.01X LOWER C. L.	LIQUID PHASE LPC
5	Copepods	73	0.39	131.4	40.6	0.41	0.1
6	Copepods	360	2.59	1,120.0	116.0	1.16	0.1
8	Copepods	180	0.00	1,972.0	16.0	0.16	0.1
12	Copepods	24	5.4*	--	-	-	-
13	Copepods	34.5	0.15	51.1	23.3	0.23	0.1
CC	Copepods	9.9	1.71	22.2	4.4	0.04	0.1

TABLE 22. Particulate phase LC 50's and LPC's for particulate phase tests with significant differences in mortality.

PIER	ORGANISM	LC 50	CONTRIBUTION TO χ^2	UPPER CONFIDENCE LIMIT	LOWER CONFIDENCE LIMIT	0.01X LOWER C. L.	LIQUID PHASE LPC
8	Copepods	215	1.74	1651	27.9	0.28	0.04
13	Copepods	27	13.2*				

* This chi square value indicates that data are too heterogeneous to plot a statistically valid line of dose versus mortality (see text).

DISCUSSION

The bioassay results will be discussed in order of completion, beginning with Pier 2. Pier 2 is a special case because it was conducted separately, different species were used, and the tests had to be repeated. Subsequent tests were conducted in sets of three and will be discussed within that format.

PIER 2

In the initial liquid and particulate phase tests (March 1978 with *Metamysidopsis elongata*) mysid survival was too low to be analyzed. In April 1978 both phases were repeated with *Acanthomysis macropsis*. Mysid survival was 90% in the control and there was no difference between treatment and control survival. In conjunction with copepod tests, additional mysid tests were conducted with *M. elongata* (December 1979) using only undiluted solutions (100%). Again, mysid survival was very high in the treatments and not significantly different from the controls. These results show that Pier 2 liquid and particulate phase solutions were not toxic to the mysids we tested.

The difference in mysid survival between the March and April bioassays was probably due to a difference in handling rather than species sensitivity. In the March test, mysids were separated from collected plankton and held overnight. The next day the animals were transported to our laboratory, counted, and distributed to the test containers. This required two handling steps and an unnecessarily long period under crowded conditions. Additionally, mysids were fed in the April but not the March bioassay. The December 1979 mysid tests confirmed that the difference in survival was not due to a difference in species sensitivity (*M. elongata* vs. *A. macropsis*).

In liquid and particulate phase tests with fish during March, there was no difference in survival between control and treatment tanks, but these data are questionable because of the low control survival (50%). In April tests, not enough fish were collected and the 10% concentration was omitted. However, the high survival (97 to 100% in 50 and 100% solutions) of *C. stigmaeus* demonstrates that liquid and particulate phase solutions of sediment from Pier 2 are not toxic to the fish we tested.

In the March and April liquid and particulate phase tests, copepod survival was too low to be analyzed. It was surprising that survival decreased even though our techniques improved. After the April bioassay we changed suppliers and survival improved dramatically. We attribute the increased survival to healthier animals. When tests were repeated in December, not enough copepods were collected, and only three replicates at the highest concentration (100%) were prepared. In liquid phase tests the difference in copepod survival between treatment and control tanks was significant. In the particulate phase there was no difference in copepod survival between treatments and controls.

We believe low survival in liquid phase tests and high survival in particulate phase tests can partly be attributed to nutrients. Unfiltered particulate phase water probably contained more nutrients than the filtered liquid phase water. It is likely that the high mortality in the liquid phase tests was caused by a combination of starvation and some other unmeasured factor. Starvation alone cannot explain the high mortality because control mortality was relatively low even though the control water was filtered and presumably contained no nutrients. Other researchers conducting sediment bioassays have observed the same low survival in the liquid phase and high survival in the particulate phase. Some have attributed

this difference to competition between organisms and particles for available toxic contaminants in the particulate phase.* Lamb and Tollefson (1973) found that toxicity of metal ions to bacteria is inversely proportional to the concentration of suspended solids. Further, Lewis et al. (1971, 1972, 1973) and Whitfield and Lewis (1976) demonstrated that marine sediments and other naturally occurring organic material in seawater could reduce the toxicity of metal ions to copepods. The three processes that can reduce the availability of heavy metal ions in solution are: ion exchange, chemical binding, or physical adsorption.

For Pier 2, even though copepod survival at 100% liquid phase was low, survival was high enough to suggest that any mortality was within allowable limits. In liquid phase tests, copepod mortality in undiluted solutions was 77%, which is much less than the mortality required to exceed the LPC (93%, derived from hypothetical "worst-case conditions"). This suggests that the sediment concentration at the dump site would be within allowable limits. This supposition is further supported by particulate phase results, which show no significant effect from the slurries prepared with the same sediment.

In the March solid phase bioassay, *Macoma nasuta* survival was low but there was no significant difference between treatment and control. With improved handling techniques in the April bioassay, survival was very high in both treatment and control tanks, and the difference was not significant. Worm survival in the March bioassay was high in both treatments and controls, and they were not significantly different.

In Pier 2 solid phase tests with mysids, low survival was attributed to poor technique (March) and starvation (April). Although minimal feeding every other day was enough to sustain the mysids in 4-day liquid and particulate phase tests, mysids in 10-day solid phase tests were probably starving. Techniques were improved by counting and sorting in one handling step as with liquid and particulate phase tests. In December solid phase tests, mysids were fed every day and control survival increased to 100% although treatment survival was only 73%. This difference in survival was statistically significant. These data show that Pier 2 sediment is toxic to *M. elongata*. However, mysids and fish in the liquid and particulate phases and clams and worms in the solid phase did not exhibit significant mortalities. Copepod survival in Pier 2 liquid phase was significantly different from the control, but survival in the particulate phase was not significantly different. The mysids appear to be a reliable test animal, but solid phase results need more interpretation. We believe the statistically significant difference could be due to chance because control survival was so high. An invalid hypothesis is accepted one time out of 20 at the 95% confidence level.

It seems improbable that mysids in the solid phase were killed by the toxicity associated with Pier 2 sediments, although toxicity cannot be dismissed. However, *M. elongata* is a sensitive test animal and the test was a long one. Another possibility is that there was an isolated, extraneous substance in this particular sediment sample that was toxic and not characteristic of the surrounding sediment. However, this is not reflected in the total concentration of heavy metals we tested (Cd, Cr, Cu, Hg). Only Chollas Creek Channel had a lower total concentration of heavy metals than Pier 2. In almost all other tests, mysid survival was greater than Pier 2 even though total sediment metal concentration was higher. In view of the low concentration of heavy metals and the lack of significant effects observed in any other species (except copepods, which appear to be unreliable test animals), we believe that the observed mortalities were not caused by toxic substances associated with Pier 2 sediments.

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*Personal communication from Richard Peddicord, US Army Corps of Engineers, 1980.

PIERS 1, 3, 4

The bioassays on Piers 1, 3, and 4 were conducted in July 1979. This was the first test series where three piers were done simultaneously, mysids were fed on a daily basis, and *P. staminea* was the test clam. Mysid survival in liquid phase tests was high and there was no difference between treatments and controls at the highest concentration tested. Mysid survival was also high in the particulate phase tests, and Pier 4 treatment (100%) was not statistically different from the control. Mysid survival in the Pier 1 and Pier 3 particulate phase treatments (100%) was significantly higher than the control and no further analysis was necessary.

In Pier 1, 3, and 4 liquid and particulate phase tests, the survival of fish and copepods in treatment and control tanks was not significantly different. In solid phase tests, control and treatment survival was not significantly different for any of the organisms tested. These data show conclusively that sediments from Piers 1, 3, and 4 were not toxic to any of the species in liquid, particulate, or solid phase tests.

PIERS 5, 6, 8

The bioassays for Piers 5, 6, and 8 were conducted in September 1979. In liquid and particulate phase tests, mysid and fish survival in treatment tanks was not significantly lower than survival in control tanks. In solid phase tests, the survival of mysids, clams, and worms was not significantly different from survival in their respective controls. Copepod survival was not significantly different between treatment and control tests in the liquid phase for Pier 5 and in the particulate phase for Piers 5 and 6. Pier 5 survival was not significantly different because of the high variance among replicates even though overall survival was low (43%). Copepod survival in treatment tanks was significantly lower than in control tanks in the liquid phase for Piers 6 and 8 and in the particulate phase for Pier 8. But the application limits were not lower than the corresponding LPC's. This indicates that the dredged sediments would not be significantly toxic to copepods. Additionally, the results with the other species show that dredged materials from Piers 5, 6, and 8 would not have an adverse environmental impact during disposal operations. Clams in Pier 8 did show a significant bioaccumulation of mercury compared with controls, and the sediment values were also quite high. However, this statistically significant difference was due, in part, to the small variability among replicate samples. The absolute difference was small (0.6 vs. 0.4 ppm). Further Pier 5 sediments were actually higher in mercury, and clams showed no significant bioaccumulation.

PIERS 10, 11, 12

The bioassays for Piers 10, 11, and 12 were conducted in November, 1979. The survival of treatment mysids and fish in liquid and particulate phase tests and clams and worms in the solid phase tests was not significantly lower than survival in their respective controls. Mysid survival in Piers 11 and 12 particulate phase treatments was significantly higher than control survival and no further analysis was necessary. Copepod survival in treatments was not significantly different from controls for Piers 10 and 11 in the liquid phase and Piers 10, 11, and 12 in the particulate phase. Copepod survival in Pier 12 liquid phase tests and mysid survival in Pier 10 and 11 solid phase tests was significantly lower than their respective controls.

The survival of copepods in control tanks for liquid and particulate phase tests was relatively low (67%). Although the manual suggests that 80% be used as the minimum survival for analyzing the results, we believe this arbitrary figure is not entirely justified and that our results are acceptable. This is particularly true with copepods, where control survival was highly variable even after our techniques had improved. We firmly believe that much of this variability can be attributed to inherent differences in field-caught animals from different environmental conditions. Arnott and Ahsanullah (1979) have reported the difficulties in maintaining field-caught copepods and reduced their acute toxicity tests from 96 to 24 hours for this reason. Sosnowski et al. (1979) found significant differences in the response to copper among six field populations of *A. tonsa*. The variable mortality in field-caught populations reflected the biological condition of the animals and did not result from test conditions since they were held constant. Considering these factors, it is not unreasonable to compare relatively low control survival with treatment survival for Piers 10, 11, and 12.

Copepod survival in Pier 12 liquid phase tests was only 17% and was significantly lower than control survival. The LC 50 could not be calculated because the data were too heterogeneous to plot a statistically valid line of dose versus mortality (using a chi square "goodness of fit"). However, these results do not prove that Pier 12 liquid phase solutions caused the observed mortality. The copepod mortality in 10, 50, and 100% liquid phase tests was 47, 50, and 83%, which is well below hypothetical "worst-case" mortality of 33, 83, and 93% required to exceed the liquid phase LPC (see the discussion under the heading Materials and Methods for derivation of "worst-case" values). The comparison between actual results and hypothetical "worst-case" values suggests that Pier 12 sediment would not have a significantly toxic effect on copepods at the dump site. Low copepod survival (17%) also supports the observation that survival in liquid phase tests is consistently lower than in particulate phase tests. The conclusion that Pier 12 sediment is not toxic to marine life is further supported by the fact that no significant differences were detected between control and treatment tests for copepods in the particulate phase, for mysids in the liquid and particulate phases, and for mysids, clams, and worms in the solid phase tests.

In Pier 10 and 11 solid phase tests with mysids, control and treatment survival was significantly different. Although it is possible that the significantly different results could be due to chance (1 chance in 20 at the 95% level of confidence), we believe these differences are real because of low variability among replicates in the Pier 10 and 11 bioassays and high survival in the Pier 12 tests (95%). The low mysid survival we observed for Piers 10 and 11 could result from three sources: 1) weak test organisms; 2) the existence of toxic contaminants associated with the sediment; 3) an isolated contaminant not necessarily an integral part of the sediment, such as a battery, tin can, metal social security card, and metal shavings (all four were found in test sediments). It is unlikely that mysids used in Pier 10 and 11 solid phase tests were weak because mysid survival was very high in control tanks and Pier 12 treatment tanks.

The existence of toxic contaminants in the sediment samples could also explain the observed mysid mortality in Pier 10 and 11 solid phase tests. A ranking of the 13 pier sites on a percentage basis of the maximum allowable concentration of heavy metals shows that Piers 5, 8, and 13 are relatively more contaminated than Piers 10 and 11. Mysid survival at the three piers with a higher total metal concentration was not significantly lower than in their controls. This indicates that the heavy metals in dredged material from Piers 10 and 11 did not cause the mysid mortalities. If sediment from Piers 10 and 11 contained significant amounts of toxic heavy metals, one would expect an increase in the concentration of these metals in clam tissues exposed to these sediments for 10 days. Pier 10 clam tissues were not

significantly higher than the control in any of the four metals tested, but it is possible that contaminants we did not measure were present in lethal concentrations. Pier 11 clam tissues were significantly higher in chromium than were control tissues, but not in cadmium, copper, or mercury. This statistically significant difference was due, in part, to the small variability between replicate samples. However, the absolute difference was small (3.5 vs. 4.3 ppm). We do not believe that 4.3 ppm in clam tissues indicates significant toxicity to mysids. Further, the chromium concentration in Pier 12 clam tissues was even higher (5.4 ppm), and yet mysid survival in Pier 12 solid phase tests was greater than survival in control tanks.

We believe the most likely explanation for low mysid survival in Pier 10 and 11 solid phase tests is the presence of isolated contaminants not associated with the other sediment we collected. This is supported by the eight other tests (liquid, particulate, and solid phases conducted with worms, copepods, clams, mysids and fish) on sediments from Piers 10 and 11, where there was no significant difference in survival between control and treatment tests. Since we believe that mysids are the most sensitive and reliable animal used in these tests, there is little doubt that high mortality can be attributed to something in the test sediment. However, since we pre-selected the most contaminated sites, the majority of dredged material should be much less toxic to mysids. Further, within this "worst site" it is possible that a single isolated spot of contamination was not representative of a "worst site" but rather a toxic volume of only a few liters. This would be insignificant when considering the total volume of dredged material.

PIERS 13, JK, CHOLLAS CREEK

The bioassays for Piers 13, JK, and Chollas Creek were completed in December 1979. There was no significant difference in survival between treatment and control tanks for any of the organisms tested except copepods. The survival of copepods in treatment tanks was significantly lower than in control tanks for Pier 13 and Chollas Creek Channel in the liquid phase and for Pier 13 in the particulate phase. Pier 13 liquid phase mortalities are within acceptable ranges when the LPC (0.1%) is compared to the application limit (0.23%). In Chollas Creek liquid phase tests the application limit after 96 hours of dilution is less than the liquid phase LPC of 0.1% which was calculated for a dilution period of only 4 hours. Since the dredged material in the liquid phase would be diluted from 100% to 0.1% in 4 hours, the material present should continue decreasing in concentration and be diluted by several orders of magnitude after 96 hours. If the dilution is several orders of magnitude lower after 96 hours, our calculated application limit for Chollas Creek would be well within the acceptable range.

The LC 50 for Pier 13 particulate phase tests could not be calculated because the data were too heterogeneous; survival in 50% solutions (73%) was higher than in 10% solutions (60%). Since a statistically valid line could not be drawn (dose vs. mortality) we constructed three lines to bracket all possible combinations of variability. The resulting LC 50's and their corresponding application limits are greater than the particulate phase LPC. These calculations suggest that Pier 13 particulate phase was not toxic to *A. tonsa*. The results from liquid, particulate, and solid phase tests on Piers 13, JK, and Chollas Creek Channel indicated that the dredged material would not have an adverse environmental impact at the dump site.

There was no evidence to suggest that heavy metals from dredged material would build up in the tissues of organisms near the dump site. After 10 days' exposure, only chromium in the Pier 11 and 12 clam tissue samples and mercury in the Pier 8 tissue samples were significantly higher than in their respective controls. However, the absolute difference

between treatment and control concentrations was very small (less than 2 ppm, see Table 15). The differences resulted from very low variability among replicate samples. The chromium and mercury concentration in tissues exposed to sediments from other piers was frequently higher than for Piers 8, 11, and 12, but there were no other significant differences.

SUMMARY

Bioassays were conducted on sediments from 13 dredge sites associated with Navy activities in San Diego Bay using liquid, particulate, and solid phase tests. In liquid phase tests, there were no significant differences in survival of *M. elongata* or *C. stigmaeus* at any of the sites tested when compared to the survival of control organisms. The survival of copepods (*A. tonsa*) in treatment tanks was not significantly different from survival in control tanks for seven of the sites tested (Piers 1, 3, 4, 5, 10, 11, JK). However, there was a significant difference in the survival of copepods between control and treatment tanks with liquid phase and sediments from Piers 2, 6, 8, 12, 13, and Chollas Creek Channel. There was no statistically significant difference between survival of treatment and control copepods using sediments from Pier 5; but because of low survival (43%), these data were treated as if a difference existed. The LC 50's for Piers 2, 5, 6, 8, 13, and Chollas Creek were calculated, and all were greater than the liquid phase LPC. In one case (Pier 12) the LC 50 could not be calculated because a statistically valid line could not be fitted to the data. However, hypothetical "worst-case" data were compared to the mortality in 10, 50, and 100% liquid phase solutions and suggest that Pier 12 dredged material would not have a significantly toxic effect on these organisms during disposal operations.

In the particulate phase tests using *M. elongata* and *C. stigmaeus*, survival was not significantly lower than the control for any of the sites tested. However, there were four tests where the survival of mysids in undiluted test media (100% test solutions) was significantly higher than survival of mysids in control tanks. We believe these results are due to chance and do not mean the sediment enhances survival. Survival of *A. tonsa* in Pier 8 and 13 particulate phase treatments was significantly different from survival of copepods in controls. Although differences in survival between control and treatment organisms are statistically significant, calculated application limits based on LC 50's are greater than the particulate phase LPC. Application limits are well above levels that might indicate a significant environmental impact.

In solid phase experiments using sediments from Piers 1, 3, 4, 5, 6, 12, 13, JK, and Chollas Creek Channel there were no significant differences between treatments and controls in survival of *M. elongata*, *P. staminea* (*M. nasuta* for Pier 2), or *N. arenaceodentata*. However, the survival of *M. elongata* that had been exposed to sediments from Piers 2, 10, and 11 was significantly lower than the survival of mysids in control tanks. We feel that the statistically significant difference in mysid survival in the Pier 2 bioassay was probably due to the high survival of mysids in the control tanks (100%) rather than a real difference caused by toxic materials in the sediment. However, mortality attributed to sediment toxicity cannot be dismissed as a possibility. The differences in survival of mysids between control and treatment tanks for Piers 10 and 11 do appear to have been caused by toxic materials because survival was high in both the control and the Pier 12 tests, which were conducted simultaneously. Low survival of mysids in these tests was probably due to isolated contamination at our collection site or extraneous contaminants that we did not measure. It should be remembered that we pre-selected test sediments from the most contaminated sites

around each pier. Therefore, it is reasonable to assume that most of the sediment to be dredged will be much less toxic. Significant bioaccumulation occurred in only three instances out of a total of 52 comparisons. In each case the absolute difference was small, and we believe the statistical significance can be partly attributed to small variability among the replicates.

In general terms, solid phase tests are probably more meaningful than liquid or particulate phase tests. This concept is suggested in the manual and is the reason for the longer exposure time, more replicates, and more animals per replicate in the solid phase test. Furthermore, since plankton are short-lived and transient, significant mortalities in liquid and particulate phase tests may be insignificant at the disposal site. Most importantly, solid phase tests more closely represent conditions in the marine environment after dumping. Liquid and particulate phases were prepared by mixing, bubbling, filtering (liquid phase only), and pumping sediment solutions. During this process constituents within the sediment may have been removed or transformed and extraneous materials could have been added. *M. elongata* was the only species in which mortality was clearly related to dose of test material.

In solid phase tests, the mysid data are the most realistic. Other species used in solid phase tests were highly resistant (clams and worms). For example, the lowest survival in clam treatment tanks was 96%. The clam we used (*P. staminea*) does not appear to be sensitive to any toxic substances which might have been present in the dredged material. Even the sensitive *M. nasuta* (which we used in the Pier 2 bioassay) exhibited high survival (98%) once the techniques were refined. Similarly, the lowest survival with worms was 86%.

In liquid and particulate phase tests, the most meaningful results are also with mysids. As in the solid phase, *M. elongata* in the liquid and particulate phases was the only species whose mortality could be attributed to dose. The fish are very resistant, and the lowest survival under any test condition was 83%. The survival of copepods was highly variable and the observed mortality appeared to be caused by factors other than toxic contaminants. In liquid and particulate phase experiments, 26 bioassays were conducted with copepods; 13 tests in each phase. Mean copepod survival in the 13 liquid phase tests with undiluted medium (100%) was 50.8%. In the 13 particulate phase tests with undiluted test medium, mean copepod survival was 67.6%. By comparison, mean copepod survival in all the control tanks was 79.3%. This is very close to the minimum acceptable survival of 80% suggested in the manual. In bioassays with other species, the mean survival of fish was 98.5% in undiluted liquid phase solutions and 96.2% in undiluted particulate phase solutions. The mean survival of mysids was 87.8% in the liquid phase and 97.2% in the particulate phase using undiluted solutions of test medium. Copepod survival was much lower than that of any other species. Superficially, it appears as if some sediment is toxic to copepods. A comprehensive analysis of these data does not support this conclusion.

Although differences in copepod survival between control and treatment groups were statistically significant in 8 of the 26 tests, only the Chollas Creek liquid phase application limit was below the LPC. Even in this case, we should not expect an adverse environmental impact at the dump site. The dredged material in the liquid phase would be diluted from 100% to 0.1% after 4 hours (0.1% is the liquid phase LPC). The concentration of dredge material would continue decreasing and be diluted by several orders of magnitude in 96 hours. An appropriate 96-hour LPC would be much lower than the application limit of 0.04% calculated for Chollas Creek.

There were two cases in which copepod survival was below 50% and the LC 50 could not be calculated. In both (Pier 12 liquid and Pier 13 particulate), survival in 50% test

medium was greater than or nearly equal to survival in 10% solutions. Since survival should have been much less in the 50% solutions than in the 10% solutions, the data for Piers 12 and 13 resulted in a random scattering of points when dose vs. mortality was plotted. A straight line could not be fitted between the points and the LC 50 could not be calculated. Since the LC 50 could not be calculated, there was concern that the dredged material from these sites might have potentially toxic effects. Therefore, all copepod data were reevaluated.

A combination of factors led to the conclusion that toxic sediment was not responsible for high copepod mortalities. In every test where copepod mortality was high, the mortality of the other four test species was negligible.

When conducting toxicity tests, it is assumed that the observed mortality results from effects of the material being tested. If this assumption is true, then mortality should be proportional to dose. In most toxicity tests, the mortality increases logarithmically with dose. This means that the difference between 10 and 50% solutions of test medium should be much greater (usually more than two times greater) than the difference in mortality between 50 and 100% solutions of test medium (10 < 50 < 100%). In 24 tests using *A. tonsa* (liquid and particulate phases, Pier 2 excluded) there were six cases where copepod mortality was lower in 100% solutions than in 50 or 10% solutions of the same test medium. Additionally, there were six tests in which copepod mortality was lower in 50% solutions than in 10% solutions. Clearly, the observed copepod mortality in half of these bioassays was not related to the concentration of sediment to which they were exposed.

There are a number of procedural problems involved in conducting bioassays with such small and sensitive animals. As mentioned, we believe that part of the mortality observed in liquid phase tests can be attributed to lack of nutrients. Arnott and Ahsanullah (1979) reduced the duration of acute toxicity tests with copepods to 24 hours because of high mortality attributed to starvation in 96-hour tests. Animals with such high metabolic rates probably require feeding in the course of tests lasting more than a few days. We also observed a number of animals that became trapped at the air–water interface due to surface tension. It is not known how much this contributed to mortality, but the phenomenon has been reported by others (Arnott & Ahsanullah, 1979).

The survival of copepods in control tanks varied significantly among tests even after our techniques had been perfected. This same variability has been reported by other investigators using field-caught animals. Sosnowski et al. (1979) found differences in LC 50's that varied by almost a factor of nine in identical 72-hour tests with copper and different populations of *Acartia tonsa*. The variability was significantly greater than in cultured populations of *Acartia tonsa* and correlated with population density and nutrition. These differences in sensitivity to copper reflected differences in the biological condition of the various populations used in the tests.

The problem of copepod reliability was further compounded by mortality differences in liquid and particulate phase tests. In 9 of the 12 liquid phase tests with *A. tonsa*, survival was less than 61%. The corresponding particulate phase results with copepods were 13 to 49% higher in six cases. There were only three tests where copepod survival in the liquid phase was similar to the survival of copepods in the particulate phase. We also observed this difference between liquid and particulate phase tests using mysids, even though survival was quite high. The average for all liquid phase tests with *M. elongata* was only 88.5%, compared with 96.5% in particulate phase tests. This difference was statistically significant. Although we feel that some copepod mortality can be attributed to lack of nutrients, there must be another contributing factor since a) copepod mortality in controls was relatively low even without added nutrients and b) mysid mortality in liquid phase tests was significantly higher

than in particulate phase tests even though they were fed equivalent portions of brine shrimp nauplii. The availability of toxic heavy metal ions was probably reduced in particulate phase solutions by ion exchange, chemical binding, or physical adsorption. Other investigators have reported reduced toxicity to copepods under similar conditions (Whitfield & Lewis, 1976).

The factors discussed above suggest that the low survival of copepods in these bioassays is not necessarily related to the toxicity of dredged material from Navy Piers in San Diego Bay. However, to remove any doubt about the significance of these copepod tests, hypothetical worst-case data were derived to determine how high mortality would have to be to exceed the LPC. If after 4 hours of exposure to 10, 50, and 100% solutions of treatment solutions, copepod mortality was 60, 93, and 97% in the particulate phase or 33, 83, and 93% in the liquid phase, respectively, the resulting application limits would still be slightly greater than the LPC. After 96 hours of exposure to liquid and particulate phase solutions, copepod survival was considerably greater than the hypothetical values listed above.

CONCLUSIONS

The results of these bioassays suggest that the dredged material from Navy Piers 1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 13, JK, and Chollas Creek Channel should not have a significantly adverse effect on the marine environment during disposal operations. Any significant effects suggested by copepod results can be dismissed by allowing for initial mixing or the fact that copepod mortalities could not be related to sediment dose. The low mysid survival at Piers 2, 10, and 11 solid phase tests was significant but factors other than sediment toxicity could explain the observed mortality.

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