EPA 910/9-90-009

EFFECTS OF SEDIMENT HOLDING TIME ON SEDIMENT TOXICITY

June 1990

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EFFECTS OF SEDIMENT HOLDING TIME ON SEDIMENT TOXICITY

By

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Prepared for

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Region 10, Office of Puget Sound
1200 Sixth Avenue
Seattle, Washington 98101

EPA Contract 68-D8-0085
PTI Contract C744-11

June 1990

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LIST OF ACRONYMS

ABN	acid/base/neutral
AET	apparent effects threshold
CLP	Contract Laboratory Program
EPA	U.S. Environmental Protection Agency
GC/ECD	gas chromatography/electron capture detection
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyl
PSDDA	Puget Sound Dredged Disposal Analysis
PSEP	Puget Sound Estuary Program
TOC	total organic carbon

EXECUTIVE SUMMARY

Four of the sediment bioassays commonly used to assess the toxicity of Puget Sound sediments were used to evaluate the influence of sample holding time on the toxicity of sediment samples collected from a highly contaminated site (i.e., Station EB) and a reference area (Station CR) in the sound. Sediments were initially homogenized in the laboratory and distributed for bioassay testing within 3–5 days after field collection. All subsequent holding times were evaluated relative to the time elapsed from initial sample homogenization. The initial holding time for each bioassay ranged from 1 to 2 weeks and was used as the basis of comparison for all longer holding times (maximum = 16 weeks for all bioassays). Two kinds of evaluations were made. In the first evaluation, the influence of holding time on the absolute bioassay responses at each station was determined. In the second evaluation, the influence of holding time on the relative differences of bioassay responses between the two stations was determined. The four sediment bioassays evaluated included the following:

- 10-day amphipod mortality test
- 20-day *Neanthes* biomass test
- 15-minute Microtox test (saline extract)
- 48-hour echinoderm embryo abnormality test.

The results of the echinoderm embryo abnormality test were not evaluated because the results for the initial sediment holding period did not satisfy quality assurance and quality control specifications. Larval abnormality in the negative seawater control was 15.9 percent, which exceeded the maximum allowable value of 10 percent.

The results of the 10-day amphipod mortality test for both Stations CR and EB suggest that sediment holding times longer than 6 weeks may result in bioassay responses at individual stations that are substantially different from those observed after a 2-week holding time. The results for Station CR suggest that holding times of 5.5 and 6 weeks may also influence sediment toxicity, compared to the results obtained after a 2-week holding time. The differences observed among the various holding times were not substantially influenced by changes in the sensitivity of the test organisms or changes in the variability of the bioassay responses. Patterns based on between-station differences in the results of the amphipod test suggest that holding times of 5.5 weeks or longer may influence the results of such comparisons.

The results of the 20-day *Neanthes* biomass test suggest that sediment holding times of 6 weeks or longer may result in bioassay responses at individual stations that are different from those observed after a 1-week holding time. The differences observed among the various holding times were not substantially affected by changes in the variability of the bioassay responses. Biomass changes with increasing holding time were relatively small compared with the differences observed between the two stations. Therefore, the observed biomass differences between Stations CR and EB were relatively consistent among all holding times (i.e., differences between Stations CR and EB were significant for all holding

times). This consistency between stations was likely the result of the relatively high sensitivity and precision of the *Neanthes* biomass test.

The results of the Microtox test suggest that sediment holding times of 4 weeks or longer may result in bioassay responses at individual stations that are substantially different from those observed after a 2-week holding time. The differences observed among the various holding times were not substantially influenced by changes in the sensitivity of the test organisms or variability of the bioassay responses. Patterns based on between-station differences for various holding times exhibited a high degree of inconsistency and suggest that holding times of 4 weeks or longer may influence the results of such comparisons.

In summary, the results of this study suggest that sediment holding time can influence the results of at least three of the sediment bioassays commonly used to assess sediment toxicity in Puget Sound.

INTRODUCTION

BACKGROUND

In most studies of contaminated sediments in Puget Sound, sediment samples are stored for various periods of time after field collection and prior to laboratory toxicity testing. This storage period is termed holding time, and its influence on sediment toxicity is largely unknown. To provide accurate estimates of the toxicity of field-collected sediments, it is essential that toxicity not be substantially altered while samples are stored prior to laboratory analysis. If the toxicity of the sediments changes during storage, erroneous conclusions could be reached regarding the toxicity of those sediments in the environment.

At present, the Puget Sound Estuary Program (PSEP) recommends that sediment holding time not exceed 2 weeks for sediments that are stored at 4°C. Sediments for most of the bioassays conducted in Puget Sound are stored at that temperature. The PSEP maximum holding time represents the consensus of regional experts (PSEP 1986a) and is based largely on best professional judgment rather than conclusive empirical data.

To meet program-specific needs, Puget Sound Dredged Disposal Analysis (PSDDA) has specified that sediments can be held at 4°C for as long as 6 weeks prior to bioassay evaluations (PSDDA 1989). The PSDDA recommendation is based largely on the use of a tiered toxicity evaluation approach, which calls for initial chemical analyses, and, if necessary or desired, subsequent bioassay evaluations. The PSDDA maximum holding time of 6 weeks is also not based on conclusive empirical data.

STUDY OBJECTIVE

The objective of the present study was to evaluate the effects of sediment holding time on sediment toxicity, as estimated by four of the sediment bioassays commonly used in Puget Sound. The evaluation was conducted using sediments from a highly contaminated area and from a reference area to bracket the approximate range of sediment contamination found in the sound. The initial holding time evaluated for each bioassay (i.e., 1-2 weeks) was used as the basis for comparison with all longer holding times.

The relationship between sediment toxicity and sediment holding time was evaluated for each bioassay by testing the following two null hypotheses:

- The mean response of each bioassay did not differ between the initial holding time and each longer holding time
- The outcome of statistical comparisons of each bioassay response between the contaminated and reference stations did not differ between the initial holding time and each longer holding time.

The first hypothesis addressed whether variable holding times influenced the absolute response of each bioassay, and considered each station independently. The second hypothesis evaluated whether variable holding times affected the relative responses of each bioassay between the contaminated and reference stations.

METHODS

FIELD COLLECTION

Sediment samples were collected at two stations in Puget Sound in May 1989 aboard the *RV Kittiwake* (Figure 1). The two stations represented a contaminated area and a reference area. Bioassay responses were evaluated independently for each station and were also compared between the two stations.

Station CR was sampled at a depth of 50 meters in Carr Inlet on 5 May 1989 and was used to represent a Puget Sound reference area. This station has been used previously by PSDDA as a Puget Sound reference station (PTI 1988, 1989). Station EB was sampled at a depth of 10 meters in Elliott Bay on 8 May 1989 and was used to represent a highly contaminated area. This station is located off a major industrialized area on Harbor Island, which has been sampled during several previous studies (Gamponia et. al. 1986; Beller et. al. 1988; Johns 1988; Pastorok and Becker 1989).

At each station, approximately 20 liters of sediment was collected using a 0.1-m^2 van Veen bottom grab. After any overlying water was drained from each grab sample, the entire sediment sample was transferred to a 20-liter plastic bucket. Sediments were later homogenized in the laboratory. Samples were rejected if they were greatly disturbed or winnowed during collection. After the required amount of sediment was collected at each station, the 20-liter bucket was sealed tightly, transferred to the laboratory, and held at 4°C in the dark.

LABORATORY ANALYSIS

Sample Homogenization and Storage

Sediments were homogenized on 10 May 1989. For each station, all of the field-collected sediment was combined and thoroughly mixed in plastic buckets using plastic utensils. Mixing was considered complete when the sediment exhibited no visible heterogeneity in color or texture.

After the sediment from each station was thoroughly mixed, aliquots were taken at random, distributed to containers, and stored for chemical, physical, and bioassay analyses as described in Table 1. For each bioassay, sediments were stored in multiple containers, so that a separate container could be opened for the evaluation of each holding time. Any remaining sediment was then discarded. This procedure ensured that the results of each evaluation were not affected by sample disturbance caused by an earlier evaluation.

After sediment homogenization, every effort was made to minimize sample contamination. All subsequent chemical and bioassay analyses should therefore be considered representative of the sediment samples at the time of homogenization, rather

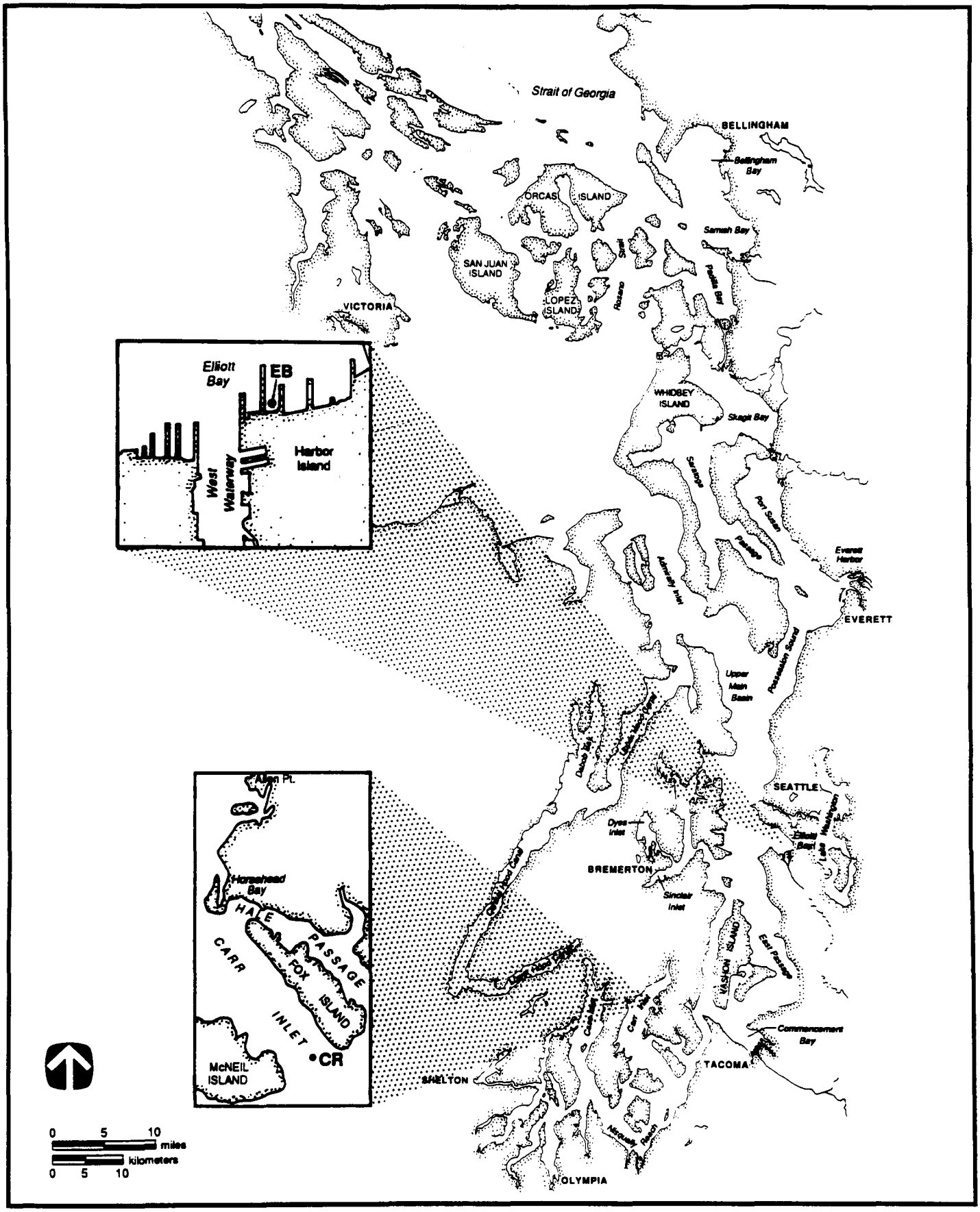


Figure 1. Location of sediment collection sites

TABLE 1. BIOASSAY AND CHEMICAL SAMPLE HOLDING CONDITIONS

Analysis	Container[a]	Preservative
Bioassays	G	4° C in nitrogen atmosphere[b]
Semivolatile organic compounds	G	Freeze
Metals	G	Freeze
Grain size	P	4° C
Total organic carbon	G	Freeze

[a] G - chemically cleaned glass; P - plastic.

[b] Each jar was filled to within 1 cm of the top, and the remaining headspace was filled with nitrogen gas before the jar was capped.

than at the time of field collection. May 10 should therefore be considered the starting time for all of the holding time experiments, and all references to sediment holding time in this report relate to the time elapsed from this initial sediment homogenization. This starting time was 5 days after sample collection at Station CR and 2 days after sample collection at Station EB. This approach was similar to that used for the PSEP bioassay comparison study (Pastorok and Becker 1989).

Chemical Analyses

Chemical analyses of sediments from Stations CR and EB were conducted for the metals and organic compounds listed in Table 2. The particle-size distribution and total organic carbon (TOC) content of the sediments were also evaluated.

Concentrations of organic compounds were determined using protocols modified from those of the U.S. Environmental Protection Agency (EPA) Contract Laboratory Program (CLP) (U.S. EPA 1986). The analyses of semivolatile compounds [including acid/base/neutral (ABN) extractables, polychlorinated biphenyls (PCBs), and pesticides] followed modified EPA CLP procedures that were consistent with the relatively low detection limits recommended by PSEP (1986b). Separate sediment subsamples were used for ABN and pesticide/PCB extraction. Ultrasonic extraction was conducted using CLP procedures. Gel permeation chromatography was conducted for all ABN extracts to reduce interference and attain the recommended detection limits. Pesticide/PCB analyses were conducted using a modified version of the EPA CLP procedure. These analyses included extract cleanup by alumina column chromatography and, when necessary, elemental sulfur cleanup, followed by gas chromatography/electron capture detection (GC/ECD) analysis. GC/ECD quantification and confirmation analyses were conducted with fused silica capillary columns rather than the packed columns commonly used in CLP procedures.

Concentrations of metals were determined by initial digestion of sediment samples using the strong-acid technique specified in EPA CLP procedures (U.S. EPA 1986). Metals concentrations in the digestates were then determined by graphite furnace atomic absorption or by direct-flame atomic absorption spectrometry (except for mercury, which was determined using cold vapor atomic absorption spectrometry).

Sediment particle-size distribution and TOC content were determined using the procedures recommended by PSEP (1986c). Particle-size distribution was determined using standard sieve and pipette techniques. TOC was determined by an elemental analyzer following sample combustion.

Bioassay Analyses

Four of the sediment bioassays commonly used in Puget Sound were used to evaluate sediment toxicity. They included the following tests:

- Amphipod mortality test
- *Neanthes* biomass test

TABLE 2. CHEMICALS ANALYZED IN TEST SEDIMENTS

Metals

antimony	copper	nickel
arsenic	lead	silver
cadmium	mercury	zinc

Phenols and Substituted Phenols

phenol	2,4-dimethylphenol
2-methylphenol	pentachlorophenol
4-methylphenol	

Low Molecular Weight Polycyclic Aromatic Hydrocarbons (LPAH)

naphthalene	phenanthrene
acenaphthylene	anthracene
acenaphthene	2-methylnaphthalene
fluorene	

High Molecular Weight Polycyclic Aromatic Hydrocarbons (HPAH)

fluoranthene	benzo(a)pyrene
pyrene	indeno(1,2,3-c,d)pyrene
benz(a)anthracene	dibenzo(a,h)anthracene
chrysene	benzo(g,h,i)perylene
benzofluoranthenes	

Chlorinated Aromatic Hydrocarbons

1,2-dichlorobenzene	1,2,4-trichlorobenzene
1,3-dichlorobenzene	hexachlorobenzene (HCB)
1,4-dichlorobenzene	

Polychlorinated Biphenyls

total PCB (mono- through decachlorobiphenyls)

Chlorinated Aliphatic Hydrocarbons

hexachlorobutadiene	hexachloroethane

Phthalate Esters

dimethyl phthalate	butyl benzyl phthalate
diethyl phthalate	bis(2-ethylhexyl)phthalate
di-n-butyl phthalate	di-n-octyl phthalate

TABLE 2. (Continued)

Miscellaneous Oxygenated Compounds	
benzyl alcohol	benzoic acid
dibenzofuran	
Organonitrogen Compounds	
N-nitrosodiphenylamine	
Pesticides	
total DDT (p,p')	aldrin
heptachlor	dieldrin
α-chlordane	γ-HCH (lindane)

- Microtox test (saline extract)
- Echinoderm embryo abnormality test.

The amphipod mortality test evaluated mortality of adult amphipods (*Rhepoxynius abronius*) following a 10-day exposure to bedded test sediments. The primary endpoints were percent mortality and percent total effective mortality. The latter endpoint was represented by the number of amphipods that died combined with the number of survivors that failed to rebury in clean sediment after the 10-day exposure period. It was assumed that failure to rebury represented effective mortality, as the affected individuals would have been rapidly consumed by predators in the environment. The methods for this test are described by Swartz et al. (1985) and PSEP (1986a). Five replicate laboratory analyses were conducted for each field sample. The sensitivity of the test organisms was evaluated using sodium pentachlorophenate (NaPCP) as the reference toxicant for positive control samples.

The *Neanthes* biomass test evaluated growth of juvenile polychaetes (*Neanthes arenaceodentata*) following a 20-day exposure to bedded test sediments. The primary endpoints were total and average biomass. Total biomass represented the pooled dry weight of surviving individuals, and thereby incorporated mortality. Average biomass represented the mean dry weight of individual survivors and did not incorporate mortality. The methods for this test are described in Johns et al. (1989). Five replicate laboratory analyses were conducted for each field sample. Positive controls were not analyzed for all holding times because this test was in the developmental stage and a separate experiment was conducted to develop appropriate positive control conditions. Therefore, potential differences among the various holding times in the sensitivity of the test organisms could not be evaluated for this test.

The Microtox test evaluated luminescence of bioluminescent bacteria (*Photobacterium phosphoreum*) following a 15-minute exposure to a saline sediment extract. The primary endpoint was percent decrease in luminescence, which represented changes in cellular metabolic state. The methods for this test are described by Beckman Instruments (1982), PSEP (1986a), and Williams et al. (1986). In the present study, two kinds of analyses were conducted for the Microtox test. In the first analysis, samples were evaluated using the dilution series recommended by Williams et al. (1986). In the second analysis, evaluations were made using four replicate samples of the highest sample dilution used in the first analysis (i.e., the 50 percent dilution). The second analysis was implemented for sediment holding times longer than 2 weeks when it was found that the test organisms were responding weakly to extracts from Station EB, and the maximum extract dilution resulted in only a 15-16 percent reduction in luminescence. It was therefore not possible to calculate EC_{50} values for statistical comparisons. The second analysis was conducted at the same time and using the same sample extract as the first analysis. For the 2-week holding period, the results based on the 50 percent dilution ($n=2$) for the first analysis were used as the basis for comparison with the results of the second analysis for all longer holding times. For each sediment holding time, the sensitivity of the test organisms was determined using phenol as the reference toxicant for positive control samples.

The echinoderm embryo abnormality test evaluated mortality and abnormality in larval sand dollars (*Dendraster excentricus*) following a 48-hour exposure to bedded test sediment. The primary endpoints were percent mortality and percent abnormality. Larval abnormality was defined as failure to develop to the normal pluteus stage after the 48-hour exposure

period. The methods for this test are described by Dinnel and Stober (1985). Five replicate laboratory analyses were conducted for each field sample. For each holding time, the sensitivity of the test organisms was determined using sodium dodecyl sulfate as the reference toxicant for positive control samples.

The sediment holding times evaluated for each of the four bioassays are presented in Table 3. For each test, the maximum holding time recommended by PSDDA (i.e., 6 weeks) was evaluated. The maximum holding time recommended by PSEP (i.e., 2 weeks) was evaluated for all of the bioassays except the *Neanthes* biomass test. That bioassay was evaluated after a holding time of 1 week, which is within the PSEP guidelines and therefore is considered appropriate as the basis for evaluating the longer holding times.

DATA ANALYSIS

To test the first null hypothesis regarding the influence of variable sediment holding time on the absolute response of each bioassay, the mean response observed for each initial holding time was compared with the mean response for each longer holding time. Pairwise comparisons were made between the results for the initial holding time and each additional holding time using the Student's *t*-test and a comparisonwise, two-tailed error rate of 0.05. Corrections to the error rate for multiple comparisons were not made because each pairwise comparison was considered a test of an independent null hypothesis. Before each *t*-test was made, heterogeneity of variances was tested using the F_{max} test (Sokal and Rohlf 1981). If heterogeneous variances were found, the pairwise comparison was made using the approximate *t*-test (Sokal and Rohlf 1981). For the Microtox test, pairwise comparisons were made using replicated data (n=2 for the 2-week holding time, n=4 for holding times longer than 2 weeks) for the maximum sample dilutions. Statistical comparisons were not made using the information on dilution series because EC_{50} values could not be calculated. For some samples, results of all replicates of the Microtox test were zero percent, so no standard deviation could be determined and a *t*-test could not be conducted. In such cases, comparisons between samples were made using the nonparametric Mann-Whitney *U*-test.

To test the second null hypothesis regarding the influence of variable sediment holding time on the relative responses of each bioassay between Stations CR and EB, the mean responses observed at the two stations for each holding time were compared using the same statistical techniques described for testing the first null hypothesis. The results of the pairwise comparisons were then examined to determine if the statistical outcome of between-station comparisons varied as a result of different holding times.

The variability of the responses of each bioassay for the various holding times was evaluated to determine whether response variability was affected by sediment holding time to the extent that it could influence the statistical comparisons. Response variability was estimated using the coefficient of variation [i.e., [(standard deviation ÷ mean) x 100].

Potential variability among the various holding times in the sensitivity of the test organisms was evaluated by examining the LC_{50} values observed for the positive control samples.

TABLE 3. SEDIMENT HOLDING TIMES EVALUATED FOR THE FOUR SEDIMENT BIOASSAYS

Bioassay	Holding Times (Weeks from Initial Sediment Homogenization)[a]
Amphipod mortality test	2.0, 5.5, 6.0, 11.0, 12.5, 16.0
Neanthes biomass test	1.0, 6.0, 11.0, 16.0
Microtox test	2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0
Echinoderm embryo abnormality test	2.0, 6.0, 11.0, 16.0

[a] Initial sediment homogenization occurred on 10 May 1989. This date was 5 days after sample collection at Station CR and 2 days after sample collection at Station EB.

RESULTS AND DISCUSSION

CHEMICAL ANALYSES

Results of all chemical analyses are presented in Appendix A (Table A-1). In this section, chemical concentrations are discussed relative to the 1988 Puget Sound apparent effects threshold (AET) values for bioassays (Barrick et al. 1988). These AET values are based on three sediment bioassays [i.e., the amphipod mortality, oyster larvae abnormality, and Microtox (saline extract) tests]. AET values provide an estimate of the concentration of each chemical above which adverse biological effects are always predicted in Puget Sound.

Sediment from Station CR was relatively fine-grained (i.e., 52.8 percent fine-grained material), with a moderate level of TOC content (i.e., 1.3 percent). Sediment from Station EB was coarser than sediment from Station CR (i.e., 47.2 percent fine-grained sediment), but had a higher TOC content (i.e., 2.1 percent).

Sediment from Station CR was relatively uncontaminated. No metal or organic compound exceeded any of its 1988 Puget Sound bioassay AET values. Although several polycyclic aromatic hydrocarbons (PAH) were detected (i.e., phenanthrene, anthracene, and chrysene), concentrations of these compounds were all less than 100 parts per billion (ppb). Sediment from Station EB was highly contaminated with both metals and organic compounds (Table 4). Four metals exceeded at least one 1988 Puget Sound bioassay AET value, and copper and mercury exceeded all three bioassay AET values. Nineteen organic compounds exceeded at least one bioassay AET value, and six of these compounds exceeded all three values. The six organic compounds exceeding all three bioassay AET values included four PAH compounds [i.e., benzofluoranthenes, benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene] and two phenols (2-methylphenol and pentachlorophenol).

SEDIMENT BIOASSAYS

Detailed results of all four sediment bioassays are presented in Appendix A (Tables A-2 to A-8). This section summarizes and discusses those results relative to the various sediment holding times. The results for each kind of bioassay are discussed separately.

Amphipod Mortality Test

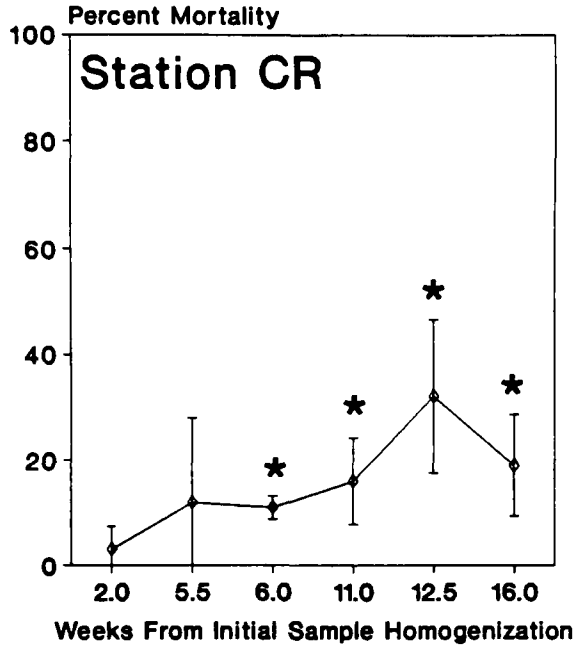
Within-Station Comparisons—For Station CR, 10-day amphipod mortality for the 2-week sediment holding time was 3 percent (Figure 2). Mortality then increased to 12 percent after a 5.5-week holding time, but was not significantly different ($P > 0.05$) from the value observed for the 2-week holding time. The elevated mortality and relatively high variability observed for the 5.5-week holding time was due primarily to a single replicate exhibiting a mortality of 40 percent, whereas mortality in the remaining four replicates ranged from 0 to 10 percent. After a 6-week holding time, mortality was 11 percent and was significantly different ($P \leq 0.05$)

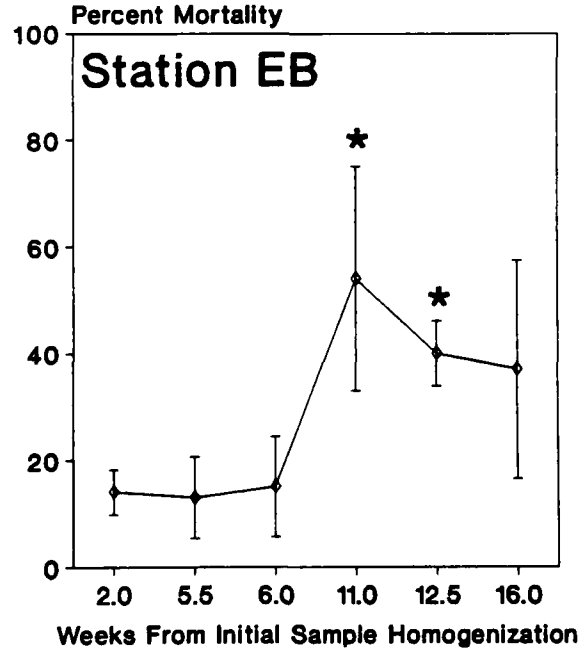
TABLE 4. CHEMICAL CONTAMINANTS IN ELLIOTT BAY SEDIMENT EXCEEDING 1988 BIOASSAY AET VALUES

Chemical	Concentration at Station EB[a]	AET Exceedances[b]
Metals		
Arsenic	112	A
Copper	1,490	A,M,O
Mercury	3.5	A,M,O
Zinc	1,010	A
Organic Compounds		
Low molecular weight polycylic aromatic hydrocarbons (LPAH)		
Total LPAH	9,400	A,M,O
Acenaphthene	780	M,O
Fluorene	790	M,O
Phenanthrene	4,800	M,O
Anthracene	1,900	M,O
High molecular weight polycyclic aromatic hydrocarbons (HPAH)		
Total HPAH	52,000	A,M,O
Fluoranthene	8,100	M,O
Pyrene	12,000	M,O
Benz(a)anthracene	4,000	M,O
Chrysene	3,300	M,O
Benzofluoranthenes	10,000	A,M,O
Benzo(a)pyrene	8,900	A,M,O
Indeno(1,2,3-c,d)pyrene	1,600	M,O
Dibenzo(a,h)anthracene	710	A,M,O
Benzo(g,h,i)perylene	3,600	A,M,O
Phthalates		
Dimethyl phthalate	110	M
Butyl benzyl phthalate	320	M
Bis(2-ethylhexyl)phthalate	6,100	M,O
Phenols		
2-methylphenol	78	A,M,O
Pentachlorophenol	1,900	A,M,O
Total PCBs	1,460	M,O

[a] Metals concentrations are reported in mg/kg dry weight. Concentrations of organic compounds are reported in µg/kg dry weight.

[b] A - amphipod mortality test
M - Microtox test (saline extract)
O - oyster larvae abnormality.





* Significantly different ($P \leq 0.05$) from the value observed for 2.0 weeks.

Figure 2. Comparisons of mean percent mortality and holding time for the amphipod mortality bioassay (bars represent standard deviations)

from the value observed for the 2-week holding time. Unlike the results for the 5.5-week holding time, the results for the 6-week holding time exhibited relatively low variability, as mortality in all five replicates ranged from 10 to 15 percent. For holding times of 11, 12.5, and 16 weeks, mortality increased to 16 and 32 percent, and then declined to 19 percent. All three of these values were significantly different ($P \leq 0.05$) from the value observed for the 2-week holding time.

For Station EB, amphipod mortality for the 2-week sediment holding time was 14 percent (Figure 2). Mortality remained relatively constant at 13 and 14 percent for holding times of 5.5 and 6 weeks, respectively. Both of these values were not significantly different ($P > 0.05$) from the value observed for the 2-week holding time. Mortality peaked at 54 percent for the 11-week holding time, and then declined to 40 and 37 percent for holding times of 12.5 and 16 weeks. The values observed for holding times of 11 and 12.5 weeks were significantly different ($P \leq 0.05$) from the value observed for the 2-week holding time, whereas the value observed for the 16-week holding time was not significantly different ($P > 0.05$) from the value observed for the 2-week holding time.

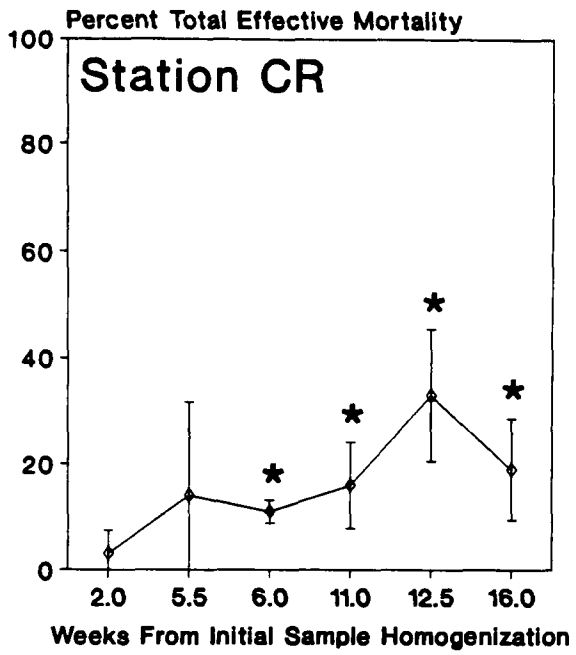
For both Stations CR and EB, total effective mortality of amphipods exhibited patterns identical to those described for mortality (Figure 3).

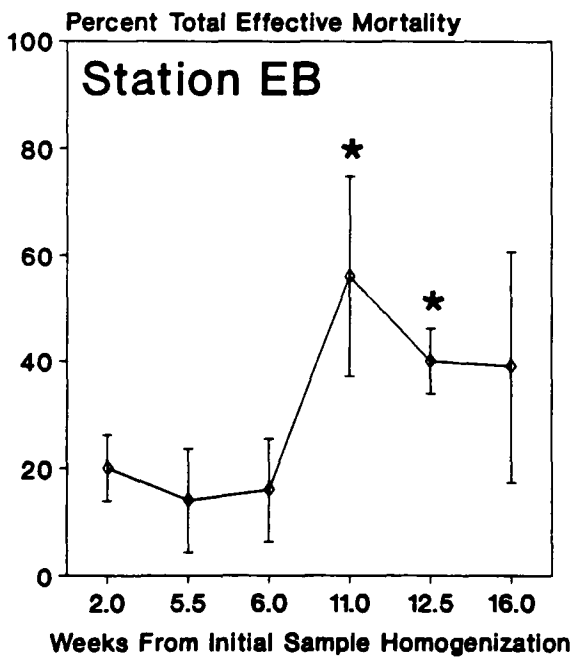
Although the coefficients of variation differed among the various sediment holding times for both bioassay endpoints at both Stations CR and EB (Figure 4), there did not appear to be a consistent relationship between response variability and holding time.

The results of the positive controls for the various sediment holding times are presented in Figure 5. As indicated by the observed LC_{50} values, the sensitivity of the test organisms was relatively consistent for all holding times except 12.5 weeks. Organism sensitivity appeared to be considerably lower for the 12.5-week holding time. However, this apparent reduced sensitivity did not prevent the observed bioassay response from being among the highest observed during the study. This pattern suggests that variations in organism sensitivity did not substantially influence the differences in bioassay responses observed among the various holding times.

Between-Station Comparisons—The results of comparisons of mortality and total effective mortality between Stations CR and EB for each sediment holding time are presented in Table 5. For both endpoints, differences between the two stations were significant ($P \leq 0.05$) for holding times of 2 and 11 weeks and were not significant ($P > 0.05$) for holding times of 5.5, 6, 12.5, and 16 weeks.

Summary—The results of the amphipod mortality test suggest that sediment holding times longer than 6 weeks may result in bioassay responses that are substantially different from those observed after a 2-week holding time. For both Stations CR and EB, most bioassay responses for holding times greater than 6 weeks were significantly different ($P \leq 0.05$) from the responses observed after the 2-week holding time. Patterns based on between-station differences for holding times longer than 6 weeks were not as consistent as absolute bioassay responses. For both bioassay endpoints, between-station differences were significant ($P \leq 0.05$) for the 2-week holding time, but not significant ($P > 0.05$) for two of the three holding times greater than 6 weeks.





* Significantly different ($P \leq 0.05$) from the value observed for 2.0 weeks.

Figure 3. Comparisons of mean percent total effective mortality and holding time for the amphipod mortality bioassay (bars represent standard deviations)

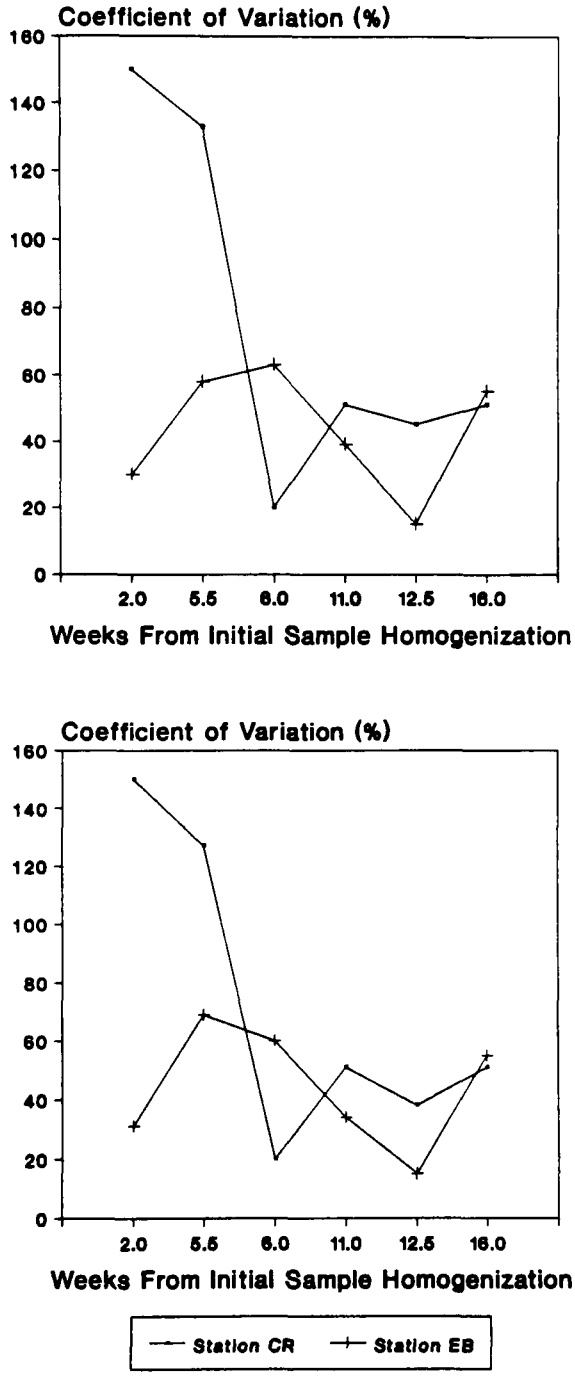


Figure 4. Comparisons of coefficient of variation and holding time for the mortality (above) and total effective mortality (below) endpoints of the amphipod mortality bioassay

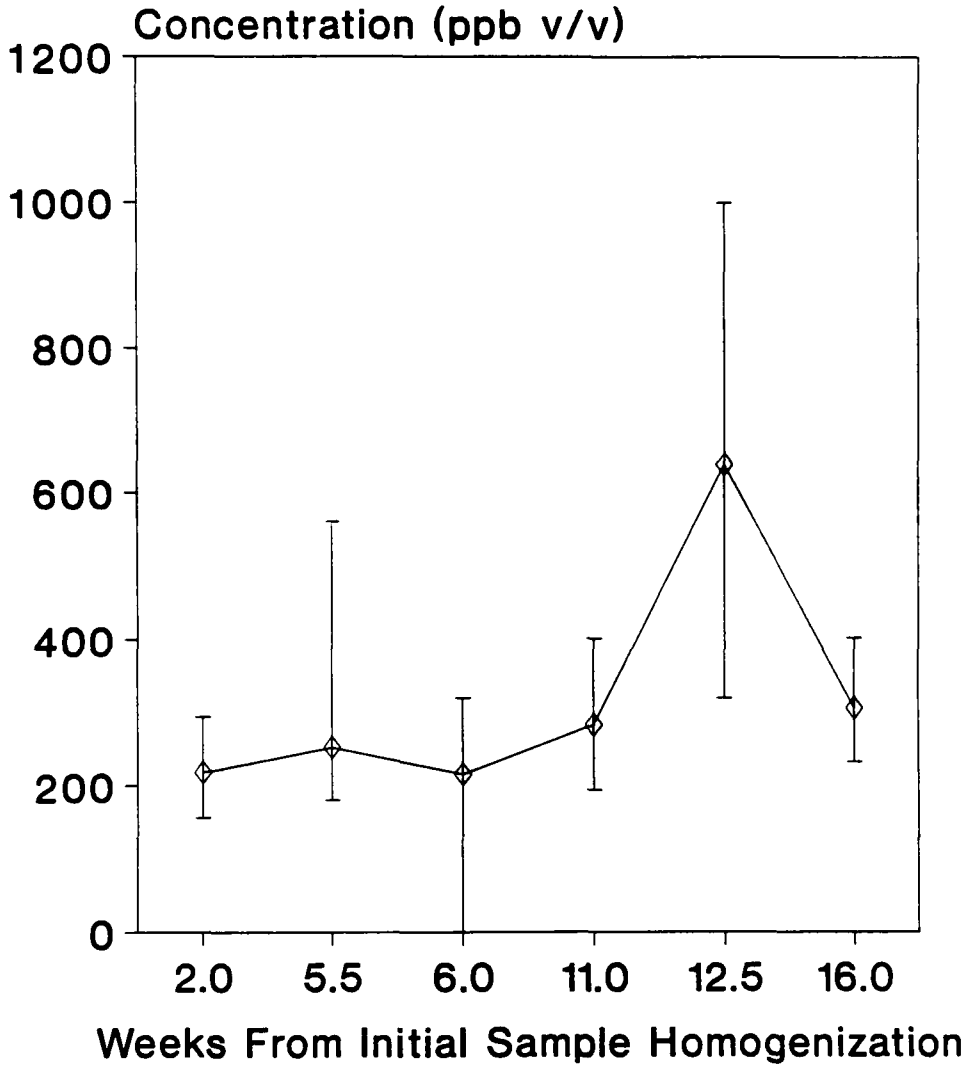


Figure 5. Comparison of LC_{50} values and holding time for the positive control samples (reference toxicant = NaPCP) evaluated for the amphipod mortality test (bars represent 95 percent confidence limits)

TABLE 5. COMPARISONS OF OBSERVED RESPONSES OF THE AMPHIPOD MORTALITY TEST BETWEEN STATIONS CR and EB[a]

Date[b]	Weeks from Initial Sediment Homogenization	Difference Between Stations CR and EB[c]	
		Percent Mortality	Percent Total Effective Mortality
May 24	2.0	11*	17*
June 16	5.5	1 *ns*	0 *ns*
July 20	6.0	4 *ns*	5 *ns*
June 25	11.0	38*	40*
August 5	12.5	8 *ns*	7 *ns*
August 30	16.0	18 *ns*	20 *ns*

[a] Comparisons were made using a *t*-test.

[b] Date bioassay was initiated. All tests were conducted in 1989.

[c] * - $P \leq 0.05$
ns - $P > 0.05$.

For sediment holding times of 5.5 and 6 weeks, the absolute bioassay responses for Station EB differed little from those found after the 2-week holding time. By contrast, the 5.5- and 6-week responses for Station CR increased by approximately 10 percent above the relatively low mortality found for the 2-week holding time. However, only the responses observed for the 6-week holding time were significantly different ($P \leq 0.05$) from the value observed for the 2-week holding time.

Patterns based on between-station differences for sediment holding times of 5.5 and 6 weeks were consistently different from the results found for the 2-week holding time. For both endpoints, differences between stations were significant ($P \leq 0.05$) for the 2-week holding time, but not significant ($P > 0.05$) for holding times of 5.5 and 6 weeks. However, it should be noted that the difference between stations observed for the mortality endpoint after the 2-week holding time (i.e., 11 percent) was relatively small.

Neanthes Biomass Test

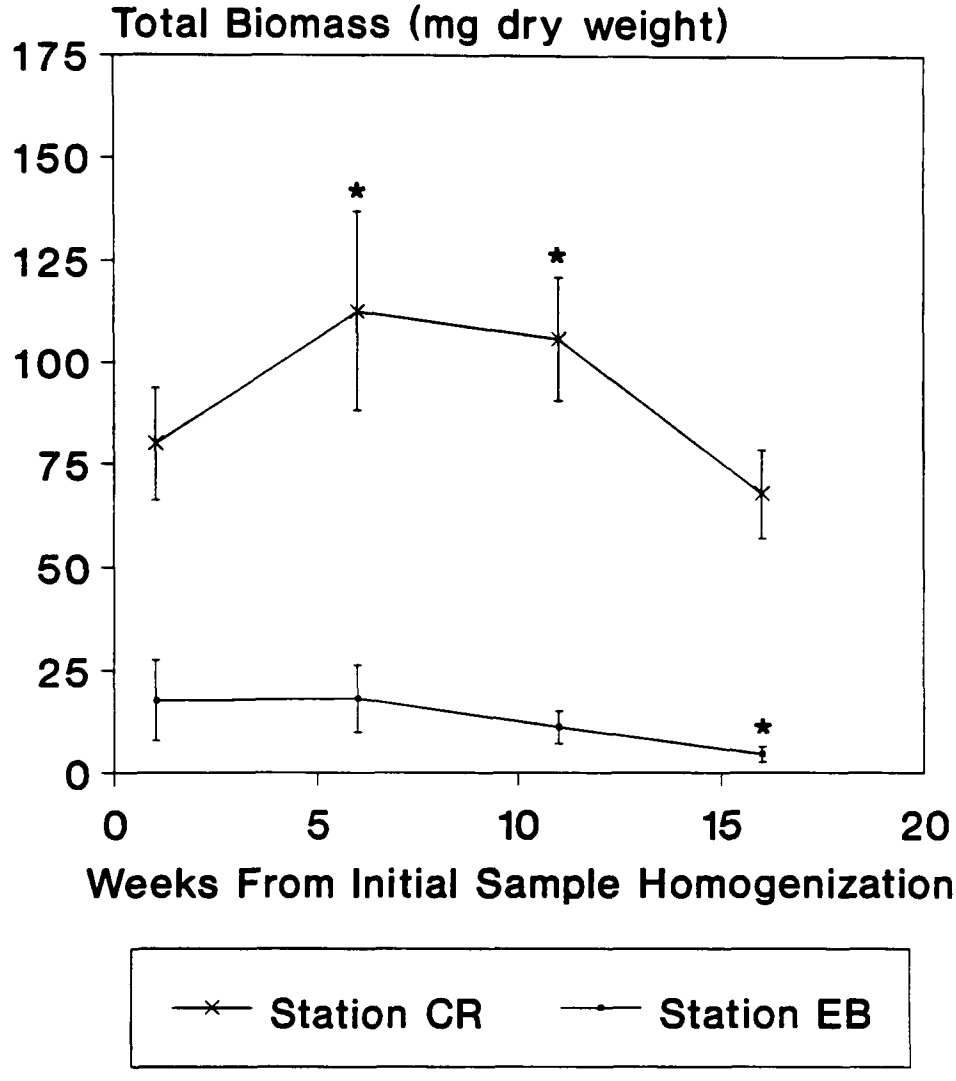
Within-Station Comparisons—Neither initial total biomass nor initial average biomass exhibited significant differences ($P > 0.05$; analysis of variance) among the four sediment holding times evaluated for the *Neanthes* test. Comparisons of final biomass values among the different holding times were therefore not biased by different initial biomass values.

After the 20-day exposure period, total biomass for Station CR for the 1-week sediment holding time was 79.9 mg (Figure 6). Total biomass peaked at a value of 112.5 mg for the 6-week holding time, and then steadily declined to 105.8 and 67.8 mg for holding times of 11 and 16 weeks. Only the values observed for holding times of 6 and 11 weeks were significantly different ($P \leq 0.05$) from the value observed for the 1-week holding time.

For Station EB, total biomass exhibited a pattern similar to that found for Station CR. Total biomass for the 1-week sediment holding time was 17.7 mg (Figure 6). Total biomass peaked at a value of 18.1 mg for the 6-week holding time, and then steadily declined to 11.1 and 4.5 mg for holding times of 11 and 16 weeks. Only the value observed for the 16-week holding time was significantly different ($P \leq 0.05$) from the value observed for the 1-week holding time.

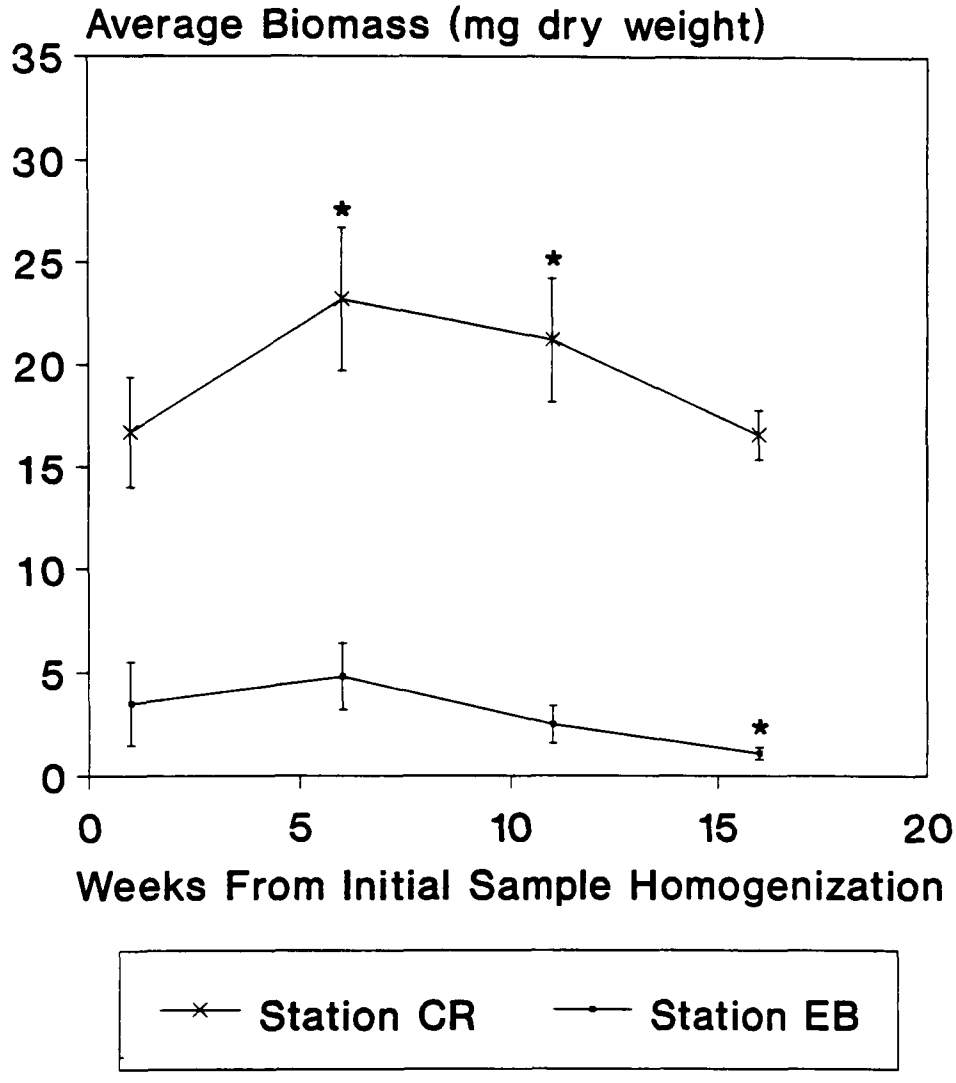
For both Stations CR and EB, average biomass exhibited patterns identical to those described for total biomass (Figure 7). This similarity between endpoints was partly the result of the relatively low mortality observed for most sediment holding times. For Station CR, mortality values for holding times of 1, 6, 11, and 16 weeks were 4, 4, 0, and 0 percent, respectively. For Station EB, mortality values for the four holding times were 0, 24, 8, and 16 percent, respectively.

The coefficients of variation exhibited relatively small differences among the various sediment holding times for both bioassay endpoints at both Stations CR and EB (Figure 8). However, a general negative relationship between response variability and holding time was evident.



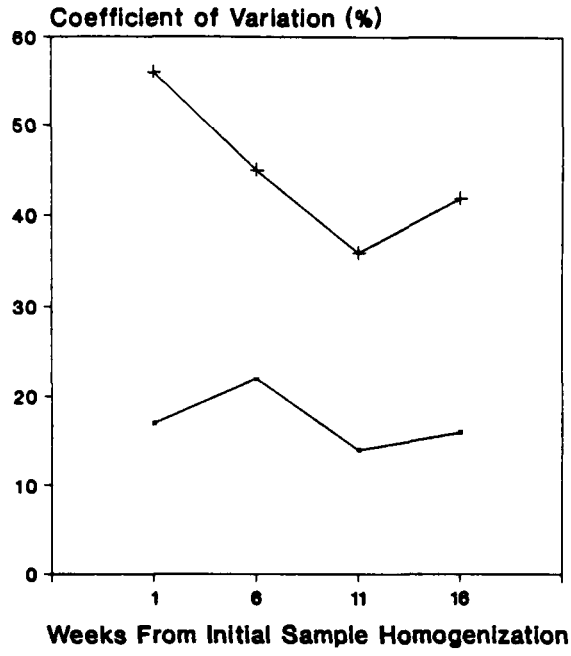
* Significantly different ($P \leq 0.05$) from the value observed for 1.0 week.

Figure 6. Comparisons of mean total biomass and holding time for the *Neanthes* biomass test (bars represent standard deviations)



* Significantly different ($P \leq 0.05$) from the value observed for 1.0 week.

Figure 7. Comparisons of mean average biomass and holding time for the *Neanthes* biomass test (bars represent standard deviations)



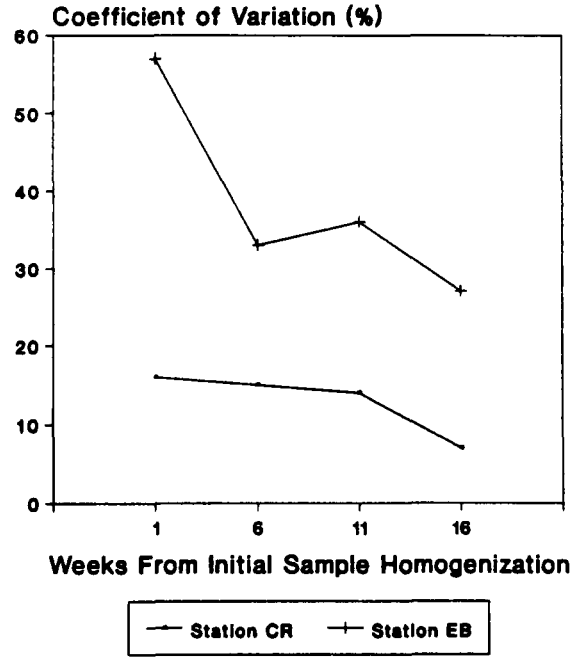


Figure 8. Comparisons of coefficient of variation and holding time for the mortality (above) and average (below) biomass endpoints of the *Neanthes* biomass test

Between-Station Comparisons—The results of comparisons of total and average biomass between Stations CR and EB for each sediment holding time are presented in Table 6. For both endpoints, differences between the two stations were significant ($P \leq 0.05$) for all holding times.

Summary—The results of the 20-day *Neanthes* biomass test suggest that sediment holding times of 6 weeks or longer may result in bioassay responses at individual stations that are different from those observed after a 1-week holding time. For Station CR, *Neanthes* biomass for holding times of 6 and 11 weeks was significantly different ($P \leq 0.05$) from the biomass observed after the 1-week holding time. For Station EB, *Neanthes* biomass for the 16-week holding time was significantly different ($P \leq 0.05$) from the biomass observed for the 1-week holding time.

Patterns based on between-station differences in *Neanthes* biomass were consistent for all of the sediment holding times evaluated. In all cases, differences between stations were significant ($P \leq 0.05$). This consistency is likely the result of both the high sensitivity (i.e., large differences between responses for Stations CR and EB) and the precision (i.e., relatively low standard deviations) of the test. These results suggest that although absolute bioassay responses may vary with holding times, between-station differences may not be affected if the magnitude of bioassay responses at the test site is considerably higher than the magnitude of responses found at the reference site. However, if response magnitudes do not differ substantially between test and reference sites (as was the case for the amphipod mortality test), variability of absolute responses as a result of different holding times could influence the statistical significance of between-station differences in sediment toxicity.

Microtox Test

Within-Station Comparisons—After the 15-minute exposure period, decrease in luminescence for Station CR for the 2-week sediment holding time was 9.3 percent (Figure 9). The bioassay response declined to 0.7 percent for the 4-week holding time and then increased steadily to 10.7, 12.8, and 13.6 percent for holding times of 6, 8, and 10 weeks. The response then declined to 1.5, 0, and 0 percent for holding times of 12, 14, and 16 weeks. The responses for holding times of 4 weeks and 10-16 weeks were significantly different ($P \leq 0.05$) from the response observed for the 2-week holding period.

For Station EB, decrease in luminescence for the 2-week sediment holding time was 15.9 percent (Figure 9). The bioassay response then exhibited a somewhat erratic pattern. Relatively high values of 30.1 and 42.8 percent were found for holding times of 4 and 10 weeks, respectively, whereas moderate values of 12.1, 16.0, and 13.4 percent were found for holding times of 6, 8, and 12 weeks, respectively. Finally, low values of 0 and 0.8 percent were found for holding times of 14 and 16 weeks, respectively. The values observed for holding times of 4-6 weeks and 10-16 weeks were significantly different ($P \leq 0.05$) from the value observed for the 2-week holding time.

Although the coefficients of variation differed among the various sediment holding times for both Stations CR and EB (Figure 10), there did not appear to be a consistent relationship between response variability and holding time.

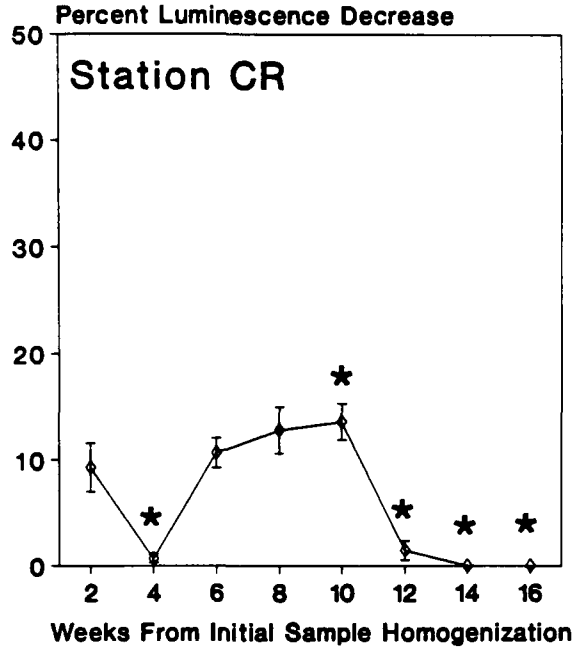
TABLE 6. COMPARISONS OF OBSERVED RESPONSES OF THE *NEANTHES* BIOASSAY BETWEEN STATIONS CR AND EB[a]

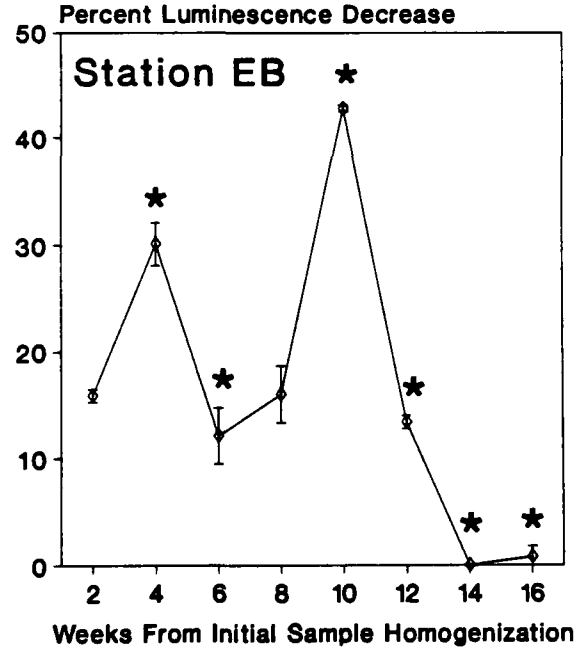
Date[b]	Weeks from Initial Sediment Homogenization	Difference Between Stations CR and EB[c]	
		Total Biomass (mg dry weight)	Average Biomass (mg dry weight)
May 18	1.0	62.2*	13.2*
June 23	6.0	94.4*	18.4*
July 28	11.0	94.7*	18.7*
August 29	16.0	63.3*	15.5*

[a] Comparisons were made using a *t*-test.

[b] Date bioassay was initiated. All tests were conducted in 1989.

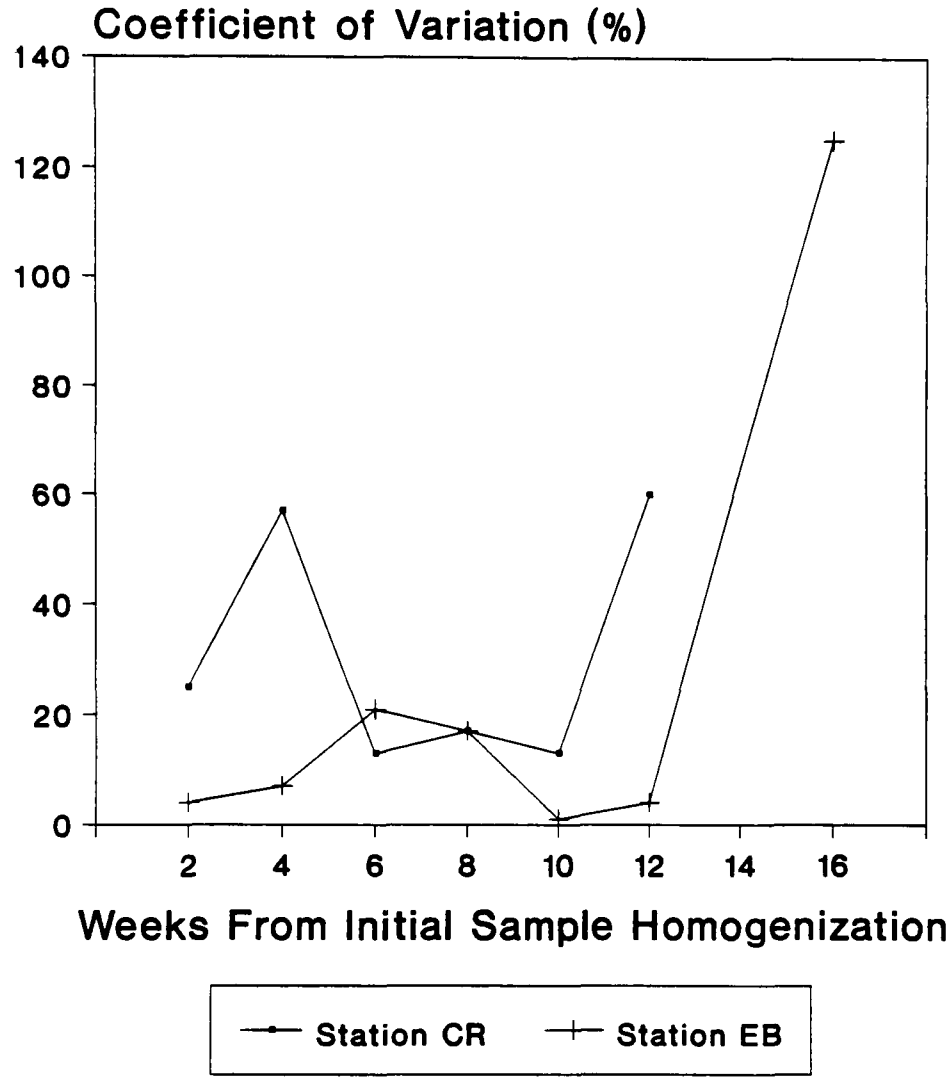
[c] * - $P \leq 0.05$.





* Significantly different ($P \leq 0.05$) from the value observed for 2.0 weeks.

Figure 9. Comparisons of mean decrease in luminescence and holding time for the Neanthes biomass test (bars represent standard deviations)



Note: Coefficients of variation could not be determined for weeks 14 and 16 at Station CR and for week 14 at Station EB because the standard deviation was zero.

Figure 10. Comparisons of coefficient of variation and holding time for the luminescence endpoint of the Microtox bioassay

The results of the positive controls for the various sediment holding times are presented in Figure 11. As indicated by the observed LC_{50} values, the sensitivity of the test organisms was relatively consistent for all holding times. This pattern suggests that variations in organism sensitivity did not substantially influence the differences in bioassay responses observed among the various holding times.

Between-Station Comparisons—Results of comparisons of decrease in luminescence between Stations CR and EB for each sediment holding time are presented in Table 7. Differences between the two stations were significant ($P \leq 0.05$) for holding times of 4, 10, 12, and 16 weeks, and were not significant ($P > 0.05$) for holding times of 2, 6, 8, and 14 weeks.

Summary—The results of the Microtox test suggest that sediment holding times of 4 weeks or longer may result in bioassay responses that are substantially different from those observed after a 2-week holding time. For both Stations CR and EB, bioassay responses for most (i.e., 11 of 14 cases) holding times of 4-12 weeks were significantly different ($P \leq 0.05$) from the response observed for the 2-week holding time.

Patterns based on between-station differences for various sediment holding times exhibited a high degree of inconsistency. Differences between Stations CR and EB were not significant ($P > 0.05$) for the 2-week holding time. By contrast, differences between stations were significant ($P \leq 0.05$) for holding times of 4, 10, 12, and 16 weeks. These results suggest that holding times of ≥4 weeks may influence between-station differences in sediment toxicity.

Echinoderm Embryo Abnormality Test

The results of the echinoderm embryo abnormality test were not considered appropriate for statistical analysis. Larval abnormality in the negative seawater control for the 2-week sediment holding time was 15.9 percent, which exceeded the maximum allowable level of 10 percent. Therefore, results for that holding time could not be considered reliable. Because the 2-week holding time was the basis of comparison for all longer holding times, quantitative evaluations of the longer holding times could not be made.

A qualitative evaluation of the results of the echinoderm embryo abnormality test showed that embryo mortality for Station CR was considerably higher after a 6-week sediment holding time (76.0 percent) than the value observed for the 2-week holding time (18.8 percent; Table A-8). By contrast, abnormality for Station CR was similar between holding times of 2 weeks (14.1 percent) and 6 weeks (10.3 percent).

For Station EB, embryo mortality was at or close to 100 percent for all sediment holding times evaluated (i.e., 2-16 weeks). Because of the low number of surviving embryos at Station EB, the abnormality endpoint could only be evaluated for the 16-week holding time.

Between-station differences could only be evaluated for the mortality endpoint because the number of surviving embryos was too low to estimate percent abnormality at Station EB during the 2- and 6-week sediment holding times. For the 2-week holding time, embryo mortality at Station EB exceeded the value at Station CR by 81.2 percent. For the 6-week holding time, mortality at Station EB exceeded the value observed at Station CR by 20.8 percent.

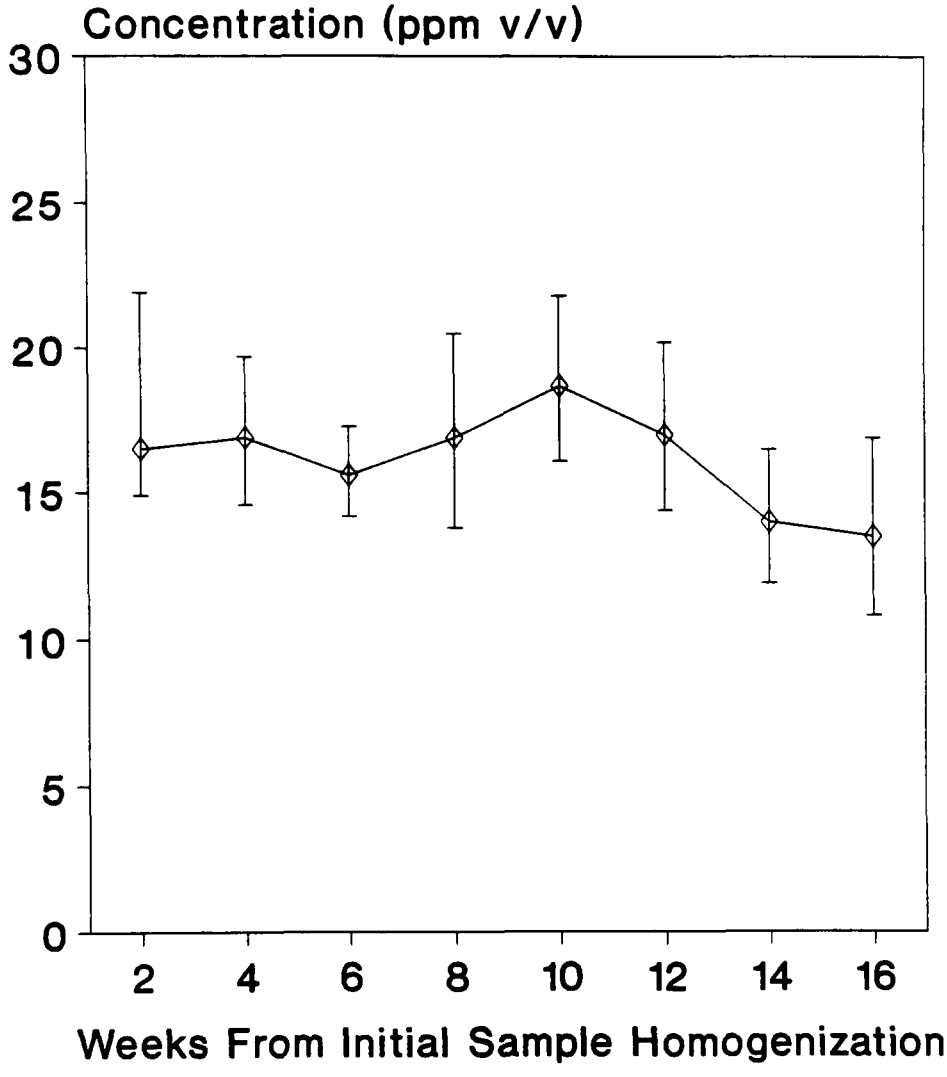


Figure 11. Comparison of LC_{50} values and holding time for the positive control samples (reference toxicant = phenol) evaluated for the Microtox bioassay

TABLE 7. COMPARISONS OF OBSERVED RESPONSES OF THE MICROTOX BIOASSAY BETWEEN STATIONS CR AND EB[a]

Date[b]	Weeks from Initial Sediment Homogenization	Percent Decrease in Luminescence[c]
May 26	2.0	6.6 *ns*
June 8	4.0	29.4*
June 21	6.0	1.4 *ns*
July 6	8.0	3.2 *ns*
July 20	10.0	29.2*
August 3	12.0	11.9*
August 18	14.0	0 *ns*
August 31	16.0	0.8*

[a] Comparisons were made using a *t*-test.

[b] Date bioassay was initiated. All tests were conducted in 1989.

[c] * - $P \leq 0.05$
ns - $P > 0.05$.

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APPENDIX A

DETAILED RESULTS OF CHEMICAL ANALYSES AND BIOASSAY EVALUATIONS

TABLE A-1. CONCENTRATIONS OF CHEMICALS OF CONCERN IN CARR INLET AND ELLIOTT BAY SEDIMENTS

Compound	Carr Inlet (Station CR)[a]	Elliott Bay (Station EB)[a]
METALS (mg/kg dry weight; ppm)		
Antimony	1.4G	10.3G
Arsenic	18.1	112
Cadmium	0.8E	1.4E
Copper	62.3	1,490
Lead	37.5	384
Mercury	0.14	3.5
Nickel	36.7	50.5
Silver	0.39E	1.2E
Zinc	111	1,010
ORGANICS (ug/kg dry weight; ppb)		
Low Molecular Weight Polycyclic Aromatic Hydrocarbons (LPAH)		
Naphthalene	14U	390
Acenaphthylene	14U	440
Acenaphthene	14U	780
Fluorene	14U	790
Phenanthrene	100	4,800
Anthracene	38	1,900
2-Methylnaphthalene	14U	260
High Molecular Weight Polycyclic Aromatic Hydrocarbons (HPAH)		
Fluoranthene	170E	8,100
Pyrene	170E	12,000
Benz(a)anthracene	83E	4,000E
Chrysene	100	3,300
Benzofluoranthenes	150E	10,000
Benzo(a)pyrene	90E	8,900
Indeno(1,2,3,-c,d)pyrene	50E	1,600
Dibenzo(a,h)anthracene	14U	910
Benzo(g,h,i)perylene	56E	3,600
Chlorinated Hydrocarbons		
1,3-Dichlorobenzene	14U	13U
1,4-Dichlorobenzene	14U	14U
1,2-Dichlorobenzene	14U	13U
Hexachlorobenzene	14U	13U

TABLE A-1. (Continued)

Compound	Carr Inlet (Station CR)[a]	Elliott Bay (Station EB)[a]
Phthalates		
Dimethyl phthalate	14U	110
Diethyl phthalate	14U	13U
Di-n-butyl phthalate	14U	140
Butyl benzyl phthalate	14U	320E
Bis(2-ethylhexyl)phthalate	64	6100
Di-n-octyl phthalate	14U	130U
Polychlorinated Biphenyls		
Total PCBs	8.2K	1460
Phenols		
Phenol	27U	170
2-Methylphenol	14U	78E
4-Methylphenol	14U	180E
2,4-Dimethylphenol	33U	31U
Pentachlorophenol	22U	1,900
Miscellaneous Extractables		
Benzyl alcohol	68U	64U
Benzoic acid	140U	128U
Dibenzofuran	14U	110
Hexachloroethane	41U	39U
Hexachlorobutadiene	14U	13U
N-Nitrosodiphenylamine	14U	13U
Pesticides		
Total DDT	2U	34
Aldrin	1U	1U
Chlordane	1.5U	1.5U
Dieldrin	2U	2U
Heptachlor	1U	1U
Lindane	1U	1U

[a] Qualifier codes used:

U - Undetected at detection limit shown
E - Estimate
G - Estimate is greater than value shown
K - Detected at less than detection limit shown.

TABLE A-2. RESULTS OF THE AMPHIPOD MORTALITY BIOASSAY FOR THE MORTALITY ENDPOINT[a]

Date[b]	Weeks from Start Date	Replicate	Station CR		Station EB	
			Number Dead	Percent Mortality[c]	Number Dead	Percent Mortality[c]
May 24	2.0	1	0	3.0 ± 4.5	3	14.0 ± 4.2
		2	0		2	
		3	0		3	
		4	1		4	
		5	2		2	
June 16	5.5	1	2	12.0 ± 16.0	1	13.0 ± 7.6
		2	0		2	
		3	1		5	
		4	8		2	
		5	1		3	
June 20	6.0	1	2	11.0 ± 2.2	4	15.0 ± 9.4
		2	3		3	
		3	2		5	
		4	2		3	
		5	2		0	
July 25	11.0	1	4	16.0 ± 8.2	12	54.0 ± 21.0
		2	5		10	
		3	2		13	
		4	1		15	
		5	4		4	
August 5	12.5	1	10	32.0 ± 14.4	6	40.0 ± 6.1
		2	2		9	
		3	6		8	
		4	7		9	
		5	7		8	
August 30	16.0	1	5	19.0 ± 9.6	12	37.0 ± 20.5
		2	6		2	
		3	3		6	
		4	1		11	
		5	4		6	

[a] Values of mean mortality in the negative controls for tests run on May 23, June 16, June 20, July 25, August 5, and August 30 were 3, 4, 5, 1, 8, and 3 percent, respectively. All of these values are less than the maximum allowable value of 10 percent (PSEP 1986).

[b] Date bioassay was initiated in 1989.

[c] Mean mortality for the five replicates ± standard deviation.

TABLE A-3. RESULTS OF THE AMPHIPOD MORTALITY BIOASSAY FOR THE ENDPOINT BASED ON TOTAL EFFECTIVE MORTALITY[a]

Date[b]	Weeks from Start Date	Replicate	Station CR		Station EB	
			Number Not Reburying	Percent Total Effective Mortality[c]	Number Not Reburying	Percent Total Effective Mortality[c]
May 24	2.0	1	0	3.0 ± 4.5	5	20.0 ± 6.1
		2	0		2	
		3	0		5	
		4	1		4	
		5	2		4	
June 16	5.5	1	2	14.0 ± 17.8	1	14.0 ± 9.6
		2	0		2	
		3	1		6	
		4	9		2	
		5	2		3	
June 20	6.0	1	2	11.0 ± 2.2	4	16.0 ± 9.6
		2	3		4	
		3	2		5	
		4	2		3	
		5	2		0	
July 25	11.0	1	4	16.0 ± 8.2	12	56.0 ± 18.8
		2	5		11	
		3	2		13	
		4	1		15	
		5	4		5	
August 5	12.5	1	10	33.0 ± 12.5	6	40.0 ± 6.1
		2	3		9	
		3	6		8	
		4	7		9	
		5	7		8	
August 30	16.0	1	5	19.0 ± 9.6	13	39.0 ± 21.6
		2	6		2	
		3	3		7	
		4	1		11	
		5	4		6	

[a] Total effective mortality = number dead + number of survivors that fail to rebury.

[b] Date bioassay was initiated in 1989.

[c] Mean value for the five replicates ± standard deviation.

TABLE A-4. RESULTS OF THE *NEANTHES* BIOASSAY FOR THE ENDPOINT BASED ON TOTAL BIOMASS

Date[a]	Weeks from Start Date	Replicate	Total Biomass (gm dry wt)			
			Station CR		Station EB	
			Replicate	Mean[b]	Replicate	Mean[b]
May 18	1.0	1	70.2	79.9 ± 13.7	22.1	17.7 ± 9.9
		2	103.5		11.8	
		3	74.4		32.2	
		4	71.8		6.5	
		5	79.6		15.9	
June 23	6.0	1	124.0	112.5 ± 24.4	21.0	18.1 ± 8.2
		2	137.4		22.9	
		3	118.7		27.0	
		4	109.6		6.5	
		5	72.6		13.2	
July 28	11.0	1	110.6	105.8 ± 15.2	12.7	11.1 ± 4.0
		2	128.5		16.6	
		3	106.1		10.9	
		4	93.4		5.8	
		5	90.4		9.4	
August 29	16.0	1	56.1	67.8 ± 10.7	3.5	4.5 ± 1.9
		2	80.4		4.8	
		3	75.7		3.3	
		4	57.7		7.6	
		5	69.0		3.1	

[a] Date bioassay was initiated in 1989.

[b] Mean biomass for the five replicates ± standard deviation.

TABLE A-5. RESULTS OF THE *NEANTHES* BIOASSAY FOR THE ENDPOINT BASED ON AVERAGE BIOMASS

Date[a]	Weeks from Start Date	Replicate	Average Biomass (gm dry wt)			
			Station CR		Station EB	
			Replicate	Mean[b]	Replicate	Mean[b]
May 18	1.0	1	14.0	16.7 ± 2.7	4.4	3.5 ± 2.0
		2	20.7		2.4	
		3	14.9		6.4	
		4	18.0		1.3	
		5	15.9		3.2	
June 23	6.0	1	24.8	23.2 ± 3.5	5.3	4.8 ± 1.6
		2	27.5		4.6	
		3	23.7		5.4	
		4	21.9		2.2	
		5	18.2		6.6	
July 28	11.0	1	22.1	21.2 ± 3.0	3.2	2.5 ± 0.9
		2	25.7		3.3	
		3	21.2		2.7	
		4	18.7		1.2	
		5	18.1		1.9	
August 29	16.0	1	15.2	16.6 ± 1.2	0.9	1.1 ± 0.3
		2	17.4		1.2	
		3	17.8		0.7	
		4	15.3		1.5	
		5	17.2		1.0	

[a] Date bioassay was initiated in 1989.

[b] Mean biomass for the five replicates ± standard deviation.

TABLE A-6. RESULTS FOR THE MICROTOX BIOASSAY BASED ON DILUTION SERIES

Date[a]	Weeks from Start Date	Concentration	Percent Decrease in Luminescence					
			Station CR			Station EB		
			Replicate 1	Replicate 2	Mean[b]	Replicate 1	Replicate 2	Mean[b]
May 23	2.0	6.25	6.6	3.5	5.1	0	0	0
		12.50	8.3	7.6	8.0	0	0	0
		25.00	11.5	10.7	11.1	5.1	2.2	3.7
		50.00	10.9	7.6	9.3	15.4	16.3	15.9
June 8	4.0	6.25	1.9	1.4	1.7	3.4	5.5	4.5
		12.50	1.5	3.2	2.4	5.7	5.1	5.4
		25.00	0	3.1	1.6	11.9	10.9	11.4
		50.00	0	0	0	24.0	23.9	24.0
June 21	6.0	6.25	19.1	15.6	17.4	0	0.3	0.2
		12.50	19.7	19.2	19.5	0	0	0
		25.00	20.4	21.6	21.0	2.9	2.4	2.7
		50.00	19.6	20.5	20.0	10.7	15.4	13.1
July 6	8.0	6.25	4.1	9.5	6.8	0	0	0
		12.50	8.0	10.4	9.2	0	0	0
		25.00	11.0	12.7	11.9	0	0	0
		50.00	14.4	15.0	14.7	12.4	13.3	12.9
July 20	10.0	6.25	11.9	11.3	11.6	1.5	4.1	2.8
		12.50	14.1	12.4	13.3	9.9	13.7	11.8
		25.00	9.5	7.9	8.7	24.3	27.3	25.8
		50.00	8.6	9.4	9.0	39.4	40.9	40.2
August 3	12.0	6.25	11.7	6.3	9.0	0	0	0
		12.50	10.5	8.6	9.6	0	0	0
		25.00	9.9	7.3	8.6	0	0	0
		50.00	12.4	7.4	9.9	12.7	15.1	13.9
August 18	14.0	6.25	0	0	0	7.6	0	3.8
		12.50	0	0	0	0	0	0
		25.00	0	0	0	0	0	0
		50.00	0	0	0	0	0	0
August 31	16.0	6.25	0	0	0	0	0	0
		12.50	0	0	0	0	0	0
		25.00	1.1	2.9	2.0	0	0	0
		50.00	0.8	2.9	1.9	0	0.5	0.3

[a] Date bioassay was initiated in 1989.

[b] Mean of the two replicate values.

TABLE A-7. RESULTS FOR THE MICROTOX BIOASSAY BASED ON REPLICATE EVALUATIONS OF THE 50 PERCENT SAMPLE DILUTION

Date[a]	Weeks from Start Date	Replicate	Percent Decrease in Luminescence			
			Station CR		Station EB	
			Replicate	Mean[b]	Replicate	Mean[b]
June 8	4.0	1	0.5	0.70 ± 0.4	27.8	30.1 ± 2.0
		2	1.0		29.2	
		3	0.2		31.5	
		4	1.1		32.0	
June 21	6.0	1	12.2	10.7 ± 1.4	15.7	12.1 ± 2.6
		2	11.1		12.4	
		3	10.8		10.7	
		4	8.8		9.7	
July 6	8.0	1	12.3	12.8 ± 2.2	20.0	16.0 ± 2.7
		2	12.9		14.3	
		3	15.6		14.3	
		4	10.2		15.5	
July 20	10.0	1	12.9	13.6 ± 1.7	43.1	42.8 ± 0.3
		2	13.6		4.25	
		3	11.9		42.7	
		4	15.9		42.8	
August 3	12.0	1	0.3	1.5 ± 0.9	13.6	13.4 ± 0.6
		2	2.0		14.0	
		3	1.5		12.5	
		4	2.3		13.5	
August 18	14.0	1	0	0	0	0
		2	0		0	
		3	0		0	
		4	0		0	
August 31	16.0	1	0	0	0.8	0.8 ± 1.0
		2	0		0.1	
		3	0		0.0	
		4	0		2.2	

[a] Date bioassay was initiated in 1989.

[b] Mean value for the four replicates ± standard deviation.

TABLE A-8. RESULTS OF THE ECHINODERM EMBRYO BIOASSAY

Date[a]	Weeks from Start Date	Replicate	Station CR Total Larvae	Station CR Percent Abnormality Replicate	Station CR Percent Abnormality Mean[c]	Station CR Mean Mortality	Station EB Total Larvae	Station EB Percent Abnormality Replicate	Station EB Percent Abnormality Mean[c]	Station EB Mean Mortality	Control Seawater[b] Percent Abnormality	Control Seawater[b] Percent Mortality	Control Sediment Percent Abnormality	Control Sediment Percent Mortality
May 23	2.0	1	14	14.3	14.1±14.6	18.8	0	-	-	100.0	15.9[d]	9.6	8.3	1.6
		2	13	23.1			0	-						
		3	1	0			0	-						
		4	1	0			0	-						
		5	3	33.3			0	-						
June 20	6.0	1	34	5.9	10.3±4.6	76.0	9	88.9	76.4±29.5	96.8	4.7	0	8.4	0
		2	27	7.4			0	-						
		3	35	17.0			3	33.3						
		4	24	8.3			6	83.3						
		5	23	13.0			1	100.0						
July 24	11.0	1	Station CR				0	-	-	99.9	1.9	19.8	1.9	0
		2	was not tested				1	0						
		3					0	-						
		4					0	-						
		5					0	-						
August 29	16.0	1	Station CR				23	91.3	83.1±12.9	99.9	7.9	9.2	8.3	0
		2	was not tested				5	80.0						
		3					22	77.3						
		4					18	100.0						
		5					3	66.7						

[a] Date bioassay was initiated in 1989.

[b] Maximum allowable abnormality = 10 percent; maximum allowable mortality = 30 percent.

[c] Mean of the five replicates ± standard deviation.

[d] Exceeds maximum allowable control value.