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Bioaccumulation in Stage I Polychaetes/Oligochaetes: A Field Feasibility Study

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13. ABSTRACT

Under the DAMOS Program, the tiered approach to monitoring dredged material at open water disposal sites in New England includes bioaccumulation analyses, which are performed with relatively large infaunal polychaetes, crustaceans and bivalves (Germano et al. 1994). While this approach provides important information about availability of contaminants for uptake by long-lived species, it is not useful for evaluation of the early environmental effects of disposal. Organisms used for standard bioaccumulation analyses are typically encountered during the final phase of recolonization (Stage III; Rhoads and Germano 1982). The initial colonizers of dredged material mounds are small polychaetes (Stage I). These small worms (<0.5 mg) are of first-order importance as food for larger predators. They are ideal "early warning" indicators for disposal site bioaccumulation monitoring; however, collecting sufficient Stage I taxa tissue for extensive chemical analysis is a formidable task and has not been accomplished.

In a Phase I feasibility study, a worm "isolator" was designed, and found to be effective at driving worms out of the sediment (Williams and Rhoads 1994). This study was the first attempt to remove large quantities of small worms from their tubes and surrounding sediment. The goals of the present Phase II bioaccumulation study were to evaluate the extraction efficiency of the worm isolators developed in the Phase I laboratory study, and to determine the yield (wet weight biomass) of worm tissue per unit sampling effort. In addition, bioaccumulation in these worms was evaluated by (1) analyzing the contaminant concentrations in an associated surface sediment sample and (2) analyzing worm tissue samples for those contaminants that were evaluated in the ambient sediment.

The results of the second phase of the bioaccumulation study indicated that Stage I organisms can be collected in sufficient quantity for chemical analysis. Three g (wet weight) of worm tissue were collected in 10 hours of sampling using the worm isolator at an estimated ambient density of approximately 600 worms/m^2 . Extrapolating to a peak density of 200,000/m^2 approximately 200g wet weight of tissue potentially could be obtained in the same time, more than enough for precise chemical analyses. As a result of the field studies, a new worm isolator was designed which would expedite the process of collecting the worms from the isolator.

Chemical results from the sediment sample were used in the Theoretical Bioaccumulation Potential (TBP) equation (EPA/ACE 1991) to predict bioaccumulation in the collected worm tissue. Tissue contaminant concentrations were much lower than predicted using the TBP; it was apparent that the current TBP model may not accurately predict bioaccumulation in Stage I organisms. Although the TBP calculation is a useful concept for extrapolating bioaccumulation potential of sediment-dwelling organisms, further empirical data need to be collected to calculate an appropriate contaminant accumulation factor for Stage I organisms.

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EXECUTIVE SUMMARY

Under the DAMOS Program, the tiered approach to monitoring dredged material at open water disposal sites in New England includes bioaccumulation analyses, which are performed with relatively large infaunal polychaetes, crustaceans, and bivalves (Germano et al. 1994). While this approach provides important information about availability of contaminants for uptake by long-lived species, it is not useful for evaluation of the early environmental effects of disposal. Organisms used for standard bioaccumulation analyses are typically encountered during the final phase of recolonization (Stage III; Rhoads and Germano 1982). The initial colonizers of dredged material mounds are small polychaetes (Stage I). These small worms (< 0.5 mg) are of first-order importance as food for larger predators. They are the ideal "early warning" indicators for disposal site bioaccumulation monitoring; however, collecting sufficient Stage I taxa tissue for extensive chemical analysis is a formidable task and has not been accomplished to date.

In a Phase I feasibility study, a worm "isolator" was designed, and found to be effective at driving worms out of the sediment (Williams and Rhoads 1994). This study was the first attempt to remove large quantities of small worms from their tubes and surrounding sediment. The goals of the present Phase II bioaccumulation study were to evaluate the extraction efficiency of the worm isolators developed in the Phase I laboratory study, and to determine the yield (wet weight biomass) of worm tissue per unit sampling effort. In addition, bioaccumulation in these worms was evaluated by (1) analyzing the contaminant concentrations in an associated surface sediment sample and (2) analyzing worm tissue samples for those contaminants that were elevated in the ambient sediment.

The results of the second phase of the bioaccumulation study indicated that Stage I organisms can be collected in sufficient quantity for chemical analysis. Three g (wet weight) of worm tissue were collected in 10 hours of sampling using the worm isolator at an estimated ambient density of approximately 600 worms/m^2. Extrapolating to a peak density of 200,000/m^2, approximately 200 g wet weight of tissue potentially could be obtained in the same time, more than enough for precise chemical analyses. As a result of the field studies, a new worm isolator was designed which would expedite the process of collecting the worms from the isolator.

Chemical results from the sediment sample were used in the Theoretical Bioaccumulation Potential (TBP) equation (EPA/ACE 1991) to predict bioaccumulation in the collected worm tissue. Tissue contaminant concentrations were much lower than predicted using the TBP; it was apparent that the current TBP model may not accurately predict bioaccumulation in Stage I organisms. Although the TBP calculation is a useful concept for extrapolating bioaccumulation potential of sediment-dwelling organisms, further empirical data need to be collected to calculate an appropriate contaminant accumulation factor for Stage I organisms.

1.0 INTRODUCTION

1.1 Purpose of the Study

Bioaccumulation analyses are a key component of the DAMOS tiered monitoring approach of dredged material disposal sites (e.g., Germano et al. 1994). Currently these analyses are performed with relatively large infaunal polychaetes, crustaceans, and bivalves. The advantage of using larger taxa is that sufficient biomass can be collected from few organisms, and separating the animals from the sediment by traditional hand-picking methods is not labor-intensive. While this method provides important information about long-term bioavailability, these organisms are inappropriate as early indicators of environmental degradation due to dredged material disposal because they are typically encountered during the final phase, or Stage III, of recolonization (Rhoads and Germano 1982). Sufficiently abundant populations for analysis may not be available until 3-6 years of recolonization, based on experience with disposal site colonization in central Long Island Sound.

Stage III colonizers may react to contaminated sediments very differently from the small, opportunistic polychaetes that constitute the initial colonizers (Stage I). Stage I taxa appear to be of first-order importance as food for predators such as demersal finfish and lobsters; they would be the ideal "early warning" indicators for disposal site bioaccumulation monitoring. However, because of the relatively large amount of tissue needed by most chemical laboratories to do body burden analyses (approximately 25-30 g), the ability to collect enough Stage I taxa tissue has been beyond the current technology. To remove the worms from their associated sediments and tubes in sufficient numbers for tissue analysis is a formidable task if done by hand, as the biomass of each individual is approximately 0.5 mg (wet weight).

In a Phase I feasibility study, SAIC investigated several potential methods for efficient removal of small worms from sediments (Williams and Rhoads 1994). That laboratory study identified one likely method of driving worms out of sediments by exposing the polychaetes to hypoxic conditions. On exposure to low-oxygen water in sediment chambers (hereafter termed worm "isolators"), the worms respond by moving to the sediment surface and ultimately crawl upward onto the sides of the isolator.

This study is the first attempt to remove large quantities of small worms from their tubes and surrounding sediment under typical field conditions in central Long Island Sound. A major goal of this field work was to determine the yield (wet weight biomass) of worm tissue per unit sampling effort and to evaluate the extraction efficiency of the worm isolators developed in the Phase I laboratory study. The unit of effort is measured in terms of hours of sampling, number of grabs taken, or number of isolators required to yield a given biomass of tissue. Secondly, bioaccumulation in these worms was evaluated by analyzing for those contaminants that appear to be elevated in the ambient sediment. Nonpolar organics are

particularly important because a Theoretical Bioaccumulation Potential (TBP) equation can be used to predict bioaccumulation in sediment-eating organisms (EPA/ACE 1991). If the analyzed tissues of pioneering worms give roughly the same values for nonpolar organics as predicted by the TBP, future analytical work may be greatly reduced.

1.2 Attributes of Stage I Organisms

Dredged material placed on the seafloor results in the formation of competition-free space and is usually accompanied by local organic enrichment of the bottom. Following cessation of dumping, benthic recolonization of dredged material occurs almost instantaneously, by dense populations of pioneering worms (Rhoads et al. 1978). Typically, these worms belong to the polychaete families Spionidae, Capitellidae, and Oweniidae, among others. Oligochaetes may also play a significant role in colonization in estuarine environments.

These pioneering worm populations have the following attributes:

- They are virtually the only numerically significant macrofauna on new disposal mounds for the first few months to years following disposal.

- They feed at, or near, the sediment surface and are physically in contact with both the solid phase and pore water, including contaminants if they are present.

- These dense worm populations are foraged upon by larger predators such as demersal fish (Lunz 1986) and crustaceans. Many of these are commercially important species.

Because of the attributes listed above, the chemical quality of these worm tissues are a potentially important aspect of disposal site monitoring and management. It would be desirable, therefore, to include such an analysis of worm tissue quality in the tiered DAMOS monitoring protocol (Germano et al. 1994). However, to date, the potential for these worms to bioaccumulate contaminants and put the food chain at risk has not been determined.

2.0 METHODS

2.1 Sampling Locations

Sampling locations in the Central Long Island Sound Disposal Area (CLIS) were chosen based on the appearance of dense assemblages of pioneering polychaetes at the MQR and FVP areas as observed in REMOTS® photographs taken in a June 1991 survey. Between the REMOTS® survey and sampling for this study (3-5 September 1991) Hurricane Bob passed over the Sound (19 August 1991). The degree of bottom scour at the sampling sites is unknown but may be comparable to the 1 to 2 cm scour depths experienced at CLIS during Hurricane Gloria in 1985. The effect of Bob on the distribution or redistribution of organisms in Long Island Sound is unknown. The sampling efforts at four areas in the central Sound are summarized in Table 2-1.

Table 2-1

Sampling Efforts at Four Central Long Island Sound Stations

Site	Station	Location	Date Sampled	Duration on Station	Number of Isolators Prepared
MQR	100E	CLIS	9/3/91	1400-1607	4
CLIS-REF	---	CLIS	9/3/91	1630-1745	1
FVP	50W	CLIS	9/4/91	0930-1015	4
New Haven Harbor	West Haven	northwest of breakwater	9/4/91	1200-1550	10
New Haven Harbor	" "	" "	9/5/91	1000-1630	25

Because the MQR, FVP, and CLIS-REF stations did not yield sufficient worm biomass for the purpose of this study, an alternate sampling location was occupied just to the northwest of the Luddington Breakwater, on the west side of New Haven Harbor (41°14′00″ N, 72°57′30″ W). This area has consistently yielded Stage I assemblages in the past, and the silt-clay sediment is filled with the shells of *Mulinia lateralis*, a bivalve known

to be a Stage I colonizer. The apparent RPD depth was estimated to be approximately 1.5 cm which is consistent with the shallow bioturbation depths of Stage I assemblages.

The temperature and salinity of the water at CLIS and West Haven Station were, respectively, 23° C and 28 ppt and 22° C and 28 ppt.

2.2 Field Protocol

At each station, a 0.1 m^2 teflon-coated Van Veen grab was used to recover bottom sediments for worm samples. The internal dimensions of the grab available for surface sediment subsamples measured 35 cm × 27.6 cm yielding an area of 966 cm^2. A plastic spatula was used to remove the upper 1-2 cm of sediment in the grab as this represented the interval containing most of the Stage I worms. The actual depth of subsampling varied according to the observed thickness of the high water content sediment located above the apparent RPD. The subsampled surface sediment ranged in volume between 350 to 800 cc/grab (mean sample volume of 562 cc per grab; n=33). This represented the volume of sediment sieved per grab to obtain a concentrated sample of Stage I worms. Prior to sieving, a small sample of the surface sediment was taken for bulk chemistry and immediately frozen and retained frozen until analyzed (see chemical analysis methods section below).

The surface subsample from the grab was washed through a 2 mm mesh stainless steel sieve to remove large shell fragments, large macrofauna, and tubes. Material passing through the 2-mm sieve was then washed through a 300-μm stainless steel sieve. The wash water was prefiltered through a CUNO Aqua-Pure® water filter cartridge (Meriden, CT) to avoid contaminating the sieved residue with suspended seston from the wash water that was pumped to the deck from the surface of Long Island Sound.

The material retained on the 300-μm sieve was transferred to each worm isolator until the 51 cc volume below the partition was filled to within 1 or 2 mm below the groove occupied by the movable partition (Figure 2-1). The FVP station (50W) required the least number of grabs to fill an isolator (approximately 1.5 grabs) as the sediment contained a significant fraction of sand. The fine-grained sediment at the MQR (100E) required 4 grabs per isolator, and the CLIS-REF required 5 grabs/isolator. The West Haven breakwater station required between 1.5 to 4.5 grabs to fill each isolator with a mean of 2.5 grabs/isolator (n=25).

The sequence of events from filling the isolator with the 300 μm sieved fraction to final removal of the worms is shown in Figure 2-2. Approximately 51 cc (bulk volume) of the 300 μm sieved residue was introduced into an isolator, which filled the isolator below the partition groove (Figure 2-2A). The partition was then closed over the sediment and the

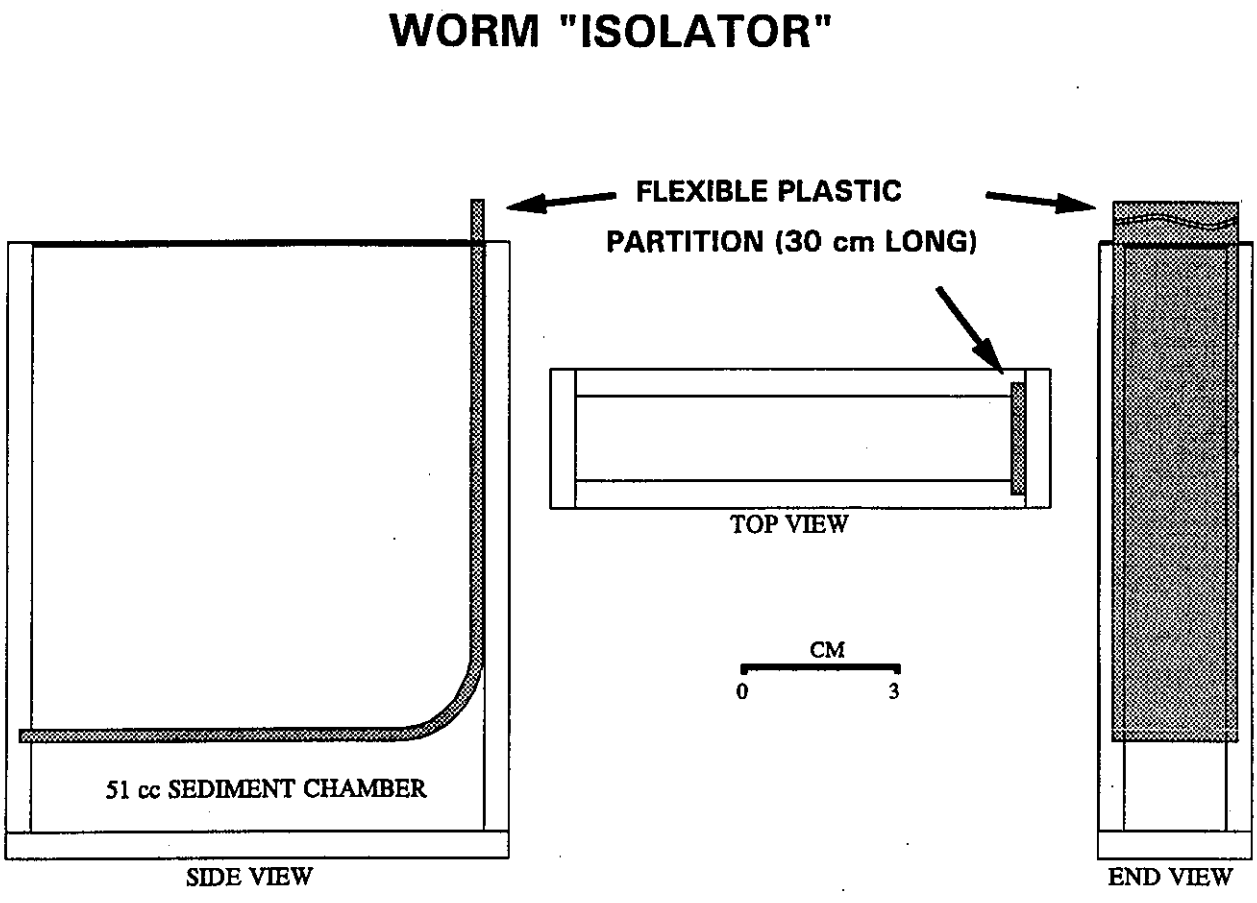

Figure 2-1. Diagram of the plexiglas worm isolator. The flexible plastic partition is capable of being moved in a milled slot, and is used to close off the 51 cc sediment/worm chamber.

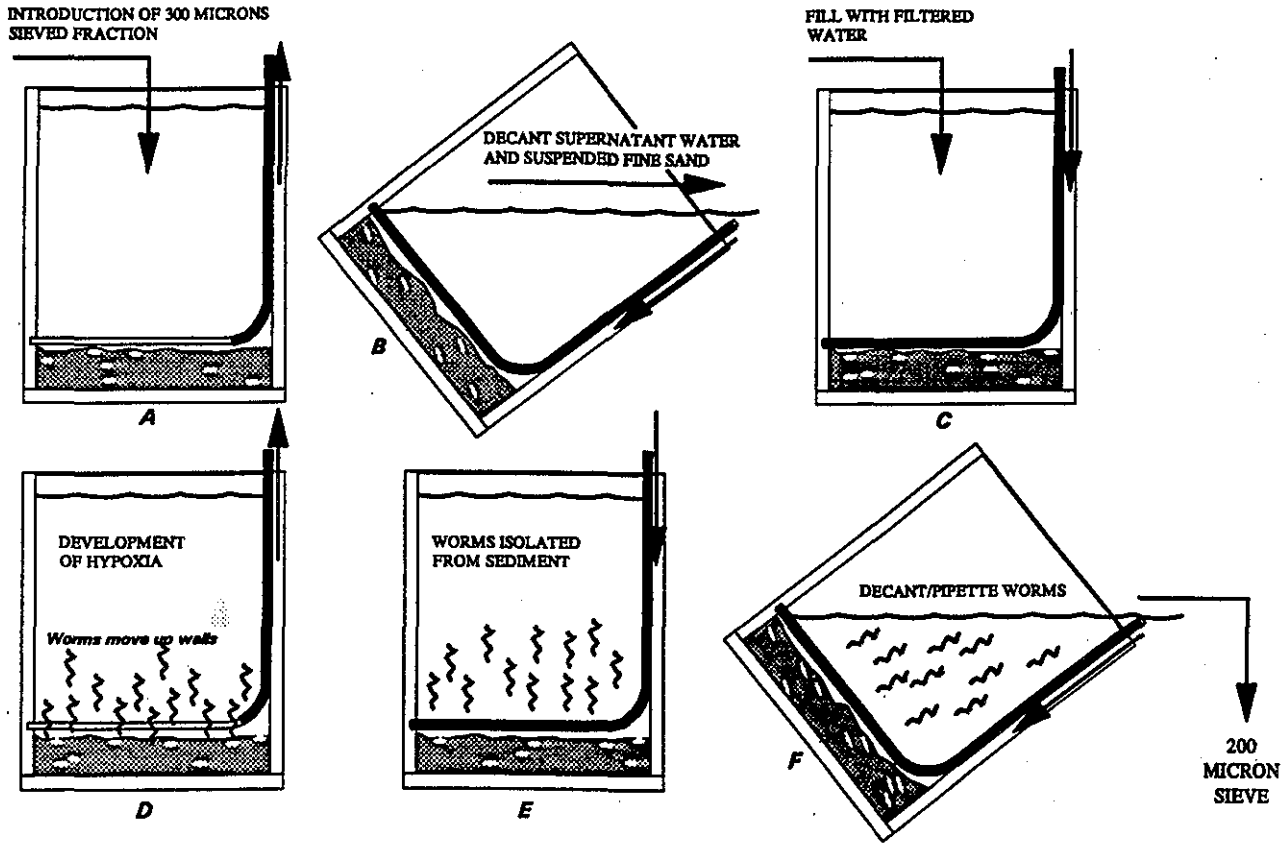

Figure 2-2. Sequence of steps leading to the isolation of Stage I worms

overlying turbid water decanted (Figure 2-2B). With the partition still in place, clean filtered seawater was added to fill the isolator. In some cases, this consisted of water deoxygenated with sodium sulphite (50, 100, 200, and 400 $mg \cdot l^{-1}$ of sodium sulphite added to the water). In other trials, filtered water without sodium sulphite was used (Figure 2-2C). Once the isolator was filled with seawater, the partition was removed and the sediment/tube/worm filtrate exposed to the overlying water column. During this step, the isolators were accumulated in a holding box and shielded from ambient light. The T=0 time for each isolator was recorded in order to determine the time for worms to crawl out of the sediment (Figure 2-2D). After 16 to 19 hours, once a dense aggregation of worms had moved above the partition groove, the partition was positioned to seal off the worms from the sediment (Figure 2-2E). The worms were either decanted or pipetted into a 200-μm fabric mesh sieve for weighing and subsequent freezing for storage (Figure 2-2F).

Estimates of bulk (blotted) wet weight of worms were made by weighing the sieve and worms and subtracting the wet tared weight of the sieve. Subsamples were taken of the worm samples for taxonomic identification and preserved in buffered formalin. The bulk of the worms were immediately frozen on dry ice and remained frozen until analyzed for tissue chemistry.

2.3 Faunal Analyses

A limited faunal analysis was performed on subsamples taken from isolators. The purpose of this analysis was to determine the relative proportion of Stage I worms present at each sampling site. An aliquot (ranging from approximately 100 to 280 specimens) from each preserved subsample was placed into a shallow dish with alcohol, and major taxa were sorted and identified under 8× to 50× magnification. The frequency of occurrence of each taxon at each study site is given in Appendix A. These data do not allow determination of field densities, only an estimate of dominance within that population of species that were driven out of the sediment (i.e., isolator) by induced hypoxia.

2.4 Chemical Analyses

The bulk surface sample was analyzed by AmTest, Inc. (Redmond, WA) in November 1991 for total organic carbon (TOC) using EPA Method 9060; total solids; pesticides and PCBs using EPA Method 8080; and semivolatile organics using EPA method 8270 (all methods from SW-846; EPA 1986). The sediment was also analyzed for total petroleum hydrocarbons (EPA Method 418.1) and total metals (EPA Method 6010). Surrogate recoveries for organics analyses were within acceptable limits, and sediment data quality was acceptable.

Two tissue samples were sent to the Geochemical and Environmental Research Group (GERG; College Station, TX). Tissues were analyzed for aliphatic hydrocarbons, aromatic

hydrocarbons, chlorinated pesticides, and total PCBs as well as 18 individual congeners in June 1992. Tissue samples were homogenized before extraction, and a subsection of the sample was taken for analysis of wet weight and dry weight. All organic analyses were performed with a gas chromatograph/mass spectrometer following the methods outlined for the NOAA National Status and Trends Program, where applicable (NOAA 1989).

Aliphatic and aromatic hydrocarbon data were accompanied with a procedural blank, a spiked blank, and a control sample; additionally, every sample was spiked with several surrogate compounds. Surrogate and spike recoveries indicated efficient extraction and accurate analysis. Organochlorine data (pesticides and PCBs) were accompanied with a blank, a spiked blank, and surrogates. The recovery rate of the spiked surrogate (PCB) was 100% ± 9.4%; tissue data quality was considered acceptable.

2.5 Calculation of TBP

In order to evaluate the analytical methods appropriate for worm tissue analysis, we used the Theoretical Bioaccumulation Potential (TBP) equation given in the testing manual, "Evaluation of Dredged Material Proposed for Ocean Disposal" (EPA/ACE 1991). The TBP is an approximation of the equilibrium concentration of the nonpolar organic chemical concentrations in tissues. The approximation of nonpolar contaminants (those that do not readily dissociate) is calculated based on the assumption that the dredged material in question is the only source of contaminants to the organisms.

The following equation allows one to estimate the whole-body TBP from three variables: TOC of the source sediment (%TOC), concentration of the nonpolar organic chemical in the sediment (C_s), and organism lipid content (%L). A range of accumulation factors (AF), or the ratio of the tissue concentration (normalized to lipid content) to the sediment concentration (normalized to TOC), has been determined through field studies of larger burrowing benthic organisms (e.g., Rubinstein et al. 1987). A range of AFs from 0.2 to 10.9 for PCBs in infaunal organisms has been determined in previous studies; a value of 4 was deemed appropriate for the TBP calculation:

$$TBP = 4(C_s / \%TOC)\ \%L$$

For the West Haven station (the only station yielding enough worms for analysis), TOC was 2.7% and the average lipid content of the dominant polychaete (*Capitella capitata*) is approximately 9% of the total wet weight of the worm, but ranges from 5 to 20% (K. Tenore, personal communication). Substituting the concentration of total petroleum hydrocarbons (TPH) as the estimate of total petroleum hydrocarbons (220 μg/g or ppm; Appendix B) for C_s, for the average lipid concentration, the TBP is estimated to be:

$$TBP = 4\ (220\ ppm / .027)\ 0.09$$

TBP= 2933 ppm (potential tissue concentration of total petroleum hydrocarbons)

The total range for the TBP using the values of 5% to 20% lipids for *C. capitata* is 1629 to 6518 ppm. It is reasonable to assume that the late summer represents a period when the detrital pool is so low in micronutrients such as essential fatty acids (Marsh and Tenore 1990) that tissue lipid reserves are probably at the 5% minimum. For this reason we estimate that the tissues could contain approximately 1600 ppm total petroleum hydrocarbons, greater than sevenfold that of the ambient sediment.

3.0 RESULTS

3.1 Recovery of Worm Biomass

3.1.1 MQR, FVP, and CLIS-REF Stations

In the course of preparing the isolators for these stations, it was clear that the abundance of Stage I worms was much lower than expected based on the June REMOTS® survey. This may be attributed to the washing away of worm populations from the elevated surface of the disposal mounds during the hurricane and/or the failure of recruitment and sustained growth of worms during the "oligotrophic" late summer period (Gremare et al. 1989, Marsh and Tenore 1990). We expected to encounter low population densities at the CLIS-REF station because of trophic group amensalism, i.e., exclusion of tube dwellers and filter feeders by bioturbating deposit feeders (Rhoads and Young 1970), but the MQR and FVP stations also appeared to have low densities of worms.

After the first day of sampling (3 September) we noted that, after a period of 4 to 5 hours, the only organisms to move up the side walls of the 5 prepared isolators were small gastropods and flatworms (Platyhelminthes). These isolators were rechecked on the following morning after a lapsed period of 14 to 16 hours. A few worms were observed at the surface of the sediment, but no worms had traversed the wall to above the partition groove. These five isolators from the MQR and CLIS-REF were pooled and preserved in formalin for taxonomic analysis. The FVP station was dominated by cossurid and capitellid polychaetes. The MQR station was dominated by cossurids and oligochaetes, and the CLIS-REF station by paraonids and *Nephtys* (Appendix A). No tissue samples were retained for chemical analysis.

We thought that the concentration of sodium sulphite used on the first day may have been too strong (400 $mg \cdot l^{-1}$), inhibiting the ability of the worms to crawl up the walls of the isolators. On the second day of sampling, the FVP (50W) was sampled, and four isolators were prepared with variable concentrations of sodium sulphite (2 isolators with 200 $mg \cdot l^{-1}$, one with 100 $mg \cdot l^{-1}$, and one with normal filtered seawater). After approximately four hours, the isolators were checked for worms. A few worms were observed on the surface of the sediment of each isolator, but none had moved onto the walls. Varying the concentration of sodium sulfite resulted in no apparent difference.

A sample of the surface sediment from FVP was decanted into a Petri dish and observed under the microscope. Specimens of *Mediomastus* sp., *Capitella* sp., and a spionid were observed, but the worms appeared to be relatively inactive. It was clear from our sampling at CLIS and at CLIS-REF that the ambient concentration of worms was too low to obtain gram quantities of tissues with any reasonable sampling effort. In the course of our sampling at MQR and FVP, we moved to other locations on these mounds and examined the

300 μm sieved residues for worms. This reconnaissance exercise convinced us that these areas had very low densities of Stage I worms, and we decided to move the sampling effort to New Haven Harbor.

3.1.2 New Haven Harbor

New Haven Harbor was sampled on the basis of past experience; from 1965 through the early 1980s, D. C. Rhoads and his students at Yale reliably collected Stage I worms and bivalves at the west end of the Luddington Breakwater in outer New Haven Harbor. This large area of sampling is approximately 25 feet deep between the west end of the breakwater and the West Haven shore. The navigation chart identifies the bottom type as "soft". A high density of active Stage I worms was present in the first grab, dominated by capitellids, the spionid *Streblospio*, and oligochaetes. Based on this result, all subsequent sampling was done at this station (10 hours).

On 4 September, ten isolators were prepared between 1245 and 1550 hours (h) at the New Haven site. Concentrations of sodium sulphite in the water introduced into the isolators were again varied, from zero (ambient filtered water) to 50, 100, 200, and 400 $mg \cdot l^{-1}$. At 1800 h the isolators were checked for worm activity. All of the isolators showed worms had moved up onto the walls except the last isolator prepared. By 2035 h, tens of worms/isolator were actively moving up the walls of all of the isolators. Based on the rate of vertical migration of the worms it was decided to wait until the following morning to collect the worms. At 0800 h on 5 September, worms were removed from the ten isolators and frozen on dry ice, representing a period of 16 to 19 hours after the worms were first introduced into the isolators.

Twenty-five isolators were prepared between 1020 and 1620 h on 5 September using the same range of concentrations of sodium sulphite. By 2000 h, sufficient densities of worms were present on the walls of the first 21 of 25 isolators to justify their removal. Because a high proportion of the worm population was contained within the horizontal part of the partition groove, we did not slide the partition closed over the sediment. Instead, a pipette was used to remove the worms from the walls and groove. This initial removal process collected approximately 0.6 g wet weight of worm tissue from the first 15 isolators (only the first 15 had sufficient biomass for weighing). Wet weights for isolators 1-5, 6-10, and 11-15 were each measured at 0.20 g (0.04 g/isolator). A total of 0.60 g of tissue was frozen on dry ice at 2145 h on this day.

At 0900 h on 6 September, more worms had moved up onto the walls and partition groove of the 25 isolators. Additional worms were removed by pipette from the first 15 isolators sampled on the previous day. The second sampling yielded an additional 0.80 g of tissue from these isolators for a cumulative total yield of 1.40 g (0.09 g/isolator). The balance of isolators 16-25 were sampled only once on 6 September and yielded a total tissue

weight of 0.90 g (0.09 g/isolator). A summary of the estimated total tissue yield per sampling effort is given in Table 3-1. We saw no significant difference in the rate of migration of the worms out of the sediment as a function of the concentration of sodium sulphite. We interpret this result to mean that the concentration of respiring biomass within an isolator results in hypoxia even when sodium sulphite is absent.

Table 3-1

Yield of Worm Tissue Per Sampling Effort

YIELD/EFFORT*

EFFORT: 90 Grab Samples

- Taken in 10 hours on station (1 grab/7 minutes)
- Approximately 9 m^2 of bottom sampled
- Approximately 50 liters of sediment, subsampled for worms
- 2.5 grabs/isolator

YIELD: Three g (wet weight) of worm tissue

- Approximately 6000 worms
- Approximately 600-700 worms/m^2
- Each isolator produced 0.09 g wet weight of tissue
- A total of 3 g (wet weight) using 35 isolators

*FIELD PERSONNEL
2 deploying grab & subsampling surface 2 sieving 1 preparing isolators 1 recorder

3.2 Sediment Chemistry

A suite of 33 metals, 19 pesticides, 7 PCBs, and 69 semivolatile organic compounds were analyzed from New Haven Harbor sediment where the worms were collected. Results were used to identify contaminants present in sufficient concentration to be of interest for the bioaccumulation study. Concentrations of metals that are typically attributed to anthropogenic sources (e.g., lead, mercury, and chromium) were low in concentration at this station; therefore, metals were not analyzed in the worm tissue.

The bulk surface sample contained no detectable concentrations of pesticides or PCBs. Eleven semivolatile compounds were detected in concentrations ranging from 78 to 23,000 μg/kg dry weight (ppb; Appendix B). The concentration of TPH (generally the sum of aliphatic and aromatic hydrocarbons) was 220 μg/g (ppm; Appendix B). These organic compounds probably represent residues of solvents and combustion products. Because these semivolatile compounds have a clear anthropogenic source, we decided to analyze only the relatively small biomass of collected worm tissues for total hexane extractable hydrocarbons.

3.3 Tissue Bioaccumulation of Hydrocarbon Contaminants

The two tissue sample results were averaged for TBP calculation purposes (Appendix C). Detection limits for tissues were lower than those for sediments, so many more hydrocarbon and organochlorine (pesticide/PCB) compounds were detected in the tissues than in the sediment (Appendix C). Worm tissues contained an average of approximately 40,000 μg/kg total alkanes (aliphatic hydrocarbons) and 3,148 μg/kg total aromatic hydrocarbons. The approximate total petroleum hydrocarbon (TPH) concentration, summing both the aliphatic and aromatic fractions, is 43,148 ppb ($\sim$43 ppm).

Individual concentrations of polycyclic aromatic hydrocarbons (PAHs) detected in both the sediment sample and the tissue samples were used for calculation of TBP. Calculated TBP values for individual semivolatile compounds ranged from 1040 to 2933 ppb. Actual tissue concentrations for semivolatile compounds ranged from 7 to 92 ppb (Appendix C). The minimum estimate for the TBP of TPH, as calculated in Section 2.5 above, was 1600 ppm (Table 3-2). Both the actual tissue PAH values, and the estimated TPH concentration in the worm tissues discussed above (43 ppm), are much lower than predicted using the TBP calculation.

Table 3-2

Calculated Theoretical Bioaccumulation Potential (TBP) as Compared to Actual Tissue Concentrations

Nonpolar Compound	Sediment Concentration (ppb)	TOC (%)	Lipids (%)	Calculated TBP (ppb)	Actual Tissue Concentration* (ppb)	Calculated AF**
Phenanthrene	87	2.7	9	1160	92	0.32
Pyrene	220	2.7	9	2933	35.9	0.05
Benzo(a)anthracene	78	2.7	9	1040	7.1	0.03
Chrysene	120	2.7	9	1600	13.1	0.03
Benzo(b)fluoranthene	120	2.7	9	1600	27.7	0.07
Benzo(k)fluoranthene	90	2.7	9	1200	12.6	0.04
Benzo(a)pyrene	120	2.7	9	1600	9.1	0.02
TPH (ppm)	220	2.7	5	1630	43	0.11

* Tissue concentrations are averaged between two samples.

** Accumulation Factor, calculated using actual sediment and tissue concentrations.

The discrepancy between the calculated bioaccumulation potential and the actual contaminant concentrations in the worm tissues may indicate a difference between the laboratory methods and matrices used to produce the chemical results. Both of the sediment and tissue samples were held longer than EPA recommended holding times for organic constituents (EPA 1988). The volatile fraction of the hydrocarbons (especially aliphatic tissue data) could be underestimated, even though the samples were frozen.

Such a large discrepancy between the TBP and the tissue concentrations suggests that differences in laboratory methods are not a sufficient explanation, and that the TBP calculation as it exists may not be an applicable tool in all environmental situations. For example, the bioaccumulation potential should vary between species because of differences in sediment residence times of the organisms (the time available to bioaccumulate), metabolic rates, species specific detoxification strategies, and the feeding and digestive efficiency. An accumulation factor (AF) of 4 was used for our TBP calculations, based on previous work. Calculating the actual AF using sediment and tissue hydrocarbon concentrations resulted in a more realistic range of 0.02 to 0.07 for all of the PAHs except phenanthrene (0.32), and an AF of 0.11 for TPH (Table 3-2). It is apparent that the AF required for accurate calculation of TBP for Stage I organisms may be lower than that for Stage III organisms.

4.0 DISCUSSION AND CONCLUSIONS

It appears that the sampling effort in central Long Island Sound may have been ill-timed due to the passage of Hurricane Bob over the Sound about two weeks prior to this cruise. Future sampling should avoid poststorm conditions as the upper 1 to 2 cm of the surface of disposal mounds may be washed away, including the Stage I worms. To optimize the "catch per unit effort" we recommend that sampling be done a few weeks after completion of a new project, preferably in the spring when there is sufficient labile detritus to fuel an exponential population growth of worms. By late summer, the detrital pool tends to consist of refractory substrates and a low inventory of micronutrients such as essential fatty and amino acids. This oligotrophic period leads to low densities of opportunists (Marsh and Tenore 1990).

Even under conditions of relatively low population densities (West Haven Station), we were able to obtain 3 g wet weight/ 10 hours at an estimated ambient density of approximately 600 worms/m^2. Under higher density conditions typically encountered at new disposal points (say, 60,000/m^2), we theoretically could have obtained 30 g of wet tissue with approximately one hour of field collection. This assumes that individual biomass values are independent of density. In fact, this is not true, as individual biomass tends to decrease under conditions of high population density. Data on *Capitella capitata* type I from Gremare et al. (1989) indicate that 10,000 worms yielded 1 g dry weight of tissue (Marsh and Tenore 1990). Converting dry weight to wet weight, 10,000 worms could yield 16 g of wet weight tissue. At a measured peak density of 205,000/m^2, 200 g wet weight of tissue potentially could be obtained.

The isolator design could benefit from one minor change. As the worms crawl vertically out of the sediment, they encounter the horizontal 2 mm deep groove for the sliding partition. As the worms attempt to cross this groove, they are diverted laterally along the groove, and many worms remain aggregated within the groove. This means that the partition cannot be closed over the sediment to decant the worms. To do so would result in a great deal of tissue damage and loss of associated body fluids and contaminants. Therefore, the worms must be removed with a pipette. The new design should take advantage of this phenomenon and utilize such grooves to direct the worms vertically rather than horizontally. This will then allow the partition to be used to seal off the sediment so that the worms on the walls can be efficiently decanted (Figure 4-1).

The technique of eliminating free oxygen in the supernatant water of an isolator by introduction of sodium sulphite does not appear to be necessary. Because biomass is concentrated into the isolators, oxygen is rapidly removed by respiration.

Chemistry results indicate that, although Stage I species may play an important role in the upper sediment column cycling of contaminants, the current TBP model may not

accurately predict bioaccumulation in these organisms. The TBP calculation is based on a simple ratio between the sediment and tissue contaminant concentrations, with an accumulation factor calculated empirically. The model has been reasonably well tested for PCBs with positive results for larger, primarily burrowing infaunal benthic organisms with longer life spans and smaller surface areas per unit volume relative to the Stage I worms (e.g., Rubinstein et al. 1987). These organisms tend to reflect an integration of sediment contaminants over a longer period of time, in contrast to the rapidly established Stage I organisms. Moreover, it is possible that Stage I organisms have different detoxification strategies to avoid or eliminate contaminant uptake (Cuomo 1985, Klerks and Levinton 1992).

The inability of the model to predict accurately hydrocarbon concentrations in the surface-dwelling polychaete and oligochaete populations indicates that the model is probably not appropriate for these shorter lived organisms. As the resulting tissue concentrations were much lower than the TBP predicted values, the worms are apparently not as efficient at bioaccumulating as compared to the longer lived burrowing Stage III organisms. Reduced bioaccumulation of metals has been shown for several organisms, including bacteria, algae, annelids, and fish (Klerks and Bartholomew 1991). Organisms coping with contaminated sediments by "under-bioaccumulating" may thus lower the bioavailability of these contaminants to larger predators. These preliminary results may lessen the concern about Stage I organisms providing a pathway for contaminants into the foodchains leading to humans.

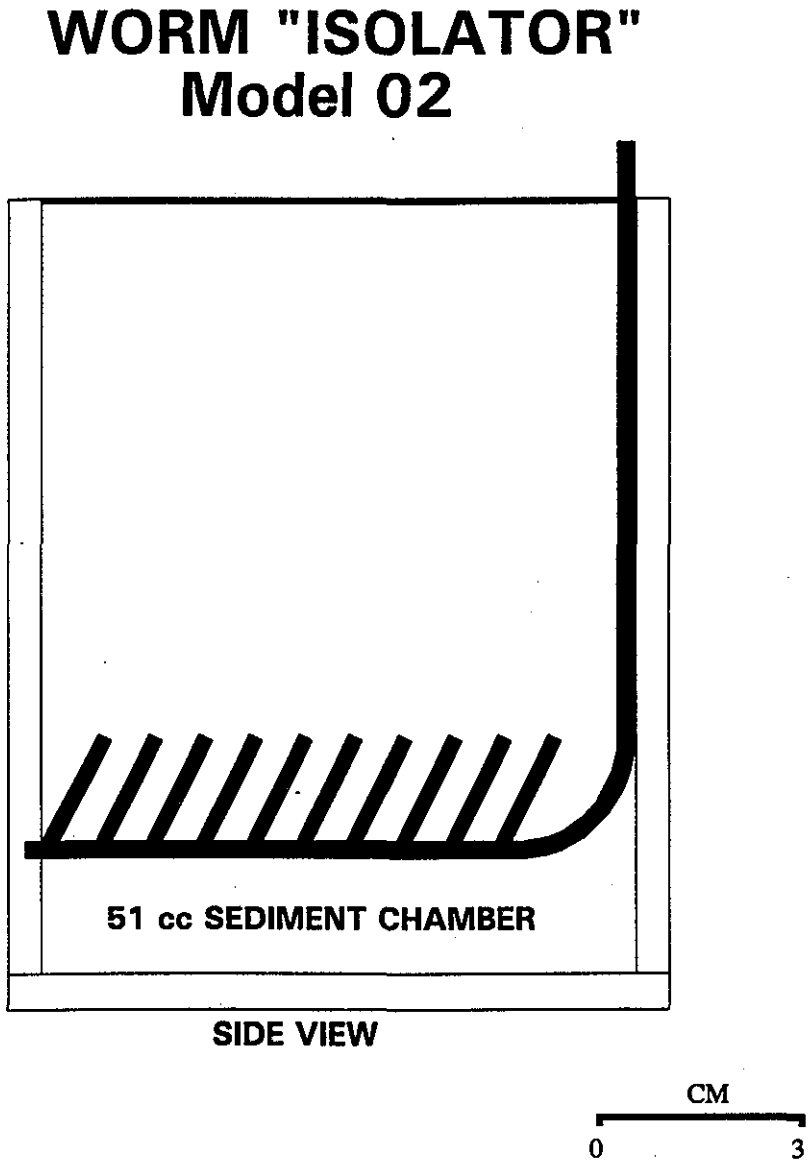

Figure 4-1. Suggested modifications to the prototype isolator. Inclined grooves leading from the sliding partition groove are cut into the walls of the isolator. These additional grooves are necessary to direct the worms away from the sliding partition groove, and are inclined so that the leading edge of the sliding partition does not hang-up on the edge of the converging grooves.

5.0 RECOMMENDATIONS

- A repeat experiment using a new isolator which does not require pipetting, and therefore increases sampling efficiency.
- Avoid sampling after major storm events.
- Recovery of additional sample tissue material to produce a more accurate estimate of lipid content.
- Consistency of laboratories and methods for both sediment and tissue matrices.
- Review of the current assumptions implicit to the theoretical bioaccumulation potential (TBP) calculation and re-evaluation for use with Stage I organisms, including the value for the accumulation factor (AF).

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APPENDIX A
Faunal Composition at Sampled Stations

CLIS-FVP

Class	Taxa	Frequency
Polychaeta	cossurids	48
	capitellids	21
	Nephtys	17
	paraonids	9
Oligochaeta	oligochaetes	18
Bivalvia	Nucula	13
	Mulinia	4
	Other	5
Other	nemertian	1
	copepods	3
	kinorhynch	1

CLIS-MQR

Class	Taxa	Frequency
Polychaeta	cossurids	26
	Nephtys	6
	capitellids	4
	paraonids	2
	ampharetid	1
	Pectinaria	1
Oligochaeta	oligochaetes	35
Bivalvia	Nucula	9
	Mulinia	5
	Other	5
Other	gastropods	3
	ostracods	2
	kinorhynchs	3

APPENDIX A (cont.)

NEW HAVEN HARBOR

Class	Taxa	Frequency
Polychaeta	capitellids	190
	Streblospio	30
	cossurids	12
	Nephtys	7
	Pectinaria	3
	cirratulids	2
	Polydora	1
	hesionid	1
Oligochaeta	oligochaetes	46
Bivalvia	tellinaceans	13
	Nucula	6
	Mulinia	1
	other	1
Other	amphipods	3
	ostracod	1
	copepod	1
	sipunculid	1

APPENDIX A (cont.)

CLIS-REF

Class	Taxa	Frequency
Polychaeta	paraonids	25
	Nephtys	19
	cossurids	8
	cirratulids	2
	pilargids	2
	capitellid	1
	phyllodocid	1
Oligochaeta	oligochaetes	4
Bivalvia	Nucula	13
	Mulinia	7
	other	5
Other	gastropod	1
	nemertian	1
	ostracods	2
	turbellarian	1

APPENDIX B

New Haven Harbor
Bulk Sediment Chemistry: Detected Results

Analyte	Result*
Conventionals (%)	
Total Organic Carbon	2.7
Total Solids	90
Total Petroleum Hydrocarbons (ppm)	220
Semivolatile Organic Compounds (ppb)	
Phenol	1100
4-Methylphenol	23000
Benzoic Acid	1400
Phenanthrene	87
Fluoranthene	190
Pyrene	220
Benzo(a)anthracene	78
Chrysene	120
Benzo(b)fluoranthene	120
Benzo(k)fluoranthene	90
Benzo(a)pyrene	120

* Results based on single surface sample.

APPENDIX C

Table 1

New Haven Harbor

Tissue Aliphatic Hydrocarbons

Sample ID:	Worms 1	Worms 2	Mean
Laboratory ID:	03418	03419	Value
Alkanes and Isoprenoids (ppb)			
C10	0	0	0.00
C11	12.23	12.9	12.57
C12	5.52	8.22	6.87
C13	22.58	26.49	24.54
C14	14.3	34.82	24.56
C15	50.77	137.13	93.95
C16	241.85	561.75	401.80
C17	1477.3	2381	1929.15
Pristane	393.8	604.1	498.95
C18	3613.7	6730.8	5172.25
Phytane	1945.6	3120.8	2533.20
C19	7267.4	14109.7	10688.55
C20	9438.1	11324.1	10381.10
C21	4432.1	3963.1	4197.60
C22	994.3	816.2	905.25
C23	162.81	172.77	167.79
C24	74.11	76.32	75.22
C25	37.01	68.24	52.63
C26	43.66	39.92	41.79
C27	98.59	130.35	114.47
C28	188.27	58.46	123.37
C29	344.03	287.65	315.84
C30	554.13	95.67	324.90
C31	730.43	321.53	525.98
C32	796.62	99.76	448.19
C33	628.06	111.65	369.86
C34	492.6	42.21	267.41
Total Alkanes	34059.9	45335.6	39697.75

APPENDIX C

Table 2

New Haven Harbor

Tissue Organochlorine Hydrocarbons

SAMPLE ID	Worms1	Worms2	Mean
LABORATORY ID	03418P	03419P	Value
Total Organochlorines (ppb)			
Total BHCs	3.4	4.5	3.95
Total Chlordanes	7.0	7.5	7.25
Total DDTs	7.0	8.1	7.55
Total PCBs	68.4	98.9	83.65
Chlorinated Pesticides (ppb)			
Alpha-BHC	0.0	0.0	0.0
HCB	0.31	0.2	0.255
Beta-BHC	0.42	0.42	0.42
Gamma-BHC	2.26	3.23	2.745
Delta-BHC	0.37	0.63	0.5
Heptachlor	0.04	0	0.02
Heptachlor Epoxide	0.58	0.32	0.45
Oxychlordane	0.38	0.68	0.53
gamma-Chlordane	2.38	2.63	2.505
alpha-Chlordane	1.85	1.8	1.825
Trans-Nonachlor	1.09	1.23	1.16
Cis-Nonachlor	0.67	0.86	0.765
Aldrin	0.0	0.0	0.0
Dieldrin	1.58	1.78	1.68
Endrin	0.0	0.0	0.0
Mirex	0.3	0.13	0.215
2,4' DDE	0.0	0.0	0.0
4,4' DDE	3.2	4.29	3.745
2,4' DDD	0.47	0.61	0.54
4,4' DDD	0.9	1.08	0.99
2,4' DDT	0.97	0.9	0.935
4,4' DDT	1.43	1.17	1.3

APPENDIX C

Table 2 (cont.)

New Haven Harbor

Tissue Organochlorine Hydrocarbons (cont.)

SAMPLE ID	Worms1	Worms2	Mean
LABORATORY ID	03418P	03419P	Value
Polychlorinated Biphenyls (ppb)			
PCB#8 (CL2)	0.0	0.0	0.0
PCB#18 (CL3)	0.3	0.3	0.3
PCB#28 (CL3)	1.3	1.6	1.45
PCB#44 (CL4)	2.0	2.1	2.05
PCB#52 (CL4)	2.1	2.7	2.4
PCB#66 (CL4)	1.5	1.7	1.6
PCB#101 (CL5)	2.9	5.6	4.25
PCB#105 (CL5)	1.0	1.5	1.25
PCB#110/77 (CL5/4)	2.6	4.6	3.6
PCB#118/108/149 (CL5/5/6)	1.7	4.0	2.85
PCB#128 (CL6)	0.6	1.0	0.8
PCB#138 (CL6)	6.3	6.7	6.5
PCB#126 (CL5)	0.4	0.0	0.2
PCB#153 (CL6)	1.4	5.5	3.45
PCB#180 (CL7)	1.2	2.5	1.85
PCB#187/182/159 (CL7/7/6)	0.8	2.4	1.6
PCB#195 (CL8)	0.7	0.9	0.8
PCB#206 (CL9)	1.1	1.2	1.15
PCB#209 (CL10)	0.9	0.7	0.8

APPENDIX C
Table 3

New Haven Harbor
Tissue Aromatic Hydrocarbons (ppb)

Sample ID:	Worms 1	Worms 2	Mean
Laboratory ID:	03418	03419	Value
Naphthalene	6.48	7.56	7.02
C1-Naphthalenes	7.73	8.81	8.27
C2-Naphthalenes	21.2	14.19	17.70
C3-Naphthalenes	43.09	43.5	43.30
C4-Naphthalenes	41.14	48.59	44.87
Biphenyl	5.0	3.58	4.29
Acenaphthylene	0.87	5.56	3.22
Acenaphthene	2.2	2.65	2.43
Fluorene	5.13	10.42	7.78
C1-Fluorenes	34.52	38.68	36.60
C2-Fluorenes	203.86	186.8	195.33
C3-Fluorenes	438.01	357.47	397.74
Phenanthrene	85.97	97.95	91.96
Anthracene	4.45	6.93	5.69
C1-Phenanthrene_anthracene	442.42	437.36	439.89
C2-Phenanthrene_anthracene	653.07	545.17	599.12
C3-Phenanthrene_anthracene	247.47	201.61	224.54
C4-Phenanthrene_anthracene	85.33	70.61	77.97
Dibenzothiophene	18.15	18.97	18.56
C1-Dibenzothiophenes	104.3	115.39	109.85
C2-Dibenzothiophenes	270.22	278.66	274.44
C3-Dibenzothiophenes	165.02	151.93	158.48
Fluoranthene	20.88	30.24	25.56
Pyrene	30.76	40.94	35.85
C1-Fluoranthene_pyrene	25.54	29.91	27.73
Benzo(a)anthracene	3.27	11	7.14
Chrysene	5.21	21.07	13.14
C1-Chrysenes	0.0	22.08	11.04
C2-Chrysenes	0.0	31.98	15.99
C3-Chrysenes	0.0	17.61	8.81
C4-Chrysenes	0.0	14.79	7.40
Benzo(b)fluoranthene	8.46	46.95	27.71
Benzo(k)fluoranthene	4.53	20.64	12.59
Benzo(e)pyrene	5.95	30.73	18.34
Benzo(a)pyrene	4.07	14.04	9.06
Perylene	1.99	4.42	3.21
Indeno(1,2,3-cd)pyrene	3.54	24.29	13.92
Dibenzo(a,h)anthracene	2.11	4.15	3.13
Benzo(g,h,i)perylene	5.75	28.97	17.36
2-Methylnaphthalene	4.53	4.53	4.53
1-Methylnaphthalene	3.2	4.28	3.74
2,6-Dimethylnaphthalene	4.45	4.66	4.56
2,3,5-Trimethylnaphthalene	6.11	8.33	7.22
1-Methylphenanthrene	99.47	103.19	101.33
Total Aromatic Hydrocarbons	3125.5	3171.2	3148.3