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Evaluation of Field Bioaccumulation as a Monitoring Tool

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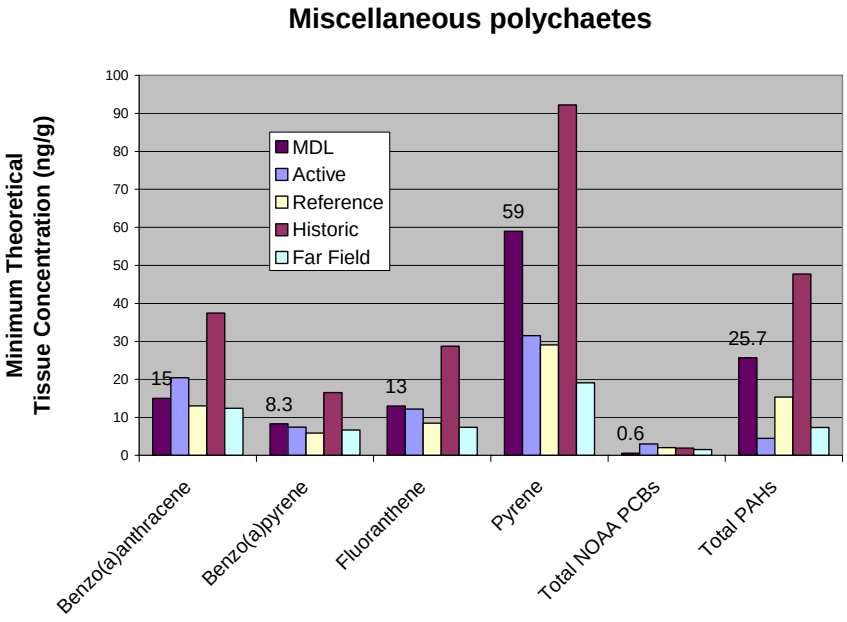

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13. ABSTRACT

Laboratory methods for measuring body burdens of low-level chemical contaminants in aquatic organisms traditionally have required sizeable amounts of tissue for analysis (i.e., 10 to 30 grams wet weight of tissue per sample). Predisposal testing of dredged material and postdisposal monitoring studies therefore have focused almost exclusively on examining bioaccumulation in large benthic macrofauna. There has long been interest, however, in evaluating bioaccumulation potential in the smaller, opportunistic benthic organisms that are typically the first to colonize new dredged material deposits in high numbers.

The following activities were undertaken as part of this desk-top study: 1) experts were interviewed about current analytical capabilities and costs, 2) published information on new developments in microscale analytical techniques was reviewed, 3) theoretical contaminant body burdens were calculated for representative small, opportunistic (i.e., Stage 1) benthic species from Long Island Sound (LIS) disposal sites and reference areas, which allowed estimates of required organism numbers for potential future studies, and 4) power analyses were employed to estimate the sample number required for a statistically valid comparison of tissue concentrations in Stage 1 organisms collected over DAMOS disposal mounds versus reference areas.

A first-order power analysis using LIS sediment chemistry data indicates that from 5 to 20 individual tissue samples would need to be collected and analyzed to reliably detect any significant differences that might exist between a disposal mound and reference area in the body burdens of various organic contaminants. The theoretical bioaccumulation calculations presented in this report, however, suggest there would be little actual difference in tissue concentrations measured at active DAMOS disposal mounds versus reference areas. In lieu of conducting field studies to test for small differences in bioaccumulation between disposal mound and reference areas, it might be more useful to develop more advanced food chain and/or risk assessment models, with laboratory exposures and/or field collections targeted toward filling any identified data gaps.

In the future, DAMOS might consider employing these microscale methods in one or more special investigative studies outside its routine monitoring efforts. Such studies could help determine, for example, whether the use of small, Stage 1 test organisms changes the outcome of field or laboratory investigations of bioaccumulation potential that have traditionally focused on larger taxa.

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EXECUTIVE SUMMARY

Laboratory methods for measuring body burdens of low-level chemical contaminants in aquatic organisms traditionally have required sizeable amounts of tissue for analysis (i.e., 10 to 30 grams wet weight of tissue per sample). Predisposal testing of dredged material and postdisposal monitoring studies therefore have focused almost exclusively on examining bioaccumulation in large benthic macrofauna. There has long been interest, however, in evaluating bioaccumulation potential in the smaller, opportunistic benthic organisms that are typically the first to colonize new dredged material deposits in high numbers.

The specific concern is that rapid bioaccumulation by these abundant, fast-growing species might result in significant trophic transfer and biomagnification of low-level contaminants, ultimately leading to significant ecological or human health risks. In response to such concerns, DAMOS scientists conducted this desk-top study to review current methods for measuring low-level contaminants in very small, sediment-dwelling organisms and to offer suggestions about how future DAMOS assessments of bioaccumulation might benefit from recent advances in analytical techniques.

The following activities were undertaken as part of this desk-top study: 1) experts were interviewed about current analytical capabilities and costs, 2) published information on new developments in microscale analytical techniques was reviewed, 3) theoretical contaminant body burdens were calculated for representative small, opportunistic (i.e., Stage 1) benthic species from Long Island Sound (LIS) disposal sites and reference areas, which allowed estimates of required organism numbers for potential future studies, and 4) power analyses were employed to estimate the sample number required for a statistically valid comparison of tissue concentrations in Stage 1 organisms collected over DAMOS disposal mounds versus reference areas.

The interviews with experts and the accompanying literature reviews indicated that "microscale" or "microextraction" analytical techniques currently exist and have been used with success to measure both lipids and environmentally realistic concentrations of bioaccumulative organic contaminants (polynuclear aromatic hydrocarbons [PAHs] and polychlorinated biphenyls [PCBs]) in small masses of tissue. Researchers at the U.S. Army Engineer Research and Development Center (ERDC), University of South Carolina (USC), and the State University of New York at Stony Brook's Marine Sciences Research Center (MSRC) have spearheaded the development and application of these microscale methods. The various research groups have reported measuring low levels of PAHs and PCBs in samples containing as few as 20 individual copepods, 3 to 15 amphipods, and 3 to 5 individuals of the small spionid polychaete *Streblospio benedicti*. The total amounts of tissue per sample required by the microscale methods ranged from

0.5 to 100 mg wet weight; these amounts are 3 to 5 orders of magnitude less than the 25,000 to 30,000 mg wet weight of tissue per sample required by the traditional methods.

The ERDC microscale approach achieved method detection limits (MDLs) adequate for measuring levels of organic contaminants likely to occur in Stage 1 polychaetes inhabiting DAMOS disposal mounds and reference areas in LIS. These detection limits were achieved through analysis of 100 mg of wet tissue per sample, and similar sample amounts would need to be collected in any future DAMOS studies if this particular set of methods were utilized. Smaller amounts of tissue per sample would be sufficient if the USC/MSRC microscale analytical methods were employed. These methods have proven useful for measuring selected PAH and PCB compounds in samples consisting of as little as 3 to 5 mg wet weight of tissue. This amount of tissue could be provided, for example, by only about 5 adult-sized individuals of the polychaete *S. benedicti*. This polychaete is a common Stage 1 colonizer of disposal mounds in LIS and a possible target species for use in any future DAMOS bioaccumulation studies.

A first-order power analysis using LIS sediment chemistry data indicates that from 5 to 20 individual tissue samples (each comprised of multiple individuals of whatever target species ultimately is chosen) would need to be collected and analyzed at both a disposal mound and reference area to reliably detect any significant differences that might exist between the two in the body burdens of various organic contaminants. The theoretical bioaccumulation calculations presented in this report, however, suggest there would be little actual difference in tissue concentrations measured at active DAMOS disposal mounds versus reference areas. In lieu of conducting field studies to test for small differences in bioaccumulation between disposal mound and reference areas, it might be more useful for DAMOS to direct limited resources toward the development of more advanced food chain and/or risk assessment models, with laboratory exposures and/or field collections targeted toward filling any identified data gaps.

Although microscale analytical methods are available, it does not necessarily mean that studies of bioaccumulation using field-collected organisms should become a routine part of DAMOS monitoring. In the future, DAMOS might consider employing these methods in one or more special investigative studies outside its routine monitoring efforts. Such studies could help determine, for example, whether the use of small, Stage 1 test organisms changes the outcome of field or laboratory investigations of bioaccumulation potential that have traditionally focused on larger taxa.

1.0 INTRODUCTION

1.1 General Background

Chemicals that resist degradation and have the potential to accumulate in organism tissues are distributed in sediments throughout the United States. Because sediments serve as both sinks and reservoirs for persistent bioaccumulative chemicals, their potential risks to ecological resources and human health must be evaluated frequently as part of the environmental assessment and sediment management activities undertaken by a variety of federal agencies. These agencies include the U.S. Environmental Protection Agency (EPA), the U.S. Army Corps of Engineers (USACE), National Oceanic and Atmospheric Administration (NOAA), and the U.S. Geological Survey (USGS). Bioaccumulation testing is used frequently to determine the biological availability of sediment-associated contaminants and their potential for long-term accumulation in aquatic food webs.

As noted in a recent EPA report, "Decision-making processes predicated on bioaccumulation are complicated by numerous factors, including site-specific issues and the variability in chemical bioavailability due to seasonal physicochemical conditions or anthropogenic changes to the environment. It is no longer sufficient to know only whether chemicals accumulate, because bioaccumulation itself is not an effect but a process. Regulatory managers must know whether the accumulation of chemicals is associated with or responsible for adverse effects to aquatic organisms and organisms that prey on them, including humans" (EPA 2000). The many complex issues underlying these statements are the subjects of on-going research within numerous monitoring and regulatory programs, as documented in several recent publications (Bridges et al. 1996; EPA 1998, 2000).

For the purpose of evaluating dredged sediments proposed for open-water disposal, potential adverse effects due to bioaccumulation must be assessed as part of the regulatory process whenever elevated concentrations of anthropogenic contaminants are suspected or known to be present. The relevant guidance manuals specify a tiered approach to testing sediments proposed for dredging (i.e., the "Green Book" methods described in EPA/USACE 1991; 1998). In the first and second tiers, historical and/or newly collected chemistry data are used to determine whether persistent bioaccumulative chemicals are present in the sediment. If the potential for bioaccumulation appears to exist based on a suite of assessments, then actual 28-day laboratory exposures of benthic test organisms and analysis of their tissues may follow in the third tier. A fourth tier exists for special situations in which a decision could not be made in any earlier tier (McFarland 1998).

In practice, using the specified tiered approach to evaluate bioaccumulation associated with dredged material disposal has raised complex technical and regulatory problems (Bridges et al. 1996). For example, the Tier II screening test used to calculate the "Theoretical

Bioaccumulation Potential" (TBP) of neutral organic chemicals is based on a relatively simple equilibrium partitioning model that does not account for metabolism of compounds, disequilibrium and non-constancy of exposure, organism feeding behavior, or numerous other processes that can influence bioaccumulation (EPA 1998). Using the default biota-sediment accumulation factor (BSAF) of 4 in such calculations has been shown to result in TBP values that grossly overestimate actual body burdens measured in a variety of field- and/or laboratory-exposed organisms (McFarland et al. 1994; McFarland 1995). Similar results were found in previous DAMOS Program investigations using Stage 1 benthic organisms collected in Long Island Sound (Rhoads et al. 1996).

Interpreting Tier III and IV bioaccumulation test results also has proven to be problematic because of a reliance on a number of subjective evaluation factors (Bridges et al. 1996; Lechich 1998). Generally speaking, body burdens determined using equilibrium partitioning models and/or laboratory bioassays can be poor predictors of actual field conditions (Maruya et al. 1997; Ferguson and Chandler 1998). However, due to the same factors that have constrained past DAMOS investigations (e.g., need for large amount of tissue, high cost and logistical difficulty of collection), the number of studies that have attempted to make direct measurements of bioaccumulation in real systems is fairly limited (Farrington et al. 1986; Foster and Wright 1988; Maruya et al. 1997; Ferguson and Chandler 1998; Brunson et al. 1998; Moore 2001). To evaluate the predictive capabilities of laboratory bioaccumulation studies and the current dredged material testing protocol, we need both more bioaccumulation measurements in the benthic organisms inhabiting aquatic disposal sites and a better understanding of the effects of specific concentrations of contaminants of concern on the biological receptors.

1.2 Evaluation of Bioaccumulation under the DAMOS Program

The DAMOS Program was established to characterize the physical behavior and monitor the environmental effects of dredged material placed at open-water disposal sites in New England, after the material has been evaluated through a defined regulatory process and classified as suitable for such placement. Given the uncertainties of TBP calculations, combined with the recognition that predredge evaluations can miss contaminant "hot spots" and/or capping materials may not always efficiently cover sediments of concern, bioaccumulation analyses have been incorporated as a component of the DAMOS tiered monitoring and decision-making framework (Germano et al. 1994).

Bioaccumulation analyses are undertaken as part of the routine monitoring of capped disposal mounds after a mature infaunal community has developed. The analyses verify that contaminants are not migrating through the cap and accumulating in the tissues of resident organisms to the point where they could become biomagnified within the

food chain and thereby pose significant ecological and human health risks. Bioaccumulation monitoring is not part of routine monitoring for unconfined, open-water disposal mounds in the DAMOS program, because numerous past DAMOS investigations that used the current guidelines for sediment characterization to determine suitability for open-water disposal (the "Green Book"; EPA/USACE 1991) have revealed no adverse ecological effects. Although the possibility exists that contaminant hot spots may be missed during the evaluation of sediment deemed suitable for unconfined open-water disposal, the probability is extremely low. No monitoring results to date have suggested that hot spots have been missed.

In the initial phases of the program, DAMOS investigators deployed mussels for in-situ bioaccumulation monitoring to investigate short-term and long-term water column impacts of open-water dredged material disposal (Feng 1980; 1982 a and b; 1983; Arimoto and Feng 1983). Because mussels are filter-feeders not commonly found in the soft-bottom communities inhabiting muddy dredged material deposits, they are not ideal test organisms for studying bioaccumulative effects of residual sediment contaminants. Subsequently, bioaccumulation monitoring under DAMOS and numerous other programs has tended to rely on examining contaminant body burdens in relatively large infaunal polychaetes, crustaceans, and bivalves (SAIC 1990; EPA/USACE 1991; 1998; EPA 2000). Testing has been restricted to these larger organisms, because traditional analytical techniques have required sizeable amounts of tissue for accurate detection of trace contaminant concentrations (e.g. soxhlet extraction requires 20 to 25 grams wet weight or more (EPA 1996) and ultrasonic techniques use 10 grams of wet tissue (EPA 1984)).

Obtaining the required biomass for tissue contaminant analysis usually involves labor-intensive collection, sieving and hand-picking of numerous sediment grab or box core samples. In addition to high field costs, it is often difficult to collect a sufficient number of individuals of the same target species (i.e., the same type of tissue) at all locations of interest across a study area or between years, because of normal spatial and temporal variability in natural benthic populations. When the dominant species changes from place to place and year to year, it is almost impossible to make valid comparisons between disposal mounds and reference areas or to look within a particular disposal site at long-term trends in biological tissue uptake.

Using only larger organisms to evaluate bioaccumulation potential has other drawbacks. Such organisms typically tend to become abundant over dredged material deposits during the later phases of the benthic recolonization process, up to several years following the initial placement event. Measuring contaminant concentrations in these "Stage 3" organisms (*sensu* Rhoads and Germano 1982) therefore could delay detection of adverse ecological effects until well after the fact. Proactive disposal site management is predicated on monitoring environmental indicators that respond in a more immediate manner, thereby

providing an early warning of undesirable impacts (Fredette et al. 1990).

Given these considerations, there has been interest for some time in evaluating the bioaccumulation potential in the small benthic organisms that are typically the first to colonize new dredged material deposits. Many of these "Stage 1" organisms are opportunistic polychaetes that have high population growth and turn-over rates. They colonize new dredged material deposits in high numbers and live at the sediment surface, where they are readily preyed upon by secondary consumers such as crustaceans and fish. Some are surface deposit-feeders that ingest sediment particles, particulate organic matter, and associated chemical contaminants (Rhoads et al. 1978; Rhoads and Germano 1982; 1986).

Such characteristics have engendered questions about the bioaccumulation potential of these organisms, even though chemicals of concern are typically present at relatively low concentrations in the dredged material. The specific concern is that rapid bioaccumulation by these abundant, fast-growing organisms might result in significant trophic transfer of low-level contaminants. Ultimately, this could result in food-chain biomagnification that might pose significant ecological or human health risks.

During the mid-1990s, DAMOS investigators attempted to address this ongoing concern by developing and testing a "worm isolator" device designed to collect large numbers of sediment-free Stage 1 organisms (principally polychaetes and oligochaetes) over dredged material mounds. The goal was to gather enough tissue mass for subsequent analysis of contaminant body burdens. Although the study was hampered by several factors, only a few grams of tissue were collected after 10 hours of concentrated field effort. Because the standard analytical methods at the time required tissue amounts on the order of 10 to 30 grams wet weight, the worm isolator was judged to have made insufficient gains in time/cost efficiency (Rhoads et al. 1996).

1.3 Objectives and Approach

Our objectives in this study were twofold: 1) to review current approaches for analyzing low levels of chemical contaminants in the tissues of sediment-dwelling organisms, and 2) to assess whether there has been sufficient change in technical capabilities to modify the current protocols regarding the use of field bioaccumulation as a monitoring tool under the DAMOS Program. Accurate measurement of contaminant tissue concentrations in the very small benthic organisms that initially colonize disposal mounds in high numbers was of particular interest. Such measurements, if feasible, would facilitate a more complete assessment of the environmental impacts of dredged material disposal on the food web.

We utilized the following multi-step approach to address the study objectives:

- We interviewed experts to provide information on current analytical capabilities and costs.

- We gathered and reviewed written sources of information (such as conference proceedings, technical reports, and journal articles) on new developments in microscale analytical techniques.
- We calculated theoretical contaminant body burdens in representative Stage 1 species using recent sediment chemistry data from Long Island Sound disposal sites and reference areas,. This enabled us to estimate the number of such organisms that would need to be collected should a study be undertaken.
- We used the same data to estimate the number of samples required for a statistically valid comparison of tissue concentrations in Stage 1 organisms collected over disposal mounds versus reference areas.

This report is organized to present our results in each of the above four areas. Based on the gathered information, we also provide some considerations and recommendations regarding the overall feasibility of including field bioaccumulation more routinely as a monitoring tool under the DAMOS Program.

2.0 RESULTS

2.1 Expert Interviews

Various individuals with expertise in environmental toxicology and chemistry were identified and contacted by members of our team over the period 18 March to 2 June 2005 (Table 2-1). Two face-to-face interviews were conducted at the Marine Biological Laboratory in Woods Hole, MA, while the other interviews were conducted through a combination of telephone calls and emails.

Some of the experts (e.g., Dr. Carol Reinisch and Dr. Norman Wainwright) have been involved in developing bioassays at the cellular and subcellular levels, allowing for extremely small sample sizes. The U.S. Army Corps of Engineers' Dredging Operations and Environmental Research (DOER) Program also has been investigating this emerging area of research and has written a technical report on biomarker-based analysis for contaminants in sediments or soil (Inouye and McFarland 2000). The biomarker-based analysis involves either individual cell-based assays with endpoint criteria ranging from death to enzyme induction, or DNA arrays which result in messenger RNA-forming proteins that reflect the specific effect of a toxicant on synthesis and activity. Another cell-based technique consists of noting cellular aberrations such as leukemia in exposed bivalves (Harper et al. 1994).

Although these new biotechnology-based methods can quantify the relationship of exposure to cellular or cDNA-mRNA function (or dysfunction), they do not directly address the question of primary interest to DAMOS: are contaminants building up in the tissues of exposed organisms to the point where the food chain is at risk? However, several of the other interviewees indicated that analytical techniques have in fact been developed over the past ten years to allow quantification of low concentrations of contaminants in small amounts of tissue, as described in the following section.

2.2 Description of Microscale Analytical Techniques

So-called "microscale" or "microextraction" analytical techniques have been developed primarily to examine bioaccumulation of hydrophobic organic contaminants, such as pesticides, PCBs, and PAHs, in small tissue samples. Because these classes of compounds concentrate in organism lipids, they are most likely to become biomagnified within aquatic food chains and therefore are commonly of the most interest to resource managers.

As summarized in Table 2-2, we identified three main groups of researchers in the U.S. that have spearheaded the development and use of microscale techniques in bioaccumulation studies involving aquatic organisms: 1) the U.S. Army's Engineer Research and Development Center (ERDC) in Vicksburg, MS, 2) the University of South Carolina (USC) in Columbia, SC, and 3) the Marine Sciences Research Center (MSRC) at the State University of New York at Stony Brook. These groups

developed microscale techniques specifically in response to the need for validated analytical methods capable of analyzing contaminants both in field-collected meiobenthic organisms and in the small organisms utilized in some of the laboratory-based testing protocols (e.g., 10-day acute toxicity test with amphipods).

ERDC Microscale Approach: ERDC researchers developed this approach specifically because some bioaccumulation studies result in tissue samples of very small wet weight (i.e., 50 to 500 mg wet weight) for body residue analysis, and, as indicated above, the traditional analytical methods are designed to address trace levels of contaminants in significantly larger sample sizes (as much as 20 to 25 g wet weight [equivalent to 20,000 to 25,000 mg wet weight] of tissue sample; EPA 1996). To date, the ERDC approach has been applied to analysis of PAHs and PCBs (Jones et al. 2005; Millward et al. 2005).

The ERDC microscale methods have been developed from standard EPA analytical methods (EPA 1996), compensating for a lower initial tissue mass by additional concentration of the final extract volume. Specifically, the cleanup stage is scaled down to reflect smaller tissue masses and lower solvent volumes, and the final extract is concentrated beyond the traditional 1 mL to either 100 µL (PAH microscale method) or 40 µL (PCB microscale method).

In a validation study involving the analysis of 100 mg wet weight per sample of spiked fish tissue, the ERDC researchers achieved Method Detection Limits (MDLs) of 6 to 59 ng/g (ppb) for PAHs and 0.5 to 1.7 ng/g for PCBs (note: these MDLs are defined as the minimum concentration of a target analyte that can be measured and reported with 99% confidence that the concentration is greater than zero, and were determined according to standard procedures (EPA 1996)). These MDLs are not significantly different from those of the traditional methods. The accuracy of the microscale methods, determined through analysis of a tissue Standard Reference Material (SRM), likewise was acceptable and comparable to that of the traditional methods. It was concluded that for bioaccumulation and toxicity testing protocols, the microscale methods based on analysis of about 100 mg wet weight of tissue per sample would in many cases offer adequate analytical sensitivity, precision, and accuracy.

USC/MSRC Approach: The "microextraction" or "micromass" techniques utilized by both of these research groups share a common origin and therefore are similar. The method originally was developed at USC (Wirth et al. 1994). In this technique, small masses of tissue are placed in vials to which solvent and 1-mm diameter glass beads are added. Extraction is accomplished by rapid mixing of the vials ("bead beating") using a mini-bead beater device, followed by centrifuging the extract and directly injecting the separated fraction(s) into the appropriate detector. Analysis of PCBs and pesticides is typically accomplished

using gas chromatography with electron capture detection (GC-ECD) (Wirth et al. 1994; Fay et al. 2000). Analysis of PAHs is accomplished using gas chromatography with either flame ionization detection (GC-FID) or selective ion monitoring (GC-SIM) (Ferguson and Chandler 1998; Fay et al. 2000; Klosterhaus et al. 2002).

Wirth et al. (1994) first reported success with these microextraction techniques in measuring body burdens of the PCB mixture Aroclor 1254 in samples consisting of as few as 20 copepods (approximately 0.18 mg wet weight of tissue). However, these Aroclor 1254 body burdens were fairly high (approximately 5.9×10^4 ng/g wet weight, or 3.9×10^5 ng/g dry weight, of tissue) and therefore readily detectable in a small amount of tissue. Klosterhaus et al. (2002) later advanced the technique by measuring PAH concentrations as low as 38 ng/g wet weight (equivalent to 250 ng/g dry weight) in samples consisting of 50 copepods (representing a total of approximately 0.4 to 0.6 mg wet weight of tissue). In the same study, a total lipid microtechnique combining the standard Bligh-Dyer extraction method with a colorimetric quantification method also was employed to measure small amounts of total lipids (1 to 50 µg) in tissue weighing between 0.16 and 1.6 mg wet weight. Fay et al. (2000) measured selected PAH and PCB compounds in samples containing from three to fifteen amphipods (1 to 7 mg wet weight of tissue). Finally, Ferguson and Chandler (1998) successfully analyzed the PAH compounds fluoranthene, benzo[a]anthracene, and benzo[a]pyrene in samples consisting of three to five individuals of the polychaete *Streblospio benedicti* (3 to 5 mg wet weight of tissue) collected from an uncontaminated site in South Carolina. These investigators measured concentrations ranging from 83 to 543 ng/g dry weight.

Overall, these results indicate that it is feasible to detect and quantify environmentally realistic concentrations of both organic chemical contaminants and lipids (allowing results to be lipid-normalized for comparison among samples or studies) in relatively small amounts of benthic invertebrate tissue. The amounts of wet tissue per sample required by the microscale methods (0.2 to 7 mg) are 4 to 6 orders of magnitude less than those required by the traditional methods (25,000 to 30,000 mg).

2.3 Calculation of Theoretical Bioaccumulation Potential

The studies cited above demonstrate that microscale methods are viable for evaluating bioaccumulation in organisms exposed to a range of contaminant concentrations in field-collected or laboratory-manipulated sediments. Generally speaking, in studies where contaminant concentrations in tissues are exceedingly low and/or insufficient amounts of tissue are collected, standard analytical methods may not be sensitive enough to detect the analytes of interest, resulting in most or all of the results being reported as “non-detects”. It is therefore desirable to have some prior knowledge of the contaminant concentrations likely to occur in sediments and resident organisms relative to the detection limits of the

proposed analytical methods. This greatly facilitates estimating the amount of tissue that needs to be collected.

To estimate the amount of tissue to be collected and thereby help determine whether the microscale approach could be usefully applied in future DAMOS studies, we performed some calculations of TBP in small benthic organisms known to inhabit disposal mounds in Long Island Sound (LIS). We placed some boundaries on this exercise by examining only a select subset of organic contaminants in a limited number of primarily Stage 1 species; these were considered to be adequately representative of the wider range of compounds and species in LIS sediments.

2.3.1 TBP Methods

As a first step, recent sediment chemistry data were obtained from the Environmental Impact Statement (EIS) for disposal site designation in Long Island Sound (ENSR 2001). These data were from four types of areas: 1) active disposal mounds, 2) historical disposal mounds, 3) disposal site reference areas, and 4) far-field stations.

Sediment chemistry data for seven representative organic contaminants (4,4-DDT, benzo[a]anthracene, benzo[a]pyrene, fluoranthene, pyrene, total PCBs, and total PAHs) were compiled into a database for calculation of TBP values for all stations sampled in each of the four types of areas. Total PCBs reported in the EIS were calculated from the sum of 20 specific congeners multiplied by two (NOAA 1993). Similarly, total PAHs were reported as the total of 16 priority pollutant PAH compounds (ENSR 2001). Both sums excluded individual compounds that were reported as below detection. If all of the individual chemicals were reported as undetected, the highest reported detection limit for the suite of chemicals was used for the reported sum. Chemical values reported as below detection were included in TBP calculations at one-half of the detection limit. There were no sediment data exclusions based on qualifiers or any other factor. All sediment samples also had reported concentrations of total organic carbon (TOC).

The TBP is a predictor of chemical concentrations in tissue based on the TOC-normalized sediment concentration, using an assumed lipid concentration of the organism of interest, and a biota/sediment accumulation factor (BSAF). The U.S. Army ERDC has developed a database of BSAFs for specific chemicals and organisms (USACE 2005). Where available, the average reported BSAFs based on dry weight were used for the following five representative organisms: 1) *Streblospio benedicti* (Stage 1 spionid polychaete), 2) *Neanthes virens* (larger, Stage 2 or 3 polychaete), 3) *Heteromastus filiformis* (Stage 1 capitellid polychaete), 4) *Lumbriculus variegatus* (freshwater oligochaete), and 5) miscellaneous polychaetes (general category).

Although *L. variegatus* is a freshwater organism unlikely to be found in LIS, both BSAF and percent lipid data were available for this species in the ERDC database, and it was therefore selected as a

representative oligochaete. Because of the lack of BSAFs for some species, results for *S. benedicti*, *N. virens*, and *H. filiformis* were grouped for analysis purposes.

The TBP calculation also requires an estimate of the lipid concentration. An average lipid concentration was calculated (in dry weight) for each species of interest, also from the ERDC BSAF database. Theoretical bioaccumulation potential values were calculated following McFarland (1998):

$$TBP = BSAF * [(C_s/\%TOC)/\%L]$$

Where:

C_s = concentration of contaminant in sediment (ng/g, dry weight)

%L = percent lipids, dry weight

Because the BSAF has no unit (based on the ratio of sediment to tissue concentration in the same units), the final units for TBP are ng/g dry weight (i.e., parts per billion, or ppb). Calculated TBP values were assessed relative to the method detection limits or reporting levels that have been documented for the microscale methods.

2.3.2 TBP Results

On average, the theoretical contaminant concentrations (i.e., TBP values) in polychaetes/oligochaetes inhabiting LIS disposal sites and reference areas were above the method detection limits (MDLs) determined by ERDC for both the microscale and traditional methods (Tables 2-3 to 2-5). The papers cited above for the USC/MSRC microscale methods do not provide a similar list of MDLs for inclusion in Tables 2-3 to 2-5; the potential suitability of these methods relative to the TBP values is evaluated in a later section.

The most complete datasets for calculating the TBPs in Tables 2-3 through 2-5 were for the Stage 1 oligochaete *L. variegatus* and the general category of "miscellaneous polychaetes" which includes species representing a variety of successional stages. For *L. variegatus*, only the minimum calculated TBP values were near the MDLs of the ERDC microscale method, especially for benzo[a]pyrene and total PCBs (Table 2-3).

The TBP values were generally comparable among the four types of areas sampled (Figure 2-1), with the highest values observed consistently at the historical disposal areas, reflecting higher contaminant concentrations in dredged material placed in these areas prior to implementation of present-day permitting and monitoring protocols. Predicted tissue concentrations at active disposal areas were comparable to those at reference areas for several compounds (Figure 2-1).

Calculations for miscellaneous polychaetes showed similar patterns (Figure 2-2), although the *minimum* theoretical tissue concentrations for the active, far-field, and reference areas were commonly near or below the MDLs for most of the investigated analytes. In particular, the ERDC microscale approach

might have difficulty detecting benzo[a]pyrene in tissue samples containing the minimum predicted concentrations shown in Table 2-4. The microscale MDL for pyrene was higher than that for the traditional method (Tables 2-3 to 2-5).

The results for several other representative polychaete species suggest that, of the selected chemicals, benzo[a]pyrene and total PCBs would be the most difficult to measure accurately at very low concentrations using either the ERDC microscale or traditional methods (Table 2-5). BSAFs for 4,4'-DDT were available only for *H. filiformis* and *N. virens*; however, the results show that the minimum predicted tissue concentrations of DDT would likely be below the MDL of 1 ng/g that is generally achieved using the traditional methods.

Overall, these data suggest that the ERDC microscale approach would be suitable for determining average predicted concentrations of organic contaminants in polychaetes, particularly Stage 1 colonizers, inhabiting DAMOS disposal sites and reference areas in LIS. An important caveat is that the MDLs in Tables 2-3 through 2-5 are based on analysis of 100 mg wet weight of tissue for the ERDC microscale methods and 3 to 4 grams wet weight of tissue for the traditional methods (Jones et al. 2005). Comparable amounts of tissue would need to be collected and analyzed in any future DAMOS studies for the ERDC methods to be usefully applied (discussed in greater detail below).

Although MDLs are not specifically listed, one of the USC/MSRC papers states that their microscale method is capable of quantifying PAHs from tissue samples containing as little as 10 picograms (pg) of a given PAH compound (Klosterhaus et al. 2002). Based on the predicted concentrations in Tables 2-3 through 2-5, the amount of tissue that might be needed for analysis using the USC/MSRC microscale approach can be estimated.

Assuming for the purpose of this first-order calculation that the Stage 1 colonizer *Streblospio benedicti* was the target species and fluoranthene was the target analyte, 29.3 ng/g (dry weight) is the minimum, and thus conservative, theoretical tissue concentration shown in Table 2-5 for individuals inhabiting reference areas. This concentration equates to 29.3 pg/mg dry weight. Using an experimentally derived wet weight:dry weight conversion factor of 3.2 for *S. benedicti* (Weinstein and Sanger 2003), this concentration further equates to 9.2 pg fluoranthene per mg wet tissue.

This represents an amount of fluoranthene in 1 mg of wet *S. benedicti* tissue that is just slightly below the Klosterhaus et al. (2002) stated quantification limit of 10 picograms. Using an estimated wet weight of 1 mg for an individual adult *S. benedicti* (Ferguson and Chandler 1998; Garcia-Arberas and Rallo 2004), 3 to 5 individuals collected from a reference area would theoretically contain a total of 28 to 46 pg fluoranthene, well above the minimum amount required for quantification by the Klosterhaus et al. (2002) microscale method.

The study by Ferguson and Chandler (1998) serves to validate these calculations. Using microscale methods similar to those of Klosterhaus et al. (2002), only 3 to 5 individuals per sample were needed to measure concentrations of three PAHs (fluoranthene, benzo[a]anthracene and benzo[a]pyrene) in field-collected *S. benedicti* from a pristine South Carolina estuary. The "environmentally realistic" tissue concentrations that were detected ranged from 83 ng/g to 543 ng/g (dry weight), roughly comparable to the range of theoretical concentrations predicted for *S. benedicti* inhabiting LIS sediments (Table 2-5). The earlier DAMOS bioaccumulation study of Rhoads et al. (1996), showing body burdens on the order of 7 to 92 ng/g in Stage 1 polychaetes from a relatively unpolluted location in LIS, provides additional confirmation of the realism of the theoretical concentrations in Tables 2-3 to 2-5.

Even using the minimum TBP values in Tables 2-3 to 2-5 as conservative estimators, it appears that surprisingly low numbers of *S. benedicti* (and probably other Stage 1 species, depending on their individual biomass) would need to be collected from LIS sediments using the USC/MSRC microscale approach for sample analysis. There are several reasons why *S. benedicti* might be particularly attractive as a target organism in any future DAMOS studies. This cosmopolitan estuarine polychaete is tolerant of both organic enrichment and low dissolved oxygen and is a long-term dominant of ambient soft-bottom benthic communities in western and central LIS (Strobel et al. 1995). Depending on the time of year and other factors, it is reasonable to expect individuals of this species to be available for collection at both reference and disposal site areas.

In LIS and elsewhere, *S. benedicti* is known to colonize defaunated sediments and fresh dredged material deposits in very high numbers (McCall 1977; Rhoads et al. 1978). It is a surface deposit-feeder that ingests sediment, has high growth rates, and is highly tolerant of PAHs (Chandler et al. 1997). Because of its abundance at the sediment surface, it is readily preyed upon by fish and other predators. Bioaccumulation of sediment-bound organic contaminants has been demonstrated in this species (Ferguson and Chandler 1998), resulting in the potential for transfer to higher trophic levels.

2.4 Preliminary Evaluation of Required Sample Number

2.4.1 Methods

Analytical capabilities have advanced to the point where only small amounts of tissue, on the order of 3 to 5 mg wet weight per sample, need to be collected in any future DAMOS studies. This should result in significantly less time and effort required to collect each sample compared to past investigations. However, overall study costs also depend on the number of samples requiring collection and analysis. In this section, we present some first-order calculations addressing the question of how many samples might need to be

collected and analyzed in any future DAMOS study. The calculations are based on the assumption that DAMOS would be interested in determining whether bioaccumulation of contaminants on disposal mounds is significantly greater than that in nearby reference areas unaffected by disposal.

As a first step, the EIS chemistry data employed in the TBP calculations of the preceding section were used to provide estimates of spatial variance in the chemical contaminant distribution in surface sediments in the four types of LIS areas sampled: active disposal mounds, historical mounds, far-field areas, and reference areas. The objective was to use these variance estimates in the design of a sampling plan to statistically test whether concentrations in benthic infaunal tissues from disposal sites are greater than those from nearby reference areas. A power analysis was performed for a one-tailed, two-sample *t*-test for several representative organic contaminants, including total PAHs, several individual PAH compounds (benzo[a]anthracene, benzo[a]pyrene, fluoranthene and pyrene), total PCBs, and the pesticide 4,4'-DDT. The variance estimates used in this power analysis were derived from the range of variance estimates observed for these compounds among field replicates in the EIS sediment chemistry data, normalized to total organic carbon (TOC).

The ideal sampling plan is one in which multiple grabs are taken from several stations at each site or mound, and one composite tissue sample for each station is sent to the lab for analysis. The sampling design for sediments in the EIS database included 1 to 2 stations per site or mound, with sediment chemistry results being available for three replicate grabs, plus a composite of five grabs, from each station (two grabs from each station also were archived). There were insufficient data to estimate within-mound variance based on between-station variability, so the variance for the power analysis was based on within-station variability. This variance represents smaller scale variability than desired in the ideal sampling design. However, the scale of difference among station composites was compared to the range observed among station replicates to determine the comparability of within-station and between-station (within-mound) variation.

2.4.2 Data

The variance estimates used in the power analysis were based on within-station variability, so only the individual field replicates (EIS sample type "N") were used. Historical disposal mound data were excluded because they were consistently elevated relative to levels at active mounds. The latter were used preferentially because they are much more representative of dredged material classified as suitable for disposal under present-day testing and permitting requirements. Data for the Cornfield Shoals Disposal Site (CSDS) active mounds also were excluded, as this is an exceptionally sandy, dispersive site and considered to be non-representative of the other LIS disposal sites.

Results for the station composites (EIS sample type "SC"), where available, were used to summarize the scale of differences observed between two stations within a site. These differences were assessed relative to the scale of differences observed among replicate grabs within stations (i.e., maximum replicate value minus the minimum replicate value).

2.4.3 Results

Variance estimates based on within-station variability (i.e., residual standard error, or RSE, for an ANOVA using station as the source of variability) were computed for each of seven chemical endpoints. Several stations had exceptionally high variance for one or more chemical endpoints, due to suspected outlier values, and the RSE for each chemical endpoint was estimated both with and without these questionable stations included (Table 2-6).

Differences among the replicate grabs at each station were generally on the same scale as those among the station composites within each type of area (Table 2-7). In particular, the average differences excluding the italicized non-representative or outlier stations (shaded bottom row of Table 2-7) were quite similar between replicate grabs and composites for each chemical endpoint, with the exception of PCBs. PCBs were particularly variable at a number of stations within each type of area, with 10 of the 17 observed differences among replicates exceeding 1,369 (the maximum difference among station composites for non-italicized sites). Individual station anomalies were often a result of one replicate grab having extremely low TOC that greatly inflated the normalized sediment concentration in that one sample. In general, the results in Table 2-7 suggest that the small-scale spatial variability measured by the replicate grabs per station is a reasonable surrogate for the variance among stations at a DAMOS disposal mound in LIS.

A range of estimates of within-station variance was selected for input to the power analysis. Five different variance scenarios were chosen, with RSE values being shown in units of parts per billion, normalized to total organic carbon content (ppb- TOC):

- **RSE = 1,000** represents a conservative (high) estimate for 4,4'-DDT. This is the RSE for 4,4'-DDT including one suspected outlier sample; it also may be representative of the RSE for PCBs between-stations based on Table 2-7 results.
- **RSE = 4,000** represents a low estimate for the individual PAHs, and a conservative estimate for PCBs. This RSE is approximately the middle of the range for individual PAHs excluding one suspected outlier sample; it is twice the RSE for PCBs excluding one suspected outlier sample.
- **RSE = 7,000** represents a reasonable range for the individual PAHs. This RSE is approximately the middle of the range for individual PAHs including one suspected outlier sample.
- **RSE = 30,000** represents a low estimate for Total PAHs. This is

the RSE for Total PAHs excluding one suspected outlier sample.

- **RSE = 60,000** represents a conservative estimate for Total PAHs and a highly conservative estimate for PCBs. This is the maximum RSE observed in the range for Total PAHs including one suspected outlier sample, and for PCBs including one suspected outlier sample. It is probably a reasonable RSE for Total PAHs, but excessively conservative for PCBs based on Table 2-7 results.

Results of the power analysis for these five variance scenarios are shown in Table 2-8 and Figure 2-3. For variance scenario 1 (RSE = 1000), five stations per area provide 80% probability of detecting a difference of 1,738 ppb-TOC (this is equivalent to 23 ppb dry weight using the average TOC value of 1.3%). This variance scenario is applicable for 4,4'-DDT and possibly PCBs. For the worst case, variance scenario 5 (RSE = 60,000), 20 stations per area provides 80% probability of detecting a difference of approximately 48,000 ppb-TOC (equivalent to 624 ppb dry weight using the average TOC value of 1.3%). This variance scenario is likely applicable for Total PAHs but appears to be excessively conservative for the other chemical endpoints evaluated. If the true RSE is 1000 to 7000 (the range of RSE that appears likely for the individual PAHs, PCBs, and 4,4'-DDT), then 20 stations per area would provide a minimum detectable difference of 802 ppb-TOC (10 ppb dry weight) to 5,616 ppb-TOC (73 ppb dry weight).

If the organic carbon content of a study site is highly variable, it can increase the between-station variability of the TOC-normalized sediment concentrations. The variance of TOC-normalized sediment concentrations will be inflated if sediment dry weight concentrations are negatively correlated with sediment TOC; conversely, variance will be diminished if they are positively correlated. If information indicates highly variable TOC at a proposed study site, the sampling plan should err on the side of more stations per site to improve the power of the statistical test. Along these same lines, if the organic carbon content is expected to be higher at the disposal sites than at the reference sites, then the differences of TOC-normalized sediment concentrations (and presumably the tissue concentrations) between the two sites will be diminished. In this situation, the sampling plan should again err on the side of more stations per site to improve power to detect a smaller difference between the two sites.

These recommendations for a tissue sampling plan are based on the presumption that infaunal tissue concentrations are primarily affected by the sediment chemical and TOC concentrations, and that the variance of TOC-normalized sediment chemical concentrations is a reasonable surrogate for the variance of tissue chemical concentrations at these sites.

Table 2-1.

Experts Contacted and Interviewed

Name	Affiliation	Expertise	Date and Contact type*	Summary
Dr. Carol Reinisch	Marine Biological Laboratory, Woods Hole, MA	Marine epidemiology and toxicology	March 8 (F)	Uses cellular anomalies rather than body burdens to assess exposure effects
Dr. David Moore	Weston Solutions, Inc., Carlsbad, CA	Aquatic toxicology	March 14 (E)	Provided referral to former colleagues at ERDC engaged in research on microanalysis methods
Dr. Roderic Millward	Analytical Services, Inc., Vicksburg, MS	Bioassay and bio-accumulation methods	March 15 (E)	Co-developer of ERDC microscale analytical methods for detection of PCBs and PAHs in small (approximately 100 mg) tissue masses.
Dr. Norman Wainwright	Marine Biological Laboratory, Woods Hole, MA	Biochemistry	March 15 (F)	Has developed microarrays for very sensitive detection of microorganisms; possible application to marine bioassays (indirect measure of bioeffects of contaminants); significant research and development would be needed
Dr. Todd Bridges	Environmental Chemistry Branch, ERDC, Vicksburg, MS	Bioassay and bio-accumulation methods	March 16 (E)	Key role in developing microscale analytical methods for detection of PCBs and PAHs in very small (approximately 100 mg) tissue masses.
Dr. Richard Pruell	U.S.EPA Narragansett, RI	Environmental chemistry	March 24 (E)	Described significant advances in analytical techniques that allow low level detection using smaller tissue mass
Dr. Josephine Aller	MSRC, Stony Brook, NY	Biochemistry and benthic processes	April 4 (E)	Referral to Dr. Anne McElroy
Dr. P. Lee Ferguson	University of South Carolina	Chemistry and aquatic toxicology	May 24 (E)	Key role in the development and use of microextraction techniques to measure PCBs and PAHs in Stage 1 polychaetes
Dr. Anne McElroy	MSRC, Stony Brook, NY	Environmental toxicology and chemistry	April 4 (E) June 2 (E)	Key role in developing microextraction techniques for assessing uptake of PCBs, PAHs, benzenes in small tissue masses (as few as 3 amphipods) using a “bead beater”

* E = contact by email and/or telephone; F = face-to-face

Table 2-2.

Summary of Microscale Analytical Techniques

Research Group	Method	Synopsis	Tissue Type and Amount Per Sample (wet weight)	Key references
Engineer Research and Development Center (ERDC)	Microscale approach for quantitative detection of PAHs and PCBs in small tissue masses	Modification of EPA standard methods with scaled-down cleanup step and concentration of final extracts	100 mg (fish tissue)	Jones et al. (2005) Millward et al. (2005)
University of South Carolina (USC)	Microextraction/micromass technique for measuring lipids and body burdens of PCBs and PAHs in small tissue masses	Efficient extraction using glass microbeads in a small vial ("bead beating"), followed by centrifugation and direct injection into GC/ECD (no cleanup step)	0.18 mg (20 individual copepods)	Wirth et al. (1994)
			3 to 5 mg (3 to 5 individuals of *S. benedicti*)	Ferguson and Chandler (1998)
			0.4 to 0.6 mg (50 copepods)	Klosterhaus et al. (2002)
Marine Sciences Research Center (MSRC)	Same as above	Same as above	1 to 7 mg (3 to 15 amphipods)	Fay et al. (2000)

Table 2-3.

TBP Results for *Lumbriculus variegatus* Compared to Method Detection Limits (MDL) of Microscale Methods (MM) and Traditional Methods (TM)

Type of Area	Analyte Name	MDLs (ng/g)[1]		TBP Tissue Concentration Estimates (ng/g)			
		MM	TM	Min	Max	Mean	StdDev
Active Mound	4,4'-DDT[2]	NA	NA	NA[3]	NA	NA	NA
	Benzo[a]anthracene	15	19	90.1	2500.6	391.9	551.2
	Benzo[a]pyrene	8.3	23	29.3	939.9	140.1	202.9
	Fluoranthene	13	18	157.9	5071.2	1038.0	1211.5
	Pyrene[4]	59	27	209.0	6629.0	1426.1	1594.2
	Total NOAA PCBs	0.6	1	10.5	753.3	209.5	192.7
	Total PAHs	25.7	27.7	NA	NA	NA	NA
Far-Field	4,4'-DDT	NA	NA	NA	NA	NA	NA
	Benzo[a]anthracene	15	19	54.5	1300.0	261.6	253.1
	Benzo[a]-)pyrene	8.3	23	26.3	373.5	96.7	80.8
	Fluoranthene	13	18	95.6	3097.2	583.4	553.8
	Pyrene	59	27	126.5	4099.2	875.3	833.4
	Total NOAA PCBs	0.6	1	5.3	18267.4	651.0	3216.4
	Total PAHs	25.7	27.7	NA	NA	NA	NA
Historical Mound	4,4'-DDT	NA	NA	NA	NA	NA	NA
	Benzo[a]anthracene	15	19	165.2	652.6	324.5	146.3
	Benzo[a]pyrene	8.3	23	65.3	218.9	109.8	38.0
	Fluoranthene	13	18	372.1	2001.4	745.3	400.9
	Pyrene	59	27	611.9	2330.2	1047.5	462.4
	Total NOAA PCBs	0.6	1	6.7	9478.6	745.6	2342.1
	Total PAHs	25.7	27.7	NA	NA	NA	NA
Reference Area	4,4'-DDT	NA	NA	NA	NA	NA	NA
	Benzo[a]anthracene	15	19	57.6	767.4	203.1	146.8
	Benzo[a]pyrene	8.3	23	23.2	279.8	79.4	51.5
	Fluoranthene	13	18	110.1	1950.1	484.8	330.4
	Pyrene	59	27	193.0	3204.0	716.4	542.1
	Total NOAA PCBs	0.6	1	7.2	320.2	45.2	57.2
	Total PAHs	25.7	27.7	NA	NA	NA	NA

[1]from Jones et al. (2005) based on analysis of 100 mg wet tissue for microscale method (MM) and 3 to 4 g wet tissue for traditional method (TM);[2] No microscale MDLs for this analyte;[3] No BSAFs available for this analyte; [4]Microscale detection limit > traditional for pyrene

Table 2-4.

TBP Results for Miscellaneous Polychaetes Compared to Method Detection Limits (MDL) of Microscale Methods (MM) and Traditional Methods (TM)

Type of Area	Analyte Name	MDLs (ng/g)[1]		TBP Tissue Concentration Estimates (ng/g)			
		MM	TM	Min	Max	Mean	StdDev
Active Mound	4,4'-DDT[2]	NA	NA	NA[3]	NA	NA	NA
	Benzo[a]anthracene	15	19	20.4	567.0	88.9	125.0
	Benzo[a]pyrene	8.3	23	7.4	238.1	35.5	51.4
	Fluoranthene	13	18	12.2	391.5	80.1	93.5
	Pyrene[4]	59	27	31.5	999.3	215.0	240.3
	Total NOAA PCBs	0.6	1	3.0	215.7	60.0	55.2
	Total PAHs	25.7	27.7	4.5	657.8	105.0	149.6
Far-Field	4,4'-DDT	NA	NA	NA	NA	NA	NA
	Benzo[a]anthracene	15	19	12.4	294.8	59.3	57.4
	Benzo[a]pyrene	8.3	23	6.7	94.6	24.5	20.5
	Fluoranthene	13	18	7.4	239.1	45.0	42.8
	Pyrene	59	27	19.1	617.9	131.9	125.6
	Total NOAA PCBs	0.6	1	1.5	5230.7	186.4	921.0
	Total PAHs	25.7	27.7	7.3	298.9	67.2	64.1
Historical Mound	4,4'-DDT	NA	NA	NA	NA	NA	NA
	Benzo[a]anthracene	15	19	37.5	148.0	73.6	33.2
	Benzo[a]pyrene	8.3	23	16.5	55.5	27.8	9.6
	Fluoranthene	13	18	28.7	154.5	57.5	31.0
	Pyrene	59	27	92.2	351.3	157.9	69.7
	Total NOAA PCBs	0.6	1	1.9	2714.1	213.5	670.6
	Total PAHs	25.7	27.7	47.7	171.2	87.8	35.9
Reference Area	4,4'-DDT	NA	NA	NA	NA	NA	NA
	Benzo[a]anthracene	15	19	13.0	174.0	46.1	33.3
	Benzo[a]pyrene	8.3	23	5.9	70.9	20.1	13.0
	Fluoranthene	13	18	8.5	150.5	37.4	25.5
	Pyrene	59	27	29.1	483.0	108.0	81.7
	Total NOAA PCBs	0.6	1	2.1	91.7	12.9	16.4
	Total PAHs	25.7	27.7	15.3	231.3	57.0	41.3

[1]from Jones et al. (2005) based on analysis of 100 mg wet tissue for microscale method and 3 to 4 g wet tissue for traditional method; [2] No microscale MDLs for this analyte; [3] No BSAFs available for this analyte; [4]Microscale detection limit > traditional for pyrene

Table 2-5.

TBP Results for Various Stage 1 Polychaetes Compared to Method Detection Limits (MDL) of Microscale Methods (MM) and Traditional Methods (TM)

Type of Area	Analyte Name	Species	Method Detection Limits (ng/g)[1]		TBP Tissue Concentration Estimates (ng/g)			
			MM	TM	Min	Max	Mean	StdDev
Active Mound	4,4'-DDT[2]	*H. filiformis*	NA	NA	0.2	413.5	103.1	115.3
Active Mound	4,4'-DDT	*N. virens*	NA	NA	0.1	217.9	54.3	60.7
Active Mound	Benzo[a]pyrene	*S. benedicti*	8.3	23	38.9	1249.3	186.2	269.7
Active Mound	Fluoranthene	*N. virens*	13	18	226.6	7278.3	1489.7	1738.7
Active Mound	Fluoranthene	*S. benedicti*	13	18	42.0	1349.2	276.2	322.3
Active Mound	Total NOAA PCBs	*N. virens*	0.6	1	3.8	276.5	76.9	70.7
Far-Field	4,4'-DDT	*H. filiformis*	NA	NA	0.4	124.0	19.9	37.3
Far-Field	4,4'-DDT	*N. virens*	NA	NA	0.2	65.3	10.5	19.7
Far-Field	Benzo[a]pyrene	*S. benedicti*	8.3	23	34.9	496.4	128.5	107.3
Far-Field	Fluoranthene	*N. virens*	13	18	137.1	4445.2	837.4	794.9
Far-Field	Fluoranthene	*S. benedicti*	13	18	25.4	824.0	155.2	147.4
Far-Field	Total NOAA PCBs	*N. virens*	0.6	1	1.9	6704.4	238.9	1180.5
Historical Mound	4,4'-DDT	*H. filiformis*	NA	NA	0.4	369.5	108.4	138.9
Historical Mound	4,4'-DDT	*N. virens*	NA	NA	0.2	194.7	57.1	73.2
Historical Mound	Benzo[a]pyrene	*S. benedicti*	8.3	23	86.8	291.0	145.9	50.5
Historical Mound	Fluoranthene	*N. virens*	13	18	534.1	2872.5	1069.6	575.4
Historical Mound	Fluoranthene	*S. benedicti*	13	18	99.0	532.5	198.3	106.7
Historical Mound	Total NOAA PCBs	*N. virens*	0.6	1	2.5	3478.8	273.7	859.6
Reference Area	4,4'-DDT	*H. filiformis*	NA	NA	0.5	162.5	17.4	42.2
Reference Area	4,4'-DDT	*N. virens*	NA	NA	0.3	85.6	9.2	22.2
Reference Area	Benzo[a]pyrene	*S. benedicti*	8.3	23	30.8	371.8	105.6	68.4
Reference Area	Fluoranthene	*N. virens*	13	18	158.1	2798.8	695.7	474.2
Reference Area	Fluoranthene	*S. benedicti*	13	18	29.3	518.8	129.0	87.9
Reference Area	Total NOAA PCBs	*N. virens*	0.6	1	2.6	117.5	16.6	21.0

[1] from Jones et al. (2005) based on analysis of 100 mg wet tissue for microscale method and 3 to 4 g wet tissue for traditional method

[2] No microscale MDLs for this analyte

Table 2-6.

Residual Standard Error (RSE) from One-way ANOVA on Station

Chemical Endpoint	Stations Excluded	RSE
PCBs	--	58,173
4,4'-DDT	--	1,093
Total PAH	--	62,055
Pyrene	--	7,230
Fluoranthene	--	6,622
BAP	--	7,003
BAA	--	5,790
PCBs	2KW in CSDS (Far Field)	1,837
4,4'-DDT	25W in CLIS (Ref)	481
Total PAH	MDI in WLIS (Active)	33,787
Pyrene	MDI in WLIS (Active)	5,002
Fluoranthene	MDI in WLIS (Active)	3,767
BAP	MDI in WLIS (Active)	3,265
BAA	MDI in WLIS (Active)	2,836

Summary Based on All Stations:		*Represents:*
Minimum	1,093	DDT
Median	7,003	Range for individual PAHs
Maximum	62,055	Total PAHs and PCBs

Summary Excluding Outliers:		
Minimum	481	DDT excluding outlier
Median	3,265	Range for individual PAHs excluding outlier
Maximum	33,787	Total PAHs excluding outlier

Power analysis performed for the following variance scenarios:

RSE	*Represents:*
1,000	Conservative for 4,4'-DDT
4,000	Individual PAHs excluding outlier and conservative PCBs
7,000	Individual PAHs all samples
30,000	Total PAHs excluding outlier
60,000	Total PAHs and PCBS all samples

Table 2-7.

Observed Range of Difference among Replicate Grabs at each Station and among Station Composites at each Site
(Among comps=among composites)

			Benzo[a]anthracene		Benzo[a]pyrene		Fluoranthene		Pyrene		Total PAH		4,4'-DDT		Total PCBs	
Type of Area	Site	Station	Among Grabs	Among Comps	Among Grabs	Among Comps	Among Grabs	Among Comps	Among Grabs	Among Comps	Among Grabs	Among Comps	Among Grabs	Among Comps	Among Grabs	Among Comps
Active Mound	CLIS	N93	2,251		3,579		3,484		4,251		34,784		1,192		2,705	
Active Mound	CSDS	B92[1]	*2,967*	*712*	*2,633*	*1,738*	*10,026*	*1,040*	*10,026*	*528*	*20,051*	*28,688*	*9,036*	*5,978*	*7,151*	*4,656*
Active Mound	CSDS	S94[1]	*3,065*		*2,569*		*6,632*		*3,955*		*3,173*		*19,005*		*14,984*	
Active Mound	NLDS	SEA	10,101		10,391		13,174		18,333		112,696		78		1,930	
Active Mound	WLIS	MDI[2]	*43,593*		*52,412*		*48,545*		*46,880*		*459,443*		491		5,987	
Far-Field	CLIS	1KW	969	1,312	1,240	1,201	1,789	2,111	2,586	2,179	11,343	14,158	19	43	308	628
Far-Field	CLIS	2KW	1,679		2,191		2,060		2,680		21,407		54		733	
Far-Field	CSDS	2KW[2]	2,192	547	905	1,558	582	547	582	547	14,908	29	3,121	51	*397,437*	*824*
Far-Field	CSDS	4KW	5,351		5,351		5,351		5,351		18,853		539		8,779	
Far-Field	NLDS	1KE	1,314	6,000	2,276	4,292	1,383	1,667	1,524	625	14,952	33,292	7	12	135	177
Far-Field	NLDS	2KE	802		908		1,288		1,330		8,289		11		31	
Far-Field	WLIS	E5H	14,138	20,019	17,482	12,680	16,182	28,086	23,364	23,641	162,219	160,506	851	4,876	6,342	621
Far-Field	WLIS	W5H	1,157		1,204		1,389		1,944		13,370		139		2,576	
Historical Mound	CLIS	FVP[1]	*7,874*	*5,932*	*8,205*	*614*	*7,352*	*14,355*	*14,266*	*6,517*	*86,527*	*48,760*	*7,764*	*10,625*	*20,710*	*6,190*
Historical Mound	CLIS	N74[1]	*2,656*		*2,337*		*3,992*		*4,356*		*30,449*		*6,304*		*2,684*	
Historical Mound	NLDS	RLC[1]	*5,033*		*2,908*		*4,933*		*3,294*		*26,797*		*47*		*204,706*	
Historical Mound	WLIS	EB1[1]	*1,547*		*1,132*		*1,761*		*1,791*		*12,894*		*91*		*1,092*	
Reference	CLIS	25W[2]	2,454	2,416	3,372	4,499	2,874	3,009	2,841	3,012	31,387	38,848	*8,157*	*6,341*	1,384	1,369
Reference	CLIS	REF	552		716		1,127		1,367		7,048		36		332	
Reference	CSDS	RF3	784	46	1,062	569	1,247	629	1,567	629	10,980	4,307	16	78	251	1,263
Reference	CSDS	RF4	12,056		13,589		18,033		23,218		165,472		296		4,930	
Reference	NLDS	LRF	2,084	1,498	2,475	1,746	3,458	2,509	4,186	3,558	23,129	19,080	13	1	113	182
Reference	NLDS	WRF	3,258		3,184		3,639		3,111		29,977		9		106	
Reference	WLIS	STH	2,571	4,444	2,708	4,828	4,038	4,929	3,458	4,525	29,063	41,333	118	54	1,022	226
Reference	WLIS	SWR	4,283		4,862		6,006		6,796		47,914		253		1,097	
		Averages:														
		All Stations:	5,389	4,293	5,988	3,372	6,814	5,888	7,722	4,576	55,885	38,900	2,306	2,806	27,501	1,614
Excluding Italicized Stations:			3,778	4,535	4,305	3,922	4,839	5,436	6,027	4,840	42,099	38,944	403	731	2,153	638

Concentration ranges shown are in ppb (OC).

[1] These stations omitted from the variance summary used in the power analysis.

[2] These stations had potential outliers for one or more endpoints, data shown in italics were excluded from variance summary used in the power analysis.

Table 2-8.

Results of Power Analysis for One-Tailed Two-Sample t-Test (alpha=0.05)

Sample Size per Group	Pooled Variance DF	MDD with 80% power for RSE equal to: 1,000	4,000	7,000	30,000	60,000
3	4	2,509	10,036	17,563	75,268	150,536
4	6	2,014	8,058	14,101	60,434	120,868
5	8	1,738	6,953	12,168	52,148	104,296
6	10	1,554	6,216	10,878	46,618	93,237
7	12	1,419	5,676	9,934	42,573	85,146
8	14	1,315	5,259	9,203	39,440	78,881
9	16	1,231	4,923	8,614	36,919	73,838
10	18	1,161	4,644	8,127	34,830	69,661
11	20	1,102	4,408	7,715	33,063	66,127
12	22	1,051	4,206	7,360	31,542	63,084
13	24	1,007	4,029	7,050	30,214	60,429
14	26	968	3,872	6,777	29,042	58,084
15	28	933	3,733	6,533	27,997	55,994
16	30	902	3,608	6,313	27,058	54,115
17	32	874	3,494	6,115	26,207	52,414
18	34	848	3,391	5,934	25,432	50,865
19	36	824	3,296	5,769	24,723	49,445
20	38	802	3,209	5,616	24,069	48,139

MDD is minimum detectable difference in ppb (OC)
RSE is Residual Standard Error
DF is degrees of freedom ($n_1 + n_2 - 2$)

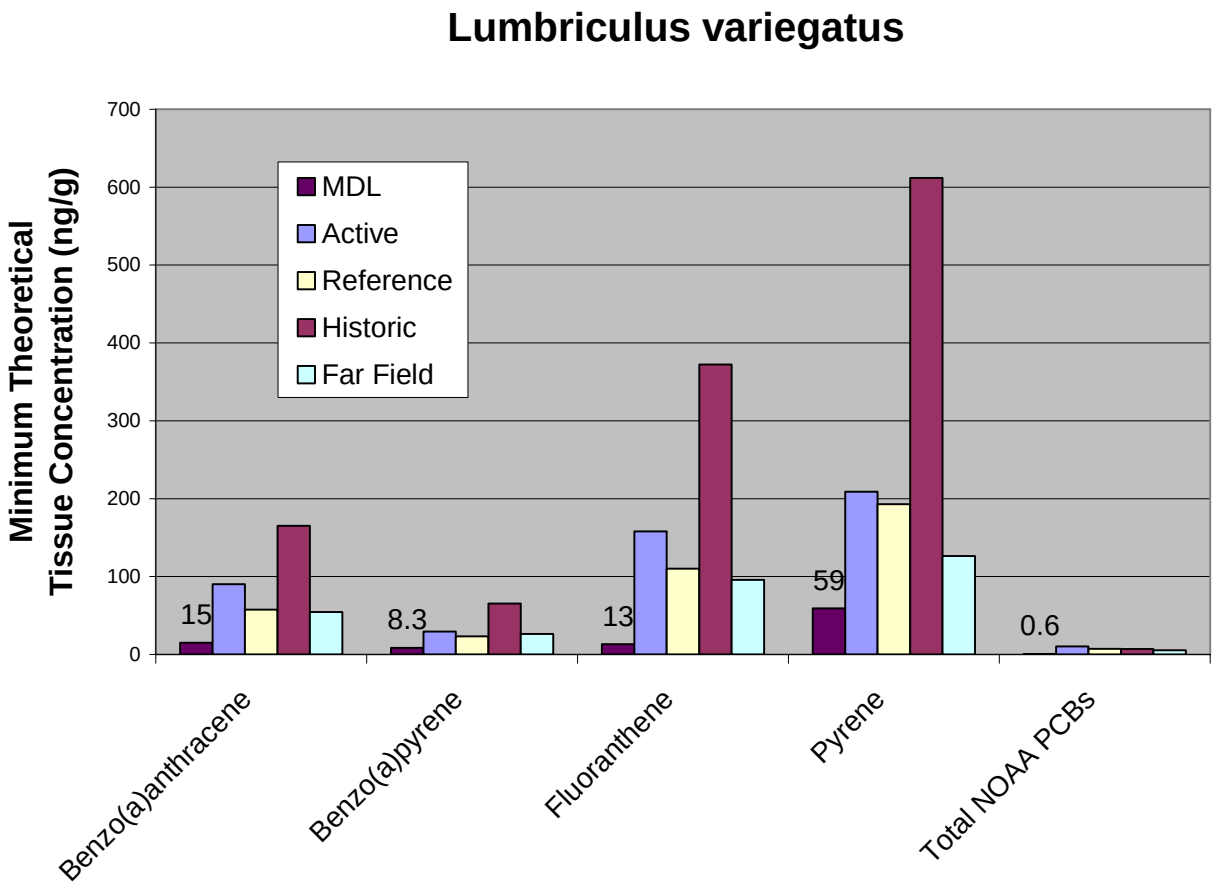

Figure 2-1. Microscale method MDLs (labeled) compared to minimum theoretical tissue concentrations of various organic contaminants in the oligochaete *Lumbriculus variegatus* for different types of areas in LIS

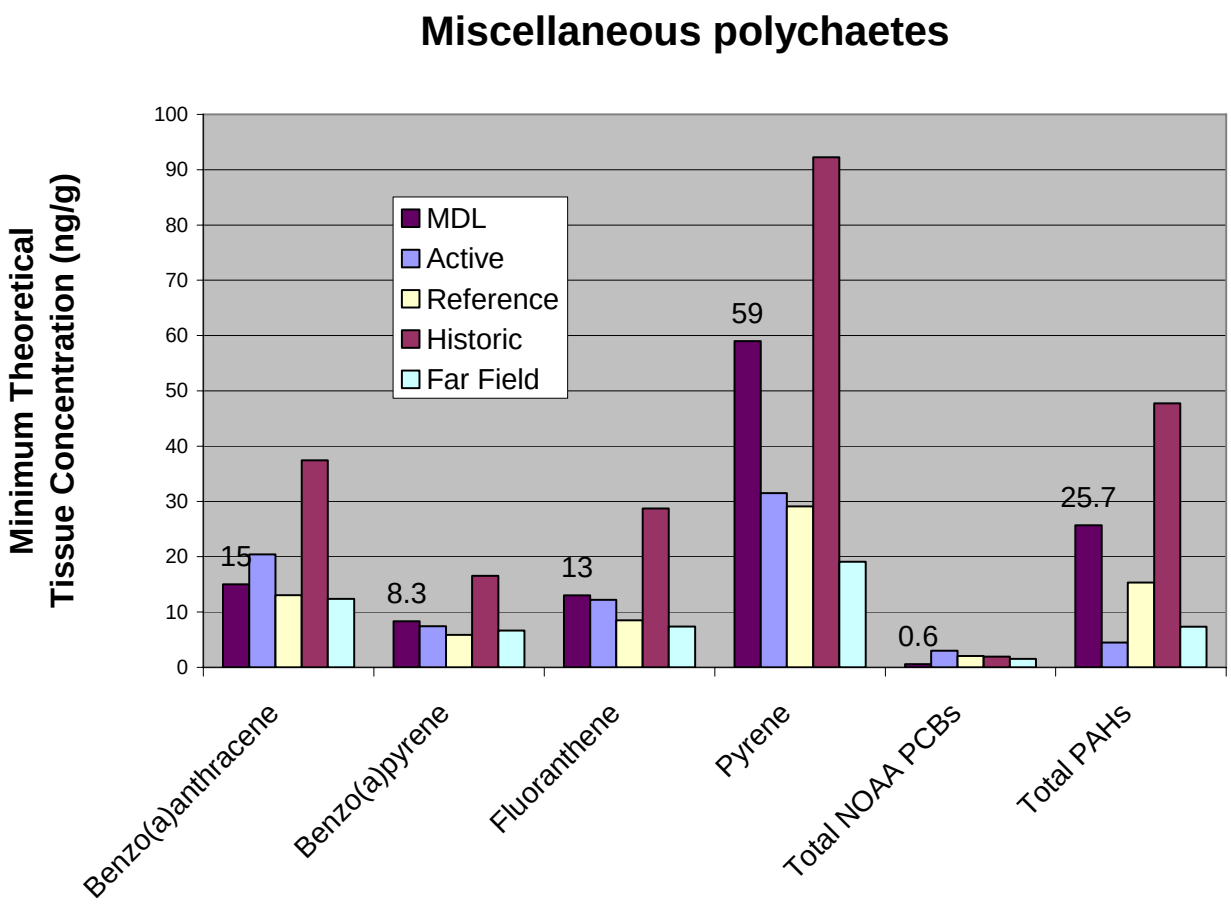

Figure 2-2. Microscale method MDLs (labeled) compared to minimum theoretical tissue concentrations of various organic contaminants in miscellaneous polychaetes for different types of areas in LIS.

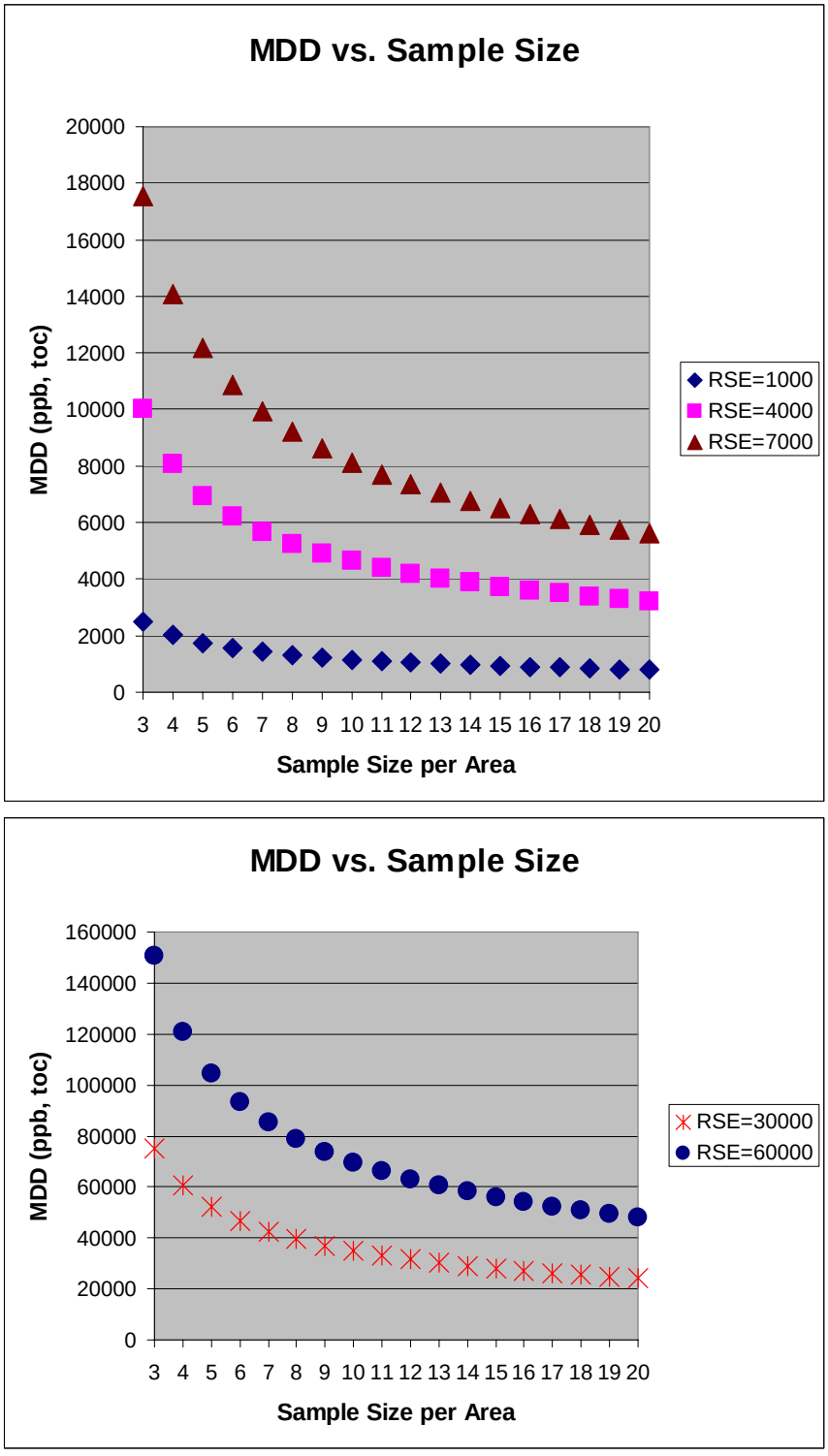

Figure 2-3. Power curves for the five variance scenarios shown in Table 2-8.

3.0 DISCUSSION AND RECOMMENDATIONS

Our interviews with experts and literature review indicate that methods for contaminant analysis in small tissue masses have advanced considerably over the past 10 to 15 years. It now appears possible to quantify relatively low "background" organic contaminant concentrations in samples comprised of just a few individuals of small aquatic organisms (e.g., 15 adult amphipods representing a total of about 7 mg wet weight of tissue per sample, or 5 adults of the Stage 1 polychaete *Streblospio benedicti* representing a total of about 5 mg wet weight of tissue per sample). Because such species typically occur at densities of hundreds to thousands of individuals per square meter, particularly when colonizing new dredged material deposits, the time and effort (and thus cost) involved in sample collection should be greatly reduced compared to years past, when up to 25,000 or 30,000 mg wet weight of tissue were required for analysis.

Given the greatly reduced biomass requirements, it may be possible to collect a sufficient amount of tissue using the traditional approach involving grab sampling, sieving and careful hand-picking of the target organism(s). This will probably require the participation of a skilled benthic taxonomist as part of the field collection team. The "worm isolator" developed by DAMOS in the mid-1990s also might prove useful for isolating sediment-free individuals of small colonizing infauna from grab samples. Decisions regarding the use of this device would benefit from the conclusions and recommendations made in the original DAMOS report (Rhoads et al. 1996), along with subsequently published bioaccumulation literature. Regardless of the collection technique, many bioaccumulation studies employ a water-only depuration step so that the collected organisms can purge their guts of sediment particles prior to extraction and analysis. Although fish typically consume whole worms including their gut contents, the depuration step allows investigations to focus more precisely on the kinetics of tissue-to-tissue contaminant transfer as opposed to sediment-to-tissue kinetics.

Our first-order power analysis indicates that from 5 to 20 samples would need to be collected at both a disposal mound and reference area to reliably detect any significant differences that might exist between the two in tissue concentrations of various organic contaminants. At an estimated cost of $500 to $1,000 per sample, the total analytical cost alone could range from $5,000 to $40,000. The ideal number of samples ultimately depends on the magnitude of the difference that investigators wish to be able to detect, and this is an important issue for planning any future DAMOS studies.

Alternately, it is worth questioning whether or not a future DAMOS study needs to employ the standard "disposal mound versus reference area" hypothesis testing approach. Our calculations of theoretical bioaccumulation potential suggest there would be little difference in tissue concentrations measured at active

disposal mounds versus reference areas. Ultimately, concerns about disposal-related contaminant bioaccumulation revolve around questions regarding food chain transfer and ecological and human health risks. It might be more advisable to direct limited resources toward the development of more advanced food chain and/or risk assessment models, with field collections targeted toward filling any identified data gaps.

There are numerous other factors that can influence contaminant bioaccumulation in benthic organisms, and that need to be considered in the planning of any future DAMOS studies (Penry and Weston 1998; Lotufo et al. 2000; Jonker et al. 2004; Rust et al. 2004a,b,c). Although not exhaustive, the following list includes several key factors:

- **Lipid Content of Tissues**: Many organic contaminants are concentrated in the lipid fraction of benthic organisms, and lipid concentrations can vary both seasonally and among species. If the objective of a study is to assess maximal or "worst-case" tissue concentrations of contaminants, then an attempt might be made to collect samples when the lipid content of benthic organisms is relatively high. In LIS, early summer is a time when the sediment detrital pool is fresh with planktonic material, and it is assumed that lipid concentrations of resident detrital-consumer organisms also may be higher than at other times of the year. Alternately, the goal of any future studies might be to avoid periods of atypically high lipid and corresponding contaminant concentrations. In late summer and fall, benthic organisms inhabiting temperate estuaries like LIS generally tend to have low inventories of fatty acids (and hence, low inventories of associated contaminants) (Marsh and Tenore 1990). This same time period can also be associated with decreased population densities of opportunists.

- **Metabolic Rates**: Metabolic rates of species of interest can vary widely and are determined by both genetics and water temperatures. High metabolic rates increase the turnover rates of the lipid pool. Varying ability to metabolize different organic compounds, such as PAHs, can greatly influence body burdens and the potential for trophic transfer within and among species. To avoid grossly underestimating total bioaccumulation of PAHs, for example, it has been strongly recommended that both parent compounds and metabolic products be measured in test organisms (McElroy et al. 1990; Driscoll and McElroy 1996). Alternately, it may be desirable to avoid using test species that are known to actively metabolize PAHs compounds, including polychaetes such as *Nereis virens* and *N. diversicolor* (Driscoll and McElroy 1996; Rust

et al. 2004a). Avoiding tissue collections during the late summer thermal peak, when metabolic rates are high, also is advised.

- **Bottom Disturbance**: The DAMOS "worm isolator" demonstration project in Long Island Sound was undertaken in early September 1991, two weeks after the passage of Hurricane Bob. If possible, a greater length of time should be allowed to pass following such an unusually high kinetic energy event to allow re-establishment of benthic populations that may have been disturbed. Contaminant gradients that typically exist under more quiescent conditions also can be obscured by such an event, making it difficult to interpret results.

- **Near-Bottom Dissolved Oxygen**: The "worm isolator" concept is based on the fact that benthic invertebrates tend to readjust their life positions according to dissolved oxygen gradients. Given an opportunity to migrate along an oxygen gradient, invertebrates will move from a low oxygen environment (i.e., reduced sediment) upward toward a higher concentration of oxygen (i.e., the walls of the "worm isolator"). This response is best displayed in benthic organisms that have been experiencing aerobic conditions at the sediment-water interface. If, prior to collection, such organisms have been exposed to low concentrations of dissolved oxygen, the migratory response seems to be reduced. In addition, it has been demonstrated that exposure to moderate hypoxia can result in significant changes in tolerance to and bioaccumulation of organic contaminants by Stage 1 organisms (Weinstein and Sanger 2003). Therefore, sampling using the "worm isolator" should be avoided during late summer and early fall, when bottom water dissolved oxygen concentrations may be low and bioaccumulation rates may be anomalously high.

All of the factors listed above should be taken into account in any future special studies or routine monitoring conducted by the DAMOS Program. If the objective is to characterize bioaccumulation when contaminant body burdens are likely to be at seasonally high levels, then sampling in late spring to early summer would probably increase the likelihood of encountering high lipid contents of tissues, increasing (but not high) bottom temperatures, high bottom water oxygen, and generally low kinetic energy in coastal New England.

Although microscale analytical methods are available, it does not necessarily mean that studies of bioaccumulation using field-collected organisms should become a routine part of DAMOS monitoring. In the present tiered monitoring approach, such studies are only called for at capped disposal mounds after mature successional assemblages have developed, as a way of verifying the

effectiveness of the cap in preventing upward migration and bioaccumulation of chemical contaminants (Germano et al. 1994). For the majority of DAMOS efforts involving routine monitoring at unconfined, open-water disposal mounds, such studies are deemed unnecessarily time-consuming and costly, based on the assumption that the current "Green Book" screening protocols are effective at minimizing any risks associated with sediment contaminants.

In the future, the DAMOS Program may be interested in conducting one or more investigative studies, outside of its routine monitoring efforts, as a way to verify the efficacy of the current screening protocols with respect to potential contaminant bioaccumulation in small, Stage 1 colonizers. Specifically, when it is deemed necessary under the current protocols, the bioaccumulation potential of dredged material proposed for ocean disposal is evaluated through laboratory tests that utilize large test organisms. By employing the microscale analytical techniques, the same dredged material could be evaluated using both the standard large and the smaller (i.e., Stage 1) test organisms, thereby facilitating an assessment of whether organism size and life history traits significantly influence the test outcome. The main value of having the microscale techniques available, however, is to facilitate any future bioaccumulation measurements that may be required under the existing tiered monitoring approach (i.e., when needed for monitoring of capped mounds). As indicated previously, it is recommended that any future bioaccumulation studies focus on acquiring and interpreting the data within a valid human and ecological risk assessment framework.

4.0 SUMMARY

- Interviews with experts and literature reviews indicate that microscale or microextraction analytical techniques exist and have been used to measure both lipids and environmentally realistic concentrations of bioaccumulative organic contaminants (PAHs and PCBs) in small tissue masses.

- Researchers at ERDC, USC and MSRC have spearheaded the development and application of the microscale analytical methods, as documented in several key published studies. Concentrations of PAHs and PCBs have been measured in samples containing as few as 20 individual copepods, 3 to 15 amphipods, and 3 to 5 individuals of the small spionid polychaete *Streblospio benedicti*. The total amounts of tissue per sample required by the microscale methods in the studies reviewed in this report range from about 0.5 to 100 mg wet weight; these amounts are 3 to 5 orders of magnitude less than the 25,000 to 30,000 mg wet weight of tissue per sample required by the traditional methods.

- The detection limits achievable with the ERDC microscale approach are sufficient for measuring predicted levels of organic contaminants in Stage 1 polychaetes inhabiting DAMOS disposal sites and reference areas in LIS. These detection limits were achieved through analysis of 100 mg of wet tissue per sample in the ERDC validation studies, and similar amounts of tissue per sample presumably would be needed in any future DAMOS studies that use these methods.

- Compared to the ERDC approach, smaller amounts of tissue per sample are sufficient for the USC/MSRC microscale analytical methods. For example, only 5 mg wet weight of tissue per sample, representing 5 individual adult *S. benedicti*, would need to be collected in any future DAMOS studies for successful analysis of even the lowest theoretical tissue concentrations of selected PAHs and PCBs (predicted using the TBP method of calculation).

- A first-order power analysis using LIS sediment chemistry data indicates that from 5 to 20 samples would need to be collected and analyzed at both a disposal mound and reference area to reliably detect any significant differences that might exist between the two in organic contaminant tissue concentrations. The number of samples ultimately depends on the magnitude of the difference that investigators wish to be able to detect.

- Our calculations of theoretical bioaccumulation potential, using actual data from LIS, suggest there would be little difference in tissue

concentrations measured at active disposal mounds versus reference areas. In lieu of conducting extensive laboratory experiments and/or field studies to test for small differences in bioaccumulation between disposal mound and reference areas, it might be more useful for DAMOS to direct limited resources toward the development of more advanced food chain and/or risk assessment models, with field collections targeted toward filling any identified data gaps.

- Although microscale analytical methods are available, it does not necessarily mean that studies of bioaccumulation using field-collected organisms should become a routine part of DAMOS monitoring. In the future, the DAMOS Program may be interested in conducting one or more investigative studies outside of its routine monitoring efforts. Such studies could help determine, for example, whether the use of small, Stage 1 organisms (in lieu of the recommended large species) changes the outcome of laboratory tests of bioaccumulation potential.

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