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Environmental Effects of Dredging

*Section 01 - Aquatic Disposal
Technical Notes
EEDP-01-37 through EEDP-01-41*

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Section 01—Aquatic Disposal
EEDP-01-34 through EEDP-01-41

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Environmental Effects of Dredging Technical Notes



Naturally Occurring Levels of Ammonia and Sulfide in Pore Water: An Assessment of the Literature

Purpose

Ammonia and sulfide are natural constituents of sediment. Both are very toxic to aquatic organisms. Consequently, their presence may bias dredged material toxicity bioassays that are designed to evaluate the toxicity of persistent contaminants such as heavy metals and petroleum and chlorinated hydrocarbons. The purpose of this technical note is to summarize published information on sediment pore water ammonia and sulfide concentrations that occur in situ. In a subsequent technical note, this exposure information will be coupled with ammonia and sulfide toxicity data to estimate the potential influence of these constituents on dredged material toxicity bioassays.

Background

In aquatic ecosystems, ammonia is derived primarily via the hydrolysis of macromolecules and subsequent deamination of amino acids (Santschi and others 1990). The molecule exists in two forms, ionized (NH_4^+) and un-ionized (NH_3) ammonia (Wajsbrodt and others 1990). Un-ionized ammonia appears to be the toxic moiety based primarily on studies with freshwater fish (Nimmo and others 1989). The proportion of total ammonia present in the un-ionized form increases with pH. For example, at pH values of 7, 8, and 9 (20 °C), the approximate percent of un-ionized ammonia is 0.4, 4.0, and 28 percent, respectively. Temperature and, to a lesser degree, ionic strength (that is, hardness or salinity) also affect the relative proportion of un-ionized ammonia (Emerson and others 1975; Thurston and others 1981; Williams, Green, and Pascoe 1986). Jones and Lee (1988) suggested that ammonia toxicity may be an important factor in many marine sediment bioassays. Ankley, Katko, and Arthur (1990) clearly demonstrated this for some freshwater sediments containing substantial amounts of anthropogenic chemicals. Ankley, Katko, and Arthur (1990) postulate that if ammonia is the causative agent in sediment toxicity bioassays, past

interpretations regarding potential environmental impacts may have been erroneous.

Sulfides are compounds containing one or more sulfur atoms connected directly to a carbon, metal, and other nonoxygen atom. In sediments, sulfides exist as insoluble precipitates and as dissolved sulfide compounds. In the presence of oxygen, sulfide rapidly oxidizes to sulfate or, in some instances, to elemental sulfur (Ponnamperuma 1972). Sulfides, therefore, are usually associated with hypoxic or anoxic conditions such as may occur in highly organic and undisturbed sediments. H_2S , the toxicologically important form of sulfide, is produced when bacteria reduce sulfates and putrefy proteins.

Sulfides in pore water may be analyzed as total sulfides (TS), as dissolved sulfides (DS), and as H_2S . TS consist of acid-soluble metallic sulfides in suspended matter plus dissolved H_2S . DS remain after the suspended solids have been removed by flocculation and settling. H_2S may be analyzed directly or calculated from the concentration of DS, sample pH, and the ionization constant for H_2S (American Public Health Association (APHA) 1980). The relationship between H_2S and pH is opposite that for NH_3 . The proportion of H_2S in DS decreases with pH. For example, at pHs 6, 7, and 8, the approximate percent of H_2S is 90, 50, and 10 percent, respectively (APHA 1980). Since most sediments are near neutral (pH 7 to 8), the proportion of H_2S in DS is 10 to 50 percent. In contrast, H_2S represents only about 6 percent of DS in seawater (Bagarinao 1992).

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Procedure

In the literature examined, approximately 40 papers contained data on the levels of ammonia and sulfide in sediment pore water. The following information was extracted from each paper: range of ammonia and sulfide concentrations observed, method of pore water extraction, method of ammonia and sulfide analysis, pH, and depth of collection.

Most papers reported ammonia and sulfide concentrations on a molar basis. To be consistent, volume-specific concentrations (for example, milligrams per liter) were converted to molar equivalents using the conversions shown below. Also provided are useful relationships for converting back to volume-specific concentrations.

1 μM NH_3 = 17 μg NH_3/L
1 μM NH_4 = 18 μg NH_4/L
1 μM H_2S = 34 μg $\text{H}_2\text{S}/\text{L}$

59 μM NH_3 = 1 ppm NH_3/L
56 μM NH_4 = 1 ppm NH_4/L
29 μM H_2S = 1 ppm $\text{H}_2\text{S}/\text{L}$

Results

Ammonia

Un-ionized ammonia (NH_3) was the most frequently reported ionic form (Table 1). Concentrations as high as 12,500 μM (430 ppm) were reported. Values between 10 and 1,000 μM NH_3 (0.17 to 17 ppm) were more common, however. The most frequently used method for obtaining pore water was centrifugation (\approx 40 percent), followed by mechanical squeezer (\approx 30 percent). Autoanalyzer and ion-selective electrode were the first and second methods of choice for analyzing NH_3 . Kjeldahl distillation and indophenol spectrophotometric methods were used to analyze NH_4 . Most pore water samples were taken from depths ranging from 0 to 30 cm of sediment where pH values were near neutral (7.0 to 8.0).

Sulfides

H_2S concentrations as high as 10,000 μM (345 ppm) have been reported. However, values between 20 and 5,000 μM (0.7 to 170 ppm) were more common. The mechanical squeezer was the most frequently used method for obtaining pore water for sulfide analysis. Analytical methods included colorimetric, titration, and ion-specific electrode. Most pore water samples were taken from the upper 30 to 40 cm of sediment where pH values were near neutral.

Discussion

Reported concentrations of ammonia and sulfide in sediment interstitial water were highly variable. Factors contributing to this variability can be ranked. Probably the most important is geographic. The data reported in Tables 1 and 2 represent sediments and environments that vary greatly in their physicochemical properties and productivity, respectively.

The next most important factor contributing to the observed variability is probably seasonality. Sediment ammonia and sulfide levels are typically low in the winter and high in late spring and early fall (Berner 1980; Feijtel, DeLaune, and Patrick 1988; Howarth and Teal 1980; Howarth and others 1983). This seasonal cycle corresponds to the annual pattern of carbon fixation by

Table 1. In Situ Concentrations of Ammonia in Sediment Pore Water					
Concentration Range, μM	Pore Water Removal Method	Method of Analysis	pH	Depth, cm	Citation
Ionic Form NH_3					
1,400-12,500	Dialysis sampler	Autoanalyzer	7.0-8.0	1-25	Boers and deBles 1991
0-6,320	Squeezer	Autoanalyzer	7.6-7.9	0-14	Murray, Grundmanis, and Smethie 1978
800-4,100	Centrifugation	Autoanalyzer	NR ¹	0-10	Klump and Martens 1981
130-3,235 ²	Centrifugation	Autoanalyzer	NR	NR	Brannon, Plumb, and Smith 1978
106-3,118 ²	Centrifugation	Ion-specific electrode	6.5-8.5	NR	Ankley, Katko, and Arthur 1990
2-1,500	Tube pressed into sediment	Ion-specific electrode	NR	0-8	Watson, Frickers, and Goodchild 1985
96-1,140	Dialysis sampler	Autoanalyzer	7.5-7.7	0-26	Viel and others 1991
110-1,540	Centrifugation	Autoanalyzer	7.0-7.9	0-30	Viel and others 1991
10-470	Centrifugation	Autoanalyzer	NR	0-10	Larat, Lasserre, and le Corre 1990
0-300	Squeezer	Ion-specific electrode	NR	0-40	Tisue, Edington, and Seils 1988
\approx 1-126.7	Squeezer	Ion-specific electrode	7.6-8.6	NR	USDI 1992
24-35 ²	Squeezer	Autoanalyzer	6.9-7.4	2	Carr, Williams, and Fragata 1989
6-79	Dialysis sampler	NR	NR	198-594	Sly 1988
3-19 ²	Hand-suction pump	Autoanalyzer	NR	NR	Oliff and others 1970
\leq 1-47	Centrifugation	Ion-specific electrode	NR	0-24	Simon 1989
0-30	Squeezer	Autoanalyzer	NR	0-30	van der Loeff 1980
¹ Not reported.					
² Concentration converted to micromoles per liter.					
<i>(Continued)</i>					

Table 1. (Concluded)					
Concentration Range, μM	Pore Water Removal Method	Method of Analysis	pH	Depth, cm	Citation
Ionic Form NH_4^+					
5,000-200,000	Squeezer	Indophenol	NR	0-10	Raaphorst and others 1990
200-2,556 ²	Centrifugation	Kjeldahl distillation	NR	0-60	Brannon and others 1976
0-2,000	Squeezer	Kjeldahl distillation	NR	0-130	Rosenfeld 1981
20-1,310	Centrifugation	Kjeldahl distillation	NR	0-60	Sholkovitz 1973
38-735 ²	Filtration	Kjeldahl distillation	7.2-7.5	NR	Ho and Lane 1973
27-631	Squeezer	Kjeldahl distillation	6.9-7.6	0-18	Aller 1980
0-398	Centrifugation	NR	NR	0-40	Grasshoff 1976
0-18	Pipette sampler	Spectrophotometric	7.4-8.6	0-20	McLachlan 1978
$\leq 1-6$	Centrifugation	Indophenol	NR	0-9	Laima 1992

phytoplankton. Confounding this seasonal influence of primary production is the recent discovery that sediment ammonia exists in different exchangeable pools which also vary seasonally (Laima 1992).

Finally, two important contributors to the observed variability are inconsistent methods for both pore water removal and chemical analysis. Methods for these activities have been shown to greatly affect results (Howes 1985, Knezovich and Harrison 1987, Pittinger and others 1988). Among the studies reviewed in this survey, two of the most popular methods for obtaining pore water are centrifugation and mechanical squeezing. In a comparison of collection methods, Schults and others (1992) concluded that centrifugation was the most accurate and precise method for analysis of organic chemical contaminants in pore water. For H_2S , centrifugation should not violate the hypoxic integrity of the sample.

Summary

Literature was reviewed for sediment pore water concentrations of ammonia and sulfides. Toxic constituents of concern are un-ionized ammonia (NH_3) and hydrogen sulfide (H_2S). Concentrations of NH_3 as high as 12,500 μM (430 ppm) have been reported. However, values between 10 and 1,000 μM (0.17 to 17 ppm) are more common. The highest concentration of H_2S was about 10,000 μM (345 ppm). Most values ranged between 20 and 5,000 μM (0.7 to 170 ppm). Factors contributing to the variable pore water concentrations

Table 2. In Situ Concentrations of Sulfides in Sediment Pore Water					
Concentration Range, μM	Pore Water Removal Method	Method of Analysis	pH	Depth, cm	Citation
Ionic Form H_2S					
0-10,080	Squeezer	Colorimetric	7.6-7.9	0-140	Murray, Grundmanis, and Smethie 1978
0-5,882 ¹	Centrifugation	Colorimetric	NR ²	0-140	Moore and Dillon 1993
0-4,920	Squeezer	Titration	6.1-7.2	0-54	Boulegue, Lord, and Church 1982
3-255	Centrifugation	Colorimetric	NR	1-16	Swider and Mackin 1989
22-287	Squeezer	NR	NR	7-24	Aller 1980
0-3 ¹	Pipette sampler	Spectrophotometric	7.4-8.6	0-20	McLachlan 1978
<2.9 ¹	Filtration	NR	6.8-7.6	0-60	USACE 1975
Dissolved Sulfides					
5-50	Squeezer	Measured on precipitated ZnS	NR	0-10	Fossing and Jorgensen 1990
0-1	Squeezer	Colorimetric	4.1-7.2	5-20	Howarth and others 1983
0-2,900	Squeezer	Titration	7.0-9.0	0-80	Krom and Sholkovitz 1977
Total Sulfides					
0-212	Squeezer and in situ sampler	NR	NR	2-20	Howes 1985
0-5 ¹	Squeezer	Ion-specific electrode	7.7-7.8	1-45	Brooks, Presley, and Kaplan 1968
0- \leq 1	Squeezer	Ion-specific electrode	7.6-8.6	NR	USDI 1992
¹ Concentration converted to micromoles per liter.					
² Not reported.					

include geographic dissimilarities, seasonal effects, different chemical methods for analyzing ammonia and sulfide, and variable techniques for obtaining pore water. Centrifugation is the method of choice for obtaining interstitial water from dredged material samples.

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Environmental Effects of Dredging Technical Notes



A Comparison of Three Lipid Extraction Methods

Purpose

This technical note summarizes results from studies that compare three commonly used lipid extraction methods: Bligh-Dyer, hexane:acetone, and dichloromethane.

Background

Organism lipid content is a critical component of both theoretical bioaccumulation potential, currently recommended in the "Green Book" (U.S. Environmental Protection Agency/U.S. Army Corps of Engineers 1991), and the sediment quality criteria (SQC) proposed by the USEPA. Since no standard extraction method exists for quantitating lipids, many different methods are used, leading to questions concerning the comparability of lipid data obtained using different extraction methods. Research examining the relationship between various extraction methodologies can help reduce uncertainty in interpreting and utilizing data obtained from different studies, leading to better environmental assessments concerning the long-term effects of dredging.

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Introduction

The role of lipids as the major compartment for neutral organic chemical partitioning in organism tissues has been well documented (Connell 1988; Esser 1986; Roberts, de Frietas, and Gidney 1977; Schneider 1982). Lipid normalization is used in the calculation of accumulation factors, which express the equilibrium distribution of neutral chemicals between sediments and aquatic biota (Ankley and others 1992; Ferraro and others 1990, 1991; Lake and others 1990; McFarland and others 1994; Young, Mearns, and Gossett 1991). A screening test used to estimate the bioaccumulation potential of neutral chemicals associated with dredged sediments relies on equilibrium partitioning to organism lipids (USEPA/USACE 1991). Additionally, the USEPA seeks to promulgate SQC that will require lipid normalization of data (USEPA 1993).

No standardized method exists for lipid determinations in environmental samples. Typically, analysts will either reserve an aliquot of a residue-analysis tissue extract for lipid analysis or run a separate tissue sample for lipid analysis concurrently. In the former case, hexane:acetone or dichloromethane is commonly used as the solvent (Ryan and others 1985, Schwartz and others 1993). In the latter case, the chloroform:methanol (Bligh-Dyer) method is commonly used, as it is specifically intended for lipid analysis and is routinely used to measure the lipid content of foods (Bligh and Dyer 1959). Often, the amount of tissue used in either of the above cases varies due to differing amounts of tissue required (or available) for chemical analysis, or to the amount of sample remaining for lipid analysis after that required for chemical analysis has been taken. Knowledge of the variability that may be introduced due to sample size or solvent used is required in order to compare lipid-normalized data obtained from different studies.

The purpose of the study described in this technical note was to assess these sources of variability by comparing percent lipid determinations made on different sample sizes of the same homogenized fish tissue. Three commonly used lipid extraction methods (Bligh-Dyer, hexane:acetone, and dichloromethane) and six sample sizes representing a 200-fold range of tissue weights were compared.

Materials and Methods

Tissue

Nine kilograms of frozen commercial whiting fish was purchased from a local supermarket, skinned, and filleted. The fillets were homogenized with a Waring blender, divided into 100-g fresh weight aliquots, and stored at -80 °C until use. For each lipid extraction method, four replicate tissue samples were used for each tissue sample size. All procedures were performed at room temperature.

Bligh-Dyer

Tissue samples of 0.5, 1, 5, 10, 50, and 100 g were analyzed for lipid content using the Bligh-Dyer method (Bligh and Dyer 1959). The samples were homogenized for 2 min in a Waring blender with chloroform and methanol in the proportion of 1 g tissue:1 ml chloroform:2 ml methanol. Solvent volumes were adjusted for each sample size to maintain the same proportions. For the 0.5-, 1-, 5-, and 10-g tissue samples, a Polytron homogenizer was used rather than a Waring blender. An additional equivalent amount of chloroform was added, and the mixture was homogenized for another 30 sec. Deionized water (1 ml water/1 g tissue) was then added, and the mixture was homogenized again for 30 sec. The final mixture proportion was 1 g tissue:2 ml chloroform:2 ml methanol:1 ml deionized water.

The mixture was filtered through Whatman No. 1 filter paper, and the remaining tissue was homogenized for 2 min with another 1 ml chloroform/1 g tissue. After filtering the mixture again, the combined filtrate was transferred to a graduated cylinder and allowed to separate. Lipid content was determined gravimetrically by measuring triplicate aliquots of the chloroform layer into tared containers, air-drying the solvent, and weighing. Percent lipid determinations were then calculated.

Hexane:Acetone Extraction

Tissue samples of 0.5, 1, 5, 10, and 50 g were analyzed for lipid content by homogenizing each sample three times with 20 ml hexane:acetone (1:1, v/v) for 2 min using a Polytron homogenizer. The three extracts were filtered and pooled. Percent lipids were calculated on triplicate extract aliquots as with the Bligh-Dyer method.

Dichloromethane Extraction

Tissue samples of 0.5, 1, 5, and 10 g were placed into 25- or 150-ml screw-capped centrifuge tubes according to sample size along with one to two times the tissue weight of anhydrous sodium sulfate. Dichloromethane in a proportion of 5 ml to 1 g tissue was added to the samples, which were rolled for 18 to 24 hr. The mixture was filtered through Whatman No. 1 filter paper, and percent lipid determinations were made for triplicate aliquots of the dichloromethane extracts as with the Bligh-Dyer method.

Statistical Analysis

All data were analyzed using PC SAS (SAS Institute 1988). Two-way analysis of variance was performed using PROC GLM, and mean comparisons were made using Fisher's Protected Least Significant Difference. The normality assumption was tested using the Shapiro-Wilk's test, and homogeneity of variances was assessed using Levene's test (Snedecor and Cochran 1989).

Results and Discussion

Percent lipid data for the three methods used are listed in Table 1. The hexane:acetone method was impractical for use with the 100-g sample size. Similarly, the dichloromethane method could not be performed using sample sizes of 50 and 100 g.

Sample Size (g)	Method		
	Bligh-Dyer	Hexane:Acetone	Dichloromethane
0.5	2.07 \pm 0.20 A ¹ b ²	1.29 \pm 0.12 A b	5.77 \pm 0.37 A a
1	1.47 \pm 0.29 B a	0.63 \pm 0.05 C b	0.28 \pm 0.05 B b
5	1.12 \pm 0.26 B a	0.93 \pm 0.14 B a	0.71 \pm 0.11 BC a
10	1.06 \pm 0.02 B a	0.92 \pm 0.06 BC a	1.14 \pm 0.37 C a
50	1.25 \pm 0.12 B a	1.00 \pm 0.08 AB a	—
100	1.39 \pm 0.19 B	—	—

¹For a given method, sample size means followed by the same uppercase letter are not significantly different from each other ($p < 0.05$).

²For a given sample size, method means followed by the same lowercase letter are not significantly different from each other ($p < 0.05$).

The data indicate that sample size has a significant effect on lipid analysis results regardless of method. The whiting tissue apparently had a percent lipid value of approximately 1 percent, since all three methods yielded results encompassing this value at one or more sample weights. Lipid determinations made using the 0.5- and 1-g sample weights were the most variable and yielded significant differences among the methods, while the 5- and 10-g sample sizes were not significantly different. The Bligh-Dyer method generally gave higher percent lipid values, yielding significantly higher results for the 1-g sample size.

Randall and others (1991) found a 3.5-fold variation among several extraction methods which included acetonitrile extraction with pentane partitioning, acetone extraction with hexane partitioning, Bligh-Dyer, and acetonitrile extraction using sample sizes of 1 to 5 g. Results from this study suggest that larger sample sizes (5 to 10 g) may yield less variable results and would be comparable using the three methods investigated.

The 0.5-g sample size for all three extraction methods yielded an aberrantly high percent lipid, indicating that this sample size is below the lower limit of practical application of the three methods. Samples of less than 5 g should probably be analyzed using a micromethod such as the method described by Gardner and others (1985).

Conclusions

Sample sizes of 5 to 10 g were optimal for the three lipid extraction methods studied, and produced similar results for all three methods. The 100-g sample size called for in the original Bligh-Dyer method (Bligh and Dyer 1959) is usually impractical for environmental studies involving small organisms, and is not necessary. However, if less than 5 g of tissue is available, a micromethod should be used.

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Environmental Effects of Dredging Technical Notes



Preliminary Protocol for Conducting 28-Day Chronic Sublethal Sediment Bioassays Using the Estuarine Amphipod *Leptocheirus plumulosus* (Shoemaker)

Purpose

This technical note describes a preliminary protocol for conducting a 28-day chronic sublethal sediment bioassay using the estuarine amphipod *Leptocheirus plumulosus*. End points for this test include survival, growth, and reproduction. This protocol provides conditions for conducting the 28-day bioassay and procedures used for sediment storage and handling, laboratory culture, preparation of test chambers, reference toxicity tests, test acceptability, and data analysis.

Background

Historically, aquatic bioassays have measured survival of sensitive species after acute exposures to high concentrations of chemicals. Data generated from these tests provided relevant information for hazard assessments, but the information generated was not based on realistic environmental contaminant levels or exposure levels. In the environment, organisms are more generally exposed to low concentrations for long periods. Animals exposed to sediments normally accumulate contaminants at a slow rate compared to animals exposed to contaminants in water (Adams 1987). Thus, researchers are developing chronic bioassays that more closely approximate field conditions and measure end points in addition to lethality.

Note: The contents of this technical note are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products.

Additional Information

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Life History

Leptocheirus plumulosus is distributed subtidally along the East Coast of the United States from Cape Cod, Massachusetts, to northern Florida (Bousfield 1973). Under laboratory conditions *L. plumulosus* matures in less than 25 days and is capable of producing multiple broods during its life span (Chesapeake Bay Program 1992). Females produce up to 40 young per brood and may live over 100 days. Males are typically larger than females.

Leptocheirus plumulosus builds U-shaped burrows in sediments ranging from fine sand to silty clay and can tolerate salinities ranging from near 0 to 33 parts per thousand (ppt) (Schlekat, McGee, and Reinharz 1992). *Leptocheirus plumulosus* feeds by filtering out suspended particles of food from the water column, scraping the surface of sediment particles, or tearing organic material into small ingestible portions. Animals can tolerate aqueous-only exposures (that is, no sediment) for extended periods if food is provided.

Regulatory Use

Amphipods represent an abundant and widely distributed component of marine and estuarine benthic communities. They serve as prey for fish, birds, and larger invertebrate species (American Society for Testing and Materials 1993). They have been shown to be among the first taxa to disappear from a pollution-impacted benthic community and are considered to be one of the more sensitive taxa in the benthic systems (Swartz, DeBen, and Cole 1979). Amphipods are recommended by the Environmental Protection Agency as appropriately sensitive test organisms for evaluating sediment quality (USEPA/USACE 1994). The amphipod *Leptocheirus plumulosus* has been used by the Maryland Department of the Environment (MDE) to evaluate sediment toxicity in the Chesapeake Bay (MDE 1991). This species is also recommended for the national dredged material testing program (USEPA/USACE 1991). *Leptocheirus plumulosus* has been proposed for evaluating the chronic sublethal effects of contaminated sediments (Chesapeake Bay Program 1992; McGee, Schlekat, and Reinharz 1993). *Leptocheirus plumulosus* is an attractive animal model for chronic sublethal bioassays because sublethal end points are easily measured with relatively high precision and because individual *L. plumulosus* maintain intimate contact with sediment through burrowing and feeding activities, tolerate a wide range in salinity, can be cultured in the laboratory (unlike all other saltwater amphipods currently considered for testing), and display a sensitivity to reference toxicants similar to other amphipod species (Schlekat, McGee, and Reinharz 1992).

Bioassay Development

This preliminary protocol was developed in response to the need for a chronic sediment bioassay using *L. plumulosus* as the animal model. Development of this protocol is in accordance with the paradigm for developing sediment toxicity bioassays described by Dillon (1994). Experiments examining nontreatment effects were conducted to evaluate test "ruggedness." Ruggedness is defined by the American Society for Testing and Materials (ASTM 1992) as the "insensitivity of a test method to departures from specified test or environmental conditions." Some of the experiments conducted in developing this protocol are outlined below:

- **Artificial seawater.** *Leptocheirus plumulosus* were exposed to a variety of commercially available artificial sea salt mixtures and reconstituted seawater (for example, Forty Fathoms, Hawaiian Brands, Instant Ocean, and GP2). Greater than 88 percent survival rate was recorded for *L. plumulosus* exposed to all artificial sea salts tested. However, growth was ~26 percent lower in animals exposed to artificial sea salts not containing trace elements (for example, Instant Ocean).
- **Diet.** A variety of diets used in culturing *L. plumulosus* were evaluated to determine the most appropriate and cost-effective artificial nutrition source. Results indicated no significant difference between amphipods fed food containing algae mixed with dry ingredients and food containing dry ingredients only. Data also suggested that this species has a preference for very fine food particles (<0.5 μm).
- **Food ration.** A range of food rations was administered to *L. plumulosus* to determine the optimal diet. Data indicated a greater than 80 percent survival rate with food rations of 0.25 \times , 0.5 \times , and 1 \times . As expected, growth and reproduction increased within increasing food ration.
- **Initial size.** Sieved size classes of *L. plumulosus* were used to determine the contaminant sensitivity of early life stages, effects of life stages on test end point sensitivity (survival, growth, and reproduction), and the cost utility of sieved size-classed animals compared to "known-age" neonates collected from gravid females. Greater than 80 percent survival was recorded for amphipods retained on 300- and 425- μm sieves. However, reproductive end points could be evaluated only in 28-day tests initiated with animals retained on a 425- μm sieve. The cost associated with collecting sieved animals was substantially less.
- **Intraspecific density.** Densities ranging from 10 to 60 amphipods per 600-ml beaker had no adverse impact on survival, which was greater than 80 percent in all treatments. However, variability in growth and reproductive end points was lower using the 20 amphipod/beaker initial stocking density.
- **Salinity.** Data collected indicated that this species can tolerate a range of salinities from 1 to 30 ppt with greater than 80 percent survival. Reproduction was higher at a salinity of 5 ppt.

Bioassay Protocol

The recommended test conditions for conducting 28-day sediment bioassays with *L. plumulosus* are summarized in Table 1 and discussed in the following paragraphs.

Table 1. Recommended Test Conditions for Conducting 28-Day Sediment Bioassays with <i>Leptocheirus plumulosus</i>	
Parameter	Conditions
Substrate	2 cm sediment (presieved to <300 µm)
Salinity	5 parts per thousand
Aeration	Trickle flow (clean filtered air)
Overlying water	Filtered natural seawater or clean artificial seawater (with trace elements)
Renewal of overlying water	Daily
Overlying water volume	500 ml
Temperature	23 ± 1 °C
Photoperiod	16:8 hr (light/dark)
Test duration	28 days
Experiment chambers	600-ml glass beakers
Initial age/size of experimental animals	Animals retained on 425-µm sieve but passing through 600-µm sieve (1 to 2 weeks old)
Feeding	Three times per week (M-W-F). Note: 1 mg Gorp/amphipod for first 2 weeks, then 2 mg Gorp/amphipod thereafter
Number of organisms/beaker	20
Number of replicate chambers/treatment	5 minimum (subject to revision upon completion of power analysis)
Water quality monitoring	Weekly (pH, DO, salinity, ammonia) Daily (temperature)
End points	Survival, growth, and reproduction
Test acceptability	Minimum mean control survival of 70 percent and reproduction in control chambers

Sediment Storage and Handling

Upon sediment arrival, portions should be analyzed for grain size, total Kjeldahl nitrogen, total organic carbon, interstitial salinity, pH, pore water

concentrations of hydrogen sulfide (H₂S), and ammonia (NH₃). When chronic bioassays are to be conducted after sediment arrival, sediments should be stored in the dark at 4 °C. Prior to test initiation, sediment should be homogenized, sieved (<300 µm), and added to the test chamber to a depth of 2 cm. Addition of animals to tests should not take place until sediments are brought to the appropriate test temperature.

Laboratory Cultures

Amphipod cultures are established using 67 animals from each of three sieved size classes (1 mm, 600 µm, and 425 µm). Cultures are maintained in 45- by 24- by 15-cm polyethylene tote boxes containing 2 cm of sieved sediment (<300 µm). Overlying water in all cultures is at 5 ppt, with a constant temperature of 23 °C ± 1 °C, placed on trickle flow aeration. Cultures are fed 2 mg Gorp/animal three times a week (Monday, Wednesday, and Friday). Gorp is a mixture of 48.5 g TetraMin, 24.0 g dried alfalfa (alfalfa tablets, Bernard Jensen International, Escondido, CA), 24.0 g wheat grass powder (Green Energy, Pines International, Inc., Lawrence, KS), and 4.5 g Neo-Novum shrimp maturation feed (Argent Chemical Laboratories, Redmond, WA), all ground to ≤0.5 mm in a food mill. Water is renewed in all culture tubs (60 percent by volume) prior to each feeding. Water quality monitoring in all cultures includes pH, salinity, dissolved oxygen (DO), and temperature.

Animal Collection

Leptocheirus plumulosus are collected from the culture using nested sieves (1 mm, 600 µm, 425 µm, and 300 µm). Animals retained on the 1-mm sieve are mature adults (≥21 days old approximately); those retained on the 600-µm sieve are subadults (<21 days old); on the 425-µm sieve, juveniles (<2 weeks old); and on the 300-µm sieve, newly released neonates (<1 week old). Sediments are gently disturbed, and the suspended sediment is poured through the nested sieves. Animals retained on the 425-µm sieve are used for chronic bioassays. The *L. plumulosus* obtained from multiple culture tubs are pooled prior to selecting animals for testing.

Preparation of Test Chambers

Sieved sediment should be added to 600-ml beakers to a depth of 2 cm (the approximate average burrow depth), 1 day prior to test initiation (but not more than 3 days), overlaid with seawater, placed on trickle flow aeration, and brought to test temperature. On the day of test initiation (before animal addition), overlying water is renewed (60 percent by volume) and water quality parameters are taken. Water renewal prior to animal addition reduces NH₃ and H₂S levels. Water quality parameters taken include pH, salinity, DO, temperature, and ammonia in overlying water at test initiation and termination.

Test Initiation

To initiate a test, 10 amphipods from pooled culture animals are randomly added to 50-ml beakers. Two of these 50-ml beakers (20 amphipods) are randomly assigned to test chambers and gently added, making sure that no animals are trapped in the surface tension of the overlying water (floating). Animals trapped in the surface tension may be freed by gently dropping water from a pipet onto the animal. Each beaker used to dispense test animals is carefully rinsed to ensure that no animals remain. Five 50-ml beakers (each containing 10 amphipods) should be retained for initial weights and used in the calculation of growth rates at test termination.

Test Conduct and Monitoring

Each test chamber should be fed at one-half food ration (1 mg Gorp/animal) for the first 2 weeks and then at full ration (2 mg Gorp/animal) for the remaining 2 weeks. This feeding regime ensures adequate food for normal growth and development while reducing the possibility of excess food contributing to poor water quality. Water should be renewed (~60 percent by volume) daily for the duration of the 28-day test. Water quality parameters should be recorded weekly for each test chamber. Water quality parameters should include pH, salinity, DO, and temperature.

Test Termination

Animals from individual test chambers will be collected via nested sieves (1 mm (adults), 600 μm (juveniles), and 300 μm (newly released neonates)). Amphipods recovered from each individual test chamber are counted and classified by sieved size class. Amphipods in the 1-mm size class are separated into gravid and nongravid categories, then fixed in a 70-percent solution of rose bengal in ethanol.

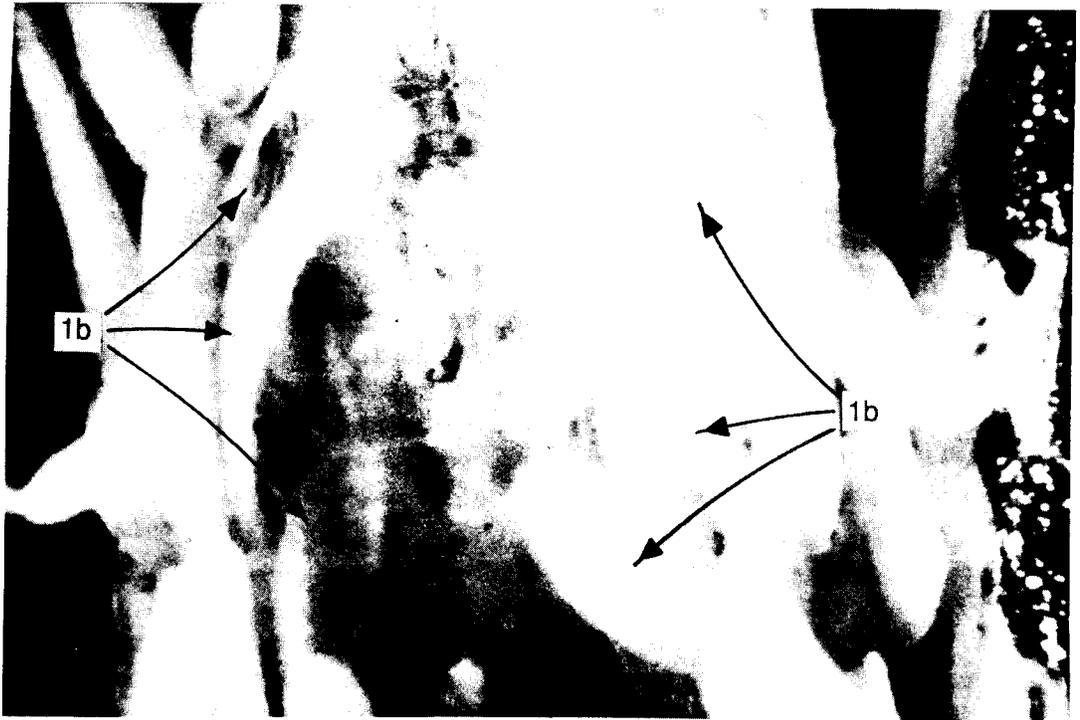
Test End Point Measurement

Survival. Amphipod survival is determined by gently prodding all animals retained on the 1-mm sieve (with Pasteur pipet) during the breakdown of each test chamber. Live animals are then counted, and the total number is divided by the number of amphipods originally placed in each beaker. Occasionally, some amphipods retained on the 600- μm sieve are noticeably larger than others. In such cases it will be necessary for the investigator to make a determination as to whether the animals should be considered adults.

Sex determination. Amphipods identified as gravid are classified as female and are used to evaluate reproduction. Amphipods identified as nongravid will be individually observed under a dissecting microscope to determine sex. Observations will be made of the ventral side of each amphipod. Penile papillae (Figure 1a) are used to identify males and setose oostegites (Figure 1b) to identify females. All adult amphipods are retained for growth end point estimates.



a. Penile papillae in adult male



b. Setose oostegites in adult female

Figure 1. Ventral view of *Leptocheirus plumulosus*

A small number of immature animals may not be sexed because distinguishing male and female organs are not apparent; these animals are grouped into an undifferentiated category.

Reproduction. Amphipod reproduction is measured by counting neonates (animals retained on 300- μm sieve) and by counting embryos stripped from brooding females. Animals are stripped by gently holding each brooding female ventral side up with a pair of forceps in a shallow petri dish containing seawater and forcing embryos out of the brood pouch with a gentle stream of seawater from a pasteur pipet.

Growth. Estimates of individual growth (by sex) are determined by placing all adult animals of a given sex and replicate on preweighed aluminum pans (dried for 24 hr at 60 °C). Animals are then dried at 60 °C for 24 hr and reweighed. Growth rate is calculated using the following equation:

$$G = \frac{DWT_{t_2} - DWT_{t_1}}{t_2 - t_1}$$

where

DWT_{t_2} = estimated individual dry weight of surviving adults at test termination

DWT_{t_1} = estimate of individual dry weight of animals at test initiation

$t_2 - t_1$ = duration of test, days

Reference Toxicant Tests

The overall health and sensitivity of culture animals should be monitored monthly using 96-hr water-only reference toxicant tests with cadmium chloride. Reference toxicant tests provide a means of biological quality control for cultures (Lee 1980; USEPA/USACE 1994, Appendix G). Animals retained on a 425- μm sieve collected from culture (see section "Animal Collection" above) are placed in holding cups (five amphipods/cup) and gently added to a range of cadmium concentrations (five replicates/concentration). No food is provided during the 96-hr tests. Using the methods described above, results in a LC_{50} of ~0.06 mg cadmium/L at a salinity of 5 ppt and a cadmium concentration range of 0.001 to 0.2 mg cadmium/L.

Test Acceptability Criteria

Greater than or equal to 70 percent survival and the presence of reproduction in all control replicates will determine test acceptability. Recommendations will be made regarding more specific levels of reproduction once additional data are collected and incorporated into our database. Also, the quality (salinity, pH, and ammonia) in overlying water should be within tolerance limits for this species.

Data Analysis

Reference Toxicant Tests. LC₅₀s should be determined for all reference toxicant tests using one of the methods described in Appendix D of USEPA/USACE (1994).

Survival, Growth, and Reproduction. Evaluation of survival, growth and reproductive data should be performed using statistical methods described in Appendix D of USEPA/USACE (1994).

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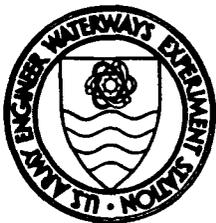
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Environmental Effects of Dredging Technical Notes



Grain Size and Total Organic Carbon Effects on Benthic Organisms

Purpose

The purpose of this technical note is to document the effects of grain size and total organic carbon (TOC) on benthic organisms and evaluate those effects in terms of their potential to confound the results of dredged material bioassays.

Background

Sediment toxicity tests must be able to assess the effects of sediment-associated contaminants without the influence of nontreatment factors (that is, sediment grain size, sediment TOC, ammonia toxicity, etc.). While nontreatment factors can affect survival in short-term acute toxicity tests, there is greater potential for such factors to affect end points measured in longer term chronic tests. Exposure in chronic bioassays generally represents a significant portion of an animal's life history and often encompasses one or more sensitive life-history stages (larval, juvenile, reproductive adults). In addition, end points measured in such tests are of a more subtle, sublethal nature (for example, growth and reproduction) and can be significantly influenced by small variations in exposure conditions (differences in grain size, TOC, etc.).

The influences of grain size and/or TOC on sediment toxicity test end points have gone largely unstudied. It has been generally assumed that the impacts of these factors on survival measured in acute toxicity tests are minimal relative to the effects of contaminants. However, as chronic tests are developed for regulatory testing, there is increasing concern over the potential influence of such factors on sublethal end points.

Additional Information

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Methods

An extensive literature search was conducted to evaluate the potential effects of grain size and TOC on benthic organisms. The literature search included five databases that yielded 131 citations. These databases were the National Technical Information Service (4 citations), SciSearch (98), Dissertation Abstracts (4), Biosis Previews (18), and Aquatic Science and Fisheries Abstracts (7). Of this total, the author identified 46 references concerning the effects of grain size and TOC on benthic organisms. Information included 21 laboratory studies, 12 field surveys, 3 studies with both laboratory and field data, and 10 general review papers.

Results

Numerous studies have presented correlations supporting a relationship between sediment grain size and/or TOC and their effects on animals (Belanger and others 1985; DeWitt 1987; Luckenbach, Huggett, and Zobrist 1988; Eleftheriou and Basford 1989, Ishikawa 1989, Bachelet and others 1992, Rakocinski and others 1993, Tanda 1990, Yates and others 1993). Results of this review were divided into laboratory and field studies describing effects on habitat selection, feeding behavior, survival, and growth rates of various animals.

Laboratory Studies

Kristensen (1988) examined habitat selection in a 30-day laboratory study with *Neanthes diversicolor*, *Neanthes virens*, and *Neanthes succinea*. Results of this study suggest that these polychaetes selected for different grain sizes, that the selection was dependent upon organic carbon content, and that selection could be influenced by the presence of other polychaetes. For example, *N. diversicolor* selected for organically rich silts when it was alone and in the presence of *N. virens*. *Neanthes virens* selected for organic-poor sand when it was alone and in combinations with *N. diversicolor* and *N. succinea*. *Neanthes succinea* selected for organically rich silts when it was alone and in the presence of *N. virens*. Grassle, Butman, and Mills (1992) conducted a study to evaluate habitat selection by *Capitella* sp. I (larvae) with respect to grain size and organic carbon content. *Capitella* sp. I (larvae) were found to actively select for mud over glass beads.

Whitlatch and Weinberg (1982) found that the polychaete *Cistenides gouldii* selected for large grain size sediment when feeding. In addition, these researchers observed that particle size selection increased with increasing worm

size. Similar results were reported by Dobbs and Scholly (1986) for the polychaete *Pectinaria koreni*. Luckenbach, Huggett, and Zobrist (1988) found that the polychaete *Paraprinospia pinnata* selectively foraged on larger particles over the duration of the 4-hr laboratory experiment.

McFarland (1981) examined the effects of grain size on survival of two polychaetes in a 10-day test. Sediment types ranged from 100 percent coarse-textured to 100 percent fine-textured sediment. *Diopatra cuprea* showed extremely high survival in all sediment types. Survival for *N. arenaceodentata* decreased as the silt/clay content increased. In a 28-day test, Dillon, Moore, and Gibson (1993) examined effects of grain size on survival and growth in the polychaete *N. arenaceodentata*. Results of this study indicate that survival was unaffected by grain size (measured as percent sand, silt, clay). However, worm weight decreased as sediment grain size increased, contradicting the earlier findings of McFarland (1981) for *N. arenaceodentata*. This apparent contradiction may be explained by differences in test duration (10 versus 28 days) and age of animals at test initiation (adults versus juveniles).

The combination of grain size and organic carbon content was found by Ott (1986) and DeWitt, Ditsworth, and Swartz (1988) to have an impact on amphipod survival. Ott (1986) found that the mortality of *Rhepoxnius abronius* was higher in sediments with silt-sized particles and low organic content. DeWitt, Ditsworth, and Swartz (1988) found that survival of the same amphipod decreased with decreasing grain size in a 10-day bioassay. DeWitt, Ditsworth, and Swartz (1988) suggested that organic content (percent total volatile solids) and sediment water content may also have played a role in observed mortality. However, these factors were not examined independently in the experiment. McFarland (1981) experimented with the grass shrimp, *Palaemonetes pugio*, and found no grain size effects in a 10-day test with grain size treatments ranging from 100 percent sand to 100 percent mud.

In other laboratory studies, Bachelet and others (1992) and Butman (1987) examined larval settlement of the bivalve *Mercenaria mercenaria* in relation to sediment grain size and TOC. Bachelet and others (1992) found larval settlement to be unrelated to grain size and TOC in 4-hr static tests using biotic and abiotic substrates (for example, a natural organic-rich mud and an abiotic, glass-bead mixture). Butman (1987) found that the organism selected for beads over mud in a static test and mud over beads in a flow-through test.

Clements and Stancyk (1984) found that the brittlestar, *Micropholis gracillima*, had a preference for small grain size sediment regardless of organic carbon coatings (bovine serum albumin and bacteria). In contrast, Moriarity (1982), Roberts and Bryce (1982), and Hammond (1983) found a deposit-feeding holothurian (echinoderm) that selected for sediment based solely on percent organics (carbon, nitrogen) regardless of particle size.

In a habitat selection study, Tanda (1990) found that juveniles of the marbled sole (*Limanda yokohamae*) and the Japanese flounder (*Paralichthys olivaceus*) preferred medium grain size sediment. It was suggested that this selection

was based upon the preference for the type of sand in which the animals could bury themselves.

Taghon (1982) found size and organic coating to play a major role in the selection of particles by deposit feeders. Cammen (1982) reported no consistent relationship in nutritional value (organic carbon, bacteria, chlorophyll *a*, and carbon-to-nitrogen ratio of organic matter) as related to particle size. However, this study examined only four sediments.

Pagano and others (1993) examined the effects of grain size on fertilization and embryological development of the sea urchin. In bioassays ranging from 72 to 120 hr, these researchers found no effect of grain size on either fertilization or embryogenesis.

In a 10-day bioassay with three freshwater invertebrates, Ankley and others (1994) found that survival of the amphipod *Hyalella azteca*, survival, reproduction, and growth of the oligochaete *Lumbriculus variegatus*, and survival of the midge *Chironomus tentans* were unaffected by sediment grain size. However, growth in the midge appeared to be influenced by grain size. Dry weights increased with increasing silicon oxide and decreased with aluminum oxide content. Ankley and others (1994) suggest that the midge was responding to the granular properties of the sediment rather than the mineralogy. Sandy sediments tend to have higher silicon oxide concentration. Similarly, other studies have shown that chironomid species perform better in sandy sediments (Dermott 1978, Winnell and Jude 1984, Ankley and others 1993).

Belanger and others (1985) examined substrate preference of adult freshwater bivalves (*Corbicula fluminea*) in a 3-day laboratory study. These results suggest that *C. fluminea* prefers fine grain sand, followed by organically enriched sand, with coarse-grained sand being the least preferred.

Field Studies

A number of field studies have examined the relationship of grain size and TOC to feeding in benthic invertebrates. Gaston (1987) found that the proportion of carnivorous polychaetes was highest in coarser sediments, and the proportion of subsurface deposit-feeders was highest in fine-grain sediment and increased with depth and percent organic carbon. In two feeding studies, Self and Jumars (1978) found an ampharetid polychaete that selectively ingested particles not based on grain size but on specific texture and specific gravity, while two spionid polychaetes (*Pseudopolydora kempii japonica* and *Pygospio elegans*) selected sediment particles based on surface texture. The degree of selectivity for specific gravity was based on worm size, and the selection for specific gravity was demonstrated with the spionids in association with the ampharetid. In another feeding study, Luckenbach, Huggett, and Zobrist (1988) found grain size selection to vary with feeding duration in the polychaete *Paraprionospio pinnata*. The longer the animal fed, the larger the grain size sediment found in gut. Petch (1986) found that the polychaete *Lumbrineris latreilli* selectively ingested small grain particles. These particles were used by

L. latreilli for construction of burrows and feeding. Whitlatch and Weinberg (1982) found that *C. gouldii* ingested a greater percentage of larger grain size particles as worm size increased. Food selection of *C. gouldii* was based on natural and experimental (abiotic) sediments. Whitlatch and Weinberg (1982) also hypothesized that this selection may be based on the presence of an organic coating on particles with increasing particle size.

In their review, Butman, Grassle, and Webb (1988) discussed numerous studies showing correlations in the distributions of soft-sediment infaunal invertebrates with grain size. Yates and others (1993) used sediment grain size as a device for predicting invertebrate densities on which shorebird densities could be based. Using regression analysis, they concluded that sediment size distribution (coarse sand, fine sand, silt, or clay), organic carbon, and inundation time could predict invertebrate density directly.

In a field survey, Belanger and others (1985) found the highest densities of the freshwater bivalve *Corbicula fluminea* in fine sand environments, followed by organically enriched fine sand, with the lowest densities found in coarse sand. Belanger and others (1985) also stated that, although the sediment preference of *Corbicula* was fine sand, the organism could use a variety of substrates during habitat selection.

Summary

The laboratory and field studies reviewed in this paper suggest that grain size and TOC affect habitat selection, feeding behavior, and survival. The objective of this review was to document the effects of grain size and TOC on benthic invertebrates, with emphasis on the potential of these factors to affect the outcome of sediment bioassays. Only a few studies to date have examined the effects of such nontreatment factors on sediment toxicity tests (DeWitt, Ditsworth, and Swartz 1988; Kristensen 1988; Dillon, Moore, and Gibson 1993; Ankley and others 1994). Ankley and others (1994) found a relationship between grain size and growth in a midge. Dillon, Moore, and Gibson (1993) found no relationship between grain size and survival in the polychaete worm *N. arenaceodentata*. However, growth decreased with increasing grain size. DeWitt, Ditsworth, and Swartz (1988) suggested that organic carbon content contributed more to mortality of the amphipod *R. abronius* than any other factor.

While there are limited data on the potential effects of grain size or TOC on sediment bioassays, there is a large body of information on field distribution and habitat selection related to grain size and TOC (Field 1971, Gage 1972, Whitlatch 1977, Elftheriou and Basford 1989, Ishikawa 1989, Rakocinski and others 1993). However, Snelgrove and Butman (1994) concluded that even distribution could not be explained solely on the basis of grain size and TOC in different environments. Along with biological factors and experimental evaluations of sediments, animal distribution must be evaluated relative to sediment transport and hydrodynamic processes.

Field studies have many more influencing factors that regulate animal distribution and selection preferences than do laboratory studies. These factors include changes in temperature and salinity, water currents, phototaxis, mobility, interspecific competition, and larval settling preferences (Gray 1974).

No study in this review considered organic carbon alone as a causal factor in affecting benthic invertebrates. However, Snelgrove and Butman (1994) believed organic carbon to be a more important factor than sediment grain size in determining field distributions of benthic invertebrates, because organic matter is a prominent source of food for deposit-feeders.

Even within a single taxon, responses to grain size and TOC are highly variable. Some polychaetes have been shown to select for smaller particles (Dorset 1961; Hylleberg 1975; Cadee 1976; Whitlatch 1980; Jumars, Self, and Nowell 1982), while others have been shown to select for larger particles (Whitlatch 1974, 1980), and still others appear to be nonselective (George 1964, Hughes 1980).

Based on this review, few studies evaluated the effects of grain size and TOC in the absence of hydrodynamic forces. Even fewer studies addressed the potential for these factors to affect the outcome of laboratory sediment toxicity tests.

Conclusions

Based on this literature review, the following conclusions were made.

- Sediment grain size and TOC can affect habitat selection, feeding behavior, and survival, with effects being species-dependent.
- Grain size/TOC effects on habitat selection may actually be a result of hydrodynamic forces in the environment.
- Only three of the 46 studies reviewed examined the potential effects of sediment grain size and TOC on laboratory bioassays.
- Additional laboratory studies are required to determine the potential effects of grain size or TOC in laboratory sediment toxicity tests.

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Environmental Effects of Dredging Technical Notes



Lower Limits of Organic Carbon Normalization: Results of Fish/Sediment/Water Equilibrium Partitioning Studies

Purpose

This technical note reports the initial results of studies measuring biota/sediment/water equilibrium partitioning of a polychlorinated biphenyl (PCB) congener. The focus of this technical note is on the validity of normalizing concentrations of neutral organic chemicals on sediment total organic carbon (TOC) when sediment TOC concentrations are low.

Background

Over the past 10 years, the U.S. Environmental Protection Agency (EPA) has aggressively pursued development of single-chemical sediment quality criteria (SQC). Equilibrium partitioning of neutral organic chemicals between the organic carbon fraction of bedded sediments and the interstitial water of the sediments provides the theoretical basis for the most popular approach to development of SQC. The solution phase of the chemical in equilibrium with the sediment is considered to represent the bioavailable fraction and to enable the conversion of existing water quality criteria (WQC) into SQC or sediment quality standards.

In this approach, sediment total organic carbon is considered to be the primary sediment phase accounting for sorption of neutral organic chemicals, and concentrations of these chemicals are therefore normalized to the TOC fraction. A chemical-unique partition coefficient (K_{oc}), applied to the TOC-normalized

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chemical concentration, is used to estimate the solution-phase concentration for calculation of the bioavailable fraction, which is then compared with WQC. Criteria documents for the pesticides endrin and dieldrin, and for several polynuclear aromatic hydrocarbons, have been released by the EPA for public review and may soon be promulgated.

State water quality standards are pass/fail criteria, as are Federal WQC for ocean disposal of dredged material. EPA has not made clear the intended purpose of SQC relative to regulation of dredging activities but has stated an intention to recommend them as water quality standards to be used by the States as applicable relevant additional requirements. Under that scenario, SQC will be applied to dredging regulation as pass/fail criteria.

The promulgation of SQCs with the intent that they be used as standards will result in confounding of effects-based testing procedures as they are now practiced in dredged sediment regulation under the Ocean Dumping Act and the Clean Water Act (Wright, Engler, and Miller 1992; Wright and Wilson 1995). Under such circumstances, it is imperative that the quality of the SQC estimations and the degree of uncertainty surrounding them be clearly understood.

The Corps of Engineers has not been directly involved in the development of SQC. For that reason Work Unit 32571, "Relationship Between Sediment Geochemistry and Biological Impacts," was initiated under the Long-term Effects of Dredging Operations Program to investigate the validity of SQC for the regulation of sediments. The research reported in this technical note continues that effort.

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Introduction

SQC predictions are dependent on the linearity of bioavailability of sediment-contaminating chemicals (that is, interstitial water concentrations) with sediment TOC content. However, several researchers have found in previous investigations that the freely dissolved fraction of several nonpolar organic chemicals is not consistently predictable from the TOC content of the sediment (Brannon and others 1993, 1995a, 1995b). The source, aromaticity, intraparticle sorption processes, and the structure and composition of humic materials have all been reported to affect the partitioning of neutral organic chemicals between sediments and water (Gauthier, Seitz, and Grant 1987; Brusseau and Rao 1989;

Grathwohl 1990). The suggested lower limit for the validity of organic carbon normalization stated in the technical documentation supporting SQC is 0.2 percent (DiToro and others 1991). This lower limit was derived by separate adsorption and desorption experiments in which the partition coefficients of several organic chemicals were normalized to the organic carbon content of sediments, and greatest departure from linearity was observed to occur at about 0.2 percent TOC in the sediments.

Sorption experiments conducted without the presence of living organisms can provide valuable information regarding the physicochemical behavior of chemicals in sediment/water systems, but do not truly address questions of bioavailability. For that reason, an exposure system (Figure 1) was designed to allow modeling of the distribution of an introduced chemical among the principal partitioning compartments represented by fish, water, and sediment (McFarland and others 1992, 1994). The partitioning data are fitted using a three-compartment closed kinetic model (Figure 2) (Gibaldi and Perrier 1982) and the simultaneous equations (Equations 1-3) describing intercompartmental distribution of the chemical as a function of time.

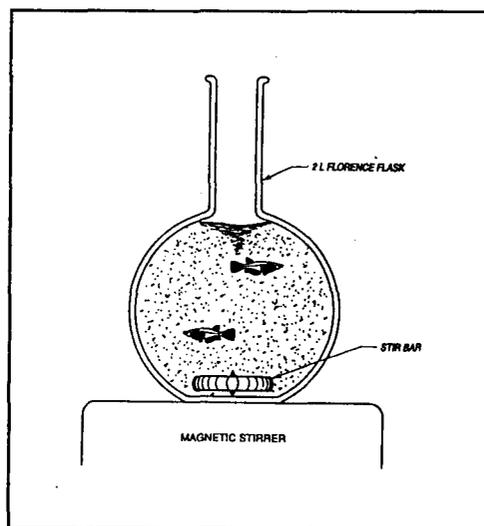


Figure 1. Exposure system

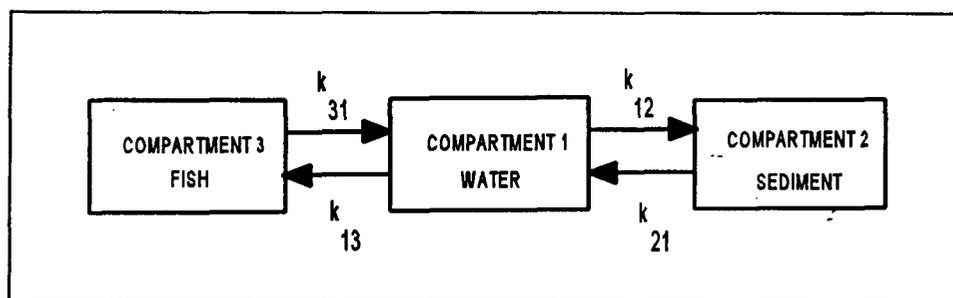


Figure 2. Three-compartment closed kinetic model

$$dX_w/dt = k_{21}X_s + k_{31}X_f - k_{12}X_w - k_{13}X_w \quad (1)$$

$$dX_s/dt = k_{12}X_w - k_{21}X_s \quad (2)$$

$$dX_f/dt = k_{13}X_w - k_{31}X_f \quad (3)$$

In this model X_w , X_f , and X_s are the mass of PCB-52 (micrograms as total radioactivity) in water, fish, and sediment, respectively, and k_{12} , k_{21} , k_{13} , and k_{31} are the intercompartmental transfer coefficients describing rates of mass transfer among the compartments. Complete derivations are given in Feldhaus,* as are solutions for the hybrid coefficients used to project mass distribution at equilibrium. This model and exposure system can be used to test the lower limit of TOC normalization for predicting bioavailability as described below.

Bioaccumulation factors (BAF) are calculated as the non-normalized ratios of the transfer coefficients:

$$BAF = (k_{13}/k_{31}) / (k_{12}/k_{21}) \cdot 0.4 \quad (4)$$

For neutral organic chemicals that partition passively, BAFs can be expected to vary inversely with sediment organic carbon content when other factors are held constant. In fact, the assumption of linearity of bioavailability with sediment TOC requires that this relationship be constant down to the lower limit of validity of TOC normalization. Biota/sediment accumulation factors (BSAFs) are calculated similarly, but normalized to lipid content (fl) in the fish and organic carbon content (foc) in the sediment:

$$BSAF = [(k_{13}/k_{31})/fl] / [(k_{12}/k_{21})/foc] \cdot 0.4 \quad (5)$$

The factor 0.4 in Equations 4 and 5 is necessary to correct for mass differences (Landrum 1983). The relationships derive from the facts that equilibrium partitioning of a chemical between an organic and an aqueous phase is described by the ratio of the forward and reverse rate constants (Kubinyi 1978) and that water is the common phase for partitioning to both sediment and organism (McFarland and others 1994). Whereas BAF is in theory linear and inversely proportional to sediment TOC (all else being constant), BSAF is a simple factorial difference in concentration or mass of chemical in organism lipids and sediment TOC. These relationships can be used to test the linearity of bioavailability of a neutral organic chemical with sediment TOC as follows:

1. If the lower limit of validity of organic carbon normalization is, in fact, 0.2 percent, then BAFs should regress linearly with TOC to that limit and BSAFs for all sediments above that level should be constant.
2. If the limit is a higher or a lower percentage of organic carbon, then that should be revealed by the TOC concentration at which a break in the linearity of BAFs occurs and above which BSAFs are constant and below which they differ.

* Jane Feldhaus. "A toxicokinetic compartmental model for the determination and prediction of a biota-sediment accumulation factor for PCB-52," Ph.D. dissertation (in preparation), Northeast Louisiana University, Monroe, LA.

3. If factors other than TOC contribute significantly to the total activity of the sediment compartment, this should be reflected by nonlinear BAFs and nonconstant BSAFs.

Materials and Methods

Freshwater sediments were collected from various locations throughout the country, air-dried, finely ground using a mortar and pestle, and analyzed for total organic carbon. TOC values were measured using a Shimadzu 5050 TOC analyzer equipped with a model SSM-5000 Solids Module. The five sediments selected for study were subsampled and analyzed for particle size distribution by sieving and for bulk mineralogy by X-ray diffraction analysis. The sediment with the highest TOC content was also subsampled and heated in a muffle furnace for 12 hr at 450 °C to drive off the carbon, thus providing a sediment with 0 percent TOC.

Stock cultures of Japanese medaka (*Oryzias latipes*) were obtained from Gulf Coast Research Laboratory, Ocean Springs, MS, and from Aquatic Research Organisms, Hampton, NY, or were cultured in the laboratory at the Waterways Experiment Station. The male medaka were maintained separately in a Living Stream on a diet of flake fish food at a temperature of 20 ± 2 °C and a photoperiod of 16 hr light:8 hr dark. Only males were used as experimental organisms in order to achieve greatest uniformity in weight and lipid content.

The experimental setup consisted of a series of 2-L florence flasks placed on individual stir plates (Figure 1). Four hundred milligrams of air-dried sediment, sieved to ≤ 500 μm , was placed in each flask, along with 2 L of charcoal-filtered aged tap water, two adult male Japanese medaka, and a Teflon-coated stir bar. The stir plates were turned on, and 100 μL [^{14}C]-PCB-52 (4.26 $\mu\text{g}/\text{mL}$ in methanol, specific activity = 0.202 $\mu\text{Ci}/\mu\text{g}$, obtained from Sigma Chemical Company, St. Louis, MO) was added to each flask, yielding an aqueous exposure concentration of 0.213 $\mu\text{g}/\text{L}$. In the initial experiment, a sample schedule of 0, 1, 2, 4, 12, 36, 72, 96, and 120 hr of exposure was used. Subsequently, the 72- and 96-hr exposures were deleted when it appeared that their inclusion was unnecessary for curve-fitting. At each sample time the flasks were taken down, and the water, sediment, and fish were extracted and analyzed for total radioactivity. Six replicate flasks were used for each time point.

A portion of the exposure water was centrifuged, and a 100-mL aliquot was extracted in a separatory funnel with 25 mL 4:1 hexane:acetone and then re-extracted with 20 mL hexane. The extracts were combined, concentrated under nitrogen to about 2 mL, and transferred to a scintillation vial. Liquid scintillation cocktail (15 mL of Packard Ultima Gold, Packard Instrument Company, Meriden, CT) was added, and the samples were counted on a Packard 2500 Tri-Carb liquid scintillation counter using a quenched calibration curve.

Upon removal from the exposure flasks, the fish were sacrificed and the intestinal tracts were removed from the fish. The fish were homogenized with

20 mL of acetone using a Brinkmann Polytron homogenizer, and the homogenates were centrifuged to collect the acetone extracts. The extraction was repeated and the extracts were combined. Two milliliters of water and 10 mL of hexane were then added for partitioning of the acetone extract between hexane and water. The hexane layers were collected and split. One aliquot was air-dried in a tared container for gravimetric determination of lipid content, and the other aliquot was concentrated under nitrogen for PCB-52 analysis as described above. The intestinal tracts were solubilized using Solvable tissue solubilizer (Dupont NEW, Boston, MA) and were analyzed for PCB-52.

The sediment from each exposure flask was collected by centrifugation and was extracted three times using 10 mL of acetone, with 20 min of sonication (Branson 2200 ultrasonic bath) each time. The acetone extracts were combined, concentrated under nitrogen, and analyzed for PCB-52 as above.

Results and Discussion

The five treatments are identified in the tables and figures by an alphanumeric code in which the letters designate the source of the sediment and the numeric suffix designates the percentage of TOC in the sediment. Mineral composition and percentage dry weight TOC of the five sediments are shown in Table 1. Sediment MSL-0.0 was a subsample of sediment SL-2.03 heated to destroy the organic carbon before use. Heating also changed the mineral composition, destroying the clay components. The sediments were all predominantly fine quartz sand. Smectite, an expandable clay mineral, constituted approximately 25 percent, by dry weight, of FP-0.331 and SL-2.03, and about 9 percent of BL-0.963. Smectite was not an identifiable component of the other sediments. Expandable clays tend to covary with sediment TOC content and have been shown to influence sorption of PCBs to a far greater extent than nonswelling clays (Uzgiris and others 1995). It was considered that their presence could influence bioavailability at low TOC concentrations. However, it is well recognized that organic carbon predominantly accounts for the sorption behavior of neutral chemicals on soils and sediments.

The data as masses of PCB-52 in each compartment of the system (0.4 g sediment, 2 L water, 1.0 g fish) at each sampling time were fitted to the model using the Gauss-Newton algorithm and PCNONLIN (Metzler and Weiner 1992). The model generally fit the data well in all treatments, with most of the variability being contributed by the sediment compartment. The fitted nonlinear regressions and means of the measured masses of PCB-52 in each compartment at each sampling interval are shown in Figure 3. The curves illustrate a typical pattern in which there is a rapid initial decline of PCB-52 in the water and a concomitant rapid uptake by the sediment, which then begins to decline in less than 24 hr. Uptake by the fish is slower, due most likely to the rate-limiting effect of gill blood flow (Karara and McFarland 1992). Partitioning of PCB-52 to all three compartments approaches an asymptote by 120 hr.

Table 1. Sediment Identification Code and Composition					
Composition	Sediments				
	MSL-0.0	NY-0.103	FP-0.331	BL-0.963	SL-2.03
TOC (%)	0.0	0.103	0.33	0.96	2.03
Quartz	Major	Major	Major	Major	Major
Na feldspar	NF ¹	Major	Minor	Minor	Minor
K feldspar	Minor	Major	Trace	Minor	Minor
Calcite	NF	Minor	Trace	Minor	Trace
Dolomite	NF	Minor	Trace	Major	Trace
Kaolinite	NF	NF	Trace	Trace	Trace
Mica	NF	Trace	Trace	Trace	Trace
Chlorite	NF	Trace	NF	NF	NF
Smectite	NF	NF	Major	Minor	Major

¹ Not found.

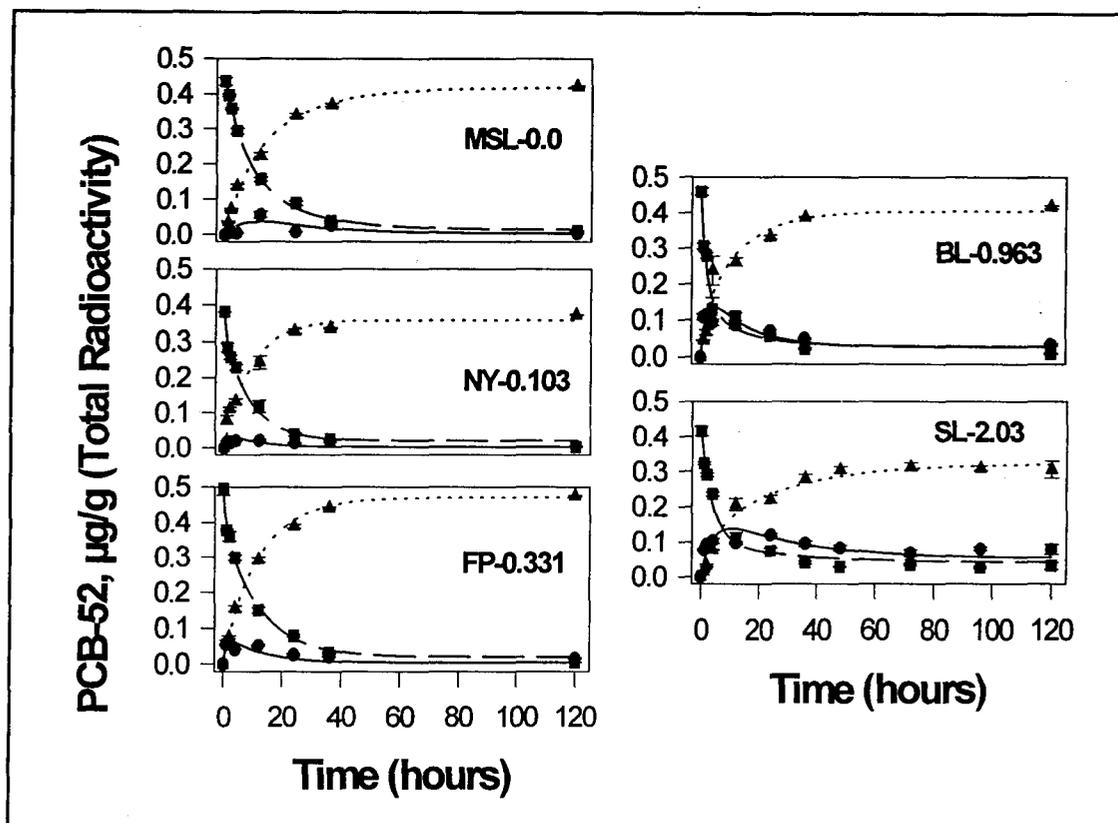


Figure 3. Mass distribution of PCB-52 over time in the five treatments. Symbols with horizontal lines are measured values (error bars). Lines are model-predicted regressions on the measured data (■, water; ●, sediment; ▲, fish)

BAFs were calculated using Equation 4 and the intercompartmental transfer coefficients for each replicate of each treatment. BSAFs were similarly calculated using Equation 5 for the four treatments having measurable organic carbon on the sediments. The grand mean (0.043415, standard error = 0.017688, N = 210) of all lipid fraction (fl) measurements was used in all BSAF calculations, and the organic carbon fraction (foc) was the TOC appropriate to the treatment, expressed as the decimal fraction.

Linearity of bioavailability with sediment organic carbon content was tested by Lack of Fit analysis on the four treatments having measurable organic carbon (Figure 4). The BAFs for each replicate of each of the four treatments were regressed on sediment TOC, and the result was a significant lack of fit ($P \leq 0.05$). The relationship between TOC content and mean BAFs and the constancy of BSAFs were assessed by statistical comparisons among the sediments using analysis of variance followed by Tukey's Honestly Significant Difference test (SAS Institute, Inc. 1988) (Table 2). Because of violations of normality or equality of variances assumptions, the BAFs and BSAFs were converted to normalized ranks (rankits) prior to analysis with Tukey's test. The BAFs fell in two groups. The lower group included the two treatments having 0.963 and 2.03 percent sediment TOC. The higher group included the three treatments with zero to 0.331 percent TOC, and these were not statistically distinguishable. The difference between the two groups was about an order of magnitude and shows clearly the influence of sediment organic carbon on the partitioning behavior of PCB-52 between sediments and fish. This result is also shown in Figure 5.

Table 2 and Figure 5 show a different grouping for the BSAFs. As expected, the two highest TOC sediments were statistically indistinguishable, confirming the validity of normalization of PCB-52 on sediment organic content in the range of 1 percent and above. However, the mean BSAF for the sediment having 0.103 percent TOC was also statistically similar to the two high-TOC treatments, while that of the 0.331 percent TOC treatment was higher and statistically different from the rest. This anomalous result was not explained by the presence or absence of swelling clays in the sediments.

It was concluded from these results that bioavailability of PCB-52 is highly variable and not linear with sediment organic carbon content at low TOC levels. It appears that the lower limit of validity of organic carbon normalization may be higher than the 0.2 percent cited in the technical document supporting equilibrium partitioning SQCs (DiToro and others 1991). Examination of Figures 4 and 5 shows what appears to be an inflection or a break point in the relationship in the region below 0.963 percent and above 0.331 percent TOC.

Clearly, more work is needed to fully understand these results. Equilibrium partitioning experiments involving additional sediments, sediment mineralogy, sediment TOC polarity, other variables, and other chemicals are ongoing or planned. It is recommended that, until definitive characterization of the phenomenon has been completed, normalization of neutral chemical concentrations on sediment TOC at levels less than 1 percent should be used cautiously in

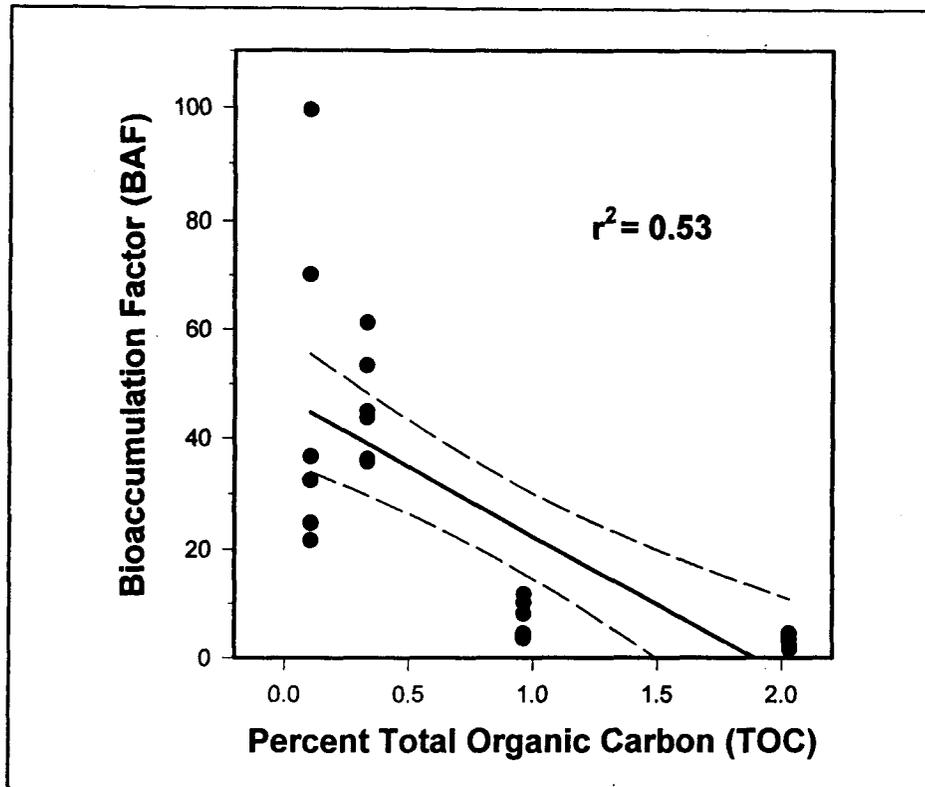


Figure 4. Linear regression and 95-percent confidence interval on bioaccumulation factors calculated for each replicate (n = 6) of the four treatments having organic carbon. (Lack of fit analysis: DF = 2, F = 4.00882, P = 0.034355)

Table 2. Fish/Sediment Ratios (BAF) and Biota/Sediment Accumulation Factors (BSAF) with Analysis of Variance Results, Means, Standard Errors (SE), and Mean Comparisons (Tukey Group)				
	BAF		BSAF	
	<i>F</i> = 16.84	<i>P</i> = 0.0001	<i>F</i> = 14.56	<i>P</i> = 0.0001
	<i>N</i> = 30	<i>R</i> ² = 0.729	<i>N</i> = 24	<i>R</i> ² = 0.686
Treatment	Mean (SE)	Tukey Group ¹	Mean (SE)	Tukey Group
MSL-0.0 ²	62.81 (12.66)	A	NA ³ (NA)	NA
NY-0.103	47.55 (12.58)	A	1.128 (0.298)	A
FP-0.331	45.88 (4.053)	A	3.498 (0.309)	B
BL-0.963	7.144 (1.394)	B	1.585 (0.309)	A
SL-2.03	2.925 (0.445)	B	1.368 (0.208)	A

¹ Within a group, means with the same letter designation do not differ significantly ($P\alpha/2 \leq 0.025$).
² Numeric suffix denotes percent TOC.
³ Not applicable.

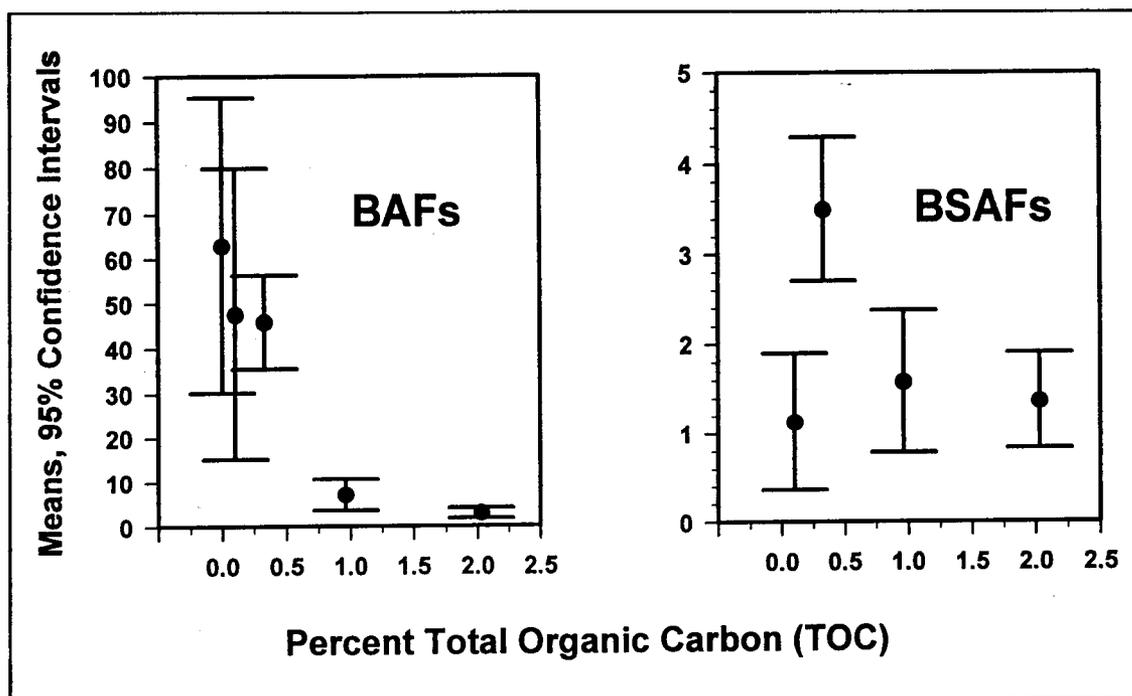


Figure 5. Bioaccumulation factor (BAF) and biota/sediment accumulation factor (BSAF) means (●) and 95-percent confidence intervals (vertical bars and caps) as a function of treatment percent TOC

estimating bioavailability and should not be used in criteria-based regulatory decision-making.

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Environmental Effects of Dredging Technical Notes



Potential Application of Geosynthetic Fabric Containers for Open-Water Placement of Contaminated Dredged Material

Purpose

The purpose of this technical note is to summarize the present state of knowledge on the use of geosynthetic fabric containers (GFCs) for placing contaminated sediments in open water, describe their benefits and potential applications, and identify issues of concern.

Background

Cost-effective placement of contaminated dredged material (assumed to be silt- and clay-sized material) is a major problem in many locations. Capping is one of several options that can be applied to the problem. A major limitation of capping projects is the thin (less than 100- to 150-mm-thick), wide (100 to 400 m) apron that forms during conventional bottom dumping of fine-grained material from split-hull barges. Locating sufficient cap volume and the cost of placing the additional capping material to cover the apron are significant problems for many capping projects. The spread of the contaminated sediment apron also poses potential problems for retaining contaminated material inside the placement site.

Another problem in disposing of contaminated dredged materials is potential water column impacts. While, in general, water quality is not a problem during conventional placement of contaminated dredged material from split-hull barges, in some cases limited mixing zones or stringent water quality standards will cause the placement process to fail state water quality standards or U.S. Environmental Protection Agency requirements.

Containing the contaminated sediments in GFCs for subsequent placement from split-hull barges offers the potential to eliminate the apron, thus substantially reducing the volume of cap material required and reducing the potential for contaminated sediments to extend beyond the site boundary. GFCs also have the potential to eliminate water quality problems at the disposal site by essentially eliminating the loss of fine sediment (silt- and clay-sized) particulates and associated contaminants to the water column. The magnitude of the contaminated sediments problem is such that considerable interest has been generated concerning application of GFCs for open-water placement of contaminated dredged material. In this technical note, when referring to sediments, the terms "fines" and "fine-grained" will follow the American Society for Testing and Materials (ASTM) and Unified Soils Classification System (USCS) definitions of fine-grained sediments—those passing the No. 200 sieve (0.074 mm), that is, silts and clays.

Additional Information

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The GFC Concept

Figure 1 illustrates the concept of barge placement of GFCs filled with dredged material. The major steps in the operation are as follows:

- a.* The barge (which often requires modifications) is lined with the appropriate geosynthetics.
- b.* Dredged material is placed (either mechanically or hydraulically) into the lined barges.
- c.* For mechanical placement, the geosynthetic fabric flap is folded over the dredged material and sewn closed, forming the GFC.
- d.* The GFC is released from the barge at the placement site.

Potential Benefits of GFCs for Placement of Contaminated Sediments

Contaminated dredged material may be defined as material that is unsuitable for unrestricted open-water placement. Materials can be unsuitable from the standpoint of potential water column impacts (both at the dredging and disposal site), where water quality standards or criteria are not met. Water column impacts are not usually a concern during placement for most materials from navigation dredging projects, unless stringent standards are imposed or unless the allowable mixing zones are tight. So, potential benthic impacts are

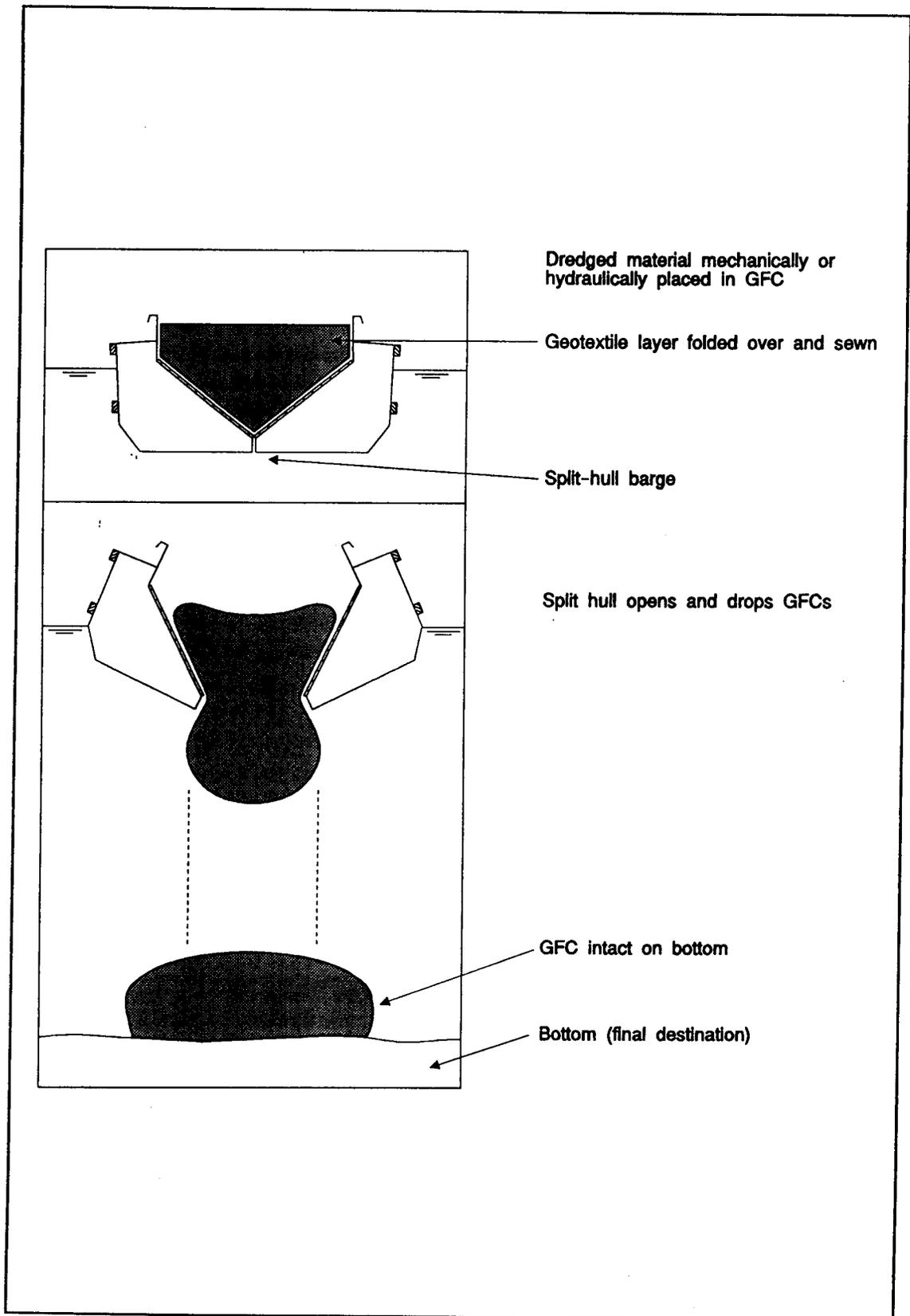


Figure 1. Concept for barge placement of geosynthetic fabric containers (GFCs)

the normal concern for most contaminated sediment placements from navigation projects. Capping, the covering of the contaminated material with a layer of clean material, may be considered as a control measure for potential benthic impacts.

GFCs have potential application for open-water placement of contaminated dredged material from two standpoints. First, GFCs can act as a control measure to reduce water column impacts. Second, the GFCs can reduce the degree of spread of the material on the bottom, which can be advantageous for capping. In fact, GFCs could eliminate the requirement to cap, though a considerable amount of investigation would be required along with other special considerations (for example, deep water, low biological activity, etc.). To understand how the GFCs may be beneficial, it is first necessary to examine the behavior of a conventional dredged material discharge from a barge or scow without containers.

Bucket or clamshell dredges remove the sediment being dredged at nearly its in situ density and place it in a barge or scow for transportation to the disposal area. Although several barges may be used so that the dredging is essentially continuous, placement occurs as a series of discrete discharges from the barge or barges. Barges are often designed with a split hull, which opens within a matter of tens of seconds, and the contents may be emptied within tens of seconds, essentially as a discrete discharge. Some fraction of the dredged material may be stripped away during its descent through the water column, and ambient water is entrained with the discharge, reducing its density.

The use of GFCs can reduce the dispersion of dredged material fines to the water column and can reduce the volume of water entrained during descent. The presence of the fabric essentially acts as a filter cloth in containing dredged solids while allowing excess water to pass through the fabric. Also, the fabric inhibits the entrainment process during descent. The reduction in entrained water results in a reduced volume of dredged material fluid fraction discharged to the water column. Use of GFCs would therefore potentially aid in meeting water quality standards or water column biological criteria for projects with stringent standards or small allowable mixing zones.

The use of GFCs would also reduce the potential spread of material on the bottom upon impact. Spreading would be limited to the elliptical configuration of the bag, with the fabric effectively preventing any larger spread and any formation of a thin apron. This reduction in footprint size could have a benefit for capping applications by reducing the volume of capping material required.

Theoretical and model studies, as well as field data, will be necessary to confirm the relative advantages of containers over conventional open-water discharge for specific site conditions and material characteristics.

Conceptually, using GFCs as part of capping projects appeals to many people. The idea of confining the contaminated material in GFCs to eliminate or

greatly reduce the losses of silt- and clay-sized particles and associated contaminants to the water column during placement (and to eliminate resuspension of contaminants during subsequent placements, during capping, or during a storm prior to capping) is appealing from an environmental standpoint. The need for less cap material can reduce capping costs and make more projects feasible in situations where suitable cap material is limited.

However, it should be noted that the Corps has performed nearly 30 capping projects using conventional hopper or barge surface-release techniques. No adverse environmental impacts have been documented, even though some losses to the water column and resuspension have occurred.

The decision to use GFCs for a capping project should therefore be justified based on economics (that is, they will lower overall project cost) and on environmental benefits. Potential advantages of using GFCs include increased site capacity, preventing material from moving offsite, and in some cases, meeting stringent water quality standards. An arbitrary decision to use GFCs for any capping project without thorough documentation of the benefits versus costs should be avoided. To allow informed decisions to be made concerning whether to use GFCs for a specific project, the following information on GFCs is presented. First, some basic information on GFCs and how they are actually used on a project is provided. Next, summaries of field applications of GFCs at Red Eye Crossing and Marina Del Rey are presented. Discussions of how GFC use impacts the various aspects of capping projects (as compared to conventional open-water placement) are also presented. The unknowns associated with use of GFCs for capping projects are described, along with required research.

Prior Experience with GFCs

Geosynthetic fabrics have been used in construction of confined disposal facilities (CDFs) for years. For CDF applications, geosynthetics have been used as liners, to help stabilize dikes, and to accelerate consolidation of sediments. GFCs filled with sediments have been placed submerged in aquatic environments since 1973. A considerable number of applications have used GFCs as shallow-water, low-energy breakwaters and as dikes to contain dredged material (Landin, Fowler, and Allen 1994; Garbarino and others 1994). Fully submerged GFCs have been used in deeper water with projects in the United States, Holland, and Japan, with most experience from European projects. For example, the Dutch used GFCs in a waterway to stabilize a bank (Fowler and Sprague 1993). Fowler, Sprague, and Toups (1995) discuss past experience with GFCs, with particular emphasis on Corps projects.

In the United States, GFCs have been used for the placement of uncontaminated dredged material on a U.S. Army Engineer District, New Orleans, project at Red Eye Crossing on the Mississippi River near Baton Rouge, LA. At this site, geosynthetic fabric bags (small GFCs containing only a few cubic meters of material) and GFCs were filled with sand and used to create soft dikes

to channel riverflow, for the purpose of reducing sedimentation (Duarte, Joseph, and Satterlee 1995). To date, the only aquatic placement of contaminated dredged material using GFCs occurred during a project for the Corps' Los Angeles District. In this project, contaminated sediments from Marina Del Rey in Venice, CA, were contained in GFCs and placed in a shallow-water habitat in the Port of Los Angeles (Fowler and others 1995b, Mesa 1995).

GFC Material and Construction

Geosynthetic fabrics are tough flat sheets consisting of synthetic fibers (such as polypropylene, polyethylene, and other polymeric materials) that can be woven, knitted, or simply pressed together. Woven and knitted sheets are termed "woven geotextiles," and sheets that are pressed, matted, or punched together are termed "nonwoven geotextiles." The sheets are resistant to corrosion and degradation from biological activity because they are made from synthetic materials. Many geotextiles are available in sheets, 5 to 8 m wide, which are easily sewn together to allow the construction of composite systems to perform specific functions. A major advantage of geotextiles is that they are pervious to water flow both across and within their manufactured plane. They are used in the construction industry to achieve some combination of reinforcement, drainage, separation, and filtration.

The use of geosynthetic fabrics (also called geotextiles) has risen steadily in the United States since about 1977. Geosynthetics, in general, and geotextiles, in particular, have come into such widespread use that the ASTM has established Committee D-35 to standardize techniques and procedures within the industry.

GFCs are formed by sewing together long sheets of geosynthetic fabric. Depending on the grain size of the dredged material, GFCs can consist solely of an outer strength layer to contain sand-sized particles. For dredged material with substantial amounts of silt- and clay-sized particles, an inner liner may be required to prevent migration of these finer particles. Together, the outer strength layer and inner liner may act as a system providing even greater resistance to rupture and filtering capabilities.

The outer strength layer of the GFCs is usually made of woven polypropylene and/or polyester yarns that are sewn together. Typically, the final shape after sewing is a cylinder or rectangular box. During filling, the GFC assumes the shape of the barge or other confining structure. When the GFC is resting on the bottom, it is nearly elliptical in shape. Seam strength is usually the limiting design factor from a strength standpoint. In woven outer layers, fabric strengths of about 175 to 193 kN/m (1,000 to 1,100 lb/in.) are possible, with seam strength about 50 to 60 percent of that value depending on the type of seam used and the machine used to do the sewing. Seams formed in the factory on large fixed machines can achieve strengths of 88 to 105 kN/m (500 to 600 lb/in.), while seams done in the field with hand-held sewing machines can be as low as 44 kN/m (250 lb/in.).

If a liner is required to reduce the migration of clay- and silt-sized particles and associated contaminants to meet water quality standards, the liners used are nonwoven fabrics that act as a filter. Liner fabric strengths range from about 35 to 75 kN/m (200 to 400 lb/in.). With the proper seam, seam strengths equal the fabric strength of nonwoven fabrics are possible.

ASTM has a number of standards that prescribe geosynthetic fabric requirements (Duarte, Joseph, and Satterlee 1995), including tensile and seam strength (ASTM D 4595) and apparent opening size (ASTM D 4751).

Stresses During Placement

Fabric and seam strength are critical because one of the major concerns associated with GFC use is their integrity during placement. It might be expected that the maximum stresses in the lining of the GFCs would occur when the GFCs impact the bottom; however, because GFCs are filled to less than full capacity (typically about 70 percent capacity), the stresses of bottom impact are not as great as those that occur when the GFCs exit the barge.

Figure 2 shows the simulated sequence of events associated with the exit of the GFC from a split-hull barge. When the GFC is partially out of the barge, maximum stresses occur when the submerged weight of the sediments (between 1.7 and 8.7 kN/m³, plus any water in the barge above the GFC, about 10 kN/m³) is supported by the fabric. The stresses in the fabric are caused by the pressure from the column of sediment in the GFC acting on the unsupported area, equal to the width and length of the split hull opening. Strain gauge testing done at Red Eye Crossing showed that stresses from bottom impacts were only about one third the stresses experienced during exit of the GFC from the barge. It is important for the containers to quickly exit the barge without hanging up. Properly designed containers should exit the barge in 1 to 4 min or less. Exit of the containers can be facilitated by a wide, quick hull opening, low friction between the containers and barge hull (liners can be used), and low strength of sediments in the containers. After the containers exit the barge, they quickly reach a terminal velocity of about 4 m/sec, in 1 sec or less.

Efficient exit of GFCs from the barge is a concern that needs research. The few model tests that have been performed at the U.S. Army Engineer Waterways Experiment Station (WES), with GFCs filled with sand and silt, have indicated that additional tests with a variety of sediments, geosynthetic fabrics, liners fabrics, barge configurations, etc., are needed to optimize the operational aspects. A computer program, which was originally developed to simulate rock scour processes and uses the distinct element method (Palmerton 1980, 1984), has been modified by the WES Geotechnical Laboratory to predict whether the GFC will exit the barge and to determine the tensile forces in the container. The program successfully modeled the container that seized during barge exit, as well as the subsequent successful deployments at Marine Del Rey. This computer program has also been used to simulate hydraulic filling of the GFC

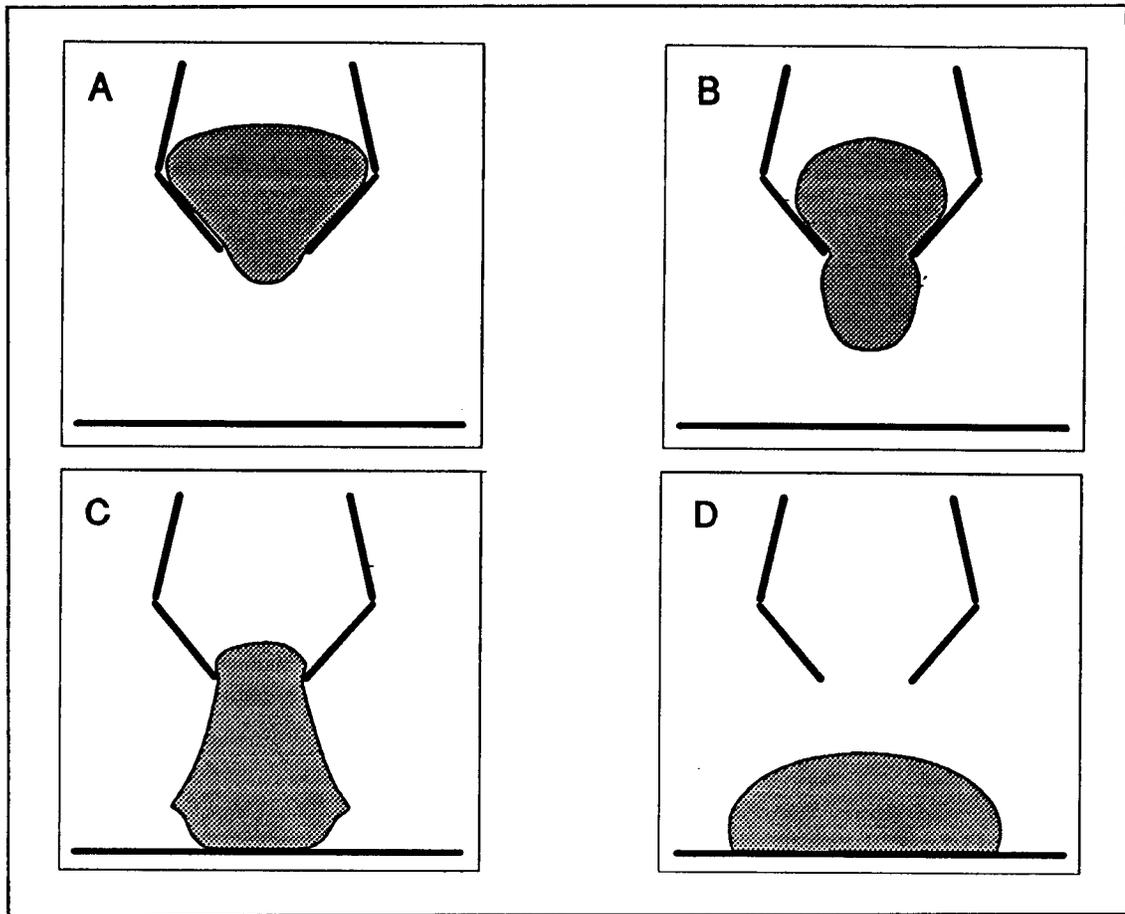


Figure 2. GFC exiting from a split-hull barge

and has been enhanced to simulate the interaction of multiple fluid-filled membranes (flexible or rigid).

Migration of Fines and Contaminants

In addition to the concerns of GFC integrity and effective exit from the barge, the other major area of concern is the ability of the GFC to prevent the migration of fines and contaminants. The ability of the fabric to retain material of a given grain size is related to the apparent opening size (AOS) of the fabric. AOS is defined as that property which indicates the approximate largest particle that can pass through a geotextile. The AOS of high-strength woven polyester fabrics that typically have been used range from about 0.2 to 0.6 mm (which corresponds to standard U.S. sieve sizes of 70 and 30, respectively).

A procedure for determining AOS is outlined in ASTM Designation D 4751 (dated 1994). The procedure involves shaking 50 g of glass beads of a given size against a geotextile that is stretched taut across a circular opening at the bottom of a pan. The AOS is the smallest size of bead that will pass through

a geotextile if 5 percent of the total weight of glass beads in the pan passes through the geotextile after 10 min of shaking.

The behavior of glass beads penetrating geotextiles in a dry vibrating environment is very different from that of soil particles (with nonspherical shapes) being carried in suspension in water. It is known from experience that the AOS of a geotextile decreases as soil particles "blind off" areas through which particles may pass. However, specific research is needed to determine the mechanism of blinding, and how AOS changes with time, soil characteristics, and thickness of geotextile.

The WES Environmental Laboratory (EL) has performed limited tests on the ability of GFCs to contain sediments and contaminants. Sediment samples, when dropped in geosynthetic bags, were found to release a small amount of fine-grained material. As part of the Marina Del Rey project, EL also performed limited testing to determine the concentration of heavy metals, water, and sediments (fine sand with 7 to 8 percent silt and clay) lost through the geosynthetic fabric. During the tests, the geosynthetic fabric was placed in a funnel, the sediments were added, then a vacuum was applied to determine if any fines or contaminants would be pulled through the fabric and liner. However, the tests were not reproducible, and were not intended to simulate field conditions. The GFCs and liners filter the materials by forming a cake on the inside. The vacuum method probably does not simulate cake formation realistically. Centrifuge testing or perhaps small-scale tests may provide better information. Standard tests on contaminants in pore water may also be applicable to the geosynthetic containers. Data on both short-term releases of fines and contaminants (during loading, transportation, and placement) and long-term releases (days, months, years) are needed. Thus, to make defensible statements on the ability of GFCs to retain fines and contaminants for projects where water column impacts are a problem or where containers would be used without caps, a considerable amount of research is required.

Logistical and Operational Considerations

With conventional dredging it takes about 10 to 15 min to bring the empty barge alongside the dredge and secure it. Then, dredging can start almost immediately at full production rate.

Use of GFCs makes the dredging and placement process considerably more complicated than with conventional dredging and placement. First, a facility is needed to prepare and assemble the containers. Following assembly, the containers are taken out to the work barge. The empty scow is brought to the work barge (usually adjacent to the mechanical dredge). The dredge's bucket crane is then used to pick up the container from the work barge and place it in the empty barge. The container (or containers if a liner is used) is then laid out in the barge, requiring a crew of about eight people to unfold and tie down the container(s) so they do not get dragged into the barge during filling. This process can take 1 to 2 hr under the best of conditions. Following filling,

filling to ensure that excess pressure from the pump does not burst the geosynthetic fabric container.

Placement of geosynthetic containers is also potentially complicated. If tight tolerances are placed on the exact location where each container is to be placed, properly positioning the barge and keeping it on station during the 30 sec to several minutes required for the container to exit the barge is not a trivial matter. In sheltered waters it is possible, but in the open ocean with conventional equipment, it will be difficult to precisely position the containers with conventional tug/barge arrangements.

At Red Eye Crossing the barges were anchored, after using survey equipment to locate them within a tolerance of a few feet. For the Marina Del Rey project, placement took place in the sheltered waters of Los Angeles/Long Beach Harbor. Meeting the tight positioning tolerances (about 10 m) required repositioning the large tug used to tow the barge, and the addition of a second smaller tug. This method of operation is probably not practical in the open ocean. Towed barges typically have long lines between the tug and the barge. Lengths of 100 to 200 m (with lengths increasing as seas become more severe) are common. Positioning the barges to tolerances greater than one barge width laterally (10 to 15 m) is difficult. To maintain steerage for a towed vessel in the open ocean, the barges have to be moving forward, making positioning difficult particularly in light of the unknown time for the containers to exit. Achieving tight positioning tolerances with GFCs in the open ocean may require specialized or modified equipment, such as powered barges that open to near-full bin width in a relatively short time.

Experience Using GFCs at Red Eye Crossing, Louisiana

Red Eye Crossing, located on the lower Mississippi River at Mile 175, is the most difficult crossing for the Corps' New Orleans District to maintain. An estimated 2 million m³ of dredged material was removed each year to maintain the 12-m-deep channel at this location. Model studies showed that underwater dikes should constrict riverflow, making the channel more self-scouring and thus substantially reducing dredging requirements. Concerns over the potential safety aspects associated with fuel barges running aground on rock dikes led the New Orleans District to construct soft dikes made from sand-filled GFCs.

Approximately 560 GFCs (14 to 44 m long, with a perimeter of 14 m) were placed in water depths of 13 to 21 m in currents up to 1.8 m/sec. The GFCs were used to construct dikes 150 to 550 m long. Dike crest width was 3 m, and with 1V:2H side slopes, heights varied from 4.5 to 9 m, producing base widths of 20 to 40 m (Figure 4). Sandy material with a D_{50} of 0.5 mm was placed in the containers, which held up to 380 m³ of material. The AOS of the material that was used corresponded to a sieve size of 70 to 30 (that is, 0.2 to 0.6 mm) (Duarte, Joseph, and Satterlee 1995).

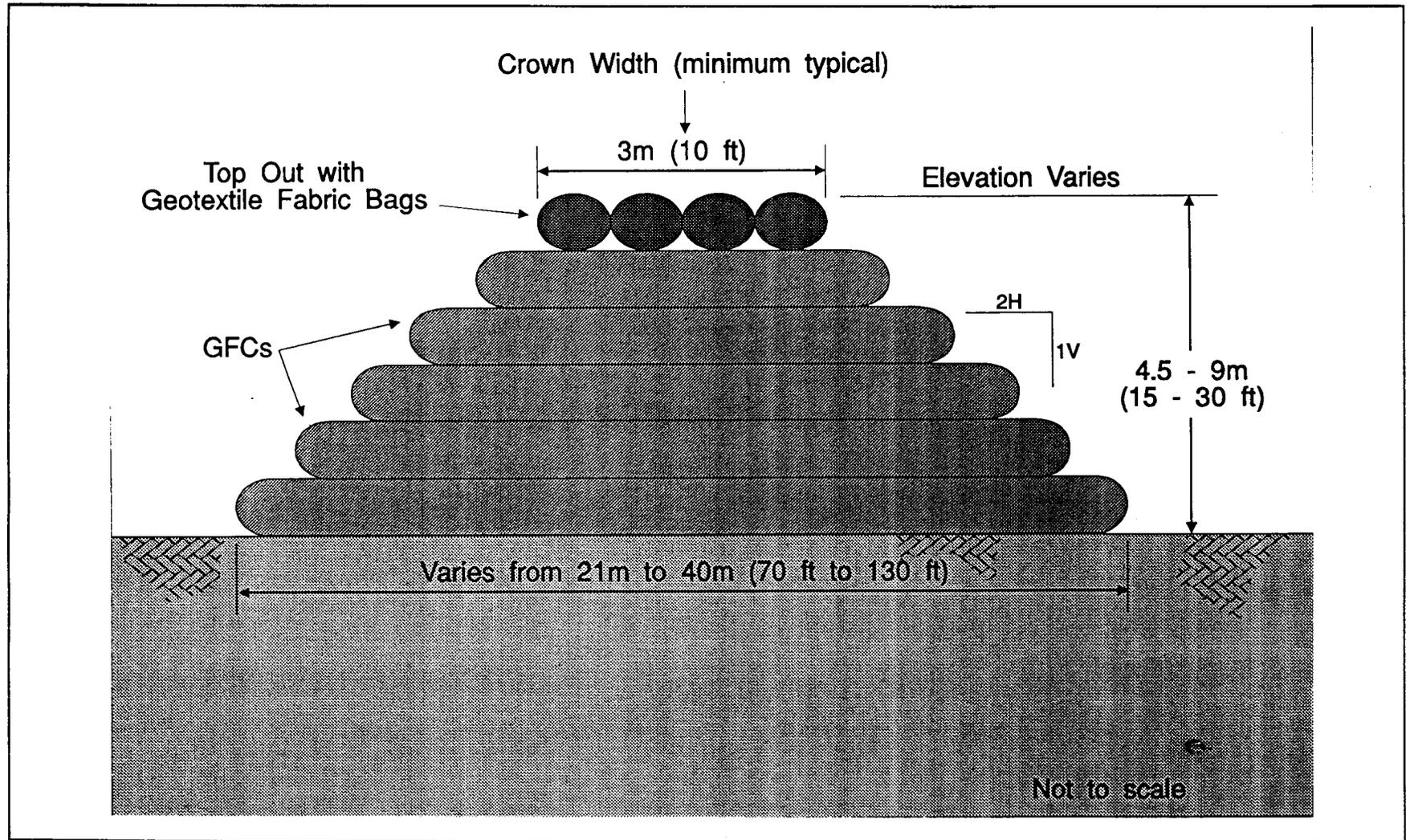


Figure 4. GFC soft dike structure used at Red Eye Crossing

GFCs secured in the barges were filled with sand using a front-end loader, which operated from a supply barge anchored adjacent to the work barge. After the GFCs were filled to 75 percent capacity, it took approximately 15 to 20 min to close the GFCs by sewing. Modified split-hull barges with a hull opening of 3 m, or 75 percent of the bin width (false bulkheads were added to reduce bin width), were used to deploy the GFCs. It took 3 to 5 min to release the GFCs from the barge. As noted above, the barges were anchored in place with positions accurately surveyed. Barge positions were offset to account for GFC displacement caused by currents of up to 1.5 m/sec. The contractor was able to accurately place the containers, as evidenced by bathymetry and side-scan sonar surveys during and after construction.

In addition to two conference papers that describe the project (Fowler and others 1995a; Duarte, Joseph, and Satterlee 1995), a report is being prepared by the New Orleans District. Additional information on the project from a geotechnical viewpoint is available from Mr. Frank Duarte (CELMN-ED-FD, (504) 862-1014). The operations point of contact on the project is Mr. James Scott, (504) 862-2905.

Experience Using GFCs at Marina Del Rey, California

In November and December 1994, 40,000 m³ of silty sand contaminated with hydrocarbons and heavy metals (chromium, lead, and zinc) were mechanically dredged from the entrance channels at Marina Del Rey and the adjacent Balona Creek entrance and placed into barges containing GFCs (Figure 5) (Fowler and others 1995b, Mesa 1995). The filled GFCs were placed in the shallow-water habitat area of Los Angeles/Long Beach Harbor. An inner liner capable of retaining 100 percent of fine particles retained on the No. 230 sieve (0.0625 mm) was a project requirement. The 16-oz (0.45-kg) nonwoven liner used on the project had an AOS that corresponds to a sieve size of 100 to 170 (0.149 to 0.088 mm).

The contaminated sediments were placed in two 2,060-m³ split-hull dump scows that were specially modified for this project. The barges were modified to meet the contract specification (based on limited small-scale model tests conducted at WES) requiring the barge hull opening to be at least one half the bin width. Barge modifications included construction of false sides to reduce the width of the bin to 6.7 m (barge bin length was 54 m with an overall height of 6.7 m). The width of the split hull opening was about 3.55 m, thus meeting the contract specification. In addition, end plates were installed at both ends of the scow bin to prevent the geosynthetic fabric from bulging at the ends and catching on the hydraulic rams. Side walls in the bins were ground down with a metal grinder to remove burrs that could tear the geotextile fabric. Cost of modifying the barges was \$250,000.

The GFCs initially used were 54 m long by 27.4 m in perimeter, with a capacity of approximately 3,000 m³. The GFCs were constructed with double

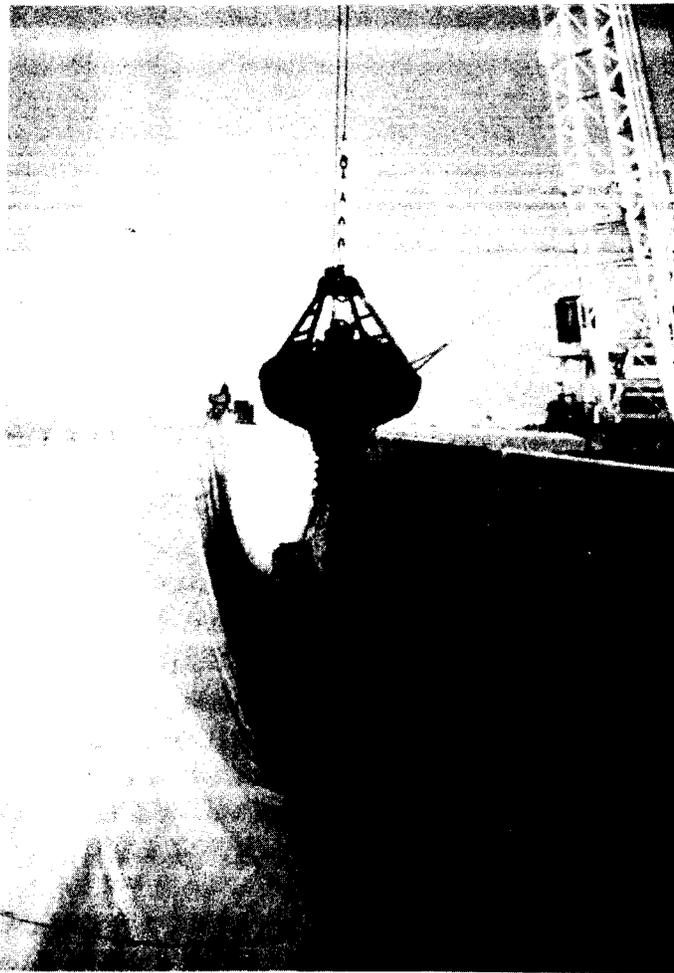


Figure 5. Filling a GFC at Marina Del Rey

liners—an outer woven polyester liner for strength and in inner nonwoven liner to provide filtration.

The inner 16-oz nonwoven fabric made it difficult to handle the containers, especially when they were saturated (for example, by rain). At the end of the filling process, the contractor had to sew up both liners; thus, the sewing process took about 2 hr. The second container also increased the time to assemble the containers initially and to deploy the containers in the barge. It was proposed that an 8-oz (0.23-kg) liner be used, since it is much easier to handle than the 16-oz liner. However, the ability of the 8-oz liner to prevent the migration of fines had not been tested, and thus it was not used for this project.

The first load consisted of 1,400 m³ of sediment placed in the 2,060-m³ scow, which filled the hopper to within 1.5 m of the top. The scow was taken to the placement site and opened fully, but the GFC would not discharge from the hopper. Some combination of arching, apparent cohesion caused by incomplete saturation, and a geotextile that pulled taut over the opening at the bottom to confine the soil and prevent movement is suspected of causing the

container to lodge in the scow. Unsuccessful attempts to free the container included bumping the scow to shake the GFC free, moving the jaws of the scow against the container to induce movement, and surcharging the container from the top to force the sand mass out. These attempts occurred over a 6-day period, after which the GFC was dislodged by injecting large quantities of water into the contained dredged material. Water was injected through diffuser pipes using the 7.6-m³/min pump of a Los Angeles County fire boat; water injection continued for about 2 hr before the GFC dislodged.

In the second barge, the volume of sediment was reduced to 460 m³, and the perimeter length of the container increased to 37 m to provide extra fabric at the bottom to allow free-fall of material into this "pouch" and facilitate container discharge from the hopper. This container discharged without incident. The volume of subsequent loads was increased by 150 to 230 m³, up to a load of 1,000 m³. At this point the containers showed a tendency to hang in the barge (as evidenced by longer exit times). Thus, the maximum practical volume was limited to about 1,000 m³.

Initially, the barges were lined with a polyester geosynthetic fabric to improve the ability of the GFCs to slide out of the barge; however, a relatively high coefficient of friction was thought to exist between the wet polyester bag and the polyester liner. As a result, the barge liners were removed after the problem with the first container was encountered. The consensus was that the placement problems were caused by the sandy nature of the material, not the liner. The material dredged from Marina Del Rey was fine sand with only 7 to 8 percent fines.

Because there were tight tolerances on where the containers had to be placed, positioning of the barge prior to releasing the GFCs at the shallow water habitat site took longer than originally expected—30 min to 1 hr. The barges were towed to the site; then, the tug released the tow and tied up alongside the barge. A second smaller tug tied up to the other side to help position the barge. The 2,000-m³ barges had a good deal of sail area and were difficult to position in high winds.

The container placement operation was monitored by divers and video, and a side-scan sonar survey was planned at the end of the project. However, the Port of Los Angeles was also disposing of contaminated material from inside the harbor in the same shallow-water habitat while the containers from Marina Del Rey were placed. This placement was by conventional bottom dumping. The mix of materials made it difficult to observe details of bag placement.

The added complexity at Marina Del Rey resulted in a total cycle time of 19 to 22 hr for dredging/placement operation. This time is broken down as follows:

Load and install containers in barge	2 hr
Dredging	6-7 hr
Sew containers	2 hr
Tow from Marina Del Rey to Port of Los Angeles	4-5 hr
Dispose of material	1 hr
Tow from the Port of Los Angeles to Marina Del Rey	4-5 hr

The time for conventional dredging was estimated to be 14 to 17 hr.

The slow pace of dredging and placement, approximately 1.5 bargeloads per day (roughly 1,500 m³ per day), resulted in the contractor removing only 42,000 m³ during the 40-day dredging period. If the use of GFCs on the project had not been required, production rate would have doubled, allowing over 85,000 m³ to be dredged.

Unit cost of the entire project, including the cost of the GFCs (\$26,000 per container including both the inner and outer liners), mobilization/demobilization, and actual dredging was about \$100/m³. If the project had used conventional split-hull barge placement, the unit cost for dredging alone would have been in the range of \$9 to \$13/m³. Assuming that 80,000 m³ had been dredged in the same 40-day time period with conventional placement, the mobilization/demobilization unit cost would have been in the range of \$10 to \$13/m³. Thus, the total unit cost for conventional placement on this project would be approximately \$20 to \$26/m³.

The Los Angeles District point of contact for additional information about the Marina Del Rey Project is Mr. Anthony Risko, (213) 894-5644.

Other Considerations for Using GFCs with Contaminated Dredged Material

While not all of the following issues are directly related to site-capacity issues for using GFCs, they should be addressed prior to using GFCs. One issue is how long after placement must capping commence. For most contaminated sediment projects, capping must be begun within 2 to 4 weeks. If capping of material placed in GFCs can be delayed longer, additional operational flexibility will be provided.

High GFC placement densities (that is, very small gaps between individual GFCs), estimated at greater than 90 percent, were achieved in the submerged dikes created at Red Eye Crossing with anchored barges. However, the

experience at Marina Del Rey showed that, even with two tugs operating inside a harbor, achieving 9-m gaps between GFCs was time consuming. To make reasonable estimates of site capacity using GFCs for contaminated sediments in open ocean sites, values of GFC placement density are needed. Practical estimates of open ocean positioning accuracy are needed, combined with field evaluations to provide placement density data.

Wave forces on GFCs, particularly in shallow water (approximately 20 m and less), should be considered. The potential for a hurricane or northeaster to move the containers prior to capping should be investigated.

While poor weather causes problems for many dredging operations, those projects using GFCs are particularly susceptible. For example, rain increases the difficulty of handling the fabric. Moderate wind and waves make precisely positioning tug-powered GFC barges more difficult. High winds, waves, and even moderate currents will make precise positioning of split-hull barges with conventional tugs essentially impossible.

Summary

GFCs can be used for contaminated dredged material placement. However, the high costs associated with using GFCs limit their use to those projects where savings in cap volume justify their use. Also, if space or site capacity is limited, containers could be worthwhile. Other applications would be those projects where more conventional options are either unavailable or extremely expensive.

Using GFCs to reduce water column impacts is probably not warranted for most projects because, in most instances, water column impacts (even with contaminated sediments and normal placement operations) are not a problem. For those projects where water column impacts are an issue, it is possible that just the outer strength container would sufficiently reduce dispersion to meet water quality standards. If a nonwoven liner is required, the 8-oz liner (which is easier to handle) may be sufficient, as opposed to the 16-oz liner used at Marina Del Rey. Testing conducted to date on migration of fines and contaminants through the fabric does not reflect actual field conditions and should be done in a more rigorous fashion.

Costs of placing dredged material using GFCs are substantially higher than conventional mechanical dredging and bottom dump barge placement because of the cost of the GFCs, labor, land facilities, and barge modifications. The cost of the GFCs (including the inner liner) is approximately \$13 to \$16/m³. The GFC manufacturer estimates that the use of GFCs increases the cost per cubic meter approximately \$33 to \$40 over the normal dredging and placement cost. For the Marina Del Rey project, the cost increase from using GFCs was approximately \$65 to \$78/m³. At Marina Del Rey (the first time GFCs were used for contaminated dredged material placement), the final unit cost (including mobilization/demobilization) was nearly \$104/m³. Note that these are

rough cost estimates; actual cost estimates for a specific project should be developed in close consultation with GFC manufacturers and dredging contractors.

The entire process of assembling, placing in the barge, unfolding, securing, and then sewing closed the GFC after filling is time consuming and labor intensive. The added time for the process could be minimized if a sufficient number of barges, tugs, and staff are available, but will probably be at least 1 to 2 hr.

Filling the GFC is relatively straightforward, but can take longer than normal mechanical dredging. The GFCs (particularly the weaker liners) can be ripped by the force of the dredged material if it falls from too far above the barge. Thus, the care required when handling the material often increases the dredge cycle time. Also, it should be noted that, to get the maximum benefit from the GFC placement, the containers have to be placed precisely, with horizontal positioning tolerances on the order of a few meters. Accomplishing this in the open ocean will be very difficult, and either the cycle time will be increased considerably or the density with which the containers can be packed will be reduced.

Using GFCs for placement of contaminated dredged material is a recent development with limited experience. Additional information is needed on the environmental effectiveness and operational feasibility of this option. Future demonstrations and evaluations should include efforts to gather additional information on the following:

- a. Methods for safe and efficient exit of the GFCs from the barge.
- b. Effectiveness of GFCs in preventing dispersion of suspended solids.
- c. Effectiveness of GFCs in inhibiting entrainment of water during descent.
- d. Quantity and quality of water that is released from the GFCs in the long term.
- e. Potential for bioturbation to degrade the GFC.

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Environmental Effects of Dredging Technical Notes



Assessment of the Genotoxic Potential of Dredged Material

Purpose

This technical note describes an approach for assessing the genotoxic potential of dredged material. The use of integrated batteries of rapid and mechanistically interpretable *in vitro* and *in vivo* assays in a tiered approach is fundamental to applied toxicology. The research described here brings this approach to the testing of sediments. Work completed to date and future work will mesh to form an advanced and cost-effective methodology. The purpose of this methodology is to increase the accuracy of environmental risk assessments and facilitate making decisions concerning open-water disposal of dredged material.

Background

A great number of the contaminants typically found in dredged material are toxic to exposed organisms through effects on DNA. Such effects are usually the result of low-level chronic exposures. These effects can result in reproductive failure of organisms, impaired growth and development of offspring, and tumors (often cancerous) in vertebrates. Collectively, such effects are called "genotoxicity" and result from damage to the genome of a cell. The damage is heritable, that is, passed on to future cell generations upon duplication of the affected cells.

Although tests of sediment genotoxicity are not routinely applied in regulatory contexts, the potential for their requirement in special circumstances is implied by the language of U.S. public law. For example, Section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972 (Public Law 92-532), which regulates disposal of dredged material in coastal regions, specifically

prohibits open-water disposal in other than trace amounts of "known carcinogens, mutagens, or teratogens or materials suspected to be carcinogens, mutagens, or teratogens by responsible scientific opinion." In addition, the emphasis in environmental toxicology over the last decade has increasingly shifted away from the catastrophic end point (death of individual organisms in acute exposures) to chronic and sublethal effects that have long-range potential to seriously affect the viability of populations of organisms. To be accurate, risk assessments involving environmental contamination must take genotoxic potential into account.

Additional Information

For additional information contact the authors, Dr. Michael E. Honeycutt, (601) 634-4300, and Dr. Victor A. McFarland, (601) 634-3721, or the manager of the Environmental Effects of Dredging Programs, Dr. Robert M. Engler, (601) 634-3624.

Approach

A tiered approach is being developed in which a battery of mechanistically related, rapid, low-cost assays are applied initially. Based on the results of these assays, decisions can be made as to whether more definitive tests are necessary at higher tiers in the evaluation. The assays are based on the approach of the U.S. Environmental Protection Agency's Health Effects Research Laboratory for assessing the genotoxic potential of chemicals to rodents and humans (Kitchin, Brown, and Kulkarni 1994).

The battery contains two types of tests: assays to assess damage to DNA and assays to assess nongenotoxic adjuncts of DNA damage. The rationale for selecting these two types of assays lies in the knowledge that cancer and other results of DNA damage are multistage events requiring alterations in protein synthesis and cell development and function. For example, the development of cancer involves processes known as initiation and promotion. Initiation can be simply defined as damage to DNA, also known as mutation. A mutation occurs when a DNA nucleotide is chemically modified, deleted, or substituted. Certain environmental contaminants act as mutagens in that they covalently bind to DNA nucleotides, chemically modifying the DNA. The cell contains DNA repair enzymes that can repair mutations under normal circumstances.

When the organism is exposed to an excessively high level of a mutagen, the DNA repair enzymes may not be able to repair all of the mutations or may misrepair some mutations by deleting the nucleotide rather than replacing it, or by substituting a wrong nucleotide for the mutated one. Depending on the location of the mutation, the number of mutations, and whether the mutation is repaired by the cellular DNA repair enzymes, a mutation may progress to tumor formation or cancer in the organism. The stage of cancer development following initiation is promotion, in which the initiated cell is

altered to allow reproduction of the cell, passing the "defect" on to daughter cells.

To adequately assess dredged material genotoxic potential, the ability of sediment contamination to cause DNA damage and its subsequent effects on exposed organisms must be ascertained. Even if analytical chemistry were capable of identifying and quantitating all the genotoxic agents present in a sediment, an assessment of genotoxic potential could not be made with analytical data alone because contaminants interact in unpredictable ways. The toxicological approach involves the use of a battery of biomarker-based *in vitro* assays on sediment extracts in the first level, or tier, of testing. These assays assess the potential for DNA damage and the subsequent biochemical and molecular changes that lead to tumor formation and other adverse somatic effects. The second level of testing is *in vivo* testing, which involves exposing fish to the dredged material and assessing genotoxic effects, thereby incorporating bioavailability of sediment-associated contaminants.

***In Vitro* Testing**

In vitro testing uses two basic types of assays for mutagenicity. Bacterial assays (Ames test and Mutatox) are designed to detect the presence of mutagenic compounds in a sample. A second type of assay (alkaline unwinding) is used to determine whether an exposed living cell has experienced mutations.

The *in vitro* testing battery also uses tests of nongenotoxic effects on adjunct systems. These assays include cytochrome P450 induction, glutathione fluctuations, ornithine decarboxylase activity, oxidative stress, and cytotoxicity. Cytochrome P450 is a family of enzymes found in most living organisms and is primarily responsible for metabolism of environmental contaminants. Exposure to certain classes of genotoxic compounds (for example, polycyclic aromatic hydrocarbons (PAHs), dioxins, and polychlorinated biphenyls) induces the formation of cytochrome P450, which has a promotional effect on initiated cells. Glutathione is a small peptide that functions as the major defense against electrophilic compounds in most vertebrate organisms. Electrophiles bind to DNA and thereby cause mutations. An organism (or cell) may be depleted of glutathione upon exposure to such compounds, leaving it vulnerable to an increased rate of mutation.

Ornithine decarboxylase is an enzyme that, when present, indicates cellular proliferation and signals possible exposure to a cancer promoter. While oxygen (O_2) is essential for life functions of all multicellular organisms, some forms of "reactive oxygen" produced during metabolism (for example, superoxide anion radicals ($O_2\cdot^-$), hydroxyl radicals ($OH\cdot$), and hydrogen peroxide (H_2O_2)) are highly reactive and can damage DNA. Subcellular biochemical changes such as these can also lead to cytotoxicity, or cell death. All of these biomarkers can be measured *in vitro* and, when used together, provide a short-term means of predicting carcinogenicity. More complete

descriptions of these and other assays that can be used to test for potential genotoxicity are provided in Honeycutt, Jarvis, and McFarland (1995a,b,c).

Sediments that are to be screened are extracted and prepared as for gas chromatography/mass spectrometry analysis. Cultured cells are dosed with the sediment extracts and are then incubated for an appropriate length of time. After incubation, the cells are assayed. The assays use two types of cultured cells, H4IIE cells and Chinese hamster ovary (CHO) cells. H4IIE is an "immortal" or continuous rat liver hepatoma cell line that contains cytochrome P450. The CHO cell line is a continuous cell line that does not contain cytochrome P450. This distinction is important because many invertebrate aquatic species do not possess well-developed cytochrome P450 systems. Thus, using both cell types gives a better indication of risk to all aquatic species than does using only one type. Also, because some chemicals, such as the PAHs, must be metabolically activated in order to exert their genotoxic effect, the use of both types of cell lines can discriminate the presence or absence of these chemicals.

***In Vivo* Testing**

In vitro testing serves to identify potentially genotoxic dredged material but does not yield information concerning bioavailability of the contaminants in the sediments. Though methods are continually being refined to predict contaminant levels in aquatic organisms (McFarland and others 1996), the genotoxic potential of dredged material must be evaluated for individual sediments on a case-by-case basis. For this purpose, *in vivo* assays will be developed to test those dredged sediments for which *in vitro* testing indicates a genotoxic potential.

Several ways to accomplish this appear to be possible. Rapidly developing larval fish (which are therefore susceptible) can be exposed to dredged material and observed for developmental abnormalities. Another possibility is development of a transgenic fish that will signal the occurrence of mutations by expression of a detectable gene product, such as firefly luciferase. A third possibility is the use of a susceptible standard fish model, such as the Japanese medaka. Exposures would necessarily be of partial lifetime duration (2 to 3 months). At the end of the exposure, the fish would be subjected to a battery of biochemical assays much like the *in vitro* screening assays. This would involve testing blood samples for alanine aminotransferase, which is indicative of cytotoxicity. Livers can be excised and analyzed for cytochrome P450 levels, DNA damage, glutathione levels, ornithine decarboxylase activity, and oxidative damage. The results of these tests can then be compared to a matrix of the effects of known carcinogenic compounds. Matrix comparisons enable interpretation of the biomarker data in terms of the effects of model genotoxic chemicals having known modes of action.

Research Efforts

The Aquatic Contaminants Team at the U.S. Army Engineer Waterways Experiment Station is currently developing and validating the *in vitro* assays that have been described in this technical note. To keep the assays rapid, inexpensive, and sensitive, multiwell fluorescence plate reader technology is being used as the basic developmental methodology whenever possible. For example, in the cytotoxicity test, H4IIE or CHO cells are plated in 96-well plates and incubated overnight. The cells are then spiked with sample extracts or chemical standards and incubated an additional 24 hr. At the end of the 24-hr exposure period, the culture medium is removed from the wells using a microplate washer. Buffer containing calcein AM is added to the cells. Calcein AM is absorbed by live cells, fluorescing green at 530 nm. The cells are read in the fluorescence plate reader, and cytotoxicity is expressed as percent viability. Similar techniques are being applied to most of the other assays in the *in vitro* genotoxicity testing battery. The development of *in vivo* genotoxicity testing methods has not yet begun.

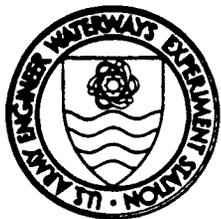
Regulatory practices are increasingly being framed in the context of risk assessment. The assessment of risk from environmental chemicals cannot be done accurately based on acute toxic responses alone. Procedures to evaluate the effects of long-term chronic exposures on growth and reproduction in whole organisms and to extrapolate such effects to populations are still in development. Even when such tests are available, their utility will be limited by high cost and diminishing resources for regulatory implementation. In addition, many of the contaminants in sediments are genotoxic and may not be detected by chronic laboratory exposures. Risk assessments that do not include the potential for genotoxic effects when that potential exists are inaccurate. The work described here is intended to address the need for less costly and more mechanistically interpretable ways to provide the basic data on which accurate risk assessments can be conducted.

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Environmental Effects of Dredging Technical Notes



Proposed New Guidance for Interpreting the Consequences of Bioaccumulation from Dredged Material

Purpose

This note describes, for consideration, modifications to current guidance for evaluating and interpreting bioaccumulation data collected during regulatory evaluations of dredged material.

Background

Evaluating the environmental consequences of contaminant bioaccumulation resulting from dredged material disposal is a complex technical and regulatory problem. This problem is magnified by the high cost of bioaccumulation testing and the lack of explicit guidance on how bioaccumulation data should be interpreted and used within a regulatory program.

Bioaccumulation is a measurable phenomenon, rather than an effect. Consideration must be given to specific information about the likelihood of biological effects (for example, reduced survival, growth, and reproduction in animals; cancer risk in humans) that are associated with contaminant residue levels in order to make objective decisions, from a regulatory standpoint, about what level of bioaccumulation constitutes an "unacceptable adverse effect."

The existing guidance attempts to overcome this problem with two approaches, both of which use low trophic level aquatic organisms and a reference-based comparison. In the first approach, the level of bioaccumulation of a specific contaminant is compared with a numerical effect limit, such as a Food and Drug Administration action level or a fish advisory. If the level of the contaminant in the organism exceeds the numerical limit, there is the potential for the dredged material disposal to have an "unacceptable adverse effect." If it does not, or there is no numerical limit, a second approach is used which involves a comparison with data collected from

animals exposed to a reference sediment. If bioaccumulation in the animals exposed to the dredged material is statistically greater than that of animals exposed to the reference, a number of subjective factors are then evaluated to determine whether dredged material disposal will result in an "unacceptable adverse effect" (U.S. Environmental Protection Agency/U.S. Army Corps of Engineers (USEPA/USACE) 1991, 1994).

The first approach is straightforward in that it uses numerical evaluation factors. However, the utility of this approach is limited by the small number of published numerical limits compared with the large number of contaminants commonly present in freshwater and marine sediments. Because the evaluation factors in the second approach are subjective, they cannot be consistently applied in the decision-making process. This has created a major problem in the interpretation of bioaccumulation data.

In response to this problem, the Corps of Engineers and the Environmental Protection Agency held a joint bioaccumulation workshop in Denver, Colorado, on August 29-31, 1995. The purpose of the workshop was to determine if more effective regulatory guidance could be developed for interpreting the effects of bioaccumulation from data currently collected during evaluations of dredged material. Workshop participants were from the Corps of Engineers, EPA, U.S. Fish and Wildlife Service, National Oceanic and Atmospheric Administration, Department of Defense, academia, and the private sector. The proceedings of this workshop are summarized in Bridges and others (1996).

Additional Information

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Discussion

Following the Denver workshop, the authors of this technical note were tasked by the Headquarters, U.S. Army Corps of Engineers, to suggest ways to improve current guidance regarding the use and interpretation of bioaccumulation data collected during evaluations of dredged material. Discussions and recommendations from the Denver workshop formed the basis for the group's subsequent considerations.

The suggested modifications, outlined below, are discussed within the four-tiered framework used in the guidance manuals for evaluation of dredged material (USEPA/USACE 1991, 1994) (Figure 1). These procedures are intended to increase the effectiveness of the regulatory process with regard to bioaccumulation. Comments regarding these suggestions should be directed to the authors.

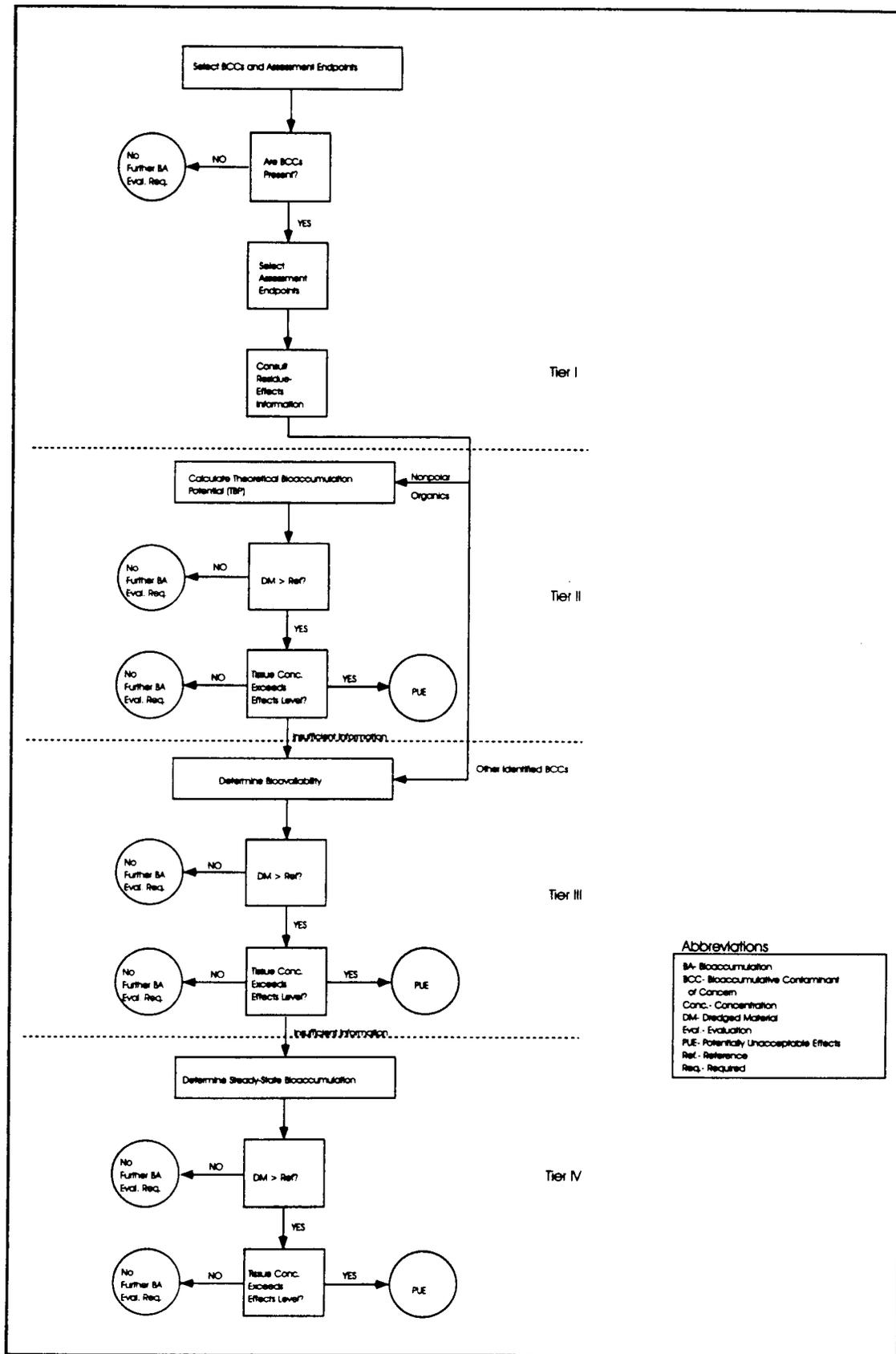


Figure 1. Flowchart describing proposed guidance for evaluating bioaccumulation data

Tier I

The purpose of Tier I, as described in existing guidance (USEPA/USACE 1991, 1994), is to determine whether a compliance decision can be reached regarding dredged material disposal on the basis of existing information, including all previously collected physical, chemical, and biological data. A primary task in Tier I is to identify the environmental contaminants of importance in the dredged material under consideration. Such an identification is necessary to select appropriate analyses in Tiers II, III, and IV.

Prepare List of Site-Specific Bioaccumulative Contaminants of Concern.

As a result of discussions at the August 1995 workshop, it became clear that bioaccumulation data are most appropriately used to evaluate the potential for contaminant effects on higher trophic level organisms (for example, fish, wildlife, and humans). For such organisms, contaminant trophic transfer, that is, the movement of contaminants from lower to higher trophic levels through the ingestion of contaminated food, represents the major route of contaminant exposure. Direct contact or ingestion of sediments is a much less important route of exposure to vertebrates in most instances.

Trophic transfer, to the extent necessary to result in adverse effects, will occur only for a subset of the contaminants found in dredged material. This is largely the result of differences in chemistry among contaminants. Trophic transfer and bioaccumulation are most likely for those organic contaminants with a $\log K_{ow} > 4$. Table 9-5 in the Ocean Testing Manual (USEPA/USACE 1991) and Table 9 in the Inland Testing Manual (USEPA/USACE 1994) should be consulted for a list of organic contaminants that meet this criterion.

Metals and metalloids are much less likely to bioaccumulate and cause adverse effects at higher trophic levels, with a few notable exceptions (methyl mercury, lead, cadmium, organotins, arsenic, and selenium). During the selection of bioaccumulative contaminants of concern (BCCs) in Tier I, the evaluation should focus on the subset of organic and inorganic contaminants described above. If none of these contaminants is present, there may be no further need to evaluate the potential for bioaccumulation in subsequent tiers. If there is reason to believe that such contaminants are present in the dredged material, the evaluation should proceed to Tiers II and/or III (Figure 1).

Select Assessment Endpoints. After the contaminants of concern have been selected, consideration should be given to the nature of the assessment and measurement endpoints that will be used during subsequent evaluation. Corps districts, in consultation with EPA regions, should select the environmental components (receptors) that are to be protected from the effects of contaminant bioaccumulation from dredged material. Examples of such assessment endpoints include ensuring the protection of (1) human health, (2) a local population (for example, striped bass), or (3) a local endangered wildlife population (bald eagles, etc.).

After the assessment endpoints have been selected, consideration must be given to how bioaccumulation data are going to be used to ensure the protection of the assessment endpoints. How will the risks to a given receptor be measured? That is, what measurement endpoints will be considered? For example, risks can be quantified in terms of the number of excess cancers produced in humans or whether residue concentrations in exposed animals exceed levels that will produce adverse effects.

What data are collected and how those data are used in the decision-making process are determined by the nature of the assessment and measurement endpoints chosen. An effective evaluation is not possible before agreement is reached on the specific assessment and measurement endpoints to be considered.

Determine Availability of Relevant Effects Data. The environmental risk posed by a sediment-associated contaminant is a function of two factors: the likelihood that the receptors (organisms) to be protected will be exposed to the contaminant (that is, bioavailable forms of the contaminant can be transported into the tissues of the receptor) and the likelihood that the contaminant, once present within the receptor, will produce harmful effects.

The bioaccumulation tests described in the Ocean and Inland Testing Manuals are tools for measuring one aspect of contaminant exposure—bioavailability. To evaluate the risk that contaminant bioaccumulation will result in “unacceptable adverse effects,” contaminant and receptor-specific residue-effects information for the contaminant of concern must be consulted. When evaluating risk to humans, and many other vertebrate species, residue information on relevant food/prey species must be used to estimate contaminant exposure before the likelihood of effects can be determined. Evaluating such information is essential to estimating the risk of adverse effects.

At this point in the bioaccumulation evaluation, three criteria should have been met. First, BCCs should have been identified and shown to be present in the dredged material. Second, one or more receptors for the contaminant should have been determined. That is, assessment and measurement endpoints have been selected. Third, residue-effects are consulted for the BCCs and receptors chosen.

With regard to the regulatory evaluation of dredged material, evaluating relevant residue-effects information for a specific BCC and receptor (considering the assessment endpoints chosen) is essential to making objective regulatory decisions concerning bioaccumulation. Evaluating such information is a necessary part of determining whether a given level of exposure will result in an adverse effect.

Some residue-effects data are available in the published literature. To ensure that future evaluations of bioaccumulation are effectively performed, the U.S. Army Engineer Waterways Experiment Station is currently developing a residue-effects database to be used by field personnel to interpret

bioaccumulation data. The database will be developed by reviewing and extracting relevant data from the published literature and will include residue-effects data for a broad range of organisms and contaminants.

Notices concerning availability of the residue-effects database will be posted on the Contaminants Bulletin Board System, which can be accessed via modem at (601) 634-4380. Technical assistance for the database will be available at (601) 634-2489.

Tier II

The tasks in Tier II are designed to provide a rapid screen for determining the potential for contaminant bioaccumulation from dredged material and for evaluating potential water column effects. Calculation of theoretical bioaccumulation potential provides an estimate of the potential for contaminants in dredged material to be bioaccumulated. Marine water quality criteria or state water quality standards are used in combination with a numerical mixing model to evaluate the potential for acute toxicity in the water column.

Collect Sediment Chemistry Data for Evaluation of Theoretical Bioaccumulation Potential (TBP). Following preparation of the site-specific list of BCCs, sediment chemistry data should be collected for these contaminants. One of the significant problems identified during the workshop regarding the statistical treatment of dredged material was the fact that adequate consideration was not being given to natural variation in contaminant concentrations; this is particularly true when laboratory tests are performed on composited samples. Considerable latitude is granted in current guidance regarding the intensity of sampling at a particular dredging project. However, current guidance does state that when important environmental contaminants are present, more intensive sampling is desirable (USEPA/USACE 1991).

When bioaccumulation is expected to be an important exposure pathway for contaminants in the material to be dredged from a particular project or project segment, care should be taken to ensure that an adequate number of replicate samples (five, for example) are collected from each of the operational units where bioaccumulation is a concern.

Evaluate TBP. TBPs should be calculated for nonpolar organic BCCs using the chemistry data described above and the most appropriate and available Biota-Sediment Accumulation Factors (BSAFs). Current predictive methods are valid only for nonpolar organics. If the dredged material contains BCCs other than nonpolar organics ($\log K_{ow} > 4$), the potential for bioaccumulation can be evaluated only through Tier III and/or Tier IV testing.

Selection of a BSAF can be approached in several ways, depending on circumstances. The Inland Testing Manual contains an up-to-date discussion describing the selection of BSAFs. The Ocean Manual is outdated in that it

recommends using a default BSAF = 4 in all TBP calculations. That factor (4) is at the 94th percentile of all BSAFs contained in the Contaminants Bulletin Board System (BBS) Database, (601) 634-4380, and is about 12-fold greater than the median BSAF (0.520) for all listings in the database, making it unreasonably conservative for predictive purposes (McFarland and Ferguson 1994, McFarland 1995). The following recommendations are given regarding the calculation of TBP:

- TBP should be calculated for a specific BCC and receptor of concern, using locally generated data if at all possible. If a Corps district has a history of conducting 28-day bioaccumulation tests using specific organisms (for example, *Nereis virens* or *Macoma nasuta*) and has data from past tests, it may be possible to generate local BSAFs. Such BSAFs can be calculated if the four components of a BSAF calculation were measured and retained:
 - Concentration of the BCCs in sediment used in the bioaccumulation test.
 - Total organic carbon (TOC) of that sediment.
 - Concentration of the BCCs in the exposed organism at the end of the test.
 - Lipid content of the organism.

If a local database from previous testing contains such data, it should be possible to generate organism/BCC-specific mean BSAFs complete with measures of variance. It is reasonable to expect that BSAFs generated in this way will provide the most accurate predictions of theoretical bioaccumulation potential in future evaluations. It is recommended that Corps districts begin to acquire these types of data as part of their dredged material evaluations, if they are not already doing so. Corps districts with the necessary data to generate local BSAFs can contact the authors of this technical note for further guidance as necessary.

- If local BSAFs are not available, the Contaminants BBS can be queried to find BSAFs that were generated in field or laboratory studies for specific organisms, chemicals, and levels of TOC in sediments. A practical approach in using the BBS to select BSAFs for a specific sediment would be as follows:
 - Begin with the concentration of a specific BCC and the TOC content of the reference sediment and the dredged material.
 - Go to the BBS and search for cases in which BSAFs are reported for the same BCC in sediments with similar TOC content.
 - Choose the reported BSAF for the organism for which TBP is to be calculated (or the organism most closely related).
- Alternatively, use the median BSAFs reported in McFarland and Ferguson (1994). Table 1 in that paper presents a statistical analysis of all the BSAF data in the Contaminants BBS Database as of November 1994. Median BSAFs (and 25th and 75th percentiles) are reported for nine categories in which the BSAF data are broken out in various ways (PCBs, PAHs, dioxins/furans, etc.).
- Separate TBP values should be calculated for each nonpolar organic BCC identified at the end of Tier I. A separate TBP value should be calculated for each chemistry value. Assuming a sample number equal to 5, this would

result in five estimates of TBP for each BCC in the dredged material and reference sediment.

A statistical analysis should then be performed to compare the dredged material and reference sediment TBP values. If the TBP value for a BCC in the dredged material is not statistically greater than the reference TBP value, no further evaluation of bioaccumulation should be necessary for that BCC. If some BCC TBP values are statistically greater than the reference TBP value, a consideration of effects should follow, as described below. In those cases when contaminant tissue concentrations are less than the detection limit of the analytical method employed, the statistical methods outlined in Clarke (1995) and Clarke and Brandon (in preparation) should be used.

Compare TBP Values with Effects Data. The likelihood for adverse effects should be evaluated for those BCCs predicted to exceed reference tissue levels. The potential for an adverse environmental effect due to bioaccumulation will be determined by evaluating information concerning the relationship between contaminant tissue concentration and relevant effects in the receptor(s) of concern (identified in Tier I). Consideration must be given to the relevance of the collected data and what extrapolation is necessary in making an effects determination (for example, worm tissue contaminant concentrations alone are insufficient to determine if a population of bald eagles will be jeopardized by disposal of dredged material). Bald eagles are more likely to be exposed to contaminants via the ingestion of tissues of higher trophic level organisms (fish and other vertebrates) rather than worms.

The residue-effects database should be consulted to reach a determination as to the potential for adverse effects. In those cases where BSAF-predicted tissue concentrations are close to or above relevant effects concentrations, or excessive uncertainty exists regarding the predicted tissue concentration, the evaluation should proceed to Tier III (Figure 1).

Tier III

Tier III testing is designed to evaluate the toxicity and bioavailability of contaminants in dredged material. Short-term toxicity tests are performed using sensitive organisms to evaluate the potential for contaminants in dredged material to produce significant lethality. Longer term bioaccumulation tests are performed to evaluate the bioavailability of contaminants in dredged material.

Perform Bioaccumulation Tests. When the information that has been accumulated in preceding tiers is insufficient to make a decision regarding bioaccumulation, bioaccumulation testing (as outlined in the Ocean and Inland Testing Manuals) may be necessary. Such testing is necessary when predictive techniques for estimating tissue concentrations are not appropriate or when the uncertainty associated with predictive techniques is excessive.

Uncertainty associated with the predicted tissue concentration is particularly important when the predicted tissue concentration is close to the level at

which effects would be expected. Bioaccumulation testing should be performed on an adequate number of replicates from a given project or project segment to ensure a satisfactory description of the mean and associated variance. Statistical comparisons should be made using the same guidance proposed in Tier II. If the concentrations of BCCs in tissue are not significantly greater than the reference concentration, no further evaluation of bioaccumulation should be necessary. If some BCC concentrations are significantly greater in animals exposed to the dredged material than for animals exposed to the reference sediment, the likelihood of effects must be evaluated (Figure 1).

Compare Tissue Concentrations with Effects Data. As discussed in Tier I, consideration of residue-effects data is essential to making objective decisions regarding dredged material disposal and management. The procedures followed here should be the same as those followed in Tier II.

Tier IV

When insufficient information has been acquired during previous tiers to allow a decision regarding dredged material disposal, Tier IV evaluations may be used. Tier IV evaluations consist of case-specific tests for evaluating the potential for significant toxicity or bioaccumulation resulting from long-term exposures to dredged material.

Perform Steady-State Bioaccumulation Test. When the information that has been accumulated in preceding tiers is insufficient for making a decision regarding bioaccumulation, steady-state bioaccumulation testing, or an evaluation of steady-state concentrations (as outlined in the Ocean and Inland Testing Manuals), may be necessary. Testing should be performed on an adequate number of replicates from the material to be dredged and the reference site. Statistical comparisons should be made using the same guidance proposed in Tiers II and III. If the concentrations of BCCs in tissues of animals exposed to the dredged material are not significantly greater than those in tissues of animals exposed to the reference sediment, no further evaluation of bioaccumulation should be necessary. If some BCC tissue concentrations are greater for animals exposed to dredged material than to the reference sediment, the likelihood of effects must be evaluated.

Compare Tissue Concentrations with Effects Data. Consideration must be given to whether or not the contaminant concentrations measured are likely to produce adverse effects. Such an evaluation will be accomplished by consulting relevant residue-effects information.

Summary

The evaluation process outlined above will provide for a more effective regulatory evaluation of the potential for "unacceptable adverse effects" due to contaminant bioaccumulation from dredged material.

This guidance is different from existing guidance in two important respects: (1) developing site-specific lists for the BCCs, assessment endpoints, and measurement endpoints will ensure that site-specific questions are well thought out and explicitly defined and (2) comparing tissue contaminant concentrations with relevant residue-effects data emphasizes the need to evaluate *effects* data in order to determine the potential for “unacceptable adverse *effects*.”

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