



# *Environmental Effects of Dredging Technical Notes*



## UPLAND ANIMAL BIOASSAYS OF DREDGED MATERIAL

**PURPOSE:** This note introduces the concept of using an upland animal as an indicator of the contaminants in dredged material (1) proposed for disposal in an upland environment or (2) already placed in an upland disposal facility. Examples of the applications of an animal bioassay procedure to estuarine and freshwater dredged material placed in an upland environment have been published in several recent papers. The text of this note is taken from a review prepared for the International Conference on Earthworms in Waste and Environmental Management, Cambridge, UK (Rhett, Simmers, and Lee In Press).

**BACKGROUND:** Animal bioassay test procedures are being evaluated, field tested, and verified under the "Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives," called the Field Verification Program (FVP). The FVP research is being conducted in conjunction with a scheduled dredging project in Black Rock Harbor (BRH) near Bridgeport, Conn. The bioassay test procedures are relatively simple and can provide information that may be required in the ecological evaluation and environmental assessment of dredged material disposal. Based on laboratory results and limited field testing, the procedures can be applied to contaminated sediment (dredged material) that requires placement in an upland environment. The concept presented in this note is the result of ongoing research under the FVP. The results of the field testing will be reported in a later Technical Note. Draft final guidance will be completed in September 1987.

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### Introduction

The Clean Water Act in the United States requires that the environmental evaluation of dredged material prior to discharge or impacting the waters of the United States include the effects of disposal on concentrations of contaminants through biological processes. This results in a need for Corps of

Engineers districts to be able to predict the contamination of animals that may be associated with potential disposal alternatives: open-water disposal, upland disposal, and wetland creation. The following is a summary of the results of bioassay procedures using the earthworm *Eisenia foetida* to evaluate the potential contaminant mobility into soil-dwelling animals. These tests were derived from proposed Organization for European Common Development (OECD) and European Economics Commission (EEC) test procedures (evaluating the effects of new chemicals) and modified to consider accumulation and sublethal effects rather than toxicity.

The availability and animal uptake of heavy metals, polychlorinated biphenyls (PCBs), and polynuclear aromatic hydrocarbons (PAHs) from contaminated dredged material placed in upland disposal environments were evaluated with a solid-phase animal bioassay. The objectives of these studies were to apply, document, and verify existing terrestrial animal contact-bioassay procedures to predict movement of contaminants into soil-dwelling animals colonizing dredged material disposal sites.

The following dredged materials were chosen for testing: a highly contaminated estuarine sediment taken prior to dredging from BRH; dewatered dredged material from the Chicago River (Ill.) used to overlay a pyritic mine spoil; and dredged material from the Buffalo River (NY) confined in an upland disposal area. Each dredged material represented a different stage of aging and plant and animal colonization. The BRH dredged material represented time = 0 with no plant or animal colonization, and the material from the Chicago and Buffalo rivers represented aging of 7 and 9 years, respectively. The dredged materials also represented cases in which prediction of contaminant mobility is essential since confined disposal sites or other upland deposits of dredged material often become highly prolific wildlife habitats.

During the summer and fall of 1983, the earthworm *Eisenia foetida* was exposed to each substrate in a laboratory experimental chamber. After 28 days, the earthworms were removed and analyzed for heavy metals, PCBs, and PAHs. Comparisons were made of sediment levels of these contaminants with animal availability, bioaccumulation, and toxicity. The test procedures were intended to evaluate the potential movement of toxic heavy metals, PCBs, and PAHs from dredged material placed in an upland (oxidized) disposal area into soil-dwelling invertebrates as a first-step evaluation of contaminant mobility into animals that may colonize the dredged material.

### General Test Description

The earthworms used for testing were purchased from a local worm grower and placed in a 1.8 m × 1.5 m × 0.3 m wood-frame container with a plywood base. The worm beds were located in a partially shaded greenhouse where temperatures did not exceed 27° C. During summer months, the worm beds were watered daily to prevent drying. The worms were fed horse manure and chicken meal mash.

When worms were needed for testing purposes, they were hand sorted, rinsed in distilled water, and placed on paper towels until any excess water had drained off. About 20 to 40 g (fresh weight) of worms were added to 6 $\times$  of test material contained in a 7.5- $\times$  plastic bucket (Figure 1). During the exposure period (usually 28 days), distilled water was added to the substrates as necessary to maintain optimum moisture. Also, a nylon insect screen material was placed over the drain holes in the base and in the lid to prevent earthworm escape.

Upon completion of the exposure period, the earthworms were sorted from the material, rinsed in distilled water, blotted with paper towels, and weighed before and after a 48-hr purging at 10° C on wet filter paper. Purged

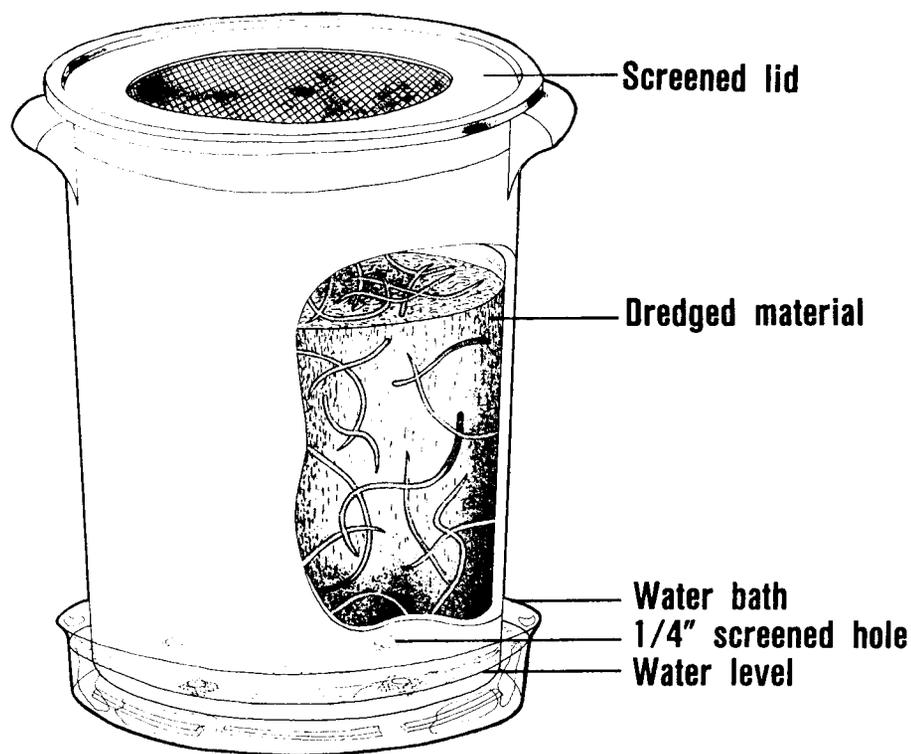


Figure 1. Setup for laboratory experimental chamber

worms were homogenized using a stainless steel Sorvall Omni-Mixer (DuPont Co.) and placed in acid-washed hexane-rinsed glassware. Samples of 5 g fresh weight for heavy metal analysis were oven-dried at 80° C for 24 hr and digested with nitric acid. Metal concentrations were measured using atomic absorption spectrometry. Organic compounds were extracted with hexane from 25-g fresh-tissue samples and measured using gas chromatography/mass spectrometry. Tabulations of test results are given in Appendix A.

### Test Results

Example 1: Highly Contaminated Estuarine Sediment. In this case the earthworm bioassay procedure was used to predict the contaminant mobility for an upland (oxidized) disposal alternative prior to a dredging project. A highly contaminated sediment from the BRH FVP dredging site was collected and transported to the WES for growth-chamber bioassay tests. In order to simulate salt leaching due to rainfall and to enhance earthworm survivability, the sediment was washed until wash water indicated 0 ppt salt. The sediment was air-dried, pulverized, and rewet with distilled water to field capacity before the animals were added. As used here, field capacity is defined as the maximum amount of water that can be held within the pores of a soil after excess water has drained, usually for 24 hr.

Initial screening tests indicated that the BRH sediment was quite toxic to the worms while a similarly prepared reference sediment collected at the mouth of BRH was not. A series of toxicity tests indicated that survival for 7 days could be obtained only if the BRH sediment were diluted. A local woodland soil at the US Army Engineer Waterways Experiment Station (WES) was chosen as the dilution medium. Mixtures of 10 percent BRH sediment and 90 percent WES soil were used for the 7-day test. About 40 g (fresh weight) of earthworms were placed in approximately 1 kg each of the following substrates: 10 percent BRH sediment + 90 percent WES soil; 100 percent BRH reference sediment; and 100 percent WES soil. The tests were conducted in a controlled-temperature growth chamber at 20° C. No supplemental food was provided during the 7-day test period.

Total worm weights recovered from each of the three substrates decreased during the test period (Table A1). Although the worms were not counted, it was apparent that the 56-percent decrease in animal weight recorded in the BRH sediment + WES soil mixture was largely due to the reduction

in numbers of worms. The reduced weights from the other two substrates (16 and 12 percent) appeared to be due to starvation rather than die-off.

Results from the analysis of the earthworm tissue for cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), and mercury (Hg) in relation to substrate levels indicated that bioaccumulation was not demonstrated (Table A2). Some accumulation of Cr, Cu, Ni, and Pb was expected due to the high concentrations of these metals in the substrate material; however, the concentrations of these elements in the worm tissues were quite low. In contrast, Cd was found to be consistently higher in the worm tissue than in the substrate material. This may have been due to a higher-than-desirable background level of Cd (4.55  $\mu\text{g/g}$ ) in the worms prior to the test period or to the potential for the earthworm to accumulate Cd from low levels in the media (Hartenstein et al. 1980). The values reported in the literature for various earthworm species (Table A3) indicated Cd levels in worm tissues that were generally greater than those of the soil.

The earthworm bioassay was successful in determining that the BRH sediment is quite toxic to earthworms under upland conditions. However, there appears to be no indication that Cd or any other toxic heavy metal will accumulate to significant elevated levels in soil-dwelling invertebrates if the material, placed in an upland disposal environment, is diluted with uncontaminated soil. Therefore, the observed toxicity appears not to be solely related to metal concentrations.

Example 2: Restoration of Pyritic Mine Spoil Using Contaminated Dredged Material. In many locations there are large areas of unvegetated mine spoil adjacent to waterways where dredging is necessary and disposal areas must be found. It was proposed that such dredged material could be used for the restoration of abandoned mine spoil areas. The Ottawa, Ill., strip-mine reclamation project was initiated in 1978 as a demonstration of the feasibility of using a cover of dewatered dredged material to reclaim pyritic surface-mine spoil (Perrier et al. 1980). The main objectives of the reclamation were abatement of erosion and acid mine drainage by using dredged material as a medium for vegetation. The dredged material used in the demonstration was found to contain toxic heavy metals (Cd, Cu, Pb, and Ni); consequently, contaminant-mobility monitoring was necessary after vegetation became established (Simmers et al. 1984).

This study was designed to determine the major routes of contaminant mobility using the earthworm as an indicator in computing the bioavailability

of metals in the leaf litter, the surface layer of dredged material (30 cm), and a deep layer of dredged material near the mine spoil (100 cm). Also, these data were needed to clarify the contaminant mobility aspects of the restoration technique in relation to management of large-scale disposal operations.

Comparisons of the earthworm tissue levels of metals from the three test media and the two dominant plants at the site are shown in Table A4. The dominant plants, smooth brome grass *Bromua inermia* and tall fescue *Festuca elatior*, the source of the thick leaf litter (duff) layer on the site, were collected and analyzed. The main source of Cd appeared to be the leaf litter layer while Cu and Ni were apparently more bioavailable in the dredged material. Lead was apparently equally available in all three media.

Although the plants did not show an appreciable uptake of Cd, the earthworms exposed to the leaf litter indicated enhanced Cd availability. This is of critical concern in the management of such sites in the future. Removal of the duff of the grassland ecosystem by fire or mechanical harvesting may be a potential solution to reduce contaminant mobility via soil invertebrates in dredged material disposal sites such as this one. These data clearly show the need to examine all components of an ecosystem in order to fully describe routes of contaminant mobility, and that the evaluation of plant uptake alone may not address the bioavailability of contaminants from the duff or leaf litter.

Example 3: Confined Disposal Site. Between 1972 and 1976, about 720,000 m<sup>3</sup> of dredged material from Buffalo Harbor was placed in an artificial lagoon on the New York shore of Lake Erie at Times Beach. The dredged material was heavily contaminated as a result of the activities of several industries including an oil refinery, steel plants, and an aniline dye chemical plant on the water front adjacent to the dredging site. The disposal operation resulted in the creation of an area composed of an aquatic, a wetland, and an upland environment, and prolific wildlife developed at the disposal site.

This site was selected for investigation because of the recognized ecological value of a continuous sediment/soil interface gradient from a pond with a maximum depth of about 2 m to a woodland at about 2 m above groundwater levels. This situation also provided an unique opportunity to study the interactions between the combination of physical conditions and biotic development on the one hand and contaminant mobility on the other hand.

Figure 2 is a sketch map indicating some of the vegetation present nine years after disposal was terminated and the location of the sampling stations (Marquenie et al. In Press).

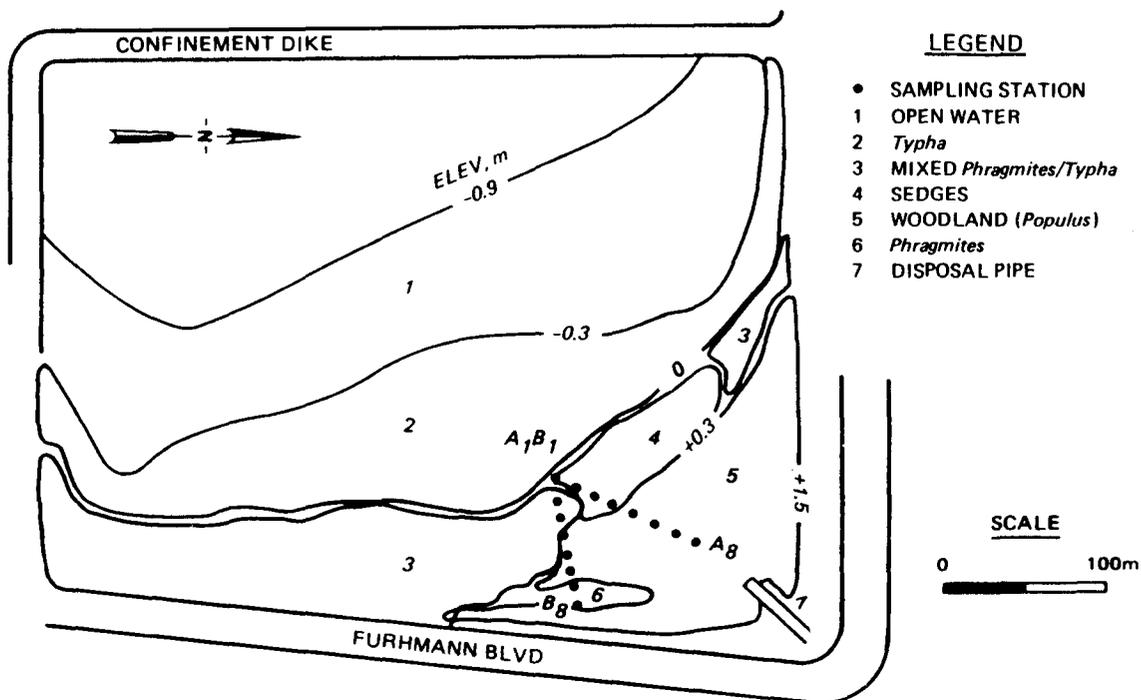


Figure 2. Locations of sampling stations and vegetative types at Times Beach confined disposal site

The results of the bioassay analyses are shown in Table A5. It is evident that the disposal site is relatively contaminated and that there appears to be a variation in the concentration of metals and organic contaminants along the transects. The highest soil concentrations of contaminants generally were found in the A<sub>2</sub>B<sub>2</sub> wetland region but also were high in the A<sub>8</sub> and B<sub>8</sub> upland regions. Exposed earthworms also showed varied values of bioaccumulation in these areas as reflected in tissue concentrations of heavy metals and total PCBs (Table A5) and individual PAHs (Table A6). Transect A was found to most accurately follow the soil/water gradient and, therefore, showed a more distinct pattern of substrate concentration and bioavailability than did Transect B.

### Conclusions

The results of the animal bioassay were found to be useful in the evaluation of the contaminant mobility from dredged material placed in upland disposal facilities and in the prediction of contaminant mobility as part of the

decision-making process prior to selection of a disposal alternative, such as upland disposal or wetland creation. The earthworm *Eisenia foetida* showed a high level of sensitivity as a bioassay animal in its ability to bioaccumulate various heavy metals, PCBs, and PAHs from a variety of contaminated substances. The animal bioassay procedure appears to be a valuable tool for predicting and evaluating the contaminant availability from dredged material of either freshwater or marine origin before or after its upland disposal.

### References

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Table A1  
Weight Changes of Earthworms after 7-day Exposure  
to Various Substrates (BRH)

<u>Substrate</u>	<u>Weight of Worms, g (wet wt)</u>		<u>Weight Loss in 7 Days, %</u>
	<u>Initial</u>	<u>Final*</u>	
10% BRH Sediment + 90% WES Soil	40	17.4 ± 5.4	56
BRH Reference Sediment	40	33.7 ± 1.8	16
WES Soil	40	35.0 ± 1.5	12

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\* Mean of 3 replications ± standard deviation.

Table A2

Contaminant Concentrations in Earthworms after Exposure to Various Substrates (BRH)

Contaminant	Type of Substrate - Contaminant Concentration, $\mu\text{g/g}$ (dry wt)						
	10% BRH Sediment + 90% WES Soil		BRH Reference Sediment		WES Soil		Background Worm Tissue*
	Substrate	Tissue*	Substrate	Tissue*	Substrate	Tissue*	
Cd	2.9	5.83 $\pm$ 0.80	2.9	4.26 $\pm$ 0.55	0.2	3.80 $\pm$ 0.34	4.55 $\pm$ 0.21
Cr	191	4.73 $\pm$ 1.55	314	6.96 $\pm$ 0.28	8.07	2.53 $\pm$ 0.06	1.90 $\pm$ 0.20
Cu	330	34.1 $\pm$ 1.4	438	33.8 $\pm$ 1.3	9.86	12.0 $\pm$ 0.60	11.25 $\pm$ 0.52
Ni	29.6	6.03 $\pm$ 2.97	45	3.07 $\pm$ 0.84	15.0	3.33 $\pm$ 1.18	0.49 $\pm$ 0.46
Pb	63.5	7.6 $\pm$ 0.13	135	5.13 $\pm$ 0.63	10.3	4.7 $\pm$ 0.10	5.23 $\pm$ 0.40
Zn	176	110.5 $\pm$ 2.3	329	112.3 $\pm$ 1.5	29.5	102.6 $\pm$ 0.6	118.5 $\pm$ 2.9
Hg	0.19	<0.050	0.475	<0.050	<0.049	<0.050	<0.050

\* Mean of 3 replications  $\pm$  standard deviation.

Table A3  
Cadmium Levels in Earthworms and Soils Reported in Literature

Reference	Locality	Species	Cadmium Levels μg/g (dry wt)	
			Tissues	Soil
Andersen (1979)	Denmark	<i>Allolobophora longa</i>	5.7 - 11.8	0.14 - 0.99
		<i>A. caliginosa</i>	6.9 - 10.9	0.14 - 0.99
		<i>A. rosea</i>	10.9 - 26.9	0.14 - 0.99
		<i>A. chlorotica</i>	10.9 - 16.2	0.14 - 0.99
		<i>Lumbricus terrestris</i>	8.8 - 16.9	0.14 - 0.99
Czarnowska and Japkiewicz (1978)	Poland	Unidentified	3.5 - 17.0	0.11 - 1.10
Gish and Christensen (1973)	USA	<i>A. species</i> and <i>L. terrestris</i> combined	5.9 - 14.4	0.66 - 1.59
Van Hook (1974)	USA	<i>A. species</i> , <i>L. species</i> and <i>Octolasion</i> species combined	3.1 - 9.3	0.23 - 0.80
Ireland (1979)	Wales	<i>Eiseniella tetraeda</i>	3.0 ± 0.3	4.0 ± 0.2
		<i>Dendrobaena veneta</i>	7.0 ± 1.0	4.0 ± 0.2
		<i>L. rubellus</i>	15.0 ± 5.0	2.0 ± 0.1
		<i>L. rubellus</i>	4.0 ± 0.1	4.0 ± 0.2
		<i>L. rubellus</i>	2.5 ± 3.0	4.0 ± 0.3
Beyer et al. (1982)	USA	<i>Aporrectodea tuberculata</i> , <i>Ap. turgida</i> , <i>Ap. longa</i> , and <i>L. terrestris</i> combined	4.8	0.1

Table A4  
Contaminant Concentrations in Earthworms, Plants, and Substrates  
from Mine Spoil Restoration Site, Ottawa, Ill.

Variable	Material Tested	Concentration, $\mu\text{g/g}$ (dry wt)			
		Cd	Cu	Pb	Ni
Background	Earthworm tissue	3.67 $\pm$ 0.51	9.55 $\pm$ 1.00	1.50 $\pm$ 0.65	2.00 $\pm$ 0.77
	Brome grass plant tissue*	0.78	8.09	6.06	4.47
	Tall fescue plant tissue*	0.73	7.83	27.16	11.00
Bioassay substrate:					
Leaf litter	Earthworm tissue	14.07 $\pm$ 5.37	9.17 $\pm$ 1.56	2.17 $\pm$ 0.46	1.87 $\pm$ 0.46
	Substrate	3.27 $\pm$ 0.73	15.66 $\pm$ 1.65	-	5.89 $\pm$ 0.20
Dredged material surface layer**	Earthworm tissue	9.03 $\pm$ 0.89	25.83 $\pm$ 4.20	2.87 $\pm$ 0.69	5.23 $\pm$ 0.61
	Substrate	10.00 $\pm$ 0.50	127 $\pm$ 8.60	620 $\pm$ 69.9	51.50 $\pm$ 3.20
Dredged material deep layer†	Earthworm tissue	8.23 $\pm$ 0.21	25.37 $\pm$ 1.03	5.27 $\pm$ 2.00	5.33 $\pm$ 0.31
	Substrate	9.18 $\pm$ 1.63	116.7 $\pm$ 10.3	585 $\pm$ 22.8	50.13 $\pm$ 2.16

\* Simmers et al. (1984)

\*\* 30 cm.

† 100 cm.

Table A5  
Contaminant Concentrations in Earthworms after Exposure to  
Various Times Beach Substrates

<u>Transect Location</u>	<u>Component</u>	<u>Concentration, <math>\mu\text{g/g}^*</math></u>					
		<u>Cd</u>	<u>Cu</u>	<u>Hg</u>	<u>As</u>	<u>PCBs (10 isomers)</u>	<u>PAHs (22 compounds)</u>
A8 (upland)	Substrate	2.10	116.0	2.10	25.0	0.462	40.93
	Tissue	8.86	27.7	0.48	21.1	3.950	21.06
A6 (transition)	Substrate	0.76	60.0	1.52	20.0	0.712	7.03
	Tissue	6.54	17.3	0.98	17.5	4.426	1.95
A2 (wetland)	Substrate	2.73	148.0	4.22	38.5	1.004	45.52
	Tissue	11.70	32.1	1.39	24.0	4.520	11.78
B2 (wetland)	Substrate	9.61	334.0	8.50	72.4	0.961	63.96
	Tissue	10.80	57.6	0.81	23.9	6.720	38.88
B6 (transition)	Substrate	5.33	228.0	4.78	58.8	0.743	32.09
	Tissue	17.60	36.2	1.14	35.3	3.620	7.46
B8 (upland)	Substrate	7.74	269.0	7.45	53.0	0.480	35.10
	Tissue	16.0	46.7	1.77	53.8	3.125	8.49
Reference	Substrate	0.39	16.5	0.74	3.40	<0.128	$\leq$ 3.49
	Tissue	3.04	10.1	0.06	8.72	<0.410	$\leq$ 0.77

\* Concentrations in substrates reported as dry weight; concentrations in tissues reported as ash-free dry weight.

Table A6  
Significant PAHs in Worm Tissue and Times Beach Substrates\*

Transect Location	Component	Concentration, $\mu\text{g/g}^{**}$							
		Pyrene	Tri-phenylene	Benzo(e) Pyrene	Benzo(b) Fluoranthene	Benzo(k) Fluoranthene	Benzo(a) Pyrene	Benzo (g,h,i) perylene	Ideno (1,2,3-c,d) Pyrene
A8	Substrate	2.9	$\leq 0.15$	1.8	3.0	1.7	3.8	3.4	3.0
	Tissue	3.9	$\leq 0.015$	1.5	2.1	1.3	2.8	1.5	1.3
A6	Substrate	0.53	$\leq 0.15$	0.32	0.43	0.25	0.56	0.26	0.47
	Tissue	0.14	0.074	0.099	0.15	0.11	0.16	0.19	0.21
A2	Substrate	2.7	$\leq 0.15$	2.1	3.5	1.9	5.2	4.5	3.1
	Tissue	0.53	1.6	0.75	1.3	0.65	1.8	1.1	1.1
B2	Substrate	2.5	$\leq 0.15$	4.0	5.6	2.9	8.6	7.6	6.7
	Tissue	$\leq 0.015$	3.6	4.2	5.4	2.6	7.4	3.2	5.0
B6	Substrate	1.5	$\leq 0.15$	2.1	2.3	1.2	3.6	3.7	2.9
	Tissue	0.093	0.57	0.65	0.54	0.24	0.93	0.93	1.6
B8	Substrate	2.1	$\leq 0.15$	2.1	2.4	1.4	3.6	3.9	3.1
	Tissue	0.15	0.69	1.1	0.67	0.37	1.3	1.5	0.83
Reference	Substrate	$\leq 0.15$	$\leq 0.15$	$\leq 0.2$	$\leq 0.025$	0.032	$\leq 0.02$	0.80	$\leq 1.01$
	Tissue	$\leq 0.15$	0.021	$\leq 0.02$	$\leq 0.002$	0.0047	$\leq 0.002$	0.160	0.029

\* Summary of preliminary data.

\*\* Concentrations in substrates reported as dry weight; concentrations in tissues reported as ash-free weight.