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DIETARY ACCUMULATION OF PCBs FROM A CONTAMINATED SEDIMENT SOURCE BY A DEMERSAL FISH SPECIES (*Leiostomus xanthurus*)

by

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Accumulation and dietary transfer of polychlorinated biphenyls (PCBs) from contaminated harbor sediments were studied in a laboratory food chain consisting of sediments, polychaetes (Nereis virens), and a predatory fish (Leiostomus xanthurus). The study was conducted in two phases to distinguish dietary uptake from PCB accumulation resulting from sediment exposure alone.

In Phase I, fish and polychaetes were separately exposed to field-collected,

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Preface

This study was conducted by the US Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Fla. (ERLGB). Financial sponsorship was primarily from the US Army Engineer District, New York, through the Environmental Laboratory (EL) of the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The WES also supported the work under the Long-Term Effects of Dredging Operations (LEDO) research program, which it is conducting under sponsorship of the Office, Chief of Engineers (OCE), Washington, DC. Broader questions being addressed under LEDO benefited from this specific study and vice versa. The study was conducted under the general supervision of Dr. Al Bourquin, Chief, Processes and Effects Branch, ERLGB. The Director of ERLGB during this study was Dr. Henry Enos. The authors of this report were Messrs. N. I. Rubinstein, W. T. Gilliam, and N. R. Gregory, ERLGB. The authors gratefully acknowledge the assistance of ERLGB personnel: Ms. T. Dunn for technical assistance, Mrs. B. Jackson for editing, and Ms. J. Seites for typing.

The New York District project manager was Mr. J. M. Mansky. Technical monitors of the LEDO program were Dr. John Hall, Operations Division, OCE; Dr. W. L. Klesch, Planning Division, OCE; and Mr. C. W. Hummer, Dredging Division, Water Resources Support Center.

The WES project manager was Dr. R. K. Peddicord, under the general supervision of Dr. C. R. Lee, Chief, Contaminant Mobility and Regulatory Criteria Group; Mr. D. L. Robey, Chief, Ecosystem Research and Simulation Division; and Dr. John Harrison, Chief, EL. The LEDO is managed in EL through the Environmental Effects of Dredging Programs (EEDP), Mr. C. C. Calhoun, Jr., Manager, and Mr. R. L. Lazor, EEDP LEDO Program Coordinator.

Commander and Director of the WES during conduct of the study and preparation of the report was COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

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DIETARY ACCUMULATION OF PCBs FROM A CONTAMINATED SEDIMENT SOURCE BY A DEMERSAL FISH (LEIOSTOMUS XANTHURUS)

Introduction

Background

1. Bioaccumulation of organic contaminants by marine organisms occurs through at least three pathways: direct partitioning from the aqueous phase via the gills; integumental sorption; and diet (Swartz and Lee 1980). Water is the probable medium of exchange for all pathways and it appears that equilibrium partitioning determines the distribution of organic contaminants between the organism and the environment. Of these three routes of uptake, direct partitioning from water across the gills is generally considered to be dominant (Hamelink et al. 1971; Scura and Theilacker 1977; Macek et al. 1979; Ellgehausen et al. 1980). However, for extremely hydrophobic compounds, such as polychlorinated biphenyls (PCBs), a number of recent studies indicate that diet is a major source of body residues at least for a number of fish species (Thomann 1981; Jensen et al. 1982; Pizza and O'Connor 1983; Thomann and Connolly 1984).

2. Because of their hydrophobic nature, PCBs have a strong affinity for particulate material; consequently, in aquatic systems they are commonly associated with bottom sediments, particularly in urbanized and industrialized areas. Previous studies have demonstrated that a variety of marine organisms incuding infaunal species can accumulate PCBs from contaminated sediments (e.g., McLeese et al. 1980; Wyman and O'Connors 1980; Fowler et al. 1978; Courtney and Langston 1978; Rubinstein et al.

1983). Many of these species are important food sources for higher trophic organisms. The relative importance of sediments as a contaminant source for the accumulation and transfer of PCBs within marine food webs remains unclear at this time. However, this question becomes extremely pertinent when evaluating the potential impact of dredged material disposal in aquatic systems.

Objective

3. The objective of this study was to determine the extent to which a contaminated sediment (collected from the field) could serve as a source of PCBs for uptake and dietary transfer in a simplified laboratory food chain consisting of sediments, polychaetes, and a predatory fish. The predator species selected for study was the spot, Leiostomus xanthurus, a commercially important demersal fish which feeds predominantly on polychaetes during its early years (Sheridan 1979). An infaunal polychaete, the sandworm, Nereis virens, was chosen as the prey species. Both of these organisms have been shown to accumulate PCBs from water and sediments (Hansen et al. 1971; McLeese et al. 1980; Rubinstein et al. 1983).

Scope

4. This study was conducted in two phases to distinguish PCB residues originating from the spot's diet from residues resulting from environmental exposure alone (direct partitioning via gills and integument). During Phase I, fish and polychaetes were allowed to establish an "apparent" steady-state concentration. Actual steady-state or equilibrium may not be achieved for PCBs within the time frame of this exposure (40 days), especially for the more highly chlorinated isomers (Shaw

and Connell 1980). In addition, during Phase I the effect of direct contact with sediments on PCB bioaccumulation potential by the spot was examined. In Phase II, we determined the dietary fraction of PCB accumulation by selectively feeding exposed and control groups of fish polychaetes having a known PCB body burden.

Methods and Materials

Organisms

5. This study was conducted at the U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Fla., from November 1982 through January 1983. Sandworms (average ($\bar{x}$) wet weight = 6 g) were purchased from the Maine Bait Co., Newcastle, Maine, and shipped via air freight to Gulf Breeze. Spot ($\bar{x}$ = 17 g) were collected (by seine) from Santa Rosa Sound, Fla. Both species were acclimated to exposure conditions in the laboratory for at least 2 weeks prior to testing.

Sediments

6. Contaminated sediment was collected from Newark Bay, N.J., shipped to Gulf Breeze by refrigerated truck, and maintained at 4°C until initiation of the study. Sediment was sieved (2-mm mesh) to remove large debris and macrofauna, thoroughly mixed to ensure uniformity, and analyzed for particle size, percentage moisture, and percentage organics (EPA/CE 1981). PCB concentrations (as Aroclor 1242 and 1254 µg/g dry weight) in sediments were measured at the beginning and end of the study.

Phase I: Sediment Exposure

7. Fish and polychaetes were separately exposed for 40 days to contaminated and control sediments (washed beach sand) in 100-ℓ glass aquaria (86 x 50 x 25 cm) receiving flowing seawater. Seawater was pumped from Santa Rosa Sound, Fla., filtered to 20 μm, and delivered to a headbox in the laboratory; temperature was maintained at 20°C ± 2°C. Water flowed by gravity from the headbox to a constant head trough where siphons delivered seawater at 30 ℓ/hr to aquaria. During the study, salinity ranged from 20 to 30 °/oo and dissolved oxygen (measured weekly using a YSI Model 57 DO meter) never fell below 5.0 mg/ℓ. Aquaria were set up and designated as follows (Figure 1):

a. Tank 1 (Exposed Worms) - A 4-cm layer (17ℓ) of contaminated sediment and 200 sandworms.

b. Tank 2 (Exposed Fish) - A 4-cm layer of contaminated sediment and 35 spot.

c. Tank 3 (Isolated Fish) - A 4-cm layer of contaminated sediment and 20 spot separated from the sediment by a nitex® screen (1-mm mesh) placed 3 cm above the substrate to isolate fish from direct contact with the sediment.

d. Tank 4 (Control Fish) - A 4-cm layer of control sediment and 40 spot.

e. Tank 5 (Control Worms) - A 4-cm layer of control sediment and 200 sandworms.

A sediment trap was placed in the effluent line of tank 2 to collect sediment resuspended by the swimming activity of the fish. This material was periodically returned to tank 2. During Phase I, fish and polychaetes were fed a maintenance diet of flake food (Tetra SM80, Tetra Werke, West Germany) at approximately 2 percent of body weight per day.

8. Prior to initiation of the sediment exposure (Phase I), three fish and three polychaetes were collected from holding aquaria and analyzed (whole body) for background concentrations of PCBs. Fish (N = 3) collected from tank 2 (sediment exposed) were analyzed for PCBs following 10 and 40 days of exposure to test sediments; fish from Tank 3 (sediment isolated) and Tank 4 (control) were analyzed on day 10 and 35. Polychaetes (N = 3) from Tank 1 (sediment exposed) and Tank 5 (control) were analyzed for PCBs on days 10, 20, and 35. Fish and polychaetes were placed in uncontaminated flowing seawater for 24 hr prior to tissue analysis to evacuate their intestinal tracts.

Phase II: Sediment and Dietary Exposure

9. Following exposure for 40 days to sediment, fish and sediment from tank 2 were equally divided into two aquaria so that exposure conditions were maintained identical to Phase I. Control fish (Tank 4) were divided similarly. During the last 2 weeks of Phase I, the diet of the spot was gradually adjusted to include increasing portions of uncontaminated (control) sandworms from tank 5. At the end of this acclimation period, fish were feeding voraciously on polychaetes. For Phase II, aquaria were redesignated as follows (Figure 1):

a. Tank A - Contaminated sediment and 13 spot (from tank 2) fed a daily ration of contaminated sandworms (from tank 1).

b. Tank B - Contaminated sediment and 13 spot (from tank 2) fed a daily ration of uncontaminated sandworms (from tank 5).

c. Tank C - Control sediment and 15 spot (from tank 4) fed a daily ration of contaminated sandworms (from tank 1).

d. Tank D - Control sediment and 15 spot (from tank 4) fed a daily ration of uncontaminated sandworms (from tank 5).

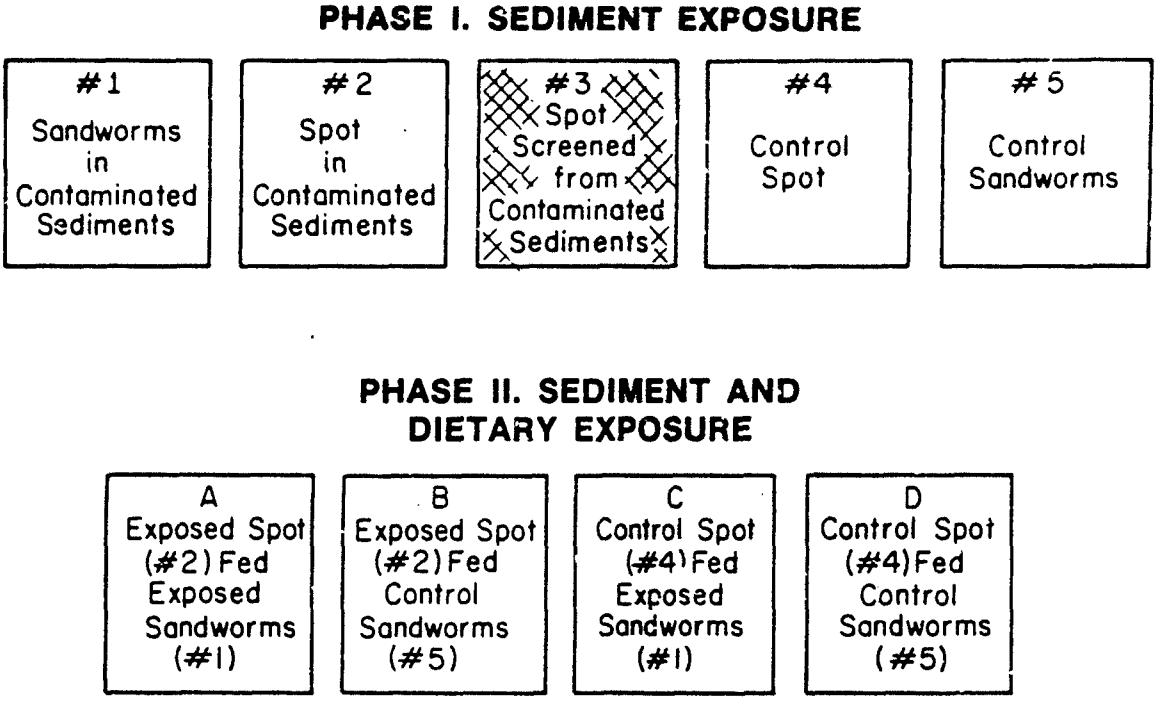

Figure 1. Exposure design for spot and sandworms in 100-ℓ aquaria during Phase I and Phase II

10. Daily food rations for all fish during Phase II were estimated at 10 percent of body weight. Sandworms were collected daily from aquaria and cut into pieces small enough for ingestion by spot. Daily samples of contaminated and control sandworms used as food were composited into weekly samples, homogenized, and analyzed for PCBs in triplicate. Sandworms used as food were not purged.

11. Fish in all aquaria consumed their respective food ration very quickly. Excess food was never observed in aquaria following a feeding event. Five fish were sampled for chemical analysis from each aquaria after 10 and 20 days of feeding. Fish were placed in flowing uncontaminated seawater and not fed for 24 hr to evacuate the digestive tract prior to PCB analysis.

Chemical Analysis

Tissues

12. Whole fish and polychaetes weighing from 3 to 15 g were cut into small pieces and slurried with an equal weight of distilled water using a polytron (Brinkman, Model PCU-2 with a PT-10 generator). Subsamples (maximum slurry weight of 16 g) were homogenized with aliquots of 10.5 and 5 ml of acetonitrile. After each homogenization, the samples were centrifuged and the supernatant was decanted. Acetonitrile extracts (20 ml) were combined with 75 ml of 2 percent aqueous Na_2SO_4 and extracted three times with 10 ml hexane. The hexane extracts were reduced to 1 to 2 ml by gentle warming under a stream of dry nitrogen. The concentrates were then transferred to a Florisil column for cleanup.

Sediments

13. Sediments were slowly air dried at room temperature to 3 to 5 percent moisture content and then ground to a fine powder using a high speed blade mill. Subsamples of up to 4 g were then extracted by the Soxhlet method of Bellar et al. (1980). Extracts were reduced to a volume of 1 to 2 ml for Florisil cleanup.

Cleanup

14. A 9-mm (outside diameter) column was packed with 4 g of activated Florisil and topped with 25 mm of anhydrous Na_2SO_4. The column was preeluted with 10 ml hexane (not collected) and the 1- to 2-ml samples immediately were introduced and eluted with several washes of hexane (total of 10 ml). This was followed by additional elutions with 10 ml hexane and 10 ml 1 percent diethylether in hexane. Elutriates originating from sediment samples were reduced to 5 ml and tumbled with 0.1 to 0.3 ml metallic Hg for 1 hr to remove sulfur interferences. All samples were then reduced to a final volume of 1 ml for analysis by gas chromatography.

15. Analysis was performed on a Hewlett-Packard 5710 gas chromatograph with an electron-capture detector operated at 300°C and a 1.8-m glass column (4 mm inside diameter x 6 mm outside diameter) packed with 3 percent OV-101 on 80/100 mesh-Supelcoport maintained at 200°C for Aroclor 1242 and at 220°C for Aroclor 1254. The carrier gas was 10 percent methane in argon at a flowrate of 60 mℓ/min.

16. PCB quantification was done by the method of Webb and McCall (1973). The reference standard, obtained from the U.S. Environmental Protection Agency, Analytical Standards Branch, Cincinnati, Ohio, was described by Sawyer (1978). Only Aroclor 1242 and 1254 isomers were quantified. Recoveries from spiked samples averaged 86 percent. Concentrations reported were not corrected for percentage recovery. Instrument detection limits for sediments (dry weight) and tissues (wet weight) were 5 ng PCB/g.

Results and Discussion

17. Test sediment contained 21.8 percent total organic carbon and 70 percent moisture. Particle-size distribution was 0 percent sand, 88 percent silt, and 12 percent clay. A net loss of PCBs in sediment was observed during the exposure period. Initial sediment concentrations which averaged 5.68 ± 0.51 µg/g (N = 5, dry weight) dropped to 4.13 ± 0.51 µg/g (N = 5) at the termination of the study. Test sediment was not acutely toxic to fish or polychaetes. No mortality was observed in spot and very few polychaetes died (< 2 percent) during the test period.

Phase I

18. Phase I was designed to expose fish to environmentally realistic concentrations of PCBs prior to dietary exposure and to provide a PCB-contaminated food source. Whole body concentrations of PCBs in spot and sandworms exposed to contaminated sediments reached an apparent equilibrium concentration during Phase I (Figure 2). In previous studies conducted with these species, steady-state concentrations of PCBs were attained within 40 days of exposure (Hansen et al. 1970; McLeese et al. 1980; Rubinstein et al. 1983). Significant differences ($p < 0.05$, ANOVA, Duncans Multiple Range Test; SAS 1982) in PCB residues between control and exposed treatments were detected in Phase I for both fish and polychaetes (Tables 1, 2). At the end of Phase I, PCB body burdens averaged 0.31 µg/g (wet weight) for fish and 0.21 µg/g (wet weight) for polychaetes (Table 2).

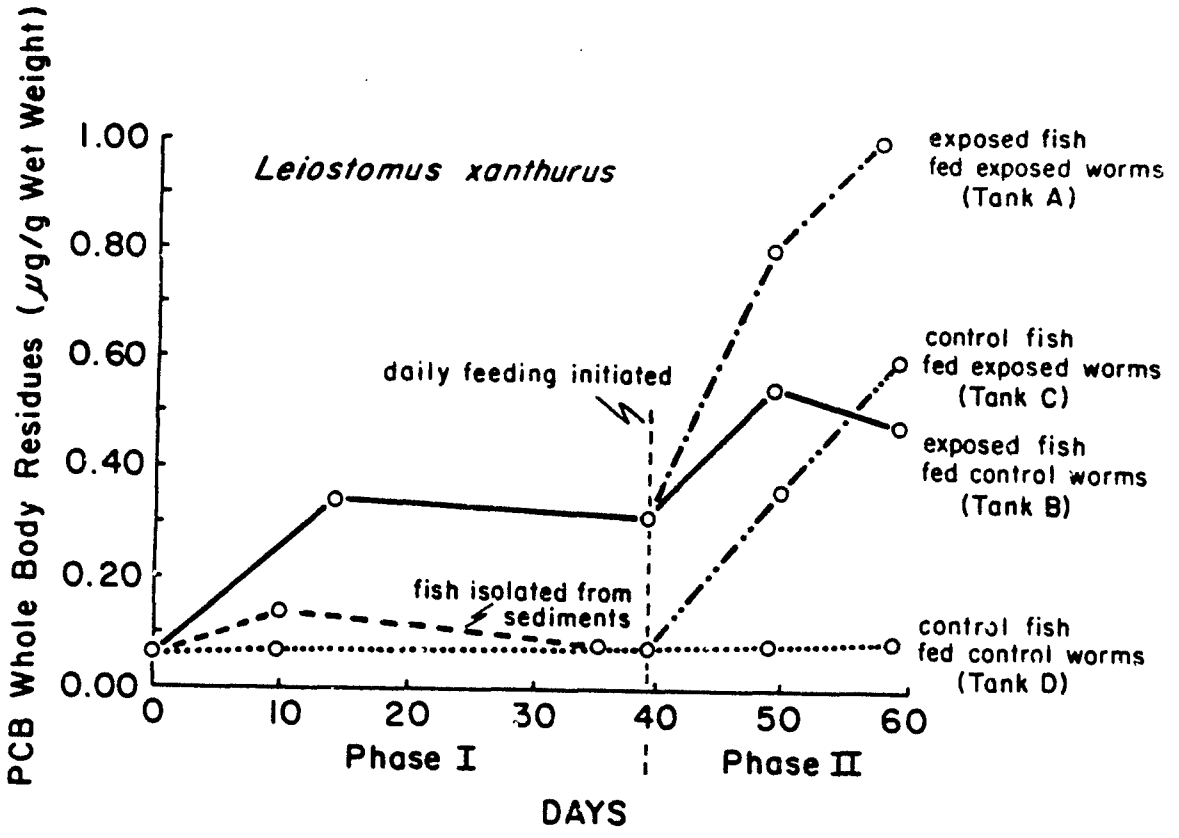

Figure 2. Average PCB whole body residues (µg/g wet weight) in spot during Phase I (N = 3) and Phase II (N = 5)

Table 1

Analysis of Variance of PCB Body-Burden Measurements

Test	Source	Degrees of Freedom	Sum of Squares	F-value	Pr > F
Phase I - Spot	Treatment	2	0.2021	108.69	0.001
	Time	1	0.0082	8.87	0.0107
	Error	13	0.0120		
Phase I - Sandworms	Treatment	1	0.0699	41.02	0.002
	Time	1	0.000004	0	0.9621
	Error	8	0.0136		
Phase II - Spot	Treatment	3	3.0938	39.41	0.0001
	Time	1	0.1198	4.58	0.0398
	Error	33	0.8635		

Table 2

Phase I - PCB Whole Body Residues (µg/g wet wt.) in Spot and Sandworms

Day 0 Background		Tank #	Sample Interval	
Phase I - PCB whole body residues (µg/g wet wt.) in spot				
0.07		2 (Sediment Exposed)	Day 14	Day 40
0.10			0.38	0.29
0.07			0.38	0.29
$\bar{x}$	0.08		0.31	0.35
SD	0.02			
		$\bar{x}$	0.36	0.31
		SD	0.04	0.04
		3 (Sediment isolated)	Day 10	Day 35
			0.19	0.06
			0.15	0.11
			0.09	0.08
		$\bar{x}$	0.14	0.08
		SD	0.05	0.02
		4 (Control)	0.12	0.07
			0.10	0.06
			*	0.07
		$\bar{x}$	0.11	0.07
		SD	0.01	0.01

Phase I - PCB whole body residues (µg/g wet wt.) in sandworms					
0.01		1 (Sediment exposed)	Day 10	Day 20	Day 35
0.01			0.17	0.28	0.21
0.01			0.23	0.21	0.22
0.01			*	0.13	0.20
$\bar{x}$	0.01	$\bar{x}$	0.20	0.20	0.21
SD	0.00	SD	0.03	0.08	0.01
		5 (Control)	0.04	0.01	0.02

*Sample lost

19. Fish isolated from direct contact with sediment (tank 3) contained significantly less PCB ($p < 0.05$, ANOVA, Duncans Multiple Range Test; SAS 1982) than fish in contact with sediment (tank 2). Average PCB concentrations in fish isolated from test sediment for 35 days were statistically indistinguishable from control fish (Figure 2). Halter and Johnson (1977) showed that a freshwater fish (fathead minnow, Pimphales promelas) in direct contact with contaminated sediment accumulated PCB residues at six times the rate of fish screened from direct contact with sediments. This is particularly interesting because a method now being considered to diminish the impact of contaminated dredged material (sediment) disposal in the marine environment involves "capping" (covering) contaminated material with a layer of clean (uncontaminated) sediment (O'Connor 1983). The data reported herein support the contention that physical isolation of contaminated sediment can effectively reduce the availability of PCBs for bioaccumulation by water column organisms. However, it is important to note that due to the use of a flow-through seawater design, the PCB distribution in the exposure system does not reflect PCB partition equilibrium between sediment and overlying water. Although this may obfuscate the ultimate contribution of water mediated uptake observed, we feel that: (a) flow-through conditions are more simulative of open ocean disposal sites where mixing and water movement over the bottom are substantial; (b) static conditions are unacceptable for bioaccumulation studies in that secondary uptake (resulting from depuration) cannot be readily quantified; and (c) flow-through conditions are preferable to meet the life support requirements of test organisms in contact with anaerobic sediments for extended periods of time.

Phase II

20. During Phase II significant differences ($p < 0.05$) in PCB whole body residues in spot were detected (ANOVA, Duncans Multiple Range Test; SAS 1982) between contaminated and control feeding regimes (Table 3).

Table 3

Phase II - PCB Whole Body Residues (µg/g wet wt.) in Spot

Days of Exposure	Days of Feeding		Tank A	Tank B	Tank C	Tank D
50	10		0.57	0.62	0.33	0.08
			0.60	0.37	0.34	0.11
			1.04	0.60	0.33	0.02
			0.88	0.58	0.29	0.10
			0.89	0.62	0.35	*
		$\bar{x}$	0.80	0.56	0.33	0.08
		SD	0.20	0.11	0.02	0.04
60	20		0.75	0.56	0.33	0.08
			0.96	0.33	0.57	0.10
			0.91	0.64	0.64	0.11
			1.54	0.56	0.58	0.10
			*	0.52	0.55	0.11
		$\bar{x}$	1.04	0.48	0.60	0.10
		SD	0.35	0.13	0.06	0.02

*Sample Lost

Fish exposed to contaminated sediments and fed a daily diet of polychaetes from the same sediments for 20 days accumulated more than twice the PCB residues than sediment-exposed fish fed control polychaetes (Figure 2). Average (N = 5) PCB body burdens on day 60 (20 days of feeding) for fish in tank A were 1.04 ± 0.35 µg/g while fish in tank B (environmental exposure only) measured 0.48 ± 0.13 µg/g. Sandworms exposed to contaminated

sediments provided the only source of PCBs for control fish during Phase II. Average PCB whole body residues measured in unpurged sandworms used as food during Phase II was 0.49 µg/g wet weight (SD = 0.09, N = 8) for sediment exposed, and 0.01 µg/g (SD = 0.004, N = 8) for control treatments (Table 4). Average PCB whole body residues in control fish maintained on a diet of contaminated polychaetes for 20 days (tank C) measured 0.60 µg/g wet weight, while control fish fed control polychaetes during the same period contained 0.01 µg/g wet weight (Table 4).

Table 4

PCB Concentrations (µg/g wet wt.) in Weekly Food Composites

(Sandworms, gut unpurged)

Treatment		Week 1	Week 2	Week 3
Exposed		0.45	0.58	0.50
		0.46	0.59	0.34
		0.47	*	0.43
	$\bar{x}$	0.46	0.59	0.43
	SD	0.01	0.01	0.08
Control		0.02	0.02	0.01
		0.01	0.02	0.01
		0.01	0.02	*
	$\bar{x}$	0.01	0.02	0.01
	SD	0.00	0.00	0.00

*Sample lost

21. On day 60 the PCB dietary contribution to whole body residues in fish was still increasing (Figure 2) and rates of uptake were similar between exposed fish (0.030 µg PCB/g per day) and control fish (0.025 µg PCB/g per day) fed contaminated polychaetes. A comparison of the regressions for PCB whole body residue vs. time for these two treatments (Tank A and Tank C) showed no significant difference ($\alpha = 0.05$) in the slopes of the lines. Fish in all treatments grew during this study period. Increases in wet weight of individual fish during phase II averaged 2.83 ± 1.28 g (N = 16) for all treatments.

Conclusions

22. These results demonstrate that contaminated harbor sediments can serve as a source of PCBs for accumulation and dietary transfer by sandworms and spot. Following 20 days of feeding the dietary contribution of PCBs accounted for 53% of the total body residue measured in spot, and this percentage appeared to be increasing (Figure 2). This observation is in agreement with previous findings by Thomann (1981), Jensen et al. (1982), and Pizza and O'Connor (1983), who identified diet as the major source of PCBs for a variety of predatory fish species. Although the relative contribution of direct partitioning across the gills is extremely high for organic compounds and produces very large bioconcentration factors, one must consider the ultimate distribution of hydrophobic compounds in the marine environment. These compounds (of which PCBs serve as an excellent model) have very low solubilities and very high partition coefficients. Consequently, little of the compound is available

for aqueous uptake compared to the amount of compound which is associated with particulate organic material that can serve as a potential food source for infaunal and epibenthic food webs.

23. Current regulations dealing with conditions for the release of contaminated material in the marine environment (i.e., Ocean Dumping Act, Public Law 92-532) utilize laboratory bioaccumulation tests as part of the permit evaluation process. Bioaccumulation testing of representative marine organisms provides a direct measure of bioavailability of sediment-associated contaminants. Results from this study support the utility of this approach by demonstrating a direct relationship between residue concentration in infauna and the potential for dietary transfer to a commercially important fish species.

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