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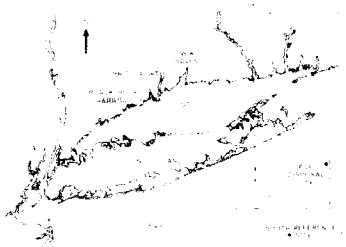

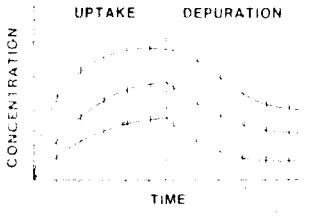

US Army Corps of Engineers

FIELD VERIFICATION PROGRAM
(AQUATIC DISPOSAL)

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BIOACCUMULATION OF CONTAMINANTS FROM BLACK ROCK HARBOR DREDGED MATERIAL BY MUSSELS AND POLYCHAETES

by

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Mussels (Mytilus edulis) and worms (Nereis virens) were exposed in laboratory studies to dredged material from Black Rock Harbor (BRH), Connecticut, to examine the bioaccumulation of organic and inorganic contaminants. Mussels were exposed in a dosing system designed to maintain a constant concentration of suspended particulates and food (algae) in seawater. Control mussels received only food (algae). Monitoring of concentrations

(Continued)

20. ABSTRACT (Continued).

of organic and inorganic contaminants showed that the system maintained constant concentrations during exposure. Exposed mussels accumulated organic compounds and some inorganic elements, reaching steady-state values between the first and second weeks of exposure. During the 28-day exposure period, mussels showed increases in concentration of two to three orders of magnitude for organic contaminants, but those metals accumulated showed increases of less than a factor of 12.

In general, the depuration of organic contaminants was rapid during the first week of depuration, and the depuration rate was inversely related to the compound's n-octanol/water partition coefficient. After the first week depuration rates decreased, and concentrations of most organic compounds remained above control values to the end of the 5-week depuration period. Iron and chromium depurated to control levels within a 2-week period.

The polychaete worm N. virens was exposed to BRH bedded sediment in glass aquaria maintained under flowing seawater. Other worms were maintained in reference sediments. Worms exposed for 28 days accumulated polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) to concentrations one to three orders of magnitude above those found in the reference organisms. Of the metals determined, only Cr and Cu were found to accumulate to concentrations higher than those in the reference worms. Concentrations of PCBs exposed to BRH sediment did not decrease during a 28-day depuration period in reference sediments, but depuration of PAHs was apparent. Chromium and copper depurated to control levels after 2 weeks.

Bioaccumulation factors for PCBs calculated for mussels and worms, when total exposure concentrations were normalized to a gram dry weight sediment basis, were generally within a factor of 1.5. This suggests that modeling bioaccumulation of some organic compounds as a partitioning of contaminants between sediments and organisms may have promise as a generalized predictive technique.

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Bioaccumulation of Contaminants from Black Rock Harbor Dredged Material by Mussels and Polychaetes"

TO: All Report Recipients

1. This is one in a series of scientific reports documenting the findings of studies conducted under the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives (referred to as the Field Verification Program or FVP). This program is a comprehensive evaluation of environmental effects of dredged material disposal under conditions of upland and aquatic disposal and wetland creation.

2. The FVP originated out of the mutual need of both the Corps of Engineers (Corps) and the Environmental Protection Agency (EPA) to continually improve the technical basis for carrying out their shared regulatory missions. The program is an expansion of studies proposed by EPA to the US Army Engineer Division, New England (NED), in support of its regulatory and dredging missions related to dredged material disposal into Long Island Sound. Discussions among the Corps' Waterways Experiment Station (WES), NED, and the EPA Environmental Research Laboratory (ERLN) in Narragansett, RI, made it clear that a dredging project at Black Rock Harbor in Bridgeport, CT, presented a unique opportunity for simultaneous evaluation of aquatic disposal, upland disposal, and wetland creation using the same dredged material. Evaluations were to be based on technology existing within the two agencies or developed during the six-year life of the program.

3. The program is generic in nature and will provide techniques and interpretive approaches applicable to evaluation of many dredging and disposal operations. Consequently, while the studies will provide detailed site-specific information on disposal of material dredged from Black Rock Harbor, they will also have great national significance for the Corps and EPA.

4. The FVP is designed to meet both Agencies' needs to document the effects of disposal under various conditions, provide verification of the predictive accuracy of evaluative techniques now in use, and provide a basis for determining the degree to which biological response is correlated with bioaccumulation of key contaminants in the species under study. The latter is an important aid in interpreting potential biological consequences of bioaccumulation. The program also meets EPA mission needs by providing an opportunity to document the application of a generic predictive hazard-assessment research strategy applicable to all wastes disposed in the aquatic environment. Therefore, the ERLN initiated exposure-assessment studies at the aquatic disposal site. The Corps-sponsored studies on environmental consequences of aquatic disposal will provide the effects assessment necessary to complement the EPA-sponsored exposure assessment, thereby allowing ERLN to develop and apply a hazard-assessment strategy. While not part of the Corps-funded FVP, the EPA exposure assessment studies will complement the Corps' work, and together the Corps and the EPA studies will satisfy the needs of both agencies.

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Bioaccumulation of Contaminants from Black Rock Harbor Dredged Material by Mussels and Polychaetes"

5. In recognition of the potential national significance, the Office, Chief of Engineers, approved and funded the studies in January 1982. The work is managed through the Environmental Laboratory's Environmental Effects of Dredging Programs at WES. Studies of the effects of upland disposal and wetland creation are being conducted by WES and studies of aquatic disposal are being carried out by the ERLN, applying techniques worked out at the laboratory for evaluating sublethal effects of contaminants on aquatic organisms. These studies are funded by the Corps while salary, support facilities, etc., are provided by EPA. The EPA funding to support the exposure-assessment studies followed in 1983; the exposure-assessment studies are managed and conducted by ERLN.

6. The Corps and EPA are pleased at the opportunity to conduct cooperative research and believe that the value in practical implementation and improvement of environmental regulations of dredged material disposal will be considerable. The studies conducted under this program are scientific in nature and will be published in the scientific literature as appropriate and in a series of Corps technical reports. The EPA will publish findings of the exposure-assessment studies in the scientific literature and in EPA report series. The FVP will provide the scientific basis upon which regulatory recommendations will be made and upon which changes in regulatory implementation, and perhaps regulations themselves, will be based. However, the documents produced by the program do not in themselves constitute regulatory guidance from either agency. Regulatory guidance will be provided under separate authority after appropriate technical and administrative assessment of the overall findings of the entire program.

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Director, Research and Development
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Assistant Administrator for
Research and Development
U. S. Environmental Protection
Agency

PREFACE

This report describes work performed by the U.S. Environmental Protection Agency (EPA), Environmental Research Laboratory, Narragansett, R.I. (ERLN), as part of the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives Program or the Field Verification Program (FVP). The FVP, sponsored by the Office, Chief of Engineers (OCE), is assigned to the Environmental Laboratory (EL), U. S. Army Engineer Waterways Experiment Station (WES), and is managed under the Environmental Effects of Dredging Programs (EEDP). The OCE Technical Monitors for FVP were Dr. John R. Hall and Dr. William L. Klesch.

The objective of the FVP is to verify existing predictive techniques for evaluating the environmental consequence of dredged material disposal under aquatic, wetland, and upland conditions. The aquatic portion of this study is being conducted by ERLN, with the wetland and upland portions conducted by WES.

The principal ERLN investigators for this aquatic study were Drs. James Lake and Gerald Hoffman, Analytical Chemists, and Mr. Steven Schimmel, Aquatic Toxicologist. Laboratory exposure system design was coordinated by Mr. Jay Sinnett and assisted by Ms. Dianne Black, Dr. Wayne Davis, and Mr. John Sewall. Organic chemical sample preparation and analyses were conducted under the supervision of Drs. Lake and Rogerson, and assisted by Mr. Curt Norwood, Ms. Sharon Pavignano, Mr. Robert Bowen, Ms. Adria Elskus, and Mr. Lawrence LeBlanc. Inorganic chemical preparation and analyses were conducted under the supervision of Dr. Gerald Hoffman, and assisted by Mr. Frank Osterman, Mr. Warren Boothman, and Mr. Dennis Migneault. Data management and data analysis were conducted by Mr. Jerfrey Rosen and Dr. James Heltshe, respectively.

The EPA Technical Director for the FVP was Dr. John H. Gentile; the

Technical Coordinator was Mr. Walter Galloway, and the Project Manager was Mr. Allan Beck.

The study was conducted under the direct WES supervision of Dr. Richard K. Peddicord and Dr. Thomas Dillon and under the general supervision of Dr. C. Richard Lee, Chief, Contaminant Mobility and Criteria Group; Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division; Dr. John Harrison, Chief, EL. The EEDP Coordinator was Mr. Robert L. Lazor. The EEDP Manager was Mr. Charles C. Calhoun.

Commanders and Directors of WES during preparation of the report were COL Tilford C. Creel, CE, and COL Robert C. Lee, CE. Technical Director was Mr. F. R. Brown.

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BIOACCUMULATION OF CONTAMINANTS FROM BLACK ROCK HARBOR DREDGED MATERIAL BY MUSSELS AND POLYCHAETES

PART I: INTRODUCTION

Background

1. The U.S. Army Corps of Engineers (CE) and the U.S. Environmental Protection Agency (EPA) are jointly conducting a comprehensive Field Verification Program (FVP) to evaluate the potential environmental impact associated with various disposal options for dredged material. The approach being used in the FVP is to evaluate and field validate assessment methodologies for predicting the environmental impacts of dredged material disposal in aquatic, upland, and wetland environments. The research, evaluation, and field verification of the upland and wetland disposal options are being conducted by the Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The application and field verification of predictive methodologies for the aquatic disposal option will be conducted by the EPA Environmental Research Laboratory (ERLN), Narragansett, R.I.

Purpose and Scope

2. The aquatic disposal option of the FVP is to be used as a site-specific case study for evaluating a hazard assessment research strategy. Hazard assessment in terms of this study is a process by which data on exposure and effects are assembled and interpreted to determine the potential for harm to the aquatic environment that could result from the ocean disposal of a particular material. To measure hazard, information on the duration and intensity of exposure (exposure assessment) of organisms to concentrations of materials disposed at the site (predicted environmental

concentration) is coupled with concentrations of the material determined from laboratory toxicity studies (effects assessment) on individual species, populations, and communities. When properly synthesized, these data provide an estimate of the probability of unacceptable adverse impact on the aquatic environment as a result of the disposal of the material. The verification of hazard assessment is comprised of two components: (a) documentation and comparison of the accuracy and precision of an individual method or protocol in the lab and field, and (b) verification of the prediction of potential impact to the aquatic environment. Within this context, hazard assessment contains parallel predictive laboratory and field verification components. The achievement of the goal of hazard assessment requires the development and verification of assessment protocols for defining exposure and effects.

3. The second research component in the aquatic portion of the FVP is an assessment of the bioaccumulation potential of available contaminants within the dredged material by the blue mussel (Mytilus edulis) and the polychaete worm Nereis virens. The focus of this study is twofold: (a) determine the qualitative and quantitative aspects of the bioavailable contaminants within BRH dredged material which are accumulated by the mussel and the worm; and (b) examine the uptake and depuration kinetics of the major contaminants within the material that constitute a potential threat to man and the ecosystem. Results of this study will contribute to the overall FVP by providing a predictive tool for predicting residues of key contaminants in the fauna at the disposal site. The accuracy of these predictive tools will be verified in the field and reported in a future report.

4. Chemicals of major environmental concern have three basic characteristics: (a) they may be acutely or chronically toxic at low concentrations; (b) they may bioaccumulate to concentrations in tissues that cause adverse effects in the species contaminated with the chemical, or otherwise make the species unsuitable for human consumption; and (c) they may depurate slowly, causing a prolonged (chronic) adverse effect or render the resource unsuitable for prolonged periods. The latter two concerns are addressed in this report. The study of uptake and depuration rates of the major bioavailable compounds and elements by the organisms allows predictions to be made of the rate and extent of chemical uptake and the time needed to depurate accumulated compounds to an acceptable concentration.

5. Appendices A and B contain organic and inorganic chemistry data, respectively. Because of the extent of the accumulated data, they were reproduced on microfiche and are enclosed in an envelope attached to the inside back cover of this report.

PART II: MATERIALS AND METHODS

Sediment Collection and Preservation

Reference Sediment

6. Reference sediment (REF) for the FVP studies was collected from the South reference site (41° 7.95'N and 72° 52.7'W), which is approximately 700 m south of the southernmost perimeter of the central Long Island Sound disposal site (Figure 1). Reference sediment was collected with a Smith-MacIntyre grab sampler (0.1 m^2) in both August and December 1982. Sediment collected on each date was returned to the laboratory, press sieved (wet) within 48 hr through a 2-mm mesh stainless steel screen, homogenized, and stored at 4° C until used for experimental purposes. Sediment was re-homogenized prior to use.

Black Rock Harbor Sediment

7. The source of the dredged material for the FVP was Black Rock Harbor (BRH), located in Bridgeport, Connecticut (Figure 2), with approximate coordinates of 73° 13'W and 41° 9'N. The study reach begins 400 m south of the fork in Cedar Creek and extends seaward for approximately 1700 m. Black Rock Harbor bottom sediments were collected at 25 locations within the study area using a 0.1-m^2 gravity box corer to a depth of 1.21 m and placed in 210-L barrels and transported in a refrigerated truck (at 4° C) to WES. The contents of the 25 barrels were emptied into a nitrogen-purged cement mixer and homogenized. The homogenized sediment was then redistributed to the 25 barrels and aliquots were taken from each for sediment chemistry analysis. Twelve barrels were kept at WES and thirteen barrels were transported to ERLN in a refrigerated truck and stored at 4° C. Prior to use the contents of each

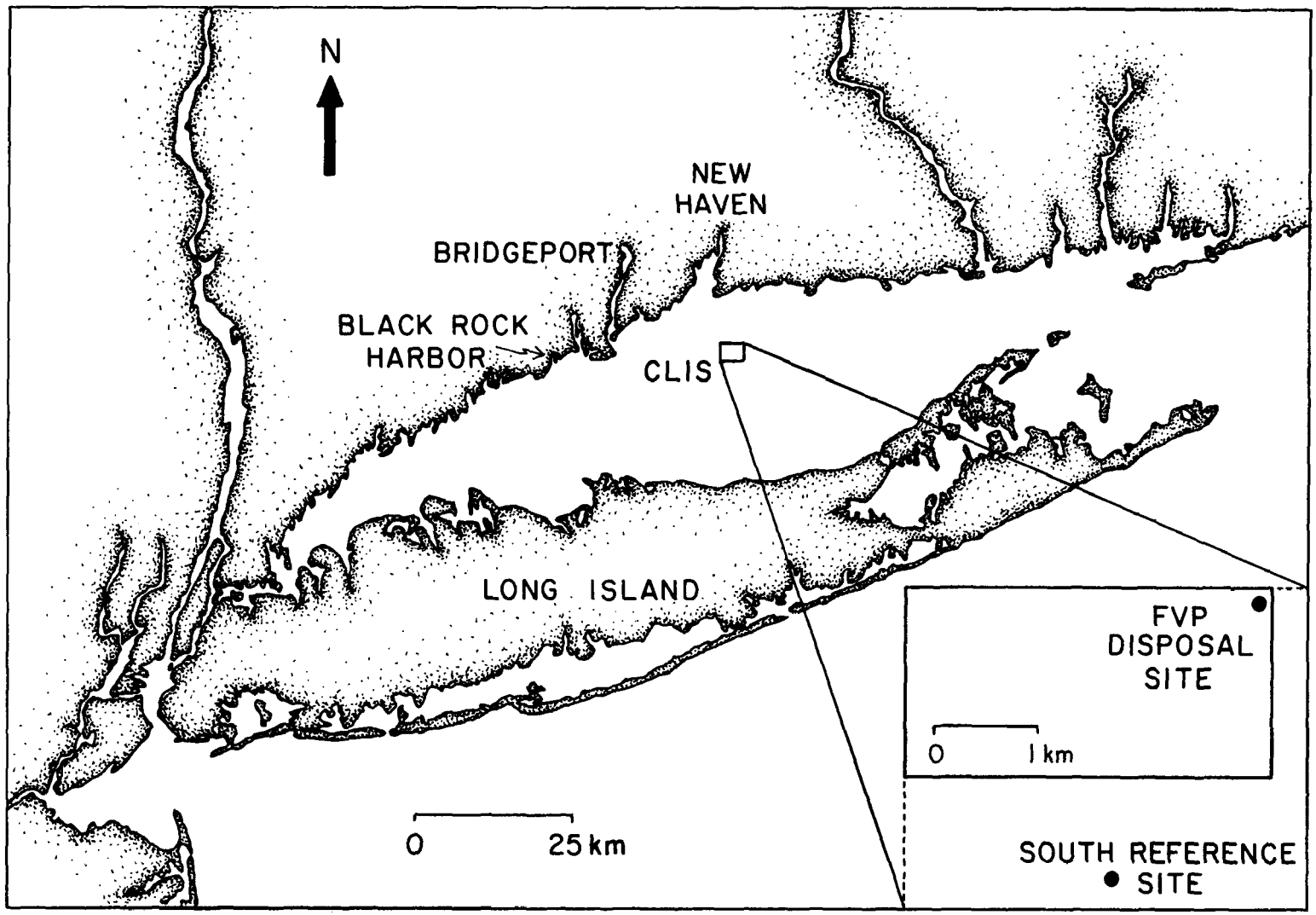

Figure 1. Central Long Island Sound disposal site and south reference site (41°7.95"N and 72°52.7"W)

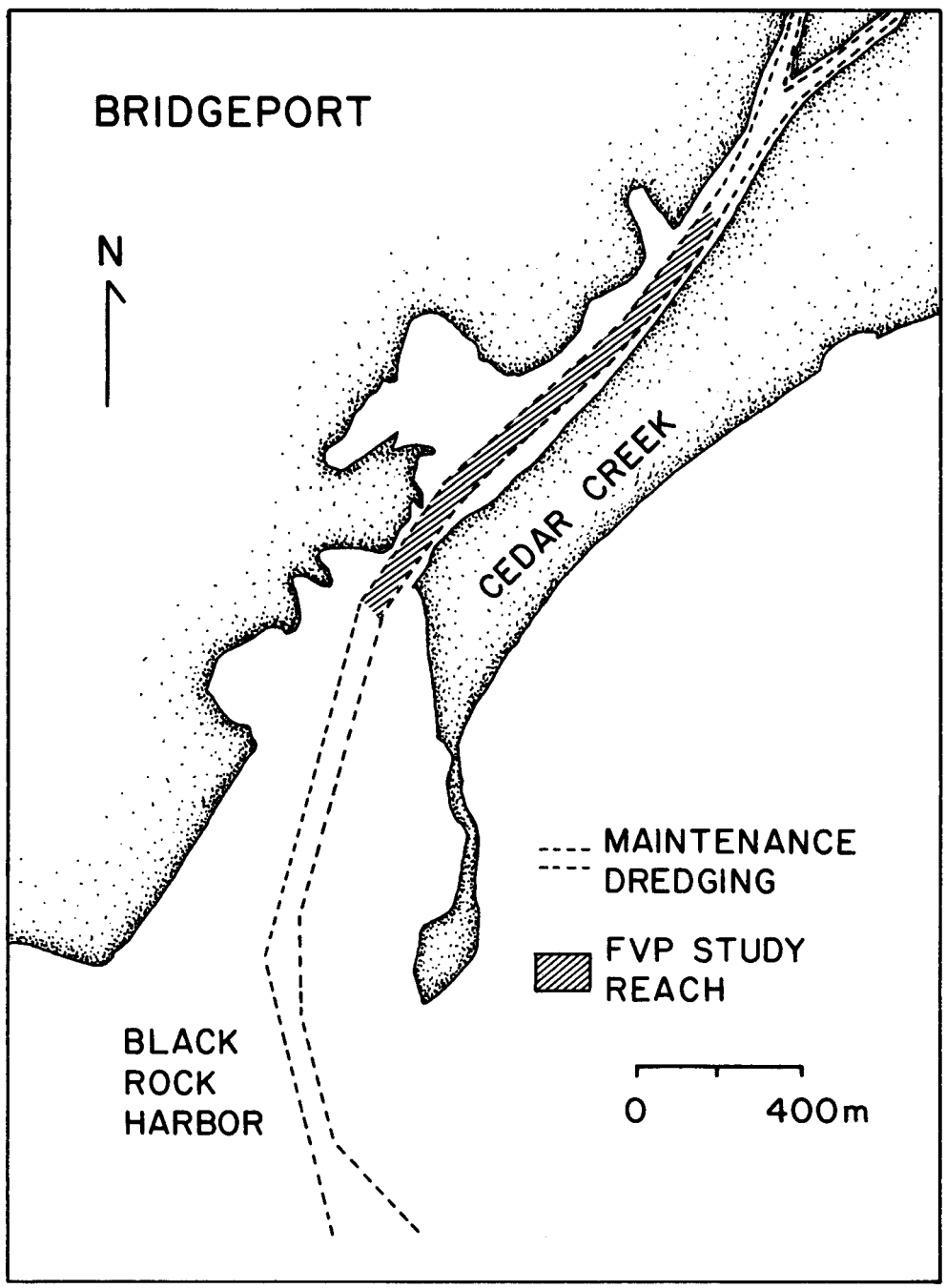

Figure 2. Black Rock Harbor, Connecticut (73°13"W and 41°90"N), source of dredged material

barrel were completely homogenized and wet seived through a 1-mm mesh seive to remove large particles. Sediment was stored in glass bottles at 4° C. To verify that the contents in the bottles were consistent, 400-ml samples were taken before the 1st, 25th, and 50th bottles for moisture content and chemical analysis.

Test Species

8. Two species of marine invertebrates were used to conduct two separate bioaccumulation studies, including depuration phases. A bivalve mollusc, the blue mussel, Mytilus edulis, and the polychaete worm, Nereis virens, were used in this study.

Mytilus edulis

9. The blue mussel is a filter-feeding bivalve mollusc that ranges along the northern Atlantic coast of the United States and Europe. In the United States, it ranges from Maine to North Carolina and on the Pacific coast from Alaska to California (Bayne 1976). Mytilus edulis was selected for this study because it is a filter-feeding mollusc, capturing food as suspended particulates. Species of Mytilus have been used extensively as a biological monitor worldwide (Farrington et al. 1983) and its biology has been studied extensively.

10. One month prior to exposure, adult mussels were collected from a well-characterized area of Narragansett Bay, Rhode Island, with relatively low background concentrations of contaminants in the sediments (Phelps et al. 1983; Phelps and Galloway 1980). Test organisms, 50 to 70 mm shell length, were temperature acclimated from 5° to 10°C at the rate of 1° C per day, then held in unfiltered flowing seawater (28 to 30 °/oo salinity) until initiation of the experiment.

Nereis virens

11. Nereis virens is a marine polychaete worm that inhabits the coastal United States from the Gulf of St. Lawrence to the Gulf of Mexico on the east coast and the central California coast on the Pacific Ocean (Pettibone 1963). They are raptorial and deposit feeders but generally opportunistic in their feeding habits. This species was selected because of its deposit-feeding habits (it will feed directly on sediment constituents), its relatively large size, and its availability. Approximately 600 adult worms were purchased from a bait dealer in Wiscasset, Maine, packed in wet seaweed, and shipped to ERLN. Upon arrival at the laboratory, they were immediately placed in sediment for testing.

Mussel Bioaccumulation Study

Sediment Dosing System

12. A sediment dosing system was constructed to provide BRH as suspended sediment for the mussel bioaccumulation study (Figure 3). The dosing system consisted of a conical-shaped slurry reservoir placed in a chilled fiberglass chamber, a diaphragm pump, a 4-L separatory funnel, and several return loops that directed the particulate slurry through a dosing valve. The slurry reservoir (40 cm diameter x 55 cm high) contained 40-L of slurry comprised of 37.7 L of filtered seawater and 2.3 L of BRH material. The slurry was changed every 2-3 days during exposure. The fiberglass chamber (94 cm x 61 cm x 79 cm high) was maintained between 4° and 10°C using an externally chilled water source. (The slurry was chilled to minimize microbial degradation during the test.) A polypropylene pipe (3.8 cm diameter) placed at the bottom of the reservoir

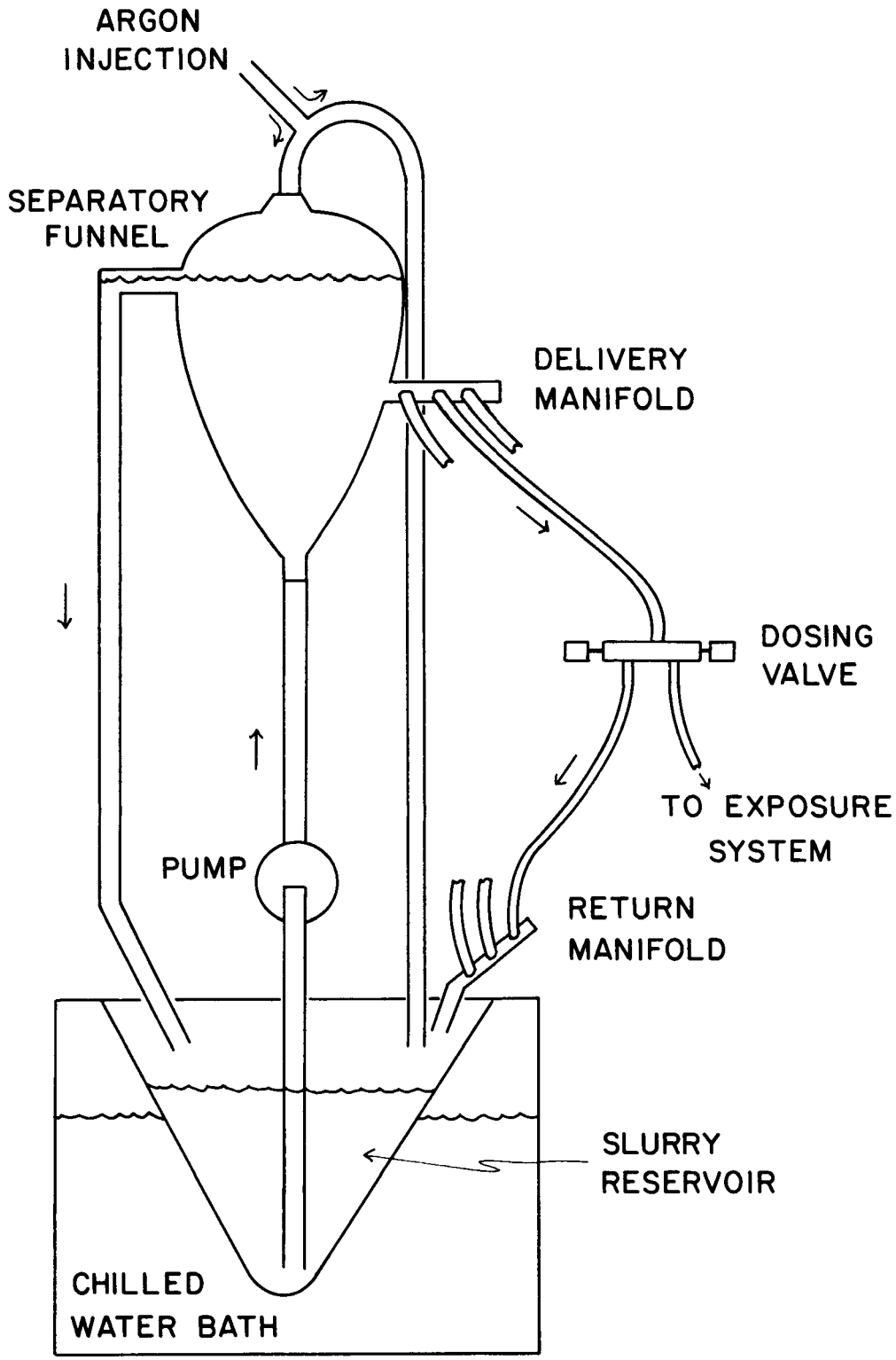

Figure 3. Sediment dosing system with chilled water bath and argon gas supply

cone was connected to the diaphragm pump (16- to 40-L/min capacity) that had a Teflon® diaphragm. This pump was used to circulate the slurry with minimal abrasion so that the physical properties and particle sizes of the material remained as unchanged as possible. The separatory funnel was connected to the pump and returned to the reservoir by polypropylene pipes. The separatory funnel served two functions: (a) to ensure that a constant head pressure was provided at the overflow, and (b) to serve as a connection for the manifold located 4 cm below the constant head level. The manifold served to distribute the slurry by directing a portion of the flow from the funnel, through 6-mm-inside diameter polypropylene tubes through the Teflon® dosing valves (Figures 3 and 4) and back to the reservoir. At the dosing valves, the slurry was mixed with Narragansett Bay seawater which had been filtered (to 15 μ) through sand filters. The valves were controlled by a microprocessor that was connected to a transmissometer (Figure 4). Under transmissometer control, the microprocessor responds by modulating the pulse length to achieve the desired setpoint of suspended sediment measured as turbidity (Sinnett and Davis 1983).

Mussel Exposure System

13. The system used to expose blue mussels to BRH material in the bioaccumulation test is shown in Figure 5. The exposure apparatus consisted of a fiberglass, resin-coated plywood tank (123-L capacity) partitioned into two components. Filtered seawater entered the mixing chamber at 2 L/min where it was vigorously combined with the BRH material and marine algae as a food source (a mixture of Phaeodactylum tricornutum and T-Isochrysis galbana). The mixture cascaded over a partition into

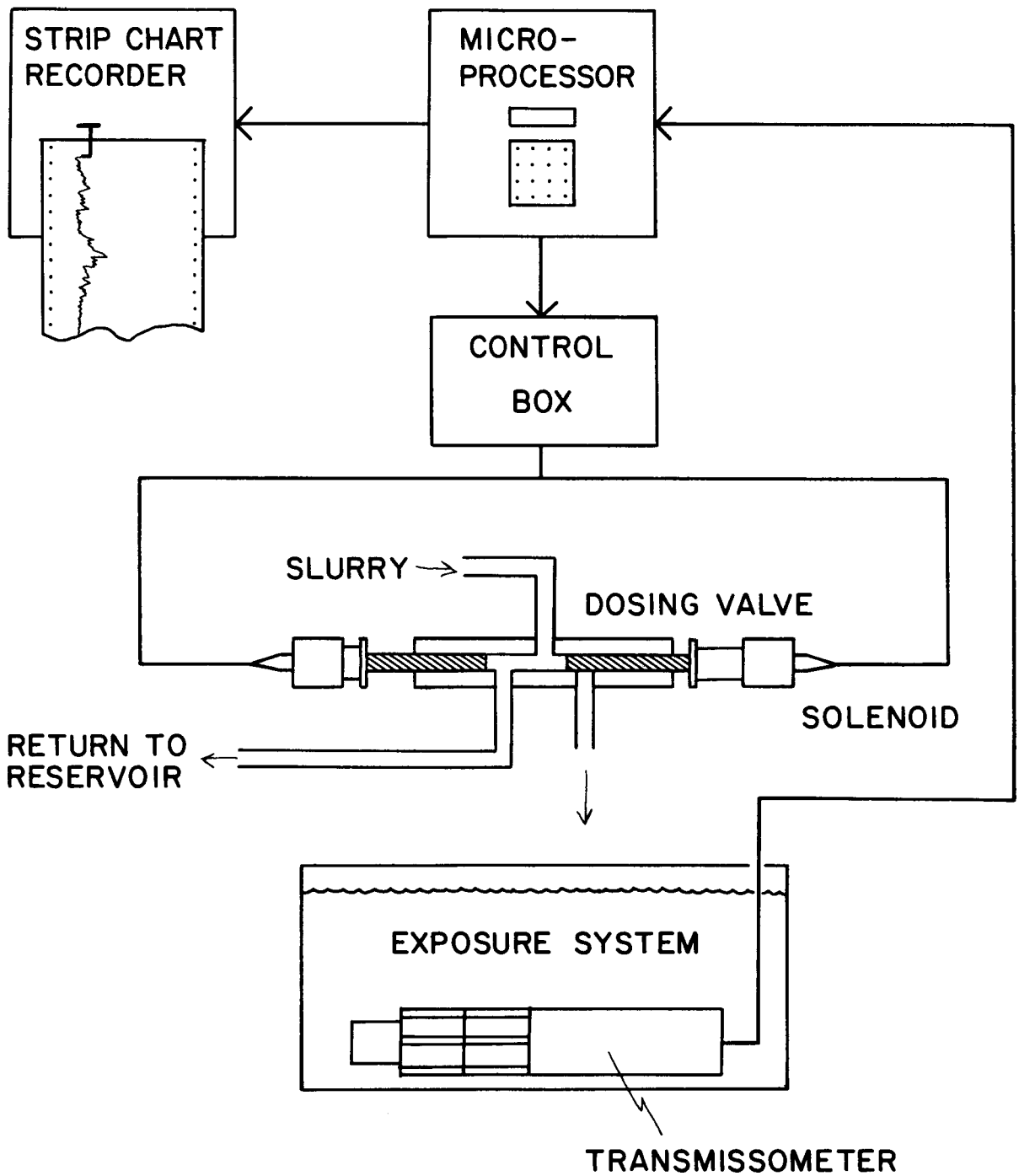

Figure 4. Suspended sediment feedback control loop and strip chart recorder

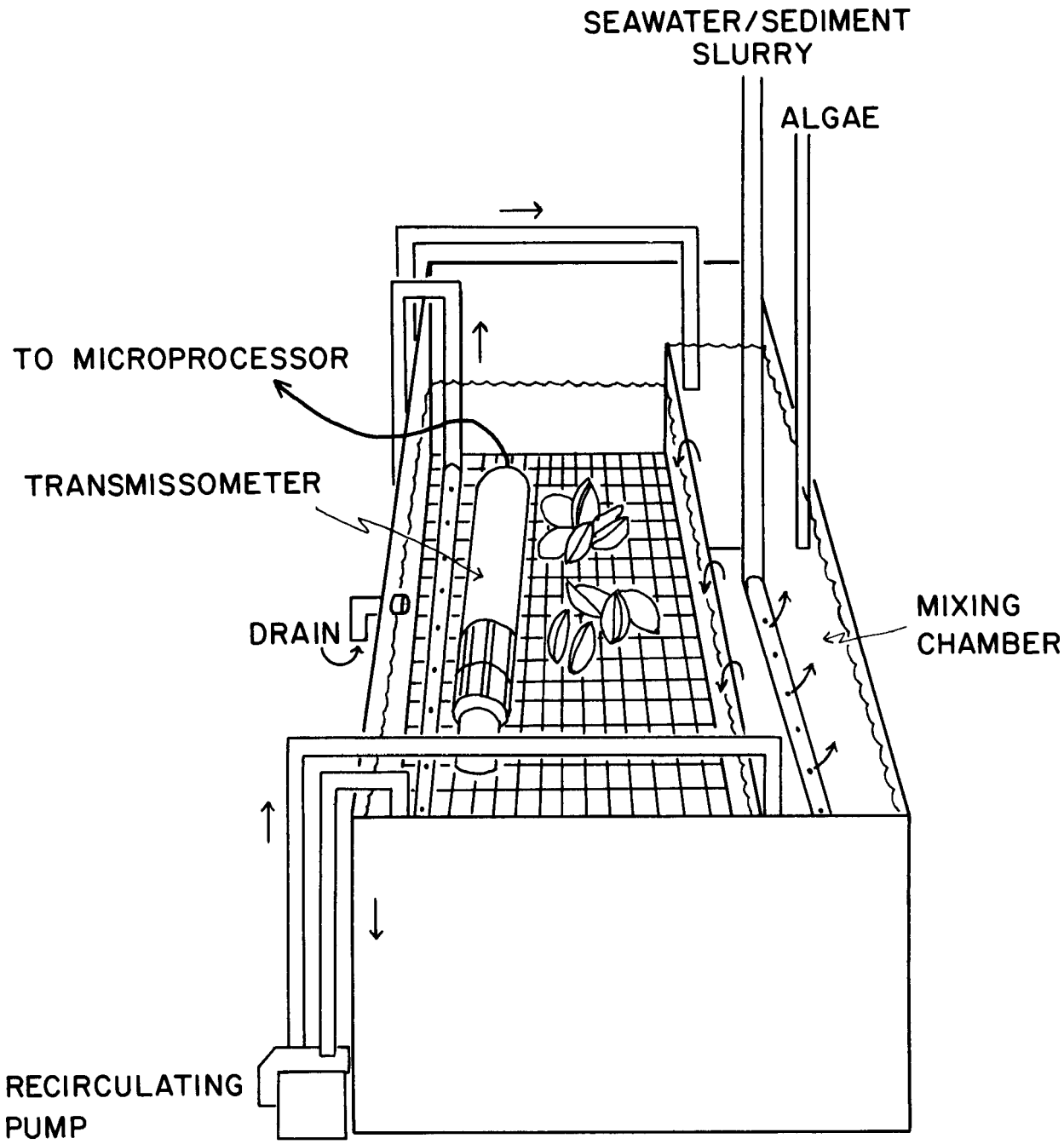

Figure 5. Blue mussel (Mytilus edulis) contaminant uptake system

the exposure chamber containing the mussels and a transmissometer which measured the amount of suspended particulates in the water. To ensure that the particles were rapidly and evenly dispersed throughout the tank, water was collected through a manifold near the transmissometer and returned to the mixing chamber at a rate of 38 L/min. Polypropylene or polyethylene plumbing materials were used throughout.

14. The sediment dosing system delivered BRH sediment directly into the mussel exposure chamber via the dosing valve which was controlled by the microprocessor and transmissometer. As the mussels removed the suspended particles to a level below the desired concentration, the microprocessor simultaneously opened the dosing valve to deliver the BRH suspension and turned on a peristaltic pump to deliver algae to the chamber. Delivery volumes by the valve and peristaltic pump were adjusted to maintain a constant ratio of sediment and algae during a microprocessor pulse. In response to a transmissometer signal every 5 min, the microprocessor modulated the pulse length to achieve an exposure concentration in the chamber of 9.5 mg/L of suspended particles, consisting of 9 mg/L sediment and 0.5 mg/L algae (30 million cells/L). This concentration of suspended sediments was estimated to be below the concentration that would stress or adversely affect the organisms during the test because a preliminary test demonstrated no appreciable mortality, histopathological responses, or adverse changes in scope for growth (SFG) after 2 weeks of exposure to 20 mg/L.

15. The control for this experiment was designed to ensure that contaminants observed in the mussels were accumulated from BRH material rather than from the seawater or the algal cultures. The control exposure

was conducted in an identical test apparatus, but no sediment was delivered to the chamber. Instead, a suspended particulate concentration of 0.5 mg/L consisting entirely of algae was maintained by the microprocessor feedback system.

Experimental Conditions

16. Whenever possible, the general bioconcentration test methods used were from Proposed Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs (American Society for Testing and Materials (ASTM) 1982). Although not specifically intended for suspended sediment testing, the general recommendations defining test animal care, handling, acclimation procedures, seawater quality, and acceptable exposure conditions were suitable for this test.

17. At the start of the bioaccumulation study, 300 mussels were initially placed in each of the BRH and control chambers. Before placing the animals in the test chamber, 20 animals were randomly selected for organic and inorganic chemical analysis to determine the baseline residues in the mussels before the exposures began. During the test, 20 mussels were sampled for chemical analysis on days 1.8, 3.5, 7, 14, 21, and 28 during exposure and 35, 40, 49, 56, 63, and 70 during depuration in the BRH chamber, and 20 mussels were sampled on days 28, 56, and 70 in the control chamber. To avoid excessive loading of the tanks, the shorter exposures were conducted after some of the mussels had been removed by sampling. Specifically, the mussels for days 1.8 and 3.5 exposures were placed in the tank on day 14 and removed on days 16 and 18 respectively. Likewise, mussels for the 7 day exposure were placed in the tank on day 21 and removed on day 28.

Since this design assumes that the exposure system operates in a consistent manner, two 14-day exposures were conducted to verify consistency, one from day 0 to day 14 and a second from day 14 to day 28.

18. Twice each week suspended particulate concentrations from the control exposure chambers were analyzed by dry weight determination and by electronic particle counting (1 to 40 u particle range). The dry weight determinations were conducted according to Standard Methods (American Public Health Association (APHA) 1976) with the following modifications. Before sample filtration, filters were washed with a 50-ml aliquot of deionized water, then with three 10-ml aliquots of deionized water. Following filtration, filters were rinsed with three 10-ml rinses of 2.4 percent ammonium formate to remove salt. Measurements of dissolved oxygen, salinity, temperature, and ammonia nitrogen were made to determine water quality and are presented in Table 1.

Worm Bioaccumulation Study

19. The worm bioaccumulation study consisted of an exposure of Nereis virens to solid phase BRH or REF materials for as long as 40 days under flowing seawater conditions. Twenty-four hours prior to introducing the animals to the exposure aquaria, approximately 9.5 L of either sediment was placed in aquaria measuring 32 cm x 38 cm x 16 cm high. Ambient temperature (9° to 13° C) seawater was then provided to each of 14 aquaria at the rate of 120 ml/min. Sediment depth in each aquarium was approximately 8 cm; seawater depth (maintained by a standpipe) was approximately 5 cm.

20. The test was initiated at time zero (T_o) by randomly placing 24 adult worms in each of 12 aquaria. Nine aquaria contained BRH

Table 1

Summary of Experimental Conditions for Bioaccumulation Study with Mytilus edulis*

Parameter	Control	Exposure
	Uptake Period	
Suspended solids dry wt, mg/L	1.72 ± 0.18 (1.45 – 2.02)	9.32 ± 0.58 (8.19 – 10.33)
Particle density, No./L	2.60 ± 0.2 X 10^7 (2.00 – 3.1 X 10^7	12.00 ± 1.3 X 10^7 (9.60 – 13.7 X 10^7)
Temperature, °C	15.7 ± 0.4 (15.0 – 16.4)	15.6 ± 0.3 (15.4 – 16.4)
Dissolved oxygen, mg/L	7.5 ± 0.6 (7.0 – 8.5)	7.6 ± 0.4 (7.1 – 8.4)
Salinity, °/oo	28.4 ± 1.8 (24 – 30)	28.4 ± 1.8 (24 – 30)
Unionized ammonia, µg/L	2.9 ± 1.29 (0.64 ± 5.40)	3.83 ± 1.68 (1.04 – 6.40)
	Depuration Period	
Suspended solids dry wt, mg/L	23.5 ± 1.17 (1.18 – 3.53)	2.48 ± 1.64 (1.16 – 4.86)
Particle density, No./L	2.8 ± 0.3 X 10^7 (2.5 – 3.2 X 10^7)	2.9 ± 0.2 X 10^7 (2.7 – 3.2 X 10^7)
Temperature, °C	15.2 ± 0.3 (15.0 – 15.8)	15.2 ± 0.3 (15.0 – 15.8)
Dissolved oxygen, mg/L	8.0 ± 0.2 (7.6 – 8.5)	8.0 ± 0.3 (7.6 – 8.4)
Salinity, °/oo	27.9 ± 1.9 (23 – 30)	27.9 ± 1.9 (23 – 30)
Unionized ammonia, µg/L	1.30 ± 0.26 (0.94 – 1.66)	1.33 ± 0.22 (0.96 – 1.52)

* Tabular values are mean and standard deviation with range denoted in parentheses.

material and three REF sediment. A subset of 15 worms was randomly selected for organic and inorganic chemical analysis to determine the contaminants in worms at T_o. Prior to chemical analysis, all worms at all sampling times were placed in Petri dishes containing filtered seawater and allowed to purge their gut contents for 14 hr.

21. For each sampling period, all the worms from a single aquarium were removed for analysis. Sampling periods during the uptake portion of the study were days 14 and 28. After 28 days, all worms in four of the five remaining BRH aquaria were removed, the sediment emptied, and the aquaria cleaned. The aquaria were then filled with REF sediment and the worms placed back into the aquaria. Sampling of these worms during the depuration phase was on days 42 and 56 (14 and 28 days of depuration). The worms in the ninth BRH aquarium were allowed to remain an additional 12 days (total of 40 days exposure) and archived at -20° C.

22. Three aquaria were each provided with REF sediment and 24 worms. The worms were sampled on days 28, 40, and 56 to determine what contaminants, if any, were obtained from the REF sediment.

23. For clarity, mussels exposed to BRH sediment and algae are referred to as "exposed mussels," while mussels exposed to algae only are referred to as "control mussels," For the worm study, worms exposed to BRH sediment are "exposed worms," while those depurated in reference sediment are referred to as "depurated worms." Worms exposed to reference sediments are referred to as "reference worms."

Chemical Analysis

Organic Sample Preparation

24. The analytical procedures described below represent the state of the art in marine organic analysis and have been intercalibrated with several oceanographic laboratories. EPA-recognized analytical methods, while available for these classes of contaminants, have been developed primarily for freshwater and wastewater systems. These methods require extensive modification and intercalibration when applied to marine systems for the types of matrices and levels of detection that are required in this study.

25. Cleaning of Glassware and Equipment. All glassware used for the collection, storage, extraction and analysis of samples was washed with Alconox®, rinsed four times with hot tap water, four times with deionized water, capped with aluminum foil, and muffled for 6 hr at 450° C. Immediately prior to use glassware was rinsed three times with an appropriate solvent.

26. Stainless steel centrifuge bottles were washed in the same manner as glassware and then rinsed twice with methanol, twice with methylene chloride and twice with hexane immediately prior to use.

27. Stainless steel tissue homogenizers were washed in the same manner as glassware and then placed in an ultrasonic bath in graduated cylinders filled first with methanol, then with methylene chloride, and finally with hexane just prior to use.

28. Glass fiber filters were placed individually in aluminum foil and muffled for 6 hr 450° C. The stainless steel filter housing was washed and rinsed with acetone and hexane prior to use.

29. Sediment. The methods that follow were used for the extraction and analysis of BRH sediment and the reference sediment from the worm dosing system. Approximately 10 gr of wet sediment was placed in a stainless steel centrifuge tube, and 50 ml of acetone was added. The mixture was homogenized for 40 sec using a brass-bearing-equipped tissue homogenizer and then centrifuged at 10,000 RPM for 5 min. The acetone was decanted into a 1-L separatory funnel containing 150 ml of pre-extracted deionized water. The extraction and centrifugation steps were repeated twice more and all extracts were combined in the separatory funnel. The aqueous layer in the separatory funnel was extracted three times with 50 ml of Freon 113 each time, and the extracts combined in a 500-ml Erlenmeyer flask. Extracts were frozen to remove water. The sample extract was then subjected to column chromatography (see Column Chromatography, paragraphs 39 and 40).

30. Water. The following procedure was used for unfiltered water samples (dissolved plus particle-bound contaminants), samples of filtered water collected after the glass fiber filter, and water taken after passage through a continuous flow centrifuge. Water samples were collected in 6-L separatory funnels. Samples were extracted twice by the addition of 100 ml Freon 113 followed by vigorous shaking. Extracts were combined in a 500-ml Erlenmeyer flask, and sodium sulfate (previously muffled at 700° C for 4 hr) was added to remove water.

31. The Freon extract was poured off and volume reduced in a round bottom flask fitted with a Kuderna-Danish evaporator, and the solvent was changed to hexane. Extracts (5 ml) were fractionated using the second silicic acid column (see Column Chromatography, paragraphs 39 and 40).

32. Suspended Particulate Material. For the mussel study suspended particulate material (SPM) was collected using a 273-mm glass fiber filter (Gelman Type AE, 0.1 micron) in a stainless steel housing (Millipore® 273 mm). Water from the exposure system tanks was allowed to gravity feed into this filtering system through Teflon® tubing.

33. Each filter was carefully removed, placed in a stainless steel centrifuge bottle, and frozen until preparation and analysis. Acetone (50 ml) was added to the centrifuge bottles containing the filter, and the filter was homogenized with a stainless steel tissue homogenizer for 20 sec at 25,000 RPM. Samples were centrifuged at 10,000 RPM for 5 min, and the acetone water layer was decanted into a 1-L separatory funnel containing 150 ml extracted deionized water. This extraction procedure was repeated two more times using 50 ml of Freon 113. The Freon was added to the separatory funnel, which was then shaken. The Freon layer was then drawn off and saved. The remaining aqueous layer was extracted again with 50 ml of Freon, and the extracts were combined. The sample extract was then subjected to column chromatography (see Column Chromatography, paragraphs 39 and 40).

34. Organisms. Mussel samples were taken for background analysis at day zero, and removed from the exposure tank at day 1.8, 3.5, 7, 14, 28, 35, 40, 49, 56, 63 and 70; control mussels were sampled on days 28, 56, and 70. At each sampling time, 20 mussels were removed using a stratified random sampling plan and stored in muffled aluminum foil in a freezer prior to analysis. From each group of 20 mussels, three replicates consisting of four individuals each were shucked into pre-weighed glass centrifuge tubes, homogenized with a tissue homogenizer for 20 sec, and

centrifuged at 25,000 RPM for 5 min. The remaining 8 organisms were archived at -20° C.

35. Dead mussels were removed daily when discovered and mortality recorded. Mortality data were analyzed by calculating survivorship functions for mussels from control and exposed treatment conditions. These survivorship functions incorporated the effect of the periodic removal of individuals for analyses other than mortality. A comparison of these functions was made using the Mantel and Haenszel (1959) Chi square test for comparing two survival distributions.

36. Worms were removed from exposure tanks for chemical analysis on days 14, 28, 42, and 56 and from the control tanks on day 28. A sample was also collected at day 0, prior to exposure. Following collection from the experimental tanks and gut depuration (see Methods paragraph 20), worms were frozen until analysis in muffled glass jars. From these samples, three replicates of 1-2 individuals each were placed into preweighed glass centrifuge tubes, homogenized with a tissue homogenizer for 20 sec and centrifuged at 25,000 RPM for 5 min.

37. Approximately 2 g of the mussel and worm homogenates was taken for inorganic analysis. A small portion (approximately 2 g) was taken for wet:dry ratio determinations. The remaining homogenate was weighed and used for organic analysis.

38. Each of the sample homogenates from above was treated as a separate sample with appropriate blanks carried through the entire procedure. To each sample was added 15 ml of acetone; the mixture was then homogenized with a tissue homogenizer for 20 sec and centrifuged at 1750 RPM for 5 min. The fluid layer was decanted into a separatory

funnel containing 150 ml of pre-extracted deionized water. The acetone extractions and centrifugation were repeated once more and the extracts were combined in the separatory funnel. The tissue homogenization, extraction, and centrifugation were repeated twice more using 25 ml of Freon 113 as the solvent. Because of the density of the Freon, the solvent was withdrawn from the bottom of the centrifuge tubes using a syringe. The Freon extracts were combined in the separatory funnel, which was then shaken and the Freon layer was drawn off and saved. The remaining aqueous layer was extracted twice more with 50 ml of Freon each time. The Freon extracts were combined and the aqueous layer was discarded. The sample extract was then subjected to column chromatography (see Column Chromatography, paragraphs 39 and 40).

39. Column Chromatography, Final Volume and Storage. To remove interfering biogenic material and some residual particulates, the combined Freon extracts were passed through the first column (2 x 25 cm of 100% activated 100-200 mesh silicic acid). For sediment samples, 2.5 cm of activated copper powder was added to the bottom of the first column to remove elemental sulfur. The column was then rinsed with 25 ml Freon followed by 50 ml of methylene chloride. The eluate was collected and volume reduced in a round bottom flask fitted with a Kuderna-Danish evaporator and 3-ball Snyder column. The solvent was exchanged to hexane as the sample approached 5 ml. Final volume reduction to 5 ml was accomplished by placing the sample in a concentrator tube fitted with a microsnyder column and placing it into a tube heater.

40. The 5-ml sample extracts were then charged onto a 0.9 x 45 cm second column of 5% water deactivated 100-200 mesh silicic acid. Three

fractions were collected from the column. Fraction 1 (PF-50) consisted of 50 ml of pentane, fraction 2 (F-2) consisted of 35 ml of 20% methylene chloride in pentane, and fraction 3 (F-3) consisted of 35 ml of methylene chloride. The PF-50 fraction is an expansion of a 1st fraction formally used by this laboratory. The PF-50 fraction is designed to include PCBs and related chlorinated pesticides of similar polarity in addition to a large portion of the petroleum hydrocarbons. Petroleum hydrocarbons (PHC) as referenced in this report include only those hydrocarbon compounds in the PF-50 fraction. The polycyclic aromatic hydrocarbons (PAH) which are collected in the F-2 fraction may also be of petroleum origin; however, these PAH compounds and the small amount of unresolved material found in the F-2 (as separated in the present study) represented only a small portion (approximately 10%) of the total petroleum hydrocarbons and were not included in petroleum hydrocarbon calculations. The F-3 fraction collected more polar material. Each column fraction was reduced in volume by a Kuderna-Danish evaporation as above, with the solvent changed to hexane. The final sample volume of 1 ml was achieved by adding 1 ml of heptane to the sample in a 10-ml concentrator tube. Glass ebullators, microsnyder columns, and a tube heater were utilized to reduce the sample to 1 ml. The extracts were then divided in half between sealed glass ampules for archival storage and screw cap vials for gas chromatographic and GC/MS analyses.

Organic Instrumental Analysis

41. Electron capture gas chromatographic analyses were conducted on a Hewlett-Packard Model 5840 gas chromatograph equipped with a 30 meter DB-5 fused silica capillary column from J & W. The chromatograph was

temperature programmed from 80° C to 290° C at 10° C/min with a 4-min hold at 80° C. Flame ionization gas chromatographic analyses were conducted on a Carlo Erba 4160 gas chromatograph equipped with an identical column. The temperature was programmed from 60° C to 325° C at 10° C/min with a 4-min hold at 60° C.

42. Gas chromatograph/mass spectrometric (GC/MS) analyses were conducted on a Finnigan Model 4500 also equipped with J & W DB-5 30 meter fused silica capillary column. The tail of the capillary column was positioned inside the mass spectrometer so that the effluent from the column was directed into the ionization volume of the mass spectrometer. The mass spectrometer was operated through a standard Incos data system and was tuned at all times to meet EPA quality assurance specifications using decafluorotriphenylphosphine. The ionizing current was typically set at 300 milliamperes and 70 EV, and the instrument operated such that 100 picograms of PAHs from naphthalene to benzopyrene gave easily quantifiable signals on their molecular ions with signal-to-noise ratios of 50:1 or better. The mass spectrometer's gas chromatograph was typically programmed from 50° C to 330° C at 10° C/min with a 2-min hold at 50° C, but was occasionally progammed at 4° C/min to permit higher chromatographic resolution.

43. All instruments were calibrated with standards each day. The concentrations of the standards used were chosen to be close to the levels of the materials of interest, and periodic linearity checks were made to ensure the proper performance of each system. When standards were not available for some compounds, response factors were calculated using mean responses of appropriate standards.

Inorganic Sample Preparation

44. Seawater. Two sets of seawater samples were collected from the mussel exposure system on day 25 of the exposure period. Approximately 1 hr before the BRH sediment slurry was renewed in the reservoir, duplicate 20-ml samples of seawater were taken from the control and exposure chambers. Two 20-ml seawater samples were also taken 3 hr after renewing the slurry from the exposure chamber only. The unfiltered samples were acidified with 2.0 ml of ultra-pure concentrated nitric acid and placed in acid-cleaned polyethylene bottles fitted with polyethylene screw caps. The acidified samples were stored at room temperature for 1 week before trace metal analysis.

45. Sediment. After the BRH sediment contained in a barrel was thoroughly homogenized (see Sediment Collection and Preservation, paragraphs 6 and 7), nine samples were taken for analysis. These samples included three from the top, three from the middle, and three from the bottom. The wet weight of all samples was determined. The samples were frozen and then freeze dried in a Virtus® lypholyzer (Model # 10-145MR-BA) for 2 days. The dry weight of each sample was then determined.

46. The dried BRH sediment samples were acidified with a total of 50-ml of concentrated HNO_3 (reagent grade). The acid was added in 10-ml aliquots since BRH sediment is very reactive to acid. All reaction was allowed to subside before the next addition of acid was made. After several days the samples were heated at 60° C for several days. The samples were subsequently evaporated down to approximately 10 ml after which 30% H_2O_2 was added in 2-ml aliquots until 50 ml had been

added. The H_2O_2 was added cautiously since BRH sediment reacts vigorously with strong oxidizing agents. The samples were evaporated down to approximately 25 ml and filtered through acid-rinsed (5% HNO_3) Whatman 41 filter paper into 250-ml volumetric flasks. The beakers were rinsed with 25-ml quantities of 5% HNO_3. The rinse solution was also filtered through the filter paper and added to the volumetric flask. The volumetric flasks were brought up to volume with 5% HNO_3. This nitric acid-hydrogen peroxide extraction procedure for sediment samples has been described by Krishmanurty et al. (1976).

47. Organisms. From each sample homogenate, described in Organic Sample Preparation, about 2 g of wet tissue was taken for inorganic analysis and placed in a tared beaker and weighed. The samples were oven dried at 110° C for 2 days, cooled in a desiccator, and weighed. Ten milliliters of concentrated reagent grade nitric acid was added to each sample, which was then allowed to digest at room temperature in a hood for 24 hr. The samples were heated at 60° C for several days until complete dissolution of the sample had occurred. The samples were then evaporated to near dryness at 90-95° C, and cooled to room temperature. Three milliliters of 30% hydrogen peroxide were slowly added in 1-ml increments since the effervescent reaction was quite vigorous. The solutions were then heated to 60° C for another day, evaporated to near dryness, and cooled to room temperature. At this point the clear and colorless solutions were transferred to 25-ml volumetric flasks with several rinses of 5% nitric acid, and were diluted to the mark with 5% nitric acid. The solutions were finally transferred to screw cap poly-

ethylene bottles. This nitric acid-hydrogen peroxide dissolution procedure has been reported by Knauer and Martin (1973).

Inorganic Instrumental Analysis

48. All flame atomization (FA) atomic absorption (AA) analysis was conducted with a Perkin-Elmer (Model #603) atomic absorption instrument. All Hg determinations were conducted by the method of Hatch and Ott (1968) using a Perkin-Elmer (Model #MHS-1) mercury/hydride system adapted to the 603 AA. The transient Hg signals were recorded with a Perkin-Elmer (Model #56) strip chart recorder. All heated graphite atomization (HGA) atomic absorption determinations were conducted with a Perkin-Elmer (Model #500) HGA unit coupled to a Perkin-Elmer (Model #5000) atomic absorption instrument retrofitted with a Zeeman HGA background correction unit. The model 500 HGA unit was equipped with an auto injector (Model # AS-40). The transient HGA-AA signals were recorded with a Perkin-Elmer strip chart recorder (Model #56) and also sent automatically to a Perkin-Elmer data station microcomputer (Model #3600). Software supplied with the data station reduced the transient signals to a peak height and peak area for each element determined. The instrument setup procedures for the FA-AA, MHS-1, and HGA-AA determinations were in accordance with procedures described in "Methods for Chemical Analysis of Water and Wastes" (EPA 1979) and are also found in the manufacturer's reference manuals.

49. The AA instruments were calibrated each time samples were analyzed for a given element. Instrument calibrations were generally checked after every five samples had been atomized into the flame unit, injected into the HGA unit, or pipetted into the MHS-1 sample reaction

flask. All samples were analyzed at least twice to determine signal reproducibility. Most were analyzed three times. Generally, for each 15 samples processed, one sample was determined by the method of standard addition, and one procedural blank sample was analyzed.

50. All elements except Hg and As were determined in the sediment samples by FA-AA. Mercury was determined only in the BRH sediment samples by the MHS-1-AA technique. Arsenic could not be determined in the sediment samples because of a chemical interference. At this time the cause of the chemical interference is under investigation.

51. All seawater samples were analyzed by HGA-AA. No chemical separation techniques were utilized to concentrate the elements of interest from the seawater matrix. All samples were analyzed by direct injection into the HGA unit (Ediger et al. 1974; Sturgeon et al. 1979; and Slavin 1980). The large non-atomic background signal was eliminated by the use of the Zeeman background correction system (Fernandez et al. 1980, and Fernandez and Giddings 1982). It was necessary to matrix match the unknown samples with the standards since chemical interferences are not corrected by the Zeeman effect. Therefore, all standards were prepared in trace metal stripped seawater and acidified in the same manner as the samples. The trace metal-free seawater was prepared by the methods of Davey et al. (1970).

52. Due to the limited size of the mussel and worm samples (2 g wet weight), only Fe and Zn could be determined by conventional FA-AA. All other elements (i.e., Mn, Cu, Pb, Cd, Cr, and As) were determined by HGA-AA. All mussel and worm samples determined by HGA-AA were matrix matched before analysis. A matrix solution containing 10% seawater and 90% 0.16

N nitric acid (V/V) was used as a diluent for both standards and samples. Samples were diluted with this matrix modification solution so that the sample extracts never exceeded 20% of the total volume of the solution analyzed. Standards were made up in an identical manner to the samples.

53. It should be noted that, unlike the As determined in BRH sediment samples, no chemical interference was detected for the As determined in the mussel samples. There is a large difference in the two sample matrixes with respect to the inorganic and organic composition which could account for the absence or presence of a chemical interference during the determination of As by HGA-AA or MHS-1 AA analysis.

PART III: RESULTS AND DISCUSSION

Mussel Test

Organic Contaminants

54. PCBs - Unfiltered Seawater. The PCB concentrations (quantitated as Aroclor-1254) in whole water samples (dissolved plus particle bound PCB compounds) taken during the exposure and depuration phases of the bioaccumulation study are shown in Table 2. The PCB concentrations found in blanks processed through the analytical procedure averaged 0.21 ng/l (Table 3). The average concentration of PCBs (as A-1254) found in control tanks was 0.52 ng/l. PCBs found in unfiltered seawater samples from the exposure tanks showed an average concentration of 112 $\pm$ 29.3 ng/l during the exposure. During the exposure the RSD* of the measurement for total PCBs was 20%, indicating that the exposure system was working well and that it delivered a relatively constant concentration of PCB contaminants to the mussels. During the depuration period the PCB concentrations in the exposure tank decreased; however, they remained elevated above those in the control tank during the depuration period (Table 2). Since the fiberglass exposure tank was cleaned with soap and water and thoroughly rinsed following the exposure period, the elevated concentrations found in the tank during depuration may reflect the further introduction of contaminants from a variety of possible sources (i.e., particulates associated with the mussels' shells or byssal threads, feces and pseudofeces, etc.).

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* RSD = Relative standard deviation = $\frac{\text{standard deviation}}{\text{mean}} \times 100$

Table 2

Mussel Dosing System; PCB Levels in Unfiltered Water

CONTROL TANK

Day	Replicate	PCB (as A-1254) ng/l (not corrected for blank levels)	
0	A	0.46	.50 ± .05
0	B	0.53	
28	A	0.23	.34 ± .16
28	B	0.45	
35	A	0.65	.65
70	A	0.66	.66 ± .00
70	B	0.66	
		.52 ± .16	

EXPOSURE TANK

Day	Replicate	PCB (as A-1254) ng/l (not corrected for blank levels)	
0	A	95.3	115. ± 27.2
0	B	134.	
8	A	116.	123. ± 9.2
8	B	129.	
14	A	97.4	80.2 ± 24.3
14	B	63.	
16	A	133.	155. ± 31.1
16	B	177.	
21	A	80.3	91.7 ± 16.1
21	B	103.	
*28	A	105.	107. ± 2.8
28	B	109.	
		112. ± 29.3	
35	A	2.27	
70	A	1.79	1.83 ± 0.06
70	B	1.87	

*At day 28 exposure ended and depuration period began.

Table 3

PCB Blank Levels - Mussel Dosing System Samples*

Day	PCB (as A-1254) ng/l
24 February 83	.18
2 March 83	.16
4 March 83	.16
9 March 83	.34
16 March 83	.24
23 March 83	.20
	.21 ± .07

* For PCB levels given (Table 2), the blank levels have not been subtracted.

55. The distribution of PCB compounds between the dissolved** and particle-bound form was examined in samples of the exposure water. Both the filters and the filtrate were extracted and analyzed. Another separation of dissolved and particulate phases was accomplished using a continuous flow centrifuge. The results of these studies are shown in Table 4. The mean PCB concentration for the filters (day 8) added to the mean PCB concentration for the dissolved compounds (day 8) is close to the value obtained for analysis of unfiltered water on day 8. These data indicate that methylene chloride method for extracting PCB compounds from the suspended particulate suspensions was as efficient as the sum of the individual extractions of the filtrate and the particles (see Methods).

56. The electron capture detection gas chromatograms from the analysis of unfiltered water, filters, filtrate, and centrifuged water taken from the dosing system on day 8 of exposure are shown in Figure 6. The chromatogram of the unfiltered water (dissolved and particle-bound contaminants) shows a distribution of PCB compounds from Cl_2 to Cl_8 with the majority of material containing four, five, and six chlorine atoms. Tentative identification of the compounds in electron capture chromatograms are shown in Table 5. The same general patterns of peaks are shown in the filter sample; however, there appears to be a relative decrease in

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** Dissolved as used in this report refers to the compounds passing through the 0.1-μ glass fiber filter and that material which passed through the continuous flow centrifuge. These compounds may be associated with surfactants or may be in colloidal forms and not truly dissolved.

Table 4

Mussel Dosing System; PCB Levels in Water-Exposure Tank

Day	Replicate	PCB (as A-1254) ng/l (not corrected for blank levels)	
Unfiltered Water			
8	A	116.	123. ± 9.2
8	B	129.	
Filtered Water			
8	A	11.1	11.6 ± .64
8	B	12.0	
Water thru Centrifuge at 14,000 RPM			
8	A	10.6	
Filter			
8	A	108.	

Table 5

Tentative Identifications of Compounds in ECD Gas Chromatograms*

Peak #	Tentative ID
1	2,3 - Dichlorobiphenyl
2	Dichlorobiphenyl
3	Dichlorobiphenyl
4	2,2',5 - trichlorobiphenyl
5	Trichlorobiphenyl
6	Trichlorobiphenyl
7	Trichlorobiphenyl
8	Trichlorobiphenyl
9	2,4',5 - trichlorobiphenyl
10	2,4,4' - trichlorobiphenyl
11	2,3,4 - trichlorobiphenyl
12	Trichlorobiphenyl
13	Trichlorobiphenyl
14	Tetrachlorobiphenyl
15	2,2',4',5, - tetrachlorobiphenyl
16	2,2',4,4' - tetrachlorobiphenyl
17	2,2',3',5 - tetrachlorobiphenyl
18	Tetrachlorobiphenyl
19	Tetrachlorobiphenyl
20	Tetrachlorobiphenyl
21	2,3',4',5 - tetrachlorobiphenyl
22**	2,3',4,5',6 - pentachlorobiphenyl, 2,3',4,4' - tetrachlorobiphenyl 2,2',3,5,6 - pentachlorobiphenyl
23	Pentachlorobiphenyl, 2,3,8 - trichlorodibenzofuran, tetrachlorodiphenyl ether
24	Tetrachlorobiphenyl, Pentachlorobiphenyl

*Since all PCB isomer standards were not available, the possibility exists that other isomers may elute with identical retention times as the PCB in this table. Therefore we prefer the conservative approach by listing identifications as tentative.

**More than one PCB isomer standard with this retention time eluted in this position.

Table 5. (Cont'd)

Peak #	Tentative ID
25	2,2',4,5,5' - pentachlorobiphenyl
26	Pentachlorobiphenyl
27	Pentachlorobiphenyl
28	Pentachlorobiphenyl, 1,1 - bis (p-chlorophenyl) - 2,2-dichloroethylene
29	Pentachlorobiphenyl
30	Pentachlorobiphenyl
31	Pentachlorobiphenyl, Hexachlorobiphenyl
32	Pentachlorobiphenyl, Hexachlorobiphenyl
33	Pentachlorobiphenyl
34	Pentachlorobiphenyl, Hexachlorobiphenyl
35	Hexachlorobiphenyl
36	2,2',4,4',5,5' - hexachlorobiphenyl
37	Pentachlorobiphenyl, Hexachlorobiphenyl
38	Hexachlorobiphenyl
39	Hexachlorobiphenyl,
40	2,2',3,3',4,5 - hexachlorobiphenyl
41	Heptachlorobiphenyl
42	2,2',3,4,4',5',6 - heptachlorobiphenyl
43	2,2',3,3',4,4' - hexachlorobiphenyl
44	Hexachlorobiphenyl
45	Heptachlorobiphenyl
46	2,3,3',4,4',5 - hexachlorobiphenyl
47	2,2',3,3',4,5',6,6'- octachlorobiphenyl
48	Heptachlorobiphenyl
49	Heptachlorobiphenyl
50	Octachlorobiphenyl
51	Octachlorobiphenyl
52	2,2',3,3',4,4',5,5' - octachlorobiphenyl
53	2,2',3,3',4,4',5,5',6 - nonachlorobiphenyl
54	Decachlorobiphenyl

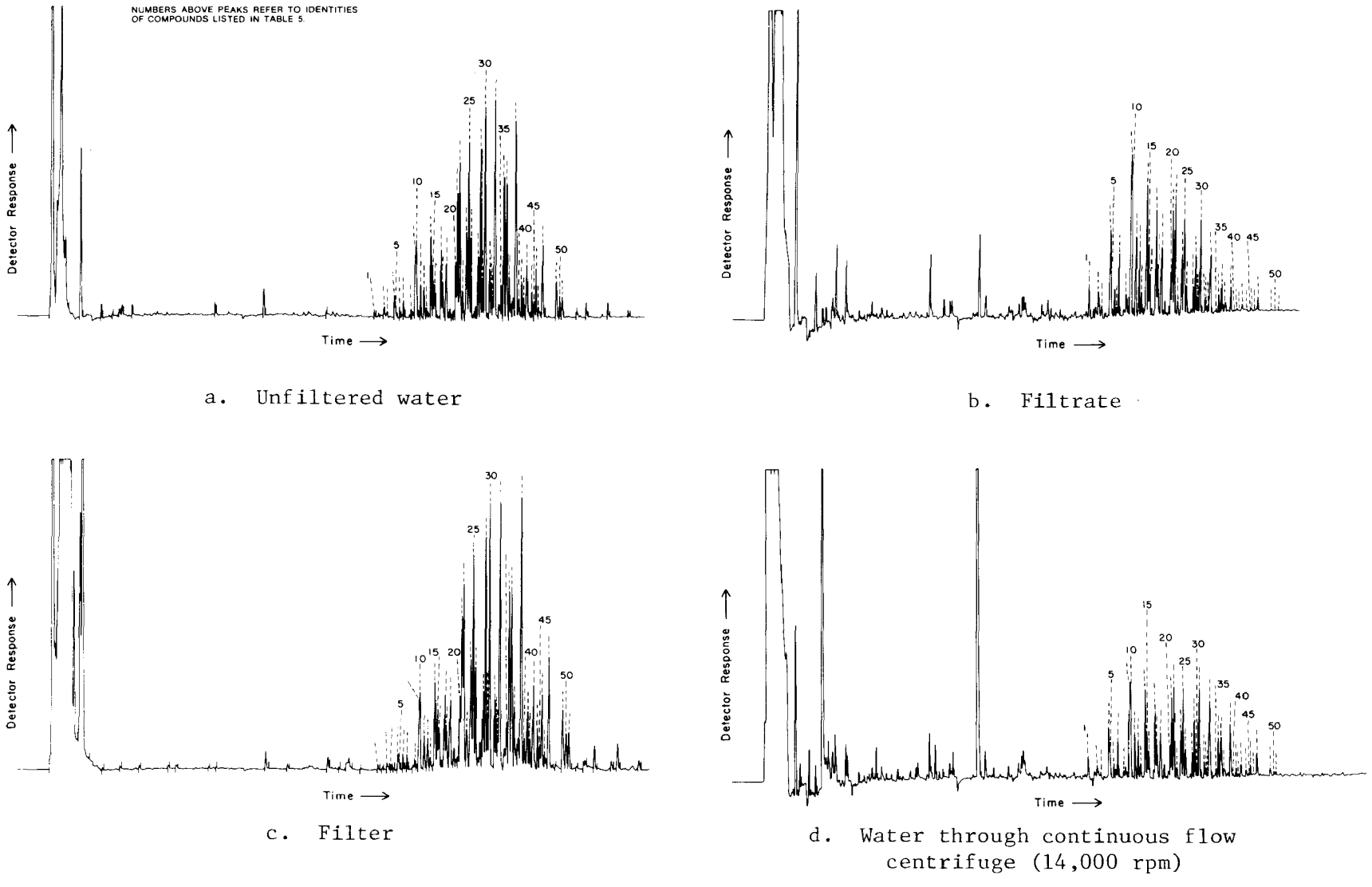


Figure 6. Capillary column electron capture gas chromatograms of PF-50 (PCB) fractions from day 8 exposure

the height of the lower molecular weight peaks in comparison with the chromatogram of the unfiltered water. The chromatogram of the filtrate shows a relative enhancement of the lower molecular weight PCB compounds when compared with the unfiltered water. The distributions found are logically consistent with the solubilities of the compounds. With lower molecular weight, more water-soluble PCB compounds were found in the filtrate and the higher molecular weight, less soluble compounds were found associated with particles.

57. In order to determine whether the distributions found on the filter and in the filtrate were artifacts of the filtration process (i.e., adsorption of less soluble PCB components on the filter while more soluble components passed into the filtrate), continuous flow centrifugation at 14,000 RPM was utilized to remove particles. Analysis of the water following passage through the centrifuge showed a distribution of PCB compounds that was very similar to the distributions found in the filtrate (Figure 7). While PCBs in the water passing though the centrifuge may still be associated with extremely fine particles or exist in colloidal form, this experiment showed that the separations were not artifacts of the filtration process.

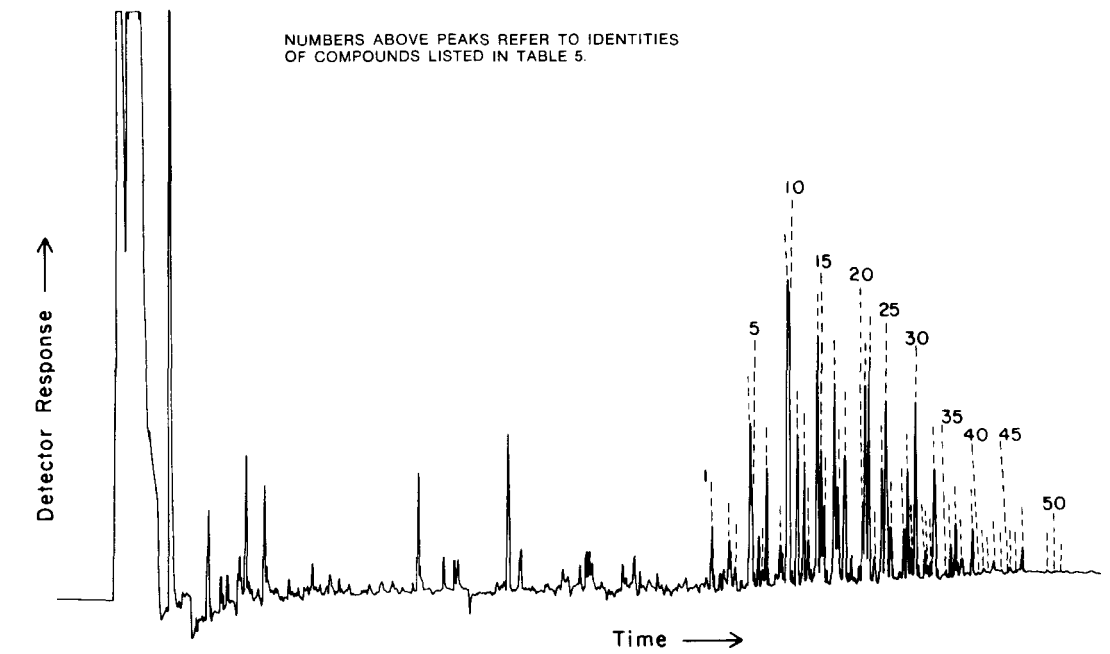
58. Data from the analysis of filtered material and filtrates were utilized to calculate sediment-water partition coefficients, Kp, where

$$Kp = \frac{Cs}{Cw}$$

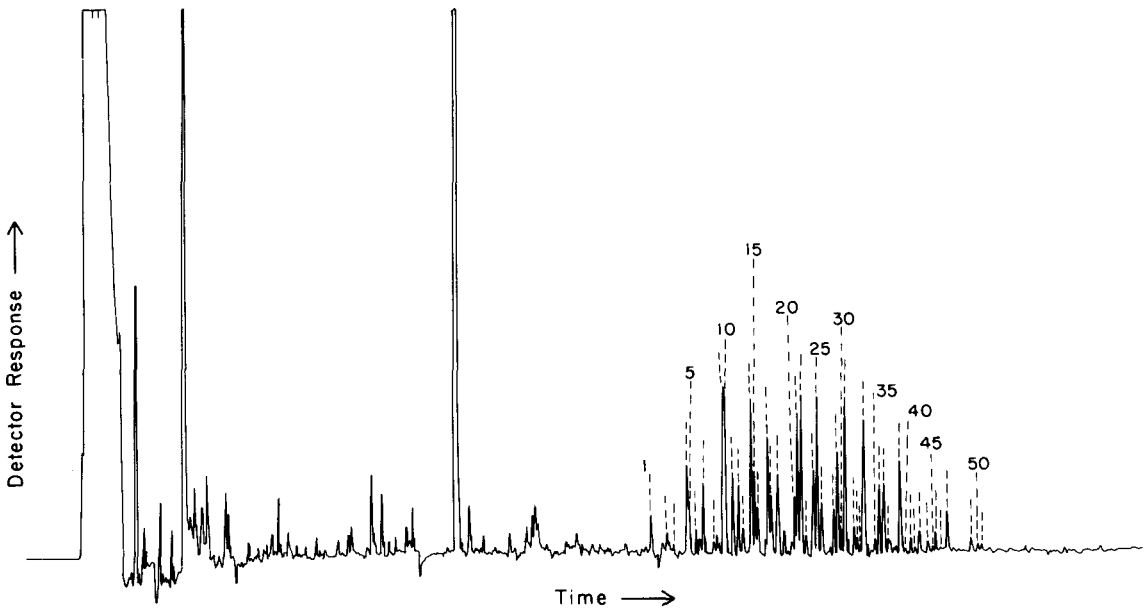
Cs= concentration of compound in sediment

Cw= concentration of compound in water

Kps were estimated for PCB compounds where Log P (Log of the n-octanol/ water partition coefficient) were known (Table 6). The estimated



a. Filtrate



b. Water through continuous flow centrifuge (14,000 rpm)

Figure 7. Capillary columm electron capture gas chromatograms of PF-50 (PCB) fractions from day 8 exposure

Table 6

Comparison of Experimental and Estimated Kps

Peak No.	PCB Compound	Log P*	Kp Experimental**	Kp Estimated†
4	2,2,'5 - trichlorobiphenyl	4.7	.99 X 10^5	.02 X 10^5
10	2,4,4' - trichlorobiphenyl	5.0	2.1 X 10^5	.036 X 10^5
25	2,2',4,5,5'-pentachlorobiphenyl	6.3	12. X 10^5	.66 X 10^5
36	2,2',4,4',5,5'-hexachlorobiphenyl	6.7	42. X 10^5	1.8 X 10^5
43	2,2',3,3',4,4'-hexachlorobiphenyl	7.0	51. X 10^5	3.2 X 10^5

* Solubility from Mackay et al.(1980b). Converted to Log P using Log P = 5.00-.670 Log S where S is solubility in μmol/l (Chiou et al. 1977).

** Kp measured as means of 3 Kps determined on day 28 from mussel exposure tank.

† Kp estimated using Log Koc = Log Kow - 0.21
Kow = n-octanol/water partition coefficient (Log P)
Koc = organic carbon/water partition coefficient from (Karickhoff et al. 1979)

and $Kp = Koc \frac{(\%OC)}{100}$ from Briggs (1973).

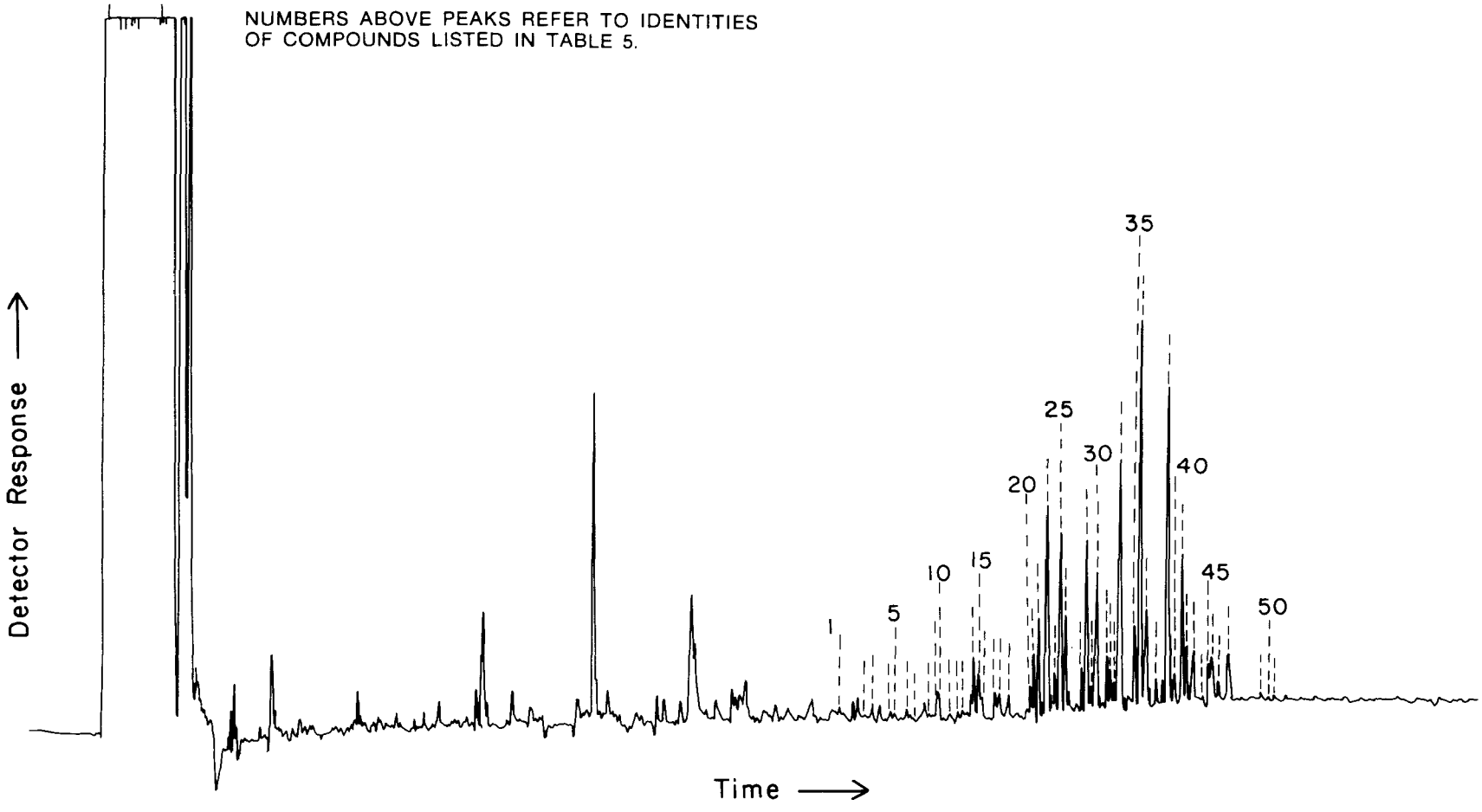


Figure 8. Capillary column electron capture gas chromatogram of PF-50 (PCB) fractions from mussel, time 0

results were considerably lower than the measured results for representative PCB isomers. This may indicate that equilibrium was not established with respect to PCBs in the aqueous and particle bound phases during the residence time of the suspensions in the dosing system.

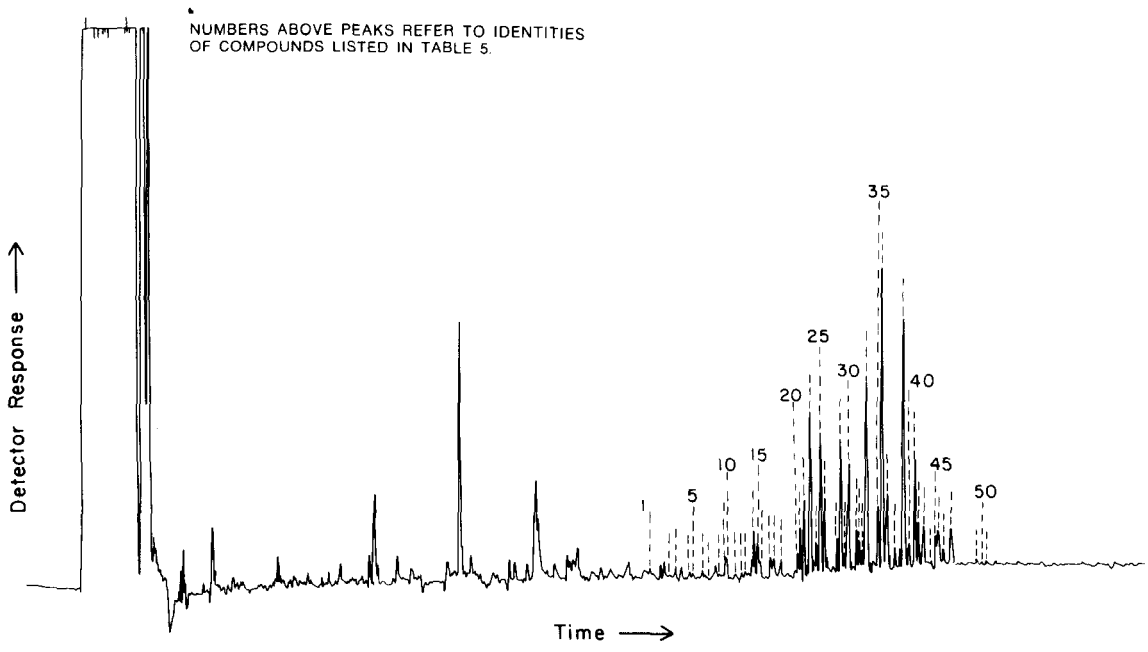
59. PCBs - Mussels. An electron capture detection (ECD) gas chromatogram from mussels taken at day 0 (Figure 8) shows a pattern of electron capturing compounds that is typical of mussel samples from lower Narragansett Bay (Lake et al. 1981). This chromatogram shows a peak consisting of 2,4,8-trichlorodibenzofuran and a tetrachlordiphenyl ether (which co-elute under the gas chromatographic conditions employed). The predominant peaks are Cl_6 PCBs.

60. Following exposure to suspensions of BRH material (day 28), more PCB peaks are evident in the ECD chromatograms of mussels and the distributions are changed considerably (Figure 9). In particular, the lower molecular weight PCB compounds consisting of PCBs with two, three, and four chlorine atoms are significantly increased, and the maximum peaks consist of Cl_5 compounds. Relative increases in some Cl_6 and Cl_7 peaks eluting in the later portions of chromatograms are also evident.

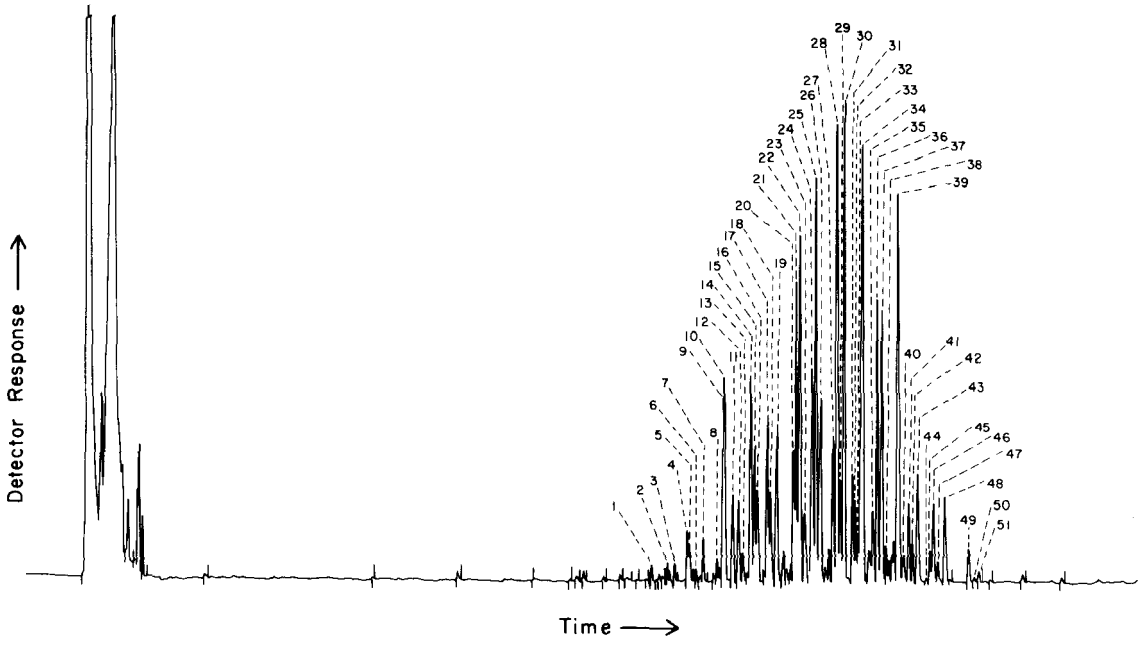
61. Comparison of this chromatogram with that of the unfiltered dosing water (Figure 10) shows that the mussels accumulated most of the different PCB isomers present in the unfiltered water. The organisms appeared to show a distribution which was very similar to that in the unfiltered water; and, as was observed in other studies (Lake et al. 1983), PCBs with seven or more chlorine atoms were not accumulated as effectively as those with four, five, and six chlorine atoms.

62. The chromatogram from mussels exposed for 28 days, and the chromatogram of mussels exposed for 28 days followed by 42 days of depuration, are shown in Figure 11. Comparison of these chromatograms shows relative decreases in lower molecular weight PCB compounds (Cl_2, Cl_3, and Cl_4 isomers) and in some Cl_6 and Cl_7 PCB isomers in the depurated sample, as well as relative increases in other Cl_6 isomers (peaks No. 36 and 39). The peaks which are becoming more prominent are the same PCB peaks that are predominant in the chromatograms from control mussels and mussels from lower Narragansett Bay.

63. Since mussels were not gut purged in the present study, the extracts of mussels include a PCB contribution from SPM in the gut of the organisms. While the significance of this material to the total PCB content of the organisms has not been determined in the present study by examining gut-purging, two facts support the contention that it is not dominant in determining the PCB distributions in mussels. First, research examining the uptake of PCBs in similar dosing studies found no differences in PCB concentrations between non gut-depurated mussels and mussels depurated for 6 hr (Pruell et al. 1983). In addition, these researchers found the SPM contained Cl_8, Cl_9, and Cl_{10} PCBs which were not observed in the mussel extracts but which would have been present if material in the gut had significantly influenced the PCB contaminants in the extracts. Secondly, the amount of SPM present in the organisms at day 28 can be calculated from the accumulation of Fe (which is not highly bioaccumulated) and the concentration of Fe in the BRH sediment. The amount of sediment accumulated multiplied by the concentration of

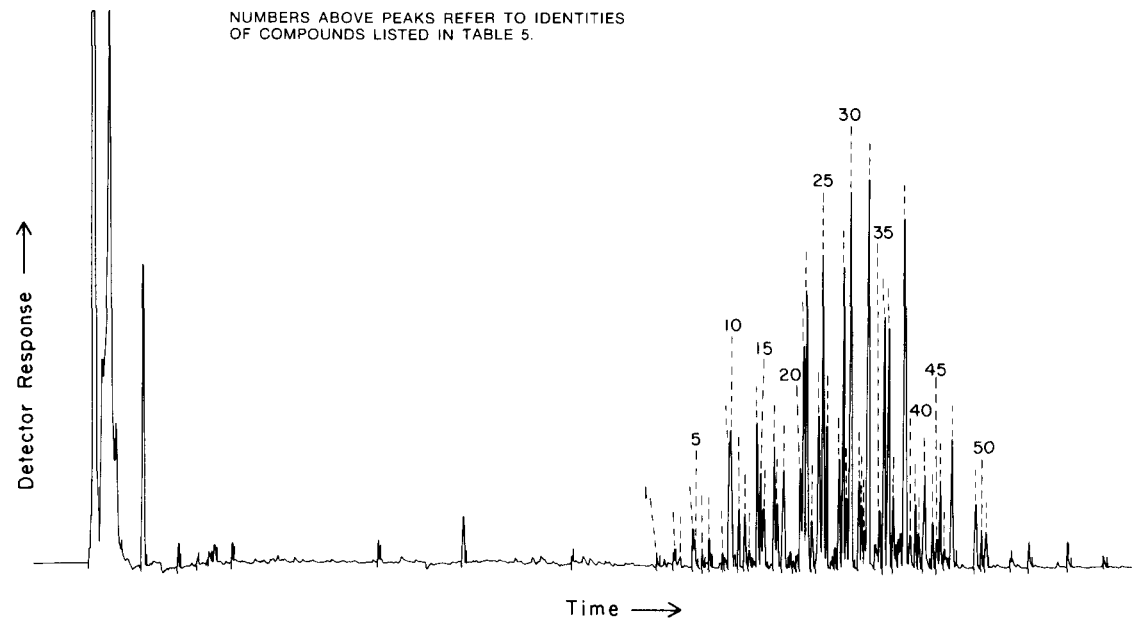


a. Time 0

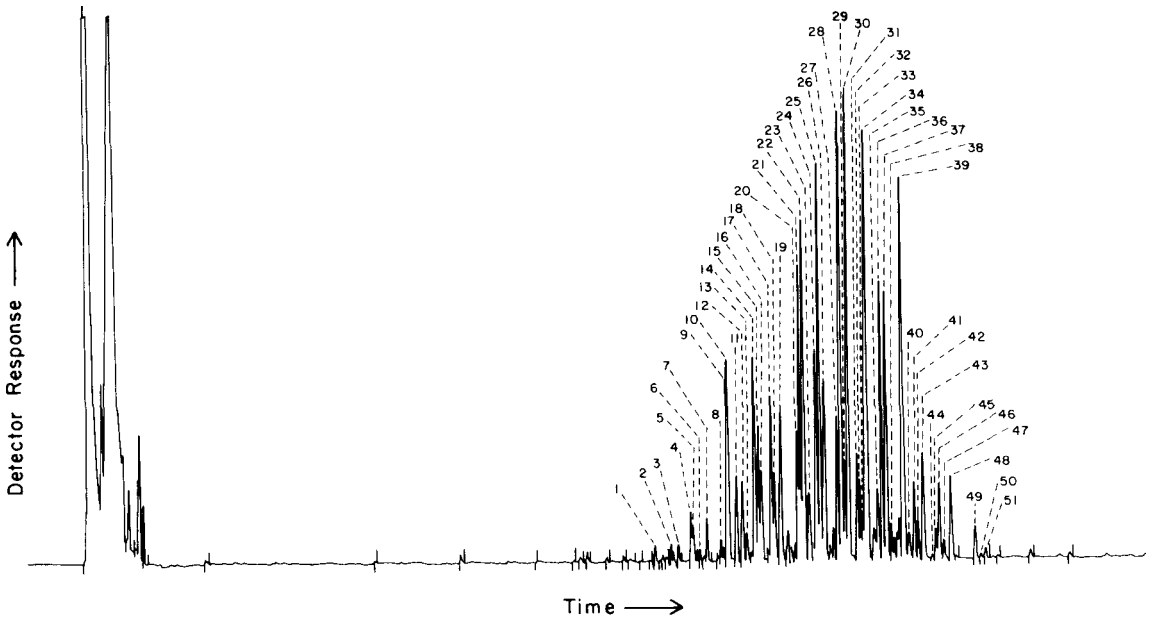


b. Day 28 exposure

Figure 9. Capillary column electron capture gas chromatograms of PF-50 (PCB) fractions from mussels

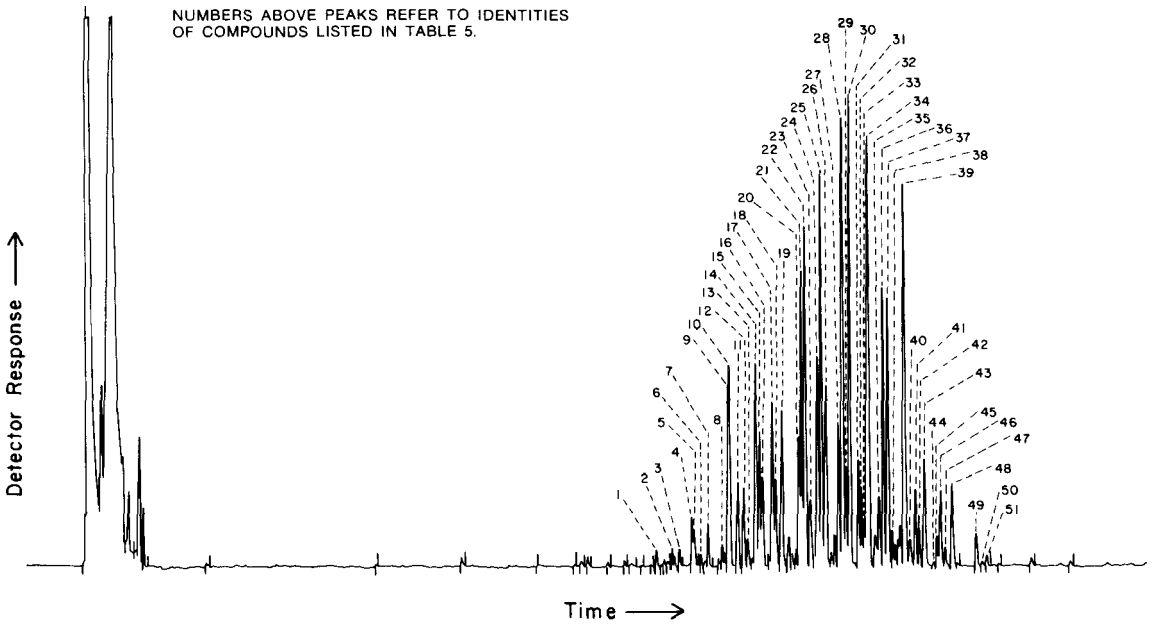


a. Unfiltered water, day 8

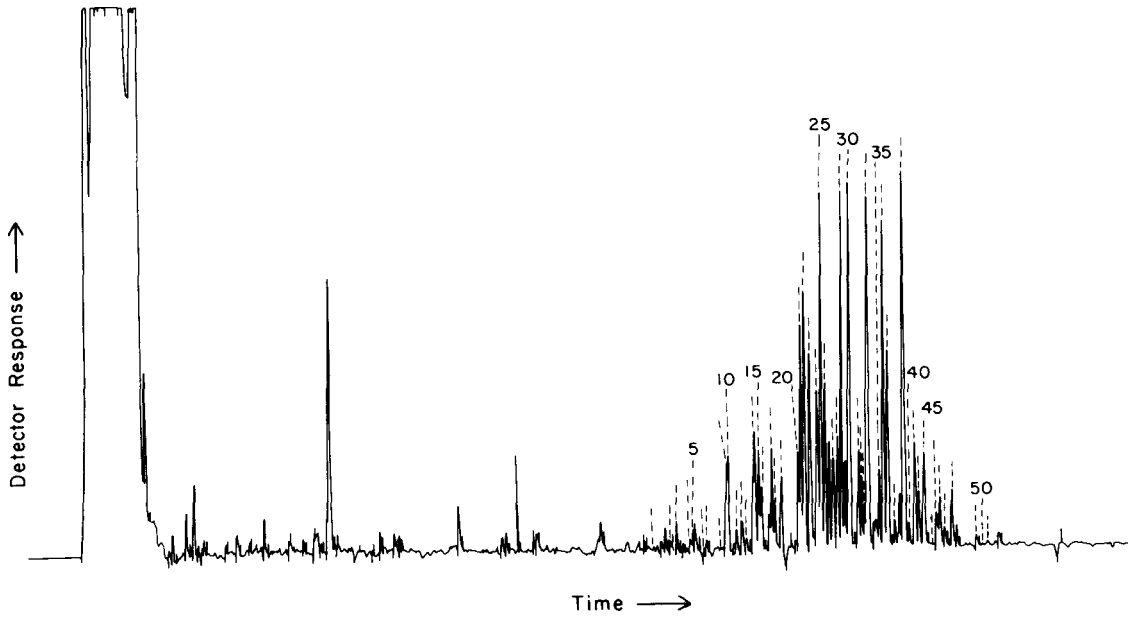


b. Mussel, day 28

Figure 10. Capillary column electron capture gas chromatograms of PF-50 (PCB) fractions from exposure



a. Day 28



b. Day 70

Figure 11. Capillary column electron capture gas chromatograms of PF-50 (PCB) fraction from exposure mussels

PCBs in the BRH material, then, gives the amount of PCBs in the SPM in the organism's guts.

Fe concentration Day 28 Mussels 500 ug/g dry wt mussel (Table 7) - Fe concentration Control Mussels 193 ug/g dry wt mussel (Table 7)

equals

Difference 307 ug/g dry wt mussel

If the assumption is made that all the Fe in the mussel at day 28 results from Fe on the SPM in the gut of the organism,

(307 ug Fe/g dry wt mussel) (1000 mg dry wt BRH sed./29600 ug/Fe) (Table 17)

equals

(10 mg dry wt BRH sed/g dry wt mussel)

(10 mg dry wt BRH sed/g dry wt mussel) (6800 ng A-1254/1000 mg dry dry wt. BRH sed)*

equals

(71 ng A-1254/g dry wt mussel)

Since the increase observed in the concentration of PCBs in the mussels is much larger than this, 28 day mussel = 2800 ng/g (Figure 12), the contribution from PCBs on SPM in the gut of the organisms appears to be almost inconsequential.

64. The concentrations of PCB in mussels exposed for 28 days were divided by the concentrations of PCBs in filtered water samples to obtain bioconcentration factors (BCFs).** These data expressed as Log BCF

* Value from Rogerson et al. (1983).

** Bioconcentration in this report refers to the process of uptake of contaminants from water.

Table 7

Average Trace Metal Concentrations for Mussels collected from the Exposure Chamber on Day 28*

Metal	Mussel 28 Day	Mussel Control
Fe	500 ± 191	193 ± 24
Zn	333 ± 84	178 ± 53
Mn	11 ± 5	12 ± 5
Cu	55 ± 18	12 ± 5
Pb	13.9 ± 4.7	5.0 ± 1.5
Cd	7.0 ± 2.0	2.6 ± 0.4
Cr	25.1 ± 10.7	2.2 ± 1.0

*The control concentrations reported for the mussels are the average of all the control samples and not just day 28. All concentrations are in µg/g dry weight.

(Table 8) were converted from dry wt to wet wt using a common wet to dry conversion factor to facilitate comparisons with estimates of Log BCFs from Geyer et al.(1982). Due to variability in the amount of water in the organism tissues, the authors prefer the use of dry weights from individual samples for calculations, as is done in the remainder of the report. The measured Log BCFs for representative PCB compounds increase with increasing Log P (decreasing aqueous solubility) as observed for Log BCFs with mussels (Ernst 1977) and fish (Veith et al. 1979). In addition, the measured values are in close agreement with estimated Log BCFs (Geyer et al. 1982).

65. The concentrations of compounds in the mussel samples at day 28 (dry weight) and the concentrations of compounds in the unfiltered water samples at day 28 were used to calculate bioaccumulation factors (BAFs).*

$$\text{BAF} = \frac{\text{concentration of individual PCB compound in mussel (dry weight)}}{\text{concentration of individual PCB compound in unfiltered water}}$$

In order to facilitate comparisons of these large values, log BAFs were calculated (Table 9). In spite of considerable differences in the n-octanol/water partition coefficients (Log Ps) for these PCB compounds, the Log BAFs appear to be quite constant. This is in contrast to BCFs (accumulation from water only) from the literature for single compound tests with dissolved components, which show increasing BCFs with

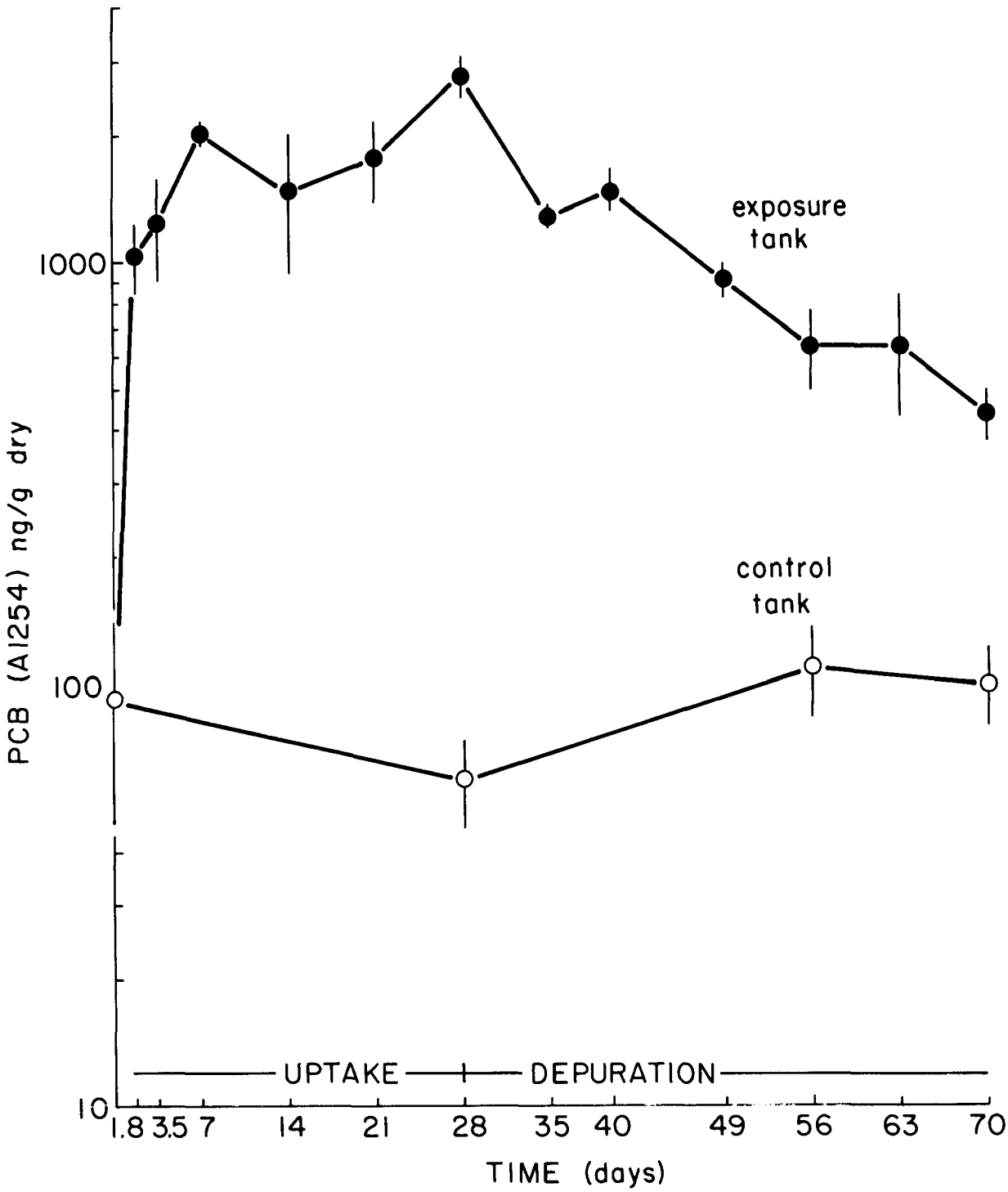


Figure 12. Concentration of total PCBs (as A-1254) in mussels exposed to BRH sediment versus time

Table 8

Estimated and Measured Bioconcentration Factors (BCF) in Mussels at Day 28

Peak No.	PCB Compound	Log P* (Log Kow)	Estimated Log BCF** (wet wt.)	Measured Log† BCF (wet wt.)
4	2,2',5 - trichlorobiphenyl	4.7	3.2	3.2
10	2,4,4', - trichlorobiphenyl	5.0	3.5	3.6
25	2,2'4,5,5'-pentachlorobiphenyl	6.3	4.6	4.2
36	2,2',4,4',5,5'-hexachlorobiphenyl	6.7	4.9	4.7
43	2,2',3,3',4,4'-hexachlorobiphenyl	7.0	5.2	4.8
	PAHs			
	Phenanthrene	4.4	3.0	2.2
	Anthracene	5.3	3.7	2.3
	Fluoranthene	4.9	3.4	2.9
	Pyrene	5.1	3.6	3.1
	Benz(a)anthracene	5.8	4.2	4.0
	Chrysene	6.4	4.7	3.9
	Benz(a)pyrene	6.2	4.5	4.2
	Perylene	6.9	5.1	3.8

* PCB solubilities from Mackay et al. (1980a). PAH solubilities from Mackay et al. (1980b). Solubility converted to Log P using Log P = 5.00 - .67 Log S where S is solubility in μmol/L (Chiou et al. 1977.)

** BCF estimated from log BF = 0.858 x Log Kow - 0.808 from Geyer et al.(1982). (BCF can be substituted for BF.)

† BCF measured from mean of concentrations in three 28-day exposed mussels divided by mean of concentrations in three 28-day filtered water samples from the exposed tank.

decreasing water solubility (increasing Log P) for mussels (Ernst 1977; Geyer et al. 1982). The uniform BAFs observed in the present study probably resulted from the presence of SPM in the dosing system.

66. The constant BAFs observed may have resulted from two processes competing for the dissolved phase contaminants. The first is re-adsorption of dissolved PCB contaminants by the SPM including algae; the second is the bioconcentration of dissolved PCB contaminants by the mussels. If these two distributions vary to approximately the same extent over the range of PCB contaminants, then constancy of BAFs could result. Another possible explanation for the relatively constant BAFs observed in this study is that the mussels accumulate individual PCB compounds by a similar constant process (i.e., transfer from particles across the lining of the gut). This method of accumulation could result in distributions which were very similar to those in the unfiltered water and filter samples if depuration rates for the individual compounds were approximately equal during accumulation. A third possible explanation for the constant BAFs observed in this study is that steady-state values were not reached for all PCB compounds during the uptake period (see discussion of Kinetics).

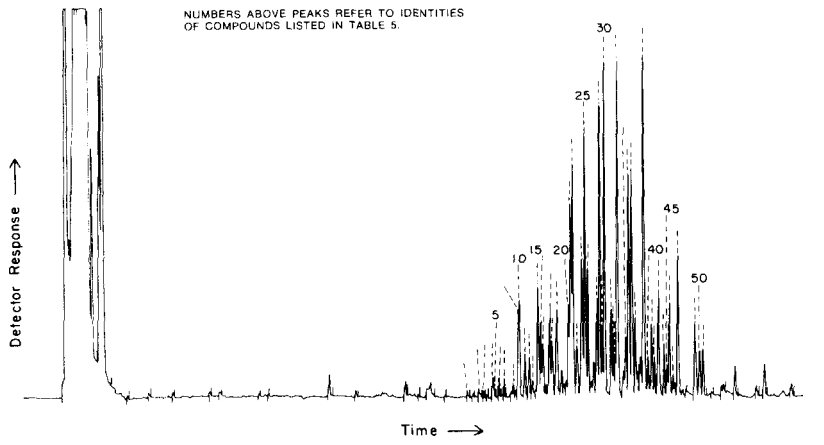
67. The distributions of dissolved (filtrate) and particle-bound (filter) PCBs were compared with those in mussels (Figure 13). The distribution in the water (filtrate) is dominated by low molecular weight compounds and the peak heights of the PCBs decrease with increasing molecular weight. The distribution on the SPM (filter) closely matches that found in mussels (Figure 14), suggesting that PCBs in the SPM influence the distribution found in the mussels.

Table 9

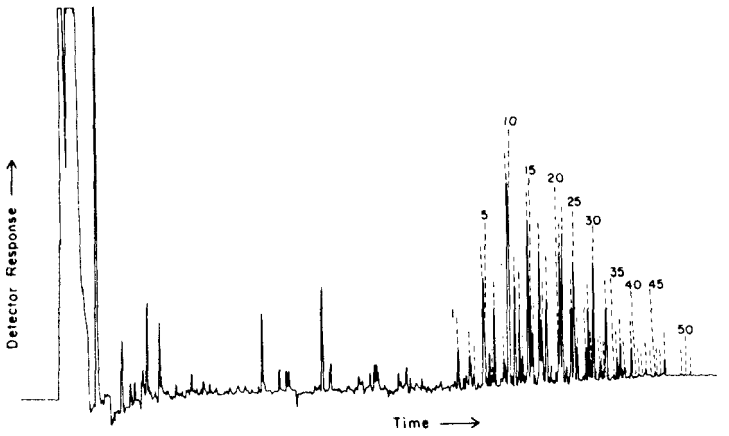
Measured Log BAF* for Each Separate PCB Peak in Exposed Mussels

Peak Number	Log BAF	Peak Number	Log BAF
1	4.3	28	4.5
2	4.4	29	4.6
3	4.5	30	4.5
4	4.4	31	4.5
5	4.4	32	4.5
6	4.1	33	4.6
7	4.4	34	4.5
8	4.6	35	4.5
9	4.5	36	4.5
10	4.4	37	4.5
11	4.4	38	3.9
12	4.4	39	4.4
13	4.4	40	4.2
14	4.4	41	4.4
15	4.5	42	4.4
16	4.5	43	4.4
17	4.4	44	3.4
18	4.4	45	4.3
19	4.5	46	4.2
20	4.5	47	4.4
21	4.4	48	4.1
22	4.4	49	3.0
23	4.4	50	3.3
24	4.5	51	3.8
25	4.4		
26	4.4		
27	4.4		

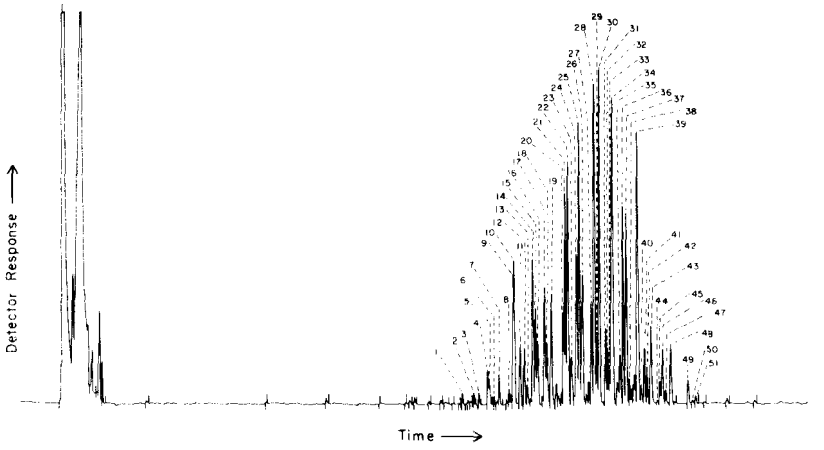
*Method for calculation of Log BAF shown in text.



a. Filter, day 8



b. Filtrate, day 8



c. Mussels, day 28

Figure 13. Capillary column electron capture gas chromatograms of PF-50 (PCB) fraction from exposure

68. Chromatograms from control mussels sampled at day 0, day 28, and day 70 (Figure 15) showed only minor changes between day 0 and day 28. Between day 28 and day 70 an increase in the height of the 2,4,8-trichlorodibenzofuran and tetrachlorodiphenyl ether peak was evident in the control mussels. This increase probably reflects an increased input of these industrial contaminants to the upper Narragansett Bay followed by down Bay transport and entrance of small amounts of these contaminants into our laboratory seawater supply. In addition, a small relative increase in some lower molecular weight PCB compounds was observed in the day 70 control. During the depuration period, a late eluting peak appeared in chromatograms. GC/MS analysis showed that it was not a chlorine or bromine-containing compound. It is probably an electron capturing biological compound. It should be noted that the PCB concentrations in control mussels remained low during the experiment and that these organisms fulfilled their intended purpose as chemical controls by accumulating background concentrations of pollutants from the control seawater.

69. PCB Kinetics. The accumulation and depuration of PCB contaminants (quantified as Aroclor®-1254) are shown in Figure 12. To determine if steady-state was reached during the uptake period, the A-1254 residue concentration in the mussels, and the time data (in days), were entered into a computerized non-linear model in accordance with proposed ASTM recommendations (ASTM 1982).

$$Y = \text{Ln Residue} = P1/(1 + P2 ** (\text{Time} - P3))$$

where Y = natural log of the residue
P1= natural log of the maximum predicted residue concentration
P2= rising slope (0<P2<1)
P3= time in days where Y=0.5*P1

Due to the folded nature of the study (see Methods paragraph 17), six data points were included at day 14. While data scatter in the two 14-day exposure concentrations are of concern (in that other curves fitting the data may be drawn if one or the other sets of "replicate" points are eliminated) when all the data are included, the predicted curve shows that steady-state values were reached during the exposure period (Figure A1). This figure graphs the model prediction utilizing the data from this study. The model prediction shows steady state had been reached by day 7.

70. The depuration* phase of the experiment showed a relatively rapid decrease in the concentration of A-1254 (Figure 12). In general, the concentrations continued to fall until the end of the study at day 70, indicating that depuration was not complete.

71. Concentrations of PCBs as A-1254 in the seawater control tanks showed some variability over the study period, but remained considerably below the levels found in the exposure tank.

72. The uptake and depuration of the individual PCB compounds by the mussels was followed by examining the concentrations of the first 51 peaks shown in Table 5 over the duration of the experiment. The plots of the uptake and depuration of the compounds with time are shown in Figures A2 through A13. The uptake of all compounds appeared to be rapid to day 7. Following that time the uptake seemed to level off.

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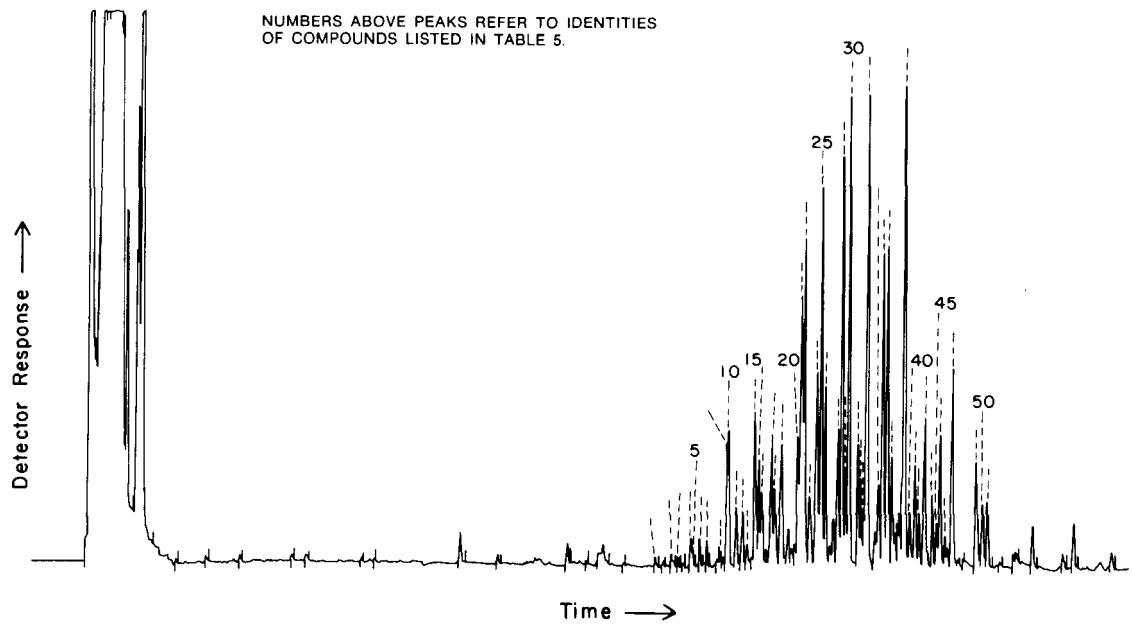
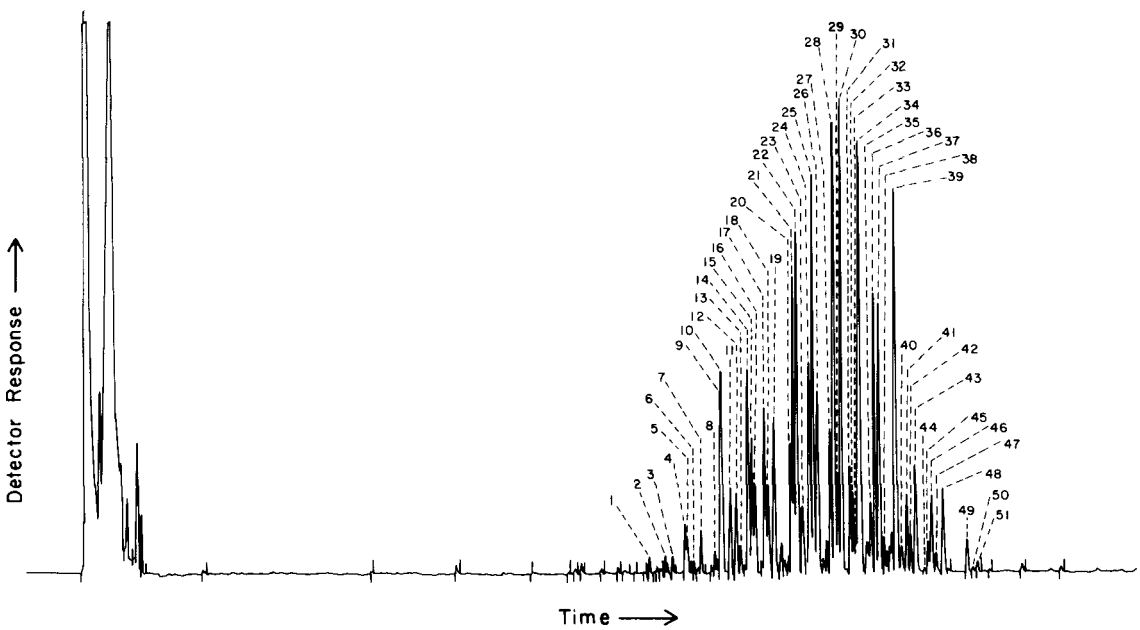
* Includes both depuration and possible metabolic breakdown of compounds.

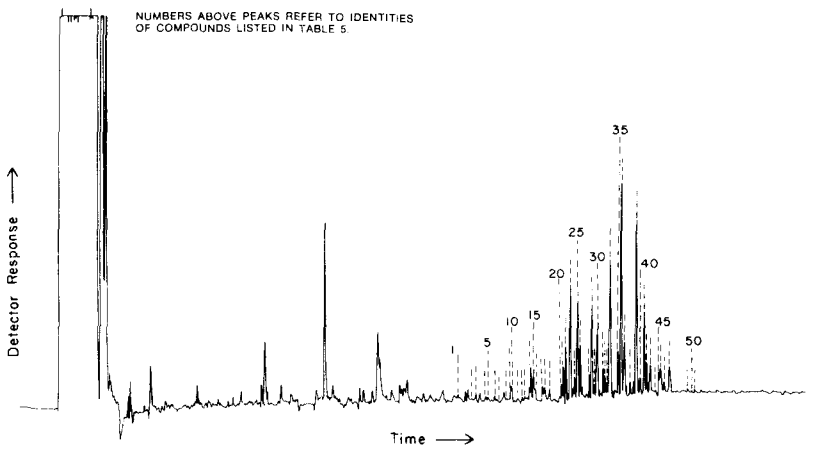


a. Filter, day 8



b. Mussels, day 28

Figure 14. Capillary column electron capture gas chromatograms of PF-50 (PCB) fraction from exposure



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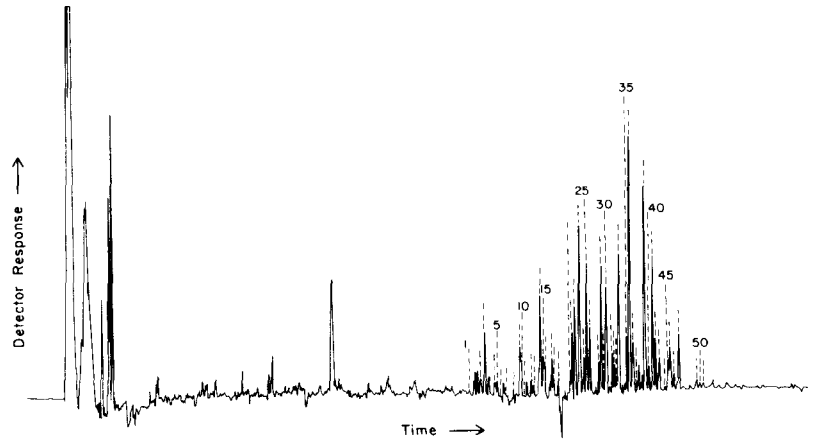
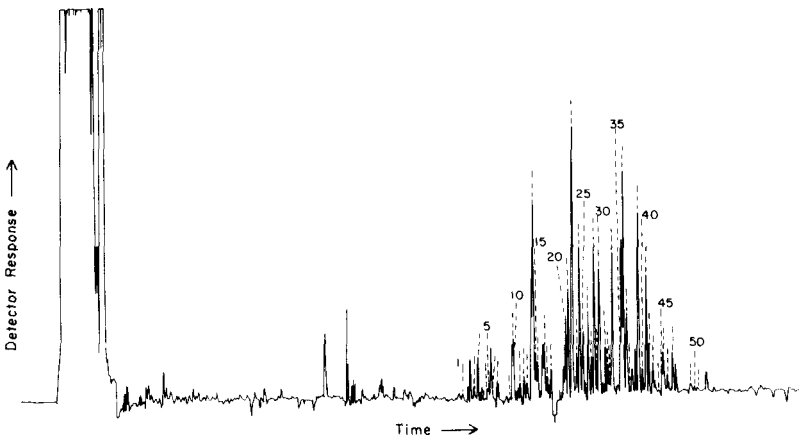


b. Control, day 28



c. Control, day 70

Figure 15. Capillary column electron capture gas chromatograms of PF-50 (PCB) fractions from mussels

73. The kinetics of the depuration phase were examined in detail. For most bioaccumulation studies, first-order kinetic expressions have been applied (Niimi and Cho 1981; Ernst 1977; Veith et al. 1979). For this process, the depuration rate is not dependent on the initial concentration. Another study found that second order kinetics were followed for the elimination of pesticides from catfish (Ellgehausen et al. 1980). In second-order processes the depuration rate is dependent on the initial concentration. Plots of ln C versus time (C= concentration) and 1/C versus time for all the peaks examined during the depuration phase were made. If first-order kinetics were followed, then the plot of ln C versus time should be linear; if second-order kinetics were followed, the plot of 1/C versus time should be linear (Glasstone and Lewis 1960; Ellgehausen et al. 1980). No clear distinction of the order of the kinetics was found in comparisons of the correlation coefficients (Table A1). In addition, scatter of the data during depuration and the impact of slightly elevated levels of PCBs in the exposure tank during depuration (Table 2) precluded a conclusive determination of the order of kinetics.

74. If first-order kinetics are assumed, as was the case in other studies on bioaccumulation (Niimi and Cho 1981; Ernst 1977; Vieth et al. 1979), differences in the depuration rates for the accumulated compounds can be examined. The slopes of lines (the first-order depuration rates) for the compounds examined in this study are shown on Table A2. A subset of eight "representative" compounds was selected from the PCB distributions in mussels for more detailed study. Analysis of covariance was used to test equality of the slopes for the eight compounds (α =0.05)

over different sections of the depuration period. This examination was made over different depuration periods (1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, and 6 weeks) to determine if the depuration rates were constant over the depuration period. The results (Table A2) show a decrease in depuration rate (demonstrated as less negative slopes) with increasing depuration time for most individual compounds. These results may demonstrate the inapplicability of first-order kinetics to describe depuration for some of these compounds. The results of the comparisons of the equality of the slopes (depuration rates) during the depuration time periods are shown in Table A2. The results show that statistical differences between some lines exist ($_{\alpha}$ =0.05) over some of the time periods. Within each depuration period there are 28 possible compound comparisons. Consequently, a very small $_{\alpha}$ level (.05/28) was chosen for each pairwise comparison. This was done to maintain a 5% level of significance for all pairwise comparisons within each depuration period. While differences between other lines were not significant at the same concentration, an observed trend showed that the lower molecular weight compounds were more rapidly depurated than the higher molecular weight compounds. Some higher molecular weight compounds appear to depurate faster than some mid range PCBs during the first weeks of depuration (up to day 56 or 28 days depuration). The slopes of the depuration lines for the different compounds converge as depuration time increases.

75. If all the depuration data are included, those compounds which are resistant to transformation (Zell and Ballschmiter 1980) and with higher chlorination have the slowest depuration rates (shown as less negative slopes in Table A2).

76. PAHs - Seawater. The concentrations of 11 polycyclic aromatic hydrocarbons (PAHs) and one chlorinated pesticide, Ethylan (1,1-dichloro-2, 2-bis (p-ethylphenyl) ethane), in unfiltered water samples (dissolved plus particle-bound compounds) taken during the exposure phase of the mussel bioaccumulation study are shown in Table 10. The levels of these contaminants in control water samples, water samples taken during depuration, and blanks were below the detection limit (<0.1 ng/L for the methods used for extraction and analysis).

77. Extracted ion current profiles (EICPs) result from the GC/MS analysis. These profiles display the concentrations of the major ion for each compound as a function of retention time on the GC column. By examining several of these plots corresponding to different times during the course of the experiment it is possible to determine what relative changes in the content of selected compounds occurred during the experiment.

78. The EICPs for the PAH and Ethylan compounds (which are reported together because they were all analyzed in the same GC/MS analyses) in an unfiltered exposure water sample from the dosing system day 8 are shown in Figure 16. The mass numbers (molecular weight/charge) of fragments characteristic of the compounds (Figure 16) are shown on the right axis.

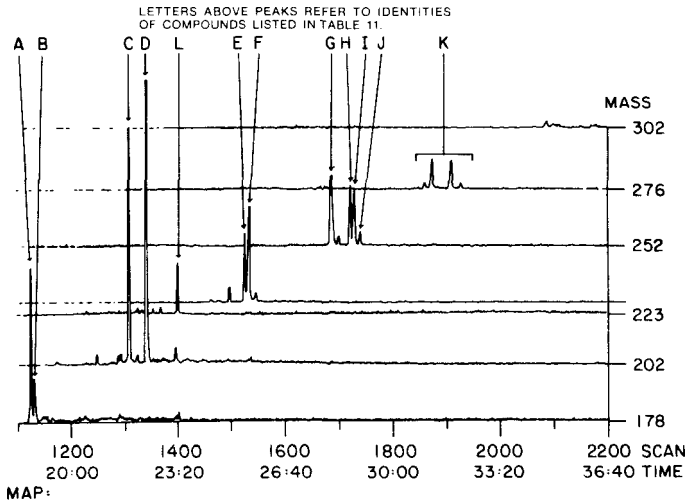
79. Examination of the unfiltered water sample EICP (Figure 16) and the data in Table 10 indicates that the relative distributions of PAH compounds and the Ethylan were fairly consistent over the exposure studies, but the total concentrations of these compounds changed (RSDs (S.D./mean x 100) for PAH compounds were up to approximately 75%). The greater

Table 10

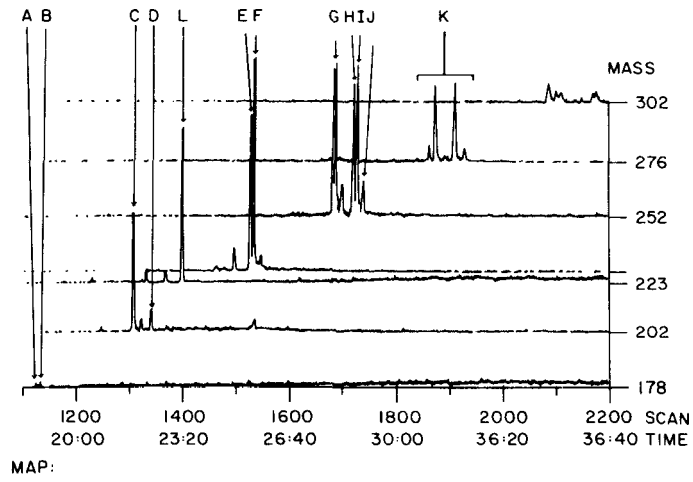
PAH and Ethylan Concentrations in Unfiltered Water Samples*

	Day			
	0	8	14	28
Phenanthrene	6.8	39.6	8.2	28.1
Anthracene	2.3	9.6	2.1	9.2
Fluoranthene	17.0	47.8	11.5	33.5
Pyrene	25.9	74.3	18.5	49.5
Benz(a)anthracene	13.7	33.2	8.7	19.4
Chrysene	20.1	45.5	13.1	29.0
Benzo(b)fluoranthene and/or Benzo(k)fluoranthene	25.4	59.3	16.8	36.7
Benzo(e)pyrene	15.7	34.6	9.4	19.7
Benzo(a)pyrene	16.1	35.9	9.7	22.3
Perylene	2.9	6.4	1.9	4.3
Sum of PAHs with MW of 276	25.2	57.8	16.8	38.4
Ethylan	0.8	1.6	0.6	1.2
SUM-PAHs	171.	444.	117.	290.

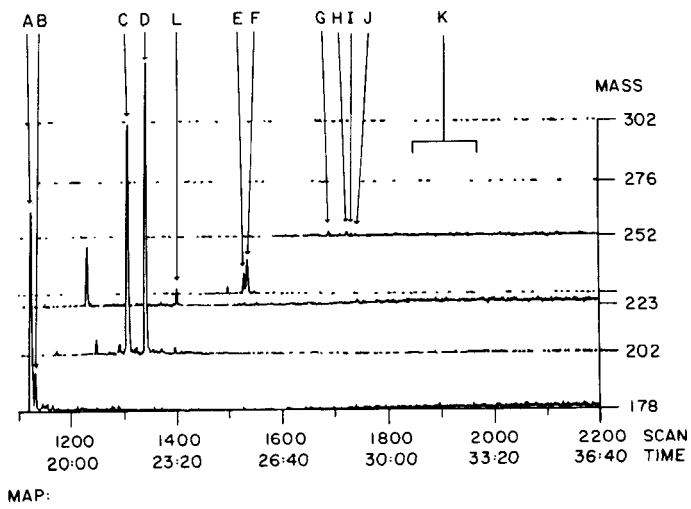
* (in Parts per Trillion)



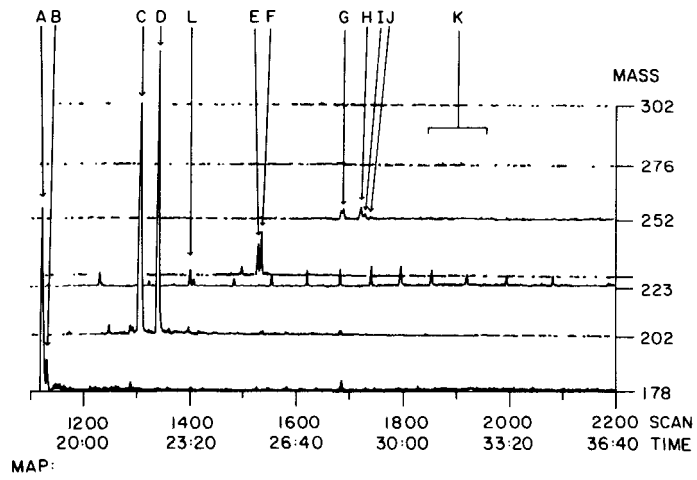
a. Unfiltered water



b. Filter



c. Filtrate



d. Water through continuous flow centrifuge

Figure 16. EICPs from GC/MS analysis of exposure tank

Table 11

PAH Compounds in Mussels

Peak	Chemical ID
A	Phenanthrene
B	Anthracene
C	Fluoranthene
D	Pyrene
E	Benz(a)anthracene
F	Chrysene
G	Benzo(b)fluoranthene and/or Benzo(k)fluoranthene
H	Benzo(e)pyrene
I	Benzo(a)pyrene
J	Perylene
K	Sum of PAHs with MW of 276
L	Ethylan

variability observed for the PAH compounds in water samples than for the PCB compounds (paragraph 54) may reflect variability of the contaminants in the BRH dredged material. It should be noted that soot particles containing high concentrations of PAH compounds may be present in contaminated sediments and that variability in the numbers of these particles in samples may substantially contribute to concentration variability.

80. The EICPs from the GC/MS analyses of unfiltered water, filters, filtrate, and water passing through the continuous flow centrifuge taken on day 8 of exposure are shown in Figure 16. The samples of unfiltered water show a pattern of peaks for the compounds of interest which is very similar to the patterns for the BRH sediment. A similar distribution is observed in the sample from the filter. The EICPs from the filtrate and the water passing through the continuous flow centrifuge show a relative enhancement of the lower molecular weight PAH compounds. As found with the PCB compounds, the PAH compounds appear to distribute in accordance with their solubilities. With lower molecular weight, more soluble PAH compounds are found in the filtrate, and with the higher molecular weight, less soluble compounds are found associated with particles.

81. Data from the analysis of filtered material and filtrates were used to calculate sediment-water partition coefficients, Kp, where

$$Kp = Cs/Cw$$

Cs = concentration of compound in sediment (dry weight)
Cw = concentration of compound in water

Kps were estimated for compounds where the Log n-octanol/water partition

coefficient (Log P) values were known. As observed for the PCB compounds, the estimated results for the PAHs were considerably below the experimental results (Table 12). This may indicate that the desorption of PAH compounds from suspended sediment was not complete (i.e., equilibrium was not reached) during the residence time of the suspensions in the dosing system.

82. PAHs - Mussels. Examination of the EICPs from mussels at day 0 showed phenanthrene, fluoranthene and pyrene, benz[a]anthracene, benzo[k] and/or benzo[b]fluoranthene, benzo[e]pyrene, and benzo[a]pyrene and perylene (Figure 17). Ethylan was not found in these background samples. At the first sampling period (day 1.8), the abundance of the above compounds had increased and anthracene, Ethylan, and some PAHs with MW 276 were apparent (Figure 17). Comparison of this EICP with one from the BRH material shows that mussels had a relatively lower concentration of peaks G, H, I, J, and K than was present in the sediment (Figure 17). This same general pattern of peaks is observed in all other mussel samples taken during exposure (Figures 17 and 18). Following 7 days of depuration, the lower molecular weight peaks A, B, C, and D had decreased considerably while peaks E through K had become more prominent (Figure 19). The selective depuration of the lower molecular weight peaks may result from the higher depuration rates associated with more water-soluble compounds (Ernst 1977). As depuration continued, general decreases in the concentrations of all compounds were observed (Figures 19 and 20).

Table 12

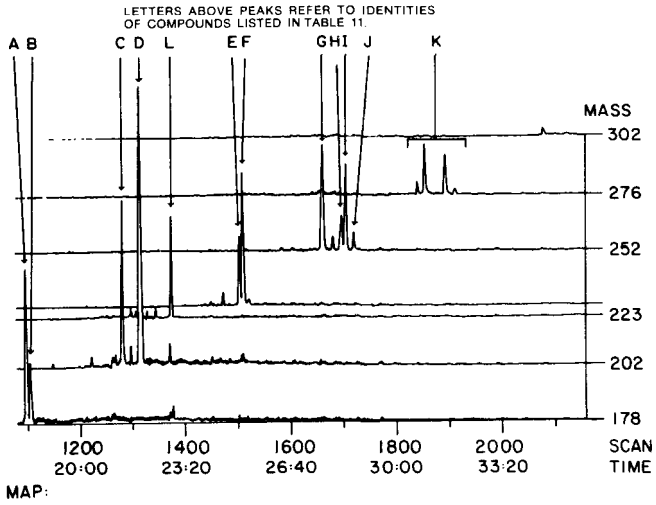
Comparison of Experimental and Estimated Sediment/Water Partition Coefficients (Kps)

PAH Compound	Log P*	Kp Experimental**	Kp Estimated†
Phenanthrene	4.4	$.27 \times 10^5$	$.009 \times 10^5$
Anthracene	5.3	$.23 \times 10^5$	$.066 \times 10^5$
Fluoranthene	4.9	$.71 \times 10^5$	$.048 \times 10^5$
Pyrene	5.1	$.79 \times 10^5$	$.031 \times 10^5$
Benz(a)anthracene	5.8	4.7×10^5	$.23 \times 10^5$
Chrysene	6.4	4.1×10^5	$.87 \times 10^5$
Benz(a)pyrene	6.2	$24. \times 10^5$	$.60 \times 10_5$
Perylene	6.9	$17. \times 10^5$	2.8×10^5

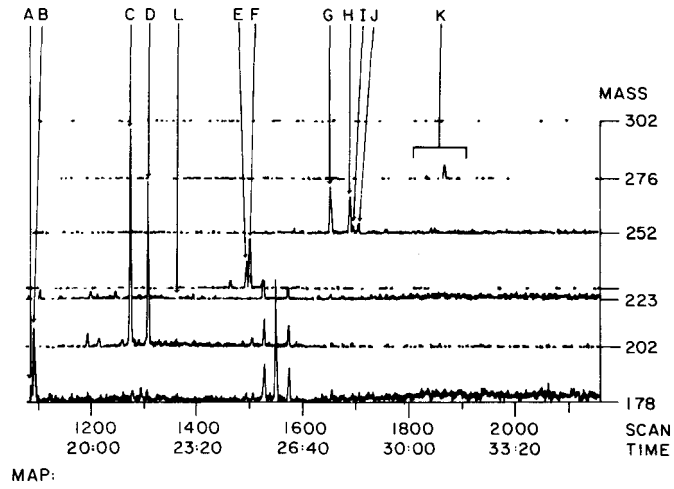
* Solubility from Mackay et al. (1980a) converted to Log P using Log P = 5.00 .67 Log S where S is solubility in µmol/L (Chiou et al. 1977).

**Kp estimated as mean of 3 Kps determined on day 28 from mussel exposure tank.

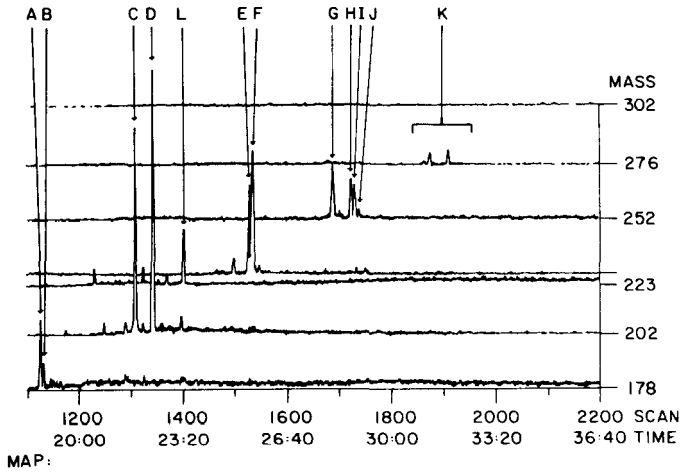
† Kp estimated as in Appendix Table A-1.



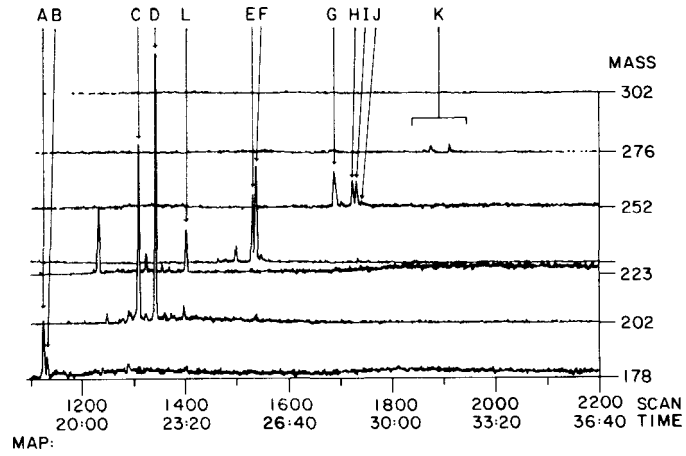
a. BRH sediments



b. Mussels, day 0

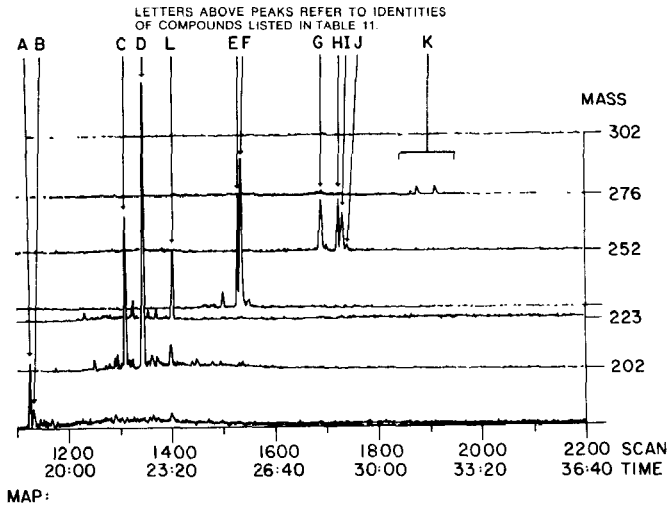


c. Exposure tank mussels, day 1.8

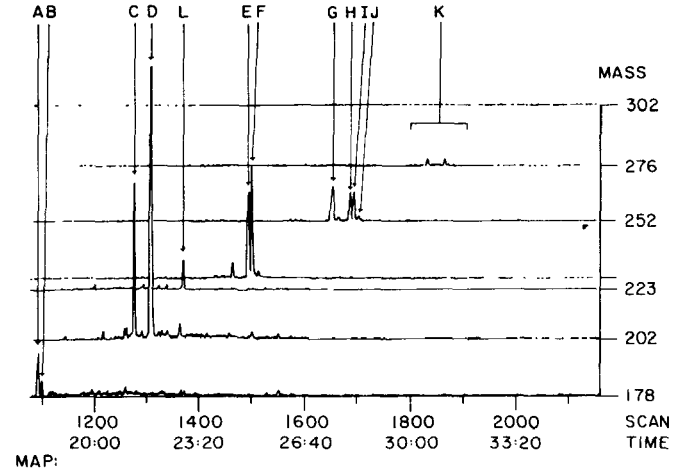


d. Exposure tank mussels, day 3.5

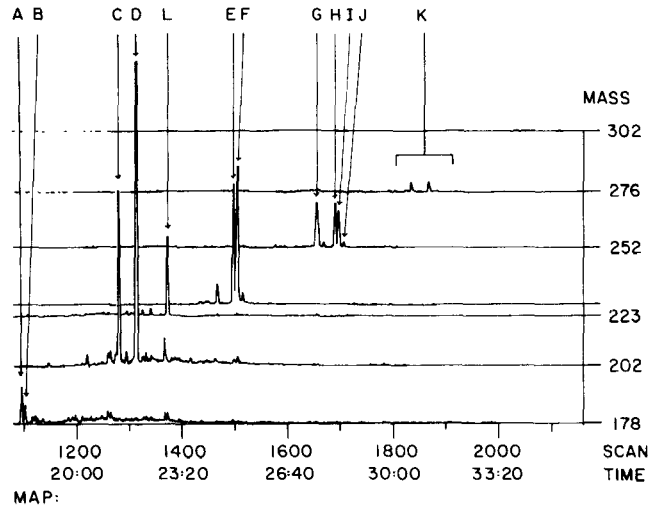
Figure 17. EICPs from GC/MS analysis



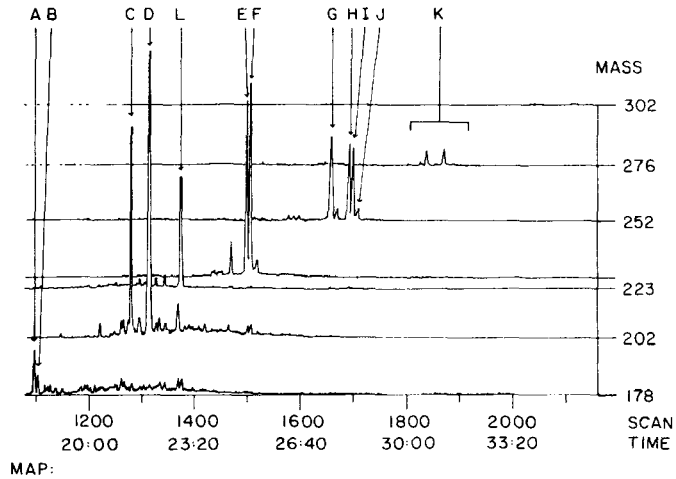
a. Day 7



b. Day 14

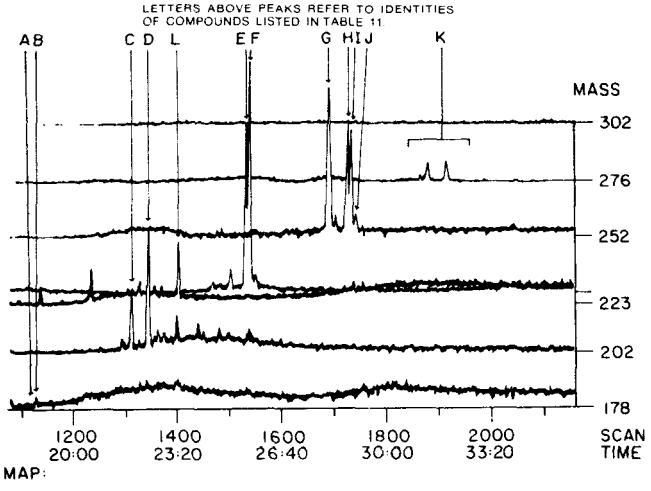


c. Day 21

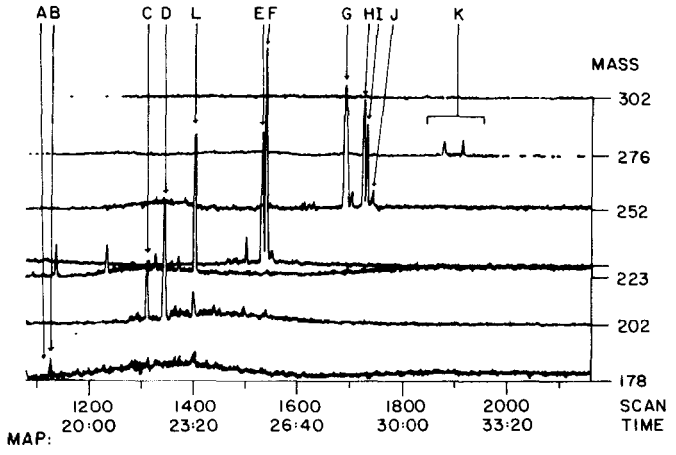


d. Day 28

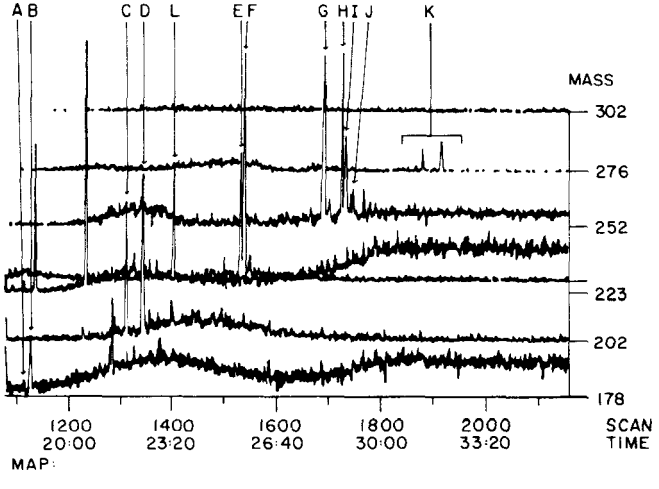
Figure 18. EICPs from GS/MS analysis of exposure tank mussels



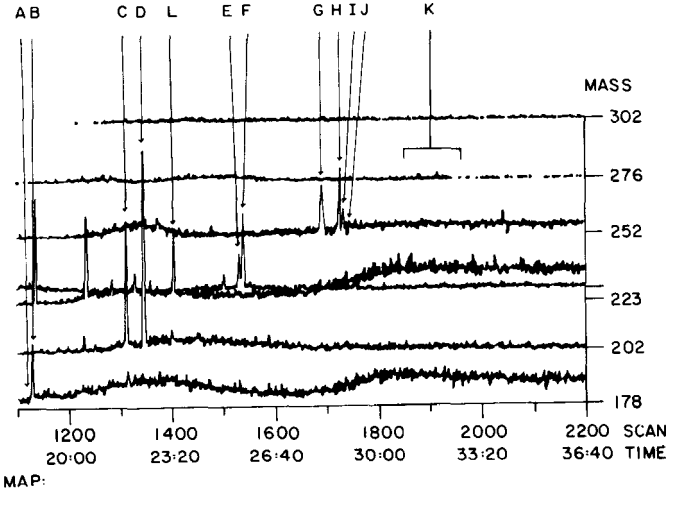
a. Day 35



b. Day 40

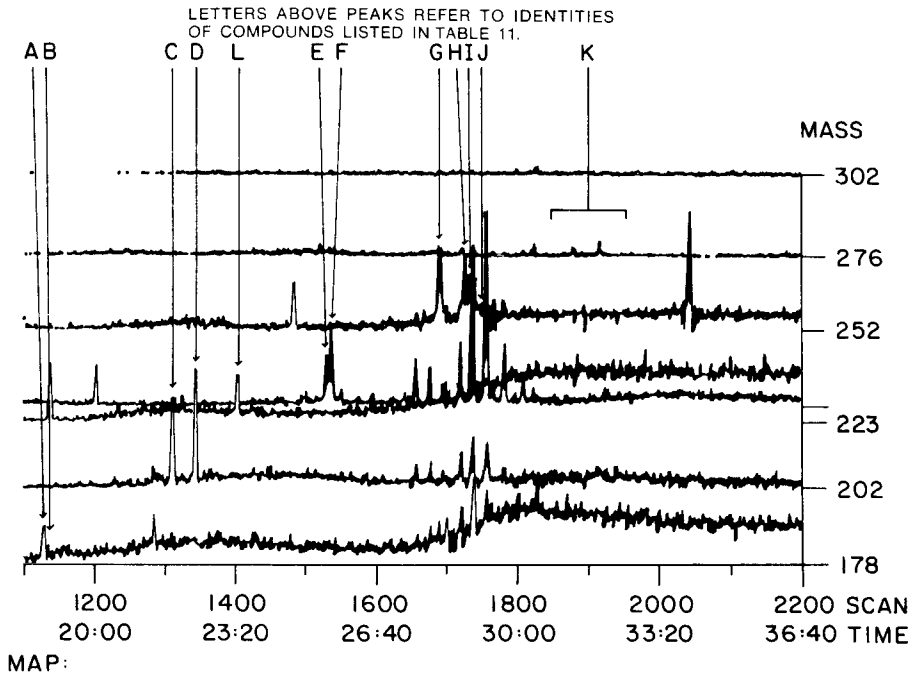


c. Day 49

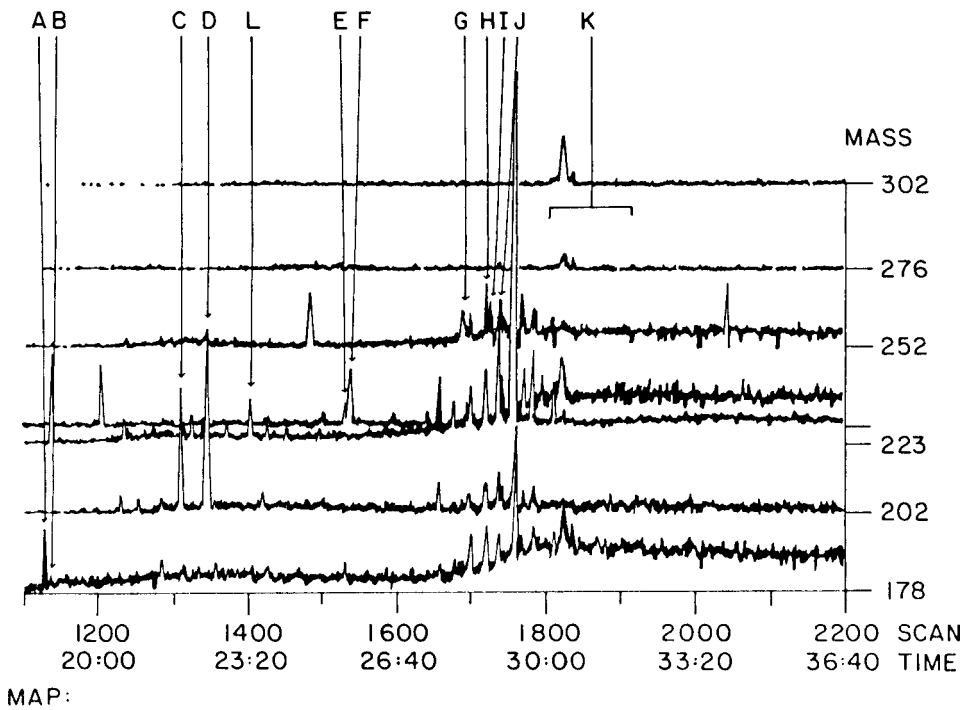


d. Day 56

Figure 19. EICPs from GC/MS analysis of exposure tank mussels



a. Day 63



b. Day 70

Figure 20. EICPs from GC/MS analysis of exposure tank mussels

Table 13

Estimated and Measured Bioconcentration Factors (BCF) in Mussels at Day 28

Peak No.	PCB Compound	Log P* (Log Kow)	Estimated Log BCF** (wet wt.)	Measured Log† BCF (wet wt.)
4	2,2',5 - trichlorobiphenyl	4.7	3.2	3.2
10	2,4,4', - trichlorobiphenyl	5.0	3.5	3.6
25	2,2'4,5,5'-pentachlorobiphenyl	6.3	4.6	4.2
36	2,2',4,4',5,5'-hexachlorobiphenyl	6.7	4.9	4.7
43	2,2',3,3',4,4'-hexachlorobiphenyl	7.0	5.2	4.8
	PAHs			
	Phenanthrene	4.4	3.0	2.2
	Anthracene	5.3	3.7	2.3
	Fluoranthene	4.9	3.4	2.9
	Pyrene	5.1	3.6	3.1
	Benz(a)anthracene	5.8	4.2	4.0
	Chrysene	6.4	4.7	3.9
	Benz(a)pyrene	6.2	4.5	4.2
	Perylene	6.9	5.1	3.8

* PCB solubilities from Mackay et al. (1980b). PAH solubilities from Mackay et al. (1980a). Solubility converted to Log P using Log P - 5.00 - .67 Log S where S is solubility in μmol/L (Chiou et al., 1977)

** BCF estimated from log BF = 0.858 x Log Kow - 0.808 from Geyer et al. (1982).

† BCF measured from mean of concentrations in three 28-day exposed mussels divided by mean of concentrations in three 28-day filtered water samples from the exposed tank.

83. The mean concentrations of PAH compounds in mussels exposed for 28 days were divided by the concentrations of PAHs in filtered seawater samples to obtain bioconcentration factors (BCFs) (Table 13). As with the PCBs (paragraph 64) these data are expressed in Log form on a wet weight basis to facilitate comparisons with estimates of Log BCFs from Geyer et al. (1982). The measured Log BCFs for the PAH compounds increase with increasing Log P (decreasing aqueous solubility) as observed for Log BCFs with mussels (Ernst 1977) and fish (Veith et al. 1979). For PAHs the measured values are not as close to the estimated values as were the PCBs (Table 13).

84. The mean concentration of PAH and Ethylan compounds in the mussels at day 28 (Table 14) and the mean concentration of these compounds in unfiltered water at day 28 (Table 10) were used to calculate bio-accumulation factors (BAFs). The Log BAFs and the Log Ps are shown in Table 15. The compounds with lower Log Ps showed lower BAFs. Benz(a) anthracene and chrysene showed the highest BAF values in the mussels while the higher molecular weight PAH compounds were accumulated less effectively. The pesticide Ethylan showed a relatively high BAF compared to the PAH compounds. Since organisms were not gut depurated prior to analysis, the PAH content of the organisms included a contribution from sediment in the gut (see discussion under PCBs, paragraph 63). The levels of PAH contaminants found in control mussels were low and remained relatively constant during the dosing period. Ethylan was not found in control samples (Table 16).

85. The uptake and depuration of total PAH compounds during the mussel exposure study are shown in Figure 21. This plot was made using

Table 14

PAH and Ethylan Concentrations in Exposed Mussels Expressed as ng/g (dry)*

	Day										
	- - - - - - - - Exposure- - - - ->| - - - - - - - - -Depuration - - - - - - - - - - ->										
Peak	0	7	14	21	28	35	40	49	56	62	70
Phenanthrene	8.69	162.	130.	246.	130.	10.7	13.6	10.1	23.2	9.65	10.9
Anthracene	.488	63.6	49.6	73.0	59.8	4.52	4.81	2.75	5.43	2.17	2.57
Fluoranthene	17.4	802	444	475	698	59.1	37.6	19.8	17.9	23.4	20.1
Pyrene	15.8	1359	811	1117	1228	175	95.3	38.8	35.0	41.7	33.3
Benz(a)anthracene	2.72	754	448	512	864	285	153	35.9	9.76	22.8	4.99
Chrysene	6.63	1005	650	856	1179	435	270	75.4	25.2	40.6	16.0
Benzo(b)fluoranthene and/or Benzo(k)fluoranthene	9.82	631	408	543	895	472	288	101	29.0	73.1	12.8
Benzo(e)pyrene	3.96	288	234	301	379	249	161	59.0	22.6	23.5	0
Benzo(a)pyrene	.532	269	216	350	392	226	120	31.1	7.01	16.1	0
Perylene	1.12	30.7	25.5	60.3	44.0	23.9	18.4	4.30	4.13	4.26	0
Sum of PAHs with MW of 276	3.66	261	170	111	348	141	82.7	21.4	7.57	20.6	5.97
Ethylan	0	452.	177.	317.	444.	142.	154.	29.0	14.5	20.3	8.8
Sum of PAH Compounds	70.8	5630	3590	4640	6220	2080	1250	399	187	278	107

* Values not corrected for blank value.

Table 15

Mussel Bioaccumulation Factors (Calculated for Day 28)

Peak	Log BAF	Log P*
Phenanthrene	3.7	4.4
Anthracene	3.8	5.3
Fluoranthene	4.3	4.9
Pyrene	4.4	5.1
Benz(a)anthracene	4.7	5.8
Chrysene	4.6	6.4
Benzo(b)fluoranthene and/or Benzo(k)fluoranthene	4.4	
Benzo(e)pyrene	4.3	
Benzo(a)pyrene	4.2	6.2
Perylene	4.0	6.9
Sum of PAHs with MW of 276	4.0	7.0
Ethylan	4.6	

* P = n-octanol/water partition coefficient obtained from solubility data in (Mackay et al. 1980a). Converted to Log P using Log P = 5.00-.67 log S where S is solubility in µmol/l (Chiou et al. 1977).

Table 16

Levels of PAH and Ethylan Compounds in Control Mussels During Study (PPb; (ng/g(dry))

	Day			
	Exposure - - - ->		Depuration - - - - - - ->	
Peak	0	28	56	70
Phenanthrene	8.69	6.36	5.70	6.22
Anthracene	.488	.697	1.20	.383
Fluoranthene	17.4	6.12	11.7	7.78
Pyrene	15.8	9.31	13.6	13.4
Benz(a)anthracene	2.72	1.13	.852	.911
Chrysene	6.63	3.12	7.95	5.80
Benzo(b)fluoranthene and/or Benzo(k)fluoranthene	9.82	3.84	1.62	2.14
Benzo(e)pyrene	3.96	2.07	4.56	3.92
Benzo(a)pyrene	.532	.621	.399	.075
Perylene	1.12	.355	.221	.075
Sum of PAHs with MW of 276	3.66	2.28	2.01	.524
Ethylan	0	0	0	0
Sum-PAHs	70.8	35.9	49.8	41.2

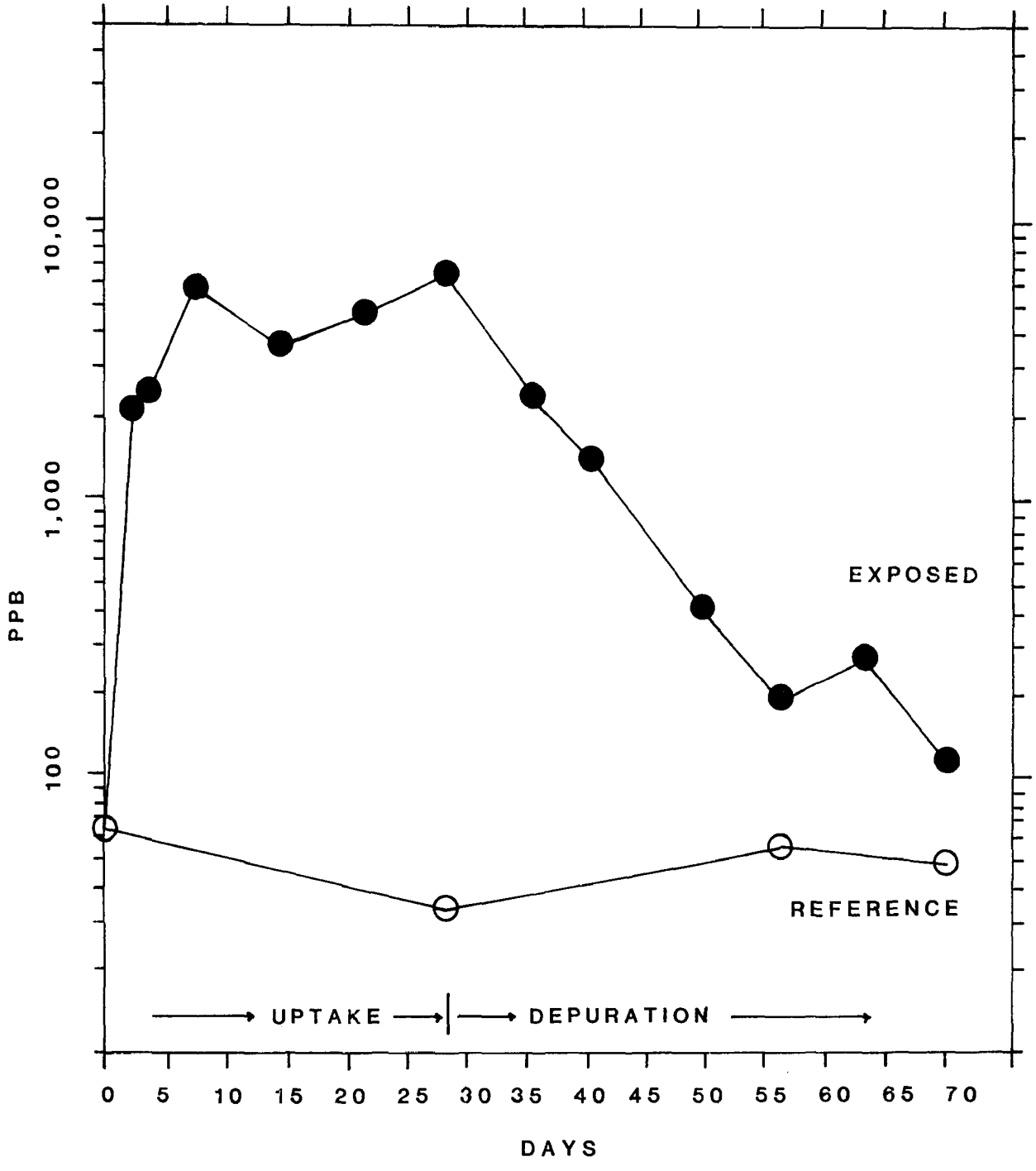


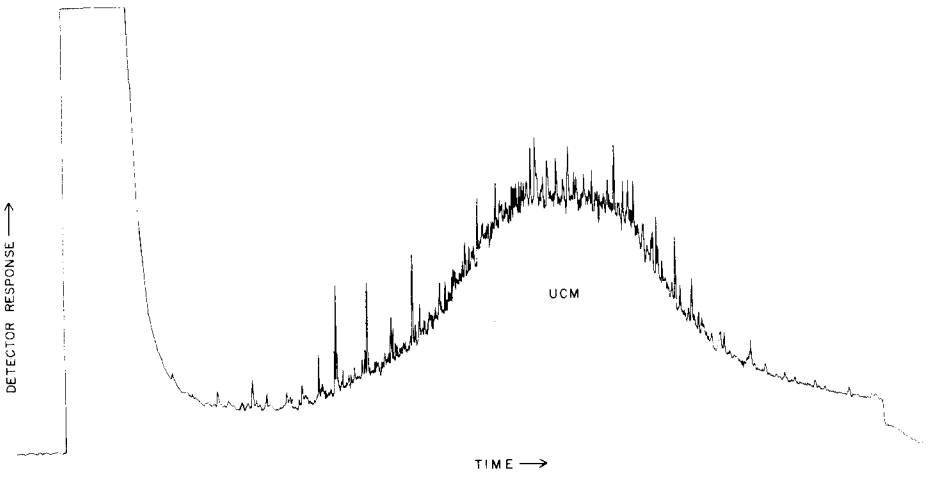
Figure 21. Concentration of sum of parent PAHs in mussels exposed to BRH sediment versus time

the sums of the concentrations of the eleven PAH compounds listed in Table 14 and shows a rapid uptake of PAH compounds to day 7 of the exposure. Following day 7 concentrations fluctuated until the end of the exposure at day 28. Depuration of the PAH compounds was rapid during the first week followed by several weeks of slower depuration. By day 70 the concentrations of most compounds had decreased to approximately two to three times their day 0 levels indicating that depuration was not complete.

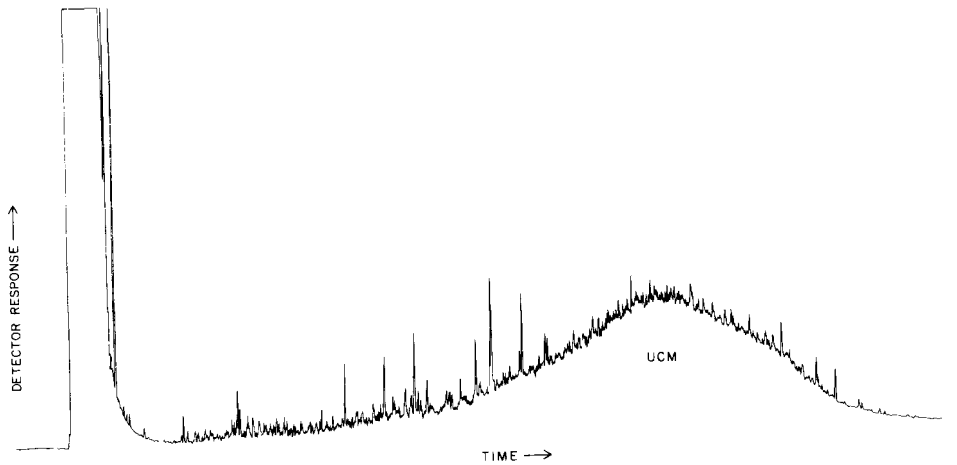
86. Figures A18 to A20 show the uptake and depuration of the PAHs and the Ethylan. Most of the curves showed maximums at day 7 or day 28. The curves for fluoranthene and pyrene showed maximum concentrations at day 7, but in general larger PAH compounds showed maximum concentrations at day 28.

87. During the depuration phase, the lower molecular weight PAH compounds appeared to be depurated more rapidly that the higher molecular weight PAH compounds. The rapid depuration of more soluble (lower Log P) compounds by mussels has been observed in another study (Ernst 1977). In general, the higher molecular weight PAH compounds (higher Log P) took longer to reach their maximum level and were depurated more slowly than the lower molecular weight (lower Log P) compounds. Ethylan which was not detected in control organisms was accumulated and depurated similar to chrysene.

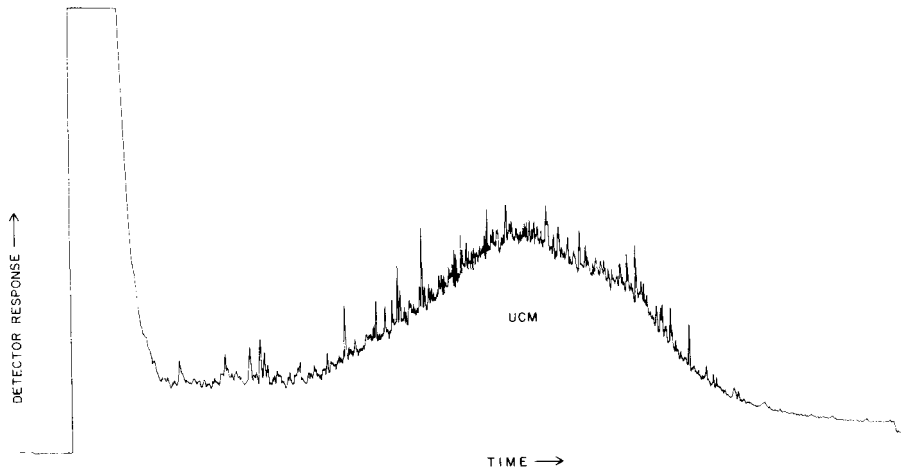
88. Petroleum Hydrocarbons - Mussels. The petroleum hydrocarbons from samples were detected as a large mound in flame ionization detection gas chromatograms (Figure 22). This mound of material, usually referred to as an unresolved complex mixture (UCM), consists of numerous petroleum



a. Exposure tank water, day 0



b. BRH sediment



c. Mussels, exposute day 28

Figure 22. Capillary column flame ionization detector gas chromatograms of PF-50 (contains mostly straight chain, branched, and cyclic saturated hydrocarbons)

hydrocarbons (i.e., alkanes, cycloalkanes). The petroleum hydrocarbons found in unfiltered water samples from the dosing system showed a distribution as an unresolved complex mixture (UCM) which was slightly lower in molecular weight, but otherwise similar to the distribution found in the BRH sediments (Figure 22). During the uptake phase the mussels accumulated a UCM which was slightly lower in molecular weight than the distribution found in the unfiltered water.

89. The petroleum hydrocarbons followed the same general pattern of concentration changes observed during the uptake and depuration for the other organic contaminants in mussels (Figure 23). By day 7 the contaminants were near their maximum values. At day 14 and day 21 lower concentrations were observed with the highest concentration found at day 28. The first week of depuration showed a rapid loss of total petroleum hydrocarbon contaminants followed by a plateau phase of decreased loss rates. This behavior for the depuration of petroleum hydrocarbons has been observed in other studies with bivalve molluscs (Lee et al. 1972; Clark and Finley 1975; Fossato 1975; Lake and Hershner 1977). Chromatograms from control mussels showed a low level of petroleum hydrocarbons (as a UCM). Petroleum hydrocarbons in mussels from the control tank showed slight concentration decreases during the study period (Figure 23).

Inorganic Contaminants

90. Sediment. The trace metal composition for the barrel of BRH sediment analyzed is given in Table 17. The wet-to-dry-weight

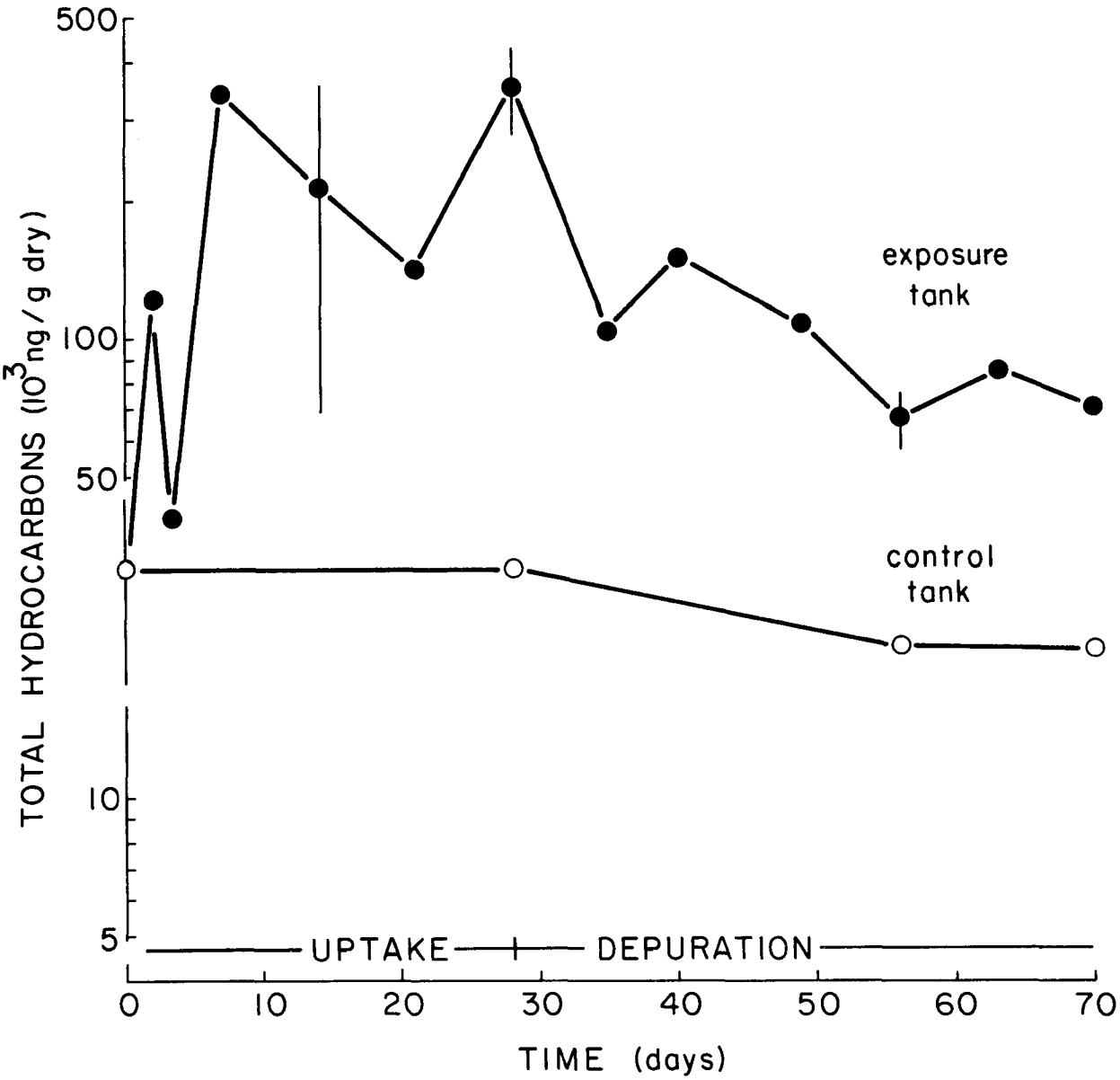


Figure 23. Concentration of total petroleum hydrocarbons in mussels exposed to BRH sediment versus time

Table 17

Average Trace Metal Concentrations for Black Rock Harbor Sediment Samples Expressed as µg/g Dry Weight

Metal	µg/g	Std. Dev.	% Std. Dev.
Fe	29600	809	2
Zn	1200	59	4
Mn	359	37	10
Cu	2380	112	4
Pb	378	16	4
Cd	23.4	0.9	4
Cr	1430	77	5
Ni	139	4	3
Hg	1.7	0.1	4
Wet/Dry	3.22	0.02	0.6

Table 18

Seawater Metal Concentrations Determined for the Black Rock Harbor Sediment Exposure and Control Chambers Expressed as µg/liter

Metal	Exposure Chamber: Prerenewal of BRH Slurry	Exposure Chamber: Postrenewal of BRH Slurry	Control Chamber
Fe	288	343	5.2
Std. Dev.	4.2	9.9	0.8
Zn	25.5	22.0	5.6
Std. Dev.	0.4	0.4	0.7
Mn	4.8	4.8	0.7
Std. Dev.	0.2	0.5	0.5
Cu	34.8	34.6	1.4
Std. Dev.	0.2	0.4	0.3
Pb	4.5	4.5	<1
Std. Dev.	0.5	0.5	
Cd	0.82	0.7	<0.2
Std. Dev.	0.1	0.2	
Cr	16.6	17.8	<0.2
Std. Dev.	0.2	0.2	

* Seawater samples were collected 2 hr before renewal of the Black Rock Harbor sediment slurry and 3 hr post-renewal of the slurry.

ratio is also given for the BRH sediment samples. No values for As are listed in this table since a chemical interference was detected during the analysis (for both HGA-AA and MHS-1 hydride generation techniques) of these sediment samples. The results indicate that BRH sediment samples are reasonably homogeneous for a given barrel if precautions are taken to re-mix each barrel before sampling.

91. Seawater. The results for the monitoring of the mussel exposure system are given in Table 18. Only the total acid leachable concentrations present in the unfiltered seawater are reported. Concentrations for the elements of interest in the control chamber were generally undetectable by direct injection of seawater samples into the HGA unit; however, seawater samples from the BRH exposure chamber could be analyzed by this method. The results show that the concentrations for most of the metals are reasonably consistent for the two sets of samples collected prerenewal and post-renewal of the BRH sediment slurry.

92. It is important to know if the concentrations of the various metals determined in the exposure chamber agree with the measured particulate concentrations that were delivered by the dosing system. The total concentration of BRH sediment delivered into the exposure chamber can be calculated from the Fe concentrations presented in Table 18 assuming Fe is conservative. Using these data, the calculated concentrations for BRH sediment are 10.8 and 12.8 mg/L for the prerenewal samples and postrenewal samples, respectively. The values actually measured by filtration of seawater samples from the exposure chamber

Table 19

Average Fe/Metal Ratios (± Standard Deviation) for the Exposure Chamber Seawater and Black Rock Harbor Sediments

Fe/Metal	BRH Sediment	Exposure Chamber Seawater
Fe/Zn	24.8 ± 0.9	17.7 ± 2.4
Fe/Mn	80.9 ± 7.9	77 ± 17
Fe/Cu	12.4 ± 0.5	9.1 ± 1.0
Fe/Pb	78.4 ± 2.6	70 ± 8
Fe/Cd	1270 ± 45	420 ± 156
Fe/Cr	20.8 ± 0.98	18.4 ± 1.9

during the course of the exposure period range from 8.19 to 10.33 mg/L. The values determined for the control chamber during this same period range from 1.45 to 2.02 mg/L. The calculated concentration of BRH sediments in the exposure chamber are, therefore, only 10 to 20 percent higher than the actual range of concentrations determined over the entire time course of the experiment. Iron was used to make these calculations because Fe has the highest concentration of any metal measured in BRH sediment and should be affected less by contamination during seawater sample analysis.

93. The inter-elemental ratios of the metals (i.e. Fe/metal) are given in Table 19. This table also contains the Fe/metal ratios of the BRH sediment samples. Theoretically the Fe/metal ratios determined in the seawater samples should agree with the Fe/metal ratios for the BRH sediment samples. There are, however, some difficulties with this concept. The seawater metal concentrations determined were analyzed at concentrations 1000 times lower than were determined in the bulk BRH sediment samples. Therefore, detection limits and contamination of seawater samples could affect the metal concentrations and their subsequent Fe/metal ratios. This is important since the measured metal concentrations are used to determine bioaccumulation factors of metals for the exposed mussel samples. The Fe/metal ratios for the exposure chamber seawater samples presented in Table 19 are the average values for the prerenewal and postrenewal of the BRH slurry. The Fe/metal ratios for the BRH sediment samples were calculated from the data presented in Table B1. The BRH sediment Fe/metal ratios are the average of the individual ratios for the nine samples. The Fe/metal ratios for the

seawater samples compare favorably with the BRH sediment ratios for all elements listed except Cd. The Fe/Cd ratio is different from the sediment ratio by a factor of 3. This indicates that a secondary source of Cd was present in the exposure tank or that the seawater samples were contaminated with Cd during collection. The detection limit for Cd in seawater using the present analytical techniques is about 0.1 to 0.3 µg/L. The concentration of Cd determined in the exposure chambers was 0.8 and 0.7 µg/L for the two sets of samples collected. Therefore, the concentration of Cd determined in the exposure chamber was very close to the analytical detection limit and this probably accounts for the factor of 3 difference in the ratio compared to the BRH Fe/Cd sediment ratio. Also, the ratio of Fe/Zn was corrected for the Zn concentration determined in the control tank (i.e., 5.4 µg/L). This Zn concentration is probably due to the large amount of PVC piping that is used in this facility to carry seawater from Narragansett Bay to the various laboratory seawater exposure experiments. Zinc is used as a catalyst in the production of PVC plastic. The concentration of Zn for Narragansett Bay at a site near the ERL-N is about 1 to 2 µg/L.

94. The concentration of Hg could not be detected in either the control chamber or the exposure chamber. The exposure chamber should have a Hg concentration of approximately 0.02 µg/L. This Hg concentration was calculated by dividing the average Fe concentration in the exposure chamber seawater samples by the Fe/Hg average ratio for the BRH sediment samples. The detection limit for Hg with our present analytical equipment is 0.05 µg/L.

95. Arsenic was not determined in the control or exposure chamber seawater samples. The As concentration for BRH sediments (provided by New England Division, Corps of Engineers) is 6.1 mg/kg. The calculated ratio of Fe/As for BRH sediments using the average Fe concentration from Table B1 is 4850. The theoretical As seawater exposure chamber concentration can be calculated by dividing the Fe seawater concentration with this calculated Fe/As ratio value. The calculated As concentration due to the addition of 10 mg/L of BRH sediment to the exposure chamber is 0.07 ug/L. The natural concentration of As in seawater is approximately 1 to 2 ug/L. Therefore, the natural seawater concentration of As is about 15 times greater than the BRH sediment As added to the exposure chamber.

96. Mussels. The average metal concentrations and standard deviations for the mussel samples collected from the BRH exposure chamber on day 28 are given in Table 7. All of the inorganic data used to calculate these averages are given in Tables B2, B3, and B4. The averages reported for the control mussel samples in this Table are the averages for all the control mussel samples and not just day 28. There is a statistically significant ($\alpha = 0.05$) difference between the means for the Cu concentrations of the control mussel samples collected at the different times during the experiment. However, for all the other metals determined, there is no statistical difference in their means for the control mussel samples collected during the course of the experiment. There is no significant difference between the mean Mn, Zn, and As in the control and exposed mussel samples for the 28-day sampling period (Student t-test, $P<0.05$). However, there is a significant difference

between the means for the 28-day control mussel samples and the 28-day exposed mussel samples for all of the other elements determined (i.e., Fe, Cr, Cu, Pb, and Cd). If the average of all of the control mussel samples (days 0, 28, 56, and 70) are compared to the 28-day exposed mussel samples, then only the mean Mn concentration for the 28-day BRH exposed mussels is not significantly different from the mean Mn concentration for all the control mussel samples.

97. During the uptake period (excluding time 0) there is no significant difference (one way analysis of variance, α =0.05) of the means for Fe, Cu, Pb, Cr, and Zn for the BRH exposed mussel samples over time. This would indicate that equilibrium was reached for these metals by the time the first set of exposed mussel samples was collected (i.e., 1.8 days). This might suggest that the mussels simply had BRH sediment in their gut at the time of collection and that the mussels depurated the BRH sediment at a constant rate during the uptake period. However, this is refuted by the following. The Fe, Cr, Cu, Pb, and Cd concentrations for the mussel samples collected from the exposure chamber during the first week of depuration (day 35) were still elevated relative to their mean concentrations in the control mussel samples. Gut depuration of BRH sediment from the exposed mussel samples should take place faster than 7 days. Also, Pb did not depurate readily from the BRH exposed mussels between day 35 and day 70.

98. The bioaccumulation factors (BAFs) for the metals determined in the 28-day exposed mussels are given in Table 20. To determine the BAF values, the following calculations are made: (a) the average metal concentrations for the control mussels are subtracted from their respective

Table 20

Metal Bioaccumulation Factors for Mussels Exposed to Black Rock Harbor Sediment*

Metal	Mussel BAF	Mussel 28 Day/Control
Fe	972	2.6
Zn	6513	1.9
Mn	-208	0.9
Cu	1243	4.6
Pb	1977	2.8
Cd	6567	2.7
Cr	1331	11.4

* The ratios reported are the day 28 mussel sample averages divided by their respective average control concentrations.

Table 21

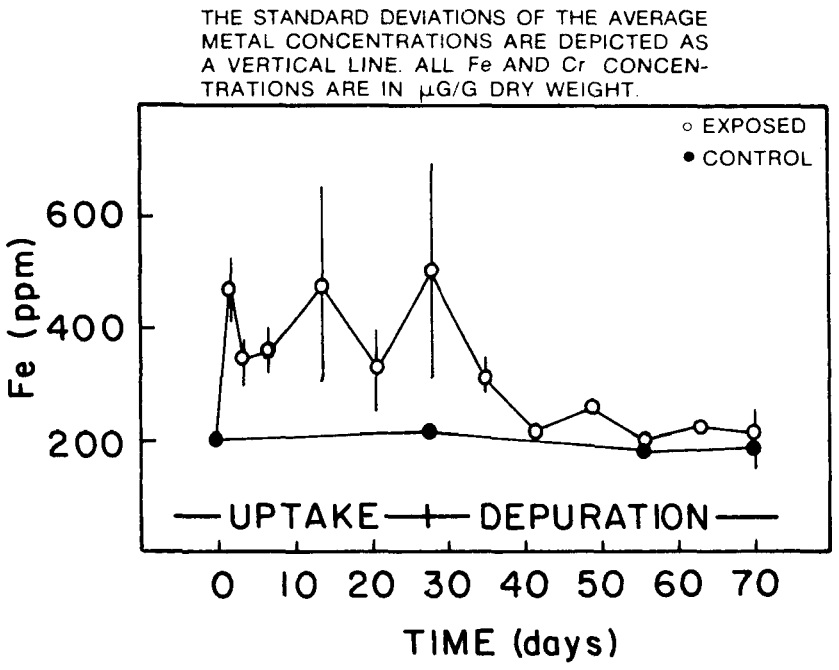
Average Fe/Metal Ratios (± Standard Deviation) for Control Mussels and Black Rock Harbor Sediment

Fe/Metal	BRH Sediments	Mussel Control
Fe/Zn	24.8 ± 0.9	1.1 ± 0.2
Fe/Mn	80.9 ± 7.9	18.2 ± 5.4
Fe/Cu	12.4 ± 0.5	17.9 ± 4.2
Fe/Pb	78.4 ± 2.6	40.7 ± 10.2
Fe/Cd	1270 ± 45	73.8 ± 9.8
Fe/Cr	20.8 ± 0.98	100 ± 33

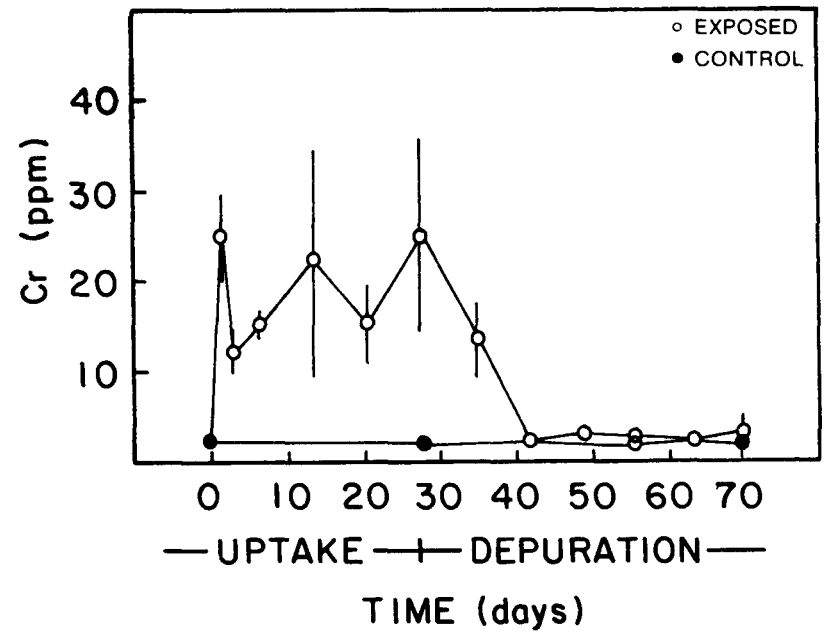
metal concentrations for day 28 BRH exposed mussels; and (b) the resultant metal concentrations are divided by their respective BRH exposure chamber seawater metal concentrations. The units of measure for the mussels are in micrograms per gram dry weight and the seawater concentration units are in micrograms per milliliter. The ratios of the average metal concentrations for the day 28 BRH exposed mussels to the average metal concentrations for the control mussel samples are also reported in Table 21. The ratios are a different method of representing the metal accumulation in the day 28 BRH-exposed mussels. The BAF values tend to give an impression of a large uptake by the mussels for the metal concentrations determined. However, the metal ratios give only the relative metal concentration increases for the BRH-exposed mussels versus the control mussel samples in this study. For example, the BAF values for Zn and Cr are 6513 and 1331, respectively. However, the day 28/control ratios are 1.9 and 11.4 for Zn and Cr, respectively. Using only the BAF values one might conclude that Zn would have a larger percent increase than Cr in exposed mussels.

99. There was no increase for the Mn concentration in the day 28 BRH-exposed mussels compared to the control mussels. However, the increase for Cr for these same mussel samples was a factor of 11. The increases for the other metal concentrations determined for the day 28 BRH-exposed mussels compared to the control mussels are generally greater by a factor of 2 to 5.

100. The uptake and depuration curves for the metals determined in the samples are given in Figures 24 to 27. There are two day 14 sets of mussel samples collected from the BRH exposure chamber (day 0

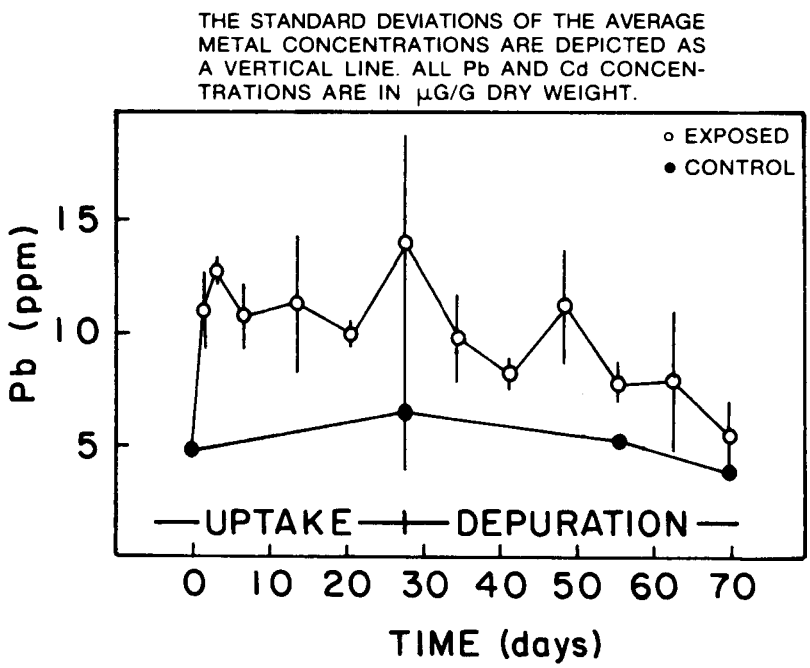


a. Fe

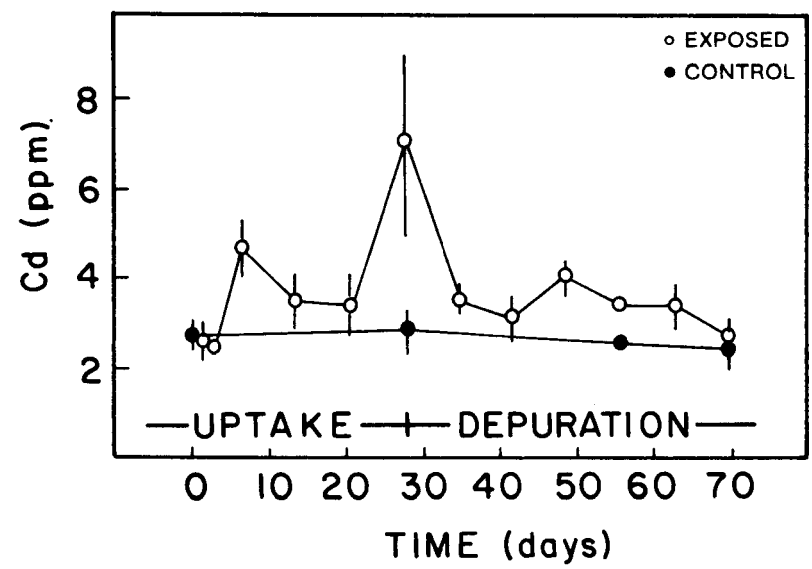


b. Cr

Figure 24. Uptake and depuration in mussels exposed to BRH sediment

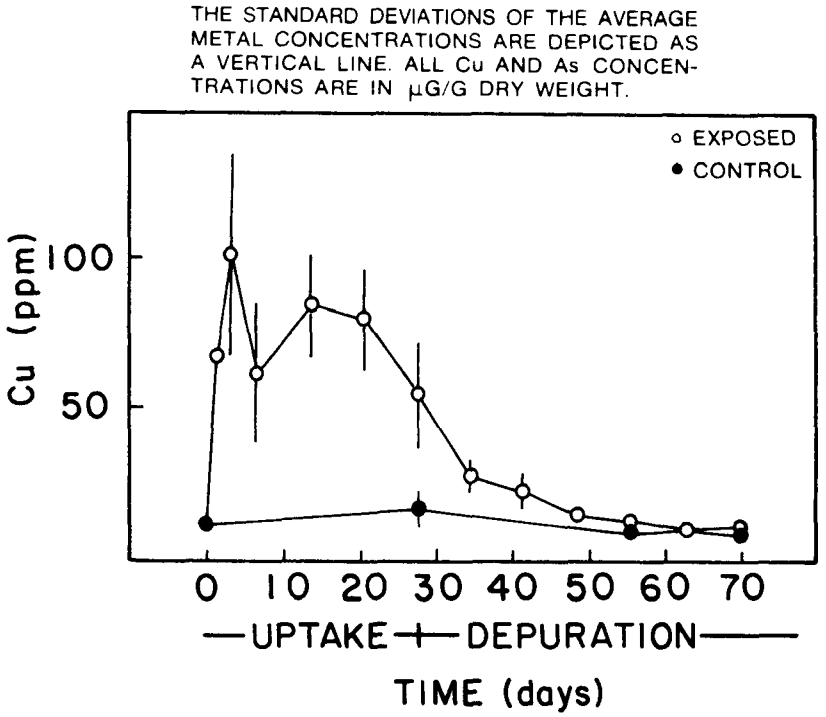


a. Pb

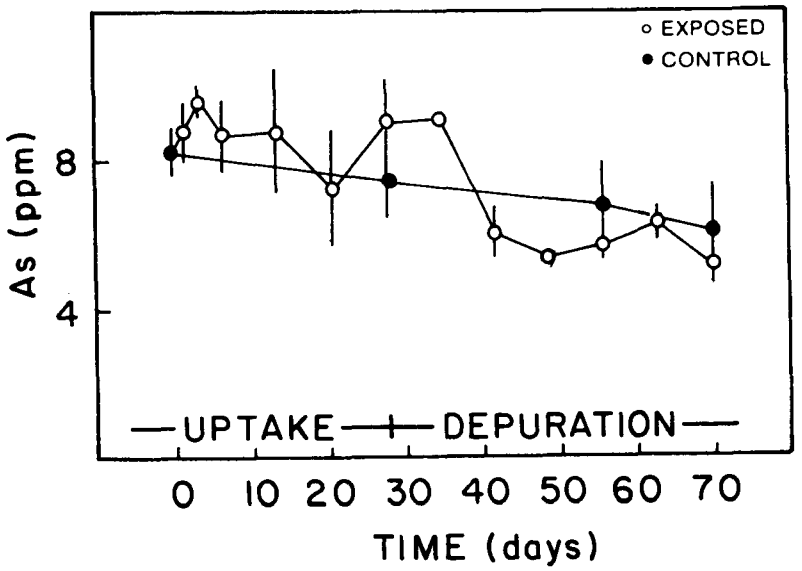


b. Cd

Figure 25. Uptake and depuration in mussels exposed to BRH sediment

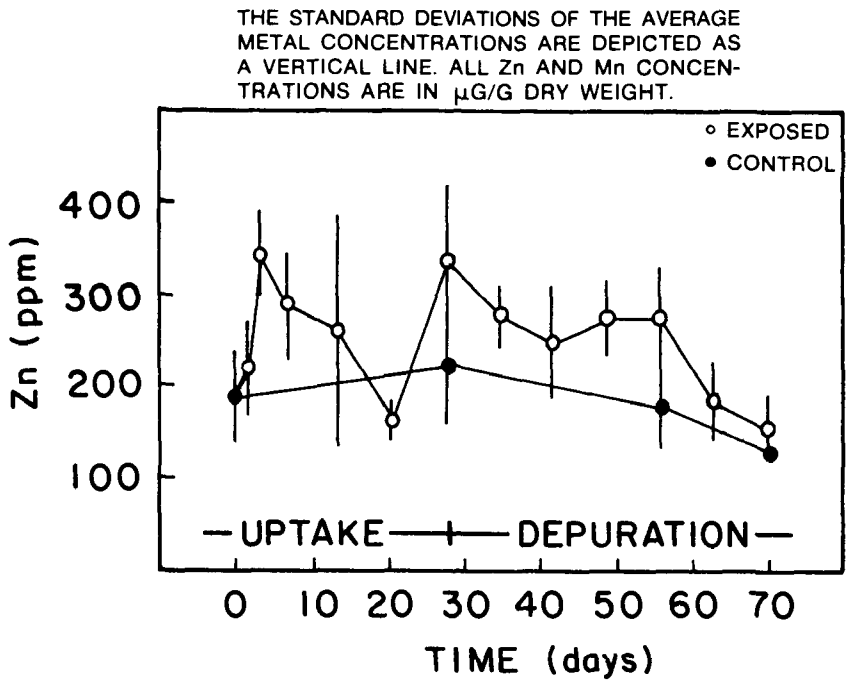


a. Cu

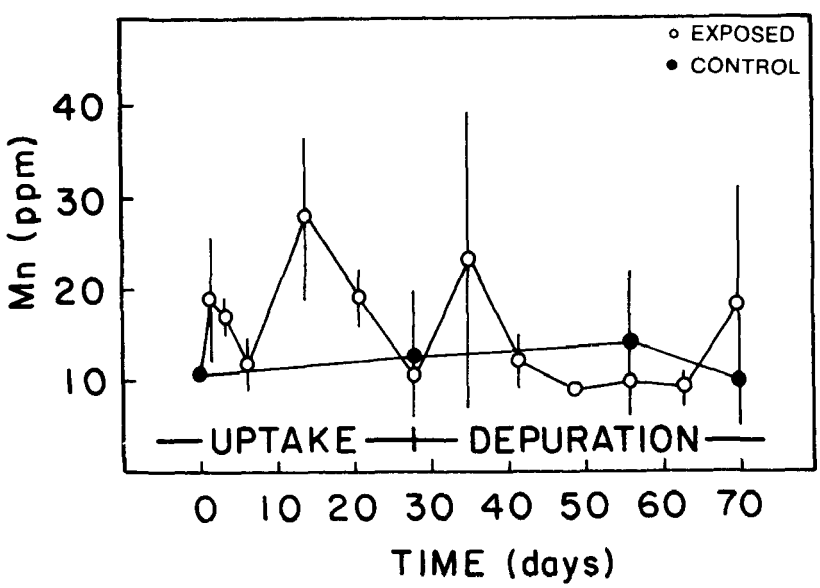


b. As

Figure 26. Uptake and depuration in mussels exposed to BRH sediment



a. Zn



b. Mn

Figure 27. Uptake and depuration in mussels exposed to BRH sediment

to 14 and day 14 to 28). These two sets have been combined to create only one average concentration for day 14 of the uptake period for the exposed mussel samples.

101. To determine if any relationships exist between any of the metals determined for the homogenized mussel samples, correlation coefficients (r) were calculated for all metals compared to the Fe concentration for each sample. Iron was chosen as the element to compare to the other metals for three reasons: (a) Fe has the highest concentration of all the metals determined in BRH sediment; (b) Fe has the smallest percent standard deviation of the means of the control mussel samples for the entire experiment; and (c) Fe should be less subject to contamination during analysis compared to any of the other metals determined.

102. The number of sample pairs (i.e., mussel concentrations of X and Y) must be considered in order to evaluate the probability of the correlation coefficient being significant due to random sampling from an uncorrelated population. All the correlation coefficients used in the following discussion are based on P values of 0.05. For example, 24 data pairs require a correlation coefficient greater than 0.381 to be at the 0.05 level of significance (Fisher 1985).

103. During the uptake period (24 sample pairs) only Mn was not significantly correlated with Fe. All the other metals showed varying degrees of correlation. The calculated correlation coefficients for Fe versus Cr, Pb, As, Zn, Cd, Cu, and Mn are 0.957, 0.827, 0.635, 0.534,

0.490, 0.461, and 0.263, respectively. None of the metals correlated with Fe for the control mussel samples or for the mussel samples collected during the depuration phase of the experiment.

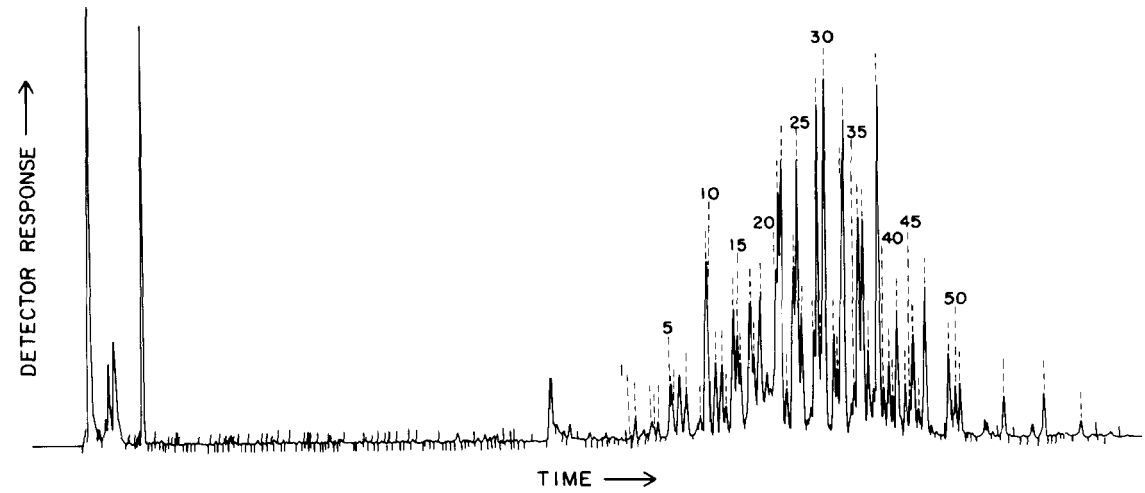
104. It is easily seen that the Fe and Cr concentrations in the mussel samples covary linearily (r=0.957) during the uptake period (see Figure 24). A comparison of the Fe/Cr ratio (see Table 21) for the control samples versus the BRH sediment samples shows that the control mussels have a ratio five times that of the sediment samples. The only other metal determined that has a Fe/metal ratio larger for the control mussels versus the BRH sediment is Cu, and this ratio difference is a factor of 2. The Fe/Cr ratio difference in the controls and BRH sediment may be an advantage in determining uptake of BRH sediment in mussels during the field verification portion of this study. The relatively low concentration of Cr in the control mussels versus the BRH sediment makes Cr an ideal choice for a metal tracer of BRH sediment.

105. The uptake/depuration plots of Pb, Cd, and Cu in the mussels from the BRH exposure chamber are given in Figures 25 and 26. All three of these metals are elevated during the uptake period compared to the control mussel samples, but there is no clear uptake pattern for any of them. The correlation coefficients of Pb, Cd, and Cu versus Fe during the uptake period are 0.827, 0.490 and 0.465, respectively. All three of these correlation coefficients are significant (P=0.05) for a population of 24 sample pairs. The Pb uptake curve also resembles many of the same features of the Cr and Fe curves during the exposure phase of the experiment.

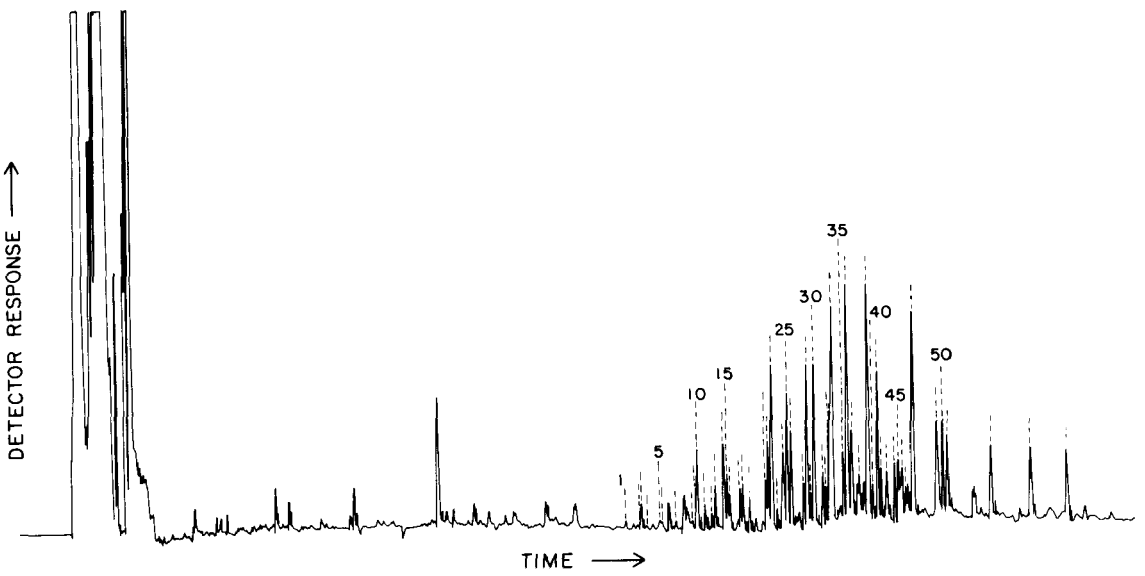
106. The maximum amount of uptake for Cu occurs early (day 3.5) into the exposure period compared to all the other metal concentrations determined and then tissue concentrations decline after 21 days into the exposure. The depuration of Cu appears to be steady in that a slow release of Cu occurs over a 3-week period. The Cu concentration declines to the average control mussel concentration on day 56 and then remains constant until the end of the experiment.

107. The metals Pb and Cd do not show the same type of depuration curve. Neither of these elements shows a steady decline in concentration during the depuration period. At day 70 (day 42 of depuration), the average Cd concentration for the exposed mussels declined to the control mussel concentration, but the average Pb concentration for the exposed mussels did not decline to the control mussel concentration.

108. The last three metals determined in the mussels, As, Zn, and Mn, have one common feature. During the uptake and depuration periods the concentrations of these metals in the exposed mussels vary around their respective concentrations in the control mussels. Several of the average concentrations for these metals in the exposed mussels are lower than the average for the controls during the uptake period. The uptake and depuration plots for these three metals are given in Figures 26 and 27. The correlation coefficients for As, Zn, and Mn versus Fe are 0.635, 0.534, and 0.263, respectively, for the uptake portion of the exposure period. Of these three metals, only As and Zn have significant correlation coefficients (P=0.05) for a population of 24 sample pairs.



a. BRH sediment



b. Reference sediment

Figure 28. Capillary column electron capture gas chromatograms of PF-50 (PCB) fraction

Organic Contaminants

109. PCBs - Sediment. The electron capture detection (ECD) chromatograms of extracts from BRH sediments taken from the exposure tank show a distribution of PCB compounds with from two to ten chlorine atoms with the predominant peaks containing five and six chlorine atoms (Figure 28). Tentative identifications of the peaks are shown in Table 5. Chromatograms from day 0 and day 40 of the exposure sediment show almost identical distributions of compounds.

110. The ECD chromatograms of reference sediment from the reference tanks show a distribution of PCBs with from two to ten chlorine atoms. The distribution of PCBs in the reference sediment shows a relatively greater abundance of higher molecular weight PCBs than is found in the exposure sediment. Chromatograms from day 0 and day 40 of the reference sediment show almost identical distributions of compounds.

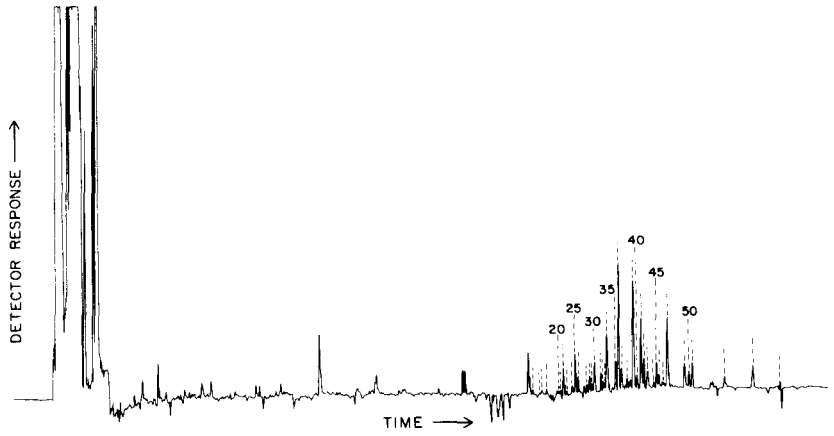
111. Sediments from the exposure and reference tanks were sampled throughout the study. Samples taken on day 0 and day 40 were analyzed and the results show only small differences in the concentrations of PCBs over the study period.

112. PCBs - Worms. The polychaete worms Nereis virens on arrival at ERLN were large but visually appeared to lose weight during exposure to BRH dredged material. Worms from the reference tank did not appear to lose weight over the duration of the experiment. Observations of worms after removal from the tanks and during the gut depuration phase (see Methods, paragraph 20) showed that the exposed

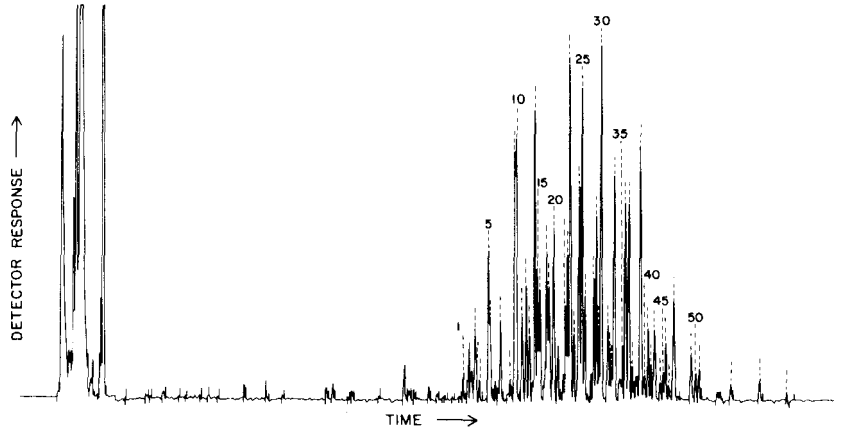
organisms appeared to process only small amounts of BRH material during the uptake phase (some worms from the exposure tank had no sediment in their guts). When the exposed worms were placed in reference sediment for depuration, they processed only small amounts of sediment. Reference worms, however, were always full of sediment prior to gut depuration.

113. Chromatograms of worms from the exposure and depuration study are shown in Figures 29 and 30. The chromatograms from the day 0 worms show three predominant peaks (36, 39, and 48) which represent compounds with structures that are resistant to degradation (Zell and Ballschmiter 1980). The distribution of these peaks maximizes at the Cl_6 PCBs. Only trace amounts of peaks No. 1 to 13 are evident. Following exposure for 14 days the organisms had accumulated a range of PCB compounds from Cl_2 to Cl_{10}. However, comparison of the chromatogram from day 14 worms with the chromatogram from BRH sediments shows that earlier eluting compounds appear to be preferentially accumulated. A peak distribution similar to that observed in the day 14 worms is found in day 28 and day 42 organisms, but by day 56 (28 days of depuration) peaks 36 and 39 are beginning to predominate. Reference worms showed only minor changes in peak patterns during the experiment.

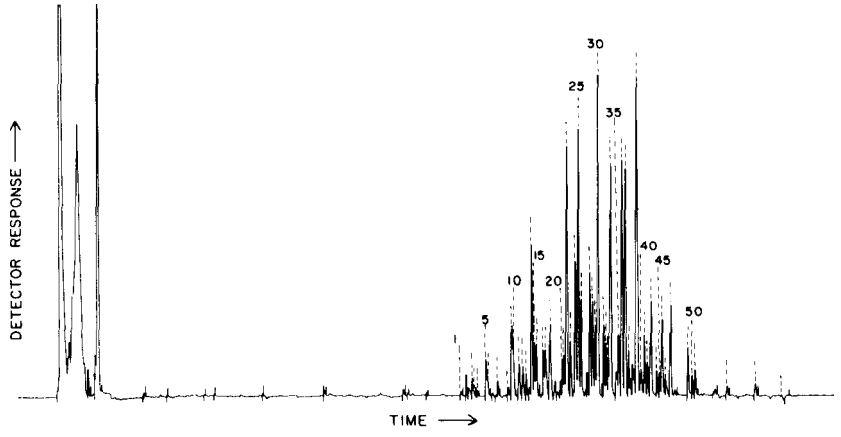
114. During the exposure to the Black Rock Harbor sediments, N. virens accumulated the PCB A-1254 (Figure 31). It is not known if the worms reached steady-state during this 28-day uptake. Some researchers have found no indication of equilibrium concentrations being approached during 32 days of exposure of worms (N. virens) to sandy sediment spiked with A-1254 (McLeese et al. 1980). Other researchers found that (a)



a. Day 0

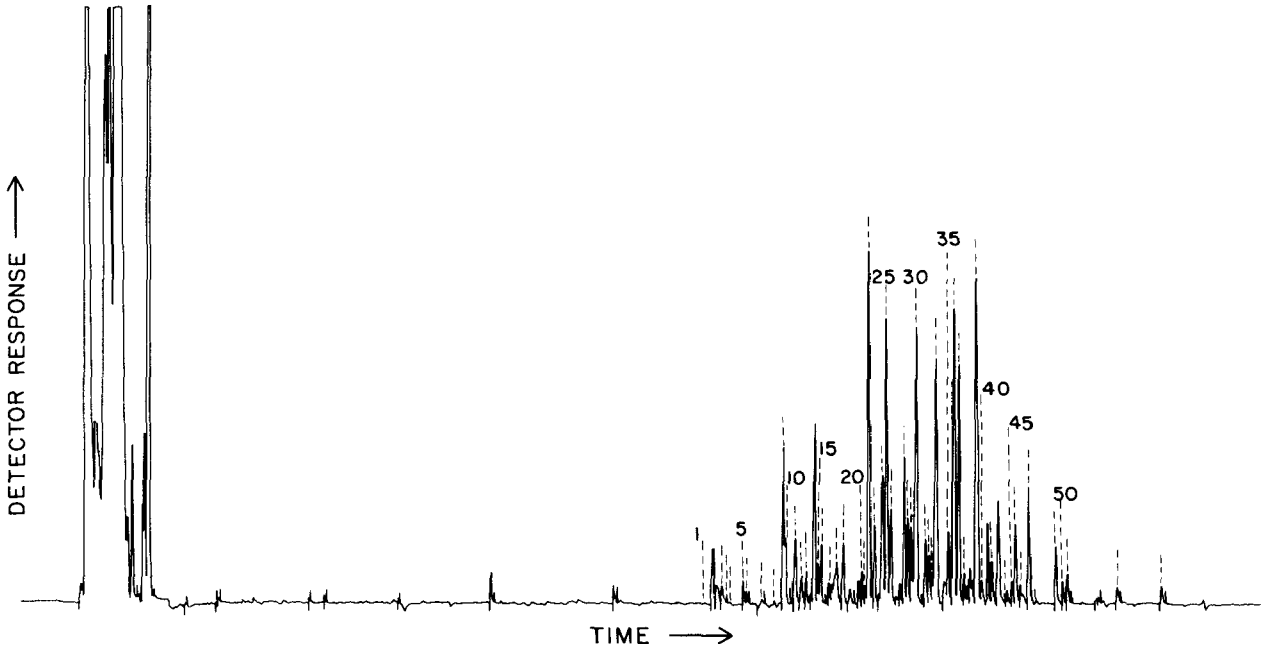


b. Day 14

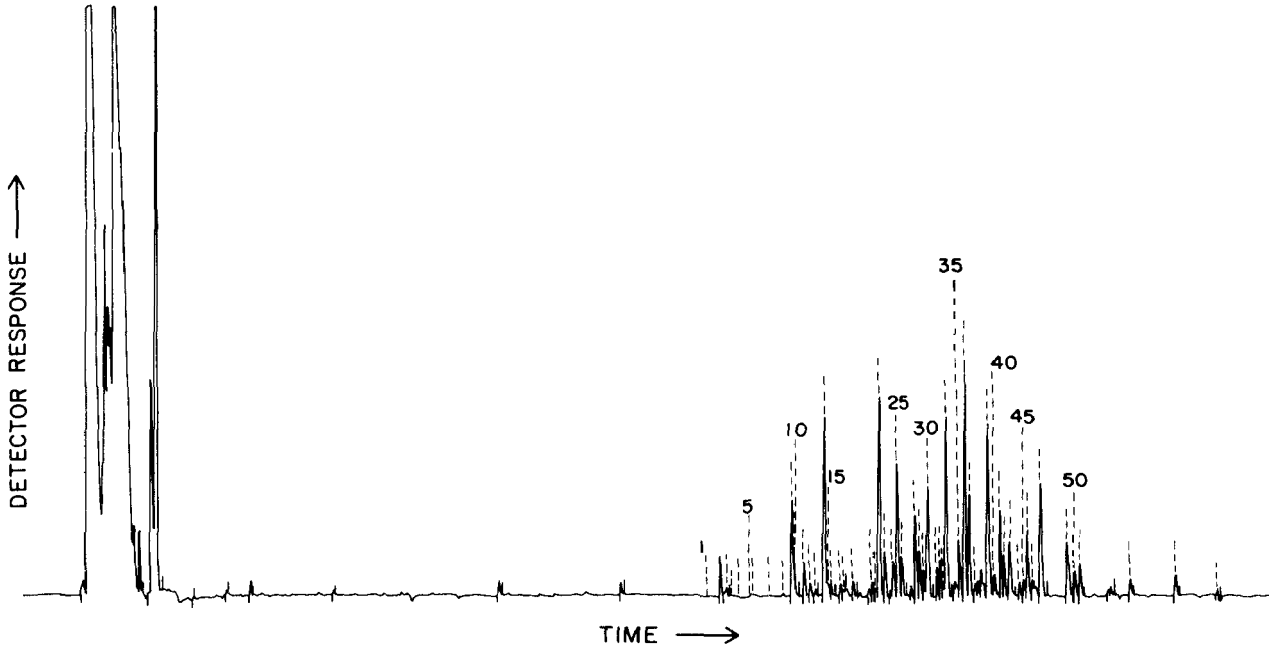


c. Day 28

Figure 29. Capillary column electron capture gas chromatograms of PF-50 (PCB) fraction for worms exposed to BRH sediment



a. Day 42



b. Day 56

Figure 30. Capillary column electron capture gas chromatograms of PF-50 fraction for worms exposed to BRH sediment

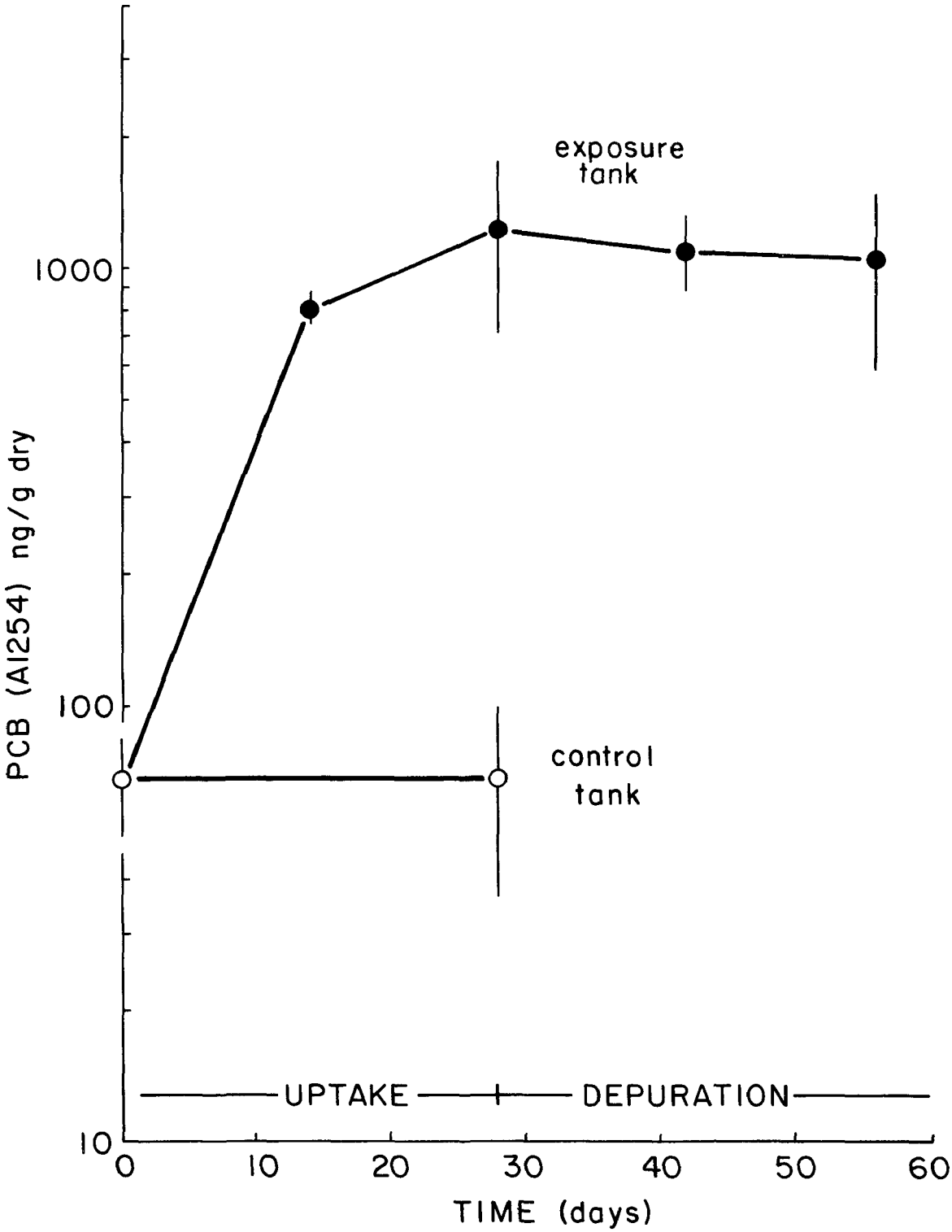


Figure 31. Concentration of total PCBs (as A-1254) in worms exposed to BRH sediment versus time

steady-state for N. virens exposed to A-1254 in naturally contaminated sediments was reached by day 30 and day 40 depending upon the sediment (Rubinstein et al. 1983); and (b) steady-state was reached between 35 days and 100 days (interpolation of graphical data) for N. diversicolor exposed to sediments spiked with different levels of PCBs (Fowler et al. 1978).

115. The bioaccumulation factors (BAFs) = $\frac{\text{PCB (worm dry wt.)}}{\text{PCB (sediment dry wt.)}}$ for A-1254 in the present study were 0.20 for the exposed worms and 1.33 for the reference organisms at day 28. Other researchers have reported BAF values of approximately 8 to 10 (dry wt.) for N. diversicolor exposed to sediments spiked with A-1254 (Fowler et al. 1978), and concentration factors of 3.8 and 10.8 for large and small N. virens exposed to A-1254 in sandy sediment after 32 days exposure, although steady-state conditions were not attained (McLeese et al. 1980). BAFs ranged from 0.157 to 1.59 for N. virens exposed to sediments naturally contaminanted with A-1254 (Rubinstein et al. 1983).

116. The present study found no depuration of A-1254 contaminants during the 28-day depuration period (Figure 31). This is in agreement with findings that there was no obvious excretion of PCB by N. virens during 21 days post-exposure (McLeese et al. 1980). In contrast, other researchers reported that N. diversicolor eliminated PCB during post-exposure depuration periods (Fowler et al. 1978).

117. The uptake and depuration of representative individual PCB peaks (identified in Table 5) are shown in Figures A14 through A17. An examination of these curves suggests that the lower molecular weight, more soluble compounds are accumulated more rapidly than the higher molecular

weight compounds, but no significant depurations were apparent. Bioaccumulation data for the individual peaks are shown in Tables 22 and 23. While the data are not conclusive, there appears to be a slight increase in BAF in the range of the Cl_5 and Cl_6 PCB peaks. The reason for the lower BAF values observed in the exposed versus the reference worms may be the result of the organic matter content of the sediments. The reference sediment contained 1.8% organic matter while the exposure sediment contained 5.9%. Other researchers have suggested that the organic content of sediments play a key role in the availability of organic pollutants to benthic organisms, with higher organic content decreasing the bioavailability of contaminants (Rubinstein et al. 1983).

118. Another reason for the relatively low exposure BAF values found in the present study may result from the low feeding rates observed for the worms during the study period. Since the organisms fed little, if at all, and appeared to lose weight during this study, a significant portion of the accumulation of PCBs that occurred may have resulted from bioconcentration of contaminants from interstitial water. This route of uptake has contributed significantly to the PCB body residues of Arenicola marina and N. diversicolor in laboratory exposures (Courtney and Langston 1978).

119. PAH - Sediment. Sediment samples collected from the exposure and reference tanks during the worm exposure study showed distributions of PAH compounds which are typical of distributions found in heavily contaminated and lightly contaminated sediments, respectively. A much more detailed analysis of these sediments can be found in Rogerson et al. (1983).

Table 22

PCB Bioaccumulation Factors, Exposed Worms Day 28

Peak No.	BAF	Peak No.	BAF
1	.10	31	.21
2	.57	32	.24
3	.27	33	.04
4	.16	34	.21
5	.30	35	.35
6	.20	36	.33
7	.15	37	.32
8	.07	38	.16
9	.20	39	.26
10	.16	40	.19
11	.19	41	.29
12	.17	42	.30
13	.39	43	.21
14	.39	44	.16
15	.20	45	.23
16	.29	46	.23
17	.13	47	.23
18	.22	48	.20
19	.19	49	.19
20	.09	50	.17
21	.07	51	.19
22	.30	52	.12
23	.38	53	.15
24	.25	54	.06
25	.28		
26	.22		
27	.36		
28	.09		
29	.22		
30	.26		

Table 23

PCB Bioaccumulation Factors of Worms in Reference Sediment - Day 28

Peak No.	BAF
17	.70
18	.96
19	1.6
20	.57
21	.43
22	.84
23	2.6
24	1.5
25	1.4
26	.97
27	1.3
28	.38
29	2.7
30	1.0
31	1.6
32	1.5
33	.57
34	1.2
35	2.9
36	1.8
37	1.1
38	2.7
39	1.6
40	4.0
41	1.8
42	2.3
43	1.5
44	.96
45	1.6
46	1.3
47	.90
48	1.9
49	1.4
50	.94
51	1.5
52	.85
53	1.5
54	1.1

120. PAH - Worms. During the exposure to BRH dredged material, the polychaete worm N. virens accumulated PAH contaminants and the pesticide Ethylan. Reference worms also contained PAH compounds; however, the concentrations of PAH compounds in the reference worms following exposure were similar to pre-exposure concentrations. Ethylan was not detected in the reference worms. The uptake and depuration of the sum of the PAH compounds identified in Table 11 are shown in Figure 32. This curve is dominated by the lower molecular weight PAH compounds.

121. Figures A21 to A23 show the uptake and depuration of the individual PAH compounds and the Ethylan during the worm exposure study. The lower molecular weight compounds (i.e., phenanthrene, anthracene, fluoranthrene, and pyrene) showed maximum uptakes at day 14, while the higher molecular weight PAHs and the Ethylan reached their maximum at day 28. Due to the limited sampling times and the fluctuating nature of the data, it cannot be determined if steady-state was reached for the accumulation of PAH and Ethylan by the worms.

122. While steady-state may not have been reached for the PAHs and Ethylan, bioaccumulation factors (concentration in worm (dry weight)/ concentration in sediment (dry weight)) were determined for comparison purposes. The data are shown in Table 24. With the exception of an unexplained increase in BAF for pyrene in the reference worms, the BAFs appear to range from almost zero to a few percent.

123. In the depuration phase the lower molecular weight PAH compounds appeared to be depurated more rapidly from worms than the higher molecular weight PAH compounds. Ethylan was accumulated by the worms from the

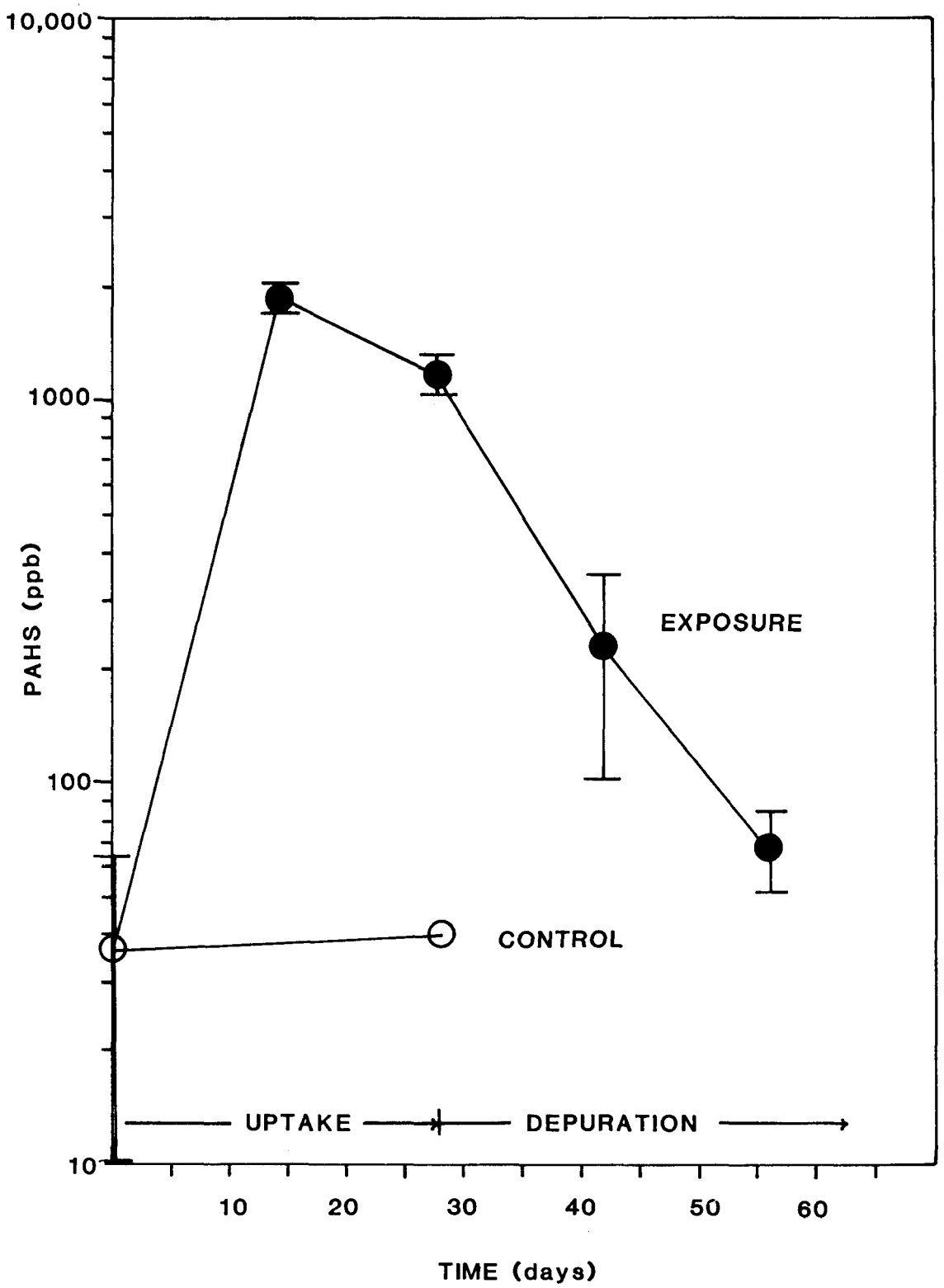


Figure 32. Concentration of sum of parent PAHs in worms exposed to BRH sediment versus time

Table 24

PAH and Ethylan Bioaccumulation Factors - Worms

Compound	Exposed Worms*	Reference Worms**
Phenanthrene	.04	.14
Anthracene	.04	.008
Fluoranthene	.05	.04
Pyrene	.04	.28
Benz(a)anthracene	.03	.004
Chrysene	.06	.01
Benzo(b)fluoranthene and/or Benzo(k)fluoranthene	.02	.005
Benzo(e)pyrene	.02	.01
Benzo(a)pyrene	.02	.005
Perylene	.07	.01
Sum of PAHs with MW of 276	.004	ND†
Ethylan	.09	ND†

* Calculated using mean (n=2) concentrations of PAH and Ethylan compounds in exposed worm samples at day 28 divided by mean (n=3) concentrations of compounds in exposure sediments (dry weight).

**Calculated using concentration of PAH and Ethylan compounds in reference worms at day 28 (n=1) divided by concentration of compounds in reference sediments (n=1)(dry weight.

† ND = not determined in reference worms.

exposure sediments and depurated similar to the higher molecular weight PAHs.

124. Petroleum Hydrocarbons - Worms. The UCM patterns found in the worms following exposure to BRH sediments, when compared to those of the sediment, were shifted toward lower molecular weight compounds (Figure 33). Only small changes were observed in these patterns during the depuration phases. The concentrations of petroleum hydrocarbons in the exposed worms during the uptake and depuration phases of the experiment are shown in Figure 34. The maximum concentration observed was at day 28. Concentrations of total petroleum hydrocarbons appeared to decrease during depuration. Only small changes in the patterns and concentrations of total petroleum hydrocarbons in the worms from the reference tank were observed.

125. Comparisons of Bioaccumulation - Mussels and Worms. The determination of bioaccumulation or bioconcentration factors for the organic compounds found in the exposed mussels and worms in this study depends upon whether the accumulation is considered to have come from the dissolved phase, the particulate phase, or both. If the mechanism of accumulation is direct uptake from the aqueous phase, then bioconcentration factors (BCFs) may be utilized. These factors are usually determined in experiments where organisms (usually fish) are exposed to known concentrations of the compound in the water, and where SPM is usually not present. BCFs are determined by dividing the concentration of contaminant in the organism at steady-state by the dissolved concentration in the exposure water. Since many organic pollutants have low aqueous solubilities and high lipid solubilities, BCFs are usually

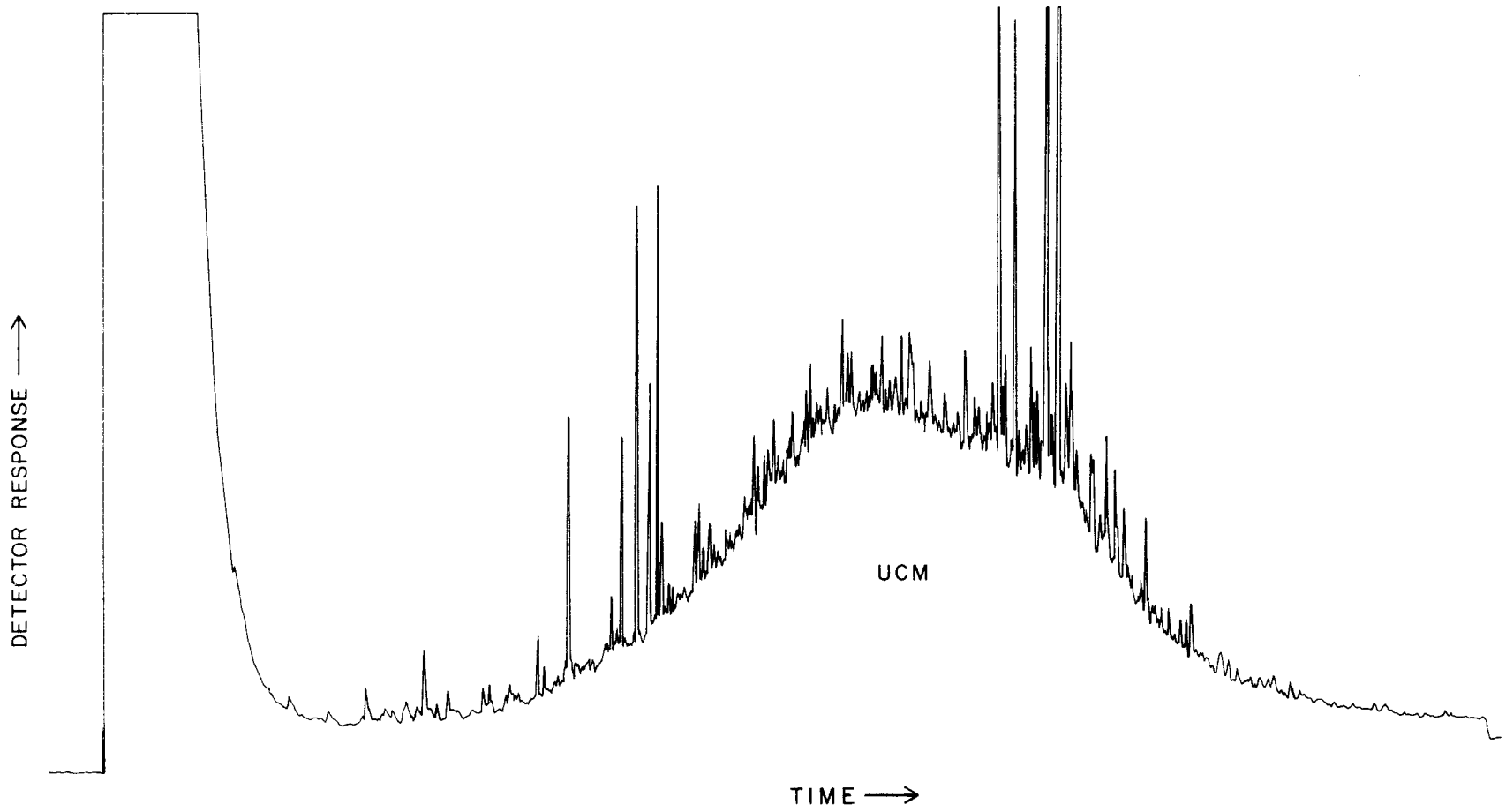


Figure 33. Capillary column flame ionization detector gas chromatogram of PF-50 fraction from worms exposed to BRH sediment for 28 days. This fraction contains mostly straight chain, branched, and cyclic saturated hydrocarbons

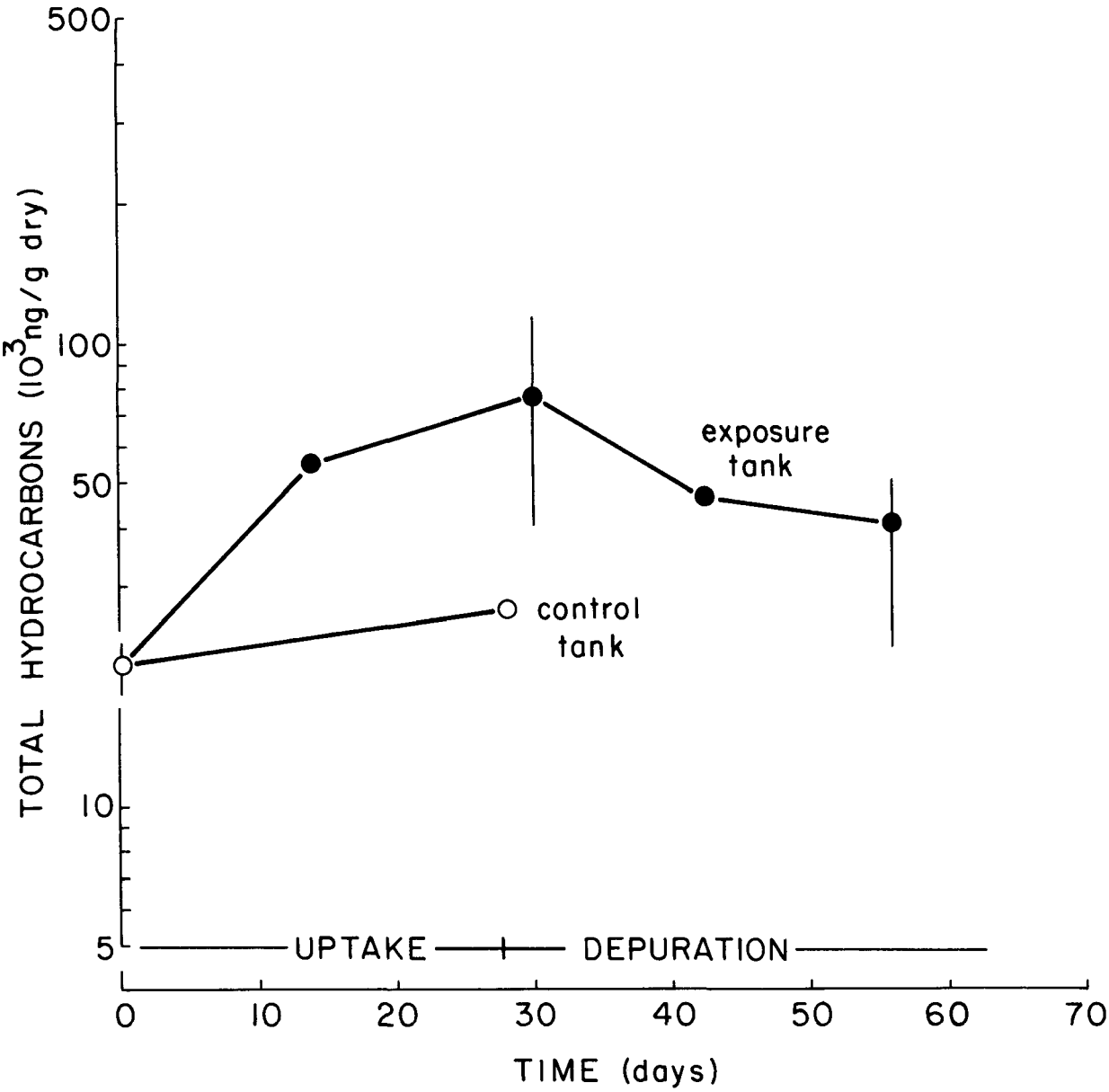


Figure 34. Concentration of total petroleum hydrocarbons in worms exposed to sediment versus time

high. BCFs calculated utilizing the concentration of contaminants in the mussels (dry wt) at day 28 divided by the concentration of contaminant in the filtrate from the exposure water show the expected high values (Table 13). In addition, a direct relationship between Log P and the Log BCF of the compound is observed, as has been found in other studies (Ernst 1977; Geyer et al. 1982).

126. With the exposure and possible accumulation from both dissolved and particulate phases, bioaccumulation factors (BAFs) defined as the concentration of contaminant in the mussels at steady-state divided by the concentration of contaminant in the unfiltered water are appropriate. In the present study, these calculations show Log BAF for PCBs and PAHs which are constant at approximately 4.2 over a range of solubilities or n-octanol/water partition coefficients (Tables 9 and 15) (see paragraph 65 for possible explanations for this constancy).

127. If the mechanism of accumulation is only direct uptake from SPM, then BAFs calculated using the concentration of contaminant in the mussels at day 28 (dry wt) divided by the concentration of contaminant in the SPM (dry wt) are appropriate. BAFs calculated this way are less than 0.3 for PCB compounds. Greater variability was found for the PAH calculations (Table 25).

128. One simplified view of bioaccumulation uses the concept of organisms as lipids and other adsorbing materials in a semi-permeable membrane. If the assumptions are made that membrane transport and the quantity and adsorption efficiences of the adsorbing materials in different organism types are approximately equal, then exposure of these "organisms" to equivalent exposure environments, until attainment of steady-state,

Table 25

Mussel Bioaccumulation Factors Calculated from Filters

PCBs		PAHs	
Peak No.	BAF*	Compound	BAF[1]
1	.18	Phenanthrene	.07
2	.23	Anthracene	.12
3	.17	Fluoranthene	.15
4	.18	Pyrene	.20
5	.19	Benzo(a)anthracene	.25
6	.10	Chrysene	.22
7	.18	Benzo(b)fluoranthene and/or	.13
8	.18	Benzo(k)fluoranthene	
9	.19	Benzo(e)pyrene	.11
10	.19	Benzo(a)pyrene	.11
11	.19	Perylene	.05
12	.20	Sum of PAHs with MW of 276	.06
13	.15		
14	.17		
15	.17		
16	.17		
17	.17		
18	.19		
19	.19		
20	.16		
21	.16		
22	.15		
23	.16		
24	.17		
25	.15		
26	.16		
27	.16		
28	.18		
29	.23		
30	.16		
31	.15		
32	.15		
33	.16		
34	.15		

(continued)

* BAFs calculated using mean mussel concentration of day 28 (n=3)/mean concentration of compounds on day 28 filter (n=3) assuming .009 g BRH sediment (dry weight)/liter.

Table 25 (Cont'd)

PCBs

Peak No.	BAF*
35	.16
36	.15
37	.15
38	.06
39	.14
40	.09
41	.15
42	.15
43	.14
44	.02
45	.12
46	.10
47	.17
48	.08

Table 26

Comparison of BAFs from Mussels and Worms

PCB Peak No.	BAF - Exposed Mussels*	BAF - Exposed Worms**
1	.71	.10
2	.22	.57
3	.28	.27
4	.21	.16
5	.24	.30
6	.11	.20
7	.24	.15
8	.33	.07
9	.26	.20
10	.24	.16
11	.24	.19
12	.25	.17
13	.23	.39
14	.20	.39
15	.27	.20
16	.27	.29
17	.25	.13
18	.25	.22
19	.27	.19
20	.25	.09
21	.24	.07
22	.24	.30
23	.24	.38
24	.25	.25
25	.24	.28
26	.24	.22
27	.25	.36
28	.27	.09
29	.35	.22
30	.24	.26
31	.24	.21
32	.24	.24

(Continued)

* Bioaccumulation factors calculated using mean (n=3) concentrations of PCBs, PAHs, and Ethylan in exposed mussels at day 28 divided by whole unfiltered water concentration at day 28 (n=3) converted to concentration/gram dry wt. BRH SPM using a .009 g (dry wt.)/liter.

** Calculated using mean (n=3) concentrations of PCBs in exposed worms at day 28 divided by mean (n=3) exposure sediment concentration (dry wts).

Table 26 (Cont'd)

PCB Peak No.	BAF - Exposed Mussels	BAF - Exposed Worms
33	.26	.04
34	.22	.21
35	.24	.35
36	.22	.33
37	.22	.32
38	.07	.16
39	.22	.26
40	.14	.19
41	.22	.29
42	.25	.30
43	.23	.21
44	.02	.16
45	.18	.23
46	.16	.23
47	.22	.23
48	.12	.20
49	.09	.19
50	.02	.17
51	.05	.19
52		.12
53		.15
54		.06

PAH Compounds	BAF - Exposed Mussels	BAF - Exposed Worms*
Phenanthrene	.04	.04
Anthracene	.06	.04
Fluoranthene	.18	.05
Pyrene	.22	.04
Benzo(a)anthracene	.40	.03
Chrysene	.37	.06
Benzo(b)fluoranthene and/or Benzo(k)fluoranthene	.22	.02
Benzo(e)pyrene	.18	.02
Benzo(a)pyrene	.16	.02
Perylene	.09	.007
Sum of PAHs with MW of 276	.08	.004
Ethylan	.33	.09

* Calculated using mean (n=2) concentration of PAH and Ethylan compounds in exposed worm samples at day 28 divided by mean (n=3) concentrations of compounds in exposed sediments (dry wts).

should result in equivalent "organism" concentrations. In the present study, the exposure environments for the mussels and worms included contaminants in both dissolved and particle bound form. For worms, the contaminants in the sediment pore water represent the dissolved phase; the particle bound phase is the sediment concentration minus the pore water concentration. Since the extraction of sediment utilized in this study included the contaminants in the pore water with the particle bound contaminants, BAFs may be calculated utilizing the measured sediment concentration as a representation of a total exposure concentration.* In a similar way the total exposure concentration for the mussels is represented by the unfiltered water samples. For comparison purposes the total content of contaminants for both worm and mussel exposures is assumed to reside on the particles. BAFs calculated as concentration in organism (dry wt) divided by the total exposure concentration (dry wt) of sediment (worms) or SPM (mussels) are shown in Table 26. The close correspondence of BAFs for PCBs for both mussels and worms suggests that bioaccumulation of relatively unreactive PCB molecules may be modeled as a partitioning of these contaminants between the organisms and either the sediment or SPM. Whether the actual bioaccumulation process occurs through direct transfer of hydrophobic organics from sediment to organism through the lining of the gut or through a dissolved intermediate phase is unknown.

129. The correspondence between measured and estimated BCF values found in the mussel studies (paragraph 64) is suggestive of a dissolved

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* Not including the concentration of contaminants in overlying water which are thought to be very small.

phase intermediate between the contaminants on the SPM and those in the mussels. However, the increase in measured BCF with increasing Log P may result in another way. The concentrations of PCB compounds in the aqueous phase decrease by orders of magnitude over the range of PCB compounds. Since organisms may accumulate particle-bound contaminants to constant concentrations (i.e., gut transfer), the division of organism concentrations by measured aqueous concentrations will give spreads of BCF values covering several orders of magnitude with BCFs increasing with decreasing compound solubility (increasing Log P). Similar relationships are possible in the pore water of the sediments and in the worms.

130. For the more reactive PAH compounds greater differences were observed in the BAFs for the worms and mussels. The worms showed smaller BAFs than the mussels (Table 26). The reason(s) for these differences are unclear, but may reflect metabolic differences of the organisms.

131. For modeling bioaccumulation, researchers have suggested utilizing normalization of sediment concentration to the organic carbon content of the sediment (site of most of adsorption of hydrophobic organic compounds) (Karickoff et al. 1979) and normalization of organism concentrations to the lipid content of the organism (most important site of storage of organic pollutants). These concentrations are utilized in thermodynamic arguments with fugacity concepts and will be the subject of future publications.

Inorganic Contaminants--Worms

132. The average concentrations and standard deviations for the day 28 worm samples collected from the BRH sediment exposure chamber are given in Table 27. All of the inorganic data used to calculate these averages are given in Table B5. Data for the time zero worm samples are not used in the following discussion since much of the Fe, Cr, and Cu data are higher than all of the other samples collected. The large concentrations of Fe, Cu, and Cr in the time zero samples are probably due to the fact that the worms were not allowed to acclimate in the reference sediment prior to the start of the experiment so that a firm baseline could have been established. The time zero worms probably represent the sediment of the Maine coastline from which they were collected since these worms had never been in BRH or REF sediment. Several data points were eliminated from the uptake, depuration, and reference worm samples. These results were rejected by application of the "Q" test for rejection of an experimental observation (Dean and Dixon 1951). Two Cd results were discarded: one from the day 28 uptake samples and one from the day 40 reference sample. Also, one value for Cr was rejected from the day 56 depuration phase of the experiment. These data points are marked with an asterisk in Table B5.

133. The means for the two control mussel Fe concentration are significantly different (Student t-test, P=0.05) for the two collection times. However, there are no significant differences in the control worm mean concentrations for Cu, Cr, Cd, and Zn for the two collection times.

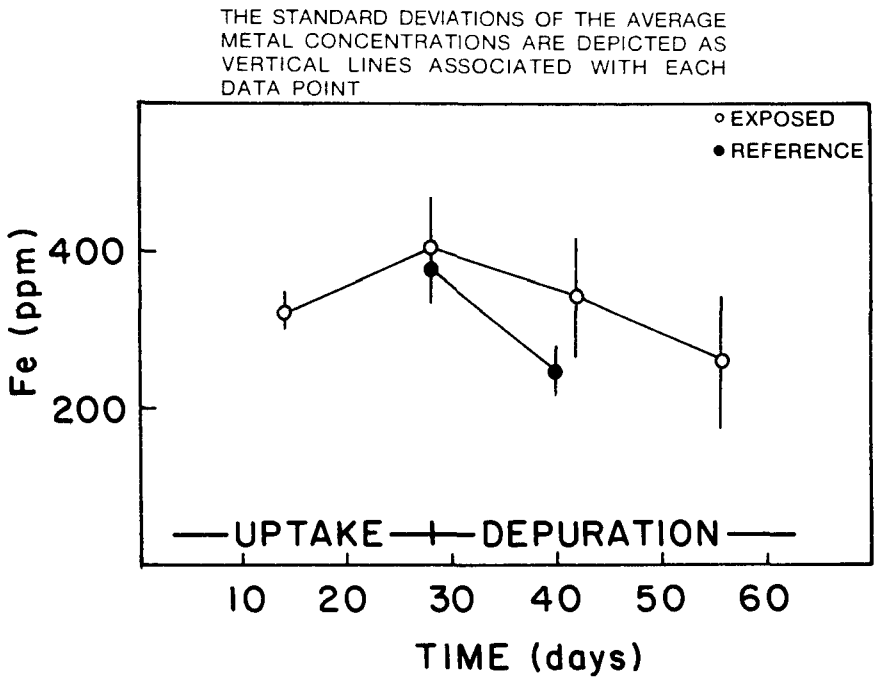
134. The Fe uptake and depuration plot for the worms is given in Figure 35. Only the average and standard deviation of the average of

Table 27

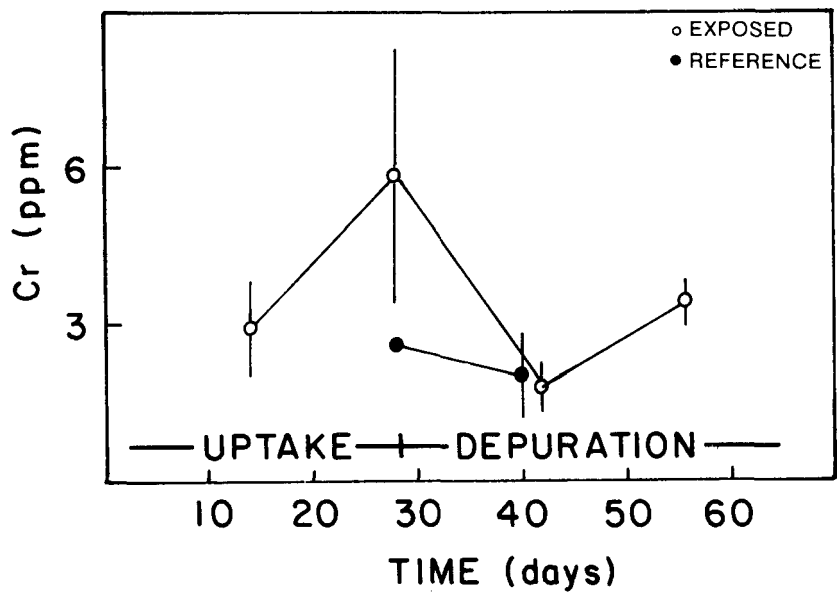
Average Trace Metal Concentration for Worms Collected from the Exposure Chamber on Day 28*

Metal	Worm 28 day	Worm Control
Fe	404 ± 68	316 ± 75
Zn	139 ± 15	109 ± 16
Cu	31.3 ± 9.9	12.2 ± 1.2
Cd	0.73 ± 0.10	0.60 ± 0.08
Cr	5.8 ± 2.4	2.3 ± 0.6

* The control concentrations reported for the worms are the average of all the control samples and not just day 28. All concentrations are in ug/g dry weight. The standard deviations of the means are also reported.



a. Fe



b. Cr

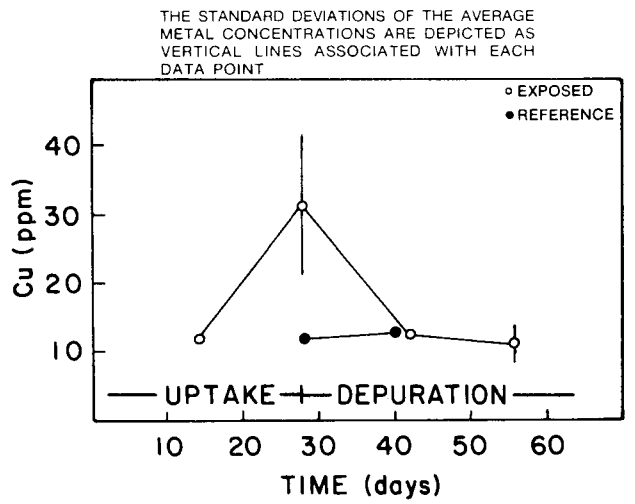
Figure 35. Uptake and depuration in worms exposed to BRH sediment

each set of exposed and control worm samples are shown in this figure. There is no significant difference in the Fe concentration of the day 28 BRH-exposed worms and the day 28 reference sediment-exposed control worms. There is no significant difference in the Fe concentrations for any of the worm samples collected from the BRH exposure chamber during uptake or depuration (one way analysis variance $\alpha = 0.05$).

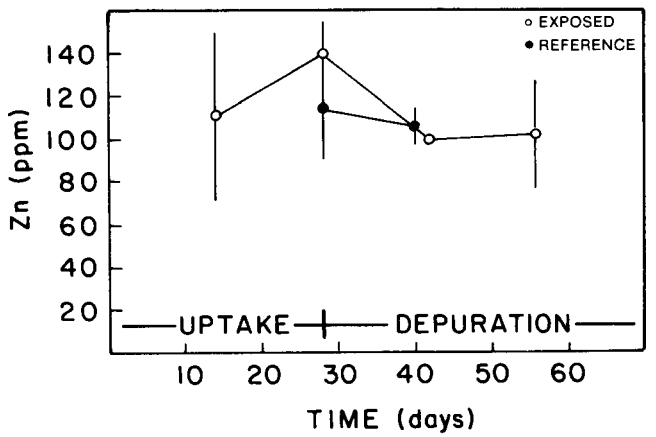
135. The Cr uptake and depuration plot for the worms is given in Figure 35. There is a large standard deviation of the concentration of Cr for the day 28 BRH-exposed worms compared to the standard deviation of the Cr concentration for the day 28 reference sediment worms. However, there is a significant difference (Student t-test, $P = 0.05$) between these two concentrations. Also, the difference between the concentrations is significant for all the worm samples collected during uptake and depuration.

136. The Cu uptake and depuration plot for the worms is given in Figure 36. Like the Cr data the Cu data have a large standard deviation of the mean for the day 28 BRH-exposed worms. Also, like Cr, the worm Cu concentration means for the uptake portion of the study from the BRH exposure chamber are significantly different from the worm Cu concentration means for the depuration portion of the study. The two control worm sample means for Cu are not significantly different from each other. The Cu concentration in BRH-exposed worms declines to the control worm concentration for the day 42 samples (14 days of depuration).

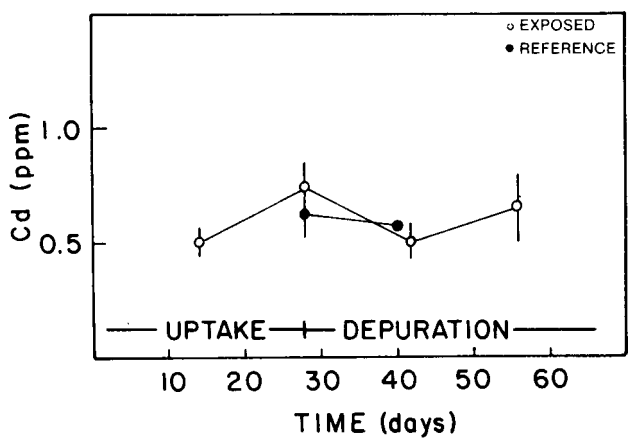
137. The Zn uptake and depuration plot for the worms is given in Figure 36. There is no significant difference between the Zn concentrations for the day 28 BRH exposed worms and the day 28 reference worms. There



a. Cu



b. Zn



c. Cd

Figure 36. Uptake and depuration in worms exposed to BRH sediment

Table 28

Metal Bioaccumulation Factors for Worms
Exposed to Black Rock Harbor Sediment*

Metal	Worm BAF	Worm 28 day/control
Fe	0.0030	1.3
Zn	0.025	1.3
Cu	0.0080	2.6
Cd	0.0058	1.2
Cr	0.0025	2.5

* The ratios reported are the 28 day worm sample average concentration divided by their average controls, respectively.

is also no significant difference between any of the Zn concentrations for any of the samples collected from the BRH exposure chamber or the reference sediment control chamber.

138. The Cd uptake and depuration plot for the worms is given in Figure 36. There is no significant difference between the Cd concentration for the day 28 BRH-exposed worms and the day 28 reference sediment control worms. The two Cd concentrations for the control samples are also not significantly different.

139. The BAF values for the metals determined in the day 28 BRH-exposed worms are given in Table 28. These BAF values were calculated for all the metals even though the Zn, Cd, and Fe data show no significant difference between the means of these elements for the BRH-exposed and the reference-exposed control worms. The BAF values were calculated as follows: (a) the average control worm metal concentration was subtracted from the average day 28 worm concentration; and (b) the corrected concentrations were then divided by the concentration of the metals determined in BRH sediment. The ratios of the average metal concentrations for the day 28 BRH-exposed worms to the average metal concentrations for the control worm samples are also reported in Table 28. These ratios are probably a better representation for the metal accumulation in the day 28 BRH-exposed worms. The ratios for Cu and Cr for the day 28 exposed worms and the reference sediment control worms are 2.6 and 2.5, respectively. These increases in Cu and Cr, while not large, are significant. The calculated ratios for Fe, Zn, and Cd are small and are not significant.

PART IV: SUMMARY

Mussel Bioaccumulation Study

Organics

140. The system utilized to dose mussels with suspensions of BRH dredged material worked well. The dose of PCB contaminants monitored in the exposure tank was quite constant over the study period, and while the concentration of total PAH compounds was found to show more variability, this variability appeared to be in total levels of PAH compounds rather than on a compound-to-compound basis. It seemed likely that the PAH variability may have resulted from the unhomogeneous distribution of soot particles (containing high concentrations of PAH compounds) in the BRH sediment. The concentrations of PAH and PCB contaminants in the control tanks were orders of magnitude below those in the exposure tanks.

141. Separation of dissolved and particle-bound PCB and PAH contaminants resulted in distributions which were logically consistent with the solubilities of the compounds. The more water-soluble compounds were found in the dissolved form while the less water-soluble compounds were found associated with the particles.

142. Based on comparisons of measured and estimated Kps for the PCB and PAH contaminants in the exposure tanks, it appeared that equilibrium conditions were not reached in the residence time of the suspensions in the dosing system.

143. During the first 7 days of exposure, mussels in the exposure tank showed a rapid uptake of PAH and PCB compounds. There was an unexplained decrease in the concentration in mussels during the next 2 weeks for most compounds, and the highest concentrations were found at day 28.

144. PCB compounds with molecular weights above Cl_7 PCB were not effectively accumulated by the mussels, and, similarly, PAHs of higher molecular weight were not accumulated as much as some lower molecular weight compounds.

145. Application of a non-linear model to the mussel data indicated that steady-state concentrations of PCBs had been reached during the 28-day exposure period. From the shapes of the uptake curves for the PAH compounds, it appeared that steady-state concentrations of PAHs also had been reached during the 28-day exposure.

146. While the dominant method of accumulation of PAHs and PCBs (i.e., uptake from water or uptake from the particles) could not be determined from these studies, measured BCFs (assuming uptake from aqueous phase only) showed increasing BCFs with increasing Log n-octanol/water partition coefficients (decreasing aqueous solubilities) and reasonable agreement with BCFs estimated from a correlation (BCF vs. Log P) in the literature. In contrast, bioaccumulation factors (BAFs; calculated using unfiltered water concentrations) for PCBs and PAHs were relatively constant over the range of PAH and PCB contaminants examined. The constancy of these BAFs suggests that similar processes determined the distributions of these compounds. In this regard, bioaccumulation in the exposure tanks may be viewed as the result of two processes competing for the dissolved phase contaminants: readsorption by the SPM, and bio-concentration of dissolved contaminants by the mussels. Alternatively, the constant bioaccumulations observed may have resulted from similar constant processes like direct transfer of contaminants through the gut.

147. In general, depuration rate in mussels appeared to be inversely related to Log P for PCB compounds; however, some higher molecular weight PCB compounds were lost at higher rates than lower molecular weight PCBs. Those compounds with recalcitrant structures appeared to be depurated most slowly. The depuration of PAH compounds also appeared to be inversely related to Log P. The concentrations of petroleum hydrocarbons measured in the mussels over the exposure and depuration period generally followed the patterns observed for the other organic contaminants.

148. Control mussels, which contained only low concentrations of contaminants, showed only small fluctuations in concentrations of organic contaminants during this study.

Inorganics

149. The acid-soluble trace metal concentrations in the seawater were generally consistent with the quantity of BRH sediment added to the exposure chamber. The total concentration of BRH sediment added to the seawater could be calculated from the Fe concentration determined in the seawater exposure chamber. The interelemental ratios determined in the exposure chamber also compared favorably with the interelemental ratios determined for BRH sediments.

150. Statistically, there was no difference for the respective means of Fe, Cr, Mn, Pb, Cd, Zn, and As concentrations over time for the control mussels collected from the control chamber. There was, however, a significant difference in the mean Cu concentrations for the control mussel samples collected over time from the control chamber. The means of Fe, Cu, Cr, Zn, Pb, Cd, and As for the day 28 BRH-exposed mussels were significantly different from their respective means for the control mussels.

151. Typically, metal BAFs over 1000 were calculated for the day 28 mussels collected from the BRH exposure chamber. A different impression of the uptake is observed in the ratios of metal concentrations in day 28 exposed mussels divided by the metal concentrations in the control mussels. The ratios which indicate only the relative metal concentration increases for BRH-exposed mussels versus the control mussel samples ranged from 0.9 to 11.

152. The uptake patterns of Fe and Cr for the exposed mussels were almost identical. The correlation coefficient for Fe versus Cr in the BRH-exposed mussels was 0.957. The relatively low concentration of Cr in the control mussels versus that in the BRH sediment makes Cr an ideal choice for a metal tracer of BRH sediment. The mussel concentrations of Fe, Cr, Cu, and Pb were all elevated during the uptake period compared to the control mussel samples; however, only Pb and Cr correlated well with Fe in the mussels during the uptake period. The concentrations of Zn, As, and Mn in the exposed mussels varied around their respective concentrations in the control mussels.

153. The depuration patterns of Fe and Cr from the mussels exposed to BRH sediment appeared to be identical. Both elements declined to control concentrations after 2 weeks of depuration. The depuration of Cu appeared to begin before the end of the exposure period. The Cu concentration in the BRH-exposed mussels fell to the control mussel concentrations after 3 weeks of depuration. Neither Pb or Cd showed a steady decline in concentration in the exposed mussels during the depuration period. The concentrations of Mn and As in the exposed mussels were generally below the concentrations of Mn and As concentrations

of the control mussels during depuration. The average Zn concentrations in the exposed mussels were elevated compared to the average control Zn concentration during the depuration period. However, the standard deviations of the average concentrations for Zn in the exposed mussels overlapped with those for the control mussels during the depuration period.

Worm Bioaccumulation Study

Organics

154. The exposure of worms Nereis virens to BRH sediment resulted in accumulation of organic compounds even though the organisms showed little evidence of feeding during the experiment. The PCBs accumulated by the worms showed a pattern which was similar to the pattern observed in the sediment. The PCB contaminants accumulated showed no apparent decreases in total concentrations over the depuration period. Exposed worms accumulated PAHs to concentrations which were orders of magnitude greater than those in the reference worms. In contrast to the PCBs, which showed no concentration decreases during the depuration period, the concentrations of PAHs in the worms fell rapidly during depuration.

155. The petroleum hydrocarbons found in worms (measured as an unresolved complex mixture) increased during the uptake phase and decreased during the depuration phase.

156. Reference worms showed relatively constant low levels of organic pollutants over the exposure and depuration study.

157. The bioaccumulation factors observed for the PCBs in the exposed worms were lower than those found for worms in the reference sediment. This may have resulted from the apparent poor health of the

exposed organisms or from decreased bioavailability of PCB contaminants in sediments with high organic carbon (i.e., Black Rock Harbor sediments). Alternatively, the lower bioaccumulation factors found for worms in Black Rock Harbor sediment may result from steady-state values not being attained during the 28-day exposure period. BAFs for PAH compounds in the worms were lower than BAFs for the PCBs, and no consistent differences between exposed and reference BAFs were found for PAHs. The differences observed in the BAFs for PCBs and PAH may reflect metabolic or bioavailability differences, or may result from steady-state values not being attained during the exposure period.

Inorganics

158. There was no significant difference between the mean Fe, Zn, and Cd concentrations in the day 28 BRH-exposed worms compared to the reference sediment exposed worms. There was a significant uptake of Cu and Cr for the day 28 BRH-exposed worms. The calculated ratio of the mean Cu and Cr concentrations for the day 28 BRH-exposed mussels and the reference sediment exposed mussel were 2.6 and 2.5, respectively. The depuration of Cu and Cr was complete 2 weeks after the exposure period.

Bioaccumulation Mussels and Worms--Organics

159. Exposure to organic contaminants in dissolved form and on SPM (mussels) and in pore water and sediments (worms) can be simplified by assuming that the total exposure concentration of PCBs resides on the particles or the sediments. When this is done the calculated BAFs for both mussels and worms are quite similar. Similar calculations with

the more reactive and possibly less available (due to incorporation in soot particles) PAHs show greater differences. Similarities in worm and mussel BAFs suggest that modeling bioaccumulation of some organics (at least less reactive compounds like PCBs) as a partitioning of contaminants between the organisms (worms and mussels) and the sediment or suspended sediment shows promise as a predictive technique for assessing the accumulation of organic contaminants from dredged material and other mixed wastes.

PART V: RECOMMENDATIONS

Mussels

160. Due to the apparent discrepancy between estimated and measured partition coefficients for organic compounds, it is recommended that partitioning studies be conducted to determine the time to equilibrium and the soluble/particulate distributions of organic and inorganic compounds under both aerobic and anaerobic conditions.

161. Due to the variability in concentrations of organics and inorganics observed in mussels during the uptake phase, the following studies are recommended:

a. Studies should be conducted to broaden our understanding of the nutritional requirements of these organisms and the potential nutritional value of the sediments.

b. Future studies should be conducted to examine the contribution of contaminants on sediment in the gut of the exposed mussels to the concentrations found in extracts of whole organisms.

162. It is recommended that a range of concentrations be used in mussel exposures to establish the constancy of bioaccumulation factors at different exposure concentrations.

Worms

163. Due to the poor feeding behavior of the worms in this exposure study, it is recommended that further studies be conducted to ensure that worms remain healthy and have adequate nutrition during exposure and depuration studies with sediments which are heavily contaminated with organic and inorganic compounds. This research should consider the utilization of standardized control sediment in exposure studies.

These standard sediments could be used to dilute toxic sediments and to serve as carriers for the nutritional needs of the organisms.

164. It is recommended that worms be held in reference sediment prior to initiation of exposure studies for a time sufficient to allow worms to adjust their contaminant levels to those of the reference sediment.

165. As adequate exposure conditions (i.e., no adverse effects) become available, it is recommended that longer term bioaccumulation studies be conducted to ensure that steady-state levels are reached for both the organic and inorganic contaminants in these organisms.

General

166. In order to determine the potential for bioaccumulation of sediment-bound contaminants, it is recommended that research be undertaken to develop a short-term abiotic test to enable prediction of the bioavailability and bioaccumulation of contaminants.

167. In order to link the laboratory bioaccumulations with potential bioaccumulations in the field at the disposal site for BRH dredged material (under the Field Verification Program), it is recommended that:

a. Field bioaccumulation samples be analyzed for the same contaminants that were accumulated in laboratory studies.

b. Where possible, the same organisms (or similar surrogate organisms) be used in field and laboratory studies.

c. If the same organisms cannot be deployed or are not indigenous at the disposal site, studies be undertaken to compare bioaccumulation between indigenous and surrogate organisms.

d. Exposure concentrations at the disposal and reference site should be determined at least seasonally to enable estimation of mean annual exposure levels in water and sediments.

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