DEVELOPMENT AND EVALUATION OF A BIOASSAY PROTOCOL FOR PREDICTING THE BIOACCUMULATION POTENTIAL OF SEDIMENT-ASSOCIATED CONTAMINANTS

Prepared For

Environmental Protection Service
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Project 6516

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March 01, 1984   Our File: 6516

Mr. H. Nelson
Environmental Protection Service
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Dear Mr. Nelson:

RE: Development and Evaluation of a Bioassay Protocol for Predicting the
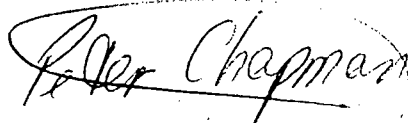   Bioaccumulation Potential of Sediment-Associated Contaminants

Please find enclosed ten copies of our final report on the above project
following your review and comment. We trust that the report completes our
present assignment to your satisfaction.

The bioassay protocol appears to have a reasonably good predictive accuracy for
comparisons between laboratory bioaccumulation of selected metals and PCBs,
and actual levels in resident biota. As such, it appears to be a useful tool for
decision-making related to ocean dumping of contaminated sediments.

E.V.S. Consultants would like to take this opportunity to thank you and the
other members of RODAC for your cooperation and assistance throughout the
study. It has been much appreciated.

Sincerely,

E.V.S. CONSULTANTS

Peter M. Chapman, Ph.D.
Vice-President

PMC/si
Attach.

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SUMMARY

This study represents an initial step towards the development of a bioassay protocol for predicting bioaccumulation from contaminated dredge spoils. The study consisted of two distinct phases: 1) a 6 month laboratory exposure of a deposit-feeding clam (Macoma balthica) to contaminated test sediments, and 2) a field sampling program to assess bioaccumulation in resident biota from the same contaminated sites tested in the laboratory. M. balthica was chosen as the test species because it had been previously shown to be a good indicator of the bioavailability of a number of sediment-associated contaminants. This species was considered to have the potential to predict the relative degree of bioavailability of contaminants to other bottom-associated species. Resident biota sampled for comparison included clams, polychaete worms, flatfish (muscle and liver) and crabs. Three contaminated test sediments representing a dry dock/ship repair facility (Vancouver Harbor), a heavily industrialized embayment (False Creek), and a pulp and paper mill effluent discharge (Powell River) were used in evaluating the protocol. A fourth sediment from a site (Trail Islands) with no known sources of contamination served as a reference. Contaminants tested included cadmium, lead and polychlorinated biphenyls (PCBs).

A statistically significant difference in the bioaccumulation of cadmium was observed in the laboratory bioassays for Powell River sediment. M. balthica did not show any significant uptake of cadmium from any of the other test sediments compared to the reference, despite high levels of cadmium present in some sediments. Results of the field studies showed levels of cadmium in polychaete worms and flatfish liver to be significantly higher at the Powell River site compared to the reference site. Thus, laboratory predictions (i.e. the high degree of bioavailability for cadmium at the Powell River site) were corroborated by the field sampling.

not in this study.
see McGreer + Reid-Y

Lead was bioaccumulated significantly by M. balthica from all test sediments compared to the reference sediment. In the field, polychaete worms from all test sites, and crab muscle from the Vancouver Harbor site showed significantly higher concentrations of lead compared to the reference site. Thus, there was general agreement between the laboratory and field results with respect to lead.

PCBs were measured in the reference and Vancouver Harbor sediments only. Both Aroclor 1254 and 1260 were bioaccumulated significantly in Vancouver Harbor compared to the reference sediments in laboratory bioassays. Analysis of resident biota showed significantly higher levels of PCBs in polychaete worms and in flatfish livers from the Vancouver Harbor site. Thus, laboratory predictions were again corroborated by field sampling.

The long-term laboratory bioassays also provided data on the chronic toxicity of some of the test sediments. For instance, mortalities in sediments from False Creek reached 40% after an exposure period of 4 months.

Our results showed that laboratory sediment bioassays using M. balthica could be generally used to predict bioaccumulation in resident biota from the same contaminated test sites. Such laboratory studies can be used to determine the suitability of solid wastes for ocean disposal with respect to bioaccumulation.

Further testing to refine the protocol is required. Aspects identified during the present study which require additional development work include: standardization of flow rates and exposure times in laboratory bioassays, and determination of the significance of various levels of bioaccumulation to resident species.

ACKNOWLEDGEMENTS

1.0   INTRODUCTION

In Canada, the ocean disposal of dredged spoils including contaminated sediments and other solid wastes is regulated by permit under the Ocean Dumping Control Act (ODCA), promulgated on December 13, 1975 as a result of an international agreement under the 1972 London Dumping Convention. Under the ODCA maximum quantities and concentrations for a number of potentially hazardous compounds (e.g. cadmium and mercury) were established. As part of the permit granting process, the ODCA also stipulated additional factors which should be taken into account, including (Schedule III, Section 1.; Subsection (6)) "Accumulations and biotransformation in biological materials or sediments." However, no guidelines or test protocols were established for assessing the biological accumulation of sediment-associated contaminants.

A number of published studies reporting the bioaccumulation of contaminants in tissues of marine organisms from polluted environments have suggested that relatively long periods of time are required to reach equilibration between environmental and tissue levels of contaminants (e.g. Bryan, 1980; Luoma and Bryan, 1979; McGreer et al., 1981). However, we know very little about the long-term potential for bioaccumulation or the ecological effects of compounds disposed in contaminated dredge spoil (Levings, 1982). Bioaccumulation of contaminants is of particular concern from the standpoint of buildup of contaminants in aquatic food chains consumed by man (Waldichuk and Buchanan, 1980; Swiss et al., 1980).

In 1977, the U.S. Environmental Protection Agency (EPA)/Corps of Engineers (COE) Technical Committee on Criteria for Dredged and Fill Material developed the first sediment bioassay implementation manual (EPA/COE, 1977). Potential bioavailability, or lack thereof, was assessed by analyzing bioassay organisms after a 10 day exposure period. No claims were made with regard to relating the concentrations of contaminants in tissues with possible levels resulting

after long-term exposures. Subsequently, scientific research and environmental legislation have indicated that no adequate chemical analytical technique is presently available which can reliably predict the availability of contaminants to biota (Bryan, 1980; Federal Register, 1980). Therefore, the most direct approach to assessing bioaccumulation potential at present is through the use of suitably designed bioassay tests.

The present study was designed to develop and evaluate a laboratory bioassay which could be used to assess the bioaccumulation potential of sediment-associated contaminants for the purposes of regulating bioaccumulation under the ODCA. Emphasis was placed on the development of a laboratory bioassay involving long-term exposure (up to 6 months) to assess bioaccumulation of cadmium, lead and PCBs. Evaluation of the protocol was carried out by comparing laboratory results with bioaccumulation in tissues of resident biota from the same contaminated sites tested under laboratory conditions. A statistically significant difference in the bioaccumulation of a contaminant from a test site compared to the reference sediment in the laboratory was interpreted as an indication of potential bioaccumulation. Resident biota from the same sites tested in the laboratory were then analyzed to assess whether they had accumulated significant levels of contaminants compared to the reference location. Corroboration of the laboratory bioassay predictions in the field sampling was taken as verification of the ability of the laboratory test to successfully predict long-term bioaccumulation in the environment.

1.1 Study Rationale

1.1.1 Choice of Test Species

It is widely recognized that certain marine species regulate levels of particular environmental contaminants in their tissues better than others (i.e. non-regulators). Those species which do not regulate a particular contaminant are ones in which higher concentrations are

manifest, and which are considered to be good 'indicators' of bioaccumulation potential (Bryan, 1980; Phillips, 1977). Species used as bioaccumulation indicators should be non-regulators of the contaminants of concern, be widely distributed, accessible, easily recognized, relatively stationary, and available at all times of the year. The bivalve Macoma balthica is one species which fits these requirements and was chosen as the test species for this study. M. balthica is a deposit-feeder which feeds primarily on sediment particles (compared to particulates suspended in the water column), and has been used extensively in laboratory and field bioaccumulation studies with contaminated sediments (e.g. Luoma and Jenne, 1977; Bryan and Hummerstone, 1977; McGreer et al., 1981; Ray et al., 1981). M. balthica is also circum-polar in its distribution and is found on all three Canadian coasts including the Arctic. Thus, development of a successful bioassay protocol for assessing bioaccumulation potential using this species could be applied Canada wide.

Field species for analysis were chosen based on several considerations. Benthic infauna (polychaetes and clams) were selected because of their close contact with contaminated sediments and potential to bioaccumulate contaminants from this source. Ideally, it would have been desirable to collect M. balthica from each location for comparison with laboratory results. However, differences in habitat and the depth distribution of this species made this impracticable. English sole (Parophrys vetulus) were collected for analysis where possible, because of its broad distribution in local waters and because it is known to bioaccumulate a variety of contaminants from polluted sediments. However, no English sole were captured in repeated trawls from one test site near a pulp and paper mill effluent discharge. The Butter sole (Isopsetta isolepis), which was abundant at this site, was used instead for assessing bioaccumulation at this one site.

1.1.2   Experimental Design

The study consisted of two separate phases:

1.   a 6 month laboratory exposure of M. balthica to two types of contaminated sediment and a reference (uncontaminated) sediment

2.   a field sampling program to assess bioaccumulation in resident biota from the same sites used in the laboratory testing.

In developing the bioassay protocol, it was desirable to use contaminated sediments representing several different types of pollution sources normally encountered in processing ocean dumping permits in the Pacific Region.   Two contaminated sites were chosen representing spoils from a dry dock/ship repair facility (Vancouver Harbor) and an industrialized embayment (False Creek). Sediments from a site near a pulp and paper mill discharge were also included as frequent dredging and ocean dumping of these sediments occurs in British Columbia. The reference sediment was collected from a site with no known pollution sources, located on the Sechelt Coast (Trail Islands).

Initially, two additional contaminated sites representing a pulp mill discharge (Woodfibre) and an abandoned mine tailings disposal site (Watts Point) were to have been tested. However, anoxic conditions were encountered in the fjord (Howe Sound) in which these sites were located, and no live organisms were captured in trawls, benthic grabs or baited traps. In addition, sediments collected from these locations smelled strongly of hydrogen sulfide and were unsuitable for use in the laboratory portion of the experiment.

The Regional Ocean Dumping Advisory Committee (RODAC) expressed a strong desire to include sediments from at least one pulp and paper mill site in the present study due to concerns over high cadmium levels which have been found in sediments at several mill sites (e.g. Sullivan, 1982).   To accommodate this requirement, resident biota

were sampled at Powell River, B.C. adjacent to a pulp and paper mill effluent discharge and relevant tissue contaminant levels determined. These data were then compared with results from a recently completed 6 month laboratory bioaccumulation experiment with M. balthica using Powell River sediments (McGreer and Reid, 1984) for evaluation purposes with respect to development of the protocol.

For the laboratory phase, the level of bioaccumulation in M. balthica for each contaminant in each contaminated test sediment was compared with levels obtained from the reference substrate. When a statistically significant difference with the reference was obtained, the contaminant and test sediment were "red flagged" as having a high bioaccumulation potential. The month at which a statistically significant difference first became apparent indicated the minimum exposure time for the laboratory bioassay. A hypothetical example of laboratory results and their interpretation are illustrated below.

```
        4 months = significant difference

        2 months = significant difference

            I month = no difference

  ┌───────────┐        ┌──────────────────┐        ┌──────────────────┐
  │ Reference │        │ Test Sediment #I │        │ Test Sediment #2 │
  └───────────┘        └──────────────────┘        └──────────────────┘

                    I month = no difference

                 2 months = no difference

              4 months = no difference
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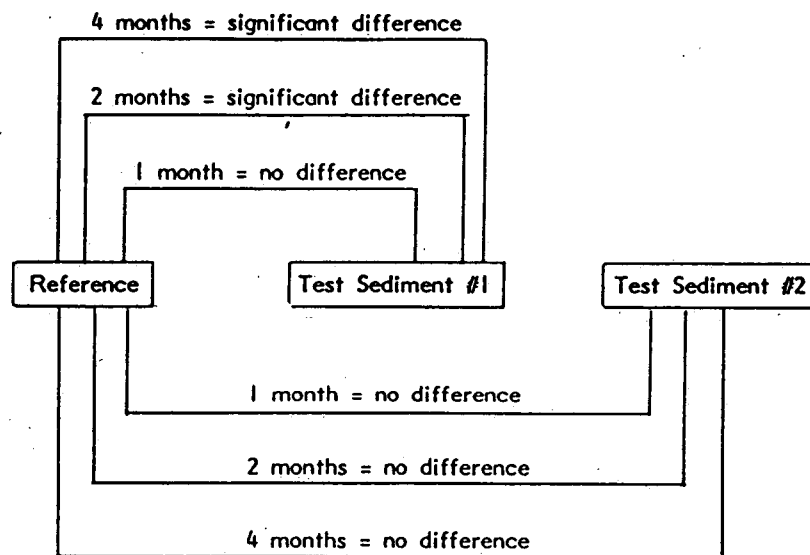
Example of Laboratory Test Results with Contaminant X

The results illustrated above for hypothetical contaminant X would be interpreted as indicating a high potential for bioaccumulation of this compound from sediment #1. Sediment from this site would thus be "red flagged" as a concern with respect to possible bioaccumulation of

contaminant X if ocean dumped. By comparison, sediment from site #2 does not show a high bioaccumulation potential for contaminant X. The results also indicate that a laboratory exposure time of at least two months is required to assess the bioaccumulation potential of this chemical.

To verify the laboratory bioassays, results from field sampling of resident biota were compared in a similar manner. That is, tissue levels at contaminated test sites were compared statistically with tissue values in biota from the reference site. Several different types of biota and tissue were compared. An illustration of how data from the field portion of the study was treated is shown below.

Crab muscle = no difference

Flatfish liver = significant difference

Flatfish muscle = no difference

| Reference Site | Test Site #1 | Test Site #2 |

Flatfish muscle = no difference

Flatfish liver = no difference

Infauna = no difference

Crab muscle = no difference
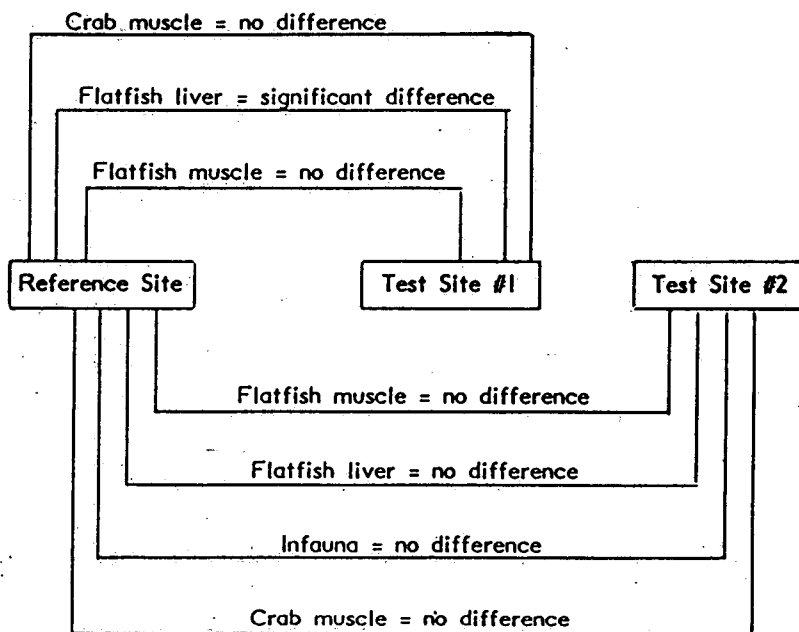
Example of Results from Analysis of
Resident Biota for Contaminant X

In the above hypothetical example, infauna and flatfish liver were found to have bioaccumulated significantly higher levels of contaminant X at Site #1. The field results also suggest that flatfish and crab muscle are not accumulation sites for contaminant X.

A comparison of laboratory with the field results shows that the potential for bioaccumulation of X identified in the laboratory bioassays for sediments #1 was confirmed by the higher levels in resident biota collected from this site.

In our example, the field results corroborated the predictions of the laboratory bioassay with respect to contaminant X, and the laboratory bioassay would be considered to be verified. A protocol using a two-month laboratory exposure would be recommended for testing of future sediments for this contaminant.

1.1.3   Extrapolation of Laboratory Test Results
         to Resident Biota

A major assumption in the adoption of the study design with M. balthica was that bioaccumulation results with this species could be used to predict bioaccumulation in other non-regulator species in the field. This assumption was tested in the present study with three groups of organisms (polychaete worms, crabs, sole). However, additional testing would be required to completely verify this hypothesis. Different animal groups (e.g. polychaete worms, clams, flatfish, crabs) have, in general, been shown to be good bioaccumulation indicators of sediment-associated contaminants (Duke et al., 1970; Konasewich et al., 1982).

2.0   METHODS

2.1   Field Collections

2.1.1   Sediments

Sediments from the False Creek (FC), Vancouver Harbor (VH) and Trail Islands (REF) site were collected using a Van Veen grab. Collections in False Creek were made approximately 300 m offshore of Ondine's Marina, on the north shore of False Creek. This site is just west of the Cambie Street Bridge which divides False Creek into

east and west basins. Samples in Vancouver Harbor were collected at a dredge spoil disposal site on the south shore of Burrard Inlet, near the foot of Granville Street. The samples were taken in a small bay immediately east of the Seabus terminal. This site had recently received dredged material from Cates Towing located in North Vancouver. Water depth at both the Vancouver Harbor and False Creek sites was approximately 9 m, and 30 m at Trail Islands at high tide. Sediments were placed in 5-gallon polyethylene buckets which had been pre-cleaned with dilute HCl and rinsed with distilled water. Only sediments which had not come into contact with the outer steel walls of the sampler were retained.

Subsamples of each sediment were placed in clean plastic Whirlpak bags and frozen for later analysis of physical characteristics and metal content. Subsamples for analysis of polychlorinated biphenyls were wrapped in pre-treated (hexane/acetone) aluminum foil prior to being frozen.

2.1.2   Seawater

Samples of seawater from near-bottom were collected with a Van Dorn water sampler. The sampler was dismantled and pre-rinsed with dilute HCl and distilled water prior to collection of each sample for metals analysis. Contents of the sampler were siphoned directly into previously cleaned polyethylene sample storage bottles supplied by the analytical sub-consultant. Separate samples for PCB analysis were collected with a second Van Dorn sampler which had been rinsed with hexane/acetone. Samples were stored in previously cleaned glass sample storage bottles.

2.1.3   Macoma balthica

Clams (Macoma balthica) were collected from Roberts Bank in the Fraser River estuary. Collections were made during periods of low tide in January and February, 1982. Sediments containing M. balthica were sieved through a 4 mm screen and clams greater than 5 mm in

length were retained for use in the laboratory tests. Approximately 4000 clams were collected for the laboratory bioassays. Clams were transported to the laboratory in polypropylene containers which had been pre-treated with dilute nitric acid and rinsed with distilled water. Clams were maintained in the laboratory in containers with sediment from the collection site and covered by seawater (27 ppt salinity) from Burrard Inlet. Aeration was supplied to each container through a single airstone. Clams were held from 2-4 weeks before the start of the six-month bioaccumulation experiments. Seawater was replaced every 48 h during the holding period.

2.1.4   Resident Biota

2.1.4.1   Benthic Infauna

Sediments at each test site were collected by Van Veen (Trail Islands, Vancouver Harbor, False Creek) or Smith-McIntyre (Powell River) grab and sieved onboard to collect resident infaunal species. Approximately five grabs were required at the Trail Islands and Powell River sites to obtain sufficient quantities of tissues for analysis. In contrast, over 30 grabs were required to collect the minimum amount of infauna from the Vancouver Harbor and False Creek sites. In addition, sediments from the Vancouver Harbor site were sampled by SCUBA divers using a hand held suction pump which passed the sediment/seawater slurry through a 2 mm screen to collect infauna. Polychaete worms (mixed species) were the major fauna at all sites and were used for tissue analysis. Sufficient numbers of clams (of any species) for analysis were found only at the Reference site.

All specimens were depurated in ambient seawater from the collection site for 24 h to clear gut contents of sediments prior to being frozen and held for chemical analysis. Tissue samples for metals were placed in clean plastic Whirlpak bags. Samples for PCB analysis were wrapped in pre-treated aluminum foil.

2.1.4.2   Flatfish

Bottom (otter) trawl sampling was undertaken to collect demersal flatfish at each test site for tissue analysis. The otter trawl used in this study was a scaled-down version of otter trawls used for commercial harvesting of bottom fish. The otter boards are approximately 25 cm x 50 cm. When deployed, the boards ensure a gape opening of 3 m x 0.5 m. The mesh in the body of the trawl net is approximately 2.0 cm on the stretch. The cod end mesh is approximately 1.0 cm on the stretch. The net was deployed from the stern of the towing vessel. When the trawl was observed to be properly filled out, it was lowered to the bottom until a ratio of 3 to 1 tow line to depth was achieved. The net, once at depth, was towed for 10 minutes at a hull speed of approximately 2.0 knots. Upon completion of one trawl, the net was retrieved, and the catch emptied into a large container half filled with seawater. Fish collected were then identified to species level. Polychaetes were sorted but not identified.

A minimum of 10 individuals of the preferred species of sole P. vetulus for tissue analyses were separated out and placed in appropriate containers. As this species was not found at the Powell River test site, a second species, I. isolepis, was retained for tissue analysis. All samples were frozen whole onboard for subsequent dissection under clean room conditions. Prior to freezing, specimens for metal analysis were wrapped in clean polyethylene bags and those for PCBs in pre-treated aluminum foil.

2.1.4.3   Crabs

Crabs (Cancer magister) for tissue analyses were obtained with the otter trawl and with baited crab traps which were fished for 5-24 h. At the Reference site crabs were also caught by SCUBA diver. Those individuals selected for organic contaminant analyses were wrapped in prepared aluminum foil and those for metal analyses in clean polyethylene bags.

2.2 Laboratory Methods

The six month laboratory portion of the study was conducted in facilities provided by the Vancouver Public Aquarium, Stanley Park, Vancouver, B.C. The Vancouver Public Aquarium draws seawater at a depth of approximately 8 m from Burrard Inlet. The seawater is passed through a sand-filtration unit before being distributed within the facility.

2.2.1 Bioaccumulation Studies

Six month continuous-flow bioassays were conducted in six (i.e. two trays per test sediment) 65 L polypropylene trays (dimensions 90 x 60 x 12 cm). Tests were conducted at 10 ± 2^oC under 10 h light/14 h dark photoperiod conditions. The laboratory area was sealed with plastic and equipped with a wall mounted air filtration unit. Entry to the facility was restricted to one doorway which was sealed with velcro tape along all sides. Prior to start-up of the test, all trays, lids and plasticware were pre-soaked in 5% nitric acid for 48 h, then rinsed twice with distilled water, and clean seawater.

The bioaccumulation experiment was initiated by placing 20 L of one type of well-mixed test sediment in each tray to a depth of 5 cm. Sediments were mixed by stirring smaller amounts thoroughly in separate buckets before placing in the experimantal trays. Seawater was then introduced into each tray at a rate of approximately 60 mL/min. The total volume of sediment and seawater was 60 L (i.e. a ratio of sediment:water of 1:2). Seawater was distributed at an equal rate to each tray from a polyethylene header tank. The level of dissolved oxygen was maintained at air saturation by aeration through two airstones in each tray. Each tray was covered with a plexiglass plate during the experiment to minimize possible airborne contamination. Clams (approximately 300 per tray) were apportioned to each tray at the start of the experiment. Individuals were spread uniformly on the surface of the sediment within each tray.

2.2.2 Experimental Sampling

Care was taken during all stages of the experimental sampling to ensure that samples were not contaminated. This was especially important for lead in seawater for which order of magnitude errors can be introduced during sampling and handling procedures (Patterson et al., 1976).

Seawater was withdrawn after 24 h, 1, 2, 4 and 6 months from each tray for analysis of dissolved cadmium, lead and PCBs. Inlet seawater to the header tank was also sampled prior to start-up and after 1, 2, 4 and 6 months. Seawater samples were withdrawn approximately 1 cm above the sediment interface using pre-cleaned polyethylene tubing fixed to a "T" bar which rested on the sides of the tray. This technique minimized sediment disturbance while sampling, and ensured that samples were collected at the same distance above the sediment on each occasion. Separate lengths of tubing pre-rinsed with dilute nitric acid and distilled water were used for each tray to prevent cross-contamination. An initial 200 mL sample was withdrawn and discarded on each occasion to further cleanse the tubing. Two 1 L samples were withdrawn for each of the metals and PCB analysis. Samples for metals analysis were stored in acid-cleaned polyethylene bottles. Samples for PCB analysis were stored in glass bottles pre-cleaned with hexane/acetone. The unfiltered samples were delivered to the analytical laboratory within three hours of collection and immediately filtered and extracted under clean room conditions.

After water samples were collected, dissolved oxygen (YSI Model 57), pH (CORNING Model 610A), salinity and temperature (YSI Model 33) were recorded. These parameters were monitored weekly in each test tray.

Reduction/oxidation potential (Eh) of the sediments was also measured monthly to provide an indication of possible changes in test sediments. Measurements were made with an Orion portable meter equipped with a combined reference/platinum electrode. The combined electrode is factory-sealed and calibration is carried out by a three way test sequence (zero, slope, maximum output).

On each sampling occasion, approximately 50 clams were removed for each replicate tissue analysis. A sufficient amount of sediment was scooped out of each tray with a plastic trowel to yield the required number of clams. Three replicate samples were analyzed on each occasion for each contaminant. Clams were depurated in clean seawater for 48 h in pre-cleaned polypropylene tubs then frozen in double sealed plastic bags prior to analysis. The effectiveness of the 48 h depuration period was assessed by comparing a group of depurated clams with a group from which the digestive tract had been removed.

2.2.3 Dissections of Resident Biota and Clams

Samples of biota were partially thawed prior to being dissected. All dissections were carried out under clean room conditions. Tissue dissections were performed using pre-cleaned plastic forceps and new stainless steel scalpel blades.

A section of dorsal muscle approximately 3 cm in length was dissected from each flatfish specimen and pooled to make one sample. The sample was split in two with one portion for analysis of metals and a second portion for PCBs. Livers were dissected out and split in a similar manner. Two replicates of each tissue sample were analyzed.

Claw meat from a minimum of 10 crabs was dissected out for analysis. Dissections were pooled and subsequently divided for analysis as described for flatfish tissue.

Clams obtained at the Reference site were shucked whole for analysis as were M. balthica from the laboratory experiment. Polychaete worms were analyzed whole.

2.3   Analytical Methodology

2.3.1   Seawater

2.3.1.1   Lead and Cadmium

Seawater sample filtration was performed in the laboratory using an in-line polypropylene filter assembly, connected with clear tygon tubing to an all-glass receiving flask. Cellulose acetate filter papers (0.45 μm) were pre-cleaned using dilute nitric acid and stored in Milli-Q deionized water.

The filtered samples were pre-concentrated using the solvent extraction procedure described by Danielsson et al. (1978). Specifically, 250 mL of seawater was transferred to a one liter glass separatory funnel equipped with a teflon stopcock and polypropylene stopper. The pH of the solution was adjusted to 5.0 ± 0.2 with the addition of a citrate buffer. Chelation of the Pb and Cd was accomplished by adding 5.0 mLs of a 1% (w/v) mixture of both APDC (ammonium pyrrolidine dithiocarbamate) and DDTC (diethylammonium diethyldithiocarbamate). The solutions were allowed to sit for five minutes to allow for complete chelation of the metals. Each solution was extracted twice with 20 and 10 mL aliquots of Freon TF (1, 1, 2-trichloro - 1,2,2-trifluoroethane). The Freon containing the metal-carbanate complexes was transferred to 50 mL polypropylene test tubes and decomposed by shaking with 200 uL of concentrated nitric acid (Baker Ultrapure). Five milliliters of Milli-Q water was pipetted into each tube, shaken for 30 seconds and allowed to separate. The aqueous layer was analyzed for Cd and Pb by graphite furnace atomic absorption spectrophotometry (AAS) (Perkin Elmer Model 2380 AA equipped with a Model HGA - 400 graphite furnace in the background correction mode). Calibration curves were prepared by

carrying synthetic seawater standards through the same extraction procedure. Analytical detection limits for seawater analyses were defined as 3X the standard deviation obtained from measurement of five reagent blanks.

2.3.1.2   Polychlorinated Biphenyls (PCBs)

The unfiltered and unpreserved seawater samples (800 mL) were extracted once with 70 mL hexane, followed by 2 x 50 mL hexane. The combined hexane extracts were filtered through sodium sulfate, chromatographed on Florisil (50 mL hexane), then evaporated to about 10 mL using a Buchi Rotavapor. The final evaporation to 1 mL was carried out under nitrogen in a centrifuge tube at 40°C. The extract was analyzed on a Hewlett Packard 5710 gas chromatograph equipped with an electron capture detector. PCB concentration as Aroclors 1242, 1254 and 1260 was determined by following the method of Webb and McCall (1973) which utilizes at least two peaks per Aroclor compound. The mean concentration for these peaks gives the result for a particular Aroclor.

2.3.1.3   Precision and Accuracy

Reagent blanks, duplicates, spikes and certified reference materials were analyzed concurrently with the samples. In the case of the metal determinations, filtration blanks and a certified reference seawater (NASS-I) were analyzed periodically with the samples. These precautions served to check both procedural contamination and determine accuracy. Precision of the analysis was monitored by extracting every sample in duplicate.

2.3.2   Tissue

2.3.2.1   Lead and Cadmium

After depuration and dissection, tissues were oven dried at 103°C to determine moisture content. The dried tissue was ground to a powder in an agate mortar and preserved for analysis.

Where sufficient quantity of tissue permitted, 0.5 g of dried ground tissue was transferred to digestion vessels equipped with special reflux caps. Digestion was performed in a stainless steel fume hood using a combination of nitric and perchloric acids. Where sample size was restricted, micro digestions were carried out using proportionately less volume of reagents.

Analysis of the extracts was performed in the same manner as for the seawater extracts, except that a chemical pre-treatment was involved. To avoid inherent matrix interferences, a modifier, consisting of 5% (w/v) ammonium phosphate was added to all standards and samples. One milliliter of sample was transferred to a pre-cleaned sample vial followed by 100 μL of matrix modifier. The solution was mixed by swirling, and aliquots were drawn off for subsequent analysis by graphite furnace AAS.

2.3.2.2   Polychlorinated Biphenyls (PCBs)

Separate aliquots of tissue were thawed, and the wet tissue was extracted with 2 x 40 mL acetonitrile, using a polytron homogenizer to maintain a slurry. The combined acetonitrile extracts were decanted into 500 mL of water adjusted to pH 14. Any PCB present was then extracted into hexane, cleaned up, concentrated and analyzed in the same manner as for the seawater samples.

2.3.2.3 Precision and Accuracy

For the determination of Cd and Pb, the QA/QC program consisted of the analysis of reagent blanks and a certified reference material (NBS - oyster tissue). The QA/QC program for PCB analysis consisted of the concurrent analysis of reagent blanks and analyte spikes.

2.3.3 Sediments

2.3.3.1 Lead, Cadmium and Other Metals

Each sample was hand blended wet to ensure homogeneity. Subsamples of the blended sediment were taken for separate moisture determination, PCB analysis and metal analysis.

Digestion of the sediment for metal analysis was carried out in 125 mL Erlenmeyer flasks equipped with reflux caps. The digestion consisted of 10 g of wet sediment followed by 10 mLs of concentrated nitric acid (HNO_3). The mixture was slowly heated until the solution became clear at which time a further 5 mL of HNO_3 was added followed by 10 mL of $HClO_4$. Heating was then continued until dense white fumes evolved and digestion was complete. After bulking to volume with deionized water, the extracts were analyzed for Cd and Pb by direct flame AAS.

Additional analysis of the extracts for metals was performed using Inductively Coupled Argon Plasma (ICAP). The ICAP is complimentary to atomic absorption and is capable of analyzing a solution for up to thirty-five elements, simultaneously.

2.3.3.2 Polychlorinated Biphenyls (PCBs)

Subsamples of previously thawed wet sediment were extracted once with acetone and once with a mixture of acetone:hexane. During the extraction, the sample flasks were agitated for two hours on a rotary shaker. Both solvent fractions were combined, washed with

organic-free water and filtered through anhydrous sodium sulfate. The dried hexane fraction was cleaned up with fluorisil, evaporated on a Buchi Rotavapor, treated to removed sulphur and analyzed for PCBs in the same manner as for the seawater samples.

2.3.3.3 Total Organic Carbon

The sediments were oven dried at 103°C and ground to a fine powder. Subsamples were leached with dilute (10% v/v) HCl, dried by vacuum filtration and analyzed for organic carbon using a Leco Induction Furnace.

2.3.3.4 Precision and Accuracy

During metal analysis, samples were analyzed concurrently with reagent blanks, duplicates and certified reference standards. Samples for PCBs were analyzed with reagent blanks and analyte spikes.

2.4 Quality Assurance/Quality Control (QA/QC)

Quality control, in the form of analyzing reagent blanks, filter blanks, reference standards and duplicate samples, was extensive for this project. The following sections discuss the QA/QC program in detail.

2.4.1 Apparatus Préparation

All glassware, plasticware, sample containers, and other utensils were pre-cleaned using a 20% nitric acid bath followed by repeated rinses with Milli-Q reagent grade water. Membrane filters, used in the filtration of the seawater samples, were cleaned in a similar manner except that 5% nitric acid was used. Containers used in the storage and preparation of PCB samples were rinsed with acetone and hexane prior to use.

2.4.2   Sample Handling

Prior to analysis of the samples, preparation stages (i.e. filtration, tissue dissection) were carried out under clean room conditions. Tissue dissections were performed using plastic forceps and stainless steel scalpels. The dissected tissues for metals analysis were placed in prepared plastic containers and stored frozen in plastic bags until analysis. The tissues for PCB analysis were stored frozen in prepared aluminum foil until analysis.

Seawater samples were filtered within 3 h of collection using an all plastic in-line filter holder (Millipore). Filter blanks were prepared by filtering Milli-Q water in the same manner as the samples. Blanks were analyzed concurrently with the samples.

2.4.3   Reagents

The reagents used in this study were either purchased as "ultra pure" grade or purified prior to use. Reagent grade Milli-Q water was used exclusively in the cleaning of all apparatus as well as the preparation of all aqueous reagents. Ultrex reagent grade nitric acid was used where appropriate in the tissue digestions and seawater extractions.

Reagent blanks were prepared and analyzed concurrently with all sample batches.

2.4.4   Sample Digestion

Both tissue and sediment samples were digested as outlined in Section 2.3. Dedicated glassware was used for both sample types after rigorous preparation as outlined in Section 2.4.1.

Accuracy of the techniques was monitored using certified reference materials closely matching the sample matrix. Oyster tissue (NBS) was used as the tissue reference while marine sediments (NRC - MESS & BCSS) were used as the sediment reference.

2.4.5   Seawater Extractions

Pre-concentration of seawater was performed as outlined in Section 2.3. The samples were extracted in duplicate as soon as possible after collection and filtering. All steps of the extraction procedure were carried out in an isolated clean room reserved exclusively for this type of work.

Filter blanks and a certified reference seawater (NRC - NASS-I) were extracted and analyzed with the samples.

2.5   Statistical Analysis

Statistical analysis of the data was carried out by Quantum Research Ltd. One-way analysis of variance (ANOVA) was used to test statistically significant differences in tissue accumulation data for M. balthica by site and by month for each month sampled. Where significant (p less than 0.05) differences were found, distinct subsets were identified by applying the Studentized Newman-Keuls test. Both programs were run on the "BMD 10 V - General Linear Hypothesis (No. 2) Revised November 3, 1975" computer program of the Health Sciences Computing Facility, UCLA. ANOVA was also used to test differences in levels of tissue contaminants in resident biota from different sites.

A standard paired t-test was used to test differences between samples of M. balthica which had been depurated and those which had digestive tracts removed. Procedures for this test followed those described by Sokal and Rohlf (1969).

3.0   RESULTS

A summary of test results is presented in Tables 1 to 19 and Figures 1 and 2. Raw data for all chemical analysis are presented in the Appendix.

3.1 Quality Assurance/Quality Control

Results of the quality control analyses including filter blanks and reference standards for seawater, sediments and tissues are given in Tables 1 to 8. A summary of the results for cadmium and lead are given below.

SUMMARY OF RESULTS OBTAINED FOR METAL ANALYSIS IN CERTIFIED REFERENCE STANDARDS

Element	Reference Standard	No. of Replicates	Certified Result	Analytical Result	C. V.
Cadmium	NASS-I Seawater	5	0.029+0.004	0.03+0.01	33.3
	NBS Oyster Tissue	7	3.5+0.4	3.51+0.24	6.8
	MESS-I Marine Sediment	3	0.59+0.10	0.6+0.1	16.7
	BCSS-I Marine Sediment	3	0.25+0.04	<0.5	-
Lead	NASS-I Seawater	5	0.039+0.006	0.05+0.01	20.0
	NBS Oyster Tissue	7	0.48+0.04	0.49+0.05	10.2
	MESS-I Marine Sediment	3	34.0+6.1	30.0+1.0	3.3
	BCSS-I Marine Sediment	3	22.7+3.4	21.0+2.0	9.5

C.V.=coefficient of variation = $\frac{\text{Standard Deviation} \times 100}{\text{Mean Result}}$

- results are expressed as micrograms of element per gram of standard

3.1.1 Reagent Blanks

Both the filtration blanks (seawater) and the digestion blanks (tissues and sediments) were near or below stated detection limits for all analyses. In all cases the extraction blanks for the PCB analysis were below detection levels. These results illustrate that procedural contamination was not a problem for any of the analyses within the stated detection limits.

3.1.2 Certified Reference Standards

Results for the reference standards presented in the summary table above show a high level of agreement between the certified result and the results obtained during this project. Although the standards are

TABLE I
QUALITY CONTROL RESULTS - FILTER BLANKS

REFERENCE STANDARD: ASL Filter Banks

DATE ANALYZED	CADMIUM	LEAD
March 3, 1983	< 0.01	< 0.05
March 24, 1983	< 0.01	< 0.05
April 29, 1983	0.01	< 0.05
June 28, 1983	< 0.01	< 0.05
September 12, 1983	< 0.01	< 0.05

Results are expressed as micrograms of element per liter of sample (ppb).

TABLE 2
QUALITY CONTROL RESULTS - SEAWATER

REFERENCE STANDARD: NRC Seawater (NASS-I)

DATE ANALYZED	CADMIUM	LEAD
March 3, 1983	0.03	0.05
March 24, 1983	0.04	0.04*
April 29, 1983	0.03	0.04*
June 28, 1983	0.03	0.05
September 12, 1983	0.04	0.06
CERTIFIED VALUES	0.29±0.004	0.039±0.006

*below usual quoted detection limit

Results are expressed as micrograms of element per liter of sample (ppb).

TABLE 3

QUALITY CONTROL RESULTS - TISSUE DIGESTION BLANKS

REFERENCE STANDARD: Tissue Digestion Blanks

DATE ANALYZED	CADMIUM	LEAD
June 15, 1983	< 0.0005/<0.0005	0.001/<0.001
November 25, 1983	< 0.0005/<0.0005	0.001/0.001
December 7, 1983	< 0.0005/<0.0005	<0.001/<0.001
December 14, 1983	< 0.0005/<0.0005	<0.001/<0.001

Results are expressed as micrograms of element per milliliter of solution (ppm).

TABLE 4

QUALITY CONTROL RESULTS - OYSTER TISSUE

REFERENCE STANDARD: NBS Oyster Tissue

DATE ANALYZED	CADMIUM	LEAD
June 15, 1983	3.26	0.42
November 25, 1983	3.50/3.70	0.56/0.53
December 7, 1983	3.85/3.65	0.43/0.46
December 14, 1983	3.20/3.40	0.50/0.52
CERTIFIED VALUES	3.5±0.4	0.48±0.04

Results are expressed as micrograms of element per milliliter of solution (ppm).

TABLE 5

QUALITY CONTROL RESULTS - SEDIMENT DIGESTION BLANKS

REFERENCE STANDARD: Sediment Digestion Blanks

DATE ANALYZED	CADMIUM	LEAD
June 14, 1983	< 0.005/<0.005	< 0.05/<0.05
September 13, 1983	< 0.005/<0.005	< 0.05/<0.05
November 22, 1983	< 0.005/<0.005	< 0.05/<0.05

Results are expressed as micrograms of element per milliliter of solution (ppm).

TABLE 6

QUALITY CONTROL RESULTS - PCB RECOVERY

The following table summarizes the blank and spike recovery data for the analysis of PCB.

SAMPLE TYPE	EXTRACTION BLANK	SPIKED STANDARD		RECOVERY OF SPIKE (%)
Seawater	<0.01	1	1254	99.6
Seawater	<0.01	1	1254	103.4
Seawater	<0.01	1	1254	92.6
Seawater	<0.01	1	1254	96.6
Tissue	<0.01	1	1242	93.8
Tissue	<0.01	0.33	1254	94.7
Tissue	<0.01	0.33	1260	90.6
Sediment	<0.01	0.10	1254	86.3

Blank and standard levels are expressed as micrograms per liter for the seawaters and micrograms per gram for both the tissues and the sediments.

TABLE 7

QUALITY CONTROL RESULTS - SEDIMENT (MESS-1)

REFERENCE STANDARD: NRC Marine Sediment (MESS-1)

DATE ANALYZED	CADMIUM	LEAD
June 14, 1983	0.6	29.0
September 13, 1983	0.5	31.0
November 22, 1983	0.6	31.0
CERTIFIED VALUES	0.59±0.10	34.0±6.1

Results are expressed as micrograms of element per milliliter of solution (ppm).

TABLE 8

QUALITY CONTROL RESULTS - SEDIMENT (BCSS-1)

REFERENCE STANDARD: NRC Marine Sediment (BCSS-1)

DATE ANALYZED	CADMIUM	LEAD
June 14, 1983	<0.5	21.0
September 13, 1983	<0.5	19.0
November 22, 1983	<0.5	23.0
CERTIFIED VALUES	0.25±0.4	22.7±3.4

Results are expressed as micrograms of element per milliliter of solution (ppm).

more suitable (i.e. dried homogeneous powder, elevated levels and known target values) than the test samples, they provide a good estimate of analytical accuracy.

3.1.3   Duplicate Analysis

Where sample size permitted, duplicate analysis was performed as a measure of intralab precision. Duplicate data are tabulated in the Appendix. Agreement between tested duplicates was generally within acceptable margins of $\pm 10\%$ irrespective of whether the values were near detection limits or somewhat elevated.

3.2   Characteristics of Test Sediments

Particle size data for test sediments are provided in Table 9, and chemical data in Tables 10 and 11. Sediment from the reference site was a sandy-mud, somewhat coarser than the two contaminated test sediments (Table 9). The finest sediment was from False Creek.

Cadmium was present at the highest concentration in sediments from False Creek (2.78 μg/dry g), and was below the limits of detection (0.25 μg/dry g) in the reference sediment (Table 10). There was little change in concentrations over the study period. Lead values were also highest in the False Creek sediment (223 μg/dry g). Lead appeared to show an increase in concentration in all three sediments at the end of six months compared to initial values.

Total PCBs were present at concentrations below the detection limit (0.01 μg/dry g) in the reference sediment and were highest in sediment from Vancouver Harbor (4.26 μg/dry g). There appeared to be a decrease in Aroclor 1254 (1.29 to less than 0.01 ppm), and a corresponding increase in Aroclor 1260 (0.58 to 2.29 ppm) over the study period in the Vancouver Harbor sediments.

TABLE 9

PHYSICAL CHARACTERIZATION OF TEST SEDIMENTS

Sediment	% Gravel Initial	Final	% Sand Initial	Final	% Silt/Clay Initial	Final
Reference	3.8	-	58.1	40.5	38.1	59.5
Vancouver Harbor	0.6	-	33.4	24.6	66.0	75.4
False Creek	0.1	-	17.2	3.9	82.7	96.1

Percentages are by dry weight: gravel >2.0 mm; sand 2.0-0.063 mm; silt/clay <0.063

TABLE 10

CHEMICAL CHARACTERIZATION OF TEST SEDIMENTS

Sediment	Cadmium Initial	Final	Lead Initial	Final	Aroclor 1254 (μg/dry g) Initial	Final	Aroclor 1260 Initial	Final	Total Organic Carbon (%) Initial	Final
Reference	<0.25	<0.25	8.9	10.6	<0.01	<0.01	<0.01	<0.01	1.75	1.80
Vancouver Harbor	0.56	0.49	185	236	1.29	<0.01	0.58	2.39	2.23	2.13
False Creek	2.78	2.92	223	258	-	-	-	-	3.51	3.68

The highest level of total organic carbon (TOC) was recorded in the False Creek sediments (3.51%), and the lowest in the Reference sediment (1.75%). There was relatively little change in TOC over the study period.

Additional chemical data on sediments sampled at the start of the experiment are provided in Table 11. The level of freon extractables (a measure of oil and grease) showed higher values in both False Creek (1700 μg/dry g) and Vancouver Harbor (1070 μg/dry g) than the Reference sediment (176 μg/dry g). Potentially toxic metals identified by ICAP which showed elevated levels in the test sediments compared to the reference site included aluminum, cadmium, chromium, copper, iron, lead, nickel, vanadium and zinc.

3.3 Release of Contaminants from Test Sediments

Results of the chemical analysis of seawater overlying test sediments are summarized in Figure 1.

3.3.1 Cadmium

Levels of dissolved cadmium were similar (0.15-0.20 μg/Kg) in seawater overlying all test sediments 24 h after start-up of the experiment. The concentration of cadmium decreased in the Reference sediment tray after 1 month, then increased to its highest level (0.4 μg/Kg) after 2 months. Vancouver Harbor sediments showed a peak release of cadmium at 1 month (1.0 μg/Kg) after which levels declined. Cadmium release from False Creek sediments appeared to reach a maximum (0.3 μg/Kg) after 2 months. The concentration of cadmium in seawater above all test sediments was at or below background seawater intake levels at months 4 and 6. Background levels of cadmium were generally less than 0.1 μg/Kg.

TABLE II

RESULTS OF TESTING

Additional Sediment Analysis

PARAMETER		REF 1, Sept. 6	V.H., Sept. 6	F.C., Sept. 6
Moisture	(%)	40.5	48.7	60.8
Freon Extractables		176.	1,060.	1,700.
Aluminum	Al	19,600.	33,600.	43,400.
Arsenic	As	<10.	<10.	<10.
Cadmium	Cd	<0.15	0.53	3.10
Calcium	Ca	9,390.	16,400.	26,500.
Chromium	Cr	19.9	75.3	64.1
Cobalt	Co	7.1	11.8	11.5
Copper	Cu	16.8	393.	153.
Iron	Fe	27,000.	42,700.	51,200.
Lead	Pb	6.6	165.	235.
Magnesium	Mg	11,200.	17,200.	21,800.
Manganese	Mn	309.	445.	560.
Mercury	Hg	0.11	0.09	0.10
Molybdenum	Mo	<1.	<1.	<1.
Nickel	Ni	14.	53.	38.
Potassium	K	2,770.	3,570.	5,200.
Sodium	Na	10,800.	15,400.	23,700.
Vanadium	V	24.8	38.2	47.9
Zinc	Zn	50.3	455.	441.

Results are expressed as micrograms per dry gram except moisture which is as percent.

REF = Reference; VH = Vancouver Harbor; FC = False Creek
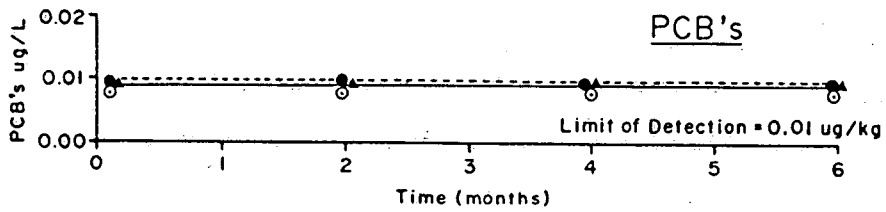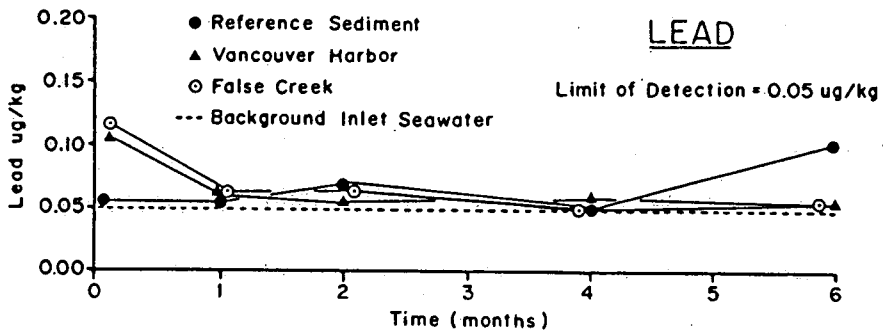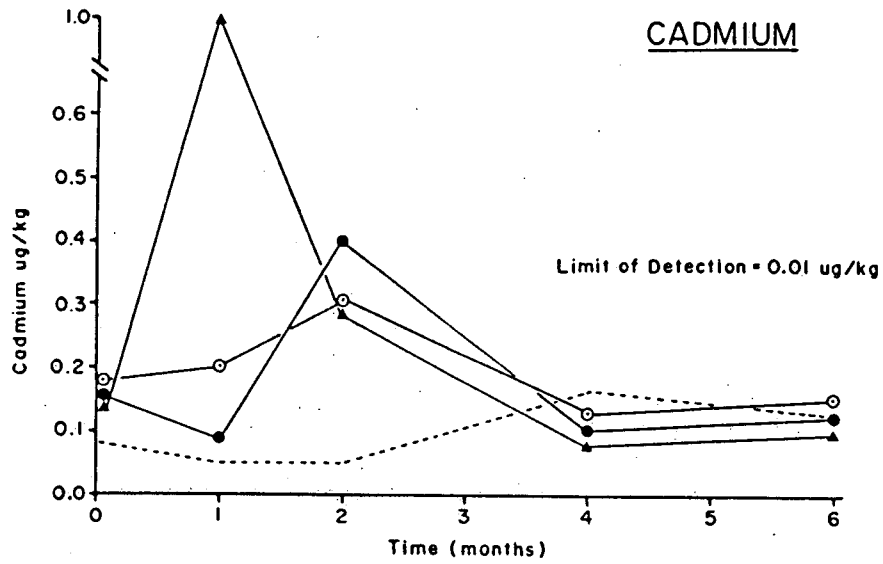
Figure I. Concentrations of dissolved metals and PCB's in seawater overlying test sediments.

3.3.2 Lead

Concentrations of dissolved lead remained at or near the analytical detection limit of 0.05 μg/Kg after an initial release by the Vancouver Harbor and False Creek sediments (Fig. 1). Levels in seawater over the Reference sediment were below detection limits on all sampling occasions with the exception of month 6. Background seawater levels were consistently below the detection limit of 0.05 μg/Kg.

3.3.3 PCBs

Background and test container seawater PCB concentrations were consistently below the analytical detection limit of 0.01 μg/Kg.

3.4 Bioaccumulation in Laboratory Bioassays

Results of a comparison to test the effectiveness of depurating M. balthica are given in Table 12. A summary of the bioaccumulation results for M. balthica from the laboratory bioassays is given in Table 13 and in Figure 2. Statistical treatment of the data is presented in Tables 14 and 15.

3.4.1 Effectiveness of Tissue Depuration

Comparative tissue levels of contaminants in depurated M. balthica are compared with clams from which digestive tracts had been removed in Table 12. Statistical comparison between 'paired' samples showed no significant differences (p less than 0.05) for any of the contaminants. These results confirmed that a 48 h depuration period was effective in removing contaminated sediment which might affect tissue analysis.

TABLE 12

RESULTS OF COMPARATIVE TISSUE ANALYSIS BETWEEN M. balthica INDIVIDUALS DEPURATED IN CLEAN SEAWATER AND INDIVIDUALS WITH DIGESTIVE TRACT REMOVED

Contaminant Tested	M. balthica Depurated for 48 h in Clean Seawater	M. balthica with Digestive Tract Removed	t-test Value	Significance at p<0.05
Cadmium	0.19 0.22 0.45 $\bar{x}$ = 0.29 SD= 0.14	0.21 0.18 0.35 $\bar{x}$ = 0.25 SD= 0.09	0.411	ns
Lead	3.01 2.93 2.91 $\bar{x}$ = 2.95 SD= 0.05	2.48 1.95 3.15 $\bar{x}$ = 2.53 SD= 0.60	1.214	ns
Aroclor 1254	0.86 1.14 1.16 $\bar{x}$ = 1.05 SD= 0.17	1.23 0.92 0.92 $\bar{x}$ = 1.02 SD= 0.18	0.212	ns
Aroclor 1260	0.39 0.48 0.38 $\bar{x}$ = 0.42 SD= 0.06	0.62 0.31 0.46 $\bar{x}$ = 0.46 SD= 0.16	-0.491	ns

$\bar{x}$ = mean; SD = standard deviation; ns = not significant

TABLE 13

MEAN ± SE AND (RANGE) OF VALUES (µg/dry g) FOR TISSUE METAL
CONCENTRATIONS IN Macoma balthica

Sediment	Time (months)	Cadmium	Lead	PCBs		
				1242	Aroclor 1254	1260
Background		0.80+0.63* (0.11-1.50)	0.24+0.07 (0.10-0.35)	<0.1+0.0 (<0.1)	0.3+0.1 (0.2-0.4)	0.2+0.0 (0.1-0.2)
Reference	1	0.19+0.04 (0.14-0.26)	1.18+0.26 (0.85-1.69)	<0.1+0.0 (<0.1)	0.21+0.08 (0.09+0.36)	<0.1+0.0 (<0.1)
	2	0.25+0.04 (0.17-0.30)	1.88+0.15 (1.59-2.07)	<0.1+0.0 (<0.1)	0.22+0.03 (0.19-0.29)	<0.1+0.0 (<0.1)
	4	0.40+0.06 (0.28+0.50)	5.27+0.66 (3.94+5.95)	<0.1+0.0 (<0.1)	0.20+0.04 (0.15+0.27)	0.29+0.12 (0.15-0.54)
	6	0.13+0.01 (0.11-0.16)	1.11+0.25 (0.83-1.61)	<0.1+0.0 (<0.1)	0.20+0.10 (<0.1-0.39)	0.25+0.15 (<0.1-0.55)
Vancouver Harbor	1	0.46+0.28 (0.6-1.02)	2.98+0.11 (2.75-3.09)	<0.1+0.0 (<0.1)	0.89+0.13 (0.76-1.14)	0.35+0.03 (0.29-0.38)
	2	0.29+0.08 (0.19-0.45)	2.95+0.03 (2.91+3.01)	<0.1+0.0 (<0.1)	1.05+0.10 (0.86-1.16)	0.42+0.03 (0.38-0.48)
	4	0.39+0.08 (0.25-0.53)	11.6+1.88 (8.51-15.0)	<0.1+0.0 (<0.1)	0.79+0.10 (0.60-0.92)	0.75+0.12 (0.54-0.95)
	6	0.14+0.02* (0.11-0.16)	0.73+0.01* (0.72-0.74)	**	**	**
False Creek	1	0.19+0.02 (0.16-0.23)	2.25+0.16 (2.02-2.55)	-	-	-
	2	0.19+0.05 (0.09-0.26)	4.30+0.44 (3.82-5.17)	-	-	-
	4	0.34+0.08* (0.26-0.41)	14.6+2.2* (12.5-16.8)	-	-	-
	6	**	**	-	-	-

n=3 with approximately 50 individuals per sample
* indicates n=2
**insufficient tissue for analysis
- not included in analysis
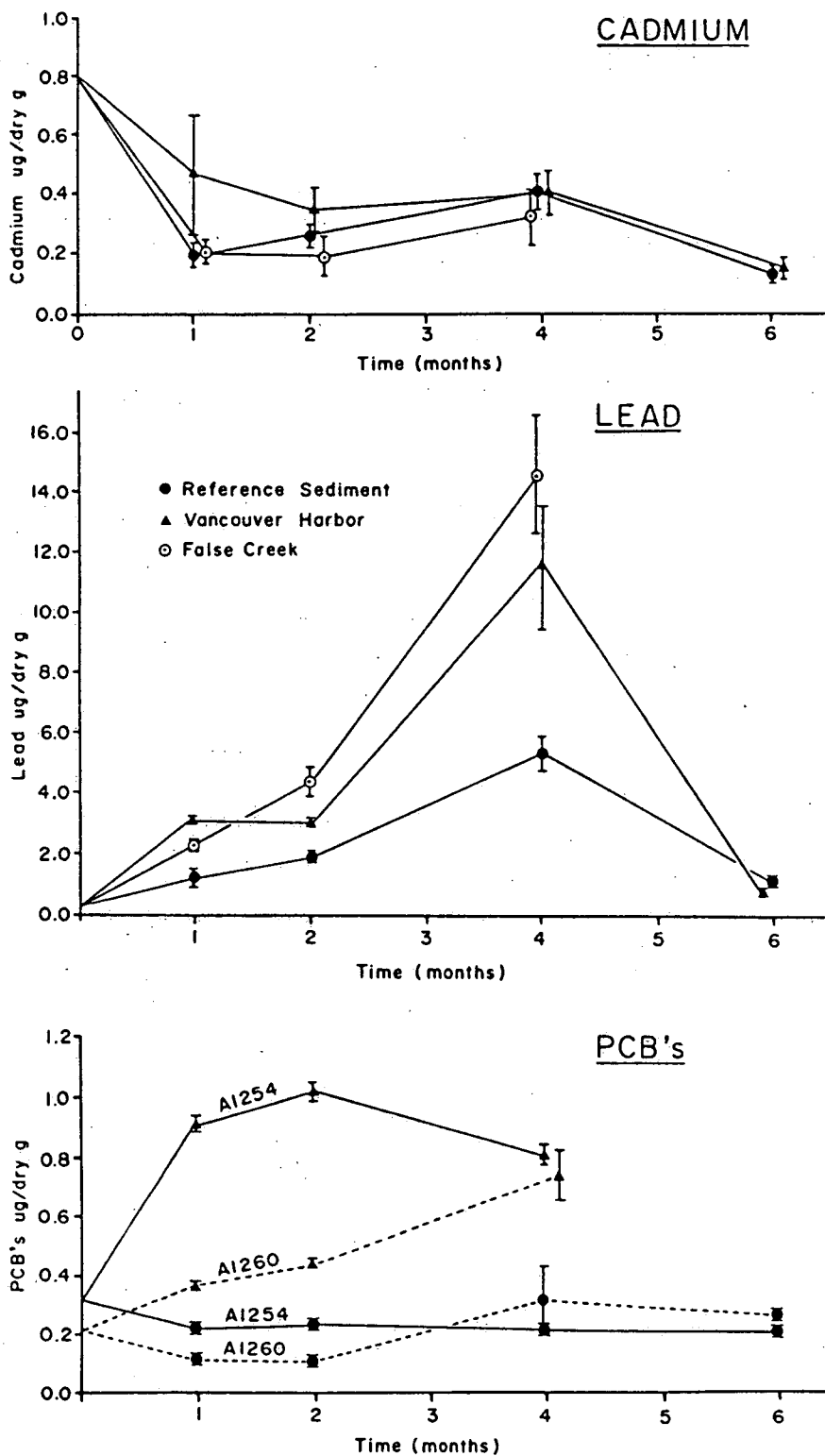
Figure 2. Bioaccumulation of cadmium, lead and PCB's in **M. balthica** exposed to test sediments in laboratory bioassays. Values shown are mean ± S.E. of three replicates.

TABLE 14

SUMMARY OF ONE WAY ANOVA* BY SITE FOR M. balthica TISSUE
CONTAMINANT CONCENTRATIONS FOR EACH MONTH SAMPLED

REF = Reference sediment; VH = Vancouver Harbor; FC = False Creek

Contaminant	Month	Distinct Homogeneous Subsets (i.e. Sites) Identified by Studentized Newman-Keuls Range Test $(p<0.05)$
Cadmium	1	ns
	2	ns
	4	ns
	6	ns
Lead	1	(REF) (FC, VH)
	2	(REF) (VH) (FC)
	4	(REF) (VH, FC)
	6	ns
Aroclor 1254	1	(REF) (VH)
	2	(REF) (VH)
	4	(REF) (VH)
	6	-
Aroclor 1260	1	(REF) (VH)
	2	(REF) (VH)
	4	ns
	6	-

*all results were significant at $p<0.05$ except where indicated
ns=not significant
-indicates insufficient tissue data for analysis

TABLE 15

SUMMARY OF ONE WAY ANOVA* BY MONTH FOR M. balthica
TISSUE CONTAMINANT CONCENTRATIONS FOR EACH SITE TESTED

Contaminant	Site	Distinct Homogeneous Subsets (i.e. Months) Identified by Studentized Newman-Keuls Range Test ($p < 0.05$)
Cadmium	Reference	ns
	Vancouver Harbor	ns
	False Creek	ns
Lead	Reference	(0, 1, 6) (1, 2, 6) (4)
	Vancouver Harbor	(0, 1, 2, 6) (4)
	False Creek	(0, 1) (1, 2) (4)
Aroclor 1254	Reference	ns
	Vancouver Harbor	(0) (1, 2, 4)
Aroclor 1260	Reference	ns
	Vancouver Harbor	(0) (1, 2) (4)

*all results were significant at $p < 0.05$ except where indicated
ns=not significant

3.4.2 Cadmium

Cadmium was not accumulated by M. balthica over background levels (Fig. 2). In fact, levels decreased in all experimental test trays. Concentrations of cadmium in M. balthica from the Reference and False Creek sediments fluctuated in a relatively narrow range between 0.2 and 0.4 µg/dry g. Statistical analysis of the data indicated no significant differences (p less than 0.05) in the bioaccumulation of cadmium amongst sites (Table 14) or over time at any one site (Table 15).

[handwritten margin note: no Powell River seds used in this study]

3.4.3 Lead

In contrast to cadmium, bioaccumulation of lead was observed in all sediments, including the Reference sediment (Fig. 2). Highest mean values were recorded in the False Creek (14.6 µg/dry g) and Vancouver Harbor (11.6 µg/dry g) sediments after 4 months. Tissue concentrations then declined sharply to near background levels in the Vancouver Harbor sediment at month 6. A similar decrease was observed after 6 months for the Reference sediment. No data were available for the False Creek sediments after 6 months as no live clams remained. These results are confirmed statistically in Tables 14 and 15. A significant difference in bioaccumulation from time zero was consistently observed after 4 months in each test sediment (Table 15).

3.4.4 PCBs

PCB levels in M. balthica from the Reference sediment were relatively stable (Fig. 2). In contrast, clams from the Vancouver Harbor test sediment showed increased levels of Aroclor 1254 and 1260 over four months. No data were available for the six month sampling period from the Vancouver Harbor sediments due to the number of mortalities in this sediment. Uptake of Aroclor 1254 was more rapid than for Aroclor 1260 but also more irregular. Aroclor 1260 showed a steady uptake over the entire study period. Highest

tissue concentrations measured were 1.05 and 0.75 μg/dry g for Aroclors 1254 and 1260 respectively (Table 13). The trends in uptake in the Vancouver Harbor sediments identified above were shown to be statistically significant (p less than 0.05) (Tables 14 and 15).

Analysis of PCBs was not included for False Creek sediments in this study as results of previous testing by the Environmental Protection Service had shown low levels of these compounds, and bioaccumulation of PCBs from False Creek sediments was not a concern.

3.5 Mortalities in Test Sediments

Relative mortalities of M. balthica in the Reference and contaminated test sediments are shown in Table 16. Mortalities reached 12% in the Reference sediment after 6 months compared to 20% in the Vancouver Harbor sediments. False Creek sediments exhibited 40% mortalities after only 4 months of exposure. The rate of mortality in the False Creek sediments resulted in no live clams remaining after the four month sampling period.

Several dozen "spat" (newly settled juvenile clams) were found in the Reference sediment at the end of six months. This observation indicated that clams had matured and spawned successfully in this container. Spat were not observed in the contaminated sediments.

3.6 Bioaccumulation in Resident Biota

A summary of tissue contaminant data for resident biota is given in Table 17. Statistically significant differences in tissue concentrations among sites are summarized in Table 18.

3.6.1 Cadmium

The highest levels of cadmium were detected in infauna (4.8 μg/dry g) and sole liver (2.7 μg/dry g) at the Powell River site. These results were significantly different compared to the Reference site (Table

TABLE 16

RELATIVE MORTALITIES FOR M. balthica IN DIFFERENT TEST SEDIMENTS DURING LABORATORY BIOASSAYS

	MONTHS					
	1	2	3	4	5	6
REFERENCE						12%
VANCOUVER HARBOR						20%
FALSE CREEK				40%		

TABLE 17

RESULTS OF CONTAMINANT TISSUE ANALYSIS FOR RESIDENT BIOTA

Bold typeface indicated highest concentration encountered for any single type of tissue.

Contaminant	Reference Site				Vancouver Harbor			
	Infauna[1]	Crab[2] Meat	Muscle	Sole[3] Liver	Infauna[1]	Crab[2] Meat	Muscle	Sole[3] Liver
Cadmium	3.46	<0.05	<0.05	0.98	0.73	<0.05	<0.05	0.98
	0.82*	0.13	<0.05	1.35	0.97	<0.05	<0.05	0.51
	x= 2.14	0.09	<0.05	1.16	0.85	<0.05	<0.05	0.74
Lead	1.98	<0.10	0.35	1.72	10.2	0.50	0.30	4.78
	3.86*	<0.10	0.10	1.82	13.2	0.40	0.40	2.04
	x= 2.92	<0.10	0.22	1.77	11.7	0.45	0.35	3.41
Aroclor 1254	0.32	<0.10	0.14	1.33	1.69	<0.10	0.21	8.5
	<0.10*	<0.10	0.19	0.33	1.64	<0.10	1.19	13.5
	x= 0.21	<0.10	0.12	0.83	1.66	<0.10	0.70	11.0
Aroclor 1260	<0.10	<0.10	<0.10	0.66	0.37	<0.10	0.21	5.59
	<0.10*	<0.10	<0.14	0.23	1.11	<0.10	0.83	9.31
	x= <0.10	<0.10	0.12	0.44	0.74	<0.10	0.52	7.45

TABLE 17 (cont'd)

Contaminant	False Creek				Powell River			
	Infauna[1]	Crab[2] Meat	Muscle	Sole[3] Liver	Infauna[1]	Crab[2] Meat	Muscle	Sole[4] Liver
Cadmium	0.70	<0.05	<0.05	0.40	4.9	<0.05	<0.05	3.0
	0.27	<0.05	<0.05	0.35	4.7	<0.05	<0.05	2.4
	0.48	<0.05	<0.05	0.38	4.8	<0.05	<0.05	2.7
Lead	3.70	<0.10	<0.10	0.85	7.5	<0.10	<0.10	2.0
	6.10	<0.10	0.35	0.85	8.5	<0.10	<0.10	0.7
	4.90	<0.10	0.23	0.85	8.0	<0.10	<0.10	1.35
Aroclor 1254		not analyzed for PCBs				not analyzed for PCBs		
Aroclor 1260		not analyzed for PCBs				not analyzed for PCBs		

[1] Infauna comprised of several species of polychaete worms except for one sample from the Reference Site (*) which was made up of two species of bivalve molluscs (Macoma nasuta; M. carlotensis)

[2] crabs analyzed were Cancer magister

[3] sole at these sites were the English sole (Parophrys vetulus)

[4] sole at this site were the Butter sole (Isopsetta isolepis)

TABLE 18

SUMMARY OF ONE WAY ANOVA BY SITE FOR MEAN TISSUE CONTAMINANT CONCENTRATIONS (µg/dry g) FOR RESIDENT BIOTA

Contaminant	Biota	Reference Site	False Creek	Vancouver Harbor	Powell River
Cadmium	Polychaete worms	3.46	0.70	0.73	4.80**
	Crab meat	0.09	<0.05	<0.05	<0.05
	Sole muscle	<0.05	<0.05	<0.05	<0.05
	Sole liver	1.16	0.38	0.74	2.7**
Lead	Polychaete worms	1.98	4.90	11.7**	8.0**
	Crab meat	<0.10	<0.10	0.45**	<0.10
	Sole muscle	0.22	0.23	0.35	<0.10
	Sole liver	1.77	0.85	3.41	1.35
Aroclor 1254	Polychaete worms	0.32	-	1.66**	-
	Crab meat	<0.10	-	<0.10	-
	Sole muscle	0.16	-	0.70*	-
	Sole liver	0.83	-	11.0**	-
Aroclor 1260	Polychaete worms	<0.10	-	0.74*	-
	Crab meat	<0.10	-	<0.10	-
	Sole muscle	0.12	-	0.52*	-
	Sole liver	0.44	-	7.45**	-

*significantly higher value from Reference Site at p<0.05
**significant at p<0.01
- not analyzed for PCBs

18).   Crab meat and sole muscle did not appear to be good bioaccumulation sites for cadmium. Polychaete worms sampled at the Reference site had higher levels of cadmium (3.46 μg/dry g) than bivalve molluscs (0.82 μg/dry g; Table 17) sampled at the same site. Of particular interest were the relatively low levels of cadmium in infauna (0.48 μg/dry g) and sole liver (0.38 μg/dry g) from False Creek despite the extremely high concentration of cadmium (3.51 μg/dry g) present in these sediments. Also noteworthy was the relatively high concentration of cadmium apparent in the polychaetes from the Reference site compared to the other sites sampled.

3.6.2   Lead

Compared to levels at the Reference site, the bioaccumulation of lead was greater for all the biota sampled at Vancouver Harbor.   The highest concentration of lead was measured in infauna (11.7 μg/dry g) from Vancouver Harbor; this value was statistically higher than levels in infauna from the Reference site. Lead was the only contaminant for which there was a statistically significant difference in concentrations in crab meat between sites (Vancouver Harbor versus the Reference site). Lead was also detected in significantly higher than background concentrations in infauna from Powell River (8.0 μg/dry g). Lead values in bivalves at the Reference site (3.86 μg/dry g) were higher than for polychaete worms (1.98 μg/dry g) at this site (Table 17).

3.6.3   PCBs

PCB Aroclors 1254 and 1260 were detected in significantly higher concentrations in infauna, sole muscle and sole liver from Vancouver Harbor compared to the Reference site (Table 18). Highest mean values were recorded in sole liver (11.0 μg/dry g) from Vancouver Harbor. A similar pattern was observed for Aroclor 1260 (Table 17). In each instance, concentrations of Aroclor 1260 were lower than for Aroclor 1254 in the same tissue.

4.0   DISCUSSION

4.1   Bioaccumulation Potential in Laboratory Bioassays
versus Resident Biota

A summary of the relationship between the laboratory bioassays and resident biota with respect to bioaccumulation is shown in Table 19. As noted previously, to evaluate bioaccumulation potential from a pulp mill site (Powell River), data from a previous 6 month laboratory bioaccumulation study with M. balthica (McGreer and Reid, 1984) are compared with resident biota obtained as part of the present study.

Laboratory bioassays with M. balthica indicated a significant degree of bioaccumulation potential for cadmium in Powell River sediments.

This predicted result was confirmed by levels of cadmium detected in resident biota (polychaete worms, sole liver) which were significantly higher than levels at the Powell River reference site. Resident biota from the other two contaminated sites did not show elevated levels of cadmium. Thus, the capacity of the laboratory bioassay for predicting the bioaccumulation potential of cadmium from contaminated sediments was confirmed.

Lead was significantly bioaccumulated by M. balthica in all three contaminated sediments compared to the Reference (Table 19). Tissue levels in resident biota from Vancouver Harbor (polychaetes, crab meat) and Powell River (polychaetes) also showed significantly higher levels of lead. However, resident biota did not show elevated concentrations of lead in False Creek. In this case, the laboratory bioassay results were not completely predictive. However, it is noteworthy that tissue lead values for polychaetes sampled in False Creek were more highly variable than at the other sites, which may be indicative of selective lead bioaccumulation by particular species or individuals.

TABLE 19

COMPARISON OF BIOACCUMULATION DATA FROM
LABORATORY BIOASSAY AND RESIDENT BIOTA

x = significantly higher bioaccumulation compared to
    reference sediment (bioasssay)/site (resident biota)
ns = no significant difference

Contaminant	Test Sediment/Site	Laboratory Bioassay	Resident Biota			
			Polychaete worms	Crab meat	Sole muscle	liver
Cadmium	Vancouver Harbor	ns	ns	ns	ns	ns
	False Creek	ns	ns	ns	ns	ns
	Powell River	x[1]	x	ns	ns	x
Lead	Vancouver Harbor	x	x	x	ns	ns[2]
	False Creek	x	ns[2]	ns	ns	ns
	Powell River	x[1]	x	ns	ns	ns
Aroclor 1254	Vancouver Harbor	x	x	ns	x	x
Aroclor 1260	Vancouver Harbor	x	x	ns	x	x

[1]significantly higher bioaccumulation compared to tissue levels at
start of experiment; data from McGreer and Reid (1984)

[2]means considerably higher than reference but not statistically significant
at $p<0.05$ due to high variability and relatively low number of replicates (n=2)

Both PCB Aroclors (1254 and 1260) were shown to have a significant bioaccumulation potential for Vancouver Harbor sediments. This prediction was corroborated by analyses of resident biota (polychaete worms, and sole muscle and liver) which all showed significant levels of bioaccumulation for Aroclors 1254 and 1260 (Table 19). Thus, the predictive capacity of the laboratory bioassay was confirmed for PCBs.

In summary, there was a high degree of success in predicting the bioaccumulation potential of the target contaminants in resident biota using laboratory bioassays with M. balthica. With respect to the numbers of contaminants and test sediments tested, the laboratory bioassays correctly predicted the bioaccumulation potential in 6 out of 7 cases (i.e. Cd in all sediments, Pb in two of three sediments, and PCB in VH sediments). For the contaminants assessed in the present study (cadmium, lead and PCBs), polychaete worms and sole liver appeared to be the most suitable tissues for assessing bioaccumulation from contaminated sediments in the field. Results of this initial study indicate that laboratory bioassays with M. balthica can be used to predict the bioaccumulation potential of sediment-associated contaminants. However, further development work is required to refine the protocol and specific aspects identified during our study are addressed below.

4.2   Aspects of the Laboratory Bioassay Protocol Requiring Standardization

The extent to which some of the test variables affect the outcome of the laboratory bioassays were not assessed as part of the present study and require evaluation. In the present study, certain variables (e.g. flow rate) were fixed based upon our previous bioassay experience to ensure the health on the test organisms. These and other test variables require standardization as part of a verified bioaccumulation protocol.

4.2.1 Flow Rate

Flow rates used in the present study resulted in a turnover time for seawater overlying test sediments of approximately 6 h. Analysis of contaminant concentrations indicated that except for cadmium, levels in the overlying seawater were at or below detection limits.

As statistical differences in levels of bioaccumulation between test sediments were observed in our bioassays, this would suggest that uptake was primarily from the sediment and not the water column. However, bioassay tests conducted with the same contaminated sediments but using slower flow rates resulted in higher final metal tissue levels with higher seawater concentrations (McGreer and Reid, 1984). A recent study on the bioaccumulation of metals from sediments by polychaete worms and flatfish (Haywood et al., 1983) found no significant increase in tissue levels after 75 days and the authors concluded that the lack of bioaccumulation may have resulted from a combination of high flow rates and low seawater metal concentrations. In contrast, the concentration of PCBs and organic compounds in seawater overlying test sediments was not considered to affect the level of bioaccumulation at a recent workshop on bioaccumulation (McFarland, 1983).

Flow rates may have to be adjusted for the assessment of different groups of contaminants in laboratory bioassays. Data on the relation of seawater concentrations of different contaminants to the degree of bioaccumulation in test species are required before standardized flow rates can be recommended as part of the bioassay protocol.

4.2.2 Exposure Times

A maximum exposure time of 6 months was tested in the present study to assess the optimum length of time required to show a "significant" difference in bioaccumulation for each contaminant and test sediment. No significant differences in the bioaccumulation of cadmium were observed at any time in the present study. These

results suggest that longer exposure times for cadmium do not increase the chances of detecting differences in bioaccumulation where this metal is not in a biologically available form. In a study by McGreer and Reid (1984), highest tissue levels of cadmium were observed after 1 month in half the test sediments and at 4 months in the other half. Differences in the rate of release of cadmium into seawater among the different sediments were also observed. A single exposure period for cadmium cannot be recommended at this time. Additional testing with a wider range of test sediments is necessary to further define this variable.

Statistically significant differences in the bioaccumulation of lead were observed at each month sampled, but maximum differences were recorded at month 4. This observation is consistent with a previous study by McGreer and Reid (1984) in which maximum levels of lead in M. balthica were observed most frequently in sediments after 4 months. It should be pointed out that in both these studies sampling occurred at 2 and 4 months, and an intermediate exposure time (e.g. 3 months) may be sufficient.

Both McGreer and Reid (1984) and the present study recorded an apparent decrease in lead tissue levels between 4 and 6 months. In each case, the decrease in lead resulted in a non-significant difference in bioaccumulation between the test and reference sediments. In the present study, tissues from the 4 month sampling period were preserved and analyzed at the same time as the 6 month samples, such that variability due to chemical analysis was minimal. The reason(s) for the decreases in lead tissue levels are unknown but could be due to adaptation and excretion of lead on the part of M. balthica in response to lead tissue levels Also, the amount of biologically available lead may have been exhausted. These results suggest that for M. balthica a 6 month exposure period is not appropriate.

PCBs were taken up relatively rapidly by M. balthica, and significant differences between test and reference sediments were observed after a 1 month exposure. Maximum differences in tissue concentrations

between the Vancouver Harbor and Reference sediment was achieved after 2 months. For PCBs, a 1 month exposure time in laboratory bioassays appears to be adequate to predict significant differences in bioaccumulation in resident biota.

4.2.3   Sediment Toxicity

Elevated mortalities compared to the Reference sediment were observed in contaminated test sediments in the present study. Sediment toxicity has also been reported in other long-term bioassay studies with contaminated test sediments, and polychaete species have been shown to be particularly sensitive (Haywood et al., 1983; McGreer and Reid, 1984). Sediment toxicity can pose a problem in long-term bioaccumulation studies where the number of mortalities reduces the amount of tissue for analysis, or prevents the study from going full term.

Several approaches have been tried to overcome the problem of sediment toxicity. Haywood et al. (1983) mixed toxic sediments with clean marine sand to reduce toxicity. This approach has also been followed in bioaccumulation studies with mine tailings (Reid and McGreer, 1983). However, this method can raise questions about the interpretation of study findings, especially where no bioaccumulation is evident. Another drawback to this method is that addition of so-called "clean" sediments can change the overall sediment chemistry within the experiment.

A second approach to solving the problem of sediment toxicity is one in which organisms are exposed to a very thin layer of sediment. This method was used by Rubenstein et al. (1983) in a 3 month exposure of polychaetes (Nereis virens), clams (Mercenaria mercenaria) and shrimp (Palaemonetes pugio). These authors reported no significant mortalities in their experiments with contaminated harbor sediments, but they also did not find significant bioaccumulation of metals.

Using such a small quantity of sediment in long-term experiments casts some doubt on whether sufficient quantities of contaminants were available for uptake.

The most practical approach to solving the problem of sediment toxicity would appear to be to use a relatively tolerant indicator species, and to provide for a sufficiently large number of individuals in the tests. Deposit-feeding bivalves (such as M. balthica) appear to be more tolerant than polychaete worms in studies conducted to date. Future bioassay testing of this type should include twice the minimum number of individuals that are required to provide sufficient quantities of tissue for chemical analysis. That is, a "safety factor" of 100% is recommended to prevent problems due to sediment toxicity.

4.2.4   Other Potential Indicator Species

The bioassay organism used in the present study (M. balthica) is a bivalve common to estuaries and shallow coastal areas. Results from laboratory tests with this species showed good agreement with bioaccumulation in infauna and sole from the coastal sites sampled. However, ocean dumping may occur in much deeper offshore waters and species in these areas may differ in their ability to bioaccumulate contaminants. It would therefore be desirable to have an indicator species normally resident in deeper ocean sediments. The marine bivalve Yoldia sp. has been used as an indicator of bioaccumulation in a deep water fjord (Alice Arm) receiving mine tailings. Tests comparing Yoldia with M. balthica as bioaccumulation indicators would provide valuable information on differences in bioaccumulation between these two species, and indicate whether more than one indicator species is required for inclusion in the bioassay protocol.

Additional testing is also required to assess the suitability of a wider range of resident biota as designated indicator species for evaluating the effectiveness of the bioassay protocol. Although English sole (P. vetulus) were the preferred species of resident biota in the present study, this species was not present at all sites due to differences in

habitat. A second species of sole, I. isolepis, analyzed in the present study also appeared to be a good bioaccumulation indicator. Bioaccumulation predicted from the laboratory bioassays with M. balthica was reflected in elevated liver concentrations in this species. As different species may be present at individual dumpsites due to variations in habitat, water depth or time of year, a number of potential resident indicator species should be screened. A list of acceptable indicator species representing a variety of habitats should then be included in the protocol for purposes of evaluating the laboratory bioassays.

4.2.5   Significance of Bioaccumulation to Resident Species

The bioassay protocol developed in this study was evaluated on the basis of statistically significant differences in bioaccumulation observed between resident biota at a test site and the Reference location. Under the protocol, where a significant difference was recorded, the sediments were "red flagged" as being a concern with respect to bioaccumulation. This may be considered an important first step in differentiating the bioaccumulation potential of various sediments. However, the ecological significance of these "statistical" differences remains to be determined. Ideally, the laboratory bioassay protocol should be able to detect sediments which have a bioaccumulation potential of ecological significance if it is to be used to protect aquatic resources.

4.3   Future Research Directions

At the present time, bioassays with contaminated sediments and appropriate indicator test species appear to provide one of the best means for assessing bioaccumulation potential. Previous studies aimed at devising reliable chemical (rather than biological) methods to predict bioaccumulation have had limited success due to the complexity of predictive relationships involved (Burton, 1979). However, efforts are continuing in this direction and progress has been made, particularly in predicting the bioaccumulation potential of

organic compounds. Gossett et al. (1983) have recently shown that the sediment and tissue concentrations of 27 selected organic compounds in sewage effluent were positively correlated with each other and with the n-octanol/water partition coefficient. These authors concluded that the n-octanol/water partition coefficients could be used to predict which organics had the greatest potential to bioaccumulate and therefore cause biological effects. A similar approach is being developed by the U.S. Army Corps of Engineers as part of their Long-Term Effects of Dredging Operations (LEDO) Program (McFarland, 1983). Efforts are being directed at evaluating the relative chemical activity of sediment molecular organic carbon and organism tissue (i.e. biological carbon) for bioaccumulating chemicals. Based on thermodynamic activity, prediction of organism accumulation is being made from the chemical analysis of sediments.

Research on the factors controlling bioavailability of metals from natural estuarine sediments has also been attempted (Luoma and Bryan, 1978; 1982). Tissue concentrations of silver, cadmium, cobalt, copper, lead and zinc have been predicted from 1 N HCl extracts of estuarine sediments (Luoma and Bryan, 1982), however this technique has not been applied to heavily contaminated sediments.

The present, preliminary bioassay/bioaccumulation test protocol appears to provide a useful approach for assessing the bioaccumulation potential of contaminated sediments. Standardization of this protocol, together with the previously noted research efforts, will provide a more realistic measure by which to judge whether sediments should be ocean dumped.

5.0 CONCLUSIONS

The following conclusions can be drawn from the experimental data obtained in this study:

1. Laboratory bioassays with appropriate indicator species can be used to predict the bioaccumulation potential of contaminants in polluted marine sediments.

2. The exposure times required to assess bioaccumulation potential in sediments vary for different contaminants. In the present study, PBCs required the shortest exposure time (I month) and. lead the longest time (4 months).

3. Of the contaminants tested, cadmium appeared to be the only one for which the concentration in seawater affected the bioaccumulation in tissues of the test species.

4. Of the resident species tested, polychaete worms (whole animals) and flatfish (livers) appeared to be the best indicators of bioaccumulation from contaminated sediments.

5. Sediments adjacent to a pulp and paper mill effluent discharge showed the greatest bioaccumulation potential for cadmium of the different types of polluted sediments tested.

6. Dredged spoil from a ship repair/dry dock facility showed the highest bioaccumulation potential for lead in both laboratory bioassays and in tissues of resident biota. This site also showed a high bioaccumulation potential for PCBs.

7. Sediment toxicity with contaminated sediments can pose a problem in long-term laboratory bioassays. Use of tolerant bioaccumulation indicator species in laboratory tests is required.

6.0   RECOMMENDATIONS

The following research needs are identified as the result of this study:

1. There is a need to refine the bioassay protocol developed herein so that specific flow rates and exposure times can be recommended for different groups of chemical contaminants. The same overall experimental design should be suitable for testing of a variety of contaminants.

2. Comparative testing between the estuarine bivalve (M.ᐧ balthica) used in this study and a deep ocean indicator species (e.g. Yoldia) should be undertaken to assess the need for a deep water laboratory bioassay indicator species.

3. There is a need to develop comparative data on the relative bioaccumulation capabilities of a wide range of resident species representative of different habitats. A list of acceptable species for evaluating bioaccumulation at ocean dumpsites should be compiled from these studies.

4. There is a need to determine the biological and ecological significance of bioaccumulation of chemical contaminants by resident biota. Due to the widespread persistence of many environmental chemicals and the protection mechanism possessed by many species, not all elevated tissue levels may be of concern.

5. The role of bioaccumulation in sediment toxicity should be investigated to determine if any cause and effect relationship exists. Such studies should include a field component to assess the biological and ecological effects of sediment toxicity.

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APPENDIX

DETAILED ANALYTICAL RESULTS

TABLE AI

RESULTS OF TESTING   SEAWATER SAMPLES

Sample Identification	Cadmium	Lead	PCB
24/02/83, Seawater	0.08/0.07	L0.05/L0.05	L0.01
" REF (1)	0.20/0.22	L0.05/L0.05	L0.01
" REF (2)	0.10/0.10	L0.05/L0.05	L0.01
" VH (1)	0.07/0.07	0.18/0.18	L0.01
" VH (2)	0.20/0.24	L0.05/L0.05	L0.01
" FC (1)	0.20/0.20	0.18/0.18	---
" FC (2)	0.15/0.16	L0.05/0.08	---
23/03/83 Seawater Inlet	0.05/0.05	L0.05/L0.05	L0.01
" REF	0.09/0.09	L0.05/L0.05	L0.01
" V.H.	1.0/1.1	L0.05/L0.05	L0.01
" F.C.	0.20/0.20	L0.05/L0.05	---
15/04/83 F.C. (field sample)	0.13/0.14	0.07/0.08	---
27/04/83, Seawater Inlet	0.05/0.05	L0.05/L0.05	L0.01
" REF	0.38/0.43	0.05/0.09	L0.01
" V.H.	0.28/0.28	L0.05/L0.05	L0.01
" F.C.	0.32/0.29	L0.05/L0.05	---
10/05/83, V.H. (field sample)	0.09/0.08	0.13/0.11	L0.01
REF, 23/06/83	0.09/0.12	L0.05/L0.05	---
V.H., 23/06/83	0.08/0.07	L0.05/L0.05	---
F.C., 23/06/83	0.13/0.13	L0.05/L0.05	---
REF, 26/06/83 (field sample)	---	---	L0.01
V.H., 23/06/83 (field sample)	---	---	L0.01
Seawater Inlet, 27/06/83	0.16/0.15	L0.05/L0.05	L0.01
S.W. Supply, Sept. 6/83	0.12/0.13	L0.05/L0.05	L0.01
REF, "	0.13/0.14	0.10/0.10	L0.01
V.H., "	0.11/0.10	L0.05/L0.05	L0.01
F.C., "	0.16/0.14	0.05/0.05	---
Oct. 11/83, Powell River (field)	0.06/0.05	0.07/0.06	---
24/11/83, REF (field sample)	0.08/0.06	L0.05/L0.05	---

PCB = Polychlorinated Biphenyls (includes aroclor 1242, 1254, and 1260)

L = Less than   Results are expressed as micrograms per liter

TABLE A2

R E S U L T S   O F   T E S T I N G

TISSUE SAMPLES

File # 160A

Sample Identification	Tissue Type	Moisture(%)	Cadmium	Lead	- - - P C B - - - -		
					1242	1254	1260
REF, Mo.1, Rep A	Clam Muscle	88.8	0.18	0.99	L0.10	0.36	L0.10
" " " B	"	89.3	0.26	0.85	"	0.09	"
" " " C	"	89.4	0.14	1.69	"	0.19	"
REF, Mo.2, Rep A	"	89.7	0.30	1.59	"	0.19	"
" " " B	"	89.2	0.17	1.98	"	0.29	"
" " " C	"	90.6	0.27	2.07	"	0.19	"
F.C., Mo.1, Rep A	"	89.5	0.19	2.02	---	---	---
" " " B	"	89.3	0.23	2.55	---	---	---
" " " C	"	89.5	0.16	2.19	---	---	---
F.C., Mo.2, Rep A	"	89.3	0.26	3.91	---	---	---
" " " B	"	90.2	0.09	3.82	---	---	---
" " " C	"	89.2	0.23	5.17	---	---	---

L = Less than

Results are expressed as micrograms per dry gram (ppm).

Table A2 (Cont'd)

RESULTS OF TESTING

TISSUE SAMPLES

Sample Identification	Tissue Type	Moisture(%)	Cadmium	Lead	PCB 1242	PCB 1254	PCB 1260
V.H., Mo.1, Rep A	Clam Muscle	89.3	0.21	3.09	L0.10	1.14	0.38
" " " B	"	90.1	1.02	2.75	"	0.76	0.29
" " " C	"	89.9	0.16	3.09	"	0.76	0.37
V.H., Mo.2, Rep S	"	91.2	0.19	3.01	"	0.86	0.39
" " " B	"	90.4	0.22	2.93	"	1.14	0.48
" " " C	"	91.0	0.45	2.91	"	1.16	0.38
Spec Analysis, Rep 1	"	94.2	0.21	2.48	"	1.23	0.62
" "	"	92.8	0.18	1.95	"	0.92	0.31
" "	"	93.5	0.35	3.15	"	0.92	0.46

L = Less than   Results are expressed as micrograms per dry gram (ppm).

Table A2 (Cont'd)

RESULTS OF TESTING   TISSUE SAMPLES   File # 160A

Sample Identification	Tissue Type	Moisture(%)	Cadmium	Lead	PCB 1242	PCB 1254	PCB 1260
REF + 4 Mos, Rep #1	Clam Muscle	88.8	0.50	5.92	L0.10	0.27	0.54
" " " Rep #2	"	87.0	0.28	3.94	L0.10	0.15	0.15
" " " Rep #3	"	88.7	0.41	5.95	"	0.18	0.18
REF + 6 Mos, Rep #1	"	86.7	0.11	0.83	"	L0.10	L0.10
" " " Rep #2	"	87.5	0.16	1.61	"	L0.10	L0.10
" " " Rep #3	"	87.2	0.13	0.89	"	0.39	0.55
REF, INFA	"	85.8	0.82	3.86	"	L0.10	L0.10
REF, SOLE - #1	Muscle	78.3	L0.05	0.35	"	0.14/0.14	L0.10/L0.10
" " #2	"	78.5	L0.05	L0.10	"	0.19	0.14
" " #1	Liver	78.9	0.98	1.72	"	1.33	0.66
" " #2	"	78.7	1.35	1.82	"	0.33	0.23

L = Less than   Results are expressed as micrograms per gram, dry weight basis

Table A2 (Cont'd)

R E S U L T S O F T E S T I N G   T I S S U E   S A M P L E S   File # 160A

Sample Identification	Tissue Type	Moisture(%)	Cadmium	Lead	P C B 1242	1254	1260
V.H. + 4 Mos, Rep #1	Clam Muscle	88.4	0.39	8.51	L0.10	0.60	0.95
" " Rep #2	"	88.1	0.53	11.4	"	0.84	0.76
" " Rep #3	"	87.0	0.25	15.0	"	0.92	0.54
V.H. + 6 Mos, Rep #1	"	86.4	0.11	0.72	---	---	---
" " Rep #2	"	85.6	0.16	0.74	---	---	---
V.H. Rep #1	Worms	81.1	0.73	10.2	L0.10	1.69	0.37
" Rep #2	"	79.3	0.97	13.2	"	1.64	1.11
V.H., SOLE #1	Muscle	80.6	L0.05	0.30	"	0.21	0.21
" " #2	"	80.8	L0.05	0.50	"	1.19	0.83
" " #1	Liver	69.8	0.98	4.78	"	8.51	5.59
" " #2	"	71.2	0.51	2.04	"	13.5	9.31
V.H., CRAB #1	Muscle	80.6	L0.05	0.50	"	L0.10	L0.10
" " #2	"	80.8	L0.05	0.40	"	L0.10/L0.10	L0.10/L0.10

L = Less than   Results are expressed as micrograms per gram, dry weight basis.

Table A2 (Cont'd)

R E S U L T S   O F   T E S T I N G

TISSUE SAMPLES   File # 160A

Sample Identification	Tissue Type	Moisture(%)	Cadmium	Lead	1242	PCB 1254	1260
F.C. + 4 Mos, Rep #1	Clam Muscle	84.3	0.26	12.5	---	---	---
" Rep #2	"	85.9	0.41	16.8	---	---	---
F.C. Rep #1	Worms	79.7	0.70	3.70	---	---	---
" Rep #2	"	79.5	0.27	6.10	---	---	---
F.C., SOLE #1	Muscle	79.8	L0.05	L0.10	---	---	---
" #2	"	80.1	L0.05	0.35	---	---	---
F.C., SOLE #1	Liver	69.8	0.40	0.85	---	---	---
" #2	"	66.5	0.35	0.85	---	---	---
F.C., CRAB-1	Muscle	87.0	L0.05/L0.05	L0.10/L0.10	---	---	---
" CRAB-2	"	87.4	L0.05/L0.05	L0.10/L0.10	---	---	---

L = Less than   Results are expressed as micrograms per gram, dry weight basis.

Table A2 (Cont'd)

<u>R E S U L T S   O F   T E S T I N G</u>

TISSUE SAMPLES

File # 160A

Sample Identification	Tissue Type	Moisture(%)	Cadmium	Lead	1242	PCB 1254	1260
Watts Pt, CRAB-1	Muscle	80.4	L0.05/L0.05	L0.10/L0.10	---	---	---
" -2	"	80.4	L0.05/L0.05	L0.10/L0.10	---	---	---
Woodfibre " -1	"	76.1	L0.05	L0.10	---	---	---
" -2	"	79.2	L0.05	L0.10	---	---	---
RB Rep #1*	Clam Muscle	88.1	0.11	L0.10	L0.01	---	0.02
" Rep #2*	"	88.9	1.50	0.28	L0.01	0.03	0.01
P.R., SOLE #1	Muscle	80.1	L0.05	L0.10	---	---	---
" " #2	"	80.4	L0.05	L0.10	---	---	---
" " #1	Liver	79.9	2.90/3.20	1.91/2.12	---	---	---
" " #2	"	78.8	2.40	0.71	---	---	---
" CRAB #1	Muscle	82.9	L0.05/L0.05	L0.10/L0.10	---	---	---
" " #2	"	82.6	L0 05/L0.05	L0.10/L0.10	---	---	---
REF, CRABS #1	Tissue	76.9	L0.05	L0.10			
" #2	"	77.4	0.13	0.10			
" "	Worms	81.5	3.46	1.98			

L = Less than   Results are expressed as micrograms per gram, dry weight basis

Table A2 (Cont'd)

RESULTS OF TESTING

TISSUE SAMPLES

File # 160A

Sample Identification	Tissue Type	Moisture(%)	Cadmium	Lead	--- P C B --- 1242	1254	1260
Powell River, INFAUNA, #1	Worms	85.8	4.90	7.50	---	---	---
" " #2	"	83.4	4.70	8.50	---	---	---

L = Less than   Results are expressed as micrograms per gram, dry weight basis.

TABLE A3

RESULTS OF TESTING

SEDIMENT SAMPLES

Sample Identification	Moisture(%)	TOC(%)	Cadmium	Lead	Iron	- - - - P C B - - - -		
						1242	1254	1260
REF, Jan 26/83	40.7	1.75	L0.25	8.9	21600.	L0.01	L0.01	L0.01
V.H., Feb/83	45.1	2.23	0.56	185.	33600.	L0.01	1.29	0.58
F.C., Feb/83	56.0	3.51	2.78	223.	42600.	---	---	---
REF, Sept. 6/83	51.7	1.80	L0.25	10.6	27500.	L0.01	L0.01	L0.01
V.H., Sept. 6/83	52.4	2.13	0.44/0.53	244./227.	39500./39300.	L0.01	L0.01	2.39
V.C., Sept. 6/83	63.4	3.68	2.92	258.	46800.	---	---	---

TOC = Total Organic Carbon

PCB = Polychlorinated Biphenyls (note different aroclors reported)

L = Less than   Results are expressed as micrograms per gram, dry weight basis except moisture and TOC, which are as percent.

TABLE A4

SUMMARY OF SEDIMENT OXIDATION-REDUCTION POTENTIAL MEASUREMENTS FOR LABORATORY BIOASSAYS

Date 1983	Depth (cm)	REF	Test Sediment[a] VH	FC	Date 1983	Depth (cm)	REF	Test Sediment[a] VH	FC
Feb. 25	sfc[b]	-50	-100	-150	May 25	sfc	+10	-70	-50
	1	-100	-200	-200		1	+20	-120	-160
	2	-100	-200	-250		2	+15	-120	-145
March 2	sfc	-20	-100	-100	June 1	sfc	+10	-50	-50
	1	-20	-140	-150		1	+20	-150	-120
	2	-60	-210	-200		2	+20	-150	-170
March 9	sfc	-60	-200	-150	June 8	sfc	+30	-50	-40
	1	-50	-180	-180		1	+85	-70	-70
	2	-80	-200	-210		2	+95	-110	-100
March 16	sfc	-50	-190	-180	June 17	sfc	+20	-75	-60
	1	-50	-190	-180		1	+30	-100	-100
	2	-65	-180	-220		2	+40	-160	-130
March 23	sfc	0	-60	-60	June 27	sfc	+10	-50	-10
	1	-15	-80	-120		1	+40	-80	-60
	2	-30	-130	-120		2	+60	-120	-80
March 30	sfc				July 6	sfc	+20	-10	+5
	1		not measured			1	+30	-120	-100
	2					2	+30	-180	-150
April 6	sfc	-45	-20	-40	July 14	sfc	+20	-20	-40
	1	-40	-130	-185		1	-10	-40	-60
	2	-45	-130	-200		2	-10	-75	-110
April 13	sfc	+20	-30	-10	July 20	sfc	+20	-50	-60
	1	-15	-110	-160		1	+10	-110	-100
	2	-30	-175	-170		2	-10	-180	-110
April 20	sfc	+35	-10	+5	July 27	sfc	0	-50	-80
	1	+40	-100	-110		1	+30	-50	-100
	2	+40	-100	-115		2	+30	-60	-100
April 26	sfc	-10	-70	-60	Aug. 4	sfc	+30	-60	-100
	1	-100	-100	-130		1	+30	-70	-80
	2	-100	-150	-140		2	+20	-100	-90
May 4	sfc	+20	-50	-60	Aug. 16	sfc	-20	-50	-80
	1	+40	-140	-80		1	-110	-110	-100
	2	+40	-140	-120		2	-155	-160	-100
May 13	sfc	+10	-10	-50	Aug. 26	sfc	+20	-20	-60
	1	+30	-110	-90		1	-60	-70	-80
	2	+35	-110	-110		2	-100	-100	-110
May 18	sfc	+10	-70	-30	Sept. 1	sfc	-15	-30	+25
	1	+15	-145	-165		1	-50	-80	-70
	2	+20	-145	-170		2	-110	-100	-100

[a]REF = reference
  VH = Vancouver Harbor
  FC = False Creek

[b]sfc = surface