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A NEW METHOD FOR EXPOSING DEPOSIT FEEDERS TO CONTAMINATED SEDIMENTS FOR FOOD CHAIN STUDIES

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The ubiquity and refractory nature of certain organic compounds, such as chlorinated pesticides and polychlorinated biphenyls (PCB's), results in their accumulation in aquatic sediments (Holdrinet et al. 1978, Peck et al. 1980, Wang et al. 1979). Their continuous release from this reservoir through physico-chemical and biogenic processes to the overlying water column results in the accumulation of xenobiotic compounds in the food chain.

Accumulation of organics within organisms can be accomplished through several routes. Direct uptake from water across gill and membrane surfaces has been suggested by some as the main mechanism (Nisbet and Sarofim 1972, Fowler et al. 1978). Others have shown that the accumulation by aquatic invertebrates and fish is a two step process: desorption from sediments into water and uptake from water by the biota (Halter and Johnson 1977). Recent studies have documented the importance of direct uptake of organics by sediment-ingesting benthos (Fowler et al. 1978, Langston 1978, Meier and Rediske 1979). However, controversy still exists as to the role water and/or sediment concentrations play regarding accumulation and biomagnification of these compounds in food chains.

In examining the uptake and behavior of organics in food chain organisms, it is important to expose test animals in such a manner that the results are reproducible, statistically significant, and applicable to natural systems. Several methods have been described in the literature. Researchers who exposed algae and/or invertebrates to contaminated water failed to consider the role of sediments in studies concerning low water soluble compounds. Often these exposures are made at artificially high concentrations of test material in water (Hattula and Karlog 1973, Melancon and Lech 1976, Sandlers and Chandler 1972). In other food chain studies, test animals were fed a contaminated synthetic processed food which did not simulate natural conditions (Zitko 1974, 1977; Hanson et al 1976; Narborne 1979).

The purpose of the study was to develop a method that would aid in food chain studies by providing: (1) A predictive capability through manipulation of the biomass-sediment ratio to achieve the desired body burden in the food organism; (2) A large number of sediment associated organisms with similar body burdens; (3) An application in a variety of situations with different substances and test organisms; and (4) Reproducible results.

MATERIALS AND METHODS

Test organisms were larvae of *Chironomus plumosus* Meigen (Diptera: Chironomidae) collected from oxidation lagoons in Fowlerville, Michigan. These midges were maintained in 38-liter aquaria according to the method described by Meier and Torres (1978) (Fig. 1). The numerous egg masses obtained from the adults of the wild collection served as the starting material for the laboratory culture. Background concentration of PCB's in the midges was insignificant at an analytical level of <0.05 μg/g wet weight.

The polychlorinated biphenyl used in this study was ^{14}C labeled 2,4,5,2',4',5' hexachlorobiphenyl packaged in crystalline form at 1.5 mg per vial.[2] The content was dissolved with 15 ml of acetone and then diluted with distilled water to a concentration of 0.1 mg/ml. This stock solution was added in specific volumes to each of the incubation vessels for the desired concentration.

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[2]Source: California Bionuclear Corporation; Specific Gravity: (mCi/mM)24; Activity per vial: 0.1 mCi.

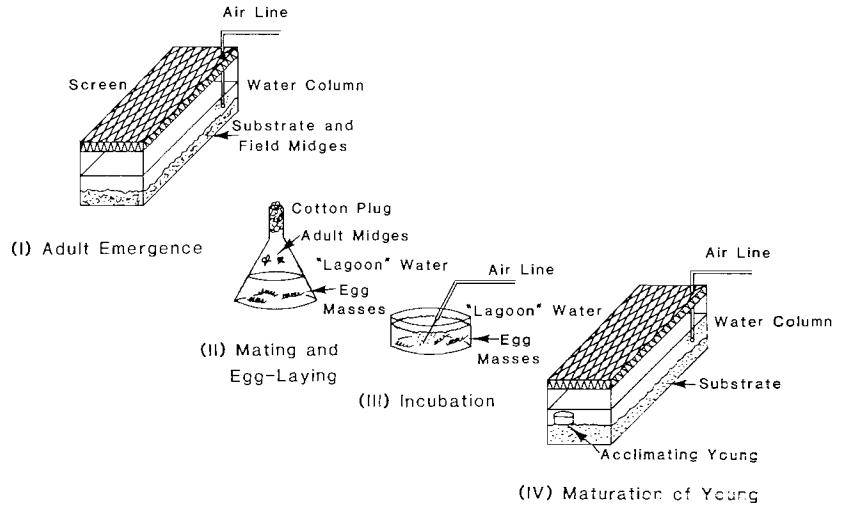

Fig. 1. Schematic representation of laboratory culture of *Chironomus plumosus*.

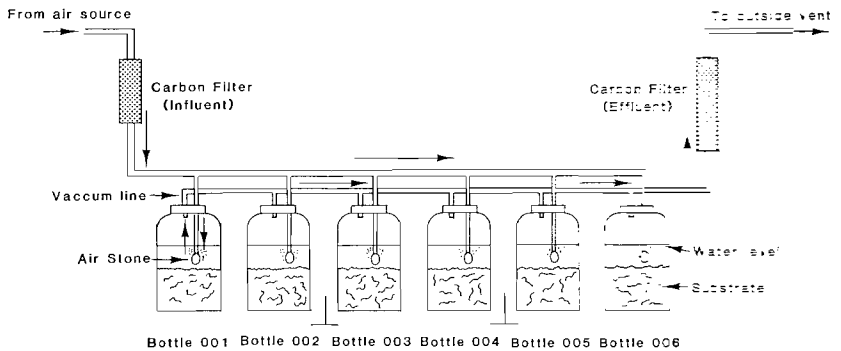

Fig. 2. Design of midge dosing chamber.

The exposure apparatus for the midges consisted of six 4-liter glass chambers (Fig. 2). Each vessel was sealed airtight with silicone rubber, except for an inlet and outlet. The incoming air was filtered through a carbon column which removed any potential contaminants (i.e. oil and PCB's) from the hydraulic pump of the aeration system. The outgoing air was passed through another carbon filter to remove any PCB passing out from the chambers. For more volatile compounds, this set up can be modified with the addition of secondary butyl alcohol and potassium hydroxide trap in series behind each chamber. These compartments may then be analyzed separately and summed for obtaining a mass balance.

Preliminary studies showed that midge larvae in the closed system readily and quickly accumulated sediment-associated PCB as a result of ingestion. These tests helped in determining the sediment to biomass ratio which most effectively and efficiently met the objectives. Hence, 25 g of paper towel (dry weight) were macerated in a blender with 1 liter of distilled water and poured in each chamber. An additional 2 liters of water containing the

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necessary amount of ^{14}C labeled PCB in solution, were added to the vessels. Each was stirred by aeration for 24 h to assure even distribution of PCB and adsorption onto the macerated paper substrate. A small amount of midge food was then added (Meier and Torres 1978). After 6 h, 20 g of III and IV instar midge larvae were transferred to each chamber. The larvae rapidly settled into the sediment and started feeding on the bacteria-enriched sediment. The flow of air to the vessels was then reduced to minimize the disturbance of the settled substrate. Each uptake experiment lasted approximately two weeks over which period the midges accumulated predictable body burdens of PCB. Food was added once every three days and the water was changed once a week.

Replicate dosing chambers contained 1, 10, and 100 μg/g of PCB-contaminated substrate. On alternating days each vessel was analyzed for [^{14}C] PCB in the water, in sediments, and in midges. These samples were appropriately treated and analyzed by the liquid scintillation method (Wang and Willis 1965). A standard quench curve was generated for converting counts-per-minute to actual disintegrations-per-minute (dpm). The dpm were converted to μg/g PCB by utilizing the specific activity of the labeled chlorobiphenyl. The concentration of PCB was plotted against time to generate the necessary uptake. The experiment was terminated when the midge population reached desired body burdens of 1, 10, and 100 μg/g. The larvae were separated from the sediments, washed, and then frozen for the food chain study (Meier and Roberts, in press). These experiments were repeated seven times to assure their reproducibility and also to obtain sufficient biomass of labeled midge larvae. Later dosings were sampled only intermittently, since dosing criteria had been established from the first experiment.

RESULTS AND DISCUSSION

Uptake of Sedimentary PCB by Chironomids: Preliminary Study

A preliminary experiment was performed to aid in determining the dosing criteria for the midge uptake study. By examining the uptake characteristics in a saturated system (30 g midge larvae to 10 g sediments) one could design future dosings so as to best achieve the goal of producing large numbers of midge larvae with similar body burdens. In the preliminary study, different compartments were examined over time to determine the fate of the PCB in the dosing chamber. The results demonstrated the efficiency by midges in stripping off sedimented PCBs and incorporating them quickly (Fig. 3). The body burden of 3 μg/g

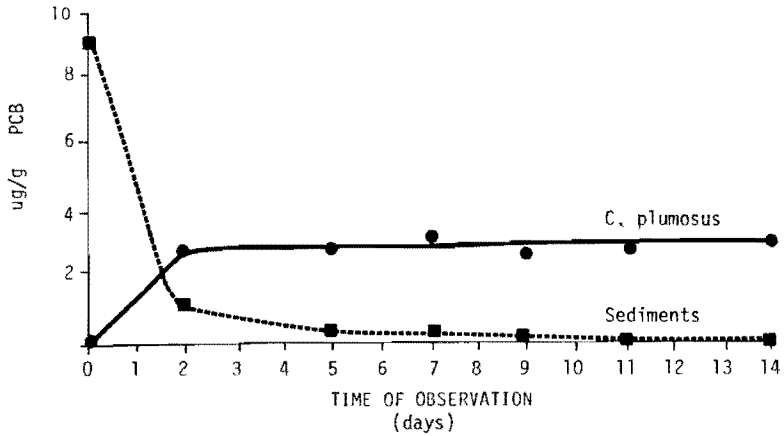

Fig. 3. Preliminary study: Plot of PCB concentration in sediments and the corresponding PCB uptake by *Chironomus plumosus* larvae in μg/g wet weight.

was established almost immediately. A mass balance performed on the sediments and larvae amounted to 95% of the added PCB. The unaccounted portion was most likely either suspended or dissolved in the water column. As the result of this investigation, dosing parameters for the actual uptake studies were set at 20 g larvae and 25 g contaminated sediment for each chamber. The limiting effect of sediment mass and PCB concentration avoided drastically overshooting the desired levels of the chemical in the insect as well as maximizing sediment-organism content.

Uptake of Sedimentary PCB by Chironomids: Actual Study

Seven separate uptake experiments, each with three different concentrations, were carried out employing this method. The results showed that benthic organisms can accumulate significant concentrations of PCB through direct uptake from contaminated sediments over a short time. In addition, the final concentrations of PCB at each dosing level for all experiments were very similar (Table 1). Besides that, large numbers of labeled food organisms were prepared for food chain studies.

The uptake of the radio-chemical followed first order rate kinetics after 48 h and showed a dose-dependent relationship between substrate concentration and body burden. The initial nonlinear portion of the uptake curve for each concentration was attributed to filling the intestinal tract with contaminated sediment (Fig. 4). Some of the contaminated larvae were placed in clean sediments for 24 h and their body burdens were reduced. The procedure of facilitated removal of contaminated gut material was not practiced in the actual dosing study since consumer-predators ingest entire organisms, including gut material.

Figure 4 summarizes graphically the range of uptake concentrations in midges for the high

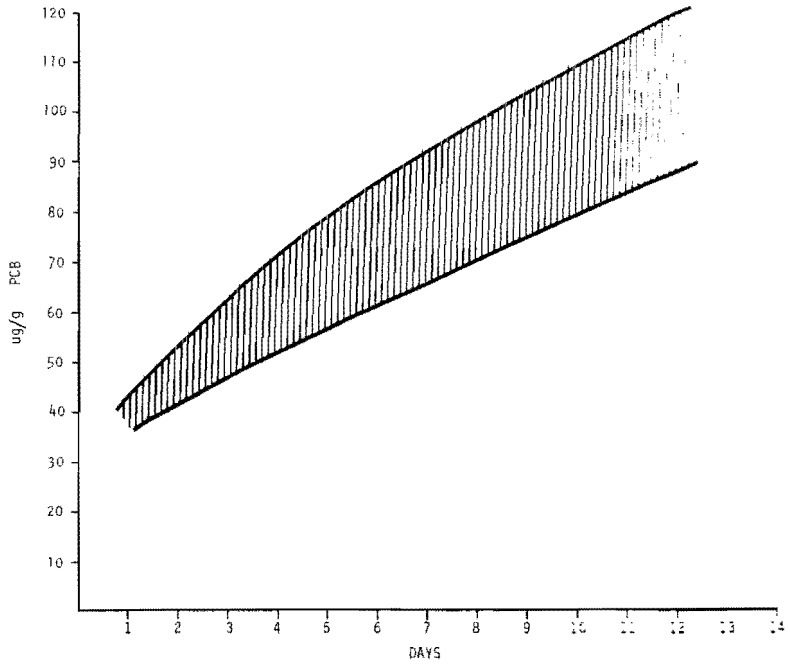

Fig. 4. Summary of sedimentary PCB uptake by *Chironomus plumosus* at a sediment concentration of 10 μg/g for all experiments.

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Table 1. Summary of the final biomass concentrations of PCB in *C. plumosus* for the seven sets of dosings. Concentrations are given on a wet-weight basis (μg/g).

	Sediment Concentration		
Date	1 μg/g	10 μg/g	100 μg/g
15/8/79	0.1213	1.2212	12.0548
5/9/79	0.1083	1.1321	10.5863
24/9/79	0.0983	0.9976	10.0041
15/10/79	0.0898	0.9296	8.8714
8/11/79	0.1146	1.1869	11.3847
11/1/80	0.0866	0.8971	9.4836
25/1/80	0.1018	1.0792	10.0616
Mean	0.1028	1.0633	10.2494
SD	0.0127	0.1217	1.3507

dosing level over time. Following the initial fast uptake, the body burdens were increasing consistently to the desired levels. The projected concentrations were reached for all levels of exposure in a two-week period for all sets of dosing. Approximately 3500 midge larvae of similar body burden and weight were obtained for each level of exposure for use in the second step uptake studies with fish.

In summary, this economical method permits the researcher to manipulate the organism-sediment ratio to assure pre-determined body burdens in test organisms. In addition, this technique is applicable in preparing large quantities of sediment ingesting organisms (natural food) that have a similar concentration for food chain studies. Finally, this method does produce reproducible results in batch experiments.

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