THE EFFECT OF SEDIMENT OIL CONTAMINATION ON THE BIOACCUMULATION AND EXCRETION OF AROCLOR 1242 BY CHIRONOMID LARVAE

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ABSTRACT

The effect of substrate oil contamination on the uptake and excretion of Aroclor 1242 by the chironomid larvae Glyptotendipes barbipes was examined. Studies were conducted under laboratory conditions using an artificial sediment substrate contaminated with 0.01, 0.1, and 1.0 ppm Aroclor 1242 and 0, 0.25, and 1.0% medicinal mineral oil. Analysis was performed by gas-liquid chromatography.

The organism accumulated PCB in a dose dependent manner with a 132 fold concentration factor over the substrate at a 0.1 ppm level. Oil contamination reduced the concentration ratio by 70-80%. No excretion of Aroclor 1242 was observed over a 1 week period.

Polychlorinated biphenyls (PCBs) are highly persistent global scare contaminants with a broad range of long-term environmental and health effects (Fishbein 1974). These compounds have been found in Great Lakes fish up to serveral times the FDA action guideline of 5.0 ppm and present a serious threat to the success of fisheries operation in many areas of the country. Body burdens of many chlorinated aryl compounds in aquatic organisms accumulate by combinations of passive absorption directly from water or by partitioning into lipids of foods. Both routes have been shown to contribute significantly to levels found in fish (Stalling and Mayer 1972, Hansen et al . 1976).

Many food web organisms such as benthic invertebrates live associated with and ingest sediment as part of their feeding behavior. Although water concentrations of PCBs tend to be in the low parts per trillion range, sediments can contain levels of parts per million due to their absorptive capabilities. Thousand-fold accumulation has been demonstrated by Sanders and Chandler (1972) and Södergren and Svensson (1973) for invertebrates via absorption from water, but accumulation from sediment feeding has not been studied. Because of the high concentrations that are present in sediments and the intimate association of benthos with the substrate, this route of uptake needs to be critically evaluated.

Petroleum-based oils are even more ubiquitous in the sediment than PCBs. These compounds also absorb onto sedimentary particles, but at environmental concentration levels which are in excess of PCBs. Biological processes naturally contribute low concentrations of hydrocarbons to the sediment (Han et al. 1968), however inputs from oil spills and discharges greatly exceed this background. In industrial areas such as the Detroit River, sediment oil concentrations range up to 4.8% (Hartung and Klingler 1968). Heavily polluted areas can be as high as 17.8% (Ludzack et al. 1957). In addition to their wide

dispersion, oils have been shown to act as concentrators of chlorinated hydrocarbons, such as PCBs, because of their high partitioning capabilities (Hartung and Klingler 1970, Sayler and Colwell 1976). Therefore, PCBs in sediments may exist both in the absorbed state and the dissolved state due to partitioning into sedimentary petroleum oils.

The availability and effect of PCBs in oily sediments to benthic organisms have not been examined. It is thought that the major mechanism of absorption of PCBs in the gastrointestinal tract is due to passive absorption based on partitioning into endogenous lipids. It is therefore expected that the availability of sedimentary PCBs will differ when they are absorbed directly on particulates, as compared with PCBs which have partitioned into sedimented non-polar solvents such as petroleum oils. In the latter case, the absorption into benthic organisms would involve competition between a two solvent system, namely sedimentary oils and endogenous lipids, for the dissolved PCBs.

The purpose of this study was to examine the effect of sediment oil contamination on the bioaccumulation and excretion of Aroclor 1242 by the chironomid larvae Glyptotendipes barbipes Staeger. This species was chosen as the test organism because it was a representative of the Chironomidae, an important fish food group, and because of its ability to tolerate a wide range of sediment PCB and oil concentrations. Experiments were conducted under laboratory conditions where midges were exposed to varying concentrations of Aroclor 1242 and mineral oil mixtures coated on an artificial sediment substrate. An artificial substrate was chosen over natural sediment because it could be easily prepared in large quantities and accurately contaminated with both PCB and oil.

MATERIALS AND METHODS

Rearing Techniques

Organisms used for stock cultures of Glyptotendipes barbipes Staeger were collected from the Ann Arbor Sewage Treatment Plant sludge lagoon system and reared according to a method described by Meier and Torres (1978). Four 38-liter rearing chambers were used. Each chamber contained 50 grams of shredded paper hang towels, 2 grams of food supplement, and 20 liters of aerated tap water. The midges were fed 1 gram of food supplement throughout their five-week life cycle. Fertile egg masses were transferred to new rearing chambers for each generation. The fourth generation, fourth instar larvae were analyzed for PCB contamination and no measurable levels were detected at an analytical limit of 0.005 ppm. Fertile egg masses from this generation were used in the bioaccumulation experiments.

Experimental Design

Experimental chambers were similar to the rearing chambers, with the exception that polyethylene sheets containing small openings for air exchange were substituted for the plywood roof. The experimental substrate was prepared by grinding the paper towels and food supplement (15:1 ratio) to a #20 mesh in a mechanical grinder. Fifty-gram portions of the substrate were placed in a flask containing pesticide grade acetone and mixtures of Aroclor 1242 and medicinal mineral oil to give the following substrate concentrations:

	Mineral Oil		
	0%	0.25%	1.0%
Aroclor 1242 (ppm)	0.01	0.01	0.01
	0.1	0.1	0.1
	1.0	1.0	1.0

The solvent was then removed by rotary evaporation at 4°C. Concentrations were verified by chemical analysis.

One hundred fifty grams of each substrate concentration were transferred to duplicate test chambers and allowed to equilibrate one week. Analyses conducted on the water in each chamber verified no Aroclor 1242 present at a level of 0.0001 ppm.

Approximately 2000 second instar midges (from four fertile egg masses) were added to each tank. Duplicate subsamples of 50-200 organisms were removed from each tank at specified intervals and placed in glass petri plates containing contamination-free substrate for 24 hrs. This facilitated gut cleaning of contaminated materials. Upon removal, the organisms were counted, towel dried, and weighed to the nearest tenth of a milligram on a Cahn Electro balance for wet weight determination. Each sample was then placed on filter paper and kept at -5°C prior to analysis. This series of experiments represented bioaccumulation studies.

After 24 days exposure, half of each culture was transferred to clean substrate in similar experimental chambers. Organisms were sampled at specified intervals and treated as before. This series of experiments represented excretion studies. The entire experiment lasted 30 days and was terminated because of emergence of adults.

Chemical Analysis

Analysis of Aroclor 1242 was conducted by extracting the filter paper containing the insect larvae in a micro soxhlet for 5 hours with an acetone/pentane mixture. The extract was concentrated in a micro Kurdenna-Danish evaporator, passed through florisil, and concentrated with 500 µL nonoane present to give an accurate final volume. Sediments were

extracted and cleaned up in a similar manner. Water samples were analyzed by liquid-liquid extraction with 15% ethyl ether in pentane and concentrated and cleaned up as before. PCB measurement was performed by gas-liquid chromatography on a Varian 2700 GC equipped with a ^{63}N electron capture detector. A 6-foot glass column (1/8 in. I.D.) of 5% OV-210 on Gas Chrom Q (80-100 mesh) was used with a nitrogen flow of 20 mL/min. Oven and injector temperatures were 200°C and the detector was operated at 300°C.

Sediment oil analysis was conducted by soxhlet extraction with ethyl ether and gravimetric weight determination.

RESULTS

The results of bioaccumulation experiments are presented in Figures 1-3. Values represent the means of duplicate samples from two experiments. In all of the experiments conducted, the uptake of Aroclor 1242 demonstrates a dose-dependent relationship between substrate concentration and body burden. The addition of mineral oil to the substrate results in a significantly lower rate of uptake and overall body burden.

In the absence of oil contamination, a linear relation was observed between exposure time and body burden for 0.01 ppm substrate level. A slight leveling was observed in the 0.1 ppm experiments with a definite steady-state condition being reached in the 1.0 ppm series. The organisms reached equilibrium at about 18-20 µg/g after 20 days exposure.

The presence of substrate oil contamination resulted in about 70-80% reduction in overall uptake in all cases. The relationship between time of exposure and body burden was linear.

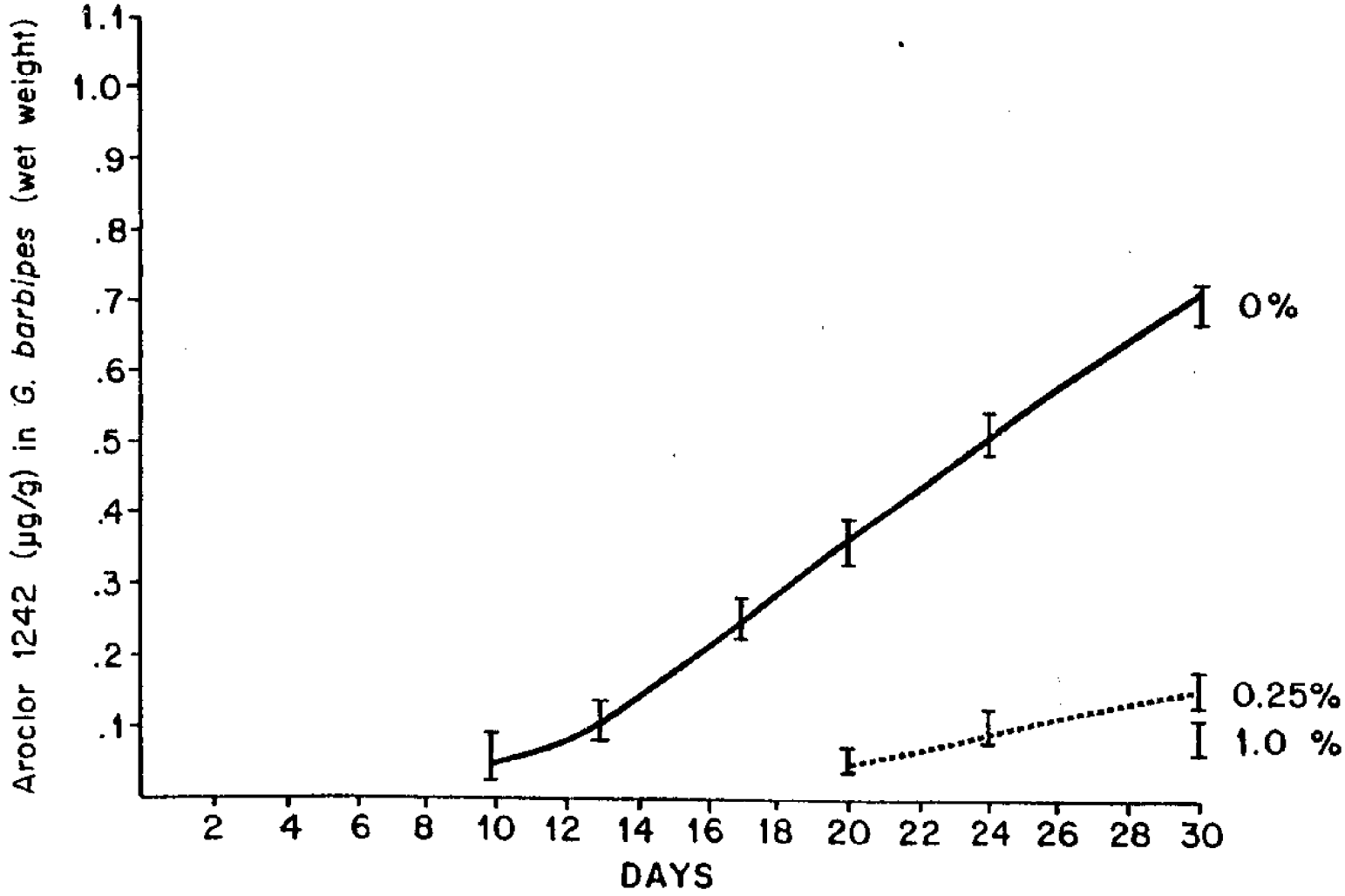

Figure 1. Accumulation of Aroclor 1242 by *G. barbipes* by substrate feeding at 0.01ppm with varying concentrations of oil. Vertical lines denote one standard deviation.

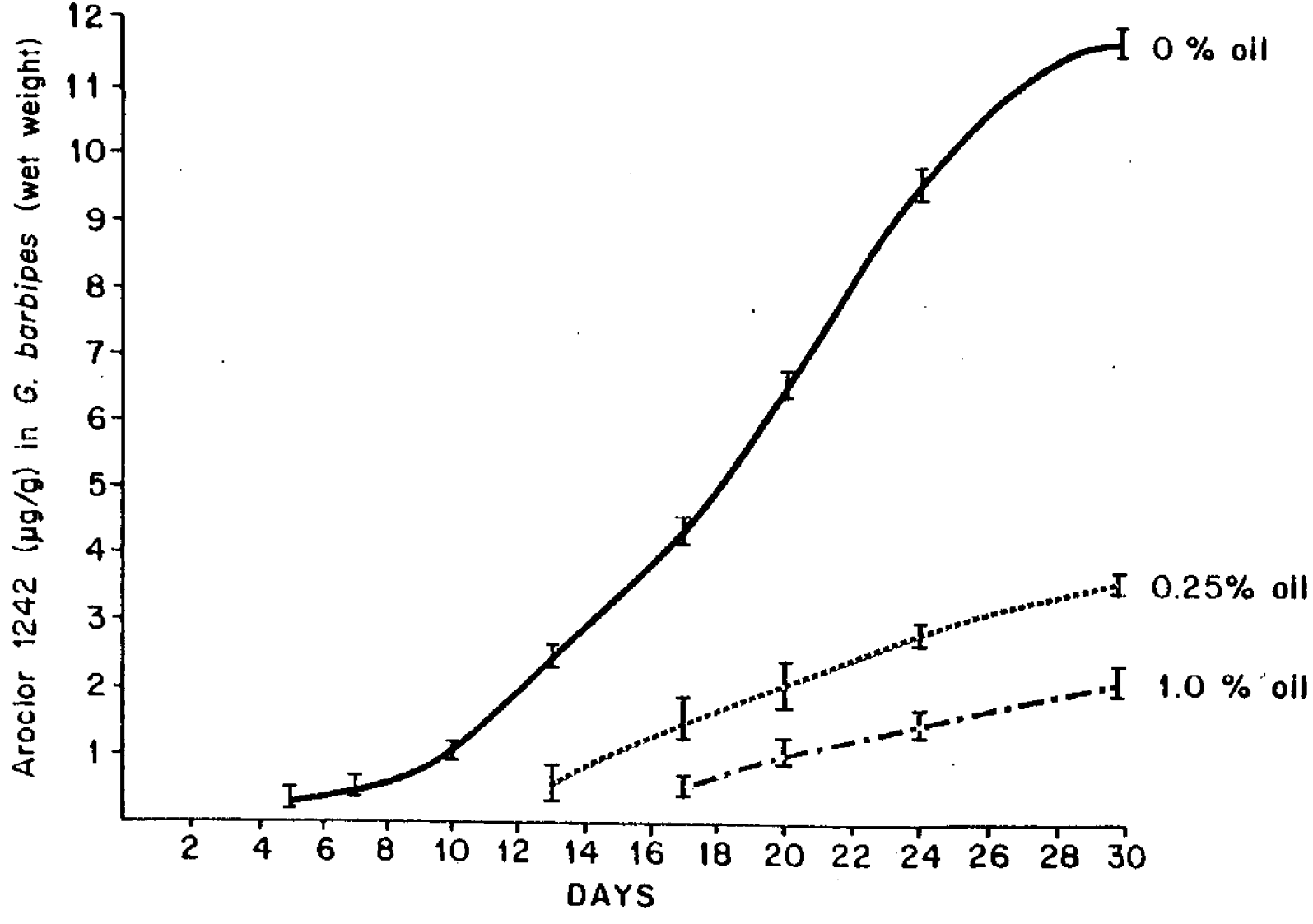

Figure 2. Accumulation of Aroclor 1242 by *G. barbipes* by substrate feeding at 0.1ppm with varying concentrations of oil. Vertical lines denote one standard deviation.

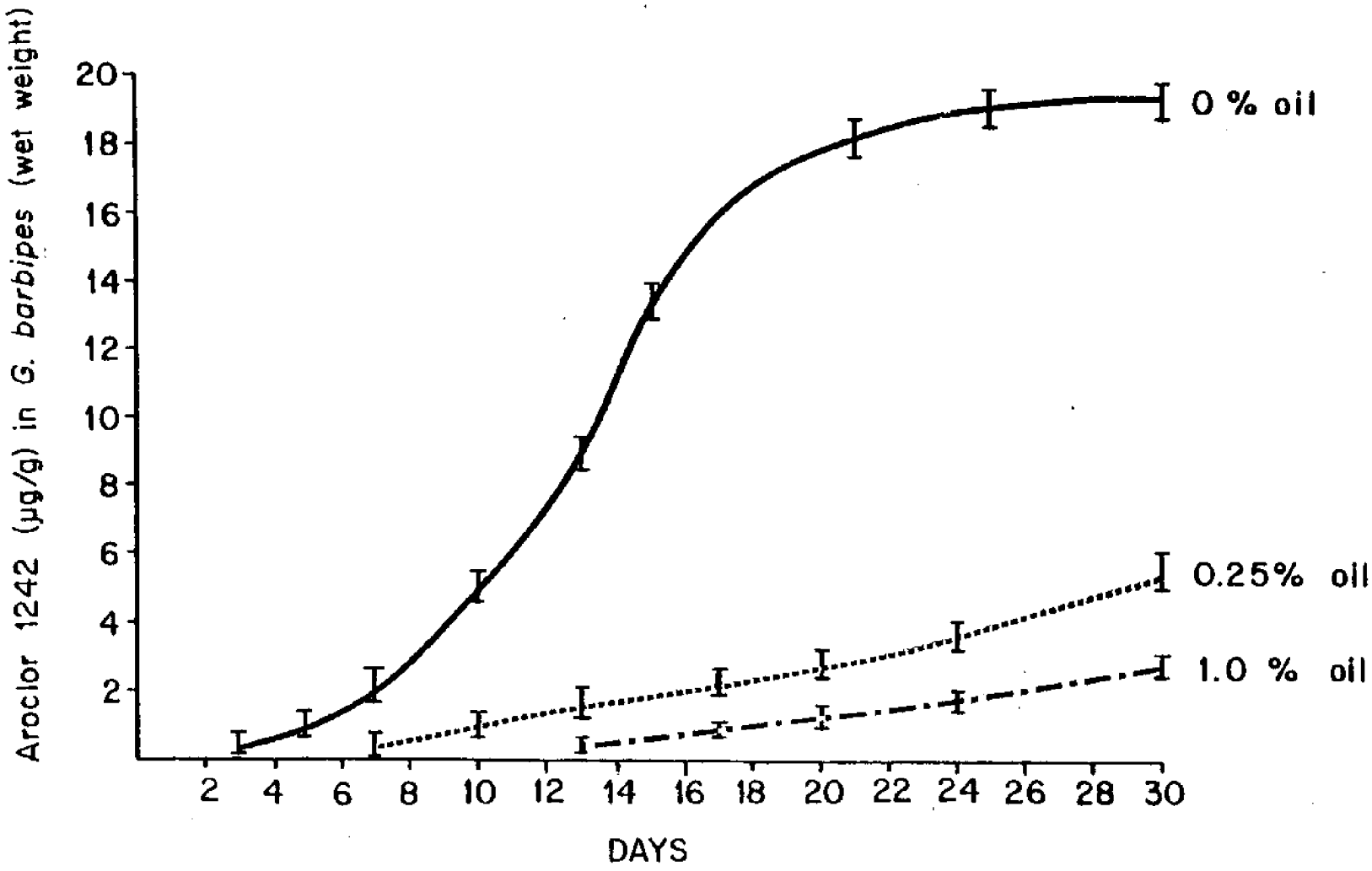

Figure 3. Accumulation of Aroclor 1242 by *G. barbipes* by substrate feeding at 1.Oppm with varying concentrations of oil.

Vertical lines denote one standard deviation.

In the absence of oil, concentration factors were 75 and 132 for 0.01 and 0.1 ppm in the substrate (Table 1). A concentration factor of 19.5 for the 1.0 ppm exposure demonstrated the attainment of equilibrium conditions. Oil contamination significantly reduced these concentration factors in all cases.

Excretion experiments revealed that no significant amount of PCBs was excreted during the one-week period of exposure to clean substrate (Table 2).

DISCUSSION

The results clearly demonstrate that the chironomid larvae, Glyptotendipes barbipes, can accumulate significant body burdens from substrate feeding alone. In comparing concentration factors for passive absorption from water vs feeding, the accumulation demonstrated in this study is considerably less. Sanders and Chandler (1972) have shown accumulation of 10,000 or more for aquatic Diptera with uptake rates being rapid and exponential. In contrast, this series of experiments has shown 100-fold accumulations with a gradual uptake rate. These differences can be explained on a basis of overall exposure as the amount of total PCBs a midge larvae can obtain by feeding on a few milligrams of substrate daily. It is much less than the total amount it is exposed to in the water on a daily basis in a dynamic bioassay. Also interesting is the attainment of a steady state condition during the 1.0 ppm experiment where a body burden of 18-20 ppm is constant during the final ten days. This shows that, at this point, an equilibrium is reached between the level present in the organism and the surrounding environment. No equilibrium is obtained in 0.01 and 0.1 ppm PCB levels which suggests that the levels obtained here are not the maximum possible.

The diameter reduction in uptake rates and overall body burden in the

TABLE 1. Summary of bioaccumulation data for G. barbipes after 30 days exposure to varying concentrations of Aroclor 1242 and medicinal mineral oil in the substrate. Results are expressed on a µg/g wet weight basis.

Substrate Concentration of Aroclor 1242 (ppm)	0% Oil		0.25% Oil		1.0 % Oil	
	µg/g ± SD[1]	Accumulation Factor[2]	µg/g ± SD[1]	Accumulation Factor[2]	µg/g ± SD[1]	Accumulation Factor[2]
0.01	0.75 ± 0.05	75	0.15 ± 0.05	15	0.10 ± 0.05	10
0.1	13.2 ± 0.5	132	3.5 ± 0.5	35	2.0 ± 0.5	20
1.0	19.5 ± 0.5	19.5	5.4 ± 0.5	5.4	2.9 ± 0.5	2.9

1 = 1 standard deviation

2 = µg/g accumulated / µg/g in sediment

TABLE 2. Summary of excretion data for G. barbipes. Organisms were harvested after 24 days exposure and fed clean substrate for 7 days. Results are expressed on a µg/g wet weight basis.

Substrate Concentration of Aroclor 1242 (ppm)	0% Oil		0.25% Oil		1.0 % Oil	
	µg/g ± SD[1] Accumulated after 24 days	µg/g ± SD[1] Excreted after 7 days	µg/g ± 1SD[1] Accumulated after 24 days	µg/g ± SD[1] Excreted after 7 days	µg/g ± SD[1] Accumulated after 24 days	g/g ± SD[1] Excreted after 7 days
0.01	0.45 ± 0.1	0.45 ± 0.1	0.1 ± 0.1	0.1 ± 0.05	--	--
0.1	9.5 ± 0.5	1.2 ± 0.2	2.7 ± 0.4	0.5 ± 0.1	1.4 ± 0.3	0.4 ± 0.15
1.0	18.0 ± 0.5	0.4 ± 0.1	3.5 ± 0.3	0.6 ± 0.15	1.9 ± 0.2	0.5 ± 0.15

1 = 1 standard deviation

- = Not detectable at a detection limit of 0.03 ppm

presence of oil contamination illustrate the effect of PCBs partitioned into oil and their availability to the benthos. Competition between the oil-PCB solvent system and endogenous lipid material in the gastrointestinal tract of the midge is of sufficient magnitude to result in a 70-80% reduction in body concentration. The competition between solvent systems is not present in oil free substrate as only molecular forces are holding PCBs to the particles. These observations are consistent with the poor absorption of fat-soluble vitamins in mammalian systems when mineral oil is substituted in the diet.

The failure of the excretion experiments to show any significant release of PCBs is not easily explained. It is known, however, that midges store fat during the last instar for an energy source for flight as an adult (Jonasson 1965). Since this series of experiments has been conducted during the last instar, the PCBs could be stored in sufficient fat reserves that little or no excretion takes place.

CONCLUSIONS

This study shows that food web organisms such as chironomid larvae can develop body burdens from substrate feeding on contaminated substrate. In addition it demonstrates that oil contamination in the substrate reduces the overall availability of PCBs to benthos. This suggests that a balancing effect exists between sediment concentration potential and bioavailability. Even though it is documented that oily sediment will concentrate high levels of non polar materials such as PCBs, this study shows that they are held in a less available form to bottom-feeding organisms.

This research provides essential information for fish-feeding experiments

where fish are fed bottom-feeding organisms with similar body burdens. These experiments will show whether sediment levels can be indirectly linked to body burdens in fish via feeding on contaminated benthos.

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