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Factors Influencing the Accumulation of Sediment-Sorbed Hexachlorobiphenyl by Midge Larvae

C. Michael Swindoll[1] and F. M. Applehans[2]

[1]Department of Zoology, University of Tennessee, Knoxville, Tennessee 37916 and [2]Department of Environmental Practice, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee 37996

A principle sink for polychlorinated biphenyls (PCBs) and other anthropogenic compounds released into the environment is the earth's waters (Halter and Johnson 1977). Due to their low water solubilities and refractory nature, PCBs are eventually deposited with sediment (Roberts and Meier 1982). The continued occurrence of PCBs in the biota indicates that sediment remains a source of hydrophobic organic compounds long after the initial source of contamination has been alleviated. Bioaccumlation of organic compounds by aquatic organisms is believed to be the result of equilibrium partitioning of the residues between the environment and biota (Hamelink et al. 1971).

This study was undertaken to elucidate the importance of abiotic and biotic factors to the bioaccumulation of sediment-sorbed hydrophobic organic compounds by benthic organisms. Hexachlorobiphenyl was chosen as the model substrate because it is extremely stable and shows no indication of being biodegradable (Hutzinger et al. 1972), and thus is representative of organic compounds which pose the greatest ecological hazard. Factors examined were substrate type, organic content of substrate, concentration, temperature and biological viability.

MATERIALS AND METHODS

Midge (Chironomus tentans) were cultured in 35-l aquaria containing approximately 5 l water. Shredded paper served as substrate for the larvae. Cultures were given 0.1 g/l Tetramin fish food (Tetre Weke, F.R.G.) twice per week, and 2 g/l Cerophyl (Cerophyl Lab. Inc., Kansas City, MO) every two months.

[2]Present address: Cyber Medics, POB 140549, Coral Gables, FL 33114
Send reprint request to C M Swindoll, NCEL, Code L71, Port Hueneme, CA 93043

Water was continuously aerated at room temperature (20-22°C). The culture received natural lighting.

Carbon-labeled 2,2',4,4',5,5'-hexachlorobiphenyl (HCB) was purchased from Pathfinder Laboratories (St. Louis, MO). Protosol, a tissue solubilizer, was purchased from New England Nuclear (Boston, MA). Ready-solv HP/b scintillation cocktail was purchased from Beckman Instruments (Fullerton, CA).

Kaolinite was purchased from Fisher Scientific (Norcross, GA),and humic acids from Aldrich Chemical Co. (Milwaulkee, WI). Sand was acid washed and seived between 0.5 and 0.25 mm seives. Natural sediment, collected from the Little River embayment of Fort Loudoun Reservoir, Knoxville, Tennessee, consisted of 49% silt, 34% clay and 17% sand; carbon content was 4.5% by dry weight. Sediment free of organic matter was obtained by combusting natural sediment in an oven at 550 ° C for 1 h; carbon content of the oxidized sediment was < 0.1% by dry weight.

Substrate contaminated with HCB was prepared prior to each experiment. Dry substrate (4.0 g) was placed in a 250-mL glass beaker. HCB dissolved in acetone was added to the substrate with microsyringe. Once the acetone had evaporated, 100 mL of distilled water was added and the beaker's contents were mixed for three minutes with a probe sonicator. The mixture was allowed to settle for 72 h prior to the experiment. Duplicate beakers were prepared for each experimental treatment.

Approximately 20 third- and fourth-instar midge were added to each beaker. Midge wet weight ranged from 5.6 to 17.2 mg; dry weight was 8.7% of wet weight. Midge were not fed during the course of the experiment. Duration of exposures ranged from 3 h to 8 days. At the end of each exposure period, 3-5 midge were randomly selected with forceps from each treatment. Each midge was rinsed for approximately 10 sec in deionized water, then blotted with a paper towel. The weight of the blotted midge was measured. The midge was then placed into a scintillation vial with 1 mL of tissue solubilizer. The vials were then placed on a mechanical shaker. Once the midge was fully digested, scintillation cocktail was added.

HCB was measured using a Tracer Analytic Model 6892 liquid scintillation counter (Elk Grove Village, IL). Counts were corrected for background, quench and counting efficiency. HCB concentrations were determined on a wet-weight basis. For each experiment, statistical comparisons (t-test) of HCB accumulation

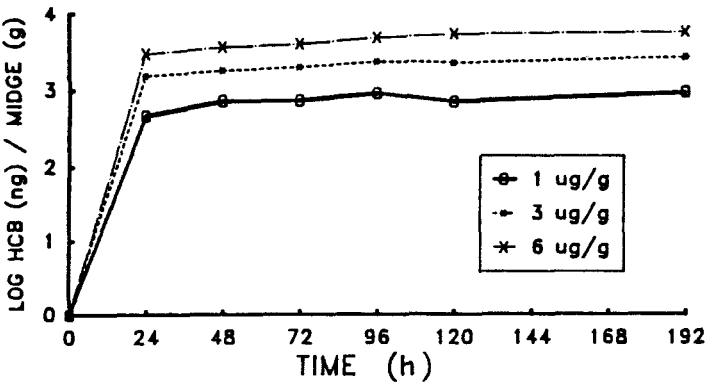

Figure 1. Mean midge HCB concentrations resulting from exposure to natural sediment initially contaminated with 1, 3 and 6 ug HCB/g.

were conducted. All statistical tests were at $\alpha = 0.05$. Steady-state concentrations of HCB in midge for each treatment were considered to have been reached when there was no significant difference in mean HCB concentrations with exposure time. Overall mortality was < 10%, and was not significantly different among controls and treatments. Dead or pupating midge were not used in the analysis.

The depuration of HCB from midge was determined following 96 h exposure to natural sediment contaminated with 1 ug HCB/g. Following exposure, midge were transferred to beakers containing either 100 mL distilled water and natural or oxidized sediment (4.0 g), or water only. Midge HCB concentrations were determined at 24-h intervals.

RESULTS AND DISCUSSION

The bioaccumulation of HCB resulting from exposure to contaminated substrate was characterized by an initial phase of rapid uptake followed by a progressively slower accumulation (Figures 1-5). The rapidity of bioaccumulation was probably the result of the HCB concentration gradient which existed when the midge were initially exposed to the contaminated substrate; midge HCB uptake slowed as equilibrium concentrations were approached.

Steady-state HCB concentrations of midge were proportional to exposure concentrations (Figure 1), which would be expected if equilibrium partitioning were responsible for bioaccumulation of sediment-sorbed compounds. Steady-state concentrations were obtained in all experiments within 4 to 5 days exposure.

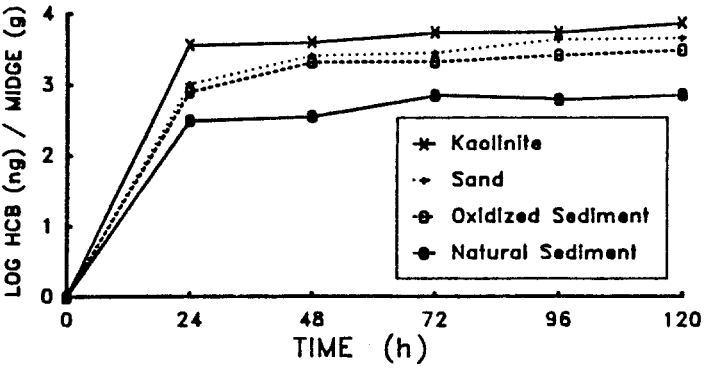

Figure 2. Mean midge HCB concentrations resulting from exposure to natural sediment, oxidized sediment, sand and kaolinite, each initially contaminated with 1 ug HCB/g.

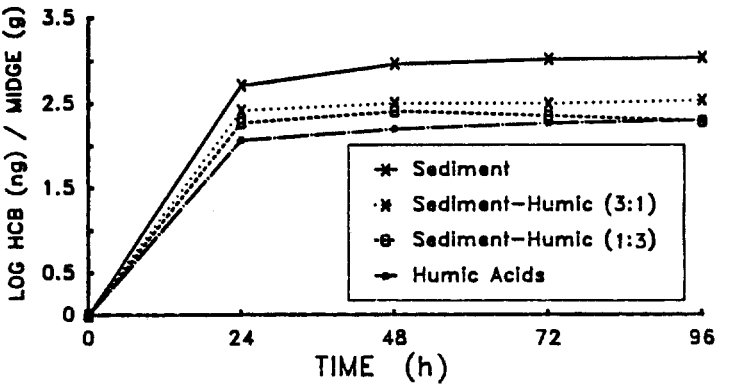

Figure 3. Mean midge HCB concentrations resulting from exposure to substrate varying in organic content, each initially contaminated with 2 ug HCB/g.

These results are consistent with those reported for midge and other chemicals (Muir et al. 1985; Roberts and Meier 1982). The proximity of midge to the contaminated sediment could have contributed to the rapidity of the equilibrium process. In addition, benthic organisms ingest sediment (Nimmo et al. 1974; Roberts and Meier 1982) which increases exposure surfaces as well as decreases distances for diffusion, and thus facilitates uptake of sediment-sorbed compounds.

HCB bioaccumulation from different contaminated substrates were significantly different and followed this sequence : natural sediment (NS) < oxidized sediment (OS) < sand (SA) < kaolinite (KA) (Figure 2),

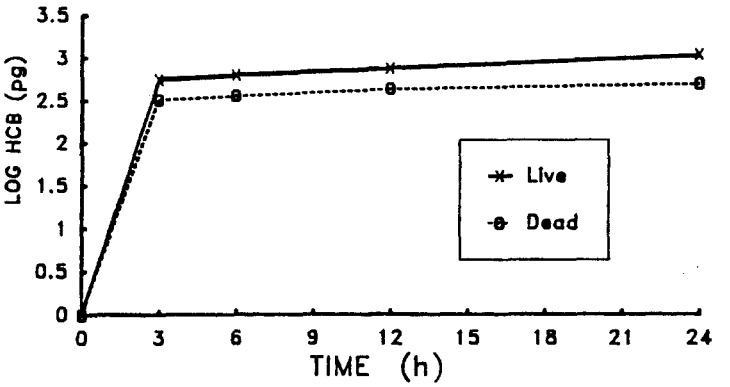

Figure 4. Mean midge HCB load resulting from exposure of live and dead midge to natural sediment initially contaminated with 1 ug HCB/g.

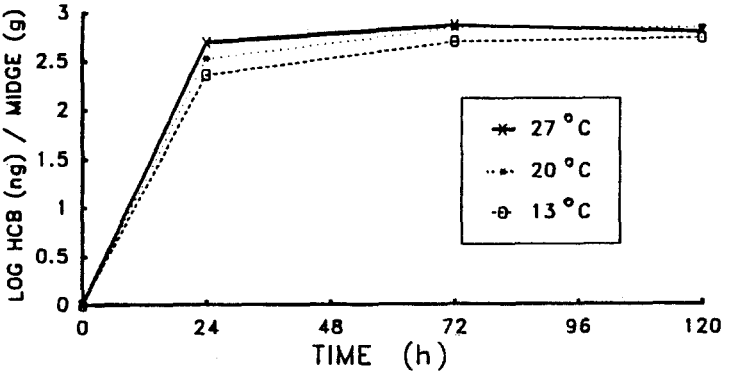

Figure 5. Mean midge HCB concentrations resulting from exposure at 13, 20 and 27 ° C to natural sediment initially contaminated with 1 ug HCB/g.

which is approximately the inverse of that of the surface area of the substrates : organic matter > KA > SA (Bailey and White 1964; Lambe and Whitman 1969). The rate of HCB uptake during the initial 24-h exposure (as ng/g/h) was 152.4, KA; 43.3, SA; 33.6, OS; and 14.1, NS. Except for the bioaccumulation resulting from exposure to sand, these data suggest that the bioavailability of HCB was inversely related to the surface area of the substrate, which is consistent with the concept of equilibrium partitioning.

To examine the influence of organic matter (OM) on the bioavailibility of HCB, the OM content of natural sediment was increased with the addition of humic acids. Organic matter had a significant influence on

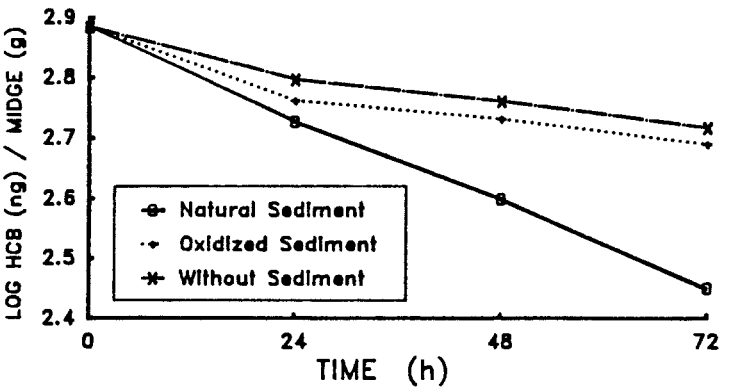

Figure 6. Mean midge HCB concentrations during depuration in the presence of natural sediment and oxidized sediment, and without sediment.

HCB accumulation, with the rate of HCB accumulation by midge inversely related to the organic content of the substrate (Figure 3). Two properties of OM appear important to the bioavailability of sorbed hydrophobic compounds: 1) the irregular structure of OM provided large surfaces for sorption; 2) OM contains both hydrophobic and hydrophilic components. The large surface area of OM competed with midge for desorbed HCB, thus decreasing the bioavailability of HCB. The hydrophilic components contributed to OM's aqueous solubility, which increased its exposure to dissolved residues. The hydrophobic portions form an intermatrix where nonpolar compounds accumulate (Goring 1967) and the desorption of residues from OM was slowed by diffusion from the hydrophobic matrix (Khan 1980). The bioaccumulation of residues sorbed to OM will be limited by the rate at which the chemical diffuses from the OM's hydrophobic matrix.

Comparison of HCB accumulation by live and dead organisms should provide information on the means of residue bioaccumulation. If equilibrium partitioning is solely responsible for the bioaccumulation of residues, then biotic processes would not be expected to influence accumulation. In this study HCB accumulation by live midge was significantly greater than that of formaldehyde-killed midge following a 24-h exposure to contaminated natural sediment (Figure 4). Accumulation by dead midge is clearly the result of equilibrium partitioning. Differences in HCB accumulation by live and dead midge probably reflects a greater absorption into live midge as a result of the circulatory system facilitating diffusion. In addition, the movements of live midge as well as

consumption of contaminated sediment would increase their exposure over that of dead midge. Thus biological activity contributed to residue uptake by maintaining a concentration gradient between midge and the environment.

Bailey and White (1964) speculate that the bioactivity of chemicals might be different at various temperatures. If so, then residue accumulation by aquatic organisms would be expected to vary with temporal and spatial differences in temperature. To examine the influence of temperature on bioaccumulation, midge were exposured to contaminated sediment at 13, 20 and 27 ° C. The initial rate of HCB uptake by midge increased with temperature; however, steady-state concentrations at the three temperatures were not significantly different (Figure 5). Due to the narrow range of temperature examined in this study, the different rates of HCB uptake are believed to be due to changes in physiological activity of midge rather than changes in affinity of sediment for HCB. An increase in physiological activity would assist the diffusion of HCB into midge, thus increasing the rate of bioaccumulation.

The rate at which aquatic organisms eliminate chemical residues is important because bioaccumulation will occur only if the rate of uptake exceeds depuration (Spacie and Hamelink 1985). In this study the nature of the environment had a significant influence on the depuration of HCB from midge (Figure 6). The initial 24-h depuration rates in the presence of NS, OS, or without substrate were 9.5, 7.9, and 5.8 ng/g/h, respectively. A slow elimination of HCB from midge would be expected due to HCB's lipophilicity (Log Kow > 6.7) and the inability of the organism to degrade the compound into soluble metabolites. The slow depuration of HCB increases the likelihood that the organism will suffer from chronic effects produced by the chemical, and increases the probability that the midge will serve as a vector for the contamination of higher trophic levels.

In summary, the data obtained in this study indicate that the physical process of equilibrium partitioning was responsible for HCB bioaccumulation from contaminated sediment. However, biological activity contributed to this partitioning process and would be expected to be important to bioaccumulation in the environment. The bioavailability of HCB was inversely related to surface area and organic content of the substrate, and proportional to concentration.

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