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# Bioaccumulation of Hexachlorobenzene in Killifish (*Fundulus similis*)

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Hexachlorobenzene (HCB) is persistent in the environment as it is highly resistant to photolysis (PLIMMER & KLINGEBIEL 1976) and is not readily degraded by soil microbes (ISENSEE et al. 1976). Recent studies in remote areas of the Pacific Ocean (Marshall Islands) have revealed HCB in atmospheric samples (ATLAS & GIAM 1980). HCB has been detected in the tissues of both marine and freshwater fish (JOHNSON et al. 1974, OFSTAD et al. 1978, NIIMI 1979). HCB has also been detected in oysters from Galveston Bay (MURRAY & GIAM, unpublished).

The use of bioaccumulation factors as an estimate of potentially harmful effects of organic chemicals has become increasingly important in studies of biological effects in aquatic systems. Likewise, the octanol-water partition coefficient seems to be a useful indicator of bioaccumulation potential in many instances (VEITH et al. 1978); however, it has not been evaluated with marine organisms. Because of this and because of our extensive experience and interest in the analysis of marine samples for low-level organic contaminants (GIAM 1976, GIAM et al. 1975a,b, 1977, 1978), we were interested in studying the relationship between bioaccumulation in marine organisms and some easily measured parameters, e.g., the octanol-water partition coefficient. As the first part of that effort, the objectives of this study were to determine the rate and extent of bioaccumulation in killifish continuously exposed to low concentrations of HCB and then to determine the rate and extent of depuration in these animals. Low concentrations were used to identify threshold levels and to more accurately approximate typical environmental exposures.

## MATERIALS AND METHODS

Solvents used in this study were Burdick and Jackson pesticide quality. Silica gel (Woelm, 70-230 mesh) was activated at 150°C for at least 24 hrs before use. Water was purified by extraction with petroleum ether in a 15-liter extractor. The petroleum ether was changed at 24-hr intervals until a 50-fold concentrate demonstrated no impurities by gas chromatography (GC).

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All glassware and equipment were washed with Micro cleaning solution (International Products Corp.), rinsed with distilled water and heated at 320°C overnight. Just prior to use, glassware was rinsed with pesticide grade petroleum ether. The petroleum ether washing (100 mL) was concentrated to about 0.1 mL and checked for contaminants by GC with an electron capture detector. If any impurities were present, rinsing was repeated as needed to obtain an acceptable blank. Procedural blanks were also performed at intervals to ensure the absence of contamination from reagents and solvents.

A Hewlett-Packard 5840A gas chromatograph with a Nickel-63 electron capture detector and a 6' x 4 mm i.d. borosilicate column packed with 5% SP-2401 was used for HCB analyses. The injector, detector, and column temperatures were 250°, 300°, and 160°C, respectively.

Specimen Collection and Dosing System. Killifish (Fundulus similis) were collected at San Luis Pass (which is a relatively clean area at the west end of Galveston Island, Texas) by seining the marsh areas and shallow inlets. Fish were placed into large ice chests containing natural sea water, chilled, and aerated during transport to the laboratory. The killifish were acclimated for two weeks in Instant Ocean, which was continuously filtered using activated charcoal; the fish were fed Oregon Moist Pellets or Tetramin fish food daily. To measure bioaccumulation, fish were placed into an exposure aquarium (Figure 1) and were continuously dosed with HCB from a sand dosing column. To prepare the column, sand (100 g) was coated with HCB by suspending it in a solution of HCB (100 mg) in benzene (150 mL) and evaporating the solvent. The spiked sand was then placed between two layers of clean sand in a glass column; both ends of the column were packed with glass wool. Water was percolated through the column in an upward direction using a peristaltic pump. By adjusting the flow rate of the peristaltic pump, concentrations in the water could be changed. Crushed oyster shell was used in the filter pump to remove particulates from the tank. For the depuration experiments, fish were transferred from the exposure aquarium to another tank equipped with a filter pump containing activated charcoal.

Analyses. At the designated sampling times, 12 fish were removed from each aquarium (control and exposure or depuration) and divided into triplicate samples (4 fish each).

Tissue was homogenized twice using 30 mL 20% acetone/acetonitrile for each extraction. Extracts were filtered and placed into a 1-L separatory funnel containing 150 mL 5% NaCl solution. The combined extracts were then extracted 3 times with petroleum ether (PE) using 25 mL each time. The sample was concentrated using a 250 mL Kuderna-Danish evaporative flask. Clean-up was accomplished by passing the sample through a column containing 10 g fully activated silica gel. The sample was eluted with 50 mL PE; the first 15 mL of eluate were discarded and the remainder was saved and

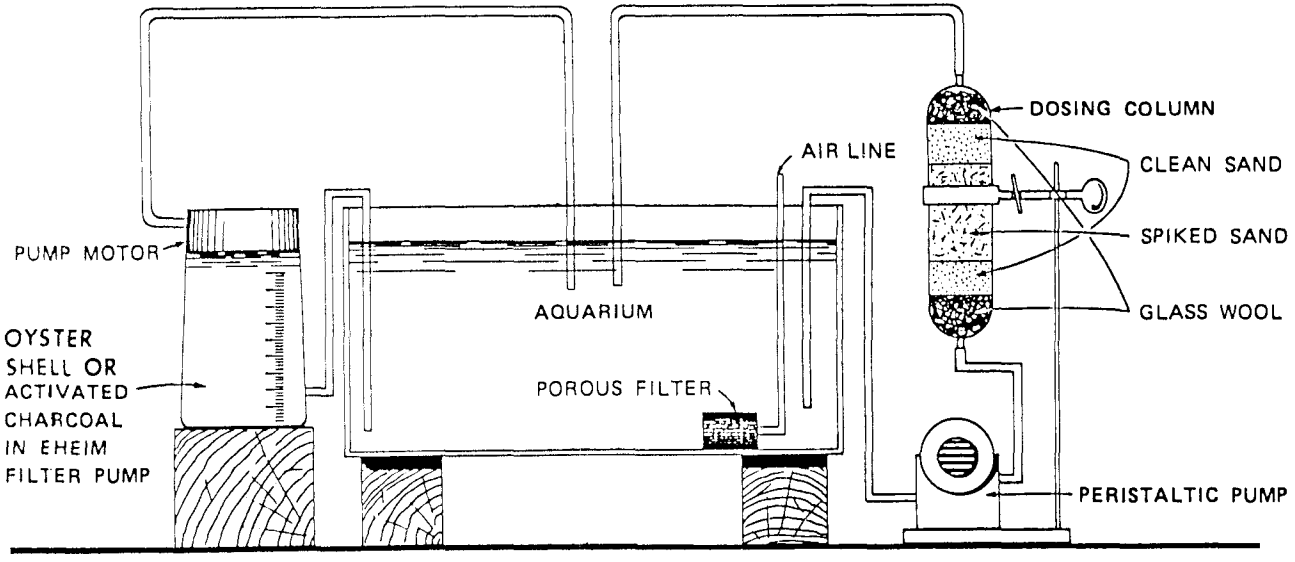


Figure 1. Exposure aquarium and sand dosing column. Hexachlorobenzene (HCB) was added to the tank from a sand dosing column as described in the text. The water was equilibrated with HCB prior to adding the fish. For depuration, exposed fish were placed in a similar aquarium without the dosing column and containing activated charcoal in the filter pump.

concentrated for analysis by GC-ECD. At each sampling time, duplicate 5.0 mL water samples were taken from test and control tanks, and extracted once with 1.0 mL isooctane. The extracts were then quantitated by GC-ECD.

## RESULTS AND DISCUSSION

The bioaccumulation factors measured in two experiments are summarized in Table 1. The values given represent average

TABLE 1. Bioaccumulation Factors for Hexachlorobenzene in Killifish

| Time, hrs | Experiment 1 B F ± S E* | Experiment 2 B F ± S E[a] |
|---|---|---|
| 1 | --- | 65±2 |
| 2 | 68±5 | 112±6 |
| 4 | --- | 155±20 |
| 6 | 215±28 | 255±71 |
| 8 | --- | 214±40 |
| 10 | --- | 365±101 |
| 12 | 206±41 | 282±39 |
| 24 | 236±33 | --- |
| 48 | --- | 607±196 |
| 72 | 128±20 | --- |
| 96 | 216±30 | 667±47 |
| 168 | 139±47 | 709±74 |
| 264 | 235±43 | --- |

[a]The bioaccumulation factor (BF) is the ratio of the concentration of HCB in killifish to that in the surrounding water. Values reported are averages of 3 individual samples ± the standard error (SE). Fish were exposed to HCB, sacrificed at various times and analyzed for HCB as described in the text. The HCB concentrations ranged from 0.04 to 0.45 µg/ℓ in experimental 1 and from 0.16 to 0.38 µg/ℓ in experiment 2.

bioaccumulation and standard error (SNEDECOR & COCHRAN 1967). The experimental data were analyzed by a curve fit method using the equation $BF = K(1-e^{-\alpha t})$, where K is the theoretical maximum bioaccumulation factor when the time of exposure (t) goes to infinity and $\alpha$ is a constant representing the rate of accumulation, excretion and metabolic turnover. Analysis of the experimental data by reiterative approximations gave the theoretical curve shown in Figure 2. Based on this analysis, the theoretical maximum bioaccumulation factor was 375. Approximately 4.8 hrs were required to reach half this value. A third experiment in which the fish were not fed during

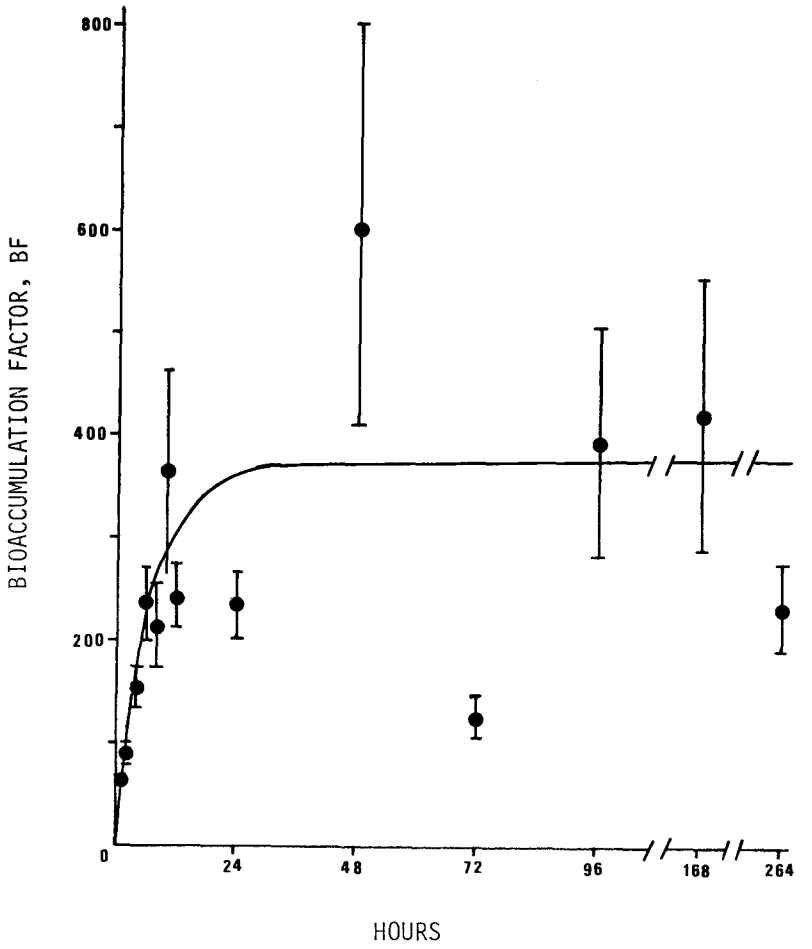


Figure 2. Bioaccumulation of hexachlorobenzene by killifish. The solid curve represents the best fit of the equation $BF = K(1-e^{-\alpha t})$ to the experimental data. Sample averages for two experiments are plotted with their standard errors. Each point represents the average of 3-6 values.

acclimation or exposure gave similar values for the maximum bioaccumulation factor (420) and the time (5.8 hrs) required to reach half this value. These values are considerably less than have been reported in several freshwater species. VEITH et al. (1979) reported bioaccumulation factors of 16,200 for HCB in the fathead minnow (Pimephales promelas), 21,900 for green sunfish (Lepomis cyanellus) and 5,500 for rainbow trout (Salmo gairdneri). ISENSEE et al. (1976) exposed catfish (Ictalurus punctatus) to 0.01, 0.09, and 0.61 µg $^{14}$C-HCB/g sand and reported bioaccumulation factors of 160±10, 680±110, and 4170±160, respectively, after 12 days exposure.

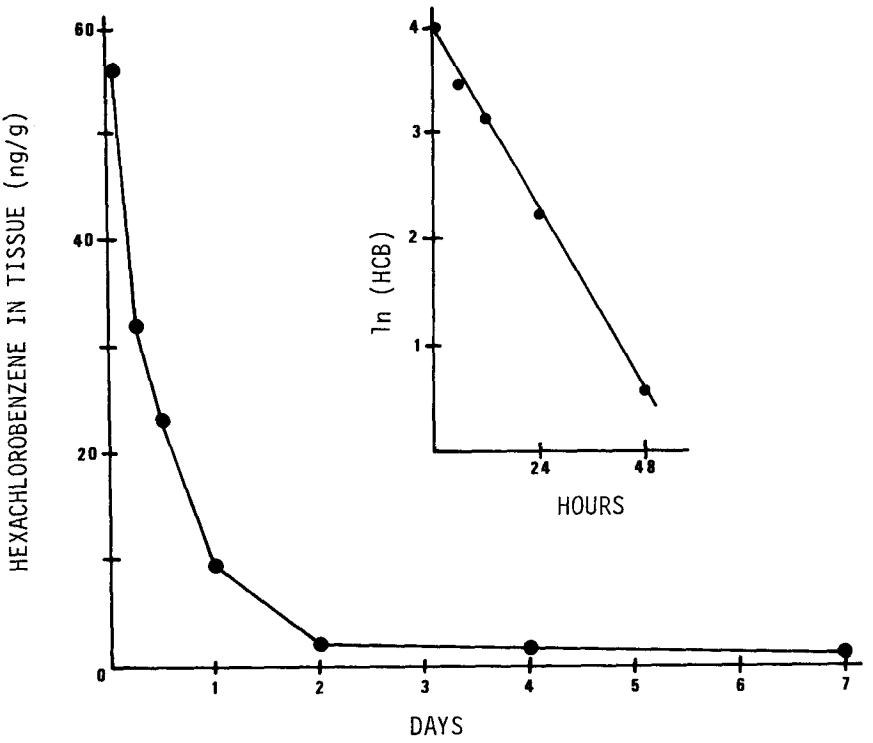


Figure 3. Depuration of hexachlorobenzene (HCB) by exposed killifish. Killifish exposed to HCB for 11 days were placed in HCB-free water and sacrificed at the indicated times. Their tissue concentrations of HCB decreased as shown; values plotted are averages of three fish.

After 11 days exposure, HCB-exposed fish were removed from the dosing tank and placed into the depuration tank. The HCB concentration decreased exponentially for two days; during this time, tissue concentration fell from about 70 ng/g to 1.8 ng/g (Figure 3). After one week, tissue concentration in the depurated fish had leveled off at 0.8 ng/g, which was also the concentration measured in control fish. The half-life ($t_{1/2}$) for loss of HCB from the whole body was calculated to be 9.3 hr. In duplicate depuration experiments in this laboratory, exponential loss of the pollutant was also observed, but at a slightly slower rate, i.e., with a half-life of about 12 hrs. The $t_{1/2}$ values reported in this study using Fundulus similis were considerably shorter than have been reported in green sunfish. SANBORN et al. (1977) compared $t_{1/2}$ values for loss of total $^{14}$C-HCB from various tissues of green sunfish; they reported a $t_{1/2}$ (days) of 10.0 in carcass; 19.6 in liver, 8.0 in stomach and pyloric caeca; 8.1 in intestine; and 8.5 in muscle. ISENSEE et al. (1976) reported that when catfish were removed from HCB-dosed water and placed into tanks without HCB for 4 days, the concentration in tissue fell to 170+29 and 750+160 ng/g when previously exposed to 0.01 and 0.09 µg HCB/g sand, respectively. Studies conducted by ISENSEE et al. (1976) and VEITH et al. (1979) indicated that the

bioaccumulation factor and $t_{1/2}$ increase with increasing concentration. It is therefore possible that the low bioaccumulation factor and $t_{1/2}$ reported in this study were related to the low concentration used for dosing.

This study has shown that HCB can be bioaccumulated by animals in the marine environment, but to a lesser extent than in fresh water animals. Under the conditions tested, the average bioaccumulation factor for HCB in F. similis was calculated as 375. When placed in clean water, F. similis will rapidly depurate the accumulated HCB. In this study, the concentration in tissue fell exponentially with a half-life of 9-12 hrs to within control levels after 48 hrs. Finally, the sand column described herein is a useful tool for continuous dosing of chemicals, such as HCB, which are poorly soluble in water.

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