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Comparative Bioaccumulation and Elimination of HCH Isomers in Short-necked Clam (*Venerupis japonica*) and Guppy (*Poecilia reticulata*)

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Pesticides applied to paddy fields have flowed into agricultural drainage attached to soil particles and in solution, and have been frequently detected in river surface water. Persistent organochlorine insecticides have been found in surface water and bottom sediments in rivers long after application (SUZUKI et al., 1974; YAMATO et al., 1980).

In Japan, HCH (1,2,3,4,5,6-hexachlorocyclohexane) had been one of the most applied insecticides to arable fields, especially to rice paddies. Residues of α-, β-, and δ-HCH have been detected as well as insecticidally active γ-HCH (lindane) in the environment because technical grade HCH had been formulated and marketed as HCH insecticide (SUZUKI et al., 1976 and 1977; YAMATO et al., 1978). In order to evaluate the environmental impact of insecticides on various organisms, model experiments using popular aquatic organisms are necessary.

In the present study, comparative bioaccumulation and elimination of 4 HCH isomers by short-necked clam (Venerupis japonica) and guppy (Poecilia reticulata) were examined.

METHODS AND MATERIALS

HCH isomers in ethanol were dissolved in sea-water at concentrations of 1, 2, 1, and 2 parts per billion (ppb) for α-, β-, γ-, and δ-HCH, respectively. The HCH water solution was fed from a reservoir with a micro pump at 10 mL/min and introduced into a glass aquarium containing 3 L of sea-water, in which shellfish were maintained. Five shellfish and 300 mL of sea-water were sampled at intervals, and the concentrations of HCH isomers were determined. In order to investigate elimination of HCH isomers from the body of shellfish, the shellfish were exposed for 10 days and transferred

to HCH-free flowing sea-water. Five shellfish were then sampled at intervals to determine concentrations of HCH isomers in their edible parts.

Also, 15 shellfish were sampled on the first and 6th days of the bioaccumulation experiment and on the third day after replacement to HCH-free water. Concentrations of HCH isomers in internal organs (IO) and tissues were determined.

In the guppy experiment, HCH isomers in ethanol were dissolved in tap water which was purified by passing through an active carbon filter. The concentrations of α-, β-, γ-, and δ-HCH in water were at 1, 2, 1, and 2 ppb, respectively. Bioaccumulation experiments were performed as in the shellfish experiments. However, a 10-L aquarium was employed in this experiment. Ten guppies and 300 mL of water were sampled at intervals to determine bioaccumulation of HCH isomers. For the elimination experiment, the guppies were exposed to HCH for 10 days, then transferred to HCH-free flowing water.

Water: A 300-mL water sample taken at various intervals was drained into a 500 mL separatory funnel, and extracted successively with 50, 30, and 30 mL of n-hexane. The combined extracts were dried by pouring them through a 2 x 5 cm column of anhydrous sodium sulfate. The separatory funnels were thoroughly rinsed with a small volume of n-hexane, and the rinsings were added to the extract. The extract was concentrated approximately to 1.5 mL using a Kuderna-Danish (K-D) evaporator. This concentrate was subjected to gas-liquid chromatography equipped with a ^{63}Ni electron capture detector (GLC-ECD) after addition of heptachlor epoxide as an internal standard.

Biological samples: Biological samples were ground with anhydrous sodium sulfate in a morter to obtain a floury mixture. The mixture was put into a 300 mL erlenmeyer flask, and was extracted with 200 mL of dichloromethane. The extract and several rinsings were filtered through a Büchner glass filter. The filtrate was concentrated using a K-D evaporator, and dichloromethane was completely removed to obtain fat. Acetonitrile partitioning and florisil clean-up were done in accordance with the official AOAC method (1975). The eluate from florisil column was concentrated to about 1.5 mL using a K-D evaporator and subjected to GLC-ECD after addition of heptachlor epoxide as an internal standard. Gas chromatographic conditions were in accordance with

the previous report (SUZUKI et al., 1974).

RESULTS AND DISCUSSION

The guppy rapidly bioaccumulated HCH isomers, and the concentrations reached a plateau on the 4th day after beginning of experiment (Fig. 1). The bioaccumulation ratio (concentration in organism/concentration in water, BR) of α-, β-, γ-, and δ-HCH were 706, 1043, 697, and 648 times on that day, respectively. The BR of β-HCH was the greatest among 4 HCH isomers. However, concentrations of HCH isomers in the guppy smoothly decreased

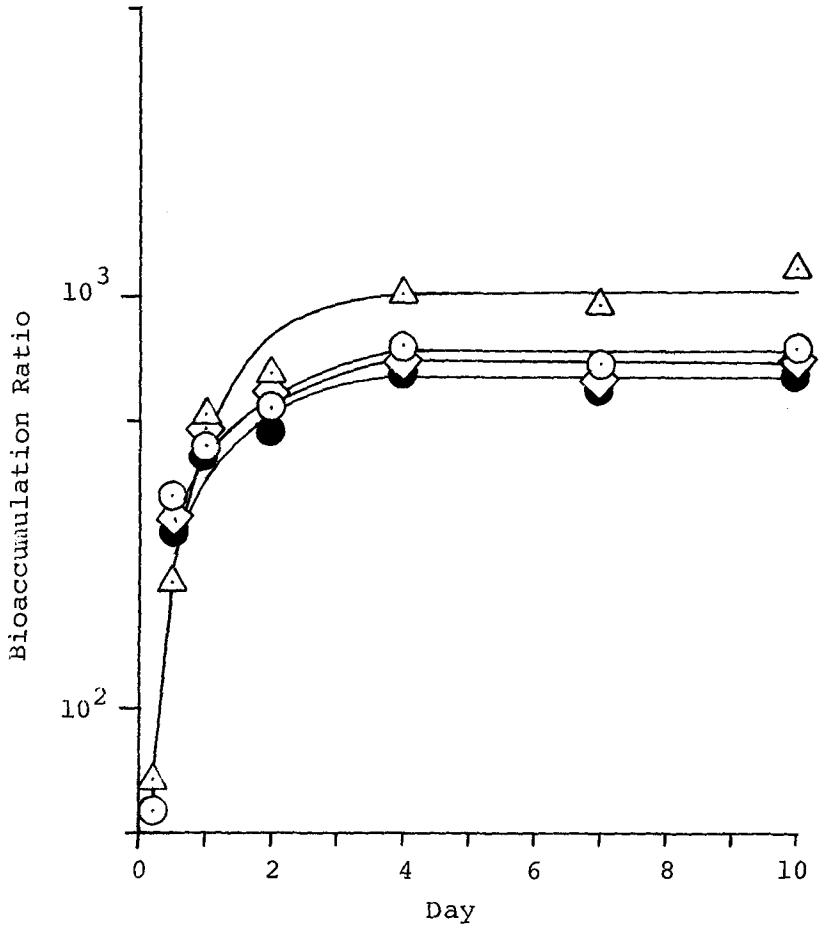

Fig. 1. Bioaccumulation of HCH isomers by guppy as a function of time. ○, α-HCH; △, β-HCH; ◇, γ-HCH; ●, δ-HCH

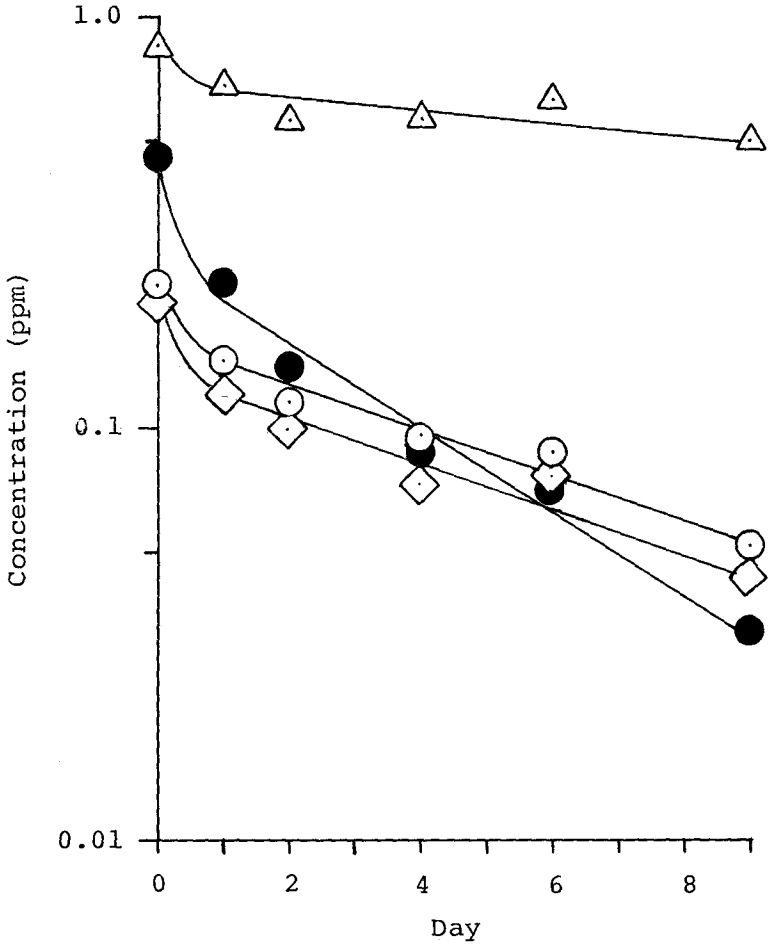

Fig. 2. Elimination of HCH isomers from guppy as a function of time. ○, α-HCH; △, β-HCH; ◇, γ-HCH; ●, δ-HCH

on first day after replacement to HCH-free water, and constantly declined afterwards (Fig. 2). Similar data were shown by CANTON et al. (1978). Elimination rates (ER) of HCH isomers were as follows: δ-HCH γ-HCH α-HCH β-HCH. Also, these results were the same as experiments with rats (KAMADA, 1971).

Short-necked clam rapidly absorbed HCH isomers, and the concentrations reached a plateau on day three (Fig. 3). The BR of α-, β-, γ-, and δ-HCH were 161, 127, 121, and 272 times, respectively, and BR of δ-HCH showed the highest value among HCH isomers. Also, the BR of δ-HCH was about twice as much as that of γ-HCH. The order of BR was as follows: δ-HCH≫β-HCH>α-HCH>γ-HCH. This order

was clearly different from the results obtained with the guppy. The BR of short-necked clam were remarkably lower than those of guppy. Perhaps this was due to the difference in body fat contents between the organisms (SUGIURA et al., 1979). Concentration of each HCH isomer in short-necked clam constantly decreased after replacement to HCH-free water (Fig. 4). ER of HCH isomers were as follows: γ-HCH>α-HCH≫δ-HCH≧β-HCH. And ER of γ-HCH was about 4 times faster than that of β- and δ-HCH. This order of ER of HCH isomers with short-necked clam were clearly different from that with guppy.

Distribution of HCH isomers in short-necked clam is

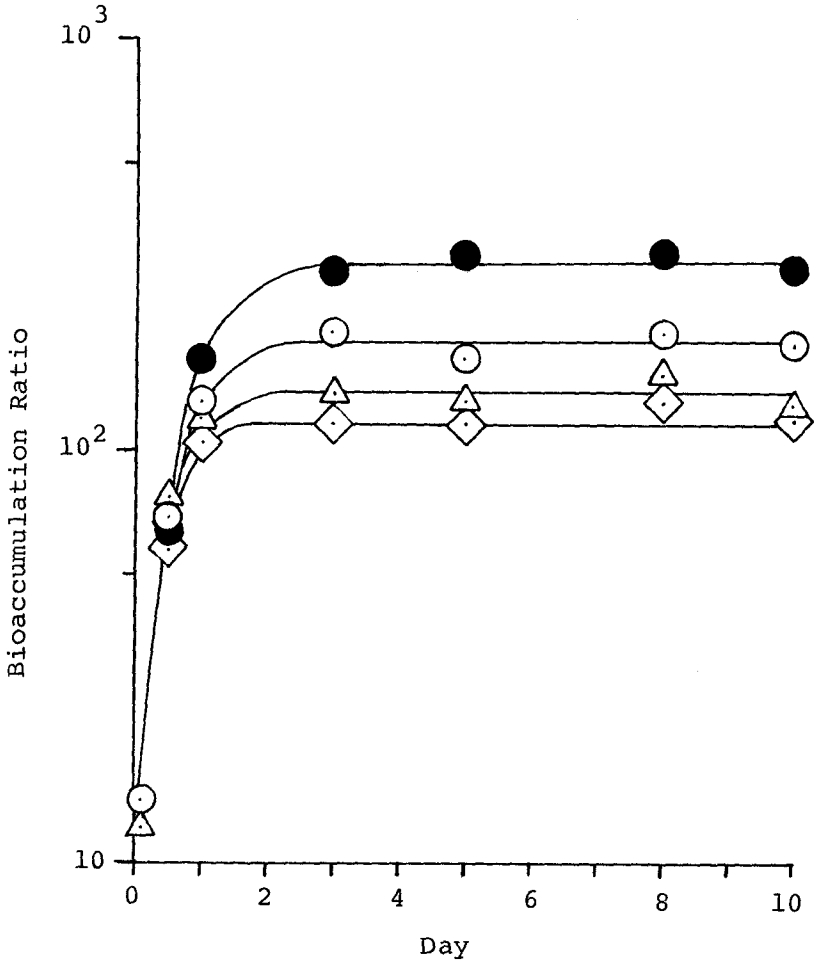

Fig. 3. Bioaccumulation of HCH isomers by short-necked clam as a function of time. ○, α-HCH; △, β-HCH; ◇, γ-HCH; ●, δ-HCH

shown in Table 1. Concentrations of HCH isomers in IO on the 6th day after beginning of experiment were twice or 3 times as much as those on the first day. In tissue, concentration of δ-HCH with 6 day-exposure was about 2.7 times as much as that with 1 day-exposure, but the concentrations of other HCH isomers on the 6th day were less than twice those on the first day. three days after replacement to HCH-free water, HCH concentrations in IO remarkably decreased. Especially, the γ-HCH concentration after 3 days-replacement to HCH-free water was 40.8% of that with 6 day-exposure, and the level of β-HCH [the most persistent among HCH isomers (SUZUKI et al., 1975)] also reduced to 59.2%. The results indicated that HCH isomers might be metabo-

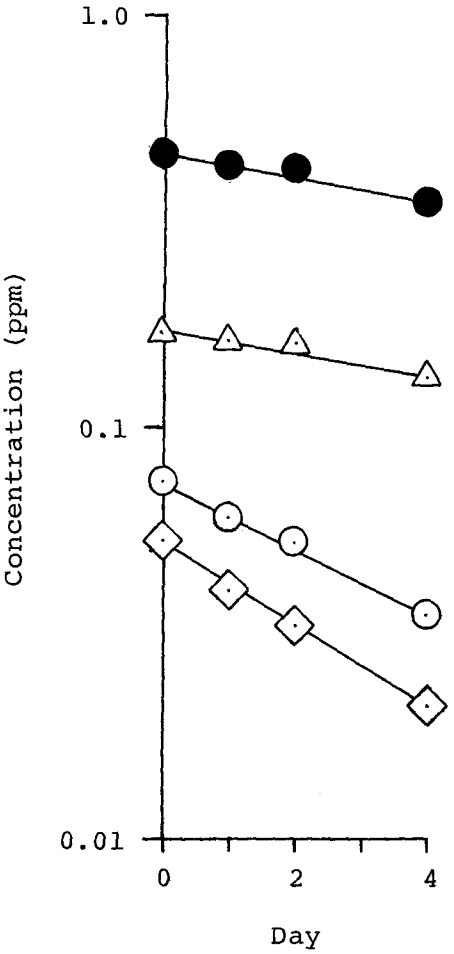

Fig. 4。 Elimination of HCH isomers from short-necked clam as a function of time。 ○, α-HCH; △, β-HCH; ◇, γ-HCH; ●, δ-HCH

Table 1. Concentration of HCH isomers in short-necked clam (ppm)

	Bioaccumulation Period		Elimination Period
	1st day	6th day	3rd day
Internal organ			
α-HCH	0.026	0.060	0.033
β-HCH	0.068	0.194	0.115
γ-HCH	0.021	0.049	0.020
δ-HCH	0.064	0.187	0.101
Tissue[a)]			
α-HCH	0.017	0.029	0.024
β-HCH	0.065	0.076	0.075
γ-HCH	0.014	0.026	0.017
δ-HCH	0.048	0.131	0.124

a) Without internal organ

lized and/or eliminated rapidly from IO. Also, elimination of HCH from IO was shown as follows: γ-HCH>δ-HCH >α-HCH>β-HCH. On the contrary, the concentrations of γ-HCH in the tissue after the elimination period was reduced to 65.4% of that on the 6th day of bioaccumulation period. The other HCH isomers did not decrease as much. Also, the ER were in order of γ-HCH>α-HCH> δ-HCH>β-HCH. This order was the same as that of the whole body.

In this experiment, bioaccumulation and elimination of δ-HCH with short-necked clam highly differed from those with guppy. In particular, δ-HCH was most concentrated among HCH isomers, because this compound is highly hydrophobic (ARMSTRONG et al., 1951), and its elimination was very slow. However, it was of considerable interest that concentration of δ-HCH decreased quickly in the internal organs of the short-necked clam after replacement to HCH-free sea-water. The phenomenon indicated that δ-HCH could be metabolized and/or easily eliminated by the short-necked clam. There was little transfer of the isomer from tissues to the internal organs. We suggest that pesticides could reside in the short-necked clam for a long time, if little transfer the internal organs occurred.

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