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Bioaccumulation of ^{14}C-hexachlorobenzene in Eggs and Fry of Japanese Medaka (*Oryzias latipes*)

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Hexachlorobenzene (HCB) is a widespread pollutant that is persistent once it enters the ecosphere. Its effects on humans and animals has been recognized, and its environmental characteristics were reviewed by Courtney (1979). It bioaccumulates in both terrestrial and aquatic animals and is not readily metabolized. Although HCB bioaccumulation in fresh water fish has been reported (Metcalf et al. 1973; Isensee et al. 1976; Giam et al. 1980; Konemann and van Leeuwen 1980), few data are available on bioaccumulation of this or other chemicals during early developmental stages of fish. In one exception, Murphy (1971) reported on the effect of body size on uptake of DDT by mosquitofish (Gambusia affinis) weighing from 70-945 mg.

We used the Japanese medaka (Oryzias latipes) to examine the rates of HCB bioaccumulation during early life stages subjected to both short term (24 h) and long term (14 day) aqueous exposure. The relatively rapid development and ease of laboratory maintenance (Yamamoto 1975) made the medaka an ideal organism for this purpose.

MATERIALS AND METHODS

Twenty adult medaka obtained from the Carolina Biological Supply Company and reared in two 20-liter aquaria served as brood stock for the experimental fish. Water temperature was 26-28°C and illumination was by fluorescent lamps (16 h L, 8 h D). The brood stock was fed with commercial tropical fish food (Tetra Min)[4] and young Daphnia magna.

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[4]Mention of trade names or commercial sources does not constitute U.S. Government endorsement.

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Fertilized eggs were taken from the surface of the abdomen of females each morning, placed in a glass basket (4 x 6 cm, with a bottom of nylon net), treated with Betadine for 10 minutes, rinsed, and then transferred in the glass basket into an incubator aquarium for hatching. At the incubation water temperature of 22°C, embryonic development took 13 days so that 13 daily samples of eggs were obtained before hatching.

Newly hatched larvae were transferred to culture tanks and fed Tetra Min and brine shrimp. Uniformly ring labeled ^{14}C-hexachlorobenzene (specific activity, 16.88 mCi/Mm) was from Pathfinder Laboratories Inc., Saint Louis, MO[4]. It was dissolved in 20% N',N',-dimethyl formamide in distilled H_2O (1:4) for application solution and 1 ml of this solution was added to the flow-through diluter system by automatic pipette. The concentration of solvent in the exposure medium was less than 0.01%. The concentration of ^{14}C-HCB in water, monitored daily during the experiments, ranged from 60 to 80 ng.l^{-1}.

All exposures of medaka eggs and fry to ^{14}C-HCB were conducted in glass flow-through aquaria (70 liters) with a flushing rate of 3-4 liters per hour. Dechlorinated (activated carbon) tap water with pH 7.8-8.0, alkalinity 105.8 ± 0.97 (SE) mg.l^{-1}, and hardness 154.8 ± 1.12 mg.l^{-1} was used for culture and exposures.

Thirteen groups of eggs with embryos (ages 1-13 days) were transferred from the incubator into the exposure tank simultaneously. Each group contained 200 eggs. The egg containers were randomly placed on a nylon net hung 3 cm beneath the water surface in the aquarium. After 24 hours of exposure all eggs were removed from the tank and residues of ^{14}C-HCB in whole eggs were analyzed immediately.

In the longer term egg uptake experiment, newly spawned eggs were put in the exposure tank each morning, and all eggs were taken out on the 13th day, just before the first group of eggs would have hatched.

To examine uptake by medaka of different ages, we transferred fish ranging from newly hatched larvae to juveniles into one 70-liter exposure tank. Newly hatched larvae were held in glass baskets and older larvae in a nylon net cage (30 x 13 x 10 cm), to avoid predation; the fish were not fed. After 24 h all fish were removed, rinsed with clean water, and frozen for analysis of ^{14}C-HCB.

In the 14-day uptake experiment, 80 larvae (weight about 25 mg each) were exposed in one aquarium and fed with Tetra Min and brine shrimp daily. Samples were taken after 1, 3, 7, 14, 24, 36 and 52 hours and 3, 4, 5, 7, 10 and 14 days; rinsed and frozen for analysis.

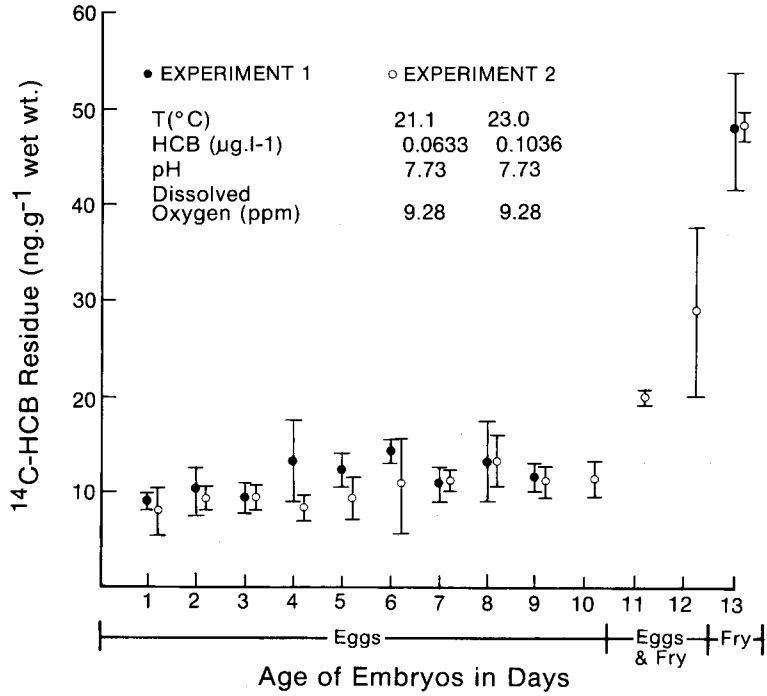

Fig. 1. 24 hour uptake of ^{14}C-HCB in different developmental stages of medaka embryos, (O. latipes). Each print represents the mean ±2SE of 3 replicates in two parallel experiments.

The ^{14}C activity of all samples was determined with a Beckman LS-355 Liquid Scintillation System Counter. Samples were corrected for background, quench, and counting efficiency. The counting efficiency for water was 92%. Water samples were mixed directly into scintillation cocktail (3a70B Research Products International) and counted overnight for ^{14}C-HCB activity.

Three replicate samples of 50 eggs with embryos and 1 to 50 fish were blotted dry on filter paper and weighed on a Cahn model 29 Electrobalance. Total $^{14}CO_2$ from samples was collected in 15 ml ^{14}C cocktail, after combustion in a Harvey Instruments model OX300 Biological Material Oxidizer. The efficiency of the oxidizer was 80%.

RESULTS AND DISCUSSION

The 24 hour accumulation of ^{14}C-HCB indicated that the uptake of HCB in medaka eggs of different ages was low, and increased only slightly during development. The amount of uptake increased after hatching and approached five times more than that of the eggs (Fig. 1). The lower uptake of ^{14}C-HCB by eggs might have been caused by protection of the egg membrane or lower metabolism of the embryos.

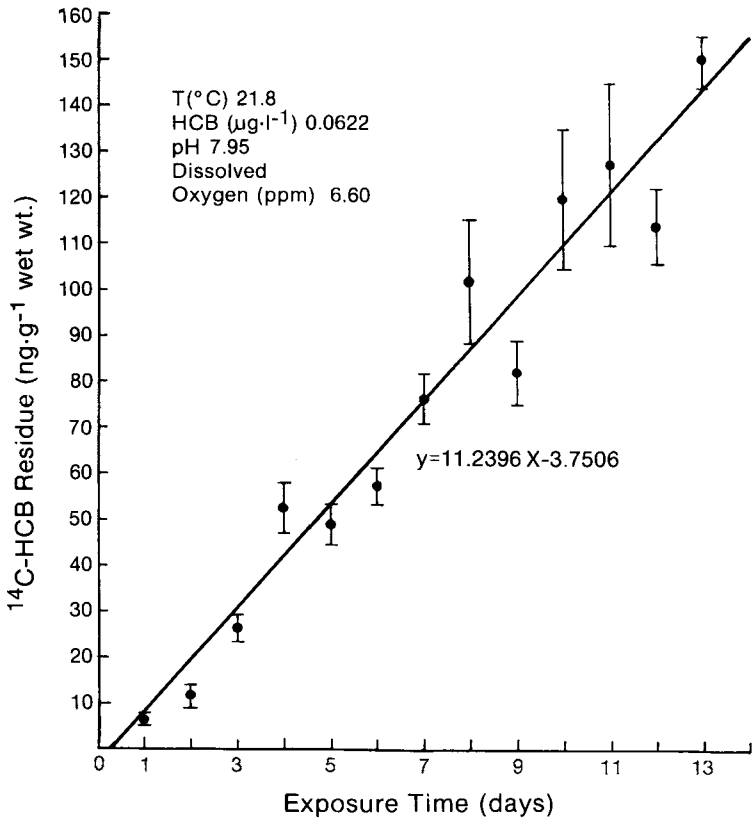

Fig 2. Residue of ^{14}C-HCB in medaka,(O. latipes) embryos. Each point represents the mean ±2SE of 3 replicates.

Akiyama (1970) reported that the resistance of medaka embryos to toxicants increased as development proceeded up to 6 days, and then gradually decreased to a minimum value on day 11, just before hatching. However, we found no similar age difference in uptake in our 24-hour experiment with ^{14}C-HCB.

The long-term accumulation of ^{14}C-HCB in eggs increased linearly with exposure time (Fig. 2). The accumulation did not reach steady state before hatching (13 days). The maximum bioconcentration factor of ^{14}C-HCB in eggs of medaka was 2186.

The 24-h accumulation of ^{14}C-HCB in fry indicated that uptake rates differed among different developmental stages and sizes of medaka (Fig. 3). The newly hatched larvae (body length of 4 mm and body weight about 0.8 mg; protolarval phase of Snyder 1981), had the lowest accumulation level among the different stages of larvae even though it was 5 to 10 times higher than that of eggs.

Uptake then increased rapidly with transition of the fish to the late mesolarval phase (body length about 7 mm, weight about 4 mg).

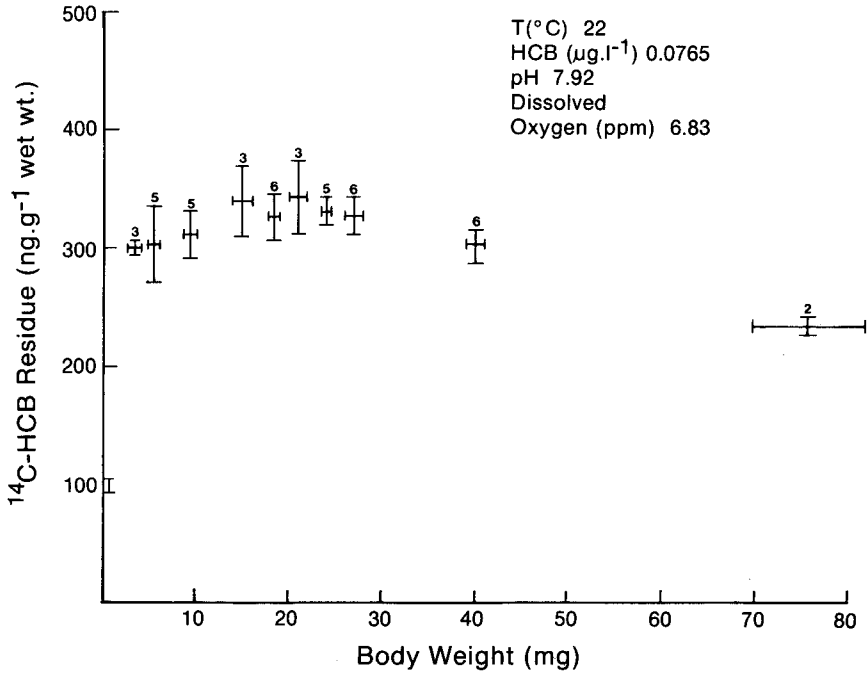

Fig. 3. 24 hour uptake of 14 C-HCB by different size medaka, (O. latipes). Each point represents the mean ± 2SE. Numbers indicate the replicates for each point.

Distinct principal rays had appeared in the median fins of these larvae and the yolk sac was completely absorbed.

The accumulation was highest in larvae 10-12 mm long and 15-30 mg in weight, which corresponded to the late metalarval and early juvenile phases. Accumulation then decreased as body size increased. The results were similar to those reported for DDT uptake by mosquitofish by Murphy (1971).

We hypothesize that the different uptake rate of ^{14}C-HCB in different stages of medaka development was associated with the intensity of metabolism of the fish. Huang (1975) reported that the rate of oxygen consumption was low in newly hatched larvae of some freshwater fishes; peaked in the second stage, during which the larva was supplied with nourishment by both the yolk sac and food captured from the environment; and then declined in subsequent stages. Murphy and Murphy (1971) also found that the ^{14}C-DDT uptake rate was related to metabolism in mosquitofish of various sizes from 70 to 950 mg. Our results of ^{14}C-HCB uptake were similar to those based on metabolism.

Long term uptake of ^{14}C-HCB by medaka fry was examined over 14 days for fish with an average initial weight of 25.3 ± 3.2 (SE) mg. Uptake of ^{14}C-HCB was initially rapid and then decreased as exposure time increased (Fig. 4). It can be represented by the

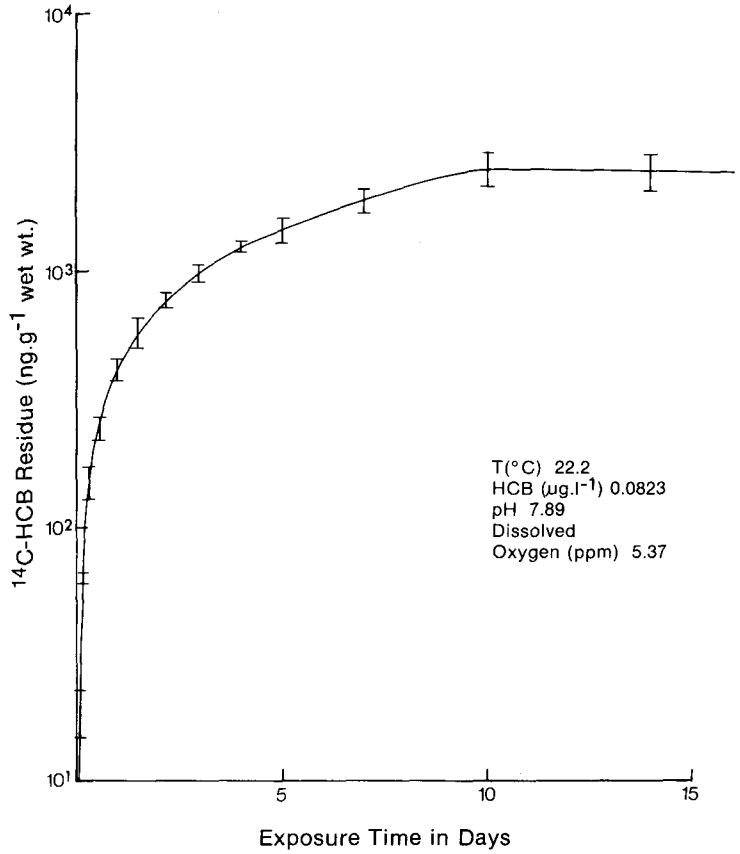

Fig. 4. Residue of ^{14}C-HCB in juvenile medaka (O. latipes) with initial mean weight of 25.3 mg. Each point represents the mean ±2SE of 2 to 4 replicates.

equation:

$$\log (Y + 12) = 2.6116 + 0.8050 \log X$$

where Y = residue of ^{14}C-HCB (ng.g^{-1} wet wt) and X = exposure time (days).

Uptake equilibrium of ^{14}C-HCB was reached after 10 days exposure and the bioaccumulation factor was 3.13×10^4. This value is higher than those reported for several freshwater fishes. Konemann and van Leeuwen (1980) reported that the accumulation equilibrium of HCB in guppy (Lebistes reticulatus) was reached after exposure for 6 days, based on lipid weight in the fish.

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), who exposed channel catfish (Ictalurus punctatus) in 0.1, 1.0, and 10.0 µg HCB.l^{-1}, reported that bioaccumulation factors varied between 0.6 and 1.6×10^4. Giam et al. (1980) reported that the maximum bioaccumulation factor for HCB was only 375 in the killifish, Fundulus similis.

Medaka eggs accumulated ^{14}C-HCB in similar and small amounts during 24-hour exposures, regardless of age, from fertilization through hatching (13 days). The accumulation of HCB in eggs was a linear function of exposure time, increasing from fertilization through age 13 days (hatching). Accumulation in eggs was one to two orders of magnitude lower than in fry and juveniles in short (24 h) versus long term (14 day) exposures. The 24-h accumulation of HCB by post yolk sac fry and juveniles ranged from 240 to 340 $ng.g^{-1}$ wet wt, decreasing in larger juveniles. Long-term exposure (14 days) of juveniles resulted in equilibrium concentrations of about 2500 $ng.g^{-1}$ wet weight after 10 days. Maximum bioconcentration factor was 3.13×10^4.

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