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Uptake and Concentration Factor of Aroclor 1254 in Aquatic Organisms

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Since the early 1900's, the manufacture and use of synthetic chemicals has become widespread. The chemical revolution began in America after World War II and by the 1970's as many as 70,000 man-made chemicals were on the market for use (HERITAGE 1978). Polychlorinated biphenyl (PCB) compounds are only one series in the long line of synthetic chemicals which have added greatly to our comfort as well as increased industrial and agricultural productivity. These halogenated aromatic hydrocarbons exhibit unique properties such as chemical inertness, resistance to acid and alkali, thermal stability, high dielectric constant, low water solubility, and high solubility in organic solvents (HUTZINGER et al. 1974; SCHNEIDER 1979). PCBs escape into the environment due to their diversity of use and persist in ecosystems throughout the world because of their stability and hydrophobic nature (RICHARDSON and WAID 1982; JENSEN 1972). Since 1976, sport and commercial fishing has been banned on much of the Hudson River due to contamination by PCBs (HERITAGE 1978). In 1981, dredging operations were initiated by New York State to remove 340,000 lb of PCB-contaminated material from hot spots on that river (PERHAM 1981).

Aquatic organisms especially, can accumulate total body concentrations of PCBs hundreds to thousands of times greater than the surrounding water. GIAM et al. (1980) reported an average bioconcentration factor of 375 in killifish exposed to hexachlorobiphenyl for 11 days. Similar exposure experiments with fish showed bioaccumulations of PCBs ranging from 37,000 to 61,190 times the amount in water (HANSEN et al. 1971; MAYER et al. 1977). SANDERS and CHANDLER (1972) ascertained that fresh water crustaceans and insects accumulated Aroclor 1254 up to 48,000 times the water concentration. BRANSON et al. (1975) reported that the bioconcentration factor of a tetrachlorobiphenyl isomer in rainbow trout muscle after 5 days exposure was 9550 ± 1610.

There are several factors that affect PCB bioaccumulation. These include: concentration in the surrounding environment; duration of exposure; temperature; solubility of the pollutant; species age, weight, feeding habits and lipid content; trophic level variations; and adsorption (RICHARDSON and WAID 1979; KALMAZ and KALMAZ 1979; NATIONAL RESEARCH COUNCIL 1979). Bioaccumulation can occur either through ingestion of contaminated food organisms or by direct absorption through the integument (KALMAZ and KALMAZ 1979; NATIONAL RESEARCH COUNCIL 1979; RICHARDSON and WAID 1979). NISBET and SAROFIM (1972) found higher concentrations of PCBs in plankton than in fish,

indicating that direct absorption is the primary route of accumulation. NARBONNE (1979a) measured the concentration coefficient of PCBs from water and from food in mullets and also reported higher accumulation from water than from food. SODERGREN and SVENSSON (1973) determined that accumulation of PCBs in mayfly nymphs followed a kinetic equation of first order with equilibrium being reached after 4-5 days. However, STALLING and MAYER (1972) showed that the uptake of Aroclor 1254 by crayfish was linear, and PCB residues did not reach equilibrium after a 28 day exposure.

COOLEY et al. (1972) reported reduced growth rates and population densities in Tetrahymena pyriformis exposed to Aroclor 1254. These authors also stated that, where exposure of these organisms to PCBs occurred, their availability as food sources would be reduced and PCBs would enter into and be translocated through aquatic food chains. The presence of PCBs can reduce phytoplankton biomass or species diversity, thus increasing the number of trophic levels or cause the decline of a species at a higher trophic level (O'CONNORS et al. 1978; RICHARDSON and WAID 1979). Therefore, this investigation was conducted to follow the uptake and concentration factor (CF) of Aroclor 1254 in selected organisms representing a simple aquatic ecosystem.

MATERIALS AND METHODS

Trophic Systems. Four organisms were used: cells of PCB-resistant Bacillus spp. (designated by numbers) previously isolated in our laboratory (NUNN 1979), mosquito larvae (Aedes aegypti), guppies (Lebistes reticulatus), and Cichlids (Cichlasoma facetum); each representing the next higher food chain system, respectively.

Growth, Uptake, and CF of ^{14}C-PCB. Uniformly labeled carbon 14-PCB 1254 (50% chlorine by weight), purchased from Amersham Corp., was utilized for all labeling experiments.

A. Bacteria. Two PCB-resistant Bacillus spp. cultures (Nos. 2 and 4) were used. Flasks of glucose basal salt broth (GBSB; HAMDY and NOYES 1975) containing 200 µg of nonradioactive Aroclor 1254 PCB/ml media, 0.1% Tween 80 (a surface active agent for PCB emulsification), and 1 µCi ^{14}C-PCB were inoculated with 2% of an 18 h active culture and incubated at 37^{0}C for 72 h. Microbial growth was monitored spectrophotometrically (510 nm) and at specific intervals, aliquots of culture medium were removed aseptically and centrifuged to obtain cells plus cell-free supernatant fractions. Aliquots of each fraction were counted for the ^{14}C-PCB activities using liquid scintillation. The direct uptake of ^{14}C-PCB (%) by the bacteria was then calculated based on the amount of radioactivity (cpm) recovered and data are an average of 3 experiments. The CF of PCB by bacterial cells was computed using the following equation:

$$CF = \frac{\text{specific activity of bacterial cells (cpm/g)}}{\text{specific activity of medium (cpm/g)}} \quad (I)$$

B. Mosquito Larvae. Aseptic larvae were obtained after hatching the eggs in a sterile environment. The eggs, prior to hatching, were disinfected (PRABHU and HAMDY 1977) and approximately 200 eggs were then transferred to a sterile beaker containing sterile distilled water, 0.1% Tween 80, and sterile diet (commercial Purina guinea pig chow). The eggs were allowed to hatch at 27°C and the direct uptake of ^{14}C-PCB from water was measured by adding 0.2 µCi of ^{14}C-PCB to 400 ml sterile, distilled water containing either 3 or 5 day old larvae, 0.1% Tween 80 and incubated for 96 h at 27° C. At intervals, samples of water and approximately 20 larvae were harvested, weighed, washed once with sterile distilled water and digested in concentrated nitric acid. Representative samples of water and the nitric acid digest were counted. Data for the CF by the larvae were calculated using the same equation (I) used for the bacterial cells. The direct uptake (%) of ^{14}C-PCB by the larvae, calculated as a function of both incubation time and age of larvae, was based on the % radioactivities (cpm) recovered. Results are reported as the average of 3 experiments.

C. Guppies. The guppies, Lebistes reticulatus, and other fish purchased from a local pet shop were reared in aquarium tanks (27°C). The lethal concentration of PCB in the guppies was determined by exposure to increasing levels (5, 10, 15 and 20 µg) of PCB Aroclor 1254/ml water. The direct uptake of ^{14}C-PCB was measured by adding 0.4 µCi ^{14}C-PCB to 800 ml sterile distilled water containing random-size guppies and 0.1% Tween 80, with incubation proceeding for 96 h at 25°C. At intervals, fish were harvested, rinsed once with distilled water, blotted dry, weighed, and digested with concentrated nitric acid. Samples were then counted and % uptake of ^{14}C-PCB calculated. Aliquots of the water at each interval were also removed and counted. The CF of the PCBs by the guppies were determined using equation (I) and data reported as the average of 3 experiments.

D. Cichlids. The Cichlasoma facetum were purchased from a local pet shop. Three µCi ^{14}C-PCB were added directly to 1 L of sterile distilled water containing the fish and 0.1% Tween 80 and incubation continued for 72 h at 25°C. Fish were sacrificed at intervals and dissected to obtain the following organs: fins, intestine, liver, spleen, head, muscle and remainder of the carcass. These organs were blotted dry, separately weighed and each digested in concentration nitric acid. Aliquots of water samples and the nitric acid digest of each organ were counted for their ^{14}C-radioactivities; the CF of PCB by each organ was determined using equation (I). The uptake and CF by each organ, at the specified time intervals, were calculated based on the amount of ^{14}C-radioactivities recovered and the average of 2 experiments is reported.

Counting System. A known weight of each sample or aliquots of the nitric acid digest was placed in a standard glass scintillation vial containing 10 ml of toluene-based scintillation fluor and counted in a Beckman LS 7500 liquid scintillation system. The instrument was programmed to count all samples to ± 2% error or 10 min, whichever occurred first and all samples were corrected for background.

RESULTS AND DISCUSSION

The data for growth, uptake and CF of ^{14}C-PCB by PCB-resistant Bacillus culture Nos. 2 and 4, following incubation in GBSB containing 200 µg non-radioactive Aroclor 1254 are presented in Fig. 1. Very little effect was noted on the growth of these organisms. Maximal uptake of 22% for culture No. 2 and 24% for culture No. 4 was reached after 72 h incubation with a CF of 28 and 21 for these bacteria, respectively. Note the increase in uptake of ^{14}C-PCB as a function of time during growth with an accompanying decrease of ^{14}C-PCB in the cell-free media.

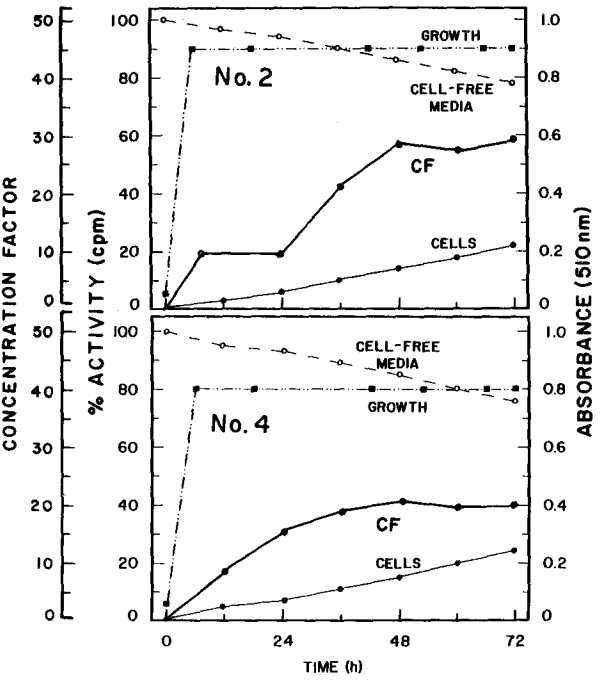

FIG. 1. Uptake and CF of ^{14}C-PCB by Bacillus spp. Nos. 2 and 4 during growth. Initial activity of cell-free media = 100%.

RICHARDSON AND WAID (1979) noted that many aquatic species of bacteria are sensitive to PCBs in low concentrations, but most appeared to be fairly resistant, and their growth rates were not greatly affected. WONG and KAISER (1975) found that concentrations up to 0.1% of Aroclor 1221, 1242, and 1254 did not inhibit the growth of lake water bacteria. SYLVESTRE (1980) isolated several types of bacteria from soil and sludge samples and grew them on para-chlorobiphenyl (pCB) or biphenyl (BP) as the sole carbon source and analyzed for an increase in the number of colony-forming units (CFU). The isolates showed an increase of about 2 log counts with an accompanying decrease in extractable pCB or BP from the medium.

When 3 and 5 day old mosquito larvae, kept at 27°C, were exposed to ^{14}C-PCB in water, the % uptake by 3 day old larvae gradually increased during incubation to 5.1% after 96 h. However, 5 day old larvae showed a maximum uptake of 6.2% at 48 h followed by a decline thereafter to 2.6% at 96 h (Fig. 2). Similarly, the CF by 3 day old larvae increased gradually to 553 after 96 h, whereas that of 5 day old larvae reached its maximum at 48 h of 638, followed by a decline at 96 h (Fig. 2). These results indicate that both time of incubation and age of the larvae affected the uptake and CF by the larvae. The decline in rates of uptake and CF by the 5 day old larvae may be due to accelerated depuration (GOOCH and HAMDY 1982).

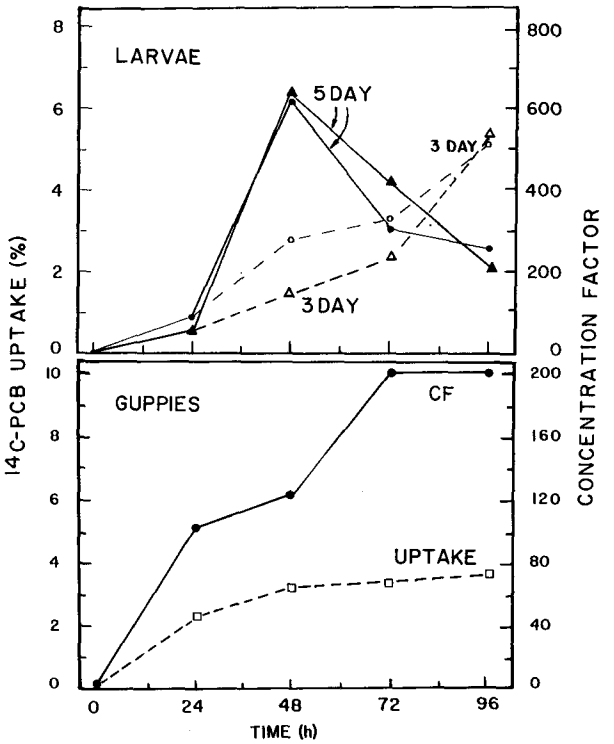

FIG. 2. Uptake and CF of ^{14}C-PCB by 3 and 5 day old mosquito larvae and guppies.

CLAYTON et al. (1977) determined amplification factors of 10^6 for PCBs in zooplankton. LOWE et al. (1972) exposed young oysters to 5 mg Aroclor 1254/L water for 24 weeks and ascertained that the greatest PCB residue (whole body) was 425 mg PCB/kg or 85,000 times the concentration in the water. NIMMO et al. (1974) studied the toxicity of Aroclor 1254 to grass shrimp and reported that after several one-week exposures to various concentrations (0.17 to 9.1 µg PCB/L water), the concentration factors ranged from 3,000 to 11,000. SANDERS and

CHANDLER (1972) found that mosquito larvae exhibited a 12,600 fold magnification of ^{36}Cl-Aroclor 1254 from water within 24 h. This accumulation continued until the 7th day when the larvae metamorphosed into pupae. On the 7th day, the magnification factor was 20,000 in the mosquito larvae.

Guppies exposed to Aroclor 1254 died after 7 days incubation in the presence of 20 µg PCB/ml water. Guppies incubated in beakers containing either 5, 10, or 15 µg Aroclor 1254/ml water lived longer but all of these died within 3 weeks. Direct uptake of ^{14}C-PCB by guppies during incubation at 27°C increased gradually to reach 3.6% after 96 h with a CF of 198 (Fig. 2) at that time.

Fig. 3 depicts data for the direct uptake and CF of ^{14}C-PCB by Cichlid organs. The head and fins acccumulated the greatest % of ^{14}C-PCB (62.5% and 17.0%, respectively) after 24 h exposure to ^{14}C-PCB in water. After 72 h exposure, uptake by the head decreased to 28% probably due to washing and/or depuration, while the uptake by fins remained fairly constant at 15.5%. On the other hand, the liver showed an increasing uptake reaching 18.6% after 72 h. The CF by various Cichlid organs tended to also increase over time. The spleen, fins, liver and muscle exhibited the highest CF values after 72 h exposure to ^{14}C-PCB in water with values ranging from 1862 in spleen, 268 in fins, 173 in liver, and 164 in muscle tissue. MORGAN (1972) established that 2 mg Aroclor 1242/L caused 100% mortality of guppies, whereas 0.2 mg/L resulted in only 25% mortality. RICHARDSON and WAID (1979) stated that the lower the solubility of a pollutant in water, the higher the CF, and this means that direct uptake of PCBs from water is a major route of entry into fish. BACHE et al. (1972) found that the concentration of PCBs progressively increased with maturity in lake trout. NARBONNE (1979b) studied the effect of age on the accumulation of Phenoclor DP6 in mullets and found that DP6 concentrations in 3.5 year old fish was always higher than those in 2.5 year old fish when both were fed a dry fish diet contaminated with 50 µg DP6/g food. Studies conducted by ZITKO (1971) and CALIFANO et al. (1980) indicated that fish accumulate PCBs on a weight-ratio basis. YOSHIDA et al. (1973) followed the distribution of ^{14}C-PCBs in carp (Cyprinus carpio) using ^{14}C-PCB with approximately 40% chlorine as a tracer. These authors found that the PCBs were localized mainly in the gall bladder, adipose tissue of the skull and hepato-pancreas, but only slight radioactivity was detected in muscle, with the exception of dark muscle. Experiments conducted by RICHARDSON and WAID (1980) concluded that in mullets, most of the PCBs (Aroclor 1248 and 1254) were retained in the head, bone, and gut of the fish with a large portion also being found in the liver. Our findings concur with these authors.

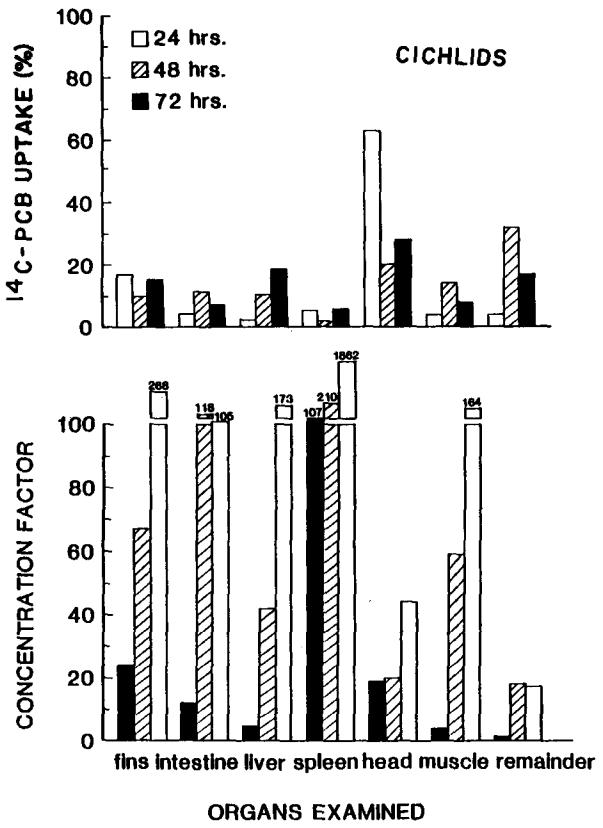

FIG. 3. Uptake, organ distribution, and CF of ^{14}C-PCB in Cichlids.

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