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Depuration and Biological Half-life of ^{14}C-PCB in Aquatic Organisms

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Polychlorinated biphenyls (PCBs) are a class of man-made halogenated aromatic hydrocarbons that are distributed throughout the environment (SCHNEIDER 1979). Because PCBs are hydrophobic and resistant to environmental degradation, aquatic plants and animals tend to accumulate them from the surrounding water environment (KALMAZ and KALMAZ 1979). Results of several investigators indicated that aquatic organisms accumulate total body concentrations of PCBs thousands of times greater than that of the surrounding water. SANDERS and CHANDLER (1972) showed that fresh water crustaceans and insects accumulated PCB (Aroclor 1254) up to 48,000 times greater than the concentration in the water. Similar exposure experiments conducted with estuarine animals showed oysters concentrating 101,000 (LOWE et al. 1972), and fish 37,000 (HANSEN et al. 1971) times the amount of PCB in the water.

The biomagnification of PCBs by organisms is affected by several factors, such as ambient concentration, species, trophic level variations, adsorption, and lipid content (KALMAZ and KALMAZ 1979). SKEA et al. (1979) studied bioaccumulation of Aroclor 1016 in Hudson River fish and found that the total quantity of PCBs accumulated by the different species of fish was closely related to the fat content and weight of the fish. Fish tissues analyzed from the River Avon showed that those fish species containing a higher percent lipid tended to accumulate more PCB (KPEKATA 1975). DEFOE et al. (1978) reported that bioconcentration in minnows was independent of the PCB concentration in the water. However, CAMP et al. (1974) found that there was a direct relationship between biological magnification and concentration of Aroclor 1254 in aquaria water for catfish.

Contaminated aquatic organisms, if transported to clean water after the exposure period, will depurate or eliminate a xenobiotic chemical from their bodies over a time span that is dependent upon differences in the species' metabolism and excretion (HAMDY and PRABHU 1978). HATTULA and KARLOG (1973) stated that the cytochrome P-450 enzyme complex, absent in fish but present in mammals and birds, dramatically affects the metabolism of PCB.

NIMMO et al. (1974) examined the accumulation and depuration of Aroclor 1254 in grass shrimp and reported that 60 to 90% of the accumulated PCB was lost from the shrimp within 4 weeks after exposure was stopped. HANSEN et al. (1971) found that after 84

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days of flushing in PCB-free water, the relative amount of Aroclor 1254 in spot fish had decreased 73%.

Therefore, this study was conducted to follow the absorption, elimination, and biological half-life (BHL) of ^{14}C-PCB (Aroclor 1254) in selected organisms representing a simple aquatic ecosystem. Factors affecting the loss of ^{14}C-PCB from labeled bacterial cells following washing were also examined.

MATERIALS AND METHODS

Trophic Systems. The following 4 organisms were used: cells of a PCB-resistant Bacillus sp. previously isolated in our laboratory representing the lowest level (NUNN 1979), mosquito larvae (Aedes aegypti), guppies (Lebistes reticulatus), and Cichlids (Cichlasoma facetum); each representing the next higher food chain system, respectively. Cichlids are a species of medium-sized tropical fish whose common name is Jack Dempsey.

Labeling of Test Organisms. Uniformly labeled carbon 14-PCB (approximately 50% chlorine by weight and with a gas chromatographic pattern closely resembling that of Aroclor 1254), purchased from Amersham Corp., was used for all isotopic labeling. An 18 h actively growing Bacillus spp. culture was inoculated into flasks containing 250 ml glucose basal salt broth (HAMDY and NOYES 1975) plus 0.1% Tween 80 (a surface active agent for PCB emulsification), 200 ppm non-radioactive Aroclor 1254, and 2 µCi ^{14}C-PCB. The culture was allowed to grow for 48 h at 37°C, and then the labeled cells were harvested by centrifugation (17,000 x g) and washed once with sterile saline.

Approximately 200 eggs of A. aegypti were sterilized by immersion in 75% ethanol for 5 min (LEA et al. 1956). The eggs were allowed to hatch in a beaker containing 400 ml distilled water (27°C), 0.1% Tween 80, and sterile diet (Purina guinea pig chow) to yield sterile larvae. When the larvae were 1 d old, 0.8 µCi of ^{14}C-PCB was added to the beaker; the larvae were then allowed to grow for 48 h. The labeled larvae were collected and washed once with distilled water.

Guppies (L. reticulatus) grown in aquarium tanks (25°C) were labeled by placing 12 fish in beakers containing 1 L distilled water, 0.1% Tween 80, 1.6 µCi ^{14}C-PCB, and incubated for 48 h. Cichlids were labeled by the same procedure used for guppies.

Counting System. A known weight of water, saline, or sample (bacterial cells or nitric acid digest of the larvae, guppies, or Cichlids) was each placed in a standard scintillation vial containing 10 ml of toluene-based scintillation fluor and counted in a Beckman LS 7500 liquid scintillation system. The instrument was set to count all samples to ± 2% error or 10 min, whichever occurred first; all samples were corrected for background.

Biological Depuration. Each labeled system was transferred to isotope-free saline (bacteria) or distilled water (larvae, guppies, and Cichlids) and incubated. Depuration rate of ^{14}C-PCB by bacterial cells was studied at 37°C. Temperatures of 27 and 25°C were used for larvae and fish, respectively. During incubation, samples of labeled bacterial cells, aliquots of the

nitric acid digest of larvae or fish, saline or distilled water were collected. The weighed larvae and/or fish were digested by heating slowly in 5 ml of concentrated nitric acid. The digest was then diluted to 25 ml with distilled water to reduce quenching when counted.

Data, reported as log of initial activities (1.0), were plotted as a function of incubation (d); and the rate of depuration (k, slope of line) was calculated for each system. The 50% biological retention time (biological half-life, BHL) of ^{14}C-PCB was also determined and reported as the time, in d, required for half of the radioactivities of ^{14}C-PCB (cpm/g) to be eliminated from each trophic system.

Confirmation of the Presence of PCB during Bacterial Depuration. This was conducted by extracting 9 ml saline from the depuration experiments with 5 ml pesticide-grade hexane. The top layer containing the PCB and hexane mixture was removed and subjected to chromatographic analyses using a Tracor Model 560 Gas Chromatograph equipped with a Pyrex glass column 6' x ¼" packed with 3% OV-1 on 80/100 Chromosorb W Hp. The carrier gas was 95% argon and 5% methane at a flow rate of 60 ml/min. The operating temperatures for the injector, column, and electron capture detector (^{63}Ni) were 150, 200, and 350°C, respectively. Chromatograms of the hexane extract were compared with those of a control system containing Aroclor 1254 PCB, but no bacteria.

Loss of ^{14}C-PCB from Bacterial Cells by Washing. Fresh and frozen *Bacillus* sp. cells, labeled with ^{14}C-PCB (same as used in depuration experiments) were used. The frozen cells were allowed to thaw and both types of labeled cells were then washed 5 times with pesticide-grade hexane. In another experiment, the labeled cells were washed 5 times with sterile 0.05 M sodium phosphate buffer (pH 7.0), immediately followed by 5 washes with pesticide-grade hexane. After each wash the cells were centrifuged (17,000 x g) and aliquots of supernatant counted to determine the % loss of ^{14}C-activities by cells as a function of wash numbers and reported as % of initial counts.

RESULTS AND DISCUSSION

Data on the depuration of ^{14}C-PCB by all 4 systems during incubation is presented in Fig. 1. Three phases of elimination were noted for the *Bacillus* culture. Rapid elimination was seen through the 7th d ($k = -0.0305$ d^{-1} for 0-3 d, and -0.0663 d^{-1} between 3-7 d) followed by a very slow rate ($k = -0.0009$ d^{-1}) to the 17th d. A BHL of 6.4 d was evident from the graph based on the 1st phase only. In larvae, 3 depuration phases were also present. A slope (k) of -0.0229 d^{-1} occurred after the 1st d, -0.0859 d^{-1} between 1-2 d, and -0.0899 d^{-1} between 2-5 d with a BHL of 4.4 d. Guppies showed 2 distinct depuration phases; the 1st exhibited a rate of -0.1308 d^{-1} and -0.1373 d^{-1} for the 2nd with a BHL of 3.3 d. The Cichlids also showed 2 phases of elimination; a rapid rate ($k = -0.1024$ d^{-1}) occurring during the 1st d followed by a slower one thereafter ($k = -0.0574$ d^{-1}) with a BHL of 5.1 d.

Table 1 summarizes all data including BHL for each system

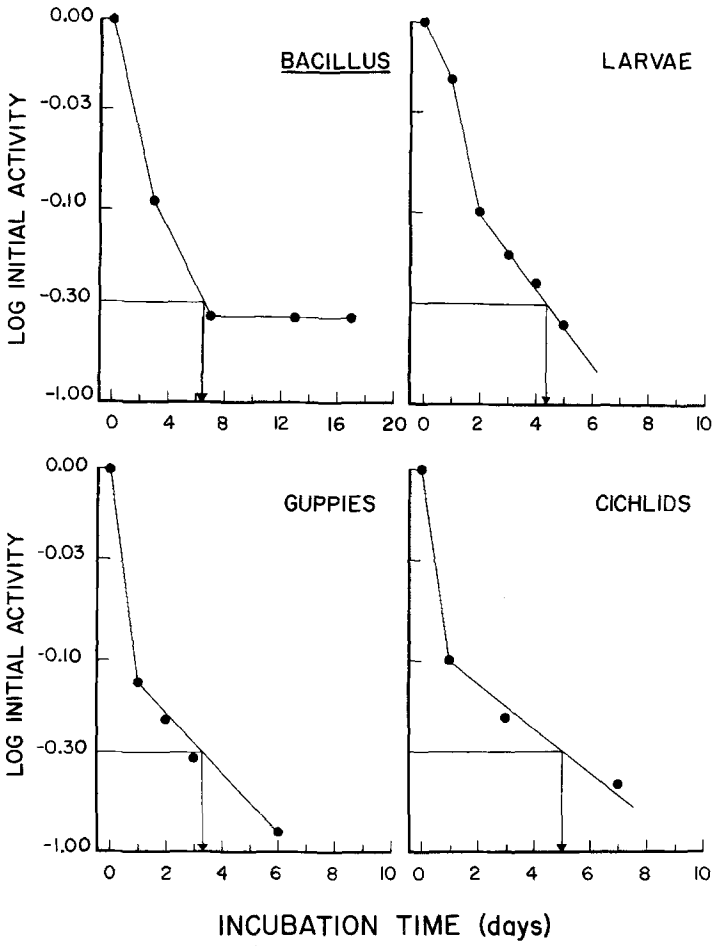

Fig. 1. Depuration and biological half-life of ^{14}C-PCB in four trophic systems. Initial activity = 1.0.

calculated from log values using regression analyses. In bacteria, using all data between 0-17 d, the BHL was 10.46 d. Whereas, using values for d 7-17 (the 2nd depuration phase), the BHL was 55.94 d. Note that all subsequent half-lives for larvae and fish based on log values using regression analyses very nearly approximate those calculated from the graphs of log values.

Several earlier studies conducted on depuration of PCBs in fish indicated that the BHL ranged from 1 to 3 months and that certain factors affected the BHL. Among these were: species differences; differences in metabolic behavior or PCB components of different chlorine content; differential solubility of PCB components, and lastly, that clearance is a combination of movement back out through the gills plus excretion in the urine and

TABLE 1. Depuration rates (k, slope of line) in various trophic levels labeled with ^{14}C-PCB.

Trophic System	Incubation Time (Days)	k value[a] $Days^{-1}$	Correlation Coefficient	Biological Half-Life (Days)	
				Graph[b]	Calculated[c]
Bacteria	0-3	-0.0305	-1.0		10.46 (0-17d)
(PCB-resistant	3-7	-0.0663	-1.0	6.4 d	
Bacillus spp.)	7-17	-0.0009	-0.8030		55.94
Mosquito Larvae	0-1	-0.0229	-1.0		4.47 (0-5d)
(*Aedes aegypti*)	1-2	-0.0859	-1.0	4.4 d	
	2-5	-0.0899	-0.9707		4.35
Guppies	0-1	-0.1308	-1.0		2.47 (0-6d)
(*Lebistes reticulatus*)	1-6	-0.1373	-0.9927	3.3 d	2.54
Cichlids	0-1	-0.1024	-1.0		4.62 (0-7d)
(*Cichlasoma facetum*)	1-7	-0.0574	-0.9988	5.1 d	4.57

[a]Slopes were calculated from graphs of initial activities *vs.* incubation time.
[b]From graph of log values. [c]Regression analyses.

feces (HUTZINGER et al. 1972; HANSEN et al. 1974; and BRANSON et al. 1975). CALAMBOKIDIS et al. (1979) found that the concentration of PCB in mussels decayed logarithmically with calculated half-lives ranging from 3 d for a PCB component with 2 and 3 chlorines to 50 d for a PCB component with 6 and 7 chlorines. These researchers established that Aroclor 1242, 1254, and 1260 had half-lives of 8, 23, and 30 d, respectively.

Our results showed a much shorter BHL for ^{14}C-PCB than others. It is possible that the size of the trophic system used, as well as differences in species and metabolic rates, may affect the data. NARBONNE (1979) reported that PCB concentration in mullets was dependent on food intake, so size of the organism is another important factor to be considered when evaluating the data.

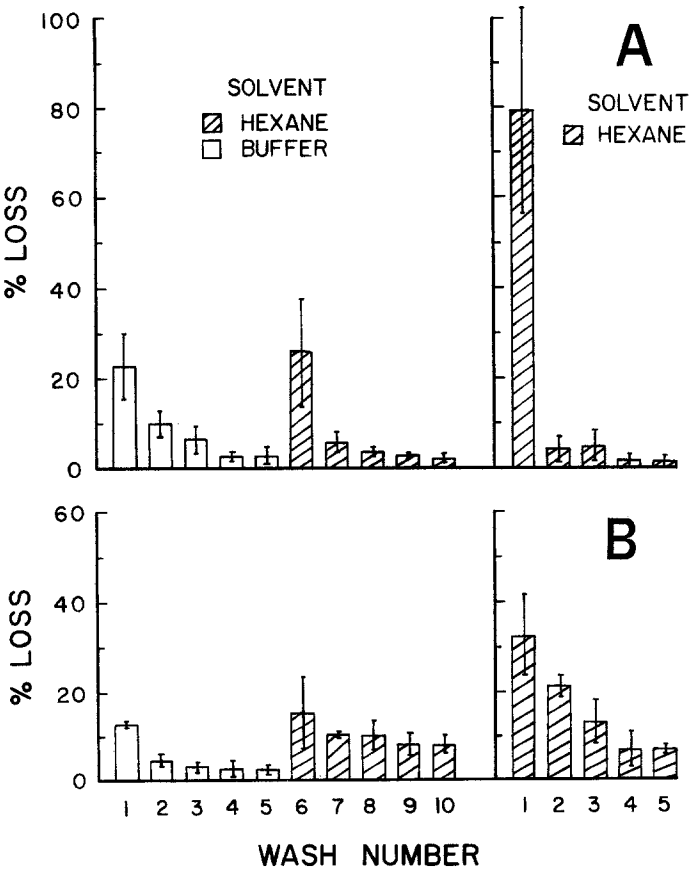

Fig. 2. Loss of ^{14}C-PCB from bacterial cells by washing. A represents data for fresh cells and B, frozen (thawed) cells.

Comparison of the chromatograms between the hexane extracts of saline during depuration by bacterial cells showed that PCB (1254) was being eliminated from the cells.

Experiments were also conducted to ascertain if depuration was due to excretion of PCB or by merely washing of PCB absorbed onto the cell wall. Data obtained (Fig. 2A & B) revealed that after 5 washes in pesticide-grade hexane, a total of 90.6 and 79.5% of the ^{14}C-PCB (based on initial activites as 100%) radioactivities were lost from the fresh and frozen cells, respectively. When the labeled cells were first washed (5 times) with 0.05 M sodium phosphate buffer, a total of 44.3 and 25.4% of the ^{14}C-PCB radioactivities were lost from the fresh and frozen cells, respectively. After the remaining 5 washes with pesticide-grade hexane, an additional 39.5% was lost from the fresh cells and 51.9% from the frozen ones. This indicated that the percent of the ^{14}C-PCB lost by washing is dependent upon the condition of the cells, the solvent and the number of washes.

This study established the depuration of PCB (Aroclor 1254) in all trophic systems used, but the rates differed in magnitude. All systems, except bacteria, followed exponential elimination with BHLs ranging from 3 to 5 d. Washing may also play a significant role during the depuration phases.

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