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Elimination Kinetics of Two Unmetabolized Polychlorinated Biphenyls in *Poecilia reticulata* after Dietary Exposure

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Persistent hydrophobic chemicals which are present in the environment can accumulate to high concentrations in living organisms. Steady state bioconcentration factors (ratios between concentrations in organism and water) or biomagnification factors (ratios between the concentrations in organism and food) measured with fish in laboratories are often used to estimate the importance of this undesirable process. In addition, to provide simplified estimates of bioconcentration or biomagnification factors, correlations between octan-1-ol/water partition coefficients (Neely et al. 1974) and solubility in water (Chiou et al. 1977) have been evaluated. Although linear relationships are usually obtained, such relationships are not found for all kinds of chemicals. In particular for extremely hydrophobic chemicals significant deviations from linearity have been found (Bruggeman et al. 1984; Zitko and Hutzinger 1976; Opperhuizen et al. 1985; Könemann and Van Leeuwen 1980).

Generally, to explain and describe bioaccumulation, models are required. Using first order kinetics, uptake and elimination in fish have been represented as:

$$\text{food} \xrightarrow{E\ f} \text{fish} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{water} \qquad (1)$$

Here, k_1 and k_2 denote the uptake (mL $d^{-1}g^{-1}$) and elimination rate constant (d^{-1}), E the uptake efficiency of the test compound by the fish from the food and f the feeding rate (g food/ g fish/ day). In this model food, water and fish are considered homogeneous compartments, while k_1, k_2 and E are considered constants, i.e., independent of exposure time and concentration (Branson et al. 1975; Bruggeman et al. 1981).

Although experimental data are usually consistent with the above model, biphasic elimination processes have also been observed, thus indicating that k_2 is not constant during the period of elimintion (Spacie and Hamelink 1982). In other studies no elimination at all was found for compounds such as PCB's, even when the fish were starved (Lieb et al. 1974).

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In the present paper elimination rates of 2,2',3,3',5,5' hexa- and 2,2',3,3',4,4',5,5' octachlorobiphenyl after dietary exposure are investigated. The data of the elimination process are described by first order kinetics. A good description of this elimination process is obtained if it is assumed that the fish is not one homogeneous kinetic compartment, but is composed of two compartments.

MATERIALS AND METHODS

Polychlorinated biphenyls were available from previous experiments (Opperhuizen et al. 1987) and were >95% pure, as confirmed by GC-ECD. GC-MS and NMR data confirmed the structures of the compounds. Analytical grade n-hexane (Merck) was redistilled before use.

Laboratory bred male guppies (Poecilia reticulata) with lengths between 14 and 20 mm and weights between 70 and 130 mg were used. A day/night cycle of 12/12 h was imposed with day-light fluorescent lamps. The aquarium water was 50% Amsterdam tap and 50% deionized water. The pH of the water was 6.9, the temperature 21 ^{o}C and the dissolved oxygen concentration was at least 8 ppm. Four glass aquaria were used containing 30 L water each.

Samples of fish (two fishes each sample) or food were homogenized in a mortar and extracted with 50 mL hexane and 50 mL water by heating under reflux, as has been described previously (Bruggeman et al. 1981). After spiking clean fishes with 1 mL of a solution of the test compounds in hexane, recoveries for both PCB's of 70% and 50% were found for fish samples and food samples, respectively. The concentrations of the test compounds were determined by injection of 1 μL of the extract in a Tracor 550 GC gas chromatograph, equipped with a linearized Electron Capture Detector. The detector was connected to a Spectra Physics 4100 computing integrator. A Dexil 300 GC column (2% on Chromosorb W AW 100-120 mesh, 2.5 m x 2 mm) was used, with Argon/Methane (95%/5%) as carrier gas.

Food containing 550±110 μg/g (wet weight) 2,2',3,3',5,5'- hexachlorobiphenyl and 530±100 μg/g 2,2',3,3',4,4',6,6'-octachlorobiphenyl was prepared by mixing dry fish food (Tetra-min) with a solution of the PCB's in hexane. The organic solvent was removed by evaporation under reduced pressure. Fish were fed this food daily for 5 days per week, with an average daily feeding rate of 0.02 g ^{food}/g fish day. During uptake and elimination periods, the water was aerated and filtered to remove PCB's from the water with activated carbon in an aquarium pump.

In experiment I, 86 fishes were fed the contaminated food for 65 days. In experiment II, 21 fishes were exposed to the contaminated food for 37 days, after which they were transferred to clean water for 140 days. During the latter period the fish were fed clean food. In experiment III 47 fishes were exposed for 65 days to contaminated food, after which they were transferred to clean water for 120 days, during which they were fed clean food. In experiment IV, 13 fishes from experiment I after being fed contamined food for 65 days and clean food for 89 days, were re-exposed to the contaminated food for

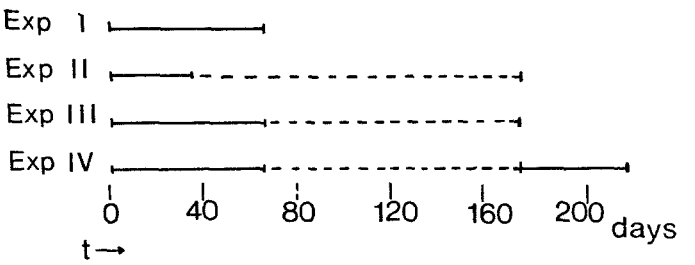


Figure 1. Schematic representation of the duration of the periods of exposure (solid line) and elimination (dashed line) during the various experiments.

another 37 days. A schematic representation of the experiments is shown in Figure 1.

RESULTS AND DISCUSSION

Analysis of food and fish samples showed that there was no detectable background contamination by the two PCB's. Fish which died spontaneously during the dietary exposure were analyzed separately, but contained no significantly different concentrations of the test compounds. In Figure 2 the uptake of the two PCB's during the initial exposure (experiment I) and during the period of re-exposure (experiment IV) is shown. From the differences in the uptake of the chemicals from the food between the two exposure experiments, it is clear that the estimated uptake efficiency is significantly higher during the period of re-exposure than during the initial exposure (T-test: $p<0.01$; $n=3$). During the re-exposure the uptake efficiency approaches 1.0, which indicates that there is almost no clearance of the chemicals in the fish. After prolonged dietary exposure (experiment I) no actual steady state concentrations in the fish ($dCf/dt = 0$) were achieved during the 65 days testing period. Hence calculation of actual biomagnification factors was impossible.

As is shown in Figure 3, no clearance of the chemicals from the fish is found after a 65 days dietary exposure (experiment III). In the same figure it is shown, however, that during the period of elimination following a 37-day period of dietary exposure a significant decrease of the concentrations of both PCB's in fish is found (experiment II, T-Test: $p<0.01$, $n_1=3, n_2=6$). In addition, from these graphs it can also be seen that the elimination process after short dietary exposures is biphasic. During the second phase, which begins, at the latest, 40 days after clearance started, no further decreases of the concentrations are found. This means that, since the uptake period was 37 days, in this experiment approximately 77 days after exposure was started, no clearance was found. This is consistent with the data from experiment III, which showed that 65 days after exposure was started no elimination was found.

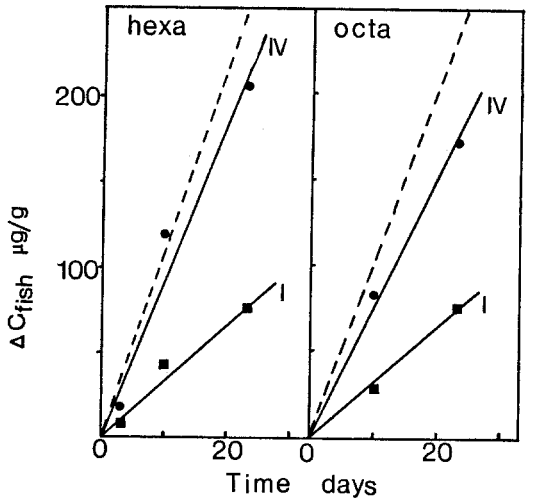


Figure 2. Increase of the concentrations of 2,2',3,3',5,5' hexa- and 2,2',3,3',4,4',6,6' octachlorobiphenyl in fish during initial exposure (experiment I) and during re-exposure (experiment IV). The solid lines represent the experimental data and the dashed line represents the curve for E=1.00 and k_2=0.

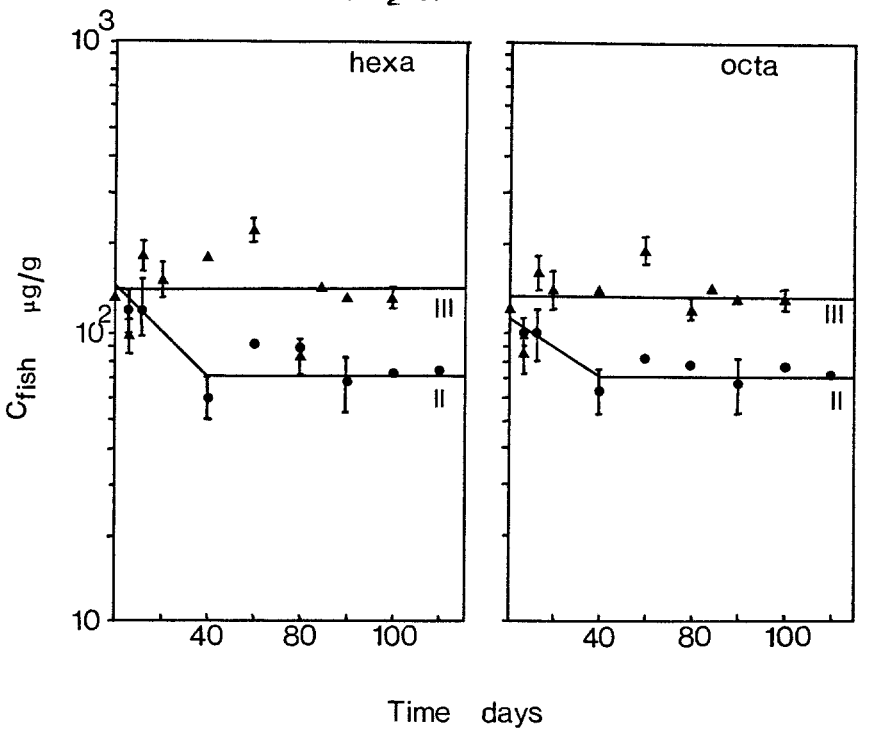


Figure 3. Concentrations of 2,2',3,3',5,5' hexa- and 2,2',3,3',4,4',6,6' octachlorobiphenyl in fish during elimination in experiment II and experiment III. 95% Confidence limits are shown only for the samples for which at least three replicate measurements were carried out.

Results which are consistent with those of the present study were reported by Hansen et al. (1976), who carried out comparable experiments with catfish. These fishes were fed a 20 mg/kg Aroclor 1242 diet for 140 days. After 84 days and 140 days the fish were allowed to eliminate the PCB mixture. After 140 days exposure, followed by 56 days elimination, some fish were re-exposed for another 56 days to the same contaminated food. Although Hansen and coworkers only suggested that the uptake rate in this re-exposure experiment had increased, it is also likely that the elimination rates had decreased.

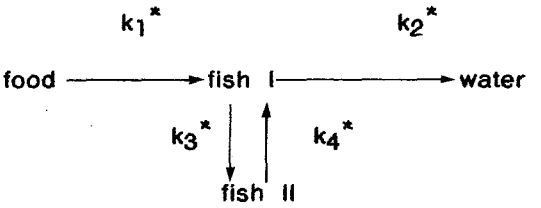
Generally, in addition to actual clearance of test compounds from the fish, growth of the fishes can lead to decreasing concentrations in long term experiments (growth dilution). This for instance has been shown by Lieb et al. (1974). In the present study, however, such growth dilution cannot help to explain the concentration decrease in experiment II, because no growth was observed throughout the experiments.

In order to obtain data which may help to describe bioaccumulation with a first order model in which fish is one homogeneous compartment, Branson et al. (1975), devised an accelerated test method. In such an accelerated method, short uptake and elimination periods are applied from which uptake and elimination rate constants are derived. This methodology has often been applied since, although data supporting the accuracy of the accelerated kinetic parameters are rather scarce (Bishop and Maki 1980). Comparison of 'kinetic' and 'steady state' bioconcentration factors for different types of test compounds can show considerable differences (Davies and Dobbs 1984), which may be explained by variations of the elimination rates after different exposure times. In a variety of studies elimination rates have been shown to be dependent on the experimental concentrations and exposure times. Hattula and Karlog (1973) found that the elimination half lives of PCB's increased with the duration of the experimental period. Melancon and Lech (1978) have shown the rate of elimination of naphthalene from rainbow trout to be lower after 4 weeks of exposure than after 8 hours exposure. In addition Grzenda et al. (1970) and Grzenda et al. (1972) measured different elimination rates of dieldrin and DDT during continued exposure of goldfish and after exposure had ceased. Zitko and Carson (1977) concluded that elimination rates of PCB's and alkylated PCB's are different after short aqueous exposure and longer dietary exposure of juvenile atlantic salmon. These differences may, however, also be explained by the differences of the exposure periods.

Furthermore, biphasic elimination periods have been found for PCB in rainbow trout by Guiney et al. (1977) and Guiney and Peterson (1980), organophosphate esters in rainbow trout fingerlings by Muir et al. (1980) for polychlorobenzenes in guppies by Konemann and Van Leeuwen (1980), for HCH isomers in guppies by Yamota et al. (1983) and for PCB's in guppies by Gooch and Hamdy (1982). Although in all studies a fast elimination period was followed by a slow period, the time of transition from one phase to the other ranged from 1 day to 21 days. This may be due to the different fish species or to the

various exposure conditions employed in the different studies.

Biphasic elimination has been described kinetically by Moriarty (1975), Könemann and Van Leeuwen (1980) and Spacie and Hamelink (1982). If it is assumed that uptake of the chemicals in dietary exposure experiments results entirely from the food, the following model can be used:



In this model, k_1^*, k_2^*, k_3^* and k_4^* are considered exchange rate constants, k_1^* being E x f, and f being constant. It is assumed that the compartments I and II are homogeneous, but independent.
Fish I and fish II represent two kinetic compartments of the whole fish. It should be noted that generally no physiological interpretation can be given to these compartments.

During the period of elimination the total concentration within the whole fish can be expressed by equation 2:

$$C_f = A\ e^{-at} + B\ e^{-bt} \qquad (2)$$

Here, a and b are the slopes of the first and the second phase of the graph of ln C_f versus time during elimination. The values of a and b correspond with the values of the exchange rate constants (Moriarty 1975). The intercepts of the ln C_f time plots correspond to the sizes of the compartments I and II. From the graphs of the elimination after 37 days exposure (experiment II) values for the parameters A, B, a and b were calculated (figure 3). Since for both PCB's no clearance in the second phase of the elimination period was found, the b-values were estimated as being zero. The a values were estimated with regression analysis from the concentrations in fish sampled during the first 40 days of exposure. These values were 0.015 for both PCB's. The values of A and B for the hexachlorobiphenyl are 70 and 70 μg/g respectively. For the octachlorobiphenyl the values are 60 and 70 μg/g. Since the fish compartment II shows no clearance, the elimination for hexachlorobiphenyl from the whole fish can be expressed by:

$$C_f = 70\ e^{-0.015\ t} + 70 \qquad (3)$$

and for octachlorobiphenyl by

$$C_f = 60^{-0.015\ t} + 70 \qquad (4)$$

The value of 70 μg/g for both test compounds represent residual concentration in the whole fish, which will not be cleared.
In the experiment III, it is found that the residual concentrations

increase to 130 µg/g for both PCB's after 65 days exposure (Figure 3). From these data it is clear that due to accumulation in compartment II, the residual concentrations in the whole fish increase with an average of approximately 2 µg/g day. Due to this increase of the contribution of the second fish compartment, it can be argued that no actual values of biomagnification factors can be measured with an accelarated test, since the elimination rate constants measured will be dependent on the exposure period. While the elimination rates tend to decrease with increasing exposure times, it may be assumed that biomagnification factors measured in accelerated test systems, underestimate biomagnification factors after long exposure times.

In the present study it is shown, that after any exposure period, residual concentrations of polychlorinated biphenyls will be found which increase with duration of the exposure, and that steady state concentrations will probably never be found in guppies even after continued dietary exposure.

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