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Measuring the Sediment/Organism Accumulation Factor of PCB-52 Using a Kinetic Model

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In aquatic systems, neutral organic chemical contaminants such as the PCBs, PAHs, dioxins, etc., primarily partition to the organic carbon (OC) of bedded sediments and the lipids of biota. Recognition of this fact led to the convention of normalizing concentrations of neutral chemicals in sediments on the basis of their total organic carbon (TOC) content and similarly normalizing concentrations in biota on the basis of their lipid content. The ratio of normalized concentrations at steady state has been called an accumulation factor (AF) (Lake et al. 1990; Rubinstein et al. 1990; Ferraro et al. 1991). Multiplying the TOC-normalized concentration of a neutral chemical in sediment by the AF provides an estimation of the steady state body burden of that chemical on a lipid basis in a sediment-exposed organism.

In theory, the AFs for individual neutral chemicals should not differ greatly from one another and can be approximated by a single value (McFarland 1984). This generalized AF for neutral organic chemicals was calculated as 1.73 based on data sets that contained PAHs and chlorobenzenes (McFarland and Clarke 1986). Theoretical expectations of constancy in the AF notwithstanding, empirical observations have produced a range of values. Most of the studies that have measured AFs, whether laboratory or field, have involved infaunal, sediment processing polychaetes, clams, and amphipods. Laboratory exposures have been continued for up to six months in order to achieve steady state tissue levels of bioaccumulating chemicals so that AFs could be calculated (Rubinstein et al. 1990; Pruell et al. 1993).

Kinetic modeling using short exposures provides an alternative to long-term exposures to achieve steady state. Numerous kinetic studies of chemical bioconcentration from water have been reported but there have been very few bioaccumulation studies in which contaminated sediments were the source of exposure. The principal criticism of kinetic modeling of bioconcentration is that uptake and elimination rates are more complex than can be accounted for by simple first-order models. Rates of elimination are not perfectly constant with time and also are affected by whether exposure is constant, variable, or is discontinued (Grzenda et al. 1970; Melancon and Lech 1979). When comparisons have been made, kinetic projections of steady-state bioconcentration of chemicals in fish have generally underestimated long-term laboratory or field exposures (Davies and Dobbs 1984). However, an advantage of kinetic modeling techniques is that short -term exposures offer the possibility of avoiding many conflicting rate-influencing variables that affect the establishment of a true equilibrium between the phases of interest.

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The purpose of this study was to test the ability of a three-compartment closed kinetic model to determine the AF for PCB-52 using a 5-d exposure. The exposure matrix involves suspended sediment and fish, rather than the usual deposited sediment and sediment-processing infaunal organisms. The model generates rate constants for intercompartmental transfer of the chemical, and an AF is calculated using these values.

MATERIALS AND METHODS

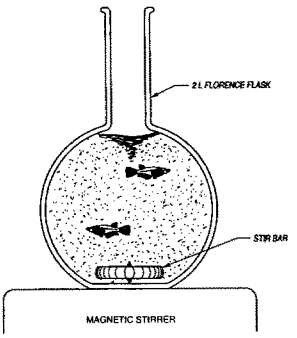

Figure 1. Exposure apparatus.

PCB-52 ([^{14}C] 2,2',5,5'-tetrachlorobiphenyl-UL) was obtained from Sigma Chemical Company, St. Louis, Missouri. A 1:50 dilution with methanol of an 11.9 μCi/mL stock solution (409 μg/mL in toluene) provided a working solution of 0.238 μCi/mL or 8.18 μg/mL of PCB-52.

Japanese Medaka, *Oryzias latipes*, were purchased from Carolina Biological Supply, Burlington, North Carolina, and were acclimated to laboratory conditions for at least 14 d at ambient temperature (≈ 23°C), in 120-L glass aquaria. Fish were maintained in filtered, aerated, dechlorinated tap water and were fed Aquarian® tablet food twice daily. A 12-hr dark/12-hr fluorescent light photoperiod was controlled by an automatic timer. Fish of both sexes were randomly used in the experiments. Air-dried and milled uncontaminated sediment from Barataria Bay, Louisiana, was stored at 4°C until use.

A 2-L Florence flask was used as the exposure vessel (Figure 1). For each exposure, sediment (400 mg), 2 L dechlorinated water and 2 fish (0.5 - 1.2 g wet weight, combined) were placed in the flask. The sediment was suspended by continuous stirring using a magnetic stir bar (rotation, ≈ 150 rpm) and 0.1 mL of the PCB solution was added to the water by pipet. Fish were not fed during exposures. At the end of the exposure time interval (1, 2, 4, 12, 24, 36, 48, 72, 96 or 120 hr) fish, sediment, and water were collected and analyzed for total radioactivity. Three replications were made at each interval. Fish were netted and rinsed with distilled water and their combined weight was taken. The fish were combined and PCB and lipids were extracted following homogenization in 20 mL acetone (x2) with a Brinkmann PCU-2-110 Polytron homogenizer. The acetone extract was partitioned between hexane and water in a conical centrifuge tube. The hexane fraction was then split to provide separate aliquots for total lipids and total radioactivity measurements. The sediment-water mixture was centrifuged and 100 mL of the supernatant were extracted with 25 mL hexane:acetone (4:1) followed by 20 mL hexane. The sediment pellet was extracted twice with 5 mL acetone and then partitioned between hexane and water. Hexane extracts of fish, sediment, and water were evaporated to near dryness in 20-mL scintillation vials. 15 mL of PCS scintillation fluid (Amersham Corp., Arlington Heights, Illinois) was added and the radioactivity counted on an LS 3801 Liquid Scintillation System (Beckman Instruments, Fullerton, California). Total recovery of PCB-52 from the water-sediment-fish system was 87%. Water, sediment and fish were spiked with PCB-52. Recovery from spiked samples was: water, 89%; sediment, 88%; and fish, 84%. The hexane extract for lipid determination was evaporated to dryness and total lipids were determined gravimetrically. Six 200-mL aliquots of the suspended sediment were centrifuged separately and the pellets were analyzed for total organic carbon (TOC) using a Model 700 TOC Analyzer with IR detection (OI Analytical

Figure 2. Three-compartment closed kinetic model for phase-distribution of PCB-52 in the exposure system.

College Station, Texas). Computer modeling was conducted using PCNONLIN® (Metzler and Weiner 1992).

RESULTS AND DISCUSSION

The weight of the fish in the exposure system varied among exposures while the weight of suspended sediment and the volume of water were constant. Therefore, the final mass of PCB-52 in fish, sediment and water was normalized to a fish wet weight of 1.0 g. The fraction of TOC in the sediment was 0.04 (±0.0058 SD), and the fraction of lipid (hexane/acetone extraction, H/A) in the fish was 0.053 (±0.022 SD).

A three-compartment closed kinetic model was used to represent PCB-52 distribution among the water, fish, and sediment (Figure 2) (Gibaldi and Perrier 1982). In this model, water represents the central compartment (compartment 1) which is reversibly connected to peripheral compartment 2 (sediment) and peripheral compartment 3 (fish). PCB-52 is taken up from the water by the sediment and by the fish. Simultaneously, some of the chemical is desorbed back to the water from the sediment and eliminated to the water by the fish. These four first-order processes are described by intercompartmental rate constants for mass transfer. This is a closed model and loss from the exposure system and direct exchange between fish and sediment were considered insignificant and are not accounted for in the model. Entrainment of suspended sediment in the guts of exposed fish contributing to total body radioactivity was also not expected to be a significant source of error based on findings with sediment-ingesting infaunal species in AF studies (Lake et al. 1990).

Simultaneous equations for the rates of change of PCB in water, fish, and sediment are:

$$dX_w/dt = k_{21}X_s + k_{31}X_f - k_{12}X_w - k_{13}X_w \quad (1)$$

$$dX_s/dt = k_{12}X_w - k_{21}X_s \quad (2)$$

$$dX_f/dt = k_{13}X_w - k_{31}X_f \quad (3)$$

where X_w, X_s, and X_f are the mass of PCB-52 (μg) in the central compartment (water), in compartment 2 (sediment), and in compartment 3 (fish), respectively, and k_{12}, k_{21}, k_{13}, and k_{31} are the intercompartmental rate constants for mass transfer of the chemical.The three differential equations (Eq 1 - 3) were fitted simultaneously to the experimental data by an iterative nonlinear least squares technique using PCNONLIN®. Values for the estimates of the rate constants are shown in Table 1. The analysis of variance for the fitted equations is shown in Table 2.

Figure 3 shows distribution of PCB-52 among the three compartments (water, sediment, and fish) over a 120-hr period. Model-generated lines are fitted to the data. Initial uptake of PCB-52 by sediment was rapid as was the decline of PCB-52 in the water. By the 24th hour the mass of PCB-52 in the water stabilized, showing only a very gradual decline thereafter. The mass of PCB-52 in the sediment decreased gradually after the initial sorption phase, and this release of the chemical is reflected in the similarly gradual uptake of PCB-52 by the fish.

The equilibrium partitioning of a chemical between an organic phase and an aqueous phase can be defined in terms of the ratio of the forward and reverse rate constants (Kubinyi 1978). The sediment/water partition coefficient can be expressed as:

$$K_s = k_{12}/k_{21} \quad (4)$$

and the fish/water partition coefficient is:

$$K_f = k_{13}/k_{31} \quad (5)$$

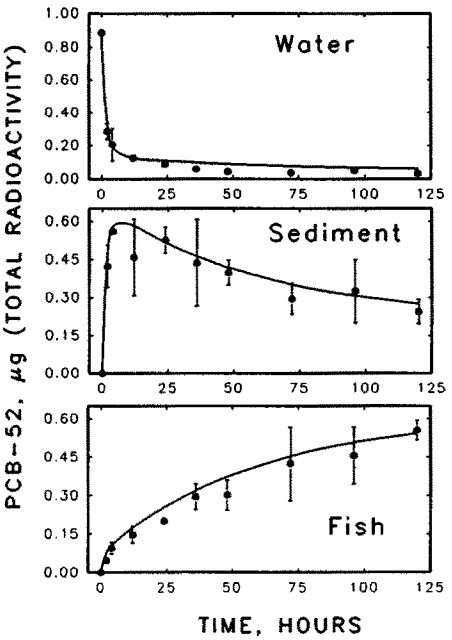

Figure 3. Distribution in μg of PCB-52 among the three compartments over time. Filled circles are means, vertical bars are ± one SD, lines are model-fitted estimates from the data.

Table 1. Least squares estimates of intercompartmental rate constants (reciprocal hours) for mass transfer of PCB-52 for the three compartment model.

Rate constant	Transfer direction	Time^{-1}, hr $\bar{x}$ ± SD
k_{12}	water to sediment	0.460 ± 0.035
k_{21}	sediment to water	0.108 ± 0.016
k_{13}	water to fish	0.066 ± 0.008
k_{31}	fish to water	0.005 ± 0.002

Because the aqueous phase is common to both partition coefficients, the ratio of the two is the proportional difference at equilibrium between mass (or concentration) of PCB-52 in the fish and in the sediment. When this ratio is normalized on sediment organic carbon and organism lipid it is the accumulation factor (AF):

$$AF = (K_f/f_{lipid})/(K_s/f_{oc}) \quad (6)$$

where f_{lipid} and f_{oc} are the fractions of lipid and OC in exposed organisms and in sediment, respectively. Substituting the rate constants (Table 1) and the fractions of organic carbon (0.04) and lipid (0.053) into equations 4-6 gives AF = 2.34 for PCB-52.

Table 2. Analysis of variance for the data fitted to the model equations.

Equation	Source	df	SS	MS	$F_{1,28}$	p	r
dX_w/dt	Model	1	2.26	2.26	--	--	--
	Error	28	0.05	0.0018	1256	<0.001	0.99
dX_s/dt	Model	1	0.89	0.89	--	--	--
	Error	28	0.24	0.0086	103	<0.001	0.86
dX_f/dt	Model	1	1.15	1.15	--	--	--
	Error	28	0.11	0.0039	295	<0.001	0.95

This value is near the mean AF of 2.65 calculated for PCB-52 in short-term (less than 30 d) laboratory exposures of the infaunal, sediment-ingesting clam, *Macoma nasuta* (Brannon et al. 1989; Ferraro et al. 1991) when lipid data of the latter study were converted to a hexane/acetone (H/A) extraction basis. A factor for conversion of chloroform/methanol lipid concentration to H/A lipid concentration equal to 1.8 was used based on data in Randall et al. (1991). In a 119-d study (*B. L. Boese personal commun.*) exposing the same species to PCB-52 in sediment, a mean AF of 4.16 was obtained when the data were similarly converted to an H/A extraction basis. In fresh water studies Ankley et al. (1992) reported AFs for total tetrachlorobiphenyls in oligochaetes equal to 1.35 (30-d exposure) and 1.10 (field exposure). In the same studies the AF reported for fathead minnows, *Pimephales promelas*, (30-d exposure) was 0.50 and for black bullhead catfish, *Ameirus melas*, (field exposure) was 2.92.

The variability in AF measurements illustrated above results from many factors and is compounded by a lack of consistency in the methods used. Failure to achieve near equilibrium conditions in the exposures is an obvious potential shortcoming of non-kinetic short-term exposures. In both laboratory and field studies the use of organisms that are not in intimate and continuous contact with sediments can fail the equilibrium criterion. Additionally, the chemical must be fully bioavailable: if desorption of the chemical from sediment binding sites is very slow, an exposed organism may never reach equilibrium bioaccumulation. Although long-term exposures represent a solution to the non-equilibrium problem, they have their own difficulties. During long-term studies, sediments can be depleted of the bioavailable fraction of a chemical, thereby reducing exposure. Nutrient quality and amount may decline and sublethal toxicity may occur, affecting the health of organisms and causing loss of lipids. Metabolic degradation of bioaccumulating chemicals may occur, reducing bioaccumulation. Induction of metabolizing enzymes caused by the chemical under investigation or by other chemicals in the sediment may exacerbate this effect. Growth during the exposure period can dilute tissue concentrations causing reduced apparent bioaccumulation. Spawning and other seasonal changes also affect bioaccumulation (Lee et al. 1989). All of these processes influence the kinetics of phase distribution of a chemical between sediment and organism, i.e., alterations in the rates of chemical transfer between phases can alter the value assigned to the endpoint, AF, which is intended to express a thermodynamic relationship.

Other sources of variability not related to length of exposure include loss of linearity in the contaminant/TOC relationship at low TOC concentrations where mineral surfaces and other factors play an increased role in sorption dynamics. In sediment-processing organisms the selective ingestion of particles according to size or nutritive value may

result in a different dose to the animal than is represented by normalizing on the basis of TOC in the whole sediment (*B. L. Boese personal commun.*). In addition, types of OC present and differences in lipid composition, life-style of the organism and presence and levels of other contaminants in the sediments may affect the AF (McElroy and Means 1988). Procedural differences in analysis of TOC and lipid also clearly influence the result.

The kinetic approach used in this study eliminates most of the sources of variability inherent in previously reported laboratory and field studies for the determination of AFs. The result for PCB-52 (AF = 2.34) is not greatly different from the theoretical value, 1.73 for neutral organic chemicals in general (McFarland and Clarke 1986). Use of a model that describes simultaneous distribution of a chemical between organism and sediment to predict the relative difference at equilibrium is a novel aspect of this approach. In previous kinetic studies using a one-compartment model and a flow-through exposure system to describe chemical uptake in deposit-feeding clams, AFs ranged from 0.149 for dioctyl phthalate to 5.6 for diphenyl ether (Foster et al. 1987). The wide range of AFs reported is contrary to theoretical expectation and was considered by the authors to be due chiefly to differences in uptake rate or bioavailability. The methods described in this paper could be used to test that interpretation.

Although the three-compartment closed model applies to non-metabolizing chemicals, it can be modified to incorporate additional compartments for those chemicals that undergo metabolism, or to account for loss from the system. We propose that by eliminating most of the conflicting rate-influencing processes in AF measurements, the approach taken here may provide a truer estimation of equilibrium phase distribution than is possible with previously used methods for measuring AFs. Additional studies are being conducted to test this hypothesis.

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