Authorized Reprint 1989 from Special Technical Publication 1027 1989
Copyright American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103

Peter F. Landrum,[1] *Warren R. Faust,*[1] *and Brian J. Eadie*[1]

Bioavailability and Toxicity of a Mixture of Sediment-Associated Chlorinated Hydrocarbons to the Amphipod *Pontoporeia hoyi*

REFERENCE: Landrum, P. F., Faust, W. R., and Eadie, B. J., **"Bioavailability and Toxicity of a Mixture of Sediment-Associated Chlorinated Hydrocarbons to the Amphipod *Pontoporeia hoyi*,"** *Aquatic Toxicology and Hazard Assessment: 12th Volume, ASTM STP 1027,* U. M. Cowgill and L. R. Williams, Eds., American Society for Testing and Materials, Philadelphia, 1989, pp. 315-329.

ABSTRACT: The toxicity and bioavailability of a mixture of 13 sediment-associated chlorinated hydrocarbons were determined for the amphipod *Pontoporeia hoyi.* The processes were traced with two radiolabeled compounds, ^{14}C-2,2′,4,4′-tetrachlorobiphenyl (2244TCB) and ^{3}H-benzo(a)pyrene (BaP). The toxicity of the chlorinated hydrocarbon mixture resulted primarily from the lindane and dieldrin components. The 72-h LC_{50} was 14.5 nmol g^{-1} as the sum of the added chlorinated hydrocarbons and 4.7 nmol g^{-1} as the sum of the lindane and dieldrin. At 144 h the LC_{50}s had dropped to 1.51 and 0.53 nmol g^{-1}, respectively. At the lowest dose, 2.4 nmol g^{-1} sediment, the time to yield 50% mortality (LT_{50}) was 119 h. The toxicokinetics for the radiolabeled compounds yielded sediment uptake clearance constants of 0.0018 and 0.018 g dry sediment g^{-1} animal h^{-1} for BaP and 2244TCB in the absence of added chlorinated hydrocarbons. At the lowest chlorinated hydrocarbons dose, the clearance constants of both BaP and 2244TCB were approximately doubled. The clearance constants were not proportional to the freely dissolved concentration of the radiolabeled compounds in the interstitial water but were presumed to be proportional to the compound desorption rates. For the chlorinated hydrocarbons, the uptake clearances were inversely proportional to the log of the octanol-water partition coefficient. The bioaccumulation factor (BAF, concentration in the organism divided by the concentration in the sediment) appeared to peak at about a log octanol-water partition coefficient of 6. However, the BAF ranged from less than 1 to greater than 30 for compounds with a log K_{ow} near 6.

KEY WORDS: bioavailability, toxicokinetics, sediments, partitioning, toxicity, LC_{50}, amphipod, mixture, chlorinated hydrocarbons, bioaccumulation, *Pontoporeia hoyi*

Contaminated sediments, considered a major problem in many areas throughout the Great Lakes Basin, are associated with biological effects such as tumors in bullheads [*1*] or changes in benthic species composition [*2*]. Further, sediment-associated contaminants are an important source of contaminants to benthic organisms [*2,3*] for potential food chain bioaccumulation. Understanding the degree of biological availability is important since sediment-associated contaminants can remain present in the biologically inhabited portions of the sediments for decades. However, the significance of these contaminated sediments as contaminant sources to the benthic food chain or to the pelagic system via resuspension is not known [*4*]. This is particularly true for mixtures of xenobiotics in sediments. Previous laboratory studies exposing *Pontoporeia hoyi* to sediment-associated polycyclic aromatic hydrocarbons (PAH) indicate that the

[1]Great Lakes Environmental Research Laboratory, NOAA, Ann Arbor, MI 48105, Contribution No. 596.

accumulation is dependent on the specific compound desorption rate from the sediment matrix with subsequent accumulation from the interstitial water. Additional accumulation occurs through ingestion of contaminated particles by *P. hoyi* [*5*]. A similar conclusion was reached for the accumulation of hexachlorobenzene by the clam *Macoma nasuta* [*6*] and oligochaetes [*7–9*].

The objectives were: (*a*) to seek a no effect level for the chlorinated hydrocarbon mixture at which to measure the toxicokinetics of these sediment-associated chlorinated hydrocarbons; (*b*) to compare the toxicokinetics for selected compounds in the mixture with previous kinetics determined on single compounds; and (*c*) to compare the uptake kinetics with partitioning between sediments and interstitial water. Measures of the partitioning between the sediment interstitial water and particules for the radioactive tracers, in combination with relationships between the bioavailability and the physical chemical properties of the different chlorinated hydrocarbons, are used to examine the equilibrium partitioning approach, one of the primary means for the development of sediment quality criteria by EPA [*10–13*].

Materials and Methods

Collection of Environmental Samples

P. hoyi were collected from Lake Michigan off Grand Haven, Michigan at a depth of 24 m with a PONAR grab sampler. The *P. hoyi* were gently removed from the sediment with a screen, placed in clean lake water, and kept cold with ice during transport to the laboratory. *P. hoyi* were kept in aquaria containing about 1 in. of sediment and 4 in. of lake water at 4°C [*14*].

Water used throughout the work was Lake Michigan surface water (collected about 1 m below the surface) and stored at 4°C.

Lake Michigan sediment used in the experiment was obtained by PONAR grab approximately 5 miles off Grand Haven, Michigan at a 45-m depth. The sediment was sieved at 1 mm to remove animals and large pieces of debris and kept at 4°C. The sediment was characterized by wet sieving a subsample to determine the amount of material, on a dry weight basis, passing an 88-μm sieve. The organic carbon content of the sediment and the sieved fractions were determined [*15*].

Dosing of Sediments

The sediment was dosed with two radioactive tracers, ^{14}C-2,2′,4,4′-tetrachlorobiphenyl (2244TCB, universally labeled, specific activity 15.33 mCi mmol^{-1}) and ^{3}H-benzo(a)pyrene (BaP, specific activity 33.1 Ci mmol^{-1}), and an additional 13 cold chlorinated hydrocarbons: hexachlorobenzene (HCB), lindane) (LIN), 2,2′,5-trichlorobiphenyl (225TCB), 2,4,4′-trichlorobiphenyl (244TCB), 2,2′,6,6′-tetrachlorobiphenyl (2266TCB), 2,2′,3,4-tetrachlorobiphenyl (2234TCB), 3,3′,4,4′-tetrachlorobiphenyl (3344TCB), 2,2′,4,4′,6,6′-hexachlorobiphenyl (224466HCB), 2,2′,4,4′,5,5′-hexachlorobiphenyl (224455HCB), dieldrin (DIEL), p,p′-DDE, p,p′-DDT, and octachlorodibenzo-p-dioxin.

A solution of chlorinated hydrocarbons was prepared by dissolving 5 to 6 mg of each the nonlabeled compounds in acetone (10.5-mL total). The mixture was filtered through glass fiber filters to remove undissolved crystals of some of the compounds. The dioxin proved to be so insoluble that it was effectively removed by the filtering; no dioxin was subsequently detected by gas chromatography in either sediments or the test organisms.

The 1-mm sieved sediment was mixed until it appeared to be homogeneous. An amount of wet sediment corresponding to 700 g dry weight was placed in a 4-L beaker, and 2500-mL lake water was added. The following solutions were added dropwise while the mixture was stirred vigorously at room temperature: 700 μL methanol containing ^{14}C-2244TCB (3.15 μCi/700 g dry sediment); 800 μL methanol containing ^{3}H-BaP (15.8 μCi/700 g dry sediment); and 7.11 mL

acetone containing the appropriate amount of the above chlorinated hydrocarbon solution for the contaminant level being prepared. The amount of acetone and methanol carriers added to each solution was held constant. The mixture was stirred at room temperature for 4 h and kept at 4°C thereafter.

The nonlabeled compounds were tested at five different dose levels (Table 1) with the radiotracers added at the same concentration for each dose level, 0.31 ± 0.02 nmol g^{-1} for 2244TCB and 0.75 ± 0.04 pmol g^{-1} for BaP. The "control" level contained the same amount of radiotracers as did all the other levels but only the natural background concentrations of the nonlabeled chlorinated hydrocarbons (Table 1).

Experimental Procedure

Two days after dosing the sediment, the overlaying water was decanted, removing most of the acetone and methanol carriers, the sediment was stirred for homogeneity, and 40-g wet sediment transferred to 400-mL beakers. The amount of acetone (<325 μL L^{-1}) and methanol (<32 μL L^{-1}) remaining were not expected to affect the toxicokinetics [*16*]. The estimate of the amount of carrier assumes 80% of the water was decanted, and there was no loss to volatilization. The following samples were taken in triplicate from each dose level at the start, middle, and end of the distribution of sediment to beakers: 2-g sediment for analysis of nonlabeled chlorinated hydrocarbons by gas chromatography (GC); 2-g sediment for wet to dry weight determination; and 5-g sediment dessicated with sodium sulfate, extracted with ethyl acetate, and counted for radioisotope activity. Lake water (250 mL) was then carefully added to each beaker, minimizing the disturbance of the sediment; on the following day (three days after dosing the sediment) an initial water sample was taken and 20 test organisms added to each beaker.

Groups of 20 *Pontoporeia hoyi* were exposed to each sediment in triplicate for each sample point, 3, 6, 12, 19, and 31 days. Thus, the total number of beakers was 15 per dose level. Two additional beakers at each concentration containing no organisms were sampled on Days 15 and 31 for radiotracer concentration in sediment interstitial water.

TABLE 1—*Concentration[a] of contaminants in dosed sediments measured by gas chromatography.*

Compound Recovery[b]	Molecular Weight	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
HCB 82.4(1.9)	284.8	0.05	0.14	1.34	1.80	6.77	17.97
LIND 82.4(2.2)	290.9	0.32	0.71	3.95	5.23	11.45	16.05
225TCB 76.6(1.4)	256	0.13	0.12	0.28	1.42	8.11	19.36
2266TCB 77.4(1.5)	290	0.02	0.17	0.27	1.70	7.77	18.19
244TCB 80.3(1.8)	256	0.02	0.22	0.88	1.30	5.72	11.74
2234TCB 81.7(1.5)	290	0.22	0.25	1.34	1.60	9.09	19.95
224466HCB 77.9(1.4)	358	ND	0.18	0.94	1.36	6.40	13.49
DIEL 82.3(1.4)	381	ND	0.13	0.60	0.81	5.11	9.92
p,p'-DDE 98.8(1.2)	318.5	ND	0.22	0.99	1.31	7.76	14.00
3344TCB 95.3(3.6)	290	ND	0.07	0.92	1.51	6.48	7.31
224455HCB 95.1(1.5)	358	0.03	0.08	0.59	0.77	6.45	10.13
p,p'DDT 101.2(1.3)	354.5	0.15	0.06	0.35	0.63	4.56	8.38
Total nmoles g^{-1}		0.94	2.4	12.5	19.9	87.9	171.6
LIND Plus DIEL Total nmoles g^{-1}		0.32	0.84	4.6	6.0	16.6	26.0

[a]Concentrations in nmoles g^{-1}. Level 0 was the natural sediment concentration.

[b]Percent recovery for spiked specimens with coefficient of variation (%) in parentheses. All concentrations are corrected for percent recovery.

Distribution of the radiolabeled compounds in the overlaying water was determined from an initial water sample of 25 mL, in duplicate, from each dose level; 2 mL were counted for total activity, 20 mL were filtered for particle bound contaminant, with a back-up filter to correct for sorption and 2 mL of filtrate counted, 15 mL was run through a C-18 Sep Pak (Waters Associates) and subsequently eluted with 3 mL of methanol that was counted to determine the amount of freely dissolved compound in the water column [*17,18*].

Three beakers from each dose level were sampled at random at each time point (3, 6, 12, 19, and 31 days) and the following information obtained:

1. *Overlying water*—total, free, bound radioisotope activity as described above and oxygen concentration [*19*].
2. *Test organisms*—the number in overlying water was recorded to check for sediment avoidance behavior, number surviving was recorded for LC_{50} determinations, and the radioisotope concentration of two individuals (wet weight basis) was measured for toxicokinetic determinations; another two individuals were measured for lipid content [*20*], and the remaining live organisms were combined weighed and extracted by homogenizing in a tissue grinder with acetone and cyclohexane for GC determination of their chlorinated hydrocarbon content.
3. *Sediment*—a sample for wet to dry weight ratio, a sample for later extraction and determination of chlorinated hydrocarbon content via GC, and a sample was dessicated, extracted, and counted for radioisotope activity as indicated above.
4. *Pore water*—on Days 15 and 31 the overlaying water was removed by aspiration from beakers having no organisms, the sediment was transferred to centrifuge tubes and centrifuged at approximately 1000 × g for 10 min, and the total, free, and bound concentration of the two radioisotopes was determined for the resulting pore water as described above for the overlying water.

Pesticide Toxicity Experiment

In a separate experiment, ten test organisms were exposed to sediment (45 g) containing various combinations of the nonlabeled chlorinated hydrocarbons to determine which were largely responsible for the observed mortality. Sediment was prepared similarly to the above procedure at the nominal concentration equivalent to Contaminant Level 3, 0.6 $\mu g\ g^{-1}$ for each constituent. The following combinations were tested: acetone carrier only; each of the PCB congeners listed above combined; and each of the following in the presence of the combined PCB congeners—HCB, DDE, DDT, LIN, and DIEL. Mortality data were collected after six and nine days exposure to the sediments. All LC_{50} values were calculated using a probit analysis routine [*21*].

Analyses

Approximately 2 g (dry) of sediments were soxhlet extracted for 24 h in a 2:1 mixture of hexane and methanol. The extracted specimens, sediments and *P. hoyi* (extraction described above), were washed twice with an equal amount of hexane-extracted distilled water. The washed extract was rotary evaporated to less than 1 mL. The sample was transferred to a 2-mL glass bottle, brought to final volume of 1 mL with cyclohexane, and stored at 4°C until analysis. Four microlitres were splitless auto-injected into a Hewlett-Packard 5880A capillary gas chromatograph fitted with a 25-m DB-1 fused silica capillary column and electron capture detector. The temperature program of the analysis ran from 100 to 270°C at 4°C min^{-1}.

Scintillation counting was performed on an LKB 1217 liquid scintillation counter using RPI scintillation cocktail 3a70B. The data were corrected for quench using the external standards ratio method after correcting for background.

Results

Sediment Characteristics

The bulk dry sediments for these studies had an organic carbon content of 1.0 ± 0.2%. The ashfree dry weight was 1.9 ± 0.2% of the dry weight. The sediment particle size was distributed between 43.6% for >88 μm and 56.4% for <88 μm on a dry weight basis. The organic carbon content of the fractions was 0.26 ± 0.06% for >88 μm and 2.1 ± 0.3% for <88 μm fraction.

The homogenity of the mixed sediments was determined through the analysis of the radiotracers for samples taken at the beginning, middle, and end of the distribution of sediment to the exposure beakers. The coefficients of variation were low 3.5 ± 2.4% (mean ± 1 SD) for the BaP and 3.1 ± 0.5% for the 2244TCB at the various dose levels. The recoveries for these radiolabeled compounds were high and averaged 103 ± 9% for BaP and 98.4 ± 7.1% for 2244TCB. Thus, because of the high recoveries and the low coefficients of variation at time zero, the sediment was assumed to be homogenously dosed.

The overlying water contained low amounts of radiolabel with higher quantities in the samples containing organisms. The overlying water in the samples with the organisms was somewhat cloudy. Greater than 75% of the activity for the ^{3}H-BaP and nearly 90% of the ^{14}C-2244TCB were either on particles or associated with dissolved organic matter. Previous studies have shown that *P. hoyi* do not obtain a substantial amount of their body burden from the overlaying water when the sediment is the major source [*5,22*].

Toxicity

No overt avoidance of the sediment was found with the chlorinated hydrocarbons. Only one or two amphipods maximum were found per beaker swimming above the sediments at any of the dose levels, and there was no pattern to the potential avoidance as a result of dose or duration. Throughout the experiment the oxygen concentration remained above 6 mg L^{-1} except for the last sample at Day 31 where the oxygen content was 5.0 ± 0.4 mg L^{-1} for animals exposed to the radiolabeled compounds only. The oxygen concentration was higher in the water where the concentration of toxicant was greater. The lipid content of the *P. hoyi* was 27.9 ± 2.2% on a dry weight basis after three days exposure, and no significant difference was found on Day 31 for the controls or on Day 6 for Level 1 exposed organisms. The one organism from Level 1, Day 12 had a higher lipid content of 46.3%. From this data, it was concluded that the organisms were not suffering from nutrient limitations.

Independent toxicity experiments with either the PCB mixture or the PCB mixture with one of the pesticides indicated that the toxicity was likely due primarily to the presence of LIN and DIEL in the chlorinated hydrocarbon mixture (Table 2). All of the other combinations of mixtures produced no mortality except one acetone control. However, the second acetone control did not replicate the high mortality.

Toxicity of the total mixture of chlorinated hydrocarbons was determined by examining the mortality data in two ways (Table 3). First, the LC_{50} was determined across the range of concentrations tested. The control, Level 0, mortality was 5% or less for all the samples except at Day 31 when the control mortality ranged from 0 to 16%. The probit calculations accounted for the percent mortality in the controls. The log concentrations used in the probit analysis were for the total number of nmol g^{-1} dry sediment for all the nonlabeled chlorinated hydrocarbons added and the total of the number of nmol g^{-1} dry sediment of LIN and DIEL only. The mixture yielded 72-h LC_{50}s of 14.45 and 4.68 nmol g^{-1} for the total for the organic contaminants and the sum of LIN and DIEL, respectively. The data had considerable variability at 72 h, and the confidence limits were not estimated by the probit analysis program [*21*]. At 144 h the respective LC_{50}s were 1.51 [0.73 to 2.21, 95% confidence interval (CI)] and 0.53 (0.26 to 0.78, 95% CI) nmol g^{-1} dry sediment from the two methods of identifying the dose.

TABLE 2—*Toxicity screen for portions of the mixture.*

Toxicants[a]	Exposure Duration, percent mortality	
	6 Days	9 Days
Control sediment only	0	0
Acetone carrier control	60	0
PCBs and HCB	0	Lost
PCBs and Lindane	90	90
PCBs and Dieldrin	20	60
PCBs and DDE	0	0
PCBs and DDT	0	0
PCBs and OCS	0	0
PCBs only	0	0

[a]The concentrations used were nominally equivalent to the amount of the compounds found at Level 3 in Table 1. The amount of carrier used to dose the sediments was the same proportion as for the kinetics experiments.

TABLE 3—*Mortality of* Pontoporeia hoyi *Exposed to a Mixture of Chlorinated Hydrocarbons.*

Dose Level[a]	Replicate	Duration of Exposure, h				
		72	144	288	456	744
0	A	20[b]	19	18	20	16
	B	20	19	19	20	16
	C	17	20	19	20	20
1	A	17	5	10	0	0
	B	19	5	1	0	0
	C	18	8	0	0	0
2	A	18	1	0	0	0
	B	20	1	0	0	0
	C	18	0	0	0	0
3	A	1	0	0	0	0
	B	1	0	0	0	0
	C	1	0	0	0	0
4		All dead at all exposure durations				
5		All dead at all exposure durations				

[a]Concentrations for each dose level can be found in Table 1 plus the concentration of the radiotracers, 0.31 nmol g^{-1} dry sediment for 2244TCB and 0.075 pmol g^{-1} dry sediment for BaP.
[b]Number alive out of 20 per group.

The second method of analyzing the data was to determine the time required for 50% mortality at the lowest dose, 2.4 nmoles g^{-1} nonlabeled chlorinated hydrocarbons (0.84 nmoles g^{-1} LIN and DIEL). The LT_{50} was 118.97 h with no confidence limits calculated by the probit analysis program [*21*]. Therefore, the toxicokinetics, discussed below, determined for the nonlabeled compounds were estimated for organisms that were responding to the toxic effects of the mixture.

Since the concentration of the compounds in the live organisms was determined for toxicokinetic purposes, the body burden that produced the resultant LC_{50} could be determined for comparison with the LC_{50}s calculated from sediment concentrations. The 72-h body burden

LC_{50} was 13.3 (12.0 to 15.1 95% CI) and 6.3 (5.7 to 7.3 95% CI) nmol g^{-1} wet weight organism for the nonlabeled chlorinated hydrocarbons and the sum of LIN and DIEL, respectively. Variabilities in the experiment were better defined on a body burden basis for the short-term response than was obtained using the sediment concentrations as a basis for calculating the LC_{50}s. For the LT_{50}, the body burden required to produce the effect was 11.8 and 4.0 nmol g^{-1} wet weight organism for the total mixture and the sum of LIN and DIEL, respectively. Variability in the data was sufficiently great that the program could not estimate the confidence intervals around the LT_{50} estimates. The amount of compound required to produce the two 50% mortality estimates are quite similar for the 72-h LC_{50} and the 118.97-h LT_{50}.

Bioaccumulation

With the extent of toxicity encountered for this mixture of chlorinated hydrocarbons, the bioaccumulation was estimated only for the radiolabeled compounds at Levels 0 and 1 and for the nonlabeled compounds at Level 1. Estimations of the uptake clearances were generally performed using a simplification of a general sediment uptake model [5].

$$dCa/dt = K_s Cse^{-\lambda t} - K_d Ca$$

where

Ca = the concentration in the animal (nmol g^{-1}),
K_s = the uptake clearance constant (g dry sediment g^{-1} wet weight organism h^{-1}),
λ = the rate constant at which compounds become biologically unavailable (h^{-1}),
K_d = the elimination rate constant (h^{-1}),
Cs = the concentration in the sediment (nmol g^{-1}) and
t = the duration of the experiment (h).

For most cases, the model could be simplified to a one- or two-compartment donor dependent model with λ equal to zero [5].

Uptake clearance from sediments for the radiolabeled compounds, determined in the presence and absence of the nonlabeled chlorinated hydrocarbons, were 0.018 ± 0.001 g dry sediment g^{-1} organism h^{-1} (note that all clearances used in this manuscript will use these units of g dry sediment g^{-1} organism h^{-1}) and 0.029 ± 0.003 for 2244TCB and 0.0018 ± 0.0002 and 0.0024 ± 0.0005 for BaP at Levels 0 and 1, respectively. Clearances for both compounds increased significantly, approximately doubled, between the two exposure conditions, with no added chlorinated hydrocarbons and the lowest level of added chlorinated hydrocarbons. Uptake clearance constants increased proportionately, implying a general change in the compound bioavailability or physiology under the differing exposure conditions.

The potential of the carrier to effect the toxicokinetics and mortality data was expected to be minimal. The "control" sediment was dosed with the same total amount of carrier as the other dose levels but exhibited minimal or no toxicity except possibly at 744 h (Table 3). The presence of low levels of water-missible solvents had previously been found not to affect kinetics data [16]. Further, the rate constant for BaP accumulation was not statistically different from previous determinations [23] that had estimated carrier concentrations of <4 μL L^{-1}.

Radiotracer concentration in interstitial water was measured for the controls, no animals, only because the samples containing animals had to be screened to insure that all organisms were found for accurate mortality data. The amount of the two radiolabeled compounds, added at constant concentration to all study levels, yielded nearly constant fractions of freely dissolved, sorbed to suspended particles and bound to dissolved organic carbon (DOC) across the concentration range of nonlabeled chlorinated hydrocarbons (Table 4). Radiotracer partitioning between the freely dissolved interstitial water concentration was not different at the various exposure levels (Table 4). Further, the partitioning was not at equilibrium when the experiment

TABLE 4—*Fractionation of 2,2′,4,4′-tetrachlorobiphenyl and benzo(a)pyrene in interstitial water and partitioning to sediment.*

Category, Sample Day	Benzo(a)pyrene	2,2′4,4′-tetrachlorobiphenyl
	15-Day Sample	
Percent freely dissolved	43.0 ± 10.3	30.2 ± 7.0
Percent dissolved organic carbon bound	44.7 ± 4.2	57.5 ± 17.5
Percent particle bound[a]	13.0 ± 5.2	14.1 ± 10.8
K_p[b]	378 ± 18.4	884 ± 147
Koc[c]	37 800 ± 1840	88 400 ± 14 700
	31-Day Sample	
Percent freely dissolved	32.7 ± 5.0	17.8 ± 10.0
Percent DOC bound	56.0 ± 10.9	51.6 ± 10.2
Percent particle bound[a]	6.4 ± 3.0	8.6 ± 2.7
K_p[b]	578 ± 42	1673 ± 107
Koc	57 800 ± 4200	167 300 ± 10 700

[a] Percent of compound remaining on unsedimented particles.
[b] Partition coefficient between the freely dissolved compound in the interstitial water and the sedimentary solid material.
[c] Partition coefficient normalized to the organic carbon content of the sediment. Organic carbon content of the sediment was 1.0 ± 0.2%.

started and increased by a factor of approximately two between the two sample times. For both 15 days and 31 days, the calculated K_p (sediment concentration/interstitial water concentration) of 2244TCB was greater than BaP by a factor of about 2.5. In spite of the higher partitioning, the 2244TCB had the higher uptake clearance by approximately an order of magnitude compared to BaP.

Average radiotracer concentrations in the organisms were not statistically different at Day 3 at exposure Levels 0, 1, and 2 but declined sharply to between 20 and 30% of the control value (Level 0) for the animals in Level 3. At this exposure the mortality was at 95%.

Uptake clearance for the nonlabeled chlorinated hydrocarbons (Table 5) ranged from the low of 0.006 for 224455HCB to a high of 0.15 for 3344TCB. Most of the data were fit to the one-compartment donor dependent simplification of the general model, while LIN appeared to reach a steady state and was fit to a two-compartment model with a resultant K_d of 0.029 ± 0.007 h^{-1}. The variability in the data for p,p′-DDE, 3344TCB and 224455HCB was so great that only an estimate of the uptake clearance could be made. The estimate employed initial rate assumptions and the slope of the uptake between time equal zero and the first sample at three days to find an estimated uptake clearance. In addition to estimating the uptake clearance, a bioaccumulation factor (BAF) was determined for each compound from Level 1 after twelve days exposure (Table 5). The uptake clearances and BAF data were determined only from live organisms, and the twelve days was the longest exposure time used for calculations. At twelve days the mortality even at Level 1 was on average greater than 50%, LT_{50} 5.5 days.

The log of the uptake clearance (K_s) for the chlorinated hydrocarbons was inversely proportional to the log K_{ow} for the chlorinated hydrocarbons (Fig. 1). There are a few outliers in the data set. These outliers occur, in part, for two reasons. First, some of the uptake clearances were only rough estimates; this is particularly true for 3344TCB. Second, BaP is from a different class of compounds than the chlorinated hydrocarbons. If just these two compounds are removed from the data set, then the regression of log K_s versus log K_{ow} is significant ($\log K_s = -0.38 \pm 0.57 - 0.22 \pm 0.10 \log K_{ow}$, $n = 12$, $r^2 = 0.33$, $p < 0.05$). Leaving these two

TABLE 5—*Uptake clearance and twelve-day bioaccumulation factor.*

Compound	Log K_{ow}	K_s[a]	BAF
Lindane	3.24[b]	0.085 ± 0.017	4.2
Dieldrin	4.56[b]	0.032 ± 0.015	12.2
225TCB	5.44[c]	0.012 ± 0.005	7.9
HCB	5.47[d]	0.043 ± 0.004	15.8
244TCB	5.51[c]	0.008 ± 0.004	3.3
p,p′-DDE	5.69[e]	0.022 ± 0.013	2.4
2234TCB	5.84[c]	0.060 ± 0.004	31.7
2266TCB	5.92[c]	0.057 ± 0.014	16.0
2244TCB[f]	5.92[c]	0.030 ± 0.003	8.8
3344TCB	5.95[c]	0.15 ± 0.07	11.1
p,p′-DDT	6.19[e]	0.014 ± 0.008	17.9
BaP[f]	6.2[d]	0.0024 ± 0.0005	0.8
224455HCB	6.77[c]	0.006 ± 0.004	3.5
224466HCB	6.88[c]	0.012 ± 0.006	3.5

[a]Uptake clearance has units of g dry sediment g^{-1} organism h^{-1}.
[b]Ref *32*.
[c]Ref *33*.
[d]Ref *34*.
[e]Ref *35*.
[f]Radiolabeled compounds.

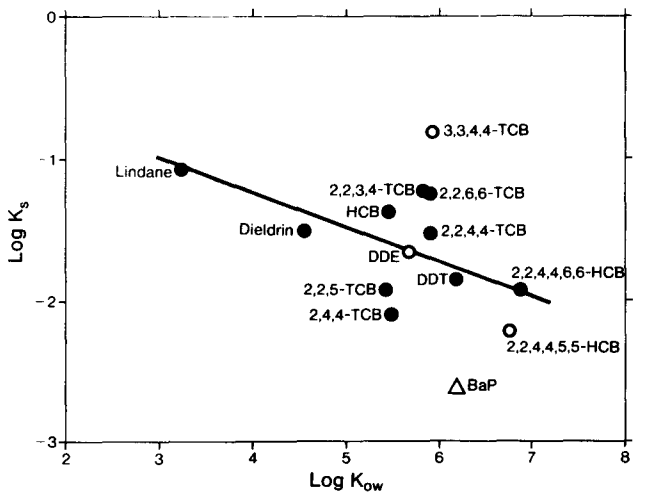

FIG. 1—*Scatter plot of log* K_s *against log* K_{ow} *shows the inverse relationship between the uptake clearance and hydrophobicity of test compounds (log* $K_s = -0.38 - 0.22 \log K_{ow}$, $r^2 = 0.33$ *when 3344TCB and BaP are omitted from the regression calculation). The uptake clearance from sediment was estimated from a one- or two-compartment donor dependent model for compounds with dark circles. The uptake clearance for the compounds with the open circles was estimated from the first uptake sample time. The open triangle represents benzo(a)pyrene, the only compound that is not a chlorinated hydrocarbon. The uptake clearance for BaP was from the one-compartment model.*

compounds as part of the data set does not statistically change the slope or the intercept but reduces the significance of the regression to $p < 0.15$.

For the BAF, an apparent "peak" was observed at about a log K_{ow} of 6. The data at the "peak" BAF ranged from 2 to 31 or about one order of magnitude (Fig. 2) for the chlorinated hydrocarbons. The 12-day BAF for BaP was 0.75 and much lower than any of the chlorinated hydrocarbons.

Discussion

At least two mechanisms of action for acute toxicity, narcosis [*24,25*] and neurotoxicity [*26*], have been attributed to various chlorinated hydrocarbons used in this study. The toxicity of the mixture was assessed by evaluating the sediment molar concentration of the nonlabeled chlorinated hydrocarbons and the molar concentration of LIN and DIEL as a pair on the assumption that the toxicity of mixtures of organic contaminants likely occur from additive molar concentrations of compounds acting with the same mechanism of action. Based on the independent experiments, the toxicity of the mixture to the amphipod, *P. hoyi*, apparently results primarily from the toxicity of the LIN and DIEL. When *P. hoyi* were exposed independently to LIN and DIEL, the ten-day LC_{50}s were 0.38 and 0.09 nmol g^{-1} dry sediment for a sediment with a mean organic carbon content of 0.3 ± 0.07% [*27*]. However, the other compounds in the mixture may contribute significantly to the toxicity. Concentrations in the organism required to produce 50% mortality in 72 h yielded a better effects estimate than the concentrations in sediment for which no confidence limits could be generated by the probit analysis program due to the variability in the data. Further, the concentration of the LIN and DIEL in the organism relative to the rest of the compounds in the mixture was greater than the LIN and DIEL concentrations in the sediment at 72 h and 50% mortality. Therefore, part of the reason LIN and DIEL exhibit greater toxicity than the other chlorinated pesticides in the mixture may occur because of enhanced bioavailability relative to the other mixture components. The concentrations in the organism required to produce toxicity were two and a half orders of magnitude lower than that

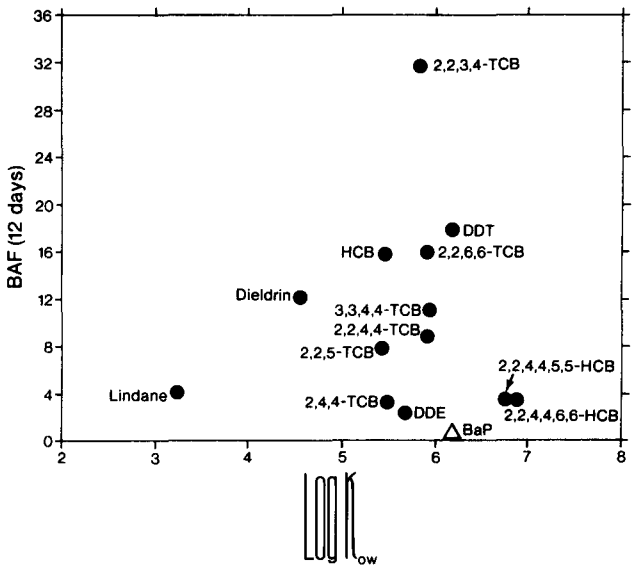

FIG. 2—*The scatter plot of the bioaccumulation factor (BAF) concentration in the organism divided by the concentration in the sediment against log* K_{ow} *tends to exhibit a peak in BAF. However, the data are quite scattered. The open triangle represents benzo(a)pyrene, the only compound that is not a chlorinated hydrocarbon.*

estimated to produce acute toxicity by a narcotic mechanism of action [*28*]. This helps confirm that the mechanism of toxic action is likely a specific mechanism of action and likely the neurotoxic action of the pesticides LIN and DIEL.

The time required to yield 50% mortality at even the lowest dose level was short, 118.97 h. The chlorinated hydrocarbon concentrations in the organisms required to yield 50% mortality at 118 h were only slightly less than that required to yield 50% mortality at 72 h with a similar result for the sum of the LIN and DIEL. Slopes of the acute mortality curves were steep and the effect of the compounds dropped off rapidly with reduction in concentration. These data further support the hypothesis of a specific mechanism of action producing the mortality.

In spite of all the data available on the toxicity of the mixture, determination of the exact compounds responsible for the mortality was not possible. Although it seems clear that inclusion of LIN and DIEL in the mixture accounts for most of the mortality, it was also not possible to determine what fraction of the toxicity was attributable to these compounds. The apparent enhanced biological availability compared to the rest of the compounds does suggest that these compounds will be ones of environmental concern.

Since the freely dissolved interstitial water radiotracer concentration was essentially constant across the various exposure levels, the freely dissolved chlorinated hydrocarbon concentrations were expected to depend on their individual partitioning to sediments and would reflect the total amount of materials at each dose. The implication of this partitioning, in this single sediment system, is that toxicity is proportional to the freely dissolved interstitial water concentration. However, the relative contribution of LIN and DIEL at the LC_{50} is about 50% lower in the sediment than in the organisms. Thus, a differential accumulation of LIN and DIEL is important for eliciting the toxic response. This differential accumulation does not support the hypothesis of equilibrium partitioning with subsequent uptake from interstitial water but supports differential desorption from the sorbed phase. Therefore, reliance on the interstitial water concentrations for prediction of a toxic response would be an inappropriate assumption.

The basic purpose of the toxicity data was to find a no effect level for the added compounds and to determine the toxicokinetics at the no effect level. Since even the lowest level of added chlorinated hydrocarbons produced effects, the toxicokinetics data for the nonlabeled compounds was that of organisms under stress. Uptake clearance for BaP, determined in the absence of added chlorinated hydrocarbons, was not different from that previously determined for *P. hoyi* from sediment [*23*]. The uptake clearance for both BaP and 2244TCB in the absence of added chlorinated hydrocarbons was about half that found in the presence of the lowest level of added chlorinated hydrocarbons. The rate for the 224455HCB at level one was not different from previous determinations in the absence of added chlorinated hydrocarbons [*5*]. However, the 224455HCB is not a very good compound for comparison of effects because the uptake estimate had to be made from the first sample point due to the extreme variability in the analytical data. Therefore, the uptake clearances for these stressed organisms are apparently an estimated factor of two greater than would be observed if no toxic effects occurred. Increased clearances imply either some stimulation of the organism or increased bioavailability. Determining which potential mechanism is responsible for the increases is not possible from the data. However, stimulation may be a reasonable mechanism because chlorinated hydrocarbons, particularly the chlorinated pesticides, are known to enhance the firing rate of neurons, and animals exhibiting toxic symptoms often show elevated activity levels [*29*]. Elevations in activity may result in increased contact with fresh interstitial water and/or increased ingestion of contaminated particles that would yield increased clearances. The potential of enhanced bioavailability as a mechanism for the increased clearances was not testable with the current data because of the mortality at higher dose levels.

The absence of proportionality between the freely dissolved compound in the interstitial water and the uptake clearance indicates that the accumulation of the compounds cannot occur strictly as a result of accumulation from sediment interstitial water. These data support our previous model of the accumulation of sediment-associated compounds by benthic organisms

(Fig. 3) [5]. Accumulation of sediment-associated compounds is thought to occur from ingestion of contaminated particles and uptake of freely dissolved compound from the sediment interstitial water. The sediment interstitial water pool of compound is perceived to be of limited size and not well mixed; as a result the concentration of compounds in the interstitial water is easily depleted by the organism. To the extent that the organism moves from one area of the sediments to another, it should encounter fresh sediment interstitial water. However, the main mechanism for maintaining the sediment interstitial water concentration is compound desorption from the particles and the dissolved organic matter pools. The particle pool is the largest, on a mass basis, and should provide the greatest mass of compound. The particle pool is, however, thought to be divided into two subpools, a readily reversible pool and a slowly reversible pool [*30,31*]. Previous data suggest that the material in the slowly reversible pool is biologically unavailable [5] over the short time scales of laboratory experiments. Thus, the rate of desorption from the rapidly reversible pool will dictate the rate of supply to the interstitial water and subsequently the rate of accumulation from the interstitial water by the biota. Accumulation through ingestion will depend on the rate of particle ingestion, gut throughput, and assimilation efficiency. Estimates for *P. hoyi* suggest that, for the more nonpolar compounds, the ingestion route could supply 50 to 100% of the accumulated dose, while the interstitial water is more important for the more water-soluble compounds with approximately 10% from the ingested sediments for compounds such as phenanthrene and anthracene [5].

If desorption rate is an important feature of sediment-associated compound accumulation by benthos, the rate of accumulation (uptake clearance) should be proportional to sediment partitioning within a compound class. This proportionality assumes that compounds within a class associate with the same sites and bind with the same mechanism. Different classes of compounds may have different binding mechanisms or binding sites for particles, and a relationship with partitioning between classes may not hold. This difference between classes is suggested from the clearance and partition data of BaP and 2244TCB in this work. Since partitioning is strongly related to the nonpolar molecular character, there should be a relationship between uptake clearance and the octanol-water partition coefficient (K_{ow}). The regression line for log K_s versus log K_{ow} for the chlorinated hydrocarbons confirms that the partitioning to sediments for a single compound class should be proportional to the hydrophobicity of the molecules while the desorption rate is inversely proportional to the hydrophobicity of the contaminants and is driving the accumulation rate. The range of the data is still large and may reflect, in part,

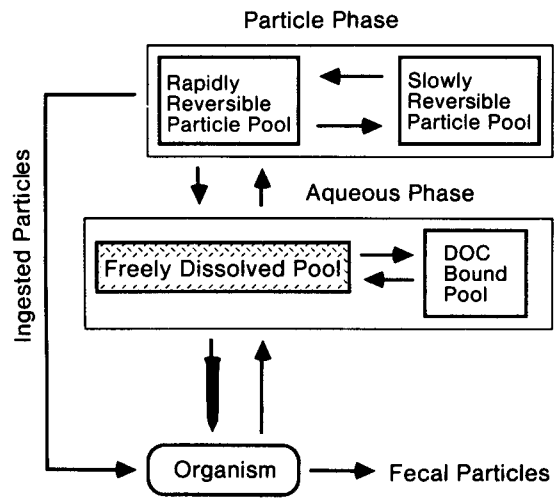

FIG. 3—*Conceptual model of the sources of sediment-associated contaminants to benthic organisms.*

differences in binding among the different chlorinated hydrocarbons, some of which are alicyclic and some aromatic.

The result of these complex interactions between accumulation and the toxicokinetics of the organisms results in a complex picture of the relative accumulation of compounds by *P. hoyi* as represented by the BAF. The BAF was quite variable for compounds at any specific log K_{ow}. This variability reflects the variability in the accumulation processes including desorption from particles as well as the variability in the ability of the organism to eliminate compounds. The end result of these interactions is a suggested peak in the BAF at approximately log K_{ow} of 6. However, even at log K_{ow} of 6 the range in BAF for the chlorinated compounds was greater than an order of magnitude. Some of this variability may have resulted from not attaining steady state since the BAFs were determined after only 12-day exposure. Thus, compound concentrations in the organism may have been at different fractions of steady state. Variability may also have been enhanced because of the effects of the compounds on the organisms. This suggested peak in BAF is similar to that found for oligochaetes feeding on both laboratory-dosed and natural sediments [*7,8*].

In summary, toxicity of the chlorinated hydrocarbon mixture was high even at the lowest concentration employed and resulted from the presence of the chlorinated pesticides, LIN and DIEL. Identification of the pesticides as the most probable toxicants was based on the body burden required to produce the toxicity and the independent experiments. In spite of the effects at the lowest level of exposure, estimates of the chlorinated hydrocarbon uptake clearances were obtained. These clearances were estimated to be about two times greater than would have occurred at a no effect level based on the BaP and 2244TCB kinetics in the presence and absence of the other added chlorinated hydrocarbons. The accumulation appeared to be driven by the desorption rate of the compounds from sediment. For a single class of compounds within a single sediment, the clearance is approximately inversely proportional to K_{ow}. The K_{ow} represents the strength of binding to sediments and therefore the inverse of the desorption rate. These data, in addition to that previously reported [*5*], indicate that the equilibrium partitioning approach to bioavailability of sediment-associated pollutants is inappropriate and the bioavailability is likely driven by the desorption rate. Further, the BAF is a complex measure of both sediment desorption, organism accumulation, and elimination and appears to result in an apparent BAF maximum for chlorinated hydrocarbons at approximately a log K_{ow} of 6.

References

[*1*] Baumann, P. C., "Cancer in Wild Freshwater Fish Populations with Emphasis on the Great Lakes," *Journal of Great Lakes Research*, Vol. 10, 1984, pp. 251-253.

[*2*] Nalepa, T. F. and Landrum, P. F., "Benthic Invertebrates and Contaminant Levels in the Great Lakes: Effects, Fates and Role of Cycling," *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus*, M. S. Evans, Ed., John Wiley & Sons, New York, NY, 1988, pp. 77-102.

[*3*] Eadie, B. J., Nalepa, T. F., and Landrum, P. F., "Toxic Contaminants and Benthic Organisms in the Great Lakes: Cycling, Fate and Effects," *Toxic Contamination in Large Lakes, Volume I: Chronic Effects of Toxic Contaminants in Large Lakes*, N. W. Schmidthe, Ed., Lewis Publishers, Chelsea, MI, Chap. 8, pp. 161-178.

[*4*] Knezovich, J. P. and Harrison, F. L., "The Bioavailability of Sediment-Sorbed Organic Chemicals: A Review," *Water, Air and Soil Pollution*, Vol. 32, 1987, pp. 233-245.

[*5*] Landrum, P. F., "Bioavailability and Toxicokinetics of Polycyclic Aromatic Hydrocarbons Sorbed to Sediments for the Amphipod, *Pontoporeia hoyi*," *Environmental Science and Technology*, Vol. 23, 1989, in press.

[*6*] Lee, H., Winson, M., Randall, R. C., Specht, D. T., and Boese, B. L., "Sediment and Water Pollutant Uptake Vector Determinations in the Deposit Feeding Clam, *Macoma nasuta*," Eighth annual meeting of the Society of Environmental Toxicology and Chemistry, Pensacola, FL, 9-12 Nov. 1987, Abstract No. 182, 1987.

[*7*] Oliver, B. G., "Uptake of Chlorinated Organics from Anthropogenically Contaminated Sediments by Oligochaete Worms," *Canadian Journal of Fisheries and Aquatic Sciences*, Vol. 41, 1984, pp. 878-883.

[*8*] Oliver, B. G., "Biouptake of Chlorinated Hydrocarbons from Laboratory-Spiked and Field Sediments by Oligochaete Worms," *Environmental Science and Technology*, Vol. 21, 1987, pp. 785-790.

[9] Frank, A. P., Landrum, P. F., and Eadie, B. J., "Polycyclic Aromatic Hydrocarbons Rates of Uptake, Depuration, and Biotransformation by Lake Michigan *Sytlodrilius heringianus*," *Chemosphere*, Vol. 15, 1986, pp. 317-330.

[10] Kadeg, R. D., Pavlou, S. P., and Duxbury, A. S., "Sediment Criteria Methodology Validation: Elaboration of Sediment Normalization Theory for Nonpolar Hydrophobic Organic Chemicals," prepared for U.S. Environmental Protection Agency, Criteria and Standards Division, Office of Water Regulation and Standards, Washington, DC, 1986.

[11] Neff, J. M., Bean, D. J., Cornaby, B. W., Vaga, R. M., Gubransen, T. C., and Scanlon, J. A., "Sediment Quality Criteria Methodology Validation: Calculation of Screening Level Concentrations from Field Data," prepared for U.S. Environmental Protection Agency, Criteria and Standards Division, Office of Water Regulation and Standards, Washington, DC, 1986.

[12] Pavlou, S. P. and Weston, D. P., Eds., "Initial Evaluation of Alternatives for Development of Sediment related Criteria for Toxic Contaminants in Marine Waters: Phase II: Development and Testing of the Sediment-Water Equilibrium Partitioning Approach," EPA Report 910/9-83-117, U.S. Environmental Protection Agency, Washington, DC, 1984.

[13] Poston, T. M. and Prohammer, L. A., "Protocol for Sediment Toxicity Testing of Nonpolar Organic Compounds," prepared for U.S. Environmental Protection Agency, Criteria and Standards Division, Office of Water Regulation and Standards, Washington, DC, 1986.

[14] Landrum, P. F., "Uptake, Depuration and Biotransformation of Anthracene by the Scud, *Pontoporeia hoyi*," *Chemosphere*, Vol. 11, 1982, pp. 1049-1057.

[15] Menzel, D. W. and Vaccaro, R. F., "The Measurement of Dissolved Organic and Particulate Carbon in Sea Water," *Limnology and Oceanography*, Vol. 9, 1964, pp. 138-142.

[16] Landrum, P. F., "The Effect of Co-contaminants on the Bioavailability of Polycyclic Aromatic Hydrocarbons to *Pontoporeia hoyi*," *Polynuclear Aromatic Hydrocarbons: Seventh International Symposium on Formation Metabolism and Measurement*, M. W. Cooke and A. J. Dennis, Eds., Battelle Press, Columbus, OH, 1983, pp. 731-743.

[17] Landrum, P. F., Nihart, S. R., Eadie, B. J., and Gardner, W. S., "Reverse-Phase Separation for Determining Pollutant Binding to Aldrich Humic Acid and Dissolved Organic Carbon of Natural Waters," *Environmental Science and Technology*, Vol. 18, 1984, pp. 187-192.

[18] Landrum, P. F., Nihart, S. R., Eadie, B. J., and Herche, L. R., "Reduction in Bioavailability of Organic Contaminants to the Amphipod, *Pontoporeia hoyi* by Dissolved Organic Matter of Sediment Interstitial Waters," *Environmental Toxicology and Chemistry*, Vol. 6, 1987, pp. 11-20.

[19] Grasshoff, K., Ehrhardt, M., and Kremling, K., *Methods of Seawater Analysis*, 2nd revised and extended ed., Verlag Chemie, Weinheim, FRG, 1983, pp. 61-72.

[20] Gardner, W. S., Frez, W. A., Chichocki, E. A., and Parish, C. C., "Micromethod for Lipid Analysis in Aquatic Invertebrates," *Limnology and Oceanography*, Vol. 30, 1985, pp. 1099-1105.

[21] *SAS Users' Guide: Statistics*, Version 5, SAS Institute, Inc., Cary, NC, 1985.

[22] Eadie, B. J., Landrum, P. F., and Faust, W., "Polycyclic Aromatic Hydrocarbons in Sediments, Porewater and the Amphipod, *Pontoporeia hoyi* from Lake Michigan," *Chemosphere*, Vol. 11, 1982, pp. 847-858.

[23] Landrum, P. F., Eadie, B. J., Faust, W. R., Morehead, N. R., and McCormick, M. J., "Role of Sediment in the Bioaccumulation of Benzo(a)pyrene by the Amphipod, *Pontoporeia hoyi*," *Polynuclear Aromatic Hydrocarbons: Eighth International Symposium on Mechanisms, Methods and Metabolism*, M. W. Cooke and A. J. Dennis, Eds., Battelle Press, Columbus, OH, 1985, pp. 799-812.

[24] Veith, G. D., Call, D. J., and Brooke, L. T., "Estimating the Acute Toxicity of Narcotic Industrial Chemicals to Fathead Minnows," *Aquatic Toxicology and Hazard Assessment: Sixth Symposium, ASTM STP 802*, W. E. Bishop, R. D. Cardwell, and B. B. Heidolph, Eds., American Society for Testing and Materials, Philadelphia, 1983, pp. 90-97.

[25] Call, D. J., Brooke, L. T., Knuth, M. L., Poirier, S. H., and Hoglund, M. D., "Fish Subchronic Toxicity Prediction Model for Industrial Organic Chemicals That Produce Narcosis," *Environmental Toxicology and Chemistry*, Vol. 4, 1985, pp. 335-341.

[26] O'Brian, R. D., *Insecticides Action and Metabolism*, Academic Press, New York, NY, 1967.

[27] Stehly, G. R., personal communication, Great Lakes Environmental Research Laboratory, Ann Arbor, MI 48105.

[28] McCarty, L. S., "The Relationship Between Aquatic Toxicity QSARS and Bioconcentration for Some Organic Chemicals, *Environmental Toxicology and Chemistry*, Vol. 5, 1986, pp. 1071-1080.

[29] Gilman, A. G., Goodman, L. S., and Gilman, A., *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 6th ed., Macmillan Publishing Co., New York, NY, 1980, pp. 1047-1055.

[30] Ditoro, D. M., Horzempa, L. M., Casy, M. M., and Richardson, W., "Reversible and Resistant Components of PCB Adsorption-Desorption: Adsorbant Concentration Effects," *Journal of Great Lakes Research*, Vol. 8, 1982, pp. 336-349.

[31] Karickhoff, S. W. and Morris, K. R., "Sorption Dynamics of Hydrophobic Pollutants in Sediment Suspensions," *Environmental Toxicology and Chemistry*, Vol. 4, 1985, pp. 469-479.

[32] Leo, A., Hansch, C., and Elkins, D., "Partition Coefficients and Their Uses," *Chemical Reviews*, Vol. 71, 1971, pp. 525-616.

[33] Burkhard, L. P., "Physical-Chemical Properties of the Polychlorinated Biphenyls: Measurement, Estimation and Application to Environmental Systems," doctoral dissertation, University of Wisconsin at Madison, 1984.

[34] Miller, M. M., Wasik, S. P., Huang, G., Shiu, W., and Mackay, D., "Relationships Between Octanol-Water Partition Coefficient and Aqueous Solubility," *Environmental Science and Technology*, Vol. 19, 1985, pp. 522-529.

[35] Verschueren, K., *Handbook of Environmental Data on Organic Chemicals*, 2nd ed. Van Nostrand Reinhold Co., New York, NY, 1983.