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A Comparative Study of the Bioconcentration and Toxicity of Chlorinated Hydrocarbons in Aquatic Macrophytes and Fish

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ABSTRACT: This study reports the uptake and elimination kinetics, the bioconcentration, and the acute toxicity of a series of chlorinated benzenes and biphenyls in a submerged aquatic macrophyte (*Myriophyllum spicatum*) and in a fish (*Poecilia reticulata*) species. The objective of this study is to investigate the relationship between the acute lethality in fish and in aquatic plants. The study shows linear relationships between the plant-water and fish-water bioconcentration factors and the 1-octanol-water partition coefficient, indicating that plant-water and fish-water exchange are largely controlled by the chemical's tendency to partition between the lipids of the plants or fish and the water. The toxicokinetics in both the plants and the fish involve "passive" transport phenomena, which can be described by a lipid-water kinetic model. Toxicity data demonstrate that the acute lethality of chlorobenzene and chlorobiphenyl congeners in fish is associated with an internal concentration in the fish of approximately 6330 μmol/L. Based on the similarity of the lethal internal concentration among the chlorobenzene congeners and between various aquatic organisms, it is hypothesized that the acute lethal toxicity of chlorobenzenes in plants and fish are similar, which would provide a basis for the extrapolation of toxicity data between fish and aquatic plants.

KEY WORDS: aquatic macrophytes, bioconcentration, kinetics, toxicity, chlorobenzene, PCB, fish, hydrophobicity

Aquatic toxicologists are usually interested in the effects of waterborne substances. A typical aquatic toxicity test therefore involves the preparation of a series of solutions with different concentrations of the tested substance. Then, a number of individuals of a certain aquatic species are exposed to each of these solutions for a defined period of time, after which a certain toxic endpoint is determined. One of the most widely used tests is the acute lethality test, where the number of dead test organisms at the end of the test is the toxic endpoint. The concentration which causes lethality to half of the individuals in the test, i.e., the LC_{50}, expresses the "toxicity" of the tested substance. These tests exist for a variety of aquatic species such as *Daphnia magna,* brine shrimp (*Artemia*), fathead minnows (*Pime-*

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phales promelas), and guppies (*Poecilia reticulata*). However, LC_{50} tests for aquatic plants are still under development and thus are relatively scarce at present.

In an effort to protect aquatic life, some of these acute lethality tests have now been incorporated in a legislative framework. For example, in the province of Ontario, an initiative has been launched which requires that municipal and industrial effluents undergo, on a regular basis, lethality tests with rainbow trout and a Daphnia species to ensure that the effluents are not toxic to life in the receiving water body. This approach is based on the premise that what is toxic to a rainbow trout is probably also toxic to other aquatic organisms, including macrophytes. In other words, it is assumed that the rainbow trout toxicity test is able to represent the toxic impacts to all organisms of the aquatic ecosystem. From a practical point of view, it may be necessary to adopt this approach since it is impossible to perform toxicity testing for every species exposed to the tested substance. However, it is conceivable that certain substances have little or no effect on rainbow trout but are toxic to other organisms, thus causing our safeguard for environmental protection to fail. It is likely that the chance of such an event increases when differences in the physiology and biochemistry between organisms are larger. Fundamental differences in physiology and biochemistry exist between plant and animal life. It is thus possible that the toxicity of a substance in a fish species is unrelated to that in plants.

To investigate the ability of lethality tests in fish to simulate the toxic impacts in aquatic plants, we will examine the mechanisms of uptake, elimination, and toxicity of a series of chlorobenzene (CB) and chlorobiphenyl (PCB) congeners in a plant and a fish species. The objective of this study is to determine if there is a similarity between the toxicokinetics and toxicity in aquatic plants and in fish. The scope of our study will be limited to a series of CB and PCB congeners. They represent a group of persistent industrial chemicals that are of environmental concern in many parts of the world. They were selected because they are nonreactive and considered to be very poorly metabolizable by many aquatic organisms including fish. By eliminating the potential of significant metabolic breakdown we attempt to facilitate the study of the toxicokinetics and toxicity mechanisms.

Experiments in Aquatic Macrophytes and in Fish

To investigate the mechanisms of chemical uptake, elimination, and bioconcentration in aquatic plants and in fish, we will briefly summarize the results of bioconcentration experiments in a submerged aquatic macrophyte species, *Myriophyllum spicatum*, and in the guppy *Poecilia reticulata*, which were performed in a similar fashion. A detailed description of the experiments and their results is presented elsewhere [*1,2*].

Bioconcentration in Myriophyllum spicatum

One hundred and twenty plants (*Myriophyllum spicatum*), with an average wet weight of 9 g and a lipid content of 0.2 ± 0.02%, were exposed for 25 days in a 150-L glass tank to an aqueous solution of 1,3,5-tri-, 1,2,4,5-tetra-, penta- and hexachlorobenzene and 2,2′,5,5′-tetra-, 2,2′,4,4′,6,6′-hexa-, 2,2′,3,3′,4,4′,5,5′-octa- and deca-chlorobiphenyl, delivered by a continuous flow generator column. During the experiment, the plants were in a submerged state, but freely floating in the water. No soils or sediment were present. Water and plant samples were collected throughout the experiment and analyzed as described by Gobas et al. [*1*]. After the 25-day uptake period, the plants were transferred to a tank that contained clean water which was continuously being filtered through an activated carbon filter to remove test chemicals eliminated by the plants. Chemical elimination from the plants was followed for up to 133 days.

Typical results of the uptake experiment are illustrated in Figure 1*a* for 2,2′,5,5′-tetrachlorobiphenyl. Figure 1*a* shows that during the uptake period, the chemical concentration in the plants increased with time to approach a constant level toward the end of the uptake period. After the uptake period, when plants were transferred to clean, uncontaminated water, a drop of the chemical concentration in the plants was observed (Fig. 1*a*). During the first 37 days of the elimination period, the concentrations of all chemicals in the plants dropped exponentially with time, corresponding to a linear decrease of logarithm of the concentration in the plant with time. During the remainder of the elimination period, the drop of the chemical concentration in the plant was somewhat slower, causing a loss of the initial linear relationship between the logarithm of the concentration in the plant and time [*1*]. The largest drop of concentration in the plant with time was observed for 1,2,4,5-tetrachlorobenzene, the smallest for octachlorobiphenyl.

Bioconcentration in Poecilia reticulata

Following a procedure similar to that described for the submerged aquatic macrophytes, 95 to 120 guppies were exposed to aqueous solutions containing CB and PCB congeners (Table 1) for up to 20 days. During this period, water and fish samples were taken and analyzed as in Ref *2*. The fish were then transferred to a depuration tank with clean water that was continuously being carbon filtered to follow the decrease of the concentration in the fish with time.

During the uptake period, the concentration of the test chemicals in the fish increased with time to approach a constant level. Figure 1*b* illustrates the increase of the concentration of 2,2′,5,5′-tetrachlorobiphenyl in the fish during the uptake period. For some of the PCB congeners, in particular those with log K_{ow} above 6.1, the duration of the uptake period was too short to reach a constant concentration in the fish. During the depuration experiment the chemical concentrations in the fish dropped exponentially, which is illustrated by the linear relationship between the logarithm of the concentration in the fish with time.

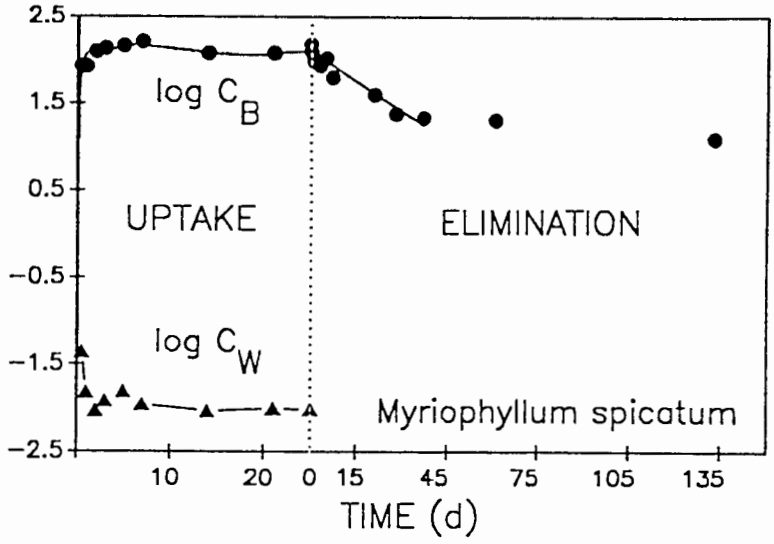

FIG. 1*A*—*Logarithms of the concentrations of 2,2′,5,5′-tetrachlorobiphenyl in the water* (▲), C_w (*μg/L*), *and in* Myriophyllum spicatum (·), C_B (*μg/L*), *during the uptake and elimination experiment. The solid line illustrates the model fit.*

TABLE 1—*The logarithm of the 1-octanol-water partition coefficient log* K_{OW}, *the rate constant for uptake from the water* k_1 ($L \cdot L^{-1} \cdot d^{-1}$), *the rate constant of elimination* k_2 (d^{-1}), *the logarithm of the bioconcentration factor log BCF, the logarithm of the lipid-weight-based bioconcentration factor log* K_L, *the logarithm of the* LC_{50} ($\mu mol/L$), *and the logarithm of the concentration at the site of action log* C_T ($\mu mol/L$) *of a series of chlorobenzenes and PCBs in the submerged aquatic macrophyte* Myriophyllum spicatum *and in the guppy* Poecilia reticulata. *NT means no acute lethality was observed.*

Compound	Log K_{OW}	k_1	k_2	Log BCF	Log K_L	Log LC_{50}	Log C_T
			MYRIOPHYLLUM SPICATUM				
1,3,5-Trichlorobenzene [*1*]	4.02 [*6*]	20	0.6	1.52	4.22		
1,2,4,5-Tetrachlorobenzene [*1*]	4.51 [*6*]	93	0.54	2.24	4.94		
Pentachlorobenzene [*1*]	5.03 [*6*]	275	0.2	3.14	5.84		
Hexachlorobenzene [*1*]	5.47 [*6*]	150	0.14	3.03	5.73		
2,2',5,5'-Tetrachlorobiphenyl [*1*]	6.10 [*7*]	450	0.09	3.70	6.40		
2,2',4,4',6,6'-Hexachlorobiphenyl [*1*]	7.00 [*7*]	500	0.02	4.40	7.10		
2,2',3,3',4,4',5,5'-Octachlorobiphenyl [*1*]	7.80 [*7*]	496	0.0008	5.79	8.49		
Decachlorobiphenyl [*1*]	8.26 [*7*]	162	0.0003	5.73	8.43		
			POECILIA RETICULATA				
Chlorobenzene	2.98 [*6*]					2.23 [*10*]	3.75
1,2-Dichlorobenzene	3.38 [*6*]					1.60 [*10*]	3.56
1,3-Dichlorobenzene	3.48 [*6*]					1.70 [*10*]	3.77
1,4-Dichlorobenzene [*8*]	3.38 [*6*]	98	1.0	1.99	3.18	1.43 [*10*]	3.39
1,2,3-Trichlorobenzene	4.04 [*6*]					1.11 [*10*]	3.86
1,2,4-Trichlorobenzene	3.98 [*6*]					1.12 [*10*]	3.79
1,3,5-Trichlorobenzene [*8*]	4.02 [*6*]	302	0.4	2.88	4.07	1.26 [*10*]	3.99
1,2,3,4-Tetrachlorobenzene	4.55 [*6*]					0.57 [*10*]	3.96
1,2,3,5-Tetrachlorobenzene [*8*]	4.65 [*6*]	1000	0.26	3.59	4.77	0.57 [*10*]	4.07
1,2,4,5-Tetrachlorobenzene	4.51 [*6*]					0.15 [*10*]	3.49
Pentachlorobenzene [*8*]	5.03 [*6*]	1738	0.11	4.20	5.39	−0.15 [*10*]	3.69
Hexachlorobenzene	5.47 [*6*]					NT [*10*]	
2,2',5,5'-Tetrachlorobiphenyl [*2*]	6.10 [*7*]	1122	0.0162	4.84	6.03	NT	
2,2',5,5'-Tetrachlorobiphenyl [*9*]	6.10 [*7*]	1202	0.015	4.90	6.09	NT	
2,2',4,4',5,5'-Hexachlorobiphenyl [*9*]	6.90 [*7*]	794	0.00398	5.30	6.49	NT	
2,2',3,3',4,4',5,5'-Octachlorobiphenyl [*9*]	7.80 [*7*]	151	0.0071	4.33	5.51	NT	
Decachlorobiphenyl [*2*]	8.26 [*7*]	41.7	0.005	3.92	5.11	NT	
Decachlorobiphenyl [*9*]	8.26 [*7*]	39.8	0.004	4.00	5.18	NT	

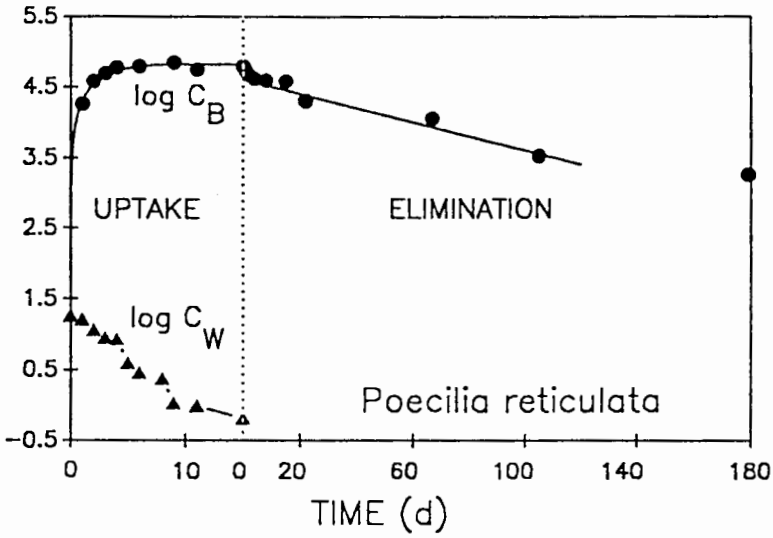

FIG. 1B—*Logarithms of the concentrations of 2,2',5,5'-tetrachlorobiphenyl in the water* (▲), C_W (*μg/L*), *and in* Poecilia reticulata (·), C_B (*μg/L*), *during the uptake and elimination experiment. The solid line illustrates the model fit.*

Toxicokinetics in Aquatic Macrophytes and Fish

Figure 1 illustrates that the plant-water and fish-water transfer of CBs and PCBs is a reversible process. The toxicant concentration in plants or fish rises when the plants or fish are introduced to the aqueous solution and declines when the chemical is no longer present in the water. The simplest description of this process can be derived by treating the plant, the fish, and the water as single, homogeneous compartments, each containing a certain chemical concentration. If, in addition, no chemical transformation occurs and chemical transfer between the plant or fish and the water is adequately represented by first order rate constants, the following two-compartment model can be proposed to describe the chemical exchange between the water and the plants or fish

$$d(V_B \cdot C_B)/dt = k_1 \cdot V_B \cdot C_W - k_2 \cdot V_B \cdot C_B \qquad (1)$$

where

C_B = the chemical concentration (μg/L) in the organism (i.e., plant or fish),
C_W = the chemical concentration (μg/L) in the water,
V_B = the volume of the plant or fish (L), and
k_1 ($L \cdot L^{-1} \cdot d^{-1}$) and k_2 (d^{-1}) = the rate constants for, respectively, chemical uptake into and chemical elimination from the plants or fish.

To fit this model to the observed time response of the chemical concentrations in the water and in the organism, Eq 1 can be integrated. This can be performed simply when the chemical concentration in the water, the volume of the plant or fish (V_B), and the rate

constants of chemical uptake and elimination do not vary with time (e.g., there is no growth), resulting in

$$C_B = C_W \cdot (k_1/k_2) \cdot \{1 - \exp(-k_2 \cdot t)\} \quad (2)$$

Equation 2 illustrates that, if the organism is exposed to a constant C_W, C_B should increase logarithmically with time to approach a constant level of $C_W \cdot (k_1/k_2)$, where (k_1/k_2) is often referred to as the bioconcentration factor BCF [3–5].

When the chemical concentration in the water is zero, such as during elimination when the organisms are exposed to clean water, integration of Eq 1 gives

$$C_B = C_{B,t=0} \cdot \exp(-k_2 \cdot t) \quad (3)$$

or

$$\log C_B = \log C_{B,t=0} - (k_2/2.303) \cdot t \quad (4)$$

where $C_{B,t=0}$ is the concentration (μg/L) in the organism at the beginning of the elimination period.

If the organism is growing and the chemical concentration in the water is not constant, such as in our uptake experiments, the model (i.e., Eq 1) can be fitted to the experimental data by a numerical integration procedure, which derives the chemical mass in the organism, i.e., X_B or $V_B \cdot C_B$ (in μg), as the sum of increments in chemical mass dX_B over time intervals dt, i.e., $X_B = \Sigma dX_B$. Each dX_B is calculated from Eq 1 as

$$dX_B = (k_1 \cdot V_B \cdot C_W - k_2 \cdot V_B \cdot C_B) \cdot dt \quad (5)$$

where dt should be chosen to be sufficiently small, and C_W and V_B at every exposure time t or Σdt, correspond with the experimentally observed values. Then, values for k_1 and k_2 are selected in an iterative fashion to produce the best agreement between calculated and observed X_B. The best fit of the observed data is the one with the k_1 and k_2 values, for which the sum of the squared differences between calculated and observed X_B is the smallest. This technique ensures that the estimates of k_1 and k_2, and thus the bioconcentration factor BCF, i.e., k_1/k_2, are not affected by the duration of the exposure period or by variations of the water concentration. This method was applied to determine k_1, k_2, and the BCF in the plants and fish, which are listed in Table 1. For this purpose, the time function of the water concentration and plant or fish volume during the experiments was established by fitting the observed values to a series of linear functions, each of which connect the observed values at two consecutive exposure times. The applicability of the model is represented by the quality of the fit, which can be expressed by the deviation, E, of the model predicted from the observed values, i.e.

$$E = \frac{\sum_{i=1}^{n} \sqrt{(C_{B,i}^0 - C_{B,i}^M)^2}/C_{B,i}^M}{n} \quad (6)$$

where C_B^0 is the observed, C_B^M is the predicted concentration in the plant or fish, and n is the number of observations. The deviation between observed and fitted concentrations ranged from 12 to 43% for the plants and 10 to 60% for the guppies, which is within the

range of experimental error associated with the plant and water analysis. This demonstrates that, considering the experimental error, the reversible organism-water two-compartment model with first order rate constants (i.e., Eq 1) satisfactorily describes the chemical exchange between the plants and water and between the fish and water.

Figure 1*b* shows that the exponential decrease of the concentration of 2,2',5,5'-tetrachlorobiphenyl in the guppies is in agreement with Eqs 4 and 5. The rate constants for chemical elimination, k_2, can thus be determined from the slope of the log C_B-time plot. The elimination rate constants agree with those derived from the uptake data. Figure 1*a* illustrates that during the first 37 days of the depuration period, the chemical concentrations in the plants also drop exponentially. However, after the first 37 days of the elimination period, the decrease of the chemical concentration in the plant tends to be somewhat slower. This does not agree with the plant-water two-compartment model. It indicates that the chemical accesses a small fraction of the plant at a slower rate than the majority of the plant. The plant may thus be more accurately represented by two compartments than by a single compartment [*11*]. However, for the purpose of this analysis, we will focus on the chemical kinetics during the initial 37 days, which represent the elimination of the majority of the chemical in the plant. During this time frame, the kinetics in the plant are satisfactorily described by a water-plant two-compartment model. Estimates of the elimination rate constant can thus be derived from the slope of the log C_B-time plot. A discussion of the elimination kinetics in plants is presented elsewhere [*1*].

This kinetic analysis demonstrates that an organism-water two-compartment model is able to give a satisfactory representation of the uptake and elimination of the investigated CBs and PCBs in both the guppies and the aquatic macrophytes. This implies that from a toxicokinetic point of view a fish and a plant can be treated as single homogeneous compartments. Studies of the anatomy and physiology of plants and fish have identified that there are many physiologically different compartments in the fish and the plant. A two- or multi-compartment model may thus be more a realistic description of the kinetics of chemicals in the plants and fish. However, the experimental detail of the uptake and elimination studies is not sufficient to distinguish between different compartments in the fish or plants. Consequently, the rate at which a chemical arrives at a target site of the fish or plant should be considered to be equal to the rate at which the chemical reaches other compartments in the organism. For the CBs and PCBs, this rate of chemical exchange is satisfactorily described by Eq 1 and the rate constants listed in Table 1.

Bioconcentration

The plant-water and fish-water bioconcentration factors are listed in Table 1 and plotted versus the 1-octanol-water partition coefficient (K_{OW}) in Fig. 2. Figure 2 demonstrates that the plant)water bioconcentration factor and the 1-octanol-water partition coefficients follow a linear relationship, i.e.,

$$\log \text{BCF} = 0.98\ [\pm 0.16] \cdot \log K_{OW} - 2.23\ [\pm 0.67]\ n = 8,\ r^2 = 0.97 \qquad (7)$$

where the confidence intervals have a 95% probability. The bioconcentration factors in the guppies also follow a linear relationship with K_{OW}, but only for chemicals which have a log K_{OW} less than 6.2, i.e.,

$$\log \text{BCF} = 1.03\ [\pm 0.24] \cdot \log K_{OW} - 1.30\ [\pm 0.58]$$
$$n = 6,\ r^2 = 0.97,\ \log K_{OW} < 6.2 \qquad (8)$$

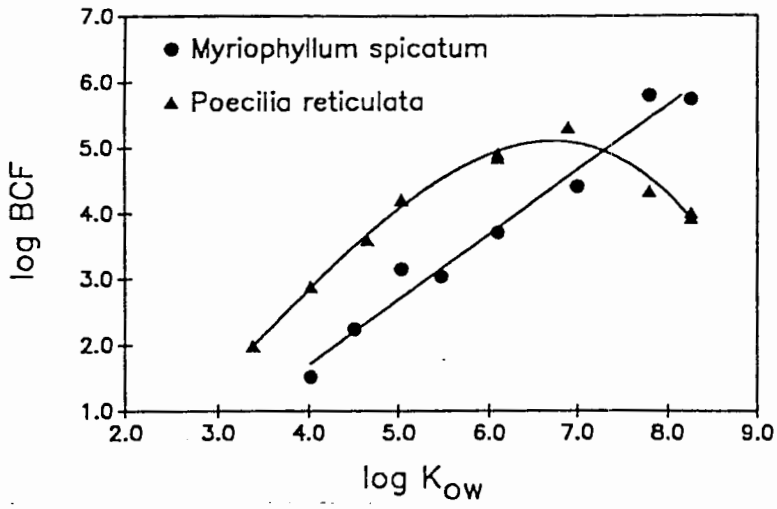

FIG. 2—*Relationship between the logarithm of 1-octanol-water partition coefficient, log* K_{OW}*, and the logarithm of the bioconcentration factor, log BCF, in* Myriophyllum spicatum *and in* Poecilia reticulata.

Equations 7 and 8 demonstrate that there is a strong relationship between the bioconcentration factors in plants and fish and the chemical's tendency to partition between water and 1-octanol. 1-Octanol is often considered to be a satisfactory surrogate phase for natural lipids. The 1-octanol-water partition coefficient therefore represents the chemical's ability to partition between lipids and water [*12*]. The excellent relationship between the BCF and K_{OW} suggests that chemical bioconcentration in the plant and fish is essentially a chemical partitioning process between the plant lipids and the water. This can be further illustrated by expressing the bioconcentration factor on a lipid weight basis as K_L. K_L is the ratio of the chemical concentration in extractable lipids of the plants or fish (C_L) over that in the water, i.e., C_L/C_W or $(C_B/C_W \cdot L_B)$ or BCF/L_B, where L_B (g/g) is the lipid content of the plant [i.e., 0.0020 (±0.00023)], or fish (i.e., approximately 0.055). Table 1 illustrates that the lipid-weight-based plant-water and fish-water bioconcentration factors are approximately equal to the 1-octanol-water partition coefficient. This suggests that chemical bioconcentration occurs predominantly in the extractable lipids of the plants and fish since the solubility of the test chemicals for 1-octanol and lipids are often similar [*12,13*]. It can thus be concluded that, in essence, bioconcentration of the investigated chemicals in the plant and fish is a thermodynamically controlled process determined by the affinity of the chemical for the plant lipids relative to that for the water. The driving force of this process is the higher solubility of the chemical in the plant and fish lipids compared to that in the water. The lipids will absorb the chemical until the ratio of the lipid/water concentrations equals the ratio of the chemical's activities or solubilities in the plant lipids and the water. This situation is often referred to as chemical equilibrium, where the chemical potential or fugacity of the chemical in lipids and water are the same [*5*]. After a chemical equilibrium has been established, there is no further net uptake of the chemical into the plant or fish. It thus appears that uptake and elimination of the CBs and PCBs in the plants and fish are passive processes, controlled by the chemical's thermodynamic gradient. Active transport, i.e., transport against the thermodynamic gradient which requires energy, is insignificant.

The linear relationship between the fish-water bioconcentration factor and the 1-octanol-water partition coefficient breaks down for chemicals with a log K_{OW} exceeding 6.2. Evidence

supports that this loss of linear correlation is caused primarily by (1) a reduction of the bioavailability of chemicals with very high K_{OW} during the experiment, and (2) the elimination of chemical by faecal egestion [2]. The reduction of the bioavailability is the result of the tendency of very hydrophobic (i.e., high K_{OW}) chemicals to sorb onto organic matter in the water introduced by the fish. As a result, a considerable fraction of the chemical in the water is in a sorbed or non-truly-dissolved state which cannot be absorbed by fish via the gills [2,14]. This sorption tendency and thus the fraction of the chemical concentration in a sorbed state tends to increase with increasing K_{OW}. Presently, there are no reliable techniques that can distinguish between sorbed and dissolved chemical in the water. As a result, water concentration measurements often reflect the total concentration of the chemical, not the truly dissolved chemical. The water concentration measurements thus overestimate the concentration in the water, which can actually be bioconcentrated by the fish, resulting in an underestimate of the bioconcentration factor and a loss of the linear BCF-K_{OW} relationship [2,15]. The loss of linear correlation due to a reduction in bioavailability is due to experimental difficulties regarding the measurement of the chemical concentrations in the water. It is not due to fundamental changes in the mechanism of the bioconcentration process if K_{OW} increases. The second factor that was identified to cause a loss of linear correlation is the chemical elimination in faecal matter [2,16]. In contrast to submerged aquatic macrophytes, guppies have the ability to eliminate chemicals not only to the water (i.e., via the gills), but also into faecal matter. The transfer of chemicals between the water and the fish during the bioconcentration experiment should therefore be described by

$$d(V_B \cdot C_B)/dt = k_1 \cdot V_B \cdot C_W - k_2 \cdot V_B \cdot C_B - k_E \cdot V_B \cdot C_B \quad (9)$$

where k_E is the rate constant (d^{-1}) for chemical elimination by faecal egestion. For chemicals with log K_{OW} less than 6.2, k_2 is much larger than k_E. Chemical elimination is therefore predominantly via the gills to the water and k_E can be ignored with respect to k_2, which simplifies Eq 9 to Eq 1. However, as we will demonstrate in more detail later, k_2 tends to drop with increasing K_{OW} and becomes smaller than k_E for chemicals with a log K_{OW} exceeding 6.2 [16]. For very hydrophobic chemicals, elimination is predominantly by faecal egestion and elimination to the water, i.e., k_2 can be ignored with respect to k_E, thus simplifying Eq 9 to

$$d(V_B \cdot C_B)/dt = k_1 \cdot V_B \cdot C_W - k_E \cdot V_B \cdot C_B \quad (10)$$

Equation 10 illustrates that for chemicals with log K_{OW} exceeding 6.2, chemical exchange is no longer between the fish and the water. Bioconcentration is therefore no longer a fish-water partitioning process, but it reflects the balance between the rates of chemical uptake from the water and chemical elimination by faecal egestion.

Factors Controlling Toxicokinetics in Plants and Fish

To explore the factors controlling the water-plant and water-fish exchange, it is interesting to plot the rate constants of chemical uptake and elimination as a function of the K_{OW} of the chemical. This has been done in Fig. 3 for the uptake rate constants in the plants and fish and in Fig. 4 for the elimination rate constants.

Figure 3 illustrates that for chemicals with a log K_{OW} below 5.5, the uptake rate constant (k_1) in the plant increases with increasing K_{OW} to approach a constant value of approximately 500 d^{-1} for chemicals with a log K_{OW} exceeding 5.5. The uptake rate constant in fish shows

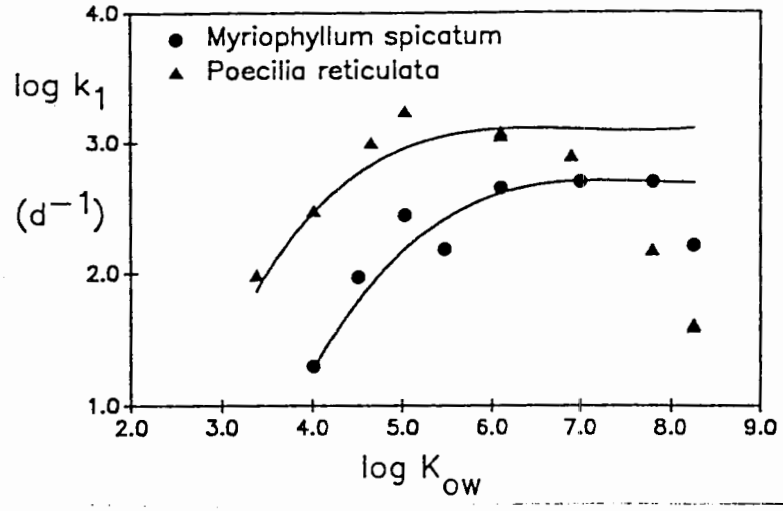

FIG. 3—*The logarithm of the uptake rate constant, log* k_1 ($L \cdot L^{-1} d^{-1}$), *versus the logarithm of the 1-octanol-water partition coefficient, log* K_{ow}, *for* Myriophyllum spicatum *and in* Poecilia reticulata. *The solid line represents the model fit, i.e., Eq 13 for the plants and Eq 15 for the guppies.*

a similar relationship with K_{ow}. However, for very high log K_{ow} chemicals (>6.2), k_1 tends to fall instead of remaining constant at a level of approximately 1200 d^{-1}. This drop of k_1 for the very high K_{ow} chemicals is believed to be caused by the incorrect measurement of the bioavailable concentrations of these chemicals in the water and is thus an artifact of the experimental procedures used.

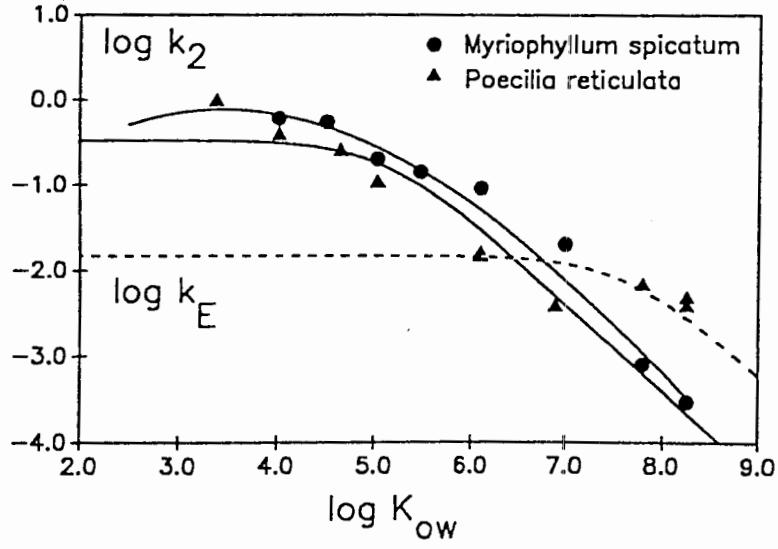

FIG. 4—*The logarithm of the elimination rate constant,* k_2 (d^{-1}), *versus the logarithm of the 1-octanol-water partition coefficient, log* K_{ow}, *for* Myriophyllum spicatum *and in* Poecilia reticulata. *The solid line illustrates the model fit, i.e., Eq 14 for the plants and Eq 16 for the guppies. The broken line represents the plot for* k_E.

Figure 4 shows that the elimination rate constant (k_2) in the plants and fish also tends to follow a "biphasic" relationship with K_{OW}. It shows that with increasing K_{OW}, the elimination rate constant drops, first slowly, but then more steeply. In particular for fish, the "biphasic" nature of the k_2-K_{OW} relationship is not as evident as that for the uptake rate constants. This may be due to the measurement of the elimination rate constants, which does not distinguish between elimination tothe water, i.e., via the gills (k_2), and elimination in faecal matter (k_E).

It has been proposed that the "biphasic" nature of the relationship between the rate constants and K_{OW} in the plants and fish is the result of the fact that chemical uptake from the water and elimination to the water involves chemical transport in aqueous and lipid parts of the plants or fish [*1,4,5,17,18*]. Examples of lipid phases in the plants and fish are the lipid bilayers of biological membranes, the plant's waxy cuticle or the mucus layers in fish. Transport in aqueous phases may involve the cytoplasm of cells or the water flow in the gill compartment of the fish. If chemical transport in water and lipid phases occurs in series, the following equations can be derived for the uptake and elimination rate constants [*1,5*]

$$1/k_1 = (V_B/D_W) + (V_B/D_L)/K_{OW} \tag{11}$$

$$1/k_2 = (L_B \cdot V_B/D_W) \cdot K_{OW} + (L_B \cdot V_B/D_L) \tag{12}$$

where D_W and D_L are transport parameters representing the transport rate in the aqueous and the lipid phases of the organisms. The derivation of Eqs 11 and 12 and an explanation of the lipid-water kinetic model for plants and fish can be found elsewhere [*1,5*]. In essence, Eqs 11 and 12 demonstrate that the uptake and elimination tend to be controlled by transport in the lipid phases when the chemical's K_{OW} is low. However, with increasing K_{OW}, chemical transport in the aqueous phases of the plant becomes more important and ultimately dominates the kinetics. Equation 11 thus predicts that with increasing K_{OW}, k_1 increases when transfer in the lipid phases (e.g., membranes) controls the uptake kinetics and then approaches a constant level (i.e., D_W/V_B) for high K_{OW} chemicals, for which transport in water phases becomes the rate-determining step. Likewise, Eq 12 illustrates that k_2 tends to be approximately constant (i.e., $D_L/V_B \cdot L_B$) for low K_{OW} chemicals, when transfer in lipid phases of the plant is the rate-determining process, and then drops with increasing K_{OW}, when transport through water phases controls the elimination process.

To test the applicability of this lipid-water kinetic model and to quantify the lipid and water phase transport parameters, Eqs 11 and 12 can be fitted to the experimental data, resulting in

Myriophyllum spicatum:

$$1/k_1 = 0.0020 + 500/K_{OW} \tag{13}$$

$$1/k_2 = 1.58 + 0.000015 \cdot K_{OW} \tag{14}$$

Poecilia reticulata:

$$1/k_1 = 0.00078 + 30/K_{OW} \tag{15}$$

$$1/k_2 = 1.0 + 0.000095 \cdot K_{OW} \tag{16}$$

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Figures 3 and 4 illustrate the excellent fit of the model to the experimental data. This indicates that the uptake and elimination kinetics in the plants and fish can be satisfactorily described by the same lipid-water kinetic model. The only lack of agreement between the model and the experimental data is for some of the very high K_{OW} chemicals. As mentioned earlier, this may be due to the fact that the measured concentration of very high K_{OW} chemicals in the water did not truly represent the chemical concentration that can be absorbed and bioconcentrated by the plants and fish.

From Eqs 13 and 14, it can be observed that the uptake and elimination kinetics of chemicals with a log K_{OW} below approximately 5.5 is predominantly controlled by transport in the lipid phases of the plant (e.g., transport across the lipid membranes). The uptake and elimination of chemicals with a log K_{OW} exceeding 5.5 are largely determined by transport in aqueous phases. The lipid and aqueous phase transfer conductivities can also be quantified. Equations 12 to 15 illustrate that in *Myriophyllum spicatum* D_W/V_B is between 133 and 500 d^{-1} and D_L/V_B is between 0.0013 and 0.0020 d^{-1} (L_B is 0.002). In the guppies, D_W/V_B is between 700 and 1300 d^{-1} and D_L/V_B is between 0.03 and 0.07 d^{-1}. The differences in the water and the lipid phase conductivities demonstrate that the plant and the guppies have their own specific water and lipid phase transport parameters. The water and lipid phase transport parameters are organism specific and reflect the differences in physiology and structure between the plant and the guppies. Based on the data from this study, it is not possible to identify the water and lipid phase transport processes in terms of actual transport processes in specific parts or tissues of the plants or fish. More detailed experiment are required to establish the nature of the lipid and aqueous phase transport processes.

Toxicity in Submerged Aquatic Macrophytes and Fish

Toxic effects in organisms, such as lethality in a LC_{50} test, are the combined result of the chemical concentration in the "target site" and the toxicity of the chemical. The toxicity of the chemical reflects the chemical's activity at the site of action. Therefore, to compare the toxicity of a chemical to that of another chemical or the chemical's toxicity in a plant to that in fish, it is necessary to determine the concentration at the site of action when the effect occurs. This can be achieved by direct measurements of the chemical concentration at the site of action. However, this is often difficult and therefore rarely performed. An alternative exists when in addition to data on toxic effects, such as LC_{50} values, the kinetics of the toxicant in the organism are available. We will demonstrate this approach for the guppy.

Chemical Toxicity in the Guppy

In a typical lethality test, guppies are exposed for up to 14 days to a constant concentration of the test chemical in the water. The concentration in the water which causes mortality to 50% of the guppies, or the LC_{50}, is often reported to express the "toxicity" of the chemical. The LC_{50} values of the CBs and PCBs are listed in Table 1 and plotted versus the 1-octanol-water partition coefficient in Fig. 5. Figure 5 illustrates that a chemical of high K_{OW} tends to cause mortality at a lower concentration than a chemical of low K_{OW}. This relationship breaks down if log K_{OW} exceeds 5.5, above which no acute lethality is observed. The reciprocal relationship between the LC_{50} and K_{OW} has often been interpreted by assigning a greater toxicity to high K_{OW} chemicals. This interpretation is not entirely correct since it equates toxic effects (i.e., 50% mortality) to chemical toxicity, thus ignoring the chemical concentration in the organism that triggered the effect. To determine the relative toxicities of the CBs, we need to consider the chemical concentration in the organism when the toxic effect

occurs. This can be achieved by considering the uptake and elimination kinetics of the CB and PCB congeners in the guppy. Earlier, it was shown that uptake and bioconcentration of CBs and PCBs in the guppy can be satisfactorily described by treating the guppy as a single homogeneous compartment. This implies that the chemical accesses the site of action at approximately the same rate as the rest of the fish, which is described by Eq 1. Since during the lethality test the chemical concentration in the water is constant, Eq 1 can be integrated to give Eq 2, which provides a means to determine the concentration at the site of action, C_T, as a result of exposure to a concentration of chemical in the water (C_W) for a period of time t. The target site concentration causing 50% mortality after 14 days of exposure can thus be estimated for each chemical by substituting the LC_{50} for C_W, 14 for t, and the appropriate rate constants (Table 1) in Eq 2, i.e.,

$$C_T = LC_{50} \cdot (k_1/k_2) \cdot \{1 - \exp(-k_2 \cdot 14)\} \quad (17)$$

In this fashion, the target site concentrations (C_T) for 50% acute lethality were calculated for the CBs, listed in Table 1 and plotted versus K_{OW} in Fig. 5.

Figure 5 shows that the target site concentrations of the CBs in the guppy are approximately similar at a level of 6330 (±2770) μmol/L. They are not dependent on K_{OW}. Since each CB congener causes 50% mortality at approximately the same concentration in the guppy, it appears that the toxicity of all CB congeners is essentially the same [*19*]. It is interesting that for a series of linear alcohols and ketones a similar internal concentration of 6000μmol/L was estimated to cause 50% mortality in fathead minnows. Based on the LC_{50} values of approximately 90 organic substances in fathead minnows, guppies, *Daphnia magna*, and the saltwater brine shrimp *Artemia*, Abernethy and Mackay [*20*] estimated that 50% mortality occurs when the chemicals reach a fairly constant volume fraction in the organisms of approximately 0.63%, which corresponds to a concentration of approximately 6000μmol/

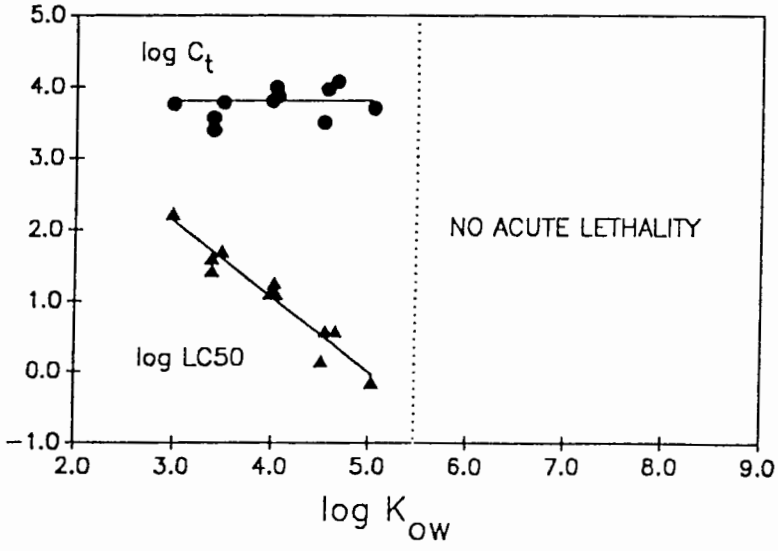

FIG. 5—*The logarithm of the LC_{50} and the internal concentration in the guppy as a function of the logarithm of the 1-octanol-water partition coefficient. For the congeners with a log K_{OW} above 5.5, no acute lethality has been observed.*

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L. These results suggest that for a certain group of organic chemicals, including the CBs and PCBs, one specific chemical concentration in the organism causes 50% lethality in all of the investigated organisms.

It appears that the internal concentration causing lethality tends to be independent of the type of chemical. It has been suggested that this mode of action is related to that of narcosis or anaesthesia, which also tends to be the result of one particular concentration or chemical activity at the site of action and fairly independent on the type of chemical [*21*]. The chemicals that cause acute lethality at a constant internal concentration are also causing narcosis and they are therefore often referred to as "narcotics." The actual mechanisms of acute lethality or narcosis are unclear. However, it is suspected that the chemical has a "physical" effect on a lipid-like target site, possibly the membrane systems of the organism [*22*]. Chemicals that cause lethality at a lower internal concentration in the organisms exert their toxic action through a different mechanism. They are often reactive chemicals, which tend to interact with specific proteins or receptors in the organism.

Estimates of the internal concentrations of narcotics suggests that the acute lethality in several aquatic organisms is associated with the same internal chemical concentration of approximately of 6000 μmol/L. It is therefore tempting to speculate that this internal concentration of approximately 6000 μmol/L is universal among aquatic organisms, including plants, thus suggesting that the toxicity of the CBs is independent on the organism and similar in plant and animal life. It is possible that the chemicals interfere with fundamental molecular processes required for the proper functioning of the cell. In that case, one specific concentration in the organism may trigger acute lethality in virtually all organisms, including plants. However, it is also conceivable that the internal lethal concentration in the plant is different from that in animal life since membranes may be the site of action and plant and animal membranes are fundamentally different. Unfortunately, there are only few LC_{50} data for aquatic macrophyte species, which makes it difficult to test this hypothesis. In absence of appropriate LC_{50} data, it is interesting to examine the results of a study by Wong et al. [*23*] regarding the effects of CBs on the primary productivity of the freshwater green algae *Ankistrodesmus falcatus*, which are summarized in Table 2. Assuming that the 4-h exposure period was sufficiently long to reach equilibrium and that the BCF can be expressed as $L_B \cdot K_{OW}$, where L_B is the lipid content of the algae (i.e., 1%), it is possible to estimate the internal concentration C_T in the algae causing the 50% reduction in primary productivity. The estimates of C_T, which are listed in Table 2, demonstrate that all CB congeners cause a 50% reduction in primary productivity at approximately the same internal concentration in the algae of 4840 (±1,430) μmol/L. Considering the error in the calculations, the internal concentration in the algae causing a 50% reduction in primary productivity is not significantly different from the internal lethal concentration in the guppies. The results further demonstrate that, similar to acute lethality in fish, congeners with a very high K_{OW}, such as hexachlorobenzene, do not demonstrate the toxic effects.

It can be concluded that in the guppy, and possibly in other aquatic organisms as well, the acute toxicity of CB congeners is essentially the same. The extent to which lethality (i.e., the effect) occurs thus reflects the concentration in the organism, which in turn is the result of the chemical concentration in the water, the rates of uptake and elimination, and the duration of chemical exposure. As demonstrated earlier, the uptake and elimination rates, and thus the relationship between the concentration in the water and that in the organism, vary between organisms such as guppies and aquatic plants and are dependent on the K_{OW} of the chemical. This explains the role of chemical properties, such as the K_{OW}, on the acute lethality and differences in "sensitivities" between organisms. For example, the LC_{50} of pentachlorobenzene is lower than that of monochlorobenzene because pentachlorobenzene has a higher bioconcentration factor, which is related to its higher K_{OW} and

TABLE 2—*The logarithm of the 1-octanol-water partition coefficient log* K_{ow}, *the logarithm of the* EC_{50} *(μmol/L), and the logarithm of the internal concentration log* C_T *(μmol/L) of a series of chlorobenzenes in the fresh water green algae* Ankistrodesmus falcatus. *NT means no reduction in primary productivity was observed.*

	ANKISTRODESMUS FALCATUS		
Compound	Log K_{ow}	Log EC_{50}	Log C_T
Chlorobenzene	2.98 [6]	0.444	3.63
1,2-Dichlorobenzene	3.38 [6]	0.136	3.51
1,3-Dichlorobenzene	3.48 [6]	0.156	3.67
1,4-Dichlorobenzene	3.38 [6]	0.136	3.51
1,2,3-Trichlorobenzene	4.04 [6]	0.033	3.56
1,2,4-Trichlorobenzene	3.98 [6]	0.033	3.50
1,3,5-Trichlorobenzene	4.02 [6]	0.05	3.72
1,2,3,4-Tetrachlorobenzene	4.55 [6]	0.019	3.83
1,2,3,5-Tetrachlorobenzene	4.65 [6]	0.014	3.80
1,2,4,5-Tetrachlorobenzene	4.51 [6]	0.023	3.87
Pentachlorobenzene	5.03 [6]	0.005	3.73
Hexachlorobenzene	5.47 [6]	NT	

thus requires a lower concentration in the water than monochlorobenzene to reach the same lethal concentration in the organism. The PCB congeners which have very high K_{OW} are unable to trigger acute lethality in the guppies because the largest possible concentration in the water, i.e., the congeners's aqueous solubilities, is too small to achieve the internal lethal concentration in the guppies.

Conclusions

This study demonstrates that the main driving force of the uptake and bioconcentration of chlorobenzenes and PCBs in the submerged macrophyte species *Myriophyllum spicatum* and in fish, i.e., *Poecilia reticulata*, is the tendency of hydrophobic chemicals to partition between the lipids of the plant or fish and the water. The mechanism of chemical uptake and elimination in the plants and the guppies is essentially the same and appears to involve passive transfer of the chemical as a result of diffusion or convection by natural fluid flow processes in the organisms (e.g., gill flow in fish). Active uptake or elimination of the chlorobenzenes and PCBs does not appear to be a significant process. In absence of metabolic transformation, the plants and fish are therefore incapable of controlling their internal concentrations of CBs and PCBs.

When plants and fish are exposed to the same concentration of chlorobenzene or PCB congeners in the water, the lipids of the plants and fish tend to approach approximately the same concentration, reflecting the chemical's lipid-water partition coefficient. Very hydrophobic chemicals with a log K_{OW} exceeding approximately 6.2 are the only exception. Their bioconcentration potential in fish is less than that in the plants. Except for experimental problems with the measurement of the bioavailable concentration in the water, this is largely the result of chemical elimination by faecal egestion.

The rate of uptake and elimination and thus the time to reach equilibrium differs between the plants and the fish. However, the dynamics of chemical exchange in the plants and fish can be described by the same lipid-water kinetic model. This model illustrates that the uptake and elimination of chemicals with a log K_{OW} less than approximately 5.5 are largely controlled by transport in lipid phases of the plants or fish, whereas for higher K_{OW} chemicals the rate determining process is in an aqueous phase.

Acute lethality tests in guppies suggest that an internal concentration of any of the chlorobenzene congeners in the fish of approximately 6330 μmol/L causes 50% lethality. This

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demonstrates that the toxicity (i.e., the activity at the site of action) of the CB and PCB congeners is the same. Although there are limited data for acute lethal effects in plants, it is conceivable that a similar internal lethal concentration applies to plants. In that case, differences in the acute lethal response in plants and fish will only reflect the uptake and elimination dynamics of the chemical in the plants and fish. Since this study suggests that the mechanism of uptake and elimination of the CBs and PCBs in the plants and the fish are similar, it is conceivable that plants and fish respond similarly to aqueous concentrations of CBs and PCBs. It should be emphasized that this similarity in toxic response may only apply to acute lethal effects, which tend to occur at relatively high concentrations in the water. There may be other, possibly nonlethal, effects that apply to fish, but not to plants or visa versa. This issue may only be resolved with continued research on the mechanisms of chemical uptake and toxicity.

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