

# RELATIONSHIP BETWEEN BIOCONCENTRATION IN FISH AND STERIC FACTORS OF HYDROPHOBIC CHEMICALS

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## ABSTRACT

Most polychlorinated naphthalenes (PCN) accumulate rapidly according to their hydrophobicity. The uptake and elimination rate constants are comparable to those of chlorinated benzenes and biphenyls.

For most PCN-congeners the resulting bioaccumulation factors show an increase with increasing hydrophobicity. For higher  $K_{d,oct}$ -values ( $>10^5$ ) however, no further increase of  $K_C$  is observed ( $K_{C,max} = 3.5 \cdot 10^4$ ).

For the two hepta- and the octachloronaphthalenes no detectable concentrations are found in the fishes, although no restricted bio-availability could be expected. Based on these observations and on data obtained from the literature, a loss of membrane permeation is suggested for hydrophobic molecules with widths over 9.5 Å.

In addition a membrane permeation model, as part of the accumulation process of hydrophobic chemicals, is proposed, which is based on diffusion and partition processes.

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## INTRODUCTION

Relationships between the molecular characteristics and uptake and elimination rates in biota have been investigated for about a decade.<sup>1,2</sup> In studies with polychlorinated biphenyls and related compounds, good relationships between bioaccumulation parameters and hydrophobicity are obtained. Hydrophobicity, which itself is directly related to the molecular structure<sup>3</sup>, is a dominant structure characteristic in various distribution processes. For this reason standardized processes, like octan-1-ol/water partitioning ( $K_{d, oct}$ )<sup>4</sup> or aqueous solubility, are often used as representative hydrophobicity parameters. Between these standardized processes and other distribution processes in which hydrophobicity is a dominant factor, often good correlations are found. For instance relationships between  $K_{d, oct}$  and Reversed Phase HPLC retention<sup>5</sup>, adsorption onto soil<sup>6</sup>, aqueous solubility<sup>7</sup>, bioaccumulation<sup>8</sup> and membrane permeability<sup>9</sup> are described. In addition, also relationship between hydrophobicity and toxicity are investigated<sup>10,11</sup>.

In several studies with extremely hydrophobic compounds however bioaccumulation and octan-1-ol/water partitioning do no longer correlate<sup>12,13,14</sup>. This lack of correlation is sometimes due to the fact that  $K_{d, oct}$  is not always a successful hydrophobicity parameter.<sup>15</sup>

In most cases however a decrease or a loss of accumulation of the hydrophobic chemicals in biota is observed. For several compounds like for hexabromobenzene, this lack of accumulation is explained by a reduced bio-availability of the chemicals, due to their extremely low aqueous solubilities.<sup>16,17</sup> In some other studies various other factors related to the molecular structure, like metabolism<sup>18,19</sup> or steric factors influencing the uptake and elimination transfer rates, are mentioned.<sup>14,15,20,21,22,23</sup>

Polychlorinated naphthalenes can be expected to bioaccumulate comparable to PCB's due to the similarity in chemical properties.<sup>24</sup> For some congeners this is confirmed in some previous studies with terrestrial<sup>25,26,27</sup> as well as with aquatic organisms.<sup>21,28</sup> On the other hand, some highly chlorinated naphthalenes show some structure similarity to octachlorodibenzo-p-dioxin, and hexabromobenzene which are not accumulated in a previous study.<sup>14</sup> For this reason, accelerated tests with fishes to study the mechanism, as well as the uptake and elimination rate kinetics and the bio-availability of PCN's could provide more specific information about the relationship between bioaccumulation and steric factors of hydrophobic chemicals.

## MATERIAL AND METHOD

**Chemicals:** Individual chloronaphthalenes, as well as an industrial mixture (Halowax 1051) were used in the experiment together with 2,3',4',5 tetrachlorobiphenyl and pentachlorobenzene.

2-Monochloronaphthalene and pentachlorobenzene were purchased from Baker. The 1,4-di, 1,8-di, 2,3-di, 2,7-di, 1,3,7-tri, 1,2,3,4,-tetra, 1,3,5,7-tetra and 1,3,5,8,-tetrachloronaphthalenes were synthesized.<sup>24</sup>

The 2,3',4',5 tetrachlorobiphenyl and octachloronaphthalene were available from

previous studies. Halowax 1051 was obtained from Kopper Corporation. After recrystallization all individual compounds were of > 99% purity as confirmed by GC-ECD. Halowax 1051, containing 1,2,3,4,5,6,7-hepta-, 1,2,3,4,5,6,8-hepta- and octachloronaphthalene was not purified.

**Solvents:** n-Pentane (Janssen), n-hexane (Baker) and toluene (Baker) were dried overnight with Molecular sieve (4A) Perflorm (Baker) and redistilled after addition of 2.0 g/2.5 liter dri-Na (sodium lead alloy) (Baker) and 2 ml propan-2-ol.

**Fish:** One year old female guppies (*Poecilia reticulata*) were used with lengths between 20-28 mm, and average weights between 330-370 mg. A 18 hours light - 6 hours night cycle was used throughout the experiment with 'daylight' fluorescenecent lamps.

**Exposure:** In one experiment all the individual chlorinated naphthalenes congeners and, in addition, pentachlorobenzene and 2,3',4',5 tetrachlorobiphenyl were administered. In the second experiment only Halowax 1051 was used.

In both experiments, a continuous flow system for saturating water with test compounds was used, which was similar to the one in a previous study.<sup>29</sup> The aquarium water was a mixture of 50% demineralized water - 50% Amsterdam tap water, at 22<sup>0</sup> C.

In the exposure period, oxygen was carefully added by capillary tubes to prevent rapid evaporation of the test compound. The oxygen content during the exposure was 7 mg/l.

Experiment 1. To 5 gram Chromosorb W (acid washed) a solution of 25 mg of each of the individual chlorinated compounds in 200 ml n-pentane was added. After removal of the solvent, the coated chromosorb was placed in the funnel of the water saturation system containing 40 l water. Ten days after the continuous flow system was started, the chromosorb was removed and 73 guppies were placed into the contaminated water.

During exposure water and fish samples were taken after 1, 2, 3, 4 and 7 days. After 7 days the remaining 23 fishes were placed into an aquarium with clean water to study the elimination process. The water was continuously filtered and aerated over activated carbon by a 'bubble-up' aquarium pump. The activated carbon was replaced every 4 days. In this period fish samples were taken after 3, 8, 18, 43, 74 and 84 days.

Experiment 2. A solution of 93 mg Halowax 1051 in 200 ml n-pentane was added to 3 g chromosorb W (acid washed). After removal of the solvent the chromosorb was

Chemical  
analysis

added to the water saturation system containing 25 l water. After 26 days the contaminated chromosorb was removed. After an additional 3 days, 35 guppies were placed into the contaminated water and left there for 7 days. During this period water and fish were sampled every day.

Extraction of Fish Samples: After sampling two or three fishes they were killed in liquid nitrogen, measured and weighed, and homogenized in a mortar. The sample was stirred and heated under reflux in a water/n-hexane or a water/toluene mixture (1:1) for 90 minutes. After cooling, the organic phase was separated. This fraction was divided in two equal portions: one for determination of the extractable lipid weight, and one for gaschromatographic analysis of the chloro-compounds.

The extractable lipid weight was determined by weighing after evaporation of the organic solvent by N<sub>2</sub>-stream. The other organic fraction of the crude extract was concentrated by evaporation and poured over columns of silica-sulphuric acid (40% by weight) and silica-sodium hydroxide (1N, 33% by weight) using 10 ml organic solvent, in order to remove lipids and other compounds. The extracts were concentrated and analyzed at GC-ECD.

The recoveries of the extraction procedures ranged from 89% to 96%, when 1 ml of a standard solution of the testcompounds in hexane was added to clean fishes after they were homogenized.

Water samples: Water samples of 100 ml were extracted two times with 25 ml organic solvent. For 250 ml and 500 ml samples two times 50 ml organic solvent were used. After concentration the extracts were analyzed at GC-ECD.

The recoveries in this extraction procedure ranged from 97% to 100%, when 1 ml of a standard solution of the testcompounds in n-hexane was added to 100, 250 or 500 ml of clean water.

Gas chromatography: A Tracor 550 gas chromatograph equipped with a <sup>63</sup>Ni-Electron Capture Detector and Tracor 560-EC-Linearizer connected to a Spectra-Physics 4100 Computing Integrator was used.

The column characteristics and GC-conditions used are summarized below:

Column	: glass, 2.5 m x 2 mm i.d.
Carrier gas	: Argon/Methane 95%/5%
Flow rate	: 20 ml/min.
Detector make up rate:	80 ml/min.

## Temperature conditions

Inlet : 300°C

Outlet : 250°C

Detector : 350°C

## Column packing

carrier	chromosorb W (130-150 mesh)	chromosorb W (150-170 mesh)
stationary phase	SE 30.3%	OV 1.3%

## Temperature programs

Initial temperatures	110°C	160°C
Initial time	12 min.	-
Rate	10°C/min.	2°C/min.
Final temperature	160°C	200°C
Final hold	12 min.	2 min.

## RESULTS AND DISCUSSION

Aqueous Solubility Water was circulated in the saturation system for 10 days before equilibrium concentrations were reached for all compounds. The measured aqueous solubilities (table 1) of the chloronaphthalenes with one to four chlorine atoms are in agreement with measured and calculated values for chlorinated benzenes and biphenyls of comparable hydrophobicity.<sup>30,31,32</sup> For the hepta's and octachloronaphthalenes the measured concentrations are higher than expected from calculations. However, similar results were previously obtained for highly chlorinated biphenyls.<sup>14,32</sup> Significant differences between calculated and experimental solubilities arise when the former is below 1 µg/l. Normally it is assumed that this is due to the incompetency of the saturatingsystems to prevent introduction of small particles or micelles into the water.

Hitherto however, it can not be ascertained that other aspects are involved. For chlorinated biphenyls for instance, there is no general agreement about the perspectives for calculating the solubility. Especially when the melting points of the compounds under investigation are high, these different perspectives of calculation can result in significant variations in predicted solubilities.<sup>32</sup>

Toxicological Effects

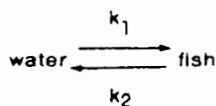
After placement into the contaminated water, most of the guppies showed loss of balance and orientation for several days. In addition, a mortality of 50% of the population exposed to

individual chloronaphthalenes within 7 days was observed. Exposure to individual PCB's at similar concentrations did not show comparable mortality, although sometimes loss of balance and orientation was observed.<sup>29</sup>

In the population exposed to the Halowax 1051 a mortality of less than 10% was observed during the 7 days exposure. They showed no disturbance of balance or orientation.

#### Bioaccumulation

Kinetics. In general the bioconcentration process is kinetically described by a first order two compartments exchange model, as initially proposed by Branson et al.<sup>2</sup>



$$\frac{dC_f}{dt} = k_1 C_w - k_2 C_f \quad (1)$$

$k_1$  and  $k_2$ : uptake and elimination rate constants ( $d^{-1}$ )

$C_f$  and  $C_w$ : concentrations in fish and water.

This model normally gives valid descriptions for bioconcentration processes in accelerated tests for hydrophobic compounds which metabolize slowly or not at all.

For constant pollution concentrations in water ( $C_w$  is constant) equation 1 can be integrated to

$$C_f(t) = K_C C_w (1 - e^{-k_2 t}) + C_f(t_0) e^{-k_2 t} \quad (2)$$

In which  $t$  is the exposure time and  $C_f(t_0)$  the concentration in fish at  $t = 0$ . When  $C_f(t_0) = 0$ , this equation is simplified to

$$C_f(t) = K_C C_w (1 - e^{-k_2 t}) \quad (3)$$

From this equation a bioconcentration factor  $K_C$  can be defined when  $t \rightarrow \infty$

$$K_C = \frac{k_1}{k_2} = \frac{C_f}{C_w} \quad (4a)$$

Simple predictions of either the bioconcentration factor and the concentrations in fish and water with variable exposure concentrations in the water can be made not until a steady-state is obtained in which

$$\frac{dC_f}{dt} = 0 \quad (4b)$$

After reaching steady-state in the fish concentrations, equation 1 can be used to calculate the bioconcentration factor described by equation 4a.

Predictions of the concentrations in the compartments as well as the uptake and elimination rate constants can be made, only when during the whole exposure period both the amounts of fish and water are constant<sup>33</sup>. It is obvious that in the present study such a method for estimating uptake and elimination rate constants could not be used because both fishes and water were sampled during the exposure period.

Therefore, an elimination period is used to determine the elimination rate constant ( $k_2$ ). During elimination, if  $C_w=0$ , equation 3 is altered into:

$$C_f(t') = C_f(t'=0)e^{-k_2 t'} \quad (5)$$

Combining this  $k_2$  value with  $K_C$  value determined at steady state give the uptake rate constants ( $k_1$ ).

In figures 1.a to 1.k the results of the analyses of the fish samples are shown. At various moments, several samples ( $n$ ) containing three fishes each, were analyzed. For all compounds the standard deviations ( $n \geq 3$ ) ranged from 5.7% up to 14.3% of the mean concentration shown in the figure 1. In figure 1.k, the standard deviation of the 2,3',4',5 tetrachlorobiphenyl concentration in the fish samples is illustrated. From the data of the fish samples, combined with the data of the aqueous concentrations, the uptake and elimination rate constants, the bioconcentration factors and the biological half-life times of the PCN's are calculated, as reported in table 1.

In tabel the concentrations based on wet weights of the fishes are used for calculation of  $k_1$ ,  $k_2$ ,  $K_C$  and  $t_{1/2}$ . For calculations based on fat-weights the concentrations of the solutes must be multiplied by the reciprocal of the fat content. This will result in a change of the proportion of  $k_1$ .

The highest concentrations in fish are found for 2-chloronaphthalenes ( $\approx 500 \mu\text{g/g}$ ), due to the high aqueous exposure concentration. This level is reached within four days. No further increase is observed for this compound due to the limited available amount in the aqueous system. Also for the di- and trichloronaphthalenes, as well as for pentachlorobenzene, no significant increase in fish concentration is observed after four days exposure. For the tetrachloronaphthalenes and the tetrachlorobiphenyl the concentrations in fish show an ongoing increase. Hence it is likely that these compounds did not reach steady-state concentrations in the fish at the end of the exposure period. For these compounds, the bioconcentration factor, as reported in tabel 1, are estimated by the ratios of pollutant concentrations in fish and water



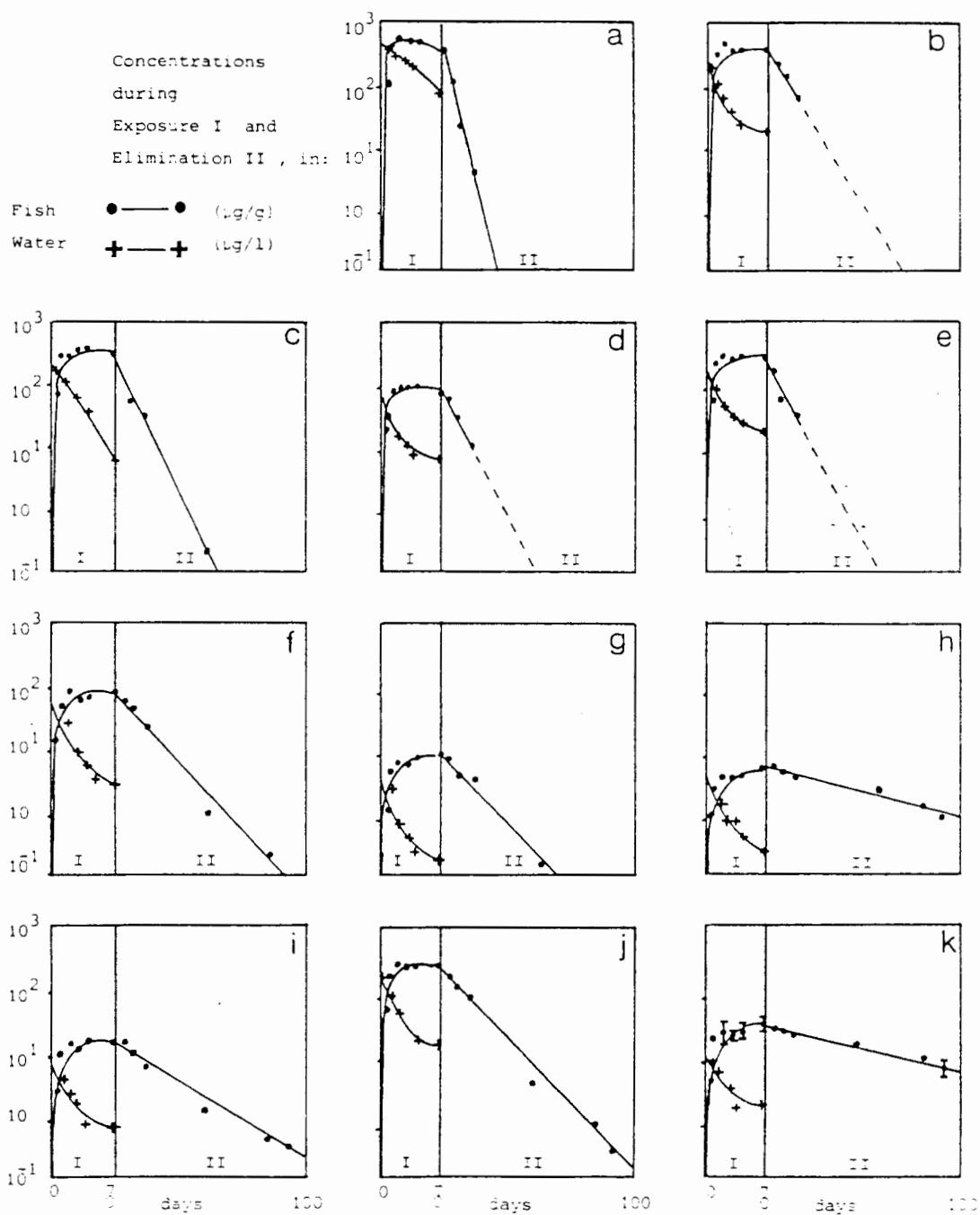


Figure 1. Concentrations in Fish and Water of: 2-mono(a)-; 1,4 di(b)-; 1,8 di(c)-; 2,3 (di(d)-; 2,7 di(e)-; 1,3,7 tri(f)-; 1,2,3,4 tetra(g)-; 1,3,5,7 tetra(h)-; 1,3,5,8 tetra(i)-chloronaphthalene, pentachlorobenzene (j) and 2,3',4',5-tetrachlorobiphenyl during exposure (I) and elimination (II).



samples at the end of the exposure period. This means that the reported bioconcentration factors are probably underestimates.

Although sufficient exposure concentrations are detected in all water samples during the whole exposure period no detectable uptake (detection limit 5 pg) of the two hepta- and the octachloronaphthalene is observed.

The reported elimination rate constants of the other compounds (table 1) are calculated by using linear regression analysis and equation 5. All compounds showed high correlation coefficient ( $r > 0.98$ ).

The uptake rate constants are calculated using equation 4a and the values of  $K_C$  and  $k_2$ . Control of these values with equation 3 (assuming  $C_W$  to be a constant) and with the method reported elsewhere<sup>33</sup> (assuming that the fish density is constant), supported the validity of these data. Only for 1,3,7- and 1,2,3,4-tetrachloronaphthalene the reported values of 3400  $d^{-1}$  and 3300  $d^{-1}$ , are probably too high, due to the relatively high  $k_2$  values (see relationships). Alternative calculations<sup>33</sup> resulted in values of 1720  $d^{-1}$  for the 1,3,7 tri- and 1340  $d^{-1}$  for the 1,2,3,4 tetrachloronaphthalene.

Table 1. Physico chemical properties and bioconcentration parameters of some chlorinated aromatic hydrocarbons.

	$S(\text{mol/l})$	$\log k_{d,\text{oct}}$	$k_c$	$k_1(d^{-1})$	$k_2(d^{-1})$	$t_{1/2}(d)$
Chloronaphthalene	$5.7 \cdot 10^{-6}$	4.19	$4.3 \cdot 10^3$	$7.3 \cdot 10^2$	$3.1 \cdot 10^{-1}$	0.3
1,4 Di "	$1.6 \cdot 10^{-6}$	4.68	$3.3 \cdot 10^3$	$1.2 \cdot 10^3$	$1.1 \cdot 10^{-1}$	6.2
1,8 Di "	$1.6 \cdot 10^{-6}$	4.41	$6.1 \cdot 10^3$	$9.8 \cdot 10^2$	$1.6 \cdot 10^{-1}$	4.3
2,3 Di "	$4.4 \cdot 10^{-7}$	4.71	$1.1 \cdot 10^4$	$1.6 \cdot 10^3$	$1.4 \cdot 10^{-1}$	5.1
1,7 Di "	$1.2 \cdot 10^{-6}$	4.81	$1.1 \cdot 10^4$	$1.6 \cdot 10^3$	$1.4 \cdot 10^{-1}$	5.1
1,3,7 Tri "	$2.8 \cdot 10^{-7}$	5.59	$1.7 \cdot 10^4$	$2.3 \cdot 10^3$	$8.4 \cdot 10^{-2}$	8.3
				$1.7 \cdot 10^3 *$		
1,2,3,4 Tetra "	$1.6 \cdot 10^{-8}$	5.94	$3.3 \cdot 10^4$	$3.3 \cdot 10^3$	$9.9 \cdot 10^{-2}$	7.0
				$1.3 \cdot 10^3 *$		
1,3,5,7 Tetra "	$1.6 \cdot 10^{-8}$	6.38	$1.4 \cdot 10^4$	$7.5 \cdot 10^2$	$2.2 \cdot 10^{-1}$	30.0
1,3,6,8 Tetra "	$5.1 \cdot 10^{-8}$	5.96	$1.5 \cdot 10^4$	$1.2 \cdot 10^3$	$4.8 \cdot 10^{-2}$	15.8
Heptachlorobenzene	$1.2 \cdot 10^{-7}$	5.46	$1.7 \cdot 10^4$	$1.4 \cdot 10^3$	$7.8 \cdot 10^{-2}$	8.9
1,2,3,4,6,8 hexa- chloronaphthalene	$3.1 \cdot 10^{-8}$	6.19	$1.1 \cdot 10^4$	$3.8 \cdot 10^2$	$1.6 \cdot 10^{-1}$	38.4

\* value estimated by the method reported in ref. 33.

**Mass balance.** The compounds in the aquatic system are distributed between water and fish by:

$$M_t = C_{fish,t} \cdot F_t + C_{w,t} \cdot W_t \quad (6)$$

In which  $M_t$  represents the total amount of test compound,  $F_t$  the wet weight of the fish and  $W_t$  the weight of the water mass, all at time  $t$ . During the experiment  $M_t$ , as well as  $F_t$  and  $W_t$  are not constants, due to sampling of fishes and water.

In this study no significant increase of the average fish weight ( $F_t$ ) is observed. So, no corrections have to be made on the kinetics as presented above.

As is shown in table 2, for some compounds mass-balance recoveries over 100% of the initially introduced amounts of testcompounds are calculated. These high values indicate probably that the estimated amounts in water and living fish at the end of the exposure period ( $t = 7$ ) are too high. The lower chlorinated naphthalenes as well as pentachlorobenzene show significant lower recoveries. For some congeners this is perhaps due to metabolism in fish or to degradation by microorganisms.

In addition it is also possible that the losses of testcompounds are caused by evaporation. This, because the most volatile compounds show the highest losses. For the 2 chloronaphthalene more than 35% of the initial amount is lost within 7 days. This loss of testcompound in both fish and water during the whole exposure period can also be observed in figure 1. a.

Table 2. Mass balance of test compounds in aquarium during the exposure period ( $\mu\text{g}$ ).

	Water* (t=0)	Removed by sampling of water	Water* (t=7)	Removed by sampling of fish	Living fish* (t=7)	Unexplained $A-(B+C+D+E)$	% recovery $(\frac{B+C+D+E}{A})$
2 monochloronaphthalene	21060	600	2930	7000	2770	7760	53%
1,4 Di "	8660	190	860	4730	2040	790	91%
1,8 Di "	9430	230	1370	4360	2040	1430	55%
2,3 Di "	2340	50	230	1250	560	240	90%
2,7 Di "	6435	185	820	2370	1740	1320	75%
1,3,7 Tri "	1910	30	80	1065	670	75	95%
1,2,3,4 Tetra "	117	2	8	70	55	- 18	115%
1,3,5,7 Tetra "	117	3	5	68	56	- 18	115%
1,3,5,8 Tetra "	310	5	20	200	120	- 36	110%
Pentachlorobenzene	6820	115	540	3620	2050	535	91%
2,3',4',5' tetra- chlorobiphenyl	470	10	45	230	200	- 15	103%

\*Estimated values.

Relationships between hydrophobicity and accumulation parameters: It is often considered that hydrophobicity is the driving force in the accumulation of chlorinated aromatic hydrocarbons. The figures below show the curves of  $k_1$ ,  $k_2$  and  $K_C$  of the test compounds, together with data of chlorobenzenes<sup>34</sup> tested previously, versus their hydrophobicity. The latter parameter is measured by reversed phase HPLC and expressed as  $K_{d,oct}$ .<sup>35</sup> The plot of  $\log k_2$  versus  $\log K_{d,oct}$  show comparable shapes for both groups of compounds, whereas the plots of  $\log k_1$  and  $\log K_C$  versus  $\log K_{d,oct}$  of chlorobenzenes and -naphthalenes show also similarity. In the plots only the intercepts are slightly different, which can be caused by differences in the experimental conditions.

In figure 2. a it is shown that the uptake rate constants of the naphthalenes in this study, are almost similar to those determined for chlorobenzenes previously.<sup>35</sup> In both studies the maximal uptake rate constant is  $\approx 1500 \text{ (d}^{-1}\text{)}$ , based on wet weight.

In addition is shown that  $k_1$  is independent of the hydrophobicity for compound with  $\log K_{d,oct}$  values  $> 4.0$ . This is in agreement with predictions based on models of membrane permeation of hydrophobic compounds, reported elsewhere.<sup>36</sup>

The rates of elimination of most chloronaphthalenes are close to that of pentachlorobenzene. Only the rate of 1,3,5,7 tetrachloronaphthalene is closer to 2,3',4',5 tetrachlorobiphenyl. The rate constant of the latter compound of this study is close to the value as reported previously.<sup>29</sup> For pentachlorobenzene a value of 0.078 is found, whereas a value of 0.11 is reported.<sup>34</sup>

In figure 2. b the 1,2,3,4 tetrachloronaphthalene is an exception of the curve. The relative high  $k_2$  value, compared to its  $K_{d,oct}$  value, indicates that metabolism is probably involved in the elimination process. Metabolism of lower chlorinated naphthalenes in aquatic organisms, is shown various times.<sup>28,37</sup> However, since no search for metabolites is incorporated in this study this potential influence is not confirmed.

As is shown in table 1 as well in figure 2. b, the elimination rate constants become extremely low for 'superlipophilic' compounds. This means that in elimination studies up to 100 days, only a very slight decrease of the concentrations will be found. So, actual excretion of the compounds becomes negligible under these conditions. Thus, during a short term uptake study, according to equation 1, a linear accumulation model can be used, as expressed by

$$C_f(t) = k_1 C_{w,t} \quad (7)$$

This, combined with the observation that also the uptake rate constants are almost identical for 'superlipophilics', means that also the bioconcentration factors becomes independent of the molecules hydrophobicity, as is shown in figure 2. c. The latter observation however, is solely a result of experimental limitations, since from a theoretical point of view the negative relationship between  $\log k_2$  and  $\log K_{d,oct}$  will hold.<sup>36</sup>

According to equation 7, the increase of the pollutants concentrations of 'superlipophilics' in fish is no longer dependent of the hydrophobicities of the compounds, but solely on the

Figure 2.a

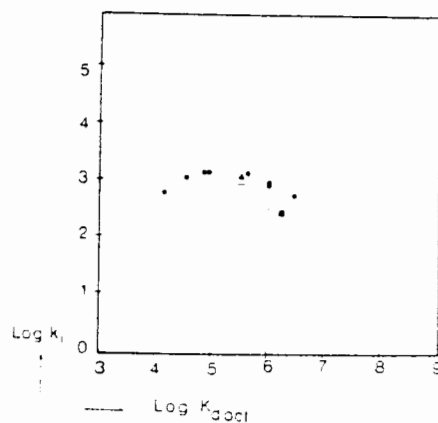


Figure 2.b

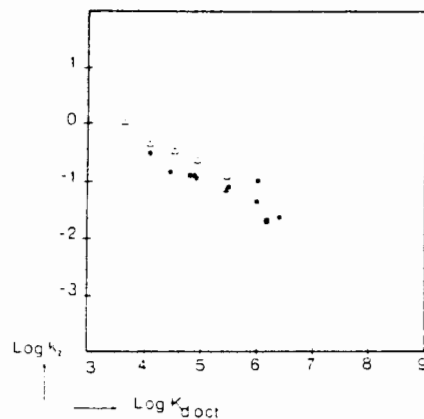
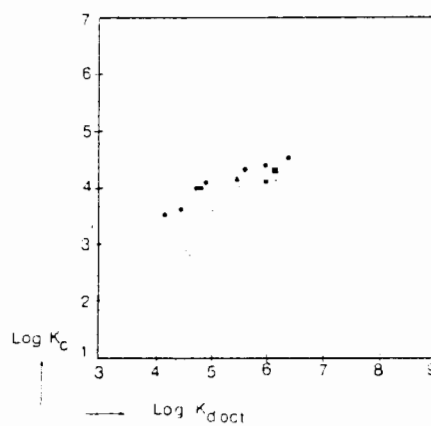


Figure 2.c



Relationship between octan-1-ol / water partition coefficients and uptake rate constants (2.a), elimination rate constants (2.b) and bioconcentration factor (2.c) of chlorobenzenes ( $\Delta$ , from ref. 34) chloronaphthalenes ( $\bullet$ ) and 2,3',4',5-tetrachlorobiphenyl ( $\blacksquare$ ).

exposure concentrations ( $C_w$ 's) and for some specific chemicals to rates of metabolism.

Since, in absence of metabolism, the fish concentrations are proportional to the exposure concentrations, plotting of the  $C_f/C_w$  ratio versus  $C_{sat}$  is useless since such plots will have slopes of  $k_1 \cdot t$  which are dominated by the exposure time.

In situations of oversaturation of the aqueous phase the result of a  $C_f/C_w - C_{sat}$  plot or a  $C_f/C_w - K_{d,oct}$  plot is even worse. This, while under such conditions  $C_f(t)$  is limited by  $k_1 C_{sat} t$ .

The resulting decrease of the  $C_f/C_w$  ratio is related to an increasing oversaturation of the waterphase ( $C_w > C_{w,sat}$ ) which will obviously give a negative slope.

Lack of uptake of several hydrophobic compounds. When bioaccumulation is considered to be a physico-chemical distribution process by equation 1, or by alternative expressions<sup>8,15</sup>, the exchange between the water phase and the organic (fish) phases are assumed to be pure diffusion processes. So the bioconcentration factor should depend only on the ratio between the activity coefficients in the involved phases and thus on the ratio of the rates of exchange in between the phases. The absolute values of the exchange rates determine only the rate of reaching steady state levels, but is unimportant for the bioconcentration factor.

For several types of hydrophobic chemicals however, these assumptions are not valid. For instance for some pharmaceuticals a mediated uptake into organisms is reported.<sup>38</sup> This phenomenon implies that the interface between the physico-chemical phases, actively participates in the overall process, so that the transfer rates are not determined by diffusion alone.

The absence of accumulation of the hepta- and octachloronaphthalenes as well as octachlorodibenzo-p-dioxin and hexabromobenzene in previous studies<sup>14,16,19</sup>, indicates that the physico-chemical equilibration across the membranes is prevented. To explain this phenomenon it is sometimes suggested that membrane permeation is impossible for molecules over 500-600 Dalton.<sup>16</sup> This, however, is not very plausible, since octachlorobenzo-p-dioxin (m.w. 400) is not accumulated, while decachlorobiphenyl (m.w. 499) shows an almost linear accumulation over time.<sup>14</sup>

The most important interfacial contact surface between the fish body and water are the gills, because of the oxygen demand of the organisms.<sup>39</sup> For most hydrophobic compounds the uptake rate across the gills is very high, due to the high ventilation volumetric rate ( $R_V$ ).<sup>40</sup> For non-accumulating compounds the lack of uptake should be caused by spatial configurations of both gills structure and hydrophobic chemical.

In the transport of chemicals across the gills at least the following steps can be distinguished:

- transport of the contaminated water into the gills
- diffusion of the contaminant from the water towards the cellmembranes
- uptake of the contaminant into the cellmembranes

Transport of contaminated water into the gills is regulated by the movement of the mouth and the operculum of the fish.<sup>41</sup> As a result, water will flow from buccal- to operculum cavity, passing the gill filaments, as illustrated in figure 3.

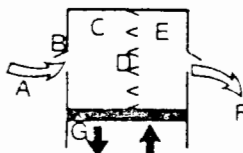


Figure 3. Water flow through the gill, from exterior (A), through the mouth (B), the buccal cavity (C), passing the gill filaments (D) and operculum cavity (E) to the exterior (F), regulated by movement of the operculum (G).

For hexabromobenzene and other highly hydrophobic chemicals it is previously suggested that uptake is limited due to the extreme low aqueous solubility.<sup>16,17</sup> However, since decachlorobiphenyl is accumulated in fish, a reduced availability in the gill's structure, is not a suitable explanation.

Diffusion of the chemicals from the water flow channel towards the cell-membranes of the epithelial cells of the gill filaments is the second step in the uptake process, which can be disturbed. At these filaments, and in particular on its secondary lamellae, exchange of the contaminant as illustrated in figure 4 is regulated by diffusion.

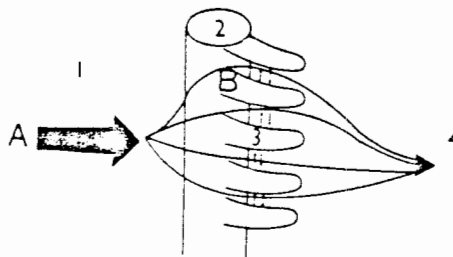


Figure 4. Flow of contaminated water (A) from buccal cavity (1) to operculum cavity (4) passing the lamellae (3) of the filaments (2), at which exchange of the contaminant (B) takes place.

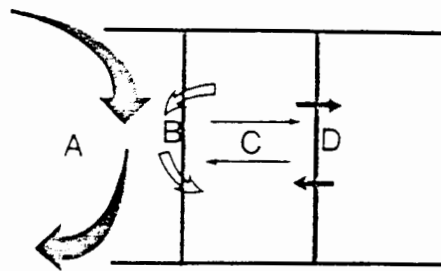


Figure 5. Solute's transfer across the gill filament-lamellae surface, resulting from waterflow (A), partitioning between water and mucus (B), diffusional transport through mucus (C) and partitioning between mucus and membrane (D).

Histologically, several pure diffusion layers are located in between the contaminated water, and the epithelial cells. In figure 5, these layers are summarized and indicated as mucus. During transfer from water to mucus, a concentration ratio of the compound at the mucus/water interfaces will arise, due to differences in the characteristics of these physico-chemical layers. This interface however, can not act as a barrier for pure diffusions.

In the diffusion layers adjacent to this interface the mass transport can be expressed with Fick's law by<sup>42</sup>:

$$J = (D/\delta) \Delta C \cdot A \quad (8)$$

Here, J denotes the flux of a contaminant with diffusion coefficient D, across surface area A, due to a concentration  $\Delta C$  over a distance  $\delta$ . In fish A and  $\delta$  are fixed by the structure of the gills, while  $\Delta C$  is due to the contaminants exposure to the gills. In addition, the transport rate is effected by the molecular characteristics of the diffusant, expressed by D.

For diffusion in homogeneous media the diffusion coefficient can be expressed in terms of molecular characteristics of the diffusant according to the Sutherland-Einstein relationship<sup>36</sup>:

$$D = \frac{R \cdot T}{N} \cdot \frac{1}{6\pi\eta} \sqrt{\frac{34 \cdot \pi \cdot N}{M \cdot v}} \quad (9)$$

where R, T and N are the gas constant, the absolute temperature and the Avogadro number,  $\eta$  the diffusion layer's viscosity, and M and v are the diffusant's molecular weight and specific volume. Since in biological diffusion layer the constituent molecules are more organized perhaps an other, more empirical relationships between D and M are more valid. For instance for a series of



structurally related non-electrolytes to diffusion coefficient in various biological membranes  $D_m$  showed a molecular weight ( $M$ ) dependence to<sup>43</sup>:

$$D_m = D_0 \cdot M^{-S_m} \quad (10)$$

where  $D_0$  is the calculated diffusion coefficient for a permeant of unit molecular weight and  $S_m$  is a parameter describing the mass selectivity of the membrane.

A similar relationship between diffusion coefficient and molecular weight was found for diffusion of non-electrolytes in soft polymers.<sup>44</sup>

Combination of the equations 8, 10 and 11 show that the contaminants transport the gills, indicated by  $J$ , will decrease at least with the cube root of the molecular weight. Since  $J$  is related to the uptake rate constant  $k_1$  of equation 1 by:

$$J = k_1 \cdot C_w \cdot F \quad (11)$$

In which  $F$  is the fish weight, the same relationship between  $k_1$  and  $M$  will hold.

Thus although it can be assumed that the uptake rate constant is influenced by molecular weight of the contaminant, it is very unlikely that total loss of uptake will result for hepta- and octachloronaphthalenes of this study.

This is supported by the observations that octa- and decachlorobiphenyls are accumulated in comparable studies.

**Uptake of the contaminant into the cell-membranes** of the epithelial cells is the last step which can disturb the uptake of the contaminant. In the gills several types of cells are located onto the mucus. Although there are cytological variations in composition, it is considered that the biochemical constructions of the bilipid membranes will show similarity.

Generally, various types of membrane permeation models are available for different types of permeants.<sup>45,46,47,48</sup>

For hydrophobic compounds often the following description is used<sup>9</sup>:

$$J = \frac{\Delta C' \cdot A'}{R_{aq} + R_m/K_m} \quad (12)$$

In which  $R_{aq}$  and  $R_m$  denote transport resistances of the permeant in water- and lipid-phases. These resistances are reciprocal to the diffusivities ( $D/s$ ) in the indicated layers (figure 6). The  $K_m$  denotes the contaminants partition coefficient between the outer- and innerside of the bilipid membrane.  $\Delta C'$  and  $A'$ , indicate that these values are not similar to those used in equation 8. For compounds with very high lipid/water partition coefficients, and thus for extremely hydrophobic compounds, it is assumed that the value of  $R_m/K_m$  will rapidly go to zero. Then, the flux across the membrane is regulated by an aqueous diffusion processes.

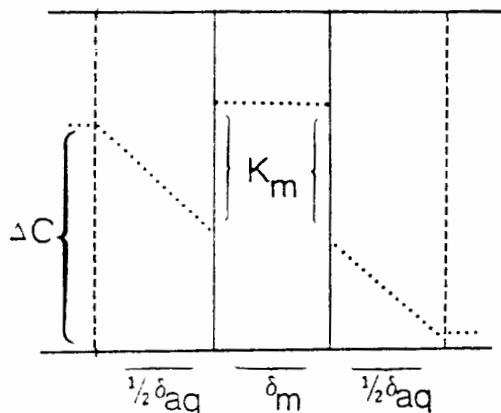


Figure 6. Concentration gradients for diffusional transport across aqueous- (aq) and lipid (m) membrane layers.

In that case, the flux across the membrane is regulated by the diffusion rate in the aqueous diffusion layer, so that the relationship between diffusivity and molecular weight as reported above for mutual diffusion becomes relevant (equations 9 and 10).

Although the viscosity ( $\eta$ ) and thickness ( $\delta$ ) of the layers, and the frictional resistance ( $r$ ) of the permeant probably are different in these layers, still no structure selectivity resulting in an absolute lack of uptake can be explained.

Since it is considered that diffusion layer control of membrane transport is caused by the value of  $K_m$ , the flux is independent to small variations in  $R_m$ . This consideration however, is probably doubtful for larger molecules, because the membrane resistance might increase.<sup>49</sup> In this view  $R_m$  not only represents the permeant's lipid diffusivity, but also the transition resistance of the permeant across the 'polar heads' of the lipids.

This transfer across the 'polar heads' is to the best of our understanding, the only part of the uptake process which can directly be related to the permeant's configuration.

A lack of membrane permeation for specific compounds is feasible when energy barriers are present which are related to steric factors of both permeant and membrane structure.

In the permeation concept of hydrophobic chemicals 'holes' in the hydrophilic outer surface of the membrane must be formed, to allow entrance of the permeant into the hydrophobic zone (figure 7). Thus, transport of a molecule is on the one hand dependent at the amount and sizes of the membranes 'holes' and on the other hand at the size of the permeant.

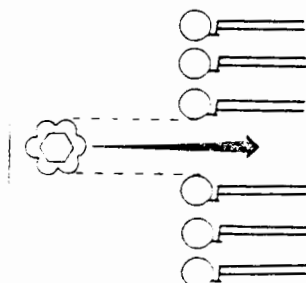


Figure 7. Schematic representation of the transfer of a hydrophobic molecule across the polar heads of a bilipid membrane in relation to effective cross section of the membrane's cavity.

The frequency to cross a 'hole' in a polar surface ( $k^*$ ) of a permeant is expressed by<sup>42</sup>:

$$k^* = B \cdot e^{-(\Delta G^*/RT)} \quad (13a)$$

In which B a constant, and  $\Delta G^*$  the activation free energy.

A relative increase of the molecular sizes compared to the sizes of the cavity, will give an actual increase of  $\Delta G^*$ , and thus a decrease of  $k^*$  (figure 8). This for instance can be achieved when the packing order of the molecule is lowered (figure 8) or molecules become larger.

A similar situation will arise when the molecule want to escape from the inner side of the bilipid layer. Then, the cross frequency ( $k^{**}$ ) will be expressed by

$$k^{**} = B' \cdot e^{-(\Delta G^{**}/RT)} \quad (14b)$$

Combining equations 14a and 14b make that the partition coefficient  $K_m$  between out- and inside of the membrane as expressed as the ratio of the cross frequency is independent of the activation energies by:

$$K_m = B'' \cdot e^{-\Delta G^0/RT} \quad (14c)$$

In which  $\Delta G^0$  is the standard free energy of the process (figure 8).

Since transfer of the permeant, due to its different affinity for out- and inside of the membrane, can only occur when 'holes' are formed by the motion of the membrane lipids, a relationship between the sizes of the 'holes' and the packing order of the lipids in the membranes might exist. Although this packing order can be influenced by various exogenic factors<sup>42</sup>, it may be considered that the membrane composition and -structure of the epithelial cell is stable during exposure.

In natural lipid membranes the packing order ranges from 20 to 100 Å<sup>2</sup> with an average around 50 Å<sup>2</sup> per lipid. If the constitution of a membrane is constant over time, it will be obvious that there is an upper limit of the size of the created 'holes'. These maximal 'hole' sizes will determine the

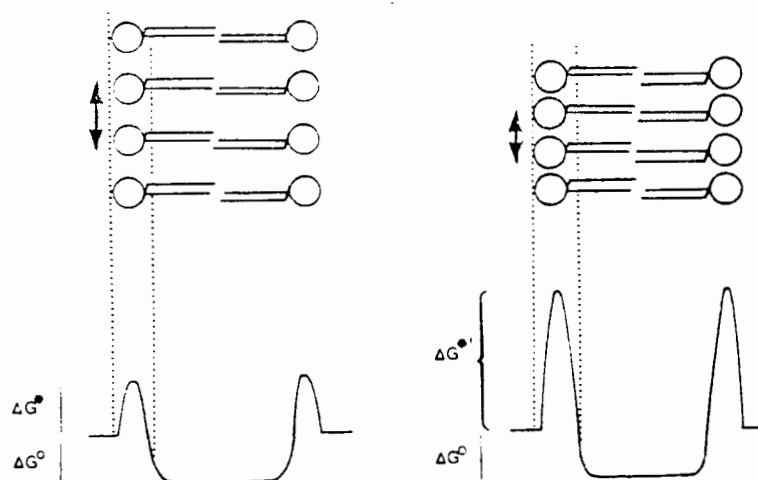


Figure 8. Schematic representation of the relationship between package density of the membrane (---) and Activation- ( $\Delta G^\ddagger$ ) and Standard Free Energy ( $\Delta G^\circ$ ) of the transfer process.

maximal sizes of permeable compounds.

To permeate across the polar surface the molecule must be small enough to pass the bores of the largest 'holes' as is illustrated in figure 7. To allow this transfer, two of the three dimensions of the molecule must be less than the cross section of the bore.

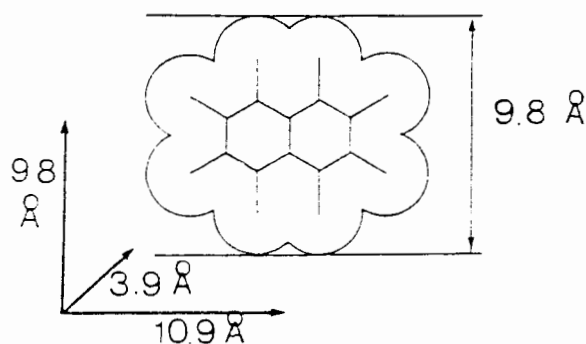


Figure 9. Three dimensions and the effective cross section of octachloronaphthalene.

For chloronaphthalene the smallest cross section is determined by the diameter of the chlorine atom<sup>50</sup>. The second smallest (effective) cross section for octachloronaphthalene is in the 1,4 direction (figure 9). This effective cross section will determine the ability to cross cavity bores. For several hydrophobic molecules, these cross sections and bioaccumulation data are represented in table 4. As is shown, compounds which were not accumulated in fish via the gills, can not pass cavities with bores under 9.5 Å, while all others can. This probably indicates a relationship between steric factors which determine the molecular cross sections, and uptake into biota. For bioaccumulation this means that for molecules with cross sections under 9.5 Å, the partitioning approach can hold, since the energy barrier is not too high. This for instance holds for all chlorobiphenyls and -benzenes, because they all have an effective cross-section under 8.7 Å. As a consequence, variation in accumulation behaviour of chlorobiphenyls must not be explained in terms of steric hindrance, e.g. due to twisting of phenyl rings<sup>20,51,52</sup>, but in terms of metabolism or changed hydrophobicity related to 2 or 6 chloro-substitution.<sup>35</sup>

In addition it seems that for several other compounds lack of uptake can be explained or expected. For instance for hexabromobenzene<sup>16</sup> and for all polybrominated biphenyls<sup>19</sup> with 6 or more bromo atoms, except 2,2',3,3',4,4' hexabromobiphenyl, membrane permeation must be limited which might explain the lack of accumulation. In addition, chloronaphthalenes, and -anthracenes, and structurally related compounds like chlorinated dibenzo-p-dioxins and dibenzofurans are of particular interest in this respect, as is illustrated in table 4. As is shown 2,3,7,8 TCDD will not be sterically hindered in permeation, in contrast to some other chlorodibenzo-p-dioxins, what is in agreement with accumulation data.<sup>53</sup>

The potential lack of permeation may perhaps allow some speculations about structure activity relationships of compounds in toxicity tests. This, because absence of permeation will normally avert toxicological action. The relative low toxicity of hepta- and octachloronaphthalene for instance as shown sometimes might be due to this phenomenon.<sup>28</sup>

A similar background can perhaps hold true for the octachlorodibenzo-p-dioxin, as well as the low toxicities of several lower chlorinated congeners.<sup>54</sup>

It must be stressed, however, that in general not only membrane permeation allows entrance of the compound into a body. Especially in the intestinal tract mediated transport can occur under several conditions.<sup>55,56,57</sup>

Micellar transport for instance is a common phenomenon.<sup>57</sup> It is likely that these routes of transport of chemicals are not restricted by steric factors of the molecule, as can be assumed for permeation, so that also toxicity is not simply limited.<sup>58</sup>

## CONCLUSIONS

Most polychlorinated naphthalenes, like other hydrophobic chemicals, accumulate rapidly. Generally this process is comparable to pure physico-chemical partition processes, like for instance octan-1-ol/water distribution.

Table 3. Structure parameters of some hydrophobic chlorinated aromatic hydrocarbons.

name	structure	molecular weight	cross-section(A)	log $K_{d,oct}$	log $K_c$	name	structure	molecular weight	cross-section(A)	log $K_{d,oct}$	log $P$
1,2 dichloro naphthalene		197	8.6	4.9	3.4	2,3,7,8 tetra chlorodibenzo p-dioxin		322	7.6	>6	>3
1,4 dichloro naphthalene		197	8.6	4.4	3.8	octachloro dibenzo-p-dioxin		460	9.8	>6	0
1,2,3,4 tetrachloro naphthalene		266	9.1	5.9	4.5	hexachloro benzene		285	8.7	5.8	>4
1,3,5,7 tetrachloro naphthalene		266	9.3	6.4	4.5	hexabromo benzene		552	9.6	>6	0
1,3,5,8 tetrachloro naphthalene		266	9.3	6.0	4.4	decachloro biphenyl		499	8.7	>6	>5
octachloro naphthalene		404	9.8	8.4	0	2,2',3,3',4,4' hexabromo biphenyl		628	9.4	>6	>1'
						decabromo biphenyl		944	9.6	>6	0'

Due to the similarity between this latter pure organic phase (octan-1-ol) and the organic phase of fish (mainly lipids) good correlations are often found between these distribution processes. For several larger molecules however such correlations no longer hold, because the interface in between the organic phase in fish and the aqueous environment no longer allows partitioning. Such lack of permeation is observed for hydrophobic chemicals with effective cross sections over 9.5 Å like for hepta- and octachloronaphthalenes.

This relationship between the steric configuration of the compounds and the lack of uptake shows also that the types and compositions of the organism's membranes can influence the bioaccumulation potential of a chemical.

Most extreme hydrophobic chemical which can permeate and have  $\log K_{d, oct}^*$  values  $> 5.0$ , show an almost linear accumulation over time, with constant uptake rate constants. So, variations of calculated fish/water concentration ratios for different compounds are only dependent on the exposure concentrations and on the initial available amount in the test system.

Exceptions to this phenomenon can arise when a highly hydrophobic chemical is metabolized. Then, the elimination rate can be significantly increased, so that the bioconcentration factor is reduced. For extreme hydrophobic chemicals, which are not metabolized, actual predictions of the accumulation rate constant lose accuracy. This because, on the one hand, long term studies ( $> 200$  days) are required to show significant alterations in fish concentrations, while on the other in such studies ageing of the fishes becomes important. The latter can include growing, change of body composition and change of internal distribution of the test compounds.

An additional problem with extreme hydrophobic chemicals is the low aqueous solubility. Proper exposure to pure solutions can almost never be achieved, because, on the one hand, such solutions can hardly be obtained, and on the other, introduction of fishes introduces massive amounts of detergents and particles, which can increase respectively decrease the bioavailability of the test compounds. For these reasons accumulation studies with dietary exposure seem to be preferable. However, since the intestinal tract is a rather complex system, in which various uptake routes can cooperate, quantitative studies with aqueous exposure of the gills will hold validity, even if the test compound in the water phase is not completely dissolved.

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