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Membrane—Water Partitioning of Polychlorinated Biphenyls in Small Unilamellar Vesicles of Four Saturated Phosphatidylcholines

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Experimental data are presented on the partition coefficients of 14 polychlorinated biphenyls (PCBs) between four species of saturated 1,2-diacyl-*sn*-glycero-3-phosphocholine membrane vesicles and aqueous buffer. Small unilamellar vesicles of dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), and diarachidoylphosphatidylcholine (DAPC) show a high affinity for extreme hydrophobic xenobiotics such as the PCBs in this study. Observed membrane—water partition coefficients K_{mw} of the PCBs, with log K_{ow} values ranging from 5.3 to 8.26, in membrane lipid vesicles were in the following order: DPPC > DMPC > DSPC > DAPC. Beside the hydrophobicity of the PCBs, the order of the K_{mw} values is primarily determined by the fluidity of the membrane bilayer. At the experimental temperature of 37 °C for every PCB, a correlation exists between log K_{mw} and the membrane lipid main phase transition temperature T_m . In comparison to the bulk solvent 1-octanol, the membrane lipid phase is highly structured and the correlation between log K_{mw} and both the molar liquid volume MLV of the PCBs and their log K_{ow} is nonlinear.

Introduction

PCBs are now widespread pollutants and have been found in food chains even far away from industrial areas (1). Due to their resistance to biotransformation and their extremely hydrophobic character, many PCBs show the ability to accumulate in food chains and to biomagnify in the highest trophic levels (2, 3). Exposure to high PCB concentrations is believed to be a major cause of many biochemical, morphological, and population dynamic effects in populations of fish, fish-eating birds and fish-eating mammals, although direct cause-linkages remain difficult to establish (4–8). It is believed that general anesthetic toxicological effects of hydrophobic compounds are the result of compound—membrane interactions (9, 10). The biochemi-

cal aspects of these interactions are poorly understood, but it is suggested that hydrophobics inserted in the membrane can alter the membrane fluidity and so interfere with biological functions of the membrane (11, 12).

Xenobiotics that enter an organism have to overcome a series of barriers before they reach their target organs or their final storage side; for hydrophobic compounds, it is mainly the adipose tissue. These barriers, such as the gills, the lungs, or the intestinal wall, consist of aqueous diffusion layers, cell membranes, and epithelial cell contents. Hydrophobics are believed to be transferred over the epithelium by passive diffusion, and uptake kinetics can be related to hydrophobicity. Concerning chemicals with log K_{ow} values higher than 5, aqueous diffusion barriers proved to be rate limiting for the permeation process (9, 13, 14). Still, unexpectedly low transport kinetics have been observed for large extremely hydrophobic molecules such as octachlorodibenzo-*p*-dioxin. This phenomenon might be explained by the highly structured character of biological membranes that may restrict the permeation of more bulky molecules (12, 15, 16). Especially for more hydrophobic solutes, processes such as membrane permeation, bioaccumulation, and biotransformation are difficult to interpret with the use of simple organic liquids like 1-octanol as model systems. In order to mimic the properties of biological membranes more accurately, phospholipid vesicles (liposomes) are often applied (10–15). Gobas et al. observed a significant stronger influence of the molar volume of the solute on the partition coefficient between water and membrane vesicles than on the partition coefficients between water and the bulk solvents hexane and 1-octanol indeed (17). Also Katz and Diamond concluded from their thermodynamic study on the transfer of 16 nonelectrolytes from water into membrane liposomes that the extra decrease in entropy during the solution of the solute in the liposomes compared to the solution in a bulk solvent is due to the stronger immobilization of the solute molecules in the more structured membrane phase (18). Omann and Lackowicz observed a reverse relation between the lipid package density of the membrane and the partitioning of chlorinated hydrocarbons in membrane vesicles (10). However, if liposomes are applied, their properties should be well characterized and they should be stable in structure and composition. These conditions are met with the use of small unilamellar vesicles, which are also easy to prepare (19, 20).

A remarkable change in partitioning of solute in membrane lipids can occur on liposomes passing through their melting point or phase main transition temperature T_m (12, 18). Below T_m , membrane lipids are in the gel phase, and above T_m , they are in the liquid crystalline phase. Since mainly van der Waals forces determine the relative stability of the gel and the liquid crystalline phases, T_m is most dependent on the characteristics of the lipid's fatty acid chain. Thus, longer chains result in higher T_m values, and double bonds in unsaturated lipids reduce T_m . However, phospholipids with a large polar headgroup, such as the phosphatidylcholines used in our study, show an intermediate ripple phase between the gel phase and the liquid crystalline phase at a corresponding pretransition temperature T_p (19).

TABLE 1

Properties of Diacyl-Saturated Phosphatidylcholines DMPC, DPPC, DSPC, and DAPC

phospholipid	alkyl chain length	partial specific vol (mL/g) ^a	phase pretransition temp (°C) ^b	phase main transition temp (°C) ^b
DMPC	C14:C14	0.962	15.3	24.0
DPPC	C16:C16	0.976	35.5	41.5
DSPC	C18:C18	0.988	51.0	54.3
DAPC	C20:C20	0.999	62.1	64.1

^a Values from ref 21 and estimated by use of the rule that molar volumes increase by 16.1 cm³/mol per added CH₂ group. ^b From reference 19.

In order to investigate the influence of the hydrophobic core in the membrane bilayer on the partitioning of PCBs, membrane-water partition coefficients of a series of PCBs were determined between small unilamellar membrane vesicles and water. Four saturated diacylphosphatidylcholines with increasing alkyl chain length were studied, and a selection of 14 PCBs with log *K*_{ow} values in a range of 5.0–8.5 was applied to represent extremely hydrophobic organic compounds. By studying the relationship between the log *K*_{mw} values of the phosphatidylcholine vesicles and log *K*_{ow} data collected from the literature, the properties of the bulk solvent 1-octanol and the membrane phases are compared.

Experimental Section

Experimental. The synthetic saturated 1,2-diacyl-*sn*-glycero-3-phosphocholine lipids dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), and diarachidoylphosphatidylcholine (DAPC) were supplied by Sigma Chemical Co., and Chromosorb G(AW) 60–80 mesh was from Chrompack. Data on the molecular weight, fatty acid chain length, partial specific volumes, phase main transition, and phase pretransition temperatures of the applied phospholipids are listed in Table 1. The experiments were conducted with 14 PCBs of which 4,4'-; 2,4,6-; 2,2',4,5'-; 2,2',3,3',4,4'-; 2,2',4,4',6,6'-; 2,2',3,4,4',5,5'-; 2,2',3,3',4,5,5',6-; and decachlorobiphenyl were purchased from Promochem; 2,2',3,3'-; 2,3,4,5-; 2,3,4,5,6-; and 2,2',3,3',6,6'-chlorobiphenyl were from Ultra Scientific; and 3,5- and 2,4,5-chlorobiphenyl were from Dr. S. Ehrendorfer. Physical-chemical properties and IUPAC Numbers of the selected PCBs are listed in Table 2 in the sequence of column elution. Recovery and internal analytical standards (1,2,3,5-tetrachlorobenzene and pentachlorobenzene) were from Ultra Scientific. The solvents acetone (Merck) and 2,2,4-trimethylpentane (Rathburn) were respectively pro analysis and HPLC grade and were distilled and controlled for purity by means of FID and ECD chromatography. They proved to be more than 99.9% pure. Phosphatidylcholines, chlorobenzenes, and PCBs were used as received from their suppliers. The water used was distilled twice.

The preparation of the membrane vesicles was adapted from the method of Richard et al. (20). Solutions of phosphatidylcholines in chloroform were evaporated to dryness under continuous stirring in a round-bottom flask under a mild stream of nitrogen. To the thin lipid film on the wall, a 1 mM EDTA buffer containing 0.02 M KH₂PO₄ and 0.15 M NaCl with pH = 7 was added. The phospholipids were hydrated at 10 °C above their phase main transition

TABLE 2

IUPAC Number and Literature Data on Aqueous Solubility (*S*_w), log *K*_{ow}, and Molar Liquid Volume (MLV) of the Selected Polychlorinated Biphenyl Congeners

structure	IUPAC No.	<i>S</i> _w ^a (nmol/L)	log <i>K</i> _{ow} ^a	MLV (cm ³ /mol) ^a
3,5-	14	2904 ^c	5.37 ^e	167
2,4,6-	30	777 ^d	5.5 ^d	190
4,4'-	15	269 ^d	5.3 ^d	172
2,4,5-	29	544 ^d	5.6 ^d	186
2,2',4,5-	49	54.8 ^d	6.1 ^d	193
2,2',3,3'-	40	103 ^d	5.6 ^d	177
2,3,4,5-	61	68.5 ^d	5.9 ^d	197
2,2',4,4',6,6'-	155	5.5 ^d	7 ^d	236
2,3,4,5,6-	116	14.5 ^d	6.3 ^d	217
2,2',3,3',6,6'-	136	2.22 ^d	6.7 ^d	228
2,2',3,3',4,4'-	128	1.66 ^d	7 ^d	212
2,2',3,4,4',5,5'-	180	0.57 ^c	7.21 ^e	214
2,2',3,3',4,4',5,5',6-	198	0.41 ^e	7.43 ^e	231
2,2',3,3',4,4',5,5',6,6'-	209	0.001 ^d	8.26 ^d	254

^a Number of decimals as given in the references. ^b Data calculated with SOFA as described elsewhere (25). ^c From ref 22. ^d From ref 23. ^e From ref 24.

temperature and briefly sonicated to obtain large multilamellar vesicles. These vesicles were passed subsequently five times through a 0.4 and 0.2 μm pore diameter polycarbonate (Nucleopore) membrane. The resulting small unilamellar vesicles maintain a narrow size distribution around 150 nm (20). The final lipid concentration was 1.0 mM for all solutions.

The experimental procedures to expose the vesicle solutions to the PCBs were similar to the procedures as described in extension elsewhere (26). In short, the applied method was as follows. Sufficient amounts of the PCBs to provide a 2–4-fold excess of solute in the solubility experiments were dissolved in pentane and added to the chromosorb. Under continuous stirring and mild vacuum, the solvent was evaporated in a rotavapor to coat the chromosorb homogeneously with the PCBs. Portions of 25 mg of coated chromosorb were placed into 10-mL vials. To each vial, 3 mL of membrane vesicle solution was added. Experiments were conducted in triplicate at 37 ± 0.5 °C, and the vials were stirred continuously during 96 h, which proved to be sufficient to reach equilibrium (26). Recoveries of the coating, rinsing, and filtering procedure were between 81% and 104% depending on the PCB congener.

Since the chromosorb does not contain any impurities, cleanup of the samples is minimized, reducing losses and time during cleanup. Chromosorb was extracted for at least 6 h but usually overnight with 5 mL of acetone and with 1.5 μg of 1,2,3,5-tetrachlorobenzene added as the recovery standard. From this sample, 100 μL was added to 3 mL of 2,2,4-trimethylpentane and analyzed by GC-ECD with 300 ng of pentachlorobenzene as the analytical internal standard for quantification of the PCBs and recovery standard. Chromosorb samples from the exposures to the EDTA buffer were extracted by 1 mL of acetone. After the addition of 300 ng of the recovery standard, the total sample was added to 2,2,4-trimethylpentane. Under a flow of nitrogen, acetone was evaporated, and the analytical standard was added. Spike recoveries were usually between 80% and 120% for the recovery standard and always higher than 95% for the analytical standard.

Sample analysis was performed on a 30-m DB-5 column with a diameter of 0.32 mm in a HP-5890 GC with a ^{63}Ni electron capture detector and on-column injector. The carrier gas was helium (30 cm/s flow and 84 kPa pressure), and the makeup gas was argon/methane. All 14 PCBs were separated by the following temperature program: 80 °C-2 min-10 °C/min → 140 °C-5 min-3 °C/min → 240 °C-30 °C/min → 325 °C-5min; temperature of the detector was 350 °C.

Quantification of PCB peaks was done by integration of the peak surfaces and multilevel calibration consisting of six levels. Calibration levels ranged from 1 to 1000 pg/injection. Samples (0.5 µL) were injected in triplicate; standard deviations of the triplicate analysis of one sample were smaller than 3%. Standard deviations of the mean due to analytical error between triple samples were within 5%, except 4,4'-dichlorobiphenyl, which possessed standard deviations up to 10% due to its irregular elution behavior. Depending on the PCB congener, the limits of detection ranged from 1.5 to 12 pg of PCB/mg of chromosorb. Standard deviations due to experimental error (chromosorb exposure and cleanup) ranged from 3% to 10% per triplicate sample.

Calculation of Membrane–Water Partition Coefficient.

A compound's membrane–water partition coefficient, K_{mw} , where the subscripts m and w denote membrane and water, respectively, is the ratio of the concentrations (mol/m³) of the compound in the membrane phase (C_m) to the concentration of the compound in the aqueous phase (C_w):

$$K_{mw} = C_m / C_w \quad (1)$$

In the experimental setup as used in this study, containing pure solute (p), solute dissolved in water (w), and solute dissolved in the membrane phase (m), a total mass (M) balance equation of the solute before (0) and after (x) the addition of membrane vesicles can be derived:

$$M_{\text{total}} = M_{w(0)} + M_{p(0)} = M_{w(x)} + M_{p(x)} + M_m \quad (2)$$

where M_{total} is the mass of solute that is introduced into the solution, and the aqueous solubility ($S_{0,0}$) without membrane vesicles is simply the difference between M_{total} and $M_{p(0)}$ divided by the volume of the water (i.e., $(M_{\text{total}} - M_{p(0)}) /$

V_w). The enhanced solubility at a particular membrane vesicle concentration (S_x) then is the difference between M_{total} and $M_{p(x)}$ divided by the volume of the membrane vesicle solution, which is considered to be equal to the volume of the water, $((M_{\text{total}} - M_{p(x)}) / V_w)$. The contribution of volume of the membrane phase (V_m in m³) to the volume in the membrane vesicle solution can be obtained from the partial specific volume of the membrane vesicles (21).

After introducing the membrane vesicles, the mass loss of pure solute from the chromosorb corresponds with the amount of compound solubilized in the membrane phase:

$$M_{p(0)} - M_{p(x)} = C_m V_m \quad (3)$$

Since the increasing solubilization capacity of a membrane vesicle solution with increasing membrane vesicle concentration is due only to the increasing volume of the hydrophobic membrane phase, the concentration of the compound in both the aqueous phase and the membrane phase remains constant. Combining eqs 1 and 3, a proper partition coefficient can be defined according to eq 4:

$$K_{mw} = C_m / C_w = (M_{p(0)} - M_{p(x)}) / C_w V_m \quad (4)$$

As the concentrations here are maximum concentrations, C_w can be considered as the solute's aqueous solubility in the buffer ($S_{0,0}$). Thus, the partitioning of a chemical between the membrane phase and the aqueous phase can easily be obtained from measurements of the mass loss of solute from the chromosorb before and after the addition of membrane vesicles.

Results and Discussion

Experimentally determined solubility data (nmol/L) in the saline phosphate buffer ($S_{0,0}$) and in the 1 mM solutions of the four saturated phosphatidylcholines are listed in Table 3 (with their standard deviations in parentheses) for all 14 PCB congeners. In comparison with $S_{0,0}$, the PCB solubilities are significantly enhanced in the membrane solutions, ranging from three times $S_{0,0}$ for the lower chlorinated isomers in the DAPC vesicles to 3000 times $S_{0,0}$ for the higher chlorinated isomers in the DPPC vesicle solutions. In the membrane vesicles, PCB 29 becomes the most soluble one, whereas in the buffer, PCB 30 has the highest solubility

TABLE 3

Experimental Solubilities (S in nmol/L) of Polychlorinated Biphenyls at 37 °C in Phosphate Buffer and in Suspensions of 1.0 mM DMPC, DPPC, DSPC, and DAPC

IUPAC No.	$S_{0,0}^a$ (nmol/L)	S_{DMPC} (nmol/L) ($\times 1000$)	S_{DPPC} (nmol/L) ($\times 1000$)	S_{DSPC} (nmol/L) ($\times 1000$)	S_{DAPC} (nmol/L) ($\times 1000$)
14	512.4 (± 22.5)	8.85 (± 0.20)	15.65 (± 0.20)	3.65 (± 0.19)	1.47 (± 0.14)
30	1101 (± 87)	47.47 (± 3.64)	156.3 (± 3.1)	27.66 (± 3.09)	10.86 (± 3.07)
15	551.9 (± 101.9)	18.12 (± 1.87)	29.92 (± 1.67)	10.08 (± 1.44)	2.80 (± 1.03)
29	1038 (± 84)	75.37 (± 7.95)	221.2 (± 8.2)	38.24 (± 7.94)	15.37 (± 4.48)
49	48.85 (± 6.98)	7.12 (± 0.86)	26.92 (± 0.79)	4.34 (± 0.78)	1.87 (± 0.58)
40	59.63 (± 10.27)	15.33 (± 0.54)	27.74 (± 0.50)	4.47 (± 0.52)	2.16 (± 0.46)
61	75.04 (± 11.53)	9.46 (± 0.59)	22.58 (± 0.54)	4.38 (± 0.54)	1.84 (± 0.57)
155	4.89 (± 1.10)	2.07 (± 0.13)	8.00 (± 0.11)	1.29 (± 0.11)	0.70 (± 0.12)
116	20.53 (± 2.28)	6.11 (± 0.55)	16.36 (± 0.49)	3.25 (± 0.50)	1.16 (± 0.35)
136	6.53 (± 1.05)	2.23 (± 0.11)	7.53 (± 0.09)	1.36 (± 0.08)	0.83 (± 0.06)
128	5.10 (± 1.09)	3.02 (± 0.14)	8.08 (± 0.12)	1.64 (± 0.12)	0.63 (± 0.11)
180	3.23 (± 0.18)	2.64 (± 0.25)	8.31 (± 0.21)	1.58 (± 0.21)	0.48 (± 0.12)
198	0.77 (± 0.42)	0.53 (± 0.04)	1.70 (± 0.02)	0.34 (± 0.02)	0.11 (± 0.02)
209	0.008 (± 0.002)	0.005 (± 0.001)	0.018 (± 0.004)	0.003 (± 0.001)	0.001 (± 0.0002)

^a Values and standard deviations (in parentheses) are for the means of triplicate experiments.

TABLE 4

Experimental Membrane–Water Partition Coefficients ($\log K_{mw}$) for PCBs in DMPC, DPPC, DSPC, and DAPC at 37 °C

IUPAC No.	$\log K_{mw}^a$			
	DMPC	DPPC	DSPC	DAPC
14	4.52 (± 0.05)	4.71 (± 0.05)	4.04 (± 0.03)	3.61 (± 0.04)
30	4.71 (± 0.07)	5.17 (± 0.03)	4.38 (± 0.07)	3.94 (± 0.02)
15	4.78 (± 0.14)	4.94 (± 0.05)	4.43 (± 0.04)	3.84 (± 0.03)
29	5.16 (± 0.10)	5.57 (± 0.07)	4.77 (± 0.06)	4.34 (± 0.03)
49	5.55 (± 0.12)	6.07 (± 0.01)	5.24 (± 0.04)	4.84 (± 0.06)
40	5.40 (± 0.05)	5.60 (± 0.03)	4.77 (± 0.03)	4.42 (± 0.06)
61	5.44 (± 0.08)	5.76 (± 0.02)	5.01 (± 0.03)	4.60 (± 0.06)
155	5.98 (± 0.08)	6.51 (± 0.02)	5.68 (± 0.04)	5.38 (± 0.08)
116	5.90 (± 0.03)	6.27 (± 0.03)	5.53 (± 0.07)	5.05 (± 0.09)
136	5.90 (± 0.02)	6.37 (± 0.01)	5.59 (± 0.02)	5.34 (± 0.02)
128	6.09 (± 0.06)	6.46 (± 0.02)	5.73 (± 0.07)	5.28 (± 0.10)
180	6.33 (± 0.04)	6.77 (± 0.01)	6.01 (± 0.02)	5.46 (± 0.06)
198	6.18 (± 0.02)	6.63 (± 0.01)	5.89 (± 0.04)	5.35 (± 0.04)
209	6.17 (± 0.12)	6.63 (± 0.03)	5.83 (± 0.02)	5.25 (± 0.02)

^a Values and standard deviations (in parentheses) are for the means of triplicate experiments.

value. Enhancement of the PCB solubility after the addition of phospholipid vesicles is in the following order: DPPC > DMPC > DSPC > DAPC. Standard deviations of the triplicate experiments for solubilities in the buffer range from 3.2 to 37.6% for PCB 30 and PCB 209, respectively. For the solubility experiments including lipid vesicles, the relative standard deviations for the DPPC experiments are considerably smaller than for the DSPC and DAPC experiments.

The experimental solubility data were determined in a physiological saline phosphate buffer containing phosphate and sodium salt but are very well comparable with the aqueous solubility data in pure water as reported in the literature (see Table 2), except for PCB 14, which showed a much lower solubility in this study than was expected from data stated in the literature. Data on aqueous solubility (S_w) are a selection by Mackay and Shiu (23), except for PCB 14 and PCB 180, which were determined by Dunnivant et al. (22), and PCB 198, which was obtained from RP-HPLC data by Brodsky and Ballschmitter (24). In our earlier experiments with micelles conducted at 25 °C, the presence of dissolved electrolytes in the buffer did not seem to have a major influence on the solubilities of the hydrophobic chemicals used in this study (26, 27).

Experimental $\log K_{mw}$ data as obtained according to eq 4 are listed in Table 4. In general, standard deviations turn out to be smaller than 0.1 log unit. Similar to the solubility results, the $\log K_{mw}$ data for DPPC are the highest, followed by DMPC, DSPC, and DAPC. For all types of phosphatidylcholines, the sequence in the $\log K_{mw}$ values of the PCBs is identical, no reversals are observed.

Some authors have mentioned the relationship between the partitioning and uptake of solutes in membranes and the viscosity of the membranes. The van't Hoff plots of the partitioning of solutes between liposomes and water show a striking difference below and above the lipid's phase main transition temperature (18). In the fluid phase above T_m , membrane–water partitioning is higher than in the more rigid gel phase. Thus at the same experimental temperature the partitioning of lindane into the fluid liquid crystalline vesicles of unsaturated phosphatidylcholines was higher than into saturated membranes in the gel phase (10).

FIGURE 1. Membrane–water partition coefficient ($\log K_{mw}$) at 37 °C as function of the lipid phase main transition temperature (T_m in °C) for PCB 15 (●), PCB 40 (▲), PCB 136 (+), and PCB 180 (■).

Oppehuizen and Sijm proposed a relation between the uptake of hydrophobic chemicals in membranes and the opportunity of solute molecules to find sufficiently large holes between the membrane lipids to penetrate into the membrane (16). The space between the membrane molecules is related to the lipid package density. The chance to find sufficiently large holes is smaller in membranes in the gel state than in the fluid crystalline state, resulting in lower partition coefficients in the gel state. In our experiments, the $\log K_{mw}$ values are higher in DMPC than in DSPC and DAPC although the volume of the hydrophobic core of the latter two is larger than in DMPC bilayers (28).

Probably as a result of lipid packing problems, diacylphospholipids that have a bulky headgroup show the ripple phase as an intermediate phase between the liquid crystalline phase and the gel phase at T_p . In this phase, the surface of the bilayer is rippled and presents a wave-like appearance (19). At the experimental temperature of 37 °C, DPPC is in the ripple phase (see Table 1) and shows the highest membrane–water partition coefficients in our experiments, probably due to larger holes that are present in the bilayer. In the work of Antunes et al., it can also be seen that the partitioning of DDT into DMPC vesicles is the highest at 24 °C where DMPC is in the ripple phase (11). At any definite temperature, phospholipids have a particular fluidity dependent on their phase main transition temperature. In Figure 1, the relationship is shown between the membrane lipid phase main transition temperatures and the $\log K_{mw}$ values of PCB 15, PCB 40, PCB 136, and PCB 180 at the experimental temperature of 37 °C. From the figure, it can be seen that at 37 °C the $\log K_{mw}$ values of the PCBs in the gel phase of DAPC are lower than in the gel phase of DSPC, probably due to its more rigid structure at 37 °C. These relationships are unique for this temperature, and the curves in the figure will shift with the environmental temperature. According to this model, the $\log K_{mw}$ values for all 14 PCBs in other phospholipids could be calculated since at least for all saturated diacylphosphatidylcholines T_m can be estimated very accurately (28).

In living organisms, all membranes are probably in the liquid crystalline phase, so the question of whether the previous discussion is more than academic is debatable. Still, the membrane lipids are not homogeneously distributed in the membrane. Even in the liquid crystalline phase lipids mix in a nonideal manner and form distinct patches (19). These patches might show a phase character differing

FIGURE 2. Relationship between the molar liquid volume (MLV) and the 1-octanol–water partition coefficient ($\log K_{ow}$) of the 14 PCBs used in this study.

FIGURE 3. Relationships between the molar liquid volume (MLV) and the membrane–water partition coefficients ($\log K_{mw}$) of the 14 selected PCBs in unilamellar vesicles of DMPC (●), DPPC (○), DSPC (□), and DAPC (■).

from the rest of the membrane bilayer. The membranes that are most important for the bioaccumulation process, the membranes of the gills and the brush border membranes of the gastrointestinal tract, interestingly show a clearly distinct membrane lipid composition compared to the usual plasma membrane. The gill membranes contain a large amount of phosphatidylethanolamine plasmalogens, a membrane lipid in which the sn-1 hydrocarbon chain is linked via a vinyl ether linkage, resulting in a higher degree of structuring (29). The luminal side of the brush border membrane consists mainly of sphingolipids, which are packed so tightly together that this membrane appears to be very impermeable, even for hydrophobics (30).

If the partition coefficient of hydrophobics between the aqueous phase and the membrane phase depends on the chance of the solute molecules to find a sufficiently large hole between the lipid molecules, a strong correlation would exist between the solute's molar liquid volume and its membrane–water partition coefficient. The number of holes will decrease dramatically with an increasing cross section of the holes, and so the number of possibilities of large molecules to find accommodating holes will decrease. In Figures 2 and 3, the relationship is shown between the molar liquid volume of the PCBs in the super-cooled liquid state (MLV in cm^3/mol as listed in Table 1) and $\log K_{ow}$ and $\log K_{mw}$ data, respectively. Data on $\log K_{ow}$ are from Table 2 and were selected by Mackay and Shiu (23) except for PCB 14, PCB 180, and PCB 198, which are obtained from

RP-HPLC experiments by Brodsky and Ballschmiter (24). Data on the MLV of our PCBs are preliminary and are calculated by using a SOFA model that is discussed in detail elsewhere (25). It is supposed that standard deviations of these MLV data are within $5 \text{ cm}^3/\text{mol}$. For the 1-octanol–water system, the $\log K_{ow}$ values for the PCBs used in this study are increasing with expanding MLV as is shown in Figure 2 and expressed by the following linear relationship with 95% confidence intervals between parentheses:

$$\log K_{ow} = 0.0312 (\pm 0.0011) \text{ MLV} - 0.022 (\pm 0.214) \quad (5)$$

$$n = 14, r^2 = 0.983, \text{SER} = 0.31, F = 794$$

As can be seen from Figure 3, above a MLV of about $230 \text{ cm}^3/\text{mol}$, $\log K_{mw}$ values start to level off in all four membrane–water systems. For the four diacylphospholipids, the membrane–water partition coefficient correlates in a parabolic way with the MLV:

$$\log K_{mw(\text{DMPC})} = -0.000041 (\pm 0.000016) \text{ MLV}^2 + 0.036 (\pm 0.004) \text{ MLV} - 0.014 (\pm 0.216) \quad (6)$$

$$n = 14, r^2 = 0.979, \text{SER} = 0.30, F = 311$$

$$\log K_{mw(\text{DPPC})} = -0.000036 (\pm 0.000017) \text{ MLV}^2 + 0.037 (\pm 0.004) \text{ MLV} - 0.019 (\pm 0.221) \quad (7)$$

$$n = 14, r^2 = 0.981, \text{SER} = 0.31, F = 342$$

$$\log K_{mw(\text{DSPC})} = -0.000024 (\pm 0.000016) \text{ MLV}^2 + 0.031 (\pm 0.004) \text{ MLV} - 0.017 (\pm 0.214) \quad (8)$$

$$n = 14, r^2 = 0.977, \text{SER} = 0.30, F = 279$$

$$\log K_{mw(\text{DAPC})} = -0.000025 (\pm 0.000017) \text{ MLV}^2 + 0.026 (\pm 0.004) \text{ MLV} - 0.021 (\pm 0.221) \quad (9)$$

$$n = 14, r^2 = 0.972, \text{SER} = 0.31, F = 223$$

$\log K_{mw}$ values were compared to the $\log K_{ow}$ data. In the general empirical linear relationship, $\log K_{mw} = B_1 \log K_{ow} + B_0$, the coefficient B_1 contains a term for the free energy required for the formation of cavities in the organic phase. If the two organic phases 1-octanol and membrane lipid differ sufficiently with respect to the required free energy, B_1 becomes a function of the molar liquid volume of the solute (17):

$$\log K_{mw} = [B_1^* + B_1^{**} \text{MLV}] \log K_{ow} + B_0 \quad (10)$$

When eq 10 is combined with the term for MLV in eq 5, it becomes clear that the resulting relationship between $\log K_{ow}$ and $\log K_{mw}$ is quadratic as shown in Figure 4. The correlations between $\log K_{ow}$ and $\log K_{mw}$ data for the four membrane lipids studied in this work follow a second-order polynomial according to

$$\log K_{mw(\text{DMPC})} = -0.288 (\pm 0.063) \log K_{ow}^2 + 4.39 (\pm 0.83) \log K_{ow} - 10.48 (\pm 2.70) \quad (11)$$

$$n = 14, r^2 = 0.927, \text{SER} = 0.18, F = 70$$

FIGURE 4. Relationship between the 1-octanol–water partition coefficient ($\log K_{ow}$) and the membrane–water partition coefficients ($\log K_{mw}$) of the 14 selected PCBs in unilamellar vesicles of DMPC (●), DPPC (○), DSPC (□), and DAPC (■). Standard deviations for triplicate experiments are expressed as error bars if large enough to be visible.

$$\log K_{mw(DPPC)} = -0.343 (\pm 0.057) \log K_{ow}^2 + 5.20 (\pm 0.76) \log K_{ow} - 12.98 (\pm 2.47) \quad (12)$$

$$n = 14, r^2 = 0.952, \text{SER} = 0.16, F = 109$$

$$\log K_{mw(DSPC)} = -0.311 (\pm 0.053) \log K_{ow}^2 + 4.74 (\pm 0.71) \log K_{ow} - 12.16 (\pm 2.31) \quad (13)$$

$$n = 14, r^2 = 0.963, \text{SER} = 0.15, F = 112$$

$$\log K_{mw(DAPC)} = -0.383 (\pm 0.063) \log K_{ow}^2 + 5.67 (\pm 0.63) \log K_{ow} - 15.53 (\pm 2.04) \quad (14)$$

$$n = 14, r^2 = 0.963, \text{SER} = 0.13, F = 144$$

The shape of the relationships between MLV and $\log K_{mw}$ of our PCBs shows a remarkable similarity with data from an earlier work of Gobas et al. on the partitioning of a series of hydrophobic organic chemicals into DMPC vesicles (17). The breakdown of the linear relationship between $\log K_{mw}$ and $\log K_{ow}$ at very high $\log K_{ow}$ values agrees with his study and occurred also in recent work with technical and biological micelles and with fish (26, 27, 31).

Conclusions

Membrane lipids show a high affinity for extreme hydrophobic xenobiotics such as PCBs. The $\log K_{mw}$ values of the PCBs were in the order DPPC (C16:C16) > DMPC (C14:C14) > DSPC (C18:C18) > DAPC (C20:C20). Hence, at least for the hydrophobic PCBs in this study, the fluidity of the membrane bilayer appears to be more important for the membrane–water partitioning process than the volume of the hydrophobic region in the membrane bilayer, which is determined by the membrane lipid's alkyl chain length. The package density and the number of opportunities for the solute molecules to find an accommodating hole in the membrane dominates the order of increasing $\log K_{mw}$ values among the diacylphospholipids in this study. For PCBs with a MLV larger than 230 cm³/mol, steric hinderance appears to become more and more important, lowering the $\log K_{mw}$. Our results support the idea that the membrane bilayer has to be regarded as a highly structured soft polymer-like phase rather than a bulk solvent like 1-octanol.

The chance to find an accommodating hole for much larger molecules might decrease so far that penetration of the membrane will be reduced to zero eventually.

Biological membranes in living organisms are in the liquid crystalline phase. Since the fluidity of the membrane appears to determine $\log K_{mw}$ values rather than the hydrophobic character of the lipids, the phosphatidylcholine DMPC seems to be a good model for biological membranes at temperatures above the phase main transition temperature. However, membranes with a very different composition might be an exception, and some of these membranes are determinant in the uptake process of xenobiotics, such as the membranes of the gills and the gastrointestinal tract. A proper understanding of the thermodynamics and mechanisms of partitioning of hydrophobics into membrane–water systems is decisive in the study of transport kinetics and fate of these compounds in living organisms. In our future work, we will try to elucidate this matter further.

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Symbols

K_{mw}	membrane–water partition coefficient
K_{ow}	1-octanol–water partition coefficient
T_m	phase main transition temperature
T_p	phase pretransition temperature
C_w	compound concentration in the aqueous phase
C_m	compound concentration in the membrane phase
M_{total}	total mass of the solute
$M_{w(0)}$	mass of the solute present in the aqueous phase before addition of membrane vesicles
$M_{p(0)}$	mass of pure solute before addition of membrane vesicles
$M_{w(x)}$	mass of the solute present in the aqueous phase after addition of membrane vesicles
$M_{p(x)}$	mass of pure solute after addition of membrane vesicles
M_m	mass of solute present in the membrane phase
V_w	volume of the aqueous phase
V_m	volume of the membrane phase
S_w	compound aqueous solubility
$S_{(0,0)}$	compound solubility at zero membrane concentration
S_x	compound solubility after addition of membrane vesicles

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