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SOLUBILITY AND MICELLE-WATER PARTITIONING OF POLYCHLORINATED BIPHENYLS IN SOLUTIONS OF BILE SALT MICELLES.

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ABSTRACT

Experimental data are presented on the enhanced solubilities and micelle/water partition coefficients of 14 polychlorinated biphenyls (PCBs) in solutions of the bile salts sodium taurocholate (TC) and sodium taurodeoxycholate (TDC) below and above the critical micellar concentration (CMC). Solubilities of PCBs in micellar solutions above the CMC depend linearly on bile salt concentration and the solubility enhancement is up to four orders of magnitude. PCB partition coefficients ($\log K_{mw}$) are independent of bile salt concentrations and are systematically higher in solutions of TDC than in solutions of TC, ranging from 4.26 to 6.24 and from 3.89 to 5.89 respectively. The partitioning behavior of the pertinent extremely hydrophobics between the aqueous and micellar phases is not completely comparable with simple organic solvent/water systems and the correlation between $\log K_{ow}$ and $\log K_{mw}$ is non-linear.

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 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].

The main storage site of PCBs in animals is the fat compartment and it is suggested that PCB uptake from food is related to the digestion of dietary fats [9]. During the normal process of fat digestion in vertebrates, bile salt micelles play a facilitatory role in the uptake of water-insoluble compounds like fatty acids, cholesterol, phospholipids and fat-soluble vitamins. Incorporation of the water-insoluble products of fat digestion into bile salt micelles allows easier movement over the unstirred water layer adjacent to the intestinal absorptive cells. Thus transport by bile salt

micelles maintains a high concentration gradient across this unstirred layer, which forms the main barrier for diffusive uptake of hydrophilic compounds [10, 11].

It has been shown that diffusion over the aqueous barriers is also the main rate limiting step in the membrane transport of extreme hydrophobic contaminants [12,13]. In their study with polynuclear aromatic hydrocarbons Rahman et al. found a direct correlation between low aqueous solubility and increased uptake efficiency of these compounds in the presence of bile [14].

The present study was designed to determine the enhanced solubilities and micelle/water partition coefficients of a series of PCBs in solutions of two common bile salts. To investigate the influence of the hydrophobicity of the bile salts on the solubility and partitioning of PCBs, the trihydroxy bile salt taurocholate (TC) and the dihydroxy bile salt taurodeoxycholate (TDC) were used. A selection of 14 PCBs with log K_{ow} values in a range of 5.0-8.5 was applied as representatives of extremely hydrophobic organic compounds. By studying the relationship between the log K_{mw} values of TC and TDC and log K_{ow} data collected from the literature, the properties of the bulk solvent n-octanol and the micellar phases of TC and TDC are compared.

METHOD

Micelle/water partitioning. Low aqueous solubilities and high n-octanol/water partition coefficients of hydrophobic chemicals result from unfavorable and weak solute-water interactions incapable of competing with the strong hydrogen bonds forces between water molecules. Aggregates of surfactants, or micelles, act as colloids and can enhance solubilities of hydrophobic compounds dramatically [15].

The main reason bile salt molecules start to aggregate into micelles is to reduce the energetically unfavorable interaction between the hydrophobic side of the bile salt monomers and the water. The gain in free energy by bringing the non-polar parts together starts to dominate over the loss in entropy due to aggregation. As a consequence the micelles consist of a hydrophilic outer layer formed by the polar substituents facing the aqueous environs and a separated hydrophobic core region which is assumed to be a distinct pseudo phase. Enhanced solubility of hydrophobic compounds in surfactant solutions is due to partitioning of compound between water and micellar phases.

Above a compound's specific threshold concentration, the CMC, bile salt monomers start to aggregate into primary micelles. The value of the CMC depends on surfactant chemistry, temperature, ionic strength and the presence of organic additives of the solution [17,18]. Above the CMC addition of more surfactant results in the incorporation of more molecules into micelles which can increase in size and in number, the number of monomers will stay constant. Under the experimental conditions the primary bile salt micelles are globular with a hydrodynamic radius of 11Å and 22Å for TC and TDC micelles respectively. At higher ionic strength the primary micelles polymerize into rodlike secondary micelles with increasing bile salt concentration [19].

Surfactant micelles do exist in relation with their aqueous environment only, hence it is impossible to measure concentrations of solute in the micellar phase directly. Commonly the solubilization potential of a surfactant is expressed by the enhanced solubility of a solute, or by the molar solubilization ratio, which is defined as the extra number of moles of solute that can be solubilized per mole of surfactant present in the solution [20].

In perspective to the application of data in environmental fate models, such as the fugacity model of Mackay, it is more convenient to use the concept that enhanced solubility of hydrophobic compounds in surfactant solutions is due to the partitioning of compound into the hydrophobic core of the micelles and to express the solubilization potential of the surfactant by the compounds micelle/water partition coefficient, K_{mw} , where de subscripts m and w denote micelle and water, respectively [21]. K_{mw} is the ratio of the concentrations (mol/m³) of the compound in the micellar phase (C_m) to the concentration of the compound in the aqueous phase at the CMC ($C_{w(cmc)}$):

$$K_{mw} = C_m / C_{w(cmc)} \quad (1)$$

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

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

M_{total} is the mass of solute that is introduced into the solution and the aqueous solubility ($S_{0,0}$) without surfactant is simply the difference between M_{total} and $M_{p(0)}$ divided by the volume of the water (i.e. $(M_{total} - M_{p(0)})/V_w$). The enhanced solubility at a particular surfactant concentration (x) then is the difference between M_{total} and $M_{p(x)}$ divided by the volume of the surfactant solution, which is considered to be equal to the volume of the water, $((M_{total} - M_{p(x)})/V_w)$.

Just before the CMC, the aqueous phase is saturated with surfactant monomers and the solubility of the solute at the CMC (S_{cmc}) is the difference between M_{total} and $M_{p(cmc)}$ divided by V_w . Since the addition of bile salt above the CMC leads to the incorporation of monomer molecules into micelles, the solubilization of compound from the solid phase into the solution is entirely due to the increase of the micellar volume in the solution. The contribution of volume of the micellar phase (V_m in m³) to the volume in the surfactant solution above the CMC, can be obtained from the partial specific volume of the surfactant, in the case of TC and TDC 403.3 cm³/mol and 396.5 cm³/mol respectively [22].

Above the CMC the concentration of the compound in the aqueous monomer solution will remain constant and the mass loss of pure solute corresponds with the amount of compound solubilized in the micellar phase:

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

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

$$K_{mw} = C_m / C_{w(cmc)} = (M_{p(cmc)} - M_{p(x)}) / C_{w(cmc)} V_m \quad (4)$$

As the concentrations here are maximum concentrations, $C_{w(cmc)}$ can be considered as the solutes aqueous solubility at the CMC (S_{cmc}). Thus the partitioning of a chemical between the micellar phase and the aqueous phase can easily be obtained from measurements of the mass loss of solute before and after the addition of surfactant.

Experimental. The sodium salts of TC and TDC were supplied by Sigma Chemical Co. and chromosorb 60-80 mesh from Chrompack. The experiments were conducted with 14 PCBs of which the 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 the 2,2',3,3',4,4',5,5',6,6'-chlorobiphenyl were purchased from Promochem; the 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 the 3,5,- and 2,4,5,-trichlorobiphenyl were from Dr. S. Ehrendorfer. Physical-chemical properties and IUPAC numbers of the selected PCBs are listed in Table I, 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 pro analysis and HPLC grade respectively, and were distilled and proved to be more than 99.9% pure by FID analysis. Bile salts, chloro-benzenes and PCBs were used as received from their suppliers. The water used was distilled twice.

An extended description of the experimental procedures is published elsewhere [23], but in short the applied method was as follows. Sufficient amounts of the PCBs 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 bile salt solution was added, with concentrations ranging from 0.0 mM to 20.0 mM TC and from 0.0 mM to 10 mM TDC. These bile salt concentrations are in accordance with reported luminal concentrations [11,24]. At the given experimental conditions bile salt critical micellar concentrations are considered to be 1.7 mM and 2.7 mM for TDC and TC respectively [18]. Solutions consist of bile salt in a phosphate buffer of 0.02 M KH_2PO_4 at physiological NaCl concentration of 0.15 M with pH=7. Experiments were conducted in triplo at $25 \pm 0.5^\circ\text{C}$ and the vials were stirred continuously during 96 hours which proved to be sufficient to reach equilibrium [23]. Recoveries of the coating, rinsing and filtering procedure showed to be between 81% and 104% depending on the PCB congener.

Table I. IUPAC number, molecular weight (MW) and literature data on aqueous solubility (S_w) and log K_{ow} of the selected polychlorinated biphenyl congeners.

Structure	IUPACno.	MW(g/mol)	S_w^a (nmol/L)	Log K_{ow}^a
3,5-	14	223.1	2904 ^b	5.37 ^d
2,4,6-	30	257.5	777 ^c	5.5 ^c
4,4'-	15	223.1	269 ^c	5.3 ^c
2,4,5-	29	257.5	544 ^c	5.6 ^c
2,2',4,5-	49	292.0	54.8 ^c	6.1 ^c
2,2',3,3'-	40	292.0	103 ^c	5.6 ^c
2,3,4,5-	61	292.0	68.5 ^c	5.9 ^c
2,2',4,4',6,6'-	155	360.9	5.5 ^c	7 ^c
2,3,4,5,6-	116	326.4	14.5 ^c	6.3 ^c
2,2',3,3',6,6'-	136	360.9	2.22 ^c	6.7 ^c
2,2',3,3',4,4'-	128	360.9	1.66 ^c	7 ^c
2,2',3,4,4',5,5'-	180	395.3	0.57 ^b	7.21 ^d
2,2',3,3',4,5,5',6-	198	429.7	0.41 ^d	7.43 ^d
2,2',3,3',4,4',5,5',6,6'-	209	498.7	0.001 ^c	8.26 ^c

^a Number of decimals as given in the references. ^b From reference 26. ^c From reference 21. ^d From reference 25.

Since the chromosorb does not contain any impurities, clean-up of the samples is minimized reducing losses and time during clean-up. Chromosorb was extracted during at least 6 hours, but usually over night, with 5 mL acetone and 1.5 μ g 1,2,3,5-tetrachlorobenzene was added as recovery standard. From this sample 100 μ L was added to 3 mL 2,2,4-trimethyl pentane and analyzed by GC-ECD with 300 ng pentachlorobenzene as analytical internal standard for quantification of the PCBs and recovery standard. Chromosorb samples from the exposures to bile salt concentrations below the CMC were extracted by 1 mL acetone. After addition of 300 ng recovery standard the total sample was added to the 2,2,4-trimethylpentane. Under a flow of nitrogen the acetone was evaporated and analytical standard was added.

Sample analysis was performed on a 30 meter DB-5 column with a diameter of 0.32 mm in a HP-5890 GC with ⁶³Ni Electron Capture Detector and on column injector. The carrier gas was helium (30 cm/sec flow and 84kPa

pressure) and the make-up gas was argon/methane. All 14 PCBs were separated by the following T-program: 80°C-2min-10°C/min-140°C-5min-3°C/min-240°C-30°C/min-325°C-5min, the temperature of the detector was 350°C.

Quantification of PCB peaks was done by integration of the peak surfaces and multi level calibration consisting of six levels. Calibration levels ranged from 1 to 1000 pg per 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 triplo samples were within 5%, except of 4,4'-dichlorobiphenyl which possessed standard deviations up to 10% due to its irregular elution behavior.

Table II. Experimental solubilities (S in nmol/L) of polychlorinated biphenyls in phosphate buffered solutions of 0.0, 2.5, 5.0, 10.0 and 20.0 mM taurocholate. CMC = 1.7 mM^a.

IUPAC	$S_{0.0}^b$		$S_{2.5}^b$		$S_{5.0}^b$		$S_{10.0}^b$		$S_{20.0}^b$	
no.	(nmol/L)		(nmol/L)		(nmol/L)		(nmol/L)		(nmol/L)	
					(x1000)		(x1000)		(x1000)	
14	266.9	(±34.2)	298.7	(±9.5)	2.41	(±0.49)	6.08	(±0.39)	16.03	(±0.57)
30	1069	(±149)	1239	(±32)	23.88	(±3.65)	56.33	(±5.09)	136.1	(±8.5)
15	437.1	(±67.3)	465.5	(±20.2)	5.31	(±0.87)	8.99	(±1.59)	28.31	(±2.16)
29	617.2	(±92.3)	701.9	(±15.0)	26.13	(±4.79)	53.05	(±3.76)	166.17	(±6.03)
49	29.05	(±5.04)	33.07	(±1.01)	3.74	(±0.69)	6.96	(±0.58)	19.52	(±1.24)
40	86.43	(±14.48)	98.66	(±1.33)	4.45	(±0.87)	11.31	(±0.73)	30.37	(±1.26)
61	49.42	(±8.09)	58.79	(±1.65)	6.90	(±1.42)	15.73	(±1.08)	39.03	(±1.64)
155	3.89	(±0.87)	5.44	(±0.57)	2.32	(±0.35)	5.22	(±0.32)	16.20	(±0.53)
116	13.33	(±2.53)	16.62	(±1.01)	3.62	(±0.68)	10.15	(±1.17)	26.72	(±1.14)
136	5.68	(±0.92)	6.73	(±0.45)	1.87	(±0.19)	5.57	(±0.27)	12.77	(±0.43)
128	5.29	(±1.56)	7.41	(±1.54)	4.36	(±0.75)	10.56	(±0.74)	29.69	(±1.40)
180	2.15	(±0.79)	2.83	(±1.26)	1.79	(±0.27)	4.88	(±0.62)	13.75	(±1.04)
198	0.68	(±0.24)	0.53	(±0.21)	0.22	(±0.06)	0.64	(±0.14)	1.54	(±0.13)
209	0.02	(±0.002)	0.05	(±0.01)	0.05	(±0.01)	0.09	(±0.01)	0.22	(±0.02)

^a From reference 18. ^b Values and standard deviations (between parentheses) are for the means of triplicate samples.

RESULTS

Experimentally determined solubility data (nmol/L) at different concentrations of TC and TDC below and above the CMC are listed in Table II and Table III respectively for all 14 PCB congeners. At bile salt concentrations above the CMC, PCB solubilities are enhanced by several orders of magnitude, ranging from sixty to more than ten thousand times the aqueous solubility at the highest bile salt concentrations for PCB 14 and PCB 209 respectively.

Table III. Experimental solubilities (S in nmol/L) of polychlorinated biphenyls in phosphate buffered solutions of 0.0, 1.0, 2.5, 5.0, and 10.0 mM taurodeoxycholate. CMC = 2.7 mM^a.

IUPAC no.	$S_{0.0}^b$ (nmol/L)		$S_{1.0}^b$ (nmol/L)		$S_{2.5}^b$ (nmol/L) (x1000)		$S_{5.0}^b$ (nmol/L) (x1000)		$S_{10.0}^b$ (nmol/L) (x1000)	
14	285.2	(±3.1)	285.4	(±51.9)	1.48	(±0.01)	7.70	(±0.05)	16.98	(±0.01)
30	1140	(±12)	1169	(±207)	24.03	(±0.18)	111.3	(±0.6)	261.1	(±0.1)
15	320.9	(±20.5)	315.4	(±62.3)	6.02	(±0.10)	28.79	(±0.28)	59.21	(±0.19)
29	642.4	(±18.4)	682.2	(±125.2)	13.98	(±0.12)	78.87	(±0.36)	163.1	(±0.1)
49	28.03	(±2.62)	32.82	(±5.74)	2.86	(±0.04)	11.25	(±0.07)	27.80	(±0.01)
40	85.24	(±9.61)	99.44	(±19.31)	3.31	(±0.03)	13.76	(±0.08)	30.57	(±0.15)
61	48.00	(±6.06)	61.78	(±8.70)	5.54	(±0.02)	21.27	(±0.14)	51.88	(±0.02)
155	3.74	(±0.62)	6.05	(±0.78)	1.91	(±0.02)	7.07	(±0.04)	18.28	(±0.04)
116	12.01	(±2.24)	18.02	(±2.32)	2.58	(±0.02)	9.38	(±0.17)	26.93	(±0.01)
136	4.85	(±0.82)	7.18	(±0.86)	1.32	(±0.02)	5.52	(±0.03)	13.20	(±0.05)
128	5.74	(±1.18)	10.49	(±1.02)	4.24	(±0.04)	17.90	(±0.19)	41.34	(±0.01)
180	2.34	(±0.23)	2.94	(±0.71)	1.65	(±0.03)	6.91	(±0.06)	16.36	(±0.01)
198	0.72	(±0.27)	0.62	(±0.13)	0.24	(±0.01)	1.02	(±0.01)	2.45	(±0.01)
209	0.02	(±0.006)	0.06	(±0.01)	0.03	(±0.01)	0.11	(±0.01)	0.27	(±0.01)

^a From reference 18. ^b Values and standard deviations (between parentheses) are for the means of triplicate samples.

PCB solubilities in the TDC solutions are considerably higher than in the TC solutions, the solubility of PCB 61 in 10 mM TDC for example, is three times as high as in 10 mM TC, for PCB 15 this is even seven times as high, as can be seen in Fig. 1. As this figure illustrates, the enhancement of the solubility above the CMC depends linearly on the bile salt concentration.

Standard deviations of the triplicate experiments for aqueous solubilities range from 2% up to 38%. At bile salt concentrations above the CMC standard deviations become extremely low in the case of TDC (between 0.01% and 2.6%), but for the experiments with TC they are between 3.5% and 30%. Due to the fact that only the pure solid that remained on the chromosorb is analyzed instead of solute extracted from the water, the percentage of error of the experimental solubility data is not correlated to the order of magnitude of these data. If aqueous solubility data are based on analyses of chemical that is extracted from the water, impurities do interfere more severely with solutes that have to be extracted from larger volumes of water, yielding higher inaccuracies for compounds possessing lower aqueous solubilities. From Table II and Table III it can be seen that even at bile salt concentrations below the CMC a small but significant ($P < 0.01$) solubility enhancement occurs, probably due to the presence of bile salt monomers in the buffer.

Fig. 1: Solubilities of PCB 15 and PCB 49 in solutions of 0.0, 2.5, 5.0, 10.0 and 20.0 mM taurocholate (open symbols) and in solutions of 0.0, 1.0, 2.5, 5.0 and 10.0 mM taurodeoxycholate (closed symbols). Standard deviations of triplicate measurements expressed as error bars if visible.

Fig. 2: Micelle/water partition coefficients (log K_{mw}) of PCB 49 and PCB 209 in solutions of 5.0, 10.0 and 20.0 mM taurocholate (open symbols) and in solutions of 2.5, 5.0 and 10.0 mM taurodeoxycholate (closed symbols).

Fig. 2 shows the micelle/water partition coefficients for PCB 49 and PCB 209 as determined by equation 4. It can be seen that the log K_{mw} for these PCBs in solutions of TDC are higher than in solutions of TC. Furthermore, it can be seen that the log K_{mw} in the solutions at different bile salt concentrations are essentially equal. Differences in log K_{mw} between solutions are smaller than two times the average of their individual standard deviations, as expressed by error bars in the figure. Average standard deviations range from 0.02 to 0.10 log units in the experiments with TDC and from 0.12 to 0.44 log units in the TC experiments. Standard deviations of the mean log K_{mw} between the solutions of different bile salt concentrations range from 0.01 to 0.07 log units for TDC and between 0.02 and 0.14 log units for TC. The values obtained are listed in Table IV.

Table IV. Experimental Micelle/Water Partition Coefficients ($\log K_{mw}$) for PCBs in TC and TDC.

IUPAC no.	$\log K_{mw}$ (TC)		$\log K_{mw}$ (TDC)	
14	3.89	(± 0.05)	4.26	(± 0.05)
30	4.23	(± 0.07)	4.84	(± 0.03)
15	3.95	(± 0.14)	4.79	(± 0.05)
29	4.51	(± 0.10)	4.87	(± 0.07)
49	4.96	(± 0.12)	5.42	(± 0.01)
40	4.64	(± 0.05)	5.01	(± 0.03)
61	5.01	(± 0.08)	5.43	(± 0.02)
155	5.60	(± 0.08)	5.97	(± 0.02)
116	5.35	(± 0.03)	5.64	(± 0.03)
136	5.45	(± 0.02)	5.76	(± 0.01)
128	5.75	(± 0.06)	6.1	(± 0.02)
180	5.82	(± 0.04)	6.24	(± 0.01)
198	5.62	(± 0.02)	6.09	(± 0.01)
209	5.89	(± 0.12)	6.11	(± 0.03)

* Values and standard deviations (between parentheses) are for the mean of $\log K_{mw}$ values in 5.0, 10.0 and 20.0 mM TC solutions and 2.5, 5.0 and 10.0 mM TDC solutions respectively.

$\log K_{mw}$ values were compared to the $\log K_{ow}$ data collected from the literature that are listed in Table I. Data on $\log K_{ow}$ are selected by Mackay and Shiu except for PCB 14, PCB 180 and PCB 198 that are obtained from RP-HPLC experiments by Brodsky and Ballschmiter [21,25]. The linear relationship between the $\log K_{ow}$ and the $\log K_{mw}$ values of TC and TDC is only weak ($r^2 = 0.84$ and 0.82 respectively). The best fit of the relationship between $\log K_{ow}$ and the experimental $\log K_{mw}$ values of PCBs with a $\log K_{ow}$ value of 5-8.5, is in the case of TC a second order polynomial as shown in Fig. 3 and equation 5:

$$\log K_{mw}(\text{TC}) = -0.31 (\pm 0.05) \log K_{ow}^2 + 4.82 (\pm 0.70) \log K_{ow} - 12.82 (\pm 2.29) \quad (5)$$

$$n = 14, r^2 = 0.96, \text{ s.e.r.} = 0.15, F = 137$$

For TDC this relationship is also shown in Fig. 3 and expressed by equation 6:

$$\log K_{mw}(\text{TDC}) = -0.28 (\pm 0.06) \log K_{ow}^2 + 4.33 (\pm 0.77) \log K_{ow} - 10.52 (\pm 2.51) \quad (6)$$

$$n = 14, r^2 = 0.94, \text{ s.e.r.} = 0.16, F = 89$$

DISCUSSION AND CONCLUSION

As the method applied here is an indirect one, the error in the final results is an accumulation of the errors from the samples of the coated chromosorb, the chromosorb after aqueous exposure, and the chromosorb after the exposure to the micellar solution. Still, the analyses themselves proved to be so accurate that the final results, at least in the TDC experiments, show the same range of errors that is achieved with the well established generator column technique for solubility measurements and the slow stirring technique for determining partition coefficients in well defined n-octanol/water systems [26, 27].

The experimental solubility data were determined in a physiological saline phosphate buffer containing phosphate and sodium salt, but fit very well in the range of aqueous solubility data in pure water as reported in the literature (see Table I.). Data on aqueous solubility (S_w) are a selection of Mackay and Shiu, except for PCB 14 and PCB 180 that were determined by Dunnivant et al. and PCB 198 that was obtained from RP-HPLC data by Brodsky and Ballschmitter [21,26,25]. The presence of dissolved electrolytes in the buffer under the experimental conditions does not seem to have a major influence on the solubilities of the hydrophobic chemicals used in this study. Below the CMC a small enhancement of solubility is seen for these extremely hydrophobic chemicals probably due to an increasing concentration of organic monomers, a phenomenon that we described previously for PCBs in solutions of Sodium Dodecyl Sulphate (SDS) and that was also observed by Kile for DDT (with a $\log K_{ow}$ of 6.19) in solutions of non ionics [23, 28].

The enhancement of the PCB solubility in solutions with bile salt concentrations above the CMC shows to depend linearly on the micellar volume, at least under our experimental conditions, indicating that this phenomenon is due indeed to the process of dissolving in the expanding hydrophobic phase of these micelles. This observation is in agreement with the results of studies with artificial surfactants [20,23]. Results from Laher and Barrowman indicate that besides expanding the micellar volume of TC by increasing the bile salt concentration also addition of lipids, thus producing larger mixed micelles, increases the solubility capacity of bile salt micelles [15].

The values of PCB partition coefficients are independent of micellar concentration and are constant for bile salt concentrations above the CMC. Constant micelle/water partition coefficients over a wide range of micellar concentrations agree with earlier observations on SDS micelles and with observations by Park and Jaff on an anionic surfactant [23,29]. The systematically higher $\log K_{mw}$ values for hydrophobic compounds in TDC solutions compared to the TC solutions might be explained by the more hydrophobic character of TDC compared to TC as expressed by

its lower CMC and a higher RP-HPLC retention index [16]. Although the structure of a bile salt micelle/PCB aggregate is not clear, the size and aggregation number of TC micelles is smaller than that of TDC micelles [19]. This may play a role in the lower solubilizing capacity of TC micelles compared to TDC micelles due to the smaller gain in terms of entropy during the transfer of PCB molecules from the aqueous phase into the micellar phase.

Fig. 3: Relationship between the *n*-octanol/water partition coefficient ($\log K_{ow}$) and the micelle/water partition coefficient ($\log K_{mw}$) for taurocholate (open squares), for taurodeoxycholate (open circles) and for sodium dodecyl sulphate (closed circles). Standard deviations for experiments at different micellar concentrations are expressed as error bars, if large enough to be visible. Data on TC and TDC are from this work and data on SDS are from reference 23.

The breakdown of the linear relationship between $\log K_{mw}$ and $\log K_{ow}$ at very high $\log K_{ow}$ values agrees with recent observations on the relatively low partitioning between water and artificial membranes and bioconcentration levels of extreme hydrophobics [30-32]. The observed non-linearity of the relationship between $\log K_{ow}$ and $\log K_{mw}$ partly may be explained by the hydration of the polar areas of the bile salt micelles [20,28]. The influence of the polar region by screening off the hydrophobic core will be stronger for the more hydrophobic chemicals. Another explanation may be the more structured nature of the micellar core in comparison to bulk solvent such as *n*-octanol.

Relatively more energy is required to form a cavity in more structured phases for larger and more hydrophobic molecules in comparison to smaller ones and the gain in terms of entropy is less than in bulk solvents, resulting in lower partition coefficients and a loss of linearity between $\log K_{ow}$ and $\log K_{mw}$. This explanation is supported by the results of Park and Jaff that demonstrated relatively higher partitioning of nonionic organics into micelles than in the more structured hemimicelles with increasing molecular size of the solute [29]. In Fig. 3 the relationship between $\log$

K_{ow} and $\log K_{mw}$ for the same series of PCBs in SDS is drawn [23]. The deviation from the ideal linear relationship for SDS is somewhat less than for the bile salts. This result may indicate that the interior of the SDS micelles, which consists of apparently more flexible saturated alkyl chains, forms an intermediate between the bulk solvent n-octanol and the more rigid environment of the steroid structure of the bile salts.

Our results indicate that if the unstirred aqueous diffusion layer present in the gastrointestinal tract of vertebrates is a major transport barrier to hydrophobic compounds like PCBs, indigenous bile salt micelles may facilitate this transport by enhancing PCB solubility during food digestion. A proper understanding of the thermodynamics and mechanisms of partitioning of hydrophobics in micelle/water and 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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