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Bioconcentration of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in guppies after aqueous exposure to a complex PCDD/PCDF mixture: relationship with molecular structure

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Abstract

Guppies (*Poecilia reticulata*) were exposed to a complex mixture of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in water for 21 days. It is the first study in which accumulation data of 15 toxic dioxins and furans were quantified simultaneously in a continuous exposure experiment. First-order uptake rate constants (k_1) and first-order elimination rate constants (k_2) were calculated for the toxic, laterally substituted congeners. Bioconcentration factors (BCFs) were calculated from both the ratios of concentrations in fish and water and from the ratios of the rate constants. Dioxins and furans were selectively accumulated in fish tissue. The non-laterally substituted congeners were either not observed in fish tissue or showed lower BCF values than their laterally 2,3,7,8-substituted isomers. The log BCF values of laterally substituted congeners ranged from 3.90 ± 0.06 to 5.27 ± 0.07 . BCF values were smaller than predicted by existing linear relationships with $\log K_{ow}$. Several phenomena were discussed with respect to the observed bioconcentration behaviour, such as limited bioavailability, reduced lipid solubility, reduced membrane permeability and biotransformation. Furthermore, the similarity between structure–activity relationships considering bioaccumulation in fishes and the Ah-affinity in mammals was discussed.

Key words: Bioconcentration; PCDD; PCDF; Fish; Hydrophobicity

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are ubiquitous contaminants in the environment. They are formed by many

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different processes, for instance by the production of chemical substances like chlorinated phenols, by the pulp and paper industry, and by many kinds of incineration processes (Hutzinger and Fiedler, 1992; Rappe, 1992). Although the emissions by, amongst others, incinerator processes can be reduced in the next few years, PCDDs and PCDFs have accumulated in sediments over the last decades and these sediments have become a sink from which dioxins and furans might become available in the future.

PCDDs and PCDFs form a large group of compounds. There exist 75 chlorinated dioxin and 135 chlorinated furan congeners, many of which are toxic, persistent and hydrophobic. In fish, LD_{50} values of 2,3,7,8-TCDD range from 3 to $16 \mu\text{g kg}^{-1}$ wet weight (Kleeman et al., 1988). Some dioxins and furans are very potent inducers of the hepatic monooxygenase enzyme system (cytochrome P450). After one month exposure of rainbow trout to food contaminated with $9 \mu\text{g kg}^{-1}$ 2,3,4,7,8-PnCDF, ethoxyresorufin-*O*-deethylase (EROD) activity remained at an increased level for 180 days (Muir et al., 1990). Congeners with few chlorine atoms are biodegradable under certain conditions (Parsons and Storms, 1989), but a number of the tetra- and higher-chlorinated congeners are persistent in the environment. Furthermore, dioxins and furans are extremely hydrophobic compounds that readily associate with organic materials when entering the aquatic environment, resulting in high sediment concentrations (Karickhoff et al., 1979). Up to 310 ng kg^{-1} dry sediment TCDD equivalents have been reported in the river Rhine delta (Evers et al., 1988). Hence, dioxins and furans present a group of compounds of great concern in the aquatic environment.

In several laboratory studies the accumulation from water, food and sediment of a small number of congeners (≤ 7) has been quantified (Muir et al., 1985; Kuehl et al., 1986; Opperhuizen et al., 1986; Batterman et al., 1989; Gobas and Schrap, 1990).

In this study, the bioconcentration of a mixture containing a large number of dioxins and furans in guppies was studied for 21 days. It is the first study that quantifies accumulation data for 15 out of the 17 toxic congeners simultaneously in a continuous exposure system. The entire experiment was performed in triplicate. The accumulation and elimination behaviour of the dioxins and furans is presented and discussed in relation to their *n*-octanol/water partition coefficients and their molecular structures.

2. Materials and methods

Chemicals

Dioxins and furans were extracted from 190 g fly-ash (pretreated with 3% HCl) from a municipal incinerator near Zaanstad, The Netherlands. The fly-ash extract was cleaned up as described below for the fish samples and concentrated in 5 ml hexane. [^{13}C]-PCDDs and [^{13}C]-PCDFs (internal standards) were obtained from Cambridge Isotopes Laboratories. All solvents used (hexane, toluene, *n*-nonane, dichloromethane, chloroform, and tetrachloromethane) were of glass distilled quality (Rathburn). Reagents used were silica gel (kieselgel 60, 70–230 mesh), aluminium

oxide (Al_2O_3 , basic 70–230 mesh), silver nitrate (AgNO_3 p.a.), sodium hydroxide (NaOH p.a.) and concentrated sulfuric acid (H_2SO_4 p.a.).

Accumulation experiment

The experiment was performed in triplicate in three separate aquaria (16 l water), each with an individual delivery system, consisting of a pump, a generator column with chromosorb and glass tubings. Chromosorb (20 g, Chrompack, P-AW 60–80 mesh) was coated with fly-ash extract (200 μl). To coat chromosorb, 200 μl of fly-ash extract was diluted to a volume of 50 ml with hexane. The diluted extract was added to the chromosorb and hexane was evaporated under nitrogen at 50°C . Coated chromosorb was placed into a glass generator column. Water (Amsterdam tap water:demineralised water, 1:1) was pumped continuously through the generator column (Veith and Comstock, 1975). The flow rate of the water was 10 l h^{-1} . The aquaria were heated with RENA 50-W electric heaters. The temperature was controlled to $24.8 \pm 0.6^\circ\text{C}$. The water was aerated through glass capillaries.

After 5 days water circulation over chromosorb, 25 adult female guppies (*Poecilia reticulata*) (average weight 0.91 ± 0.21 g, lipid content $9.7 \pm 2.4\%$ w/w) were placed into each of the three systems. The fish were held in 16 h light/8 h dark cycles with fluorescent daylight lamps. The water circulation over chromosorb was continued during the exposure experiment; every week the chromosorb was replaced with freshly coated (100 μl fly-ash extract) chromosorb. Faeces and food residues were removed from the aquaria twice a week by an aquarium vacuum cleaner device. After 0, 1, 2, 4, 8, 14 and 21 days a fish sample consisting of two guppies was taken from each system. Hence, at every sampling time three samples, consisting of two guppies each, were collected. Fish samples were stored at -18°C . Water samples (250 ml) were taken at day 0, 4, 8, 11, 15 and 21. Water was extracted immediately after sampling. Blank fish and water were sampled at regular time intervals from a fourth aquarium (blank). A control sample (clean-up procedure without fish or water) was run with each batch of six samples.

Sample analysis

Fish samples were freeze dried for 24 h and prior to extraction a mixture of 10 [^{13}C]-PCDDs and [^{13}C]-PCDFs was added as internal standard. The internal standard mixture contained [^{13}C]-2,3,7,8-TCDD, [^{13}C]-2,3,7,8-TCDF, [^{13}C]-1,2,3,7,8-PnCDD, [^{13}C]-2,3,4,7,8-PnCDF, [^{13}C]-1,2,3,6,7,8-HxCDD, [^{13}C]-1,2,3,4,7,8-HxCDF, [^{13}C]-1,2,3,4,7,8-HpCDD, [^{13}C]-1,2,3,4,6,7,8-HpCDF, [^{13}C]-OCDD and [^{13}C]-OCDF. The samples were soxhlet-extracted with toluene for 24 h. For the determination of lipid, a known fraction of the toluene extract was evaporated till constant weight. The lipid contents were quantified by weighing. For the PCDD and PCDF analysis, the toluene extract was cleaned up by open column chromatography. Silica was washed with dichloromethane and dried under a nitrogen stream during 24 h. Silica was activated under nitrogen at 180°C for 90 min. Activated silica was treated with H_2SO_4 or NaOH . First, the samples were passed over macro-columns (diameter 10 mm) with H_2SO_4 on silica (2 cm 22%, followed by 10 cm 44% w/w) and NaOH on silica (9 cm 33% w/w). The macro-columns were eluted with 50 ml hexane. After concentration of

the samples under nitrogen at 50°C, the extracts were transferred to high aspect columns (diameter 6 mm) with AgNO₃ on silica (5 cm 10% w/w) and high aspect columns with Al₂O₃ (basic, 70–230 mesh, 19 cm). Both high aspect columns were eluted with 80 ml hexane. The Al₂O₃ columns were subsequently eluted with 20 ml 10% CCl₄ and 30 ml dichloromethane. The dichloromethane extracts were concentrated under nitrogen at 50°C and transferred with hexane to a small glass vial. Water samples were extracted with hexane (three times 5 ml) and cleaned up by the same procedure with exception of the AgNO₃ column.

Quantification of fish and water extracts was performed by GC-MSD analysis (HP5890/HP5970) with on column injection, using a 60 m Supelco 2331 column; temperature program: 140°C, 40°C/min, 200°C, 4°C/min, 250°C. Because of suspected degradation of OCDF on a Supelco 2331 column (Olie et al., 1989), OCDF was analysed using a DB5 column (30 m); temperature program: 160°C, 70°C/min, 300°C. Prior to injection the extracts were evaporated to near dryness and dissolved in *n*-nonane. Two μ l were injected at an inlet pressure of 12 psi ($\approx$ 83 kPa) He and analysed with the SIM program (selected ion monitoring). In the fly-ash extract, 104 different peaks were detected of tetra and higher-chlorinated PCDDs and PCDFs by GC-MSD analysis. Lower-chlorinated PCDDs and PCDFs were not quantified. Detection limits were defined as the ratio of two times the background noise divided by the internal standard peak, multiplied by the amount of internal standard.

Data analysis

The first-order uptake and elimination rate constants of PCDDs and PCDFs were calculated by fitting the data to a first-order accumulation curve by iterative nonlinear regression analysis (SAS, 1990). The best fitting values for the uptake and elimination rate constants and the asymptotic standard errors were calculated. Uptake and elimination rate constants were calculated for 2,3,7,8-substituted PCDDs and PCDFs. For non-2,3,7,8-substituted PCDDs and PCDFs, kinetic rate constants were not calculated because only a limited number of reliable concentrations in fish were found. The reason was that the concentrations of the non-2,3,7,8-substituted congeners in fish were often below or around the detection limits.

Values of bioconcentration factors (BCFs) were calculated in three ways. First BCFs were calculated as the ratio of the concentrations in the fish at day 21 and the average concentrations in water for 21 days (Eq. 1). This method was applied to both 2,3,7,8- and non-2,3,7,8-substituted congeners. The BCF was calculated for each system and averaged ($n = 3$). Secondly, BCFs were calculated by using the concentrations in fish that were estimated by Eq. (2). In this equation, the values estimated for k_1 and k_2 by nonlinear regression analyses and the average concentrations in water were used to calculate the concentrations in fish after 21 days exposure. The BCF values were calculated by the ratio of the calculated concentrations in fish ($C_{f,calc}$) and the average concentrations in water (C_w) (Eq. 3). Finally, BCF values were calculated from k_1 and k_2 according to Eq. (4). These BCF values give the best estimate because they are not dependent on the duration of the exposure period, but represent equilibrium values. Because k_1 and k_2 were not determined for non-2,3,7,8-substituted con-

genera, Eqs. (2), (3) and (4) were applied only to the 2,3,7,8-substituted congeners. The concentrations in fish were on lipid weight basis in all BCF calculations:

$$\text{BCF} = C_{\text{f,exp}}/C_{\text{w}} \quad (1)$$

$$C_{\text{f,calc}} = k_1/k_2 \cdot C_{\text{w}} \cdot (1 - \exp(-k_2 t)) \quad (2)$$

$$\text{BCF} = C_{\text{f,calc}}/C_{\text{w}} \quad (3)$$

$$\text{BCF} = k_1/k_2 \quad (4)$$

In Eqs. (1)–(4), BCF is the bioconcentration factor (1 kg^{-1}), $C_{\text{f,exp}}$ experimentally determined concentration in fish ($\mu\text{g kg}^{-1}$), $C_{\text{f,calc}}$ calculated concentration in fish ($\mu\text{g kg}^{-1}$), C_{w} average concentration in water for each individual aquarium during the entire exposure period ($\mu\text{g l}^{-1}$), k_1 first-order uptake rate constant ($1 \text{ kg}^{-1} \text{ d}^{-1}$), k_2 first-order elimination rate constant (d^{-1}), and t time (d).

3. Results and discussion

3.1. Concentrations in water

In two blank water samples, 1,2,3,4,6,7,8-HpCDF and OCDD were detected in trace concentrations. Since they were also observed in one control sample, their presence was probably caused by contamination during GC-analysis. The concentrations of HpCDF and OCDD in the control and blank water samples were very small compared to the concentrations in the spiked water, hence this contamination is further neglected.

The average concentrations in water of the individual congeners during the experiment ranged from not detected to 2.08 ng l^{-1} for 1,3,4,7-TCDF. Of the 104 different peaks detectable in the fly-ash extract, 79 peaks of PCDDs and PCDFs were detected in the exposure water. Concentrations of the other congeners were below detection limits. In Table 1, the average concentrations of congeners that were present in water, but that were not detected in fish during the exposure period are included. The average aqueous concentrations of PCDDs and PCDFs that did accumulate in fish tissue are presented in Table 2. The water concentrations were below the reported water solubilities of PCDFs and PCDFs (Friesen et al., 1985, 1990), except for OCDD, whose concentrations exceeded the reported maximum water solubility. The latter congener is a very hydrophobic compound with large affinity for organic matter (Servos et al., 1989). The relatively large concentrations in water may be explained by adsorption to organic matter or by the formation of micells.

During the uptake experiment, the water concentrations were maintained at rather constant values. The concentrations in water are presented in Fig. 1 for four congeners. A decline in the concentrations of some hepta- and octachlorinated compounds was observed during the third week.

3.2. Concentrations in fish

During the exposure period, four out of 75 guppies died spontaneously. In control fish samples no PCDDs or PCDFs were detected. The average detection limits ranged from circa 40 to 270 pg per sample for the different congeners. This corresponds to an average concentration of 0.23–1.53 $\mu\text{g kg}^{-1}$ fish (lipid weight). The concentrations in fish at the end of the exposure period ranged from not detected to $58.65 \pm 8.29 \mu\text{g kg}^{-1}$ lipid weight (Table 2). The latter concentration was obtained for 1,2,3,4,6,7,8-HpCDD.

Table 1

Concentrations in water (C_w , AVG $\pm$ SE) during 21 days of PCDDs and PCDFs that were not detected in fish

PCDDs		C _w (ng l ⁻¹)		PCDFs		C _w (ng l ⁻¹)	
		AVG	SE			AVG	SE
TCDD	1368	0.16	0.03	TCDF	1368	0.12	0.05
	1379	0.15	0.04		1378, 1379	0.13	0.12
	1378	0.12	0.04		1246	0.07	0.01
	1369, 1247, 1248 ^a	0.15	0.06		1268, 1237, 1478	0.18	0.09
	1268	0.08	0.03		2349, 1234	0.11	0.06
	1478	0.11	0.07		2468, 1238, 1467, 1236	0.11	0.04
	1234, 1237, 1238, 1246, 1249	0.23	0.06		1278	0.06	0.03
	1236, 1279	0.12	0.03		1267, 1279	0.08	0.01
					2368	0.10	0.02
PnCDD	12479, 12468	0.42	0.08	PnCDF	2467	0.15	0.01
	12368	0.30	0.06		2347, 1239	0.13	0.03
	12478	0.17	0.02		2346	0.13	0.03
	12379	0.27	0.06		3467	0.15	0.04
	12469, 12347	0.17	0.03		13468	0.13	0.04
	12369	0.07	0.00		12468	0.09	0.03
	12489, 12467, 12346	0.16	0.02		23479	0.10	0.04
	12367	0.16	0.04		12478	0.13	0.04
	12389	0.13	0.02		12467	0.11	0.03
HxCDD	124679, 124689, 123468	0.95	0.17		23469, 12347 12346	0.13 0.07	0.03 0.04
	123679, 123689	0.88	0.16		23468, 12469	0.11	0.09
	123467	0.21	0.03				
					13489	0.05	0.01
HpCDD	1234679	0.94	0.12		23489	0.20	0.05
					12349	0.11	0.09
				HxCDF	123689, 123469	0.06	0.01
				HpCDF	1234689	0.16	0.02

^a Peaks of isomers not separated on GC-column.

After 21 days a smaller number of PCDDs and PCDFs was detected in the fish tissue than in the exposure water, namely 28 compounds instead of 79 compounds in water.

3.3. Accumulation kinetics

The accumulation kinetics of four congeners are shown in Fig. 1. No PCDDs and PCDFs were detected at the start of the experiment. Fifty percent of the average detection limits in fish samples are presented at $t = 0$ in Fig. 1. Fig. 1a is representative for the non-laterally substituted congeners. Fig. 1b–d are representative for the 2,3,7,8-substituted compounds. In general an increase in fish concentration is observed during the first week for the 2,3,7,8-substituted congeners. Plateau levels seem to be reached after 14 days exposure. The accumulation curve can be described by Eq. (2) which is the integrated form of Eq. (5):

$$dC_{\text{fish}}/dt = k_1 C_w - k_2 C_{\text{fish}} \quad (5)$$

where C_{fish} is concentration in fish ($\mu\text{g kg}^{-1}$), C_w concentration in water ($\mu\text{g l}^{-1}$), t time of exposure (d), k_1 uptake rate constant ($\text{l kg}^{-1} \text{d}^{-1}$), and k_2 elimination rate constant (d^{-1}).

From Eq. (5) it is evident that the concentration in fish will increase in time, but the

Table 2

Average PCDD and PCDF concentrations in fish (C_f) after 21 days exposure, average concentrations in water (C_w) during 21 days and water solubilities (S) from literature (Friesen et al., 1985, 1990)

PCDDs	C_f (ng g ⁻¹)		C_w (ng l ⁻¹)		S (ng l ⁻¹)	PCDFs	C_f (ng g ⁻¹)		C_w (ng l ⁻¹)		S (ng l ⁻¹)
	AVG	SE	AVG	SE			AVG	SE	AVG	SE	
2378	5.08	1.31	0.08	0.02	483	12367	0.14		0.13	0.04	
12378	36.01	8.21	0.21	0.04	4.42	23478	21.98	2.20	0.16	0.04	
123478	21.10	3.41	0.20	0.04		23467	16.47	14.94	0.40	0.06	
123678	24.68	3.10	0.23	0.07		123468	1.79		0.15	0.04	
						124678, 134679	2.10	0.30	0.32	0.06	
123789	24.15	3.05	0.28	0.07		134678	0.15		0.26	0.06	
1234678	58.65	8.29	1.31	0.17	2.40	124679	0.47	0.12	0.07	0.02	
OCDD	13.14	1.32	0.80	0.16	0.40	123479, 123478	16.11	1.94	0.21	0.05	8.25
PCDFs						123678	18.00	2.12	0.22	0.04	17.7
1347	0.68	0.30	2.08	0.73		123467	1.32	0.23	0.26	0.05	
2378	2.17	0.28	0.12	0.02		123679	0.40	0.10	0.02	0.01	
2367	0.89	0.14	0.14	0.02		234678	19.83	2.66	0.19	0.04	
12479, 12368	1.35	0.31	0.18	0.05		1234678	26.96	4.51	0.68	0.18	1.35
12479, 12347	0.70	0.28	0.14	0.04		1234679	0.13	0.09	0.17	0.05	
12348, 12378	3.00	0.76	0.14	0.04		OCDF	1.97	0.46	0.09	0.01	1.16

Fig. 1. Bioaccumulation kinetics of non-2,3,7,8 (A) and 2,3,7,8-substituted PCDDs and PCDFs (B–D). A = 1,2,4,7,8 PnCDF, B = 2,3,4,7,8 PnCDF, C = 1,2,3,4,7,8 HxCDF, and D = 1,2,3,4,6,7,8 HpCDD. ●, concentration in fish (ng kg^{-1} lipid weight); □, concentration in water (ng l^{-1}).

Table 3

Uptake rate constants (k_1) and elimination rate constants (k_2) of 2,3,7,8-substituted PCDDs and PCDFs

PCDDs	k_1 ($\text{l kg}^{-1} \text{d}^{-1}$)		k_2 (d^{-1})		PCDFs	k_1 ($\text{l kg}^{-1} \text{d}^{-1}$)		k_2 (d^{-1})	
	$\bar{x}^a$	SE ^b	$\bar{x}$	SE		$\bar{x}$	SE	$\bar{x}$	SE
2378	500	175	0.049	0.048	2378	603	116	0.292	0.069
12378	952	405	0.030	0.054	12378	395	96	0.189	0.060
					23478	1012	401	0.044	0.053
123478	868	441	0.065	0.075	123478	879	440	0.087	0.082
123678	844	327	0.050	0.053	123678	1310	518	0.078	0.062
123789	687	321	0.074	0.072	234678	1101	432	0.103	0.069
1234678	456	247	0.081	0.086	1234678	524	283	0.127	0.106
OCDD	275	122	0.119	0.084	OCDF	217	131	0.174	0.143

Uptake rate constants are based on wet weight.

^a $\bar{x}$, estimate from nonlinear regression analysis.

^b SE, asymptotic standard error of regression.

rate of increase will decline as accumulation approaches equilibrium. Finally, the rate of uptake ($k_1 C_w$) equals the rate of elimination ($k_2 C_{\text{fish}}$) and equilibrium is reached.

Uptake and elimination rate constants calculated from Eq. (2) are shown in Table 3. The standard errors are relatively high. However, one should take into account that the values concern asymptotic standard errors with a non normal distribution.

The uptake rate constants varied between 217 ± 131 and $1320 \pm 518 \text{ l kg}^{-1} \text{ d}^{-1}$. These values are comparable to uptake rate constants reported in the literature for PCDDs, PCDFs and other hydrophobic chlorinated hydrocarbons (Connell and Hawker, 1988a; Opperhuizen and Sijm, 1990). Note the low uptake rate constants of the hepta- and octachlorinated compounds (217 ± 131 to $524 \pm 283 \text{ l kg}^{-1} \text{ d}^{-1}$). Two processes may contribute to these small values. Firstly, the measured concentration in water may be an overestimation of the bioavailable concentration. Furthermore, the small uptake rate constants of the higher-chlorinated congeners might be the result of reduced membrane permeability (Opperhuizen and Sijm, 1990). This aspect is discussed further on in this paper.

Table 4

Log bioconcentration factors (BCF_1) based on lipid weight (l kg^{-1})

	Non-2,3,7,8-substituted	log BCF_1^a $n = 3$		2,3,7,8-substituted	log BCF_1^b $n = 3$		log BCF_1^b	log BCF_1^c
		AVG	SE		AVG	SE		
TCDD				2378	5.24	0.22	4.83	5.02
TCDF	1347	2.53	0.18	2378	4.19	0.07	4.33	4.33
	2367	3.73	0.03					
PnCDD				12378	5.27	0.07	5.18	5.52
PnCDF	13479, 12368	3.92	0.06	12378, 12348	4.59	0.15	4.33	4.33
	13467, 12479	3.69	0.02	23478	5.14	0.18	5.15	5.38
	12367	2.88	0.30					
	23467	4.58	0.27					
HxCDD				123478	5.01	0.06	5.01	5.14
				123678	4.94	0.19	5.05	5.24
				123789	4.93	0.09	4.88	4.98
HxCDF	123468	3.75	0.30	123478, 123479	4.91	0.09	4.99	5.04
	124678, 134679	3.65	0.18	123678	4.95	0.05	5.15	5.24
	134678	2.41	0.30	234678	4.99	0.06	4.94	5.02
	124679	3.97	0.08					
	123467	3.79	0.01					
	123679	4.21	0.15					
HpCDD				1234678	4.68	0.06	4.68	4.76
HpCDF	1234679	2.64	0.30	1234678	4.46	0.07	4.60	4.63
				OCDD	4.13	0.02	4.34	4.38
				OCDF	3.90	0.06	4.10	4.11

^a $\text{BCF}_1 = C_{\text{fish}} \text{ at 21 days} / \text{AVG } C_{\text{water}}$ (measured values); Eq. (1).

^b BCF_1 idem, however C_{fish} at 21 days calculated with fitted curve; Eq. (3).

^c $\text{BCF}_1 = k_1/k_2$ (from non-linear regression analysis); Eq. (4).

The elimination rate constants varied from 0.030 ± 0.054 to 0.292 ± 0.069 d^{-1} . These values are also in the same range as values from the literature (Connell and Hawker, 1988a; Oppenhuizen and Sijm, 1990). The elimination rate constants of PCDFs are consistently larger than those of PCDDs. The differences in elimination rate constants between PCDFs and PCDDs were also observed in rats. Rose et al. (1976) and Van den Berg et al. (1989) determined the half lives of 2,3,7,8-TCDD and 2,3,7,8-TCDF in rats as 31 and 1 days, respectively.

3.4. Bioconcentration factors

The bioconcentration factor (BCF) indicates the potential accumulation of chemicals in aquatic organisms. In this study, the log BCF values were calculated in three different ways (Table 4), in all cases based on lipid weight. As expected, the BCF values calculated according to Eq. (1) agree quite well with the results obtained after curve fitting (Eq. 3). Furthermore, the BCF values calculated for day 21 (Eqs. 1 and 3) are slightly lower than the BCF values predicted at equilibrium (Eq. 4), indicating that equilibrium was not yet completely established at the end of the experiment. BCF values obtained after curve fitting (Eqs. 3 and 4) were calculated for 15 congeners (2,3,7,8-substituted compounds). However, BCF values of 28 congeners could be calculated with the experimentally determined concentrations (Eq. 1). Hence, the latter data are discussed here. The experimentally determined bioconcentration factors (Eq. 1) vary substantially. For instance a BCF of 260 was measured for 1,3,4,6,7,8-HxCDF, while for 2,3,4,6,7,8-HxCDF the BCF was as high as 100 000. The standard errors of the log BCF values calculated for the 2,3,7,8-substituted compounds were always less than 0.2 log units, whereas the standard errors of the non-

Fig. 2. Relationship between log BCF and log K_{ow} . The straight line indicates relationship calculated by Gobas et al. (1989) (Eq. 6); the dashed line indicate the polynomial relationship calculated by Connell and Hawker (1988) (Eq. 7). Symbols indicate experimentally determined BCF values from the present study. +, non-2,3,7,8-substituted congeners, Δ , 2,3,7,8-substituted congeners.

2,3,7,8-substituted congeners were less than 0.3 log units. These values are satisfactory, considering the biological processes involved.

A clear difference between 2,3,7,8- and non-2,3,7,8-substituted dioxins and furans is observed with respect to the bioconcentration factors. Although the concentrations in water of both groups are comparable, no non-2,3,7,8-substituted PCDDs were detected in fish. Hence, no BCF values could be calculated for non-2,3,7,8-substituted dioxins. The non-2,3,7,8-substituted furans in general show BCFs values a factor 5–100 lower than their 2,3,7,8-substituted isomers. Within the group of non-2,3,7,8-substituted congeners, furans substituted simultaneously at least at the 2,3,6 and 7 positions have relatively high BCF values. The same selective retention has been observed in mammals and carp (Van den Berg et al., 1989; Kuehl et al., 1987).

3.5. Relationship between log BCF and log K_{ow} -values

For many persistent hydrophobic organic chemicals the BCF can be predicted from the *n*-octanol/water partition coefficient (K_{ow}). The bioconcentration process is considered to be a partitioning process between the lipid phase of the fish and the water phase, analogous to the octanol/water partitioning process of hydrophobic chemicals (Veith et al., 1979; Esser, 1986). Several relationships have been established between log BCF and log K_{ow} by different authors (e.g. Gobas et al., 1989). Generally, a good linear relationship exists for chemicals which are not biotransformed with log $K_{ow} < 6$. For more hydrophobic chemicals, BCFs are often smaller than expected from their log K_{ow} value. Connell and Hawker (1988b) calculated a polynomial relationship that describes the reduced bioconcentration factors of very hydrophobic compounds.

The experimentally determined BCFs of PCDDs and PCDFs are plotted against their log K_{ow} values in Fig. 2. Log K_{ow} values were obtained from the literature (Sijm et al., 1989a; Shiu et al., 1988). Out of the 28 PCDDs and PCDFs for which BCFs were determined, log K_{ow} values were available for only 15 compounds. These log K_{ow} values ranged from 6.2 to 8.1. The straight line indicates the relationship that was calculated for halogenated hydrocarbons by Gobas et al. (1989) (Eq. 6). The dashed line describes the polynomial relationship calculated by Connell and Hawker (1988b). Their equation was originally based on wetweight data. Here the equation is transformed to lipid weight based BCFs for fishes having 10% lipid weight (Eq. 7).

$$\log \text{BCF} = 0.91 \cdot \log K_{ow} + 0.65 \quad (6)$$

$$\log \text{BCF} = 6.9 \cdot 10^{-3} \cdot (\log K_{ow})^4 - 0.185 \cdot (\log K_{ow})^3 + 1.55 \cdot (\log K_{ow})^2 - 4.18 \cdot \log K_{ow} + 5.79 \quad (7)$$

From Fig. 2 it is evident that the experimentally determined BCFs are lower than predicted from the linear relationship with log K_{ow} and that a linear relationship between log BCF and log K_{ow} is absent for these PCDDs and PCDFs. The BCFs of 2,3,7,8-substituted PCDDs and PCDFs (Δ) are predicted better by the polynomial relationship. Several explanations may contribute to the phenomenon that BCFs are

smaller than expected from the K_{ow} . These explanations are discussed below. Some are of general importance for hydrophobic compounds, others apply specifically to dioxins and furans.

Equilibrium. First, a true equilibrium may not have been reached. However, the differences in log BCF values (Eq. 3 versus Eq. 1, Table 4) are only small (generally less than 0.2 log units) and cannot explain the observed absence of the log K_{ow} /log BCF relationship.

Lipid solubility. When relating BCFs to K_{ow} values, lipid solubility is considered to be analogous to *n*-octanol solubility (Veith, 1979). However, as has been proposed by Anliker and Moser (1987) and Banerjee and Baughman (1991) the lipid solubility of relatively large hydrophobic compounds decreases with increasing K_{ow} . Gobas et al. (1988) determined partition coefficients between *n*-octanol, *n*-hexane or L- α -phosphatidylcholine dimyristoyl (DMPC) membranes and water. They found that for nonpolar chemicals with log K_{ow} values > 5.5, the membrane–water partition coefficients (K_{mw}) did not show a linear increase with larger octanol/water partition coefficients. K_{mw} became dependent on molar volume, which was explained by the relatively large amount of energy needed to form cavities for the solute in the structured membranes. This suggests that accumulation of large hydrophobic compounds is limited by their lipid solubility. Reduced concentrations in fish lipid at equilibrium results in smaller BCF values. In accordance with this, Chessels et al. (1992) reported that the reduction in bioaccumulation of very hydrophobic compounds with log K_{ow} > 6, corresponded to reduced lipid solubility of these compounds. Little is known about *n*-octanol and lipid solubility of dioxins and furans, but the log K_{ow} parameter may not be a good model for log BCF values for these chemicals.

Lipid solubility is influenced by electronic charge distribution. Asymmetrical substitution patterns have high dipole moments. A weak dipole moment has been calculated for symmetrical PCDDs (Koester and Hites, 1988). Hence, a better prediction of BCFs might be obtained by combining K_{ow} and dipole moments. De Voogt et al. (1990) pointed out that the BCFs of several PCDDs could be satisfactorily predicted by simultaneously using *n*-octanol/water partition coefficients and dipole moments.

Membrane permeation. It has been suggested that compounds with large effective cross diameters are limited in membrane permeability. Opperhuizen et al. (1985) pointed out that compounds with an effective cross diameter of more than 0.95 nm would show limited membrane passage. Dioxins substituted simultaneously at the 1 and 4 or 6 and 9 positions and furans simultaneously substituted at the 1,4,6 and 9 positions meet this criterion. This results in reduced uptake rate constants, but it should have no effect on the equilibrium bioconcentration factor as was reported by Geyer et al. (1992). A limited membrane permeation by the dioxins and furans involved in this study may be indicated by the relatively low uptake rate constants observed for hepta- and octachlorinated congeners ($< 524 \text{ l kg}^{-1} \text{ d}^{-1}$ for hepta- and octachlorinated congeners).

Bioavailability. Another factor contributing to the relatively low BCF values may be reduced bioavailability. PCDDs and PCDFs are extremely hydrophobic compounds. Therefore in the aquatic environment they will sorb to particulate and dissolved organic matter (such as humics) (Servos and Muir, 1989). In the current experiment organic substances may have been introduced into the water by the fishes. Although faeces and food residues were removed from the water, it cannot be excluded that some particulate and dissolved organic matter remained in the water. So in this experiment, part of the dioxins and furans were possibly sorbed to the organic matter that was introduced in the water by the guppies. It has been shown that only truly dissolved chemicals are readily available for uptake by fishes (Black and McCarthy, 1988). In the present experiment the total concentration in water was determined, including a possibly sorbed fraction. Therefore the available water concentrations may have been overestimated, which implies an underestimation of the true bioconcentration factors. However, two observations suggest that this phenomenon was probably of only limited importance in this experiment. Firstly, the measured concentrations in water did not exceed the reported maximum water solubilities in general. Secondly, if PCDDs and PCDFs were sorbed to organic materials in the water phase, an increase in the concentrations in water would be expected during the experiment, due to the introduction of faecal materials by the fishes. No such increase was observed.

Biotransformation. The lack of accumulation of non-2,3,7,8-substituted PCDDs and PCDFs has been attributed to selective biotransformation, which results in low bioconcentration factors. It was shown that biotransformation in mammals occurs preferentially at the 2, 3, 7 and 8 positions (Pluess et al., 1987). Chlorination of these positions strongly reduces the rate of biotransformation. Sijm and Opperhuizen (1988) and Sijm et al. (1989b) have shown that biotransformation of non-2,3,7,8-substituted dioxins and furans also occurs in fishes. It is therefore likely that the relatively low BCF values of non-2,3,7,8-substituted PCDDs and PCDFs are caused by biotransformation.

Protein binding. The processes discussed above give an explanation for the relatively low BCF values of PCDDs and PCDFs. However, some congeners show larger BCF values than expected, namely the 2,3,6,7-substituted dibenzofurans. Although, 2,3,6,7-substituted furans were expected to be biotransformed rapidly, bioconcentration was also observed for these compounds. Similar observations were reported for 2,3,6,7-TCDF and 2,3,4,6,7-PnCdf in mammals (Van den Berg et al., 1986) and in addition for 1,2,3,4,6,7-HxCDF in carp (Kuehl et al., 1987). This phenomenon was explained by the idea that 2,3,6,7-substituted isomers have a structure rather similar to 2,3,7,8-TCDD, which favours binding to specific proteins (Van den Berg and Poiger, 1989). Safe (1990) reviewed a number of studies that discussed the relationships between the binding affinity for the aryl hydrocarbon receptor (Ah-receptor) and the structure of PCDDs and related compounds. Although this concerned studies with mammals, the deduced structural rules seem also to apply to the BCF values obtained in the current study for fishes. The following structural properties show a

relationship with both Ah affinity and BCF values. Dioxins: both Ah affinity and BCF values decrease with decreasing lateral substitution; Ah affinity and BCF values of completely laterally substituted congeners decrease with increasing number of non-lateral chlorine substituents. The situation regarding dibenzofurans is somewhat more complex. Because of the asymmetry of the molecule, the four possible substituted positions on the ring have a different impact on the activity. Highest binding affinities and BCF values amongst dibenzofurans are observed for 2,3,7,8-substituted tetra- to hexachlorinated dibenzofurans. Both properties decrease with decreasing lateral substitution. Substitution at the C4 and/or C6 positions results in a larger increase in Ah-affinity than substitution at the C1 and/or C9 positions. In agreement with this, non-2,3,7,8-substituted PCDFs only accumulated if they were substituted at the C4 and/or C6 position. So although these structure-activity relationships were deduced from the Ah-affinities in mammals, they correspond to the BCF values in fishes. Apparently, the bioconcentration of PCDDs and PCDFs in fishes is governed by the same structure-activity relationships. This suggests that the Ah receptor or a structurally similar protein is also present in guppies.

4. Conclusions

First-order uptake and elimination rate constants were calculated for fifteen 2,3,7,8-substituted congeners. With respect to the uptake rate constants, it can be concluded that the hepta- and octachlorinated dioxins and furans have relatively low uptake rate constants. The uptake rate constants range from 217 to 1310 l kg⁻¹ d⁻¹. The first-order elimination rate constants range from 0.030 to 0.292 d⁻¹. Elimination rate constants of PCDFs are larger than those of PCDDs in fish.

PCDDs and PCDFs are accumulated selectively. Twenty-eight PCDDs and PCDFs were determined in guppies after exposure to water containing 79 detectable PCDDs and PCDFs. The other congeners were not detected in fish. A distinction can be made between the laterally substituted (2,3,7,8-substituted) congeners and the non-laterally (non-2,3,7,8-substituted) congeners. The non-2,3,7,8-substituted isomers show lower BCF values than the corresponding 2,3,7,8-substituted isomers, which was explained by biotransformation. The log BCF values of the 2,3,7,8-substituted PCDDs and PCDFs ranged from 3.90 to 5.27. From the results it is evident that the BCF values of these persistent PCDDs and PCDFs are lower than would be predicted from their high hydrophobicity. Several processes that can contribute to the relatively low BCF values are discussed. It can be summarised that (1) the influence of equilibrium not being reached is not significant; (2) limited lipid solubility of PCDDs and PCDFs will reduce bioaccumulation; (3) hepta- and octachlorinated congeners have reduced uptake rate constants, which may be explained by reduced membrane permeability; (4) the bioaccumulation can be limited due to a reduction in bioavailability; and (5) non 2,3,7,8-substituted congeners are biotransformed. Relatively high BCF values were observed for 2,3,6,7-substituted furans. The selective accumulation of these congeners and 2,3,7,8-substituted PCDDs and PCDFs can be predicted by the same structure-activity relationships that apply to the binding affin-

ity of these compounds to the Ah-receptor in mammals, indicating that similar processes are possibly involved in the accumulation of PCDDs and PCDFs in fish species.

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