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# BIOACCUMULATION OF SOME POLYCHLORINATED DIBENZO-p-DIOXINS AND OCTACHLORODIBENZOFURAN IN THE GUPPY (Poecilia reticulata)

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## ABSTRACT

A study is reported which investigates the bioconcentration and dietary accumulation of some chlorinated dibenzo-p-dioxins congeners (PCDDs) and octachlorodibenzofuran (OCDF) in the guppy (Poecilia reticulata). Dietary bioaccumulation of the PCDD congeners and OCDF was insignificant. The bioconcentration factors of PCDDs and OCDF were approximately two orders of magnitude lower than those of polychlorinated biphenyls of similar 1-octanol-water partition coefficient. It is demonstrated that the low bioconcentration and dietary bioaccumulation factors of the PCDDs are due to rapid depuration of the chemicals from the fish. Metabolic transformation of the PCDDs in the fish is shown to be an important factor causing this rapid depuration. Metabolic transformation of the PCDDs appears to involve hydroxylation, probably mediated by the mixed function oxidase system.

## INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDDs) are a class of chemicals with aqueous solubilities and 1-octanol-water partition coefficients ($K_{OW}$) comparable to those of polychlorinated biphenyls (PCBs) [1]. Consequently, the bioaccumulation behaviour of

PCBs and PCDDs have also been expected to show similarities. However, uptake and bioaccumulation studies of PCDDs and PCBs in fish suggest otherwise. For example, Muir et al. [2,3] and Muir and Yarechewski [4] reported bioconcentration factors and dietary accumulation of 1,3,6,8-tetra-, 1,2,3,7-tetra-, 1,2,3,4,7-penta-, 1,2,3,4,7,8-hexa-, 1,2,3,4,6,7,8-hepta- and octachlorodibenzo-p-dioxins in rainbow trout (Salmo gairderni) and fathead minnow (Pimephales promelas) which are lower than those of PCB congeners with equal $K_{OW}$. Bruggeman et al. [5] observed no significant uptake or accumulation of octachlorodibenzo-p-dioxin from the water in the guppy (Poecilia reticulata). Niimi and Oliver [6] reported short half-life times and low dietary absorption efficiencies of non-2,3,7,8-substituted PCDD congeners in the rainbow trout. Kuehl et al. [7] observed a preferential uptake of PCDD congeners with chlorines in the 2,3,7 and 8 positions in the rainbow trout. A similar study in the guppy by Opperhuizen et al. [8] showed no significant uptake of 1,4 and 1,6 substituted PCDD congeners. Only 2,3,7,8-substituted PCDDs, which did not have chlorines in the 1,4 or 1,6 positions, were absorbed and bioconcentrated by the fish. In summary, bioconcentration and dietary accumulation of PCDD congeners in fish are smaller than those of PCBs of similar 1-octanol-water partition coefficients ($K_{OW}$). The only exception is 2,3,7,8-tetrachlorodibenzo-p-dioxin, for which high bioaccumulation factors of 26,707 and 9,270 were measured in the rainbow trout by respectively Mehrle et al. [9] and Branson et al. [10].

Various explanations can be proposed for the low bioaccumulation tendency of many of the PCDDs: (i) Metabolic transformation in the organisms may prevent the accumulation of high levels of the parent PCDD congeners [4,11,12]. (ii) Sorption of PCDDs to organic matter in the water column may reduce uptake via the gills [2,3]. (iii) A lack of membrane permeability, due to large molecular cross sections (i.e. larger than 0.95 nm), has been suggested to prevent bioaccumulation of PCDD congeners with chlorines in the 1,4 and/or 1,6 positions [8,13]. In addition, short experimental exposure times (no steady-state) [14] and differences in equilibrium partitioning of dioxins in 1-octanol-water and fish lipid-water systems may contribute to the low bioaccumulation factors [15].

The objective of this study is to explore some of the factors controlling the bioconcentration and dietary bioaccumulation behaviour of various dioxin congeners. For this purpose, a bioconcentration and a dietary bioaccumulation study with some PCDD congeners and octachlorodibenzofuran in the guppy (Poecilia reticulata) are reported. The

experimental data are presented and combined with bioaccumulation data from other studies in a comprehensive analysis.

## MATERIALS AND METHODS

**Materials:**

2-Monochlorodibenzo-p-dioxin (2-MCDD), 2,7-dichlorodibenzo-p-dioxin (2,7-DCDD), 1,2,4-trichlorodibenzo-p-dioxin (1,2,4-$T_3$CDD), 1,2,3,4-tetrachlorodibenzo-p-dioxin (1,2,3,4-$T_4$CDD), octachlorodibenzo-p-dioxin (OCDD), 2,4,5-trichlorobiphenyl (TCB), decachlorobiphenyl (DCB), octachlorodibenzofuran (OCDF) and mirex were obtained from Foxboro Analabs. Glass distilled n-hexane, n-pentane, toluene and dichloromethane were obtained from Caledon Laboratories, Ontario.

**Experiments :**

Dietary Bioaccumulation : 105 One-year-old male guppies with an average weight of 0.0967 (± 0.0103) g and a lipid content of 5.0 %, and 20 female guppies were added to a 40 L fish tank containing dechlorinated, filtered tap water, gravel and aquatic plants. Throughout the experiment, the water was aerated and filtered over activated carbon to remove test chemicals, eliminated by the fish, from the water. No detectable amounts of the test chemicals were observed in the water. The oxygen content of the water was approximately constant at 8.2 ppm. The water temperature was 22 (± 1) $^{o}$C. Fish were fed contaminated fish food, administered as dry flakes on the surface of the water, at a rate of 0.021 g food/g fish/day for a period of 82 days.

Contaminated fish food was prepared by adding 70 g dry fish food (Tetramin) to a 400 mL (10:1) pentane/dichloromethane solution of the test chemicals. This solution was stirred for 90 minutes at 40$^{o}$C, after which the solvent was evaporated. Independent analysis of the contaminated food, by extraction with toluene, showed concentrations in the food of 52.0 µg/g for 2-MCDD, 220 µg/g for 2,7-DCDD, 51.1 µg/g for 1,2,4-$T_3$CDD, 43.0 µg/g for 1,2,3,4-$T_4$CDD, 31.6 µg/g for OCDD, 53.5 µg/g for OCDF, 53.9 µg/g for mirex, 54.9 µg/g for TCB and 52.0 µg/g for DCB. Samples of 3 male guppies were taken after 5, 11, 19, 30, 40, 51, 61, 68, 75 and 82 days. The fish were killed in liquid nitrogen and stored in a freezer until analysis.

Bioconcentration : 170 female guppies, with an average weight of 0.079 (± 0.02) g and a lipid content of 7.5 %, were exposed to a 35 L aqueous solution of 2-MCDD, 2,7-DCDD, 1,2,4-$T_3$CDD, 1,2,3,4-$T_4$CDD, OCDD, OCDF and TCB for 10 days. The bioconcentration experiments were performed as described previously [16]. Briefly, 35 L dechlorinated, filtered tap water was added to a 40 L fish tank and recirculated continuously at a flow rate of 50 L/d through, respectively, a sedimentation tank, a 50 um Mesh glass filter (to remove particulate matter from the water) and a generator column of Chromosorb W (methanol-, pentane- and toluene -washed) impregnated with the test chemicals. After 7 d of water recirculation, the fish were added (i.e. start of the experiment, t=0). The water circulation continued for 8 d, during which samples of 10 fish were taken after 6,7 and 8 d. After 8 d, water circulation was stopped. Of the remaining fish in the tank, 70 guppies were transferred to a depuration tank, in which the water was continuously aerated and filtered over activated carbon to remove test chemicals eliminated by the fish. No detectable amounts of the test chemicals were observed in the water of the depuration tank. The other 70 fish remained in the tank to let the system equilibrate. Then, samples of 10 fish were taken from both the equilibration and depuration tank after 2.5, 5.5, 8, 14, 24, 32 and 40 hr. Fish were killed in liquid nitrogen and stored in a freezer until analysis. Throughout the entire exposure period, pure oxygen was bubbled into the water. This kept the oxygen content at 8.0 (± 0.4) ppm. The temperature of the water was 22 (± 1)$^{o}$C. Fish were fed uncontaminated dried fish food (Tetramin) twice during the initial 8 d uptake period. During the experiment, no mortality or toxic effects were observed.

**Analysis :**

Fish : After thawing, the lengths and wet weights of the fish were determined. Then, the fish were homogenized in a porcelain mortar and refluxed, while stirring, in 100 mL of n-hexane and 50 mL of water for 90 minutes. The extract was centrifuged for 20 minutes at 4000 rpm. The top hexane layer was removed and concentrated by evaporation at 40$^{o}$C to about 4 to 5 mL. The concentrate was passed through a Florisil column to remove lipids. This extract was again concentrated, followed by gaschromatographic analysis.

The recovery of the extraction procedure was above 95% for all chemicals except 1,2,3,4-$T_4$CDD, OCDD and OCDF, for which recoveries of respectively 93%, 26% and 4% were determined. The low recoveries were due to poor elution of the Florisil columns. The

Florisil of the columns were therefore refluxed for 90 minutes in 150 mL of hexane to extract 1,2,3,4-$T_4$CDD, OCDD and OCDF from the column. This extract was concentrated by evaporation and analyzed by gaschromatography. The recovery of the complete extraction procedure, including extraction of the Florisil columns, for 1,2,3,4-$T_4$CDD, OCDD and OCDF was larger than 95%.

Fish lipids : The lipid content of the fish was determined by extraction with n-hexane, as described above. The hexane was then completely evaporated from the extract, after which the lipids were determined by weight.

Water : Throughout the bioconcentration experiment, water samples of 500 mL were taken from the centre of the fish tank at the same times that fish were sampled. Each water sample was extracted twice with 125 mL n-hexane. The extract was then concentrated by evaporation at 40°C and analyzed by gaschromatography. In addition, water samples of 500 mL were taken and passed through a Sep-pak HPLC column at a flow rate of 9 mL/min, as described by Landrum et al. [17]. This method has been proposed to distinguish between chemical in true solution and chemical sorbed to particulate matter. The water passed through the Sep-pak column was extracted twice with 125 mL hexane, then concentrated and analyzed by gaschromatography. The Sep-pak column was extracted with hexane. The extract was concentrated and analyzed by gaschromatography.

Gas chromatography : All samples were analyzed on a Hewlet-Packard 5890 gas chromatograph equipped with a $^{63}$Ni-electron capture detector at 300°C, an OGSE on-column injector and a 30m J&W Scientific DB17 fused silica capillary column (film thickness: 0.25 um). Carrier gas was ultra-high-pure-grade helium at a linear velocity of 30 cm/s at 50°C. Make up gas was ultra-high pure 5% methane-argon, which was applied at a flow rate of 60 mL/min. Temperature was programmed from 50 to 300°C.

**Metabolic transformation :**

After 6 d of aqueous exposure, a water and fish sample were extracted as described above and analyzed by gaschromatography with mass detection (GC-MS). A Hewlet-Packard 5987A GC-MS equipped with a 30 m DB-17 fused silica capillary column (J&W Scientific; film thickness: 0.25 um), and a Hewlet-Packard on-column injector was used for GC-MS analysis. Carrier gas was ultra-high-pure-grade helium at a linear velocity of 30 cm/s at

50°C. Chromatographic temperatures were 40°C for 3 min., then increasing to 200°C at 25°C/min and 315°C at 7.5°C/min, at which the temperature was held constant for 10 min. Mass detection was by chemical ionization with methane as the reagent at 400mTorr. Source temperature was 200°C. The transfer line temperature was 300°C. Mass range scans from 60 to 600 g/mol and mass selective detection were performed.

## RESULTS AND DISCUSSION

### Dietary bioaccumulation

Throughout the 82 d period, in which fish were simultaneously exposed to PCDD and PCB congeners and mirex in the food, no detectable amounts of 2-MCDD, 2,7-DCDD, 1,2,4-$T_3$CDD and 1,2,3,4-$T_4$CDD were found in the fish. The detection limits for fish tissue analysis of these dioxin congeners were respectively 0.1, 0.08, 0.05 and 0.01 µg/g. The fish/food concentration ratios of these dioxin congeners were therefore lower than 0.002 for 2-MCDD, 0.002 for 2,7-DCDD, 0.001 for 1,2,4-$T_3$CDD and 0.0002 for 1,2,3,4-$T_4$CDD. Small amounts of OCDD and OCDF (i.e. 0.02 to 0.1 µg/g) were found in all fish samples.

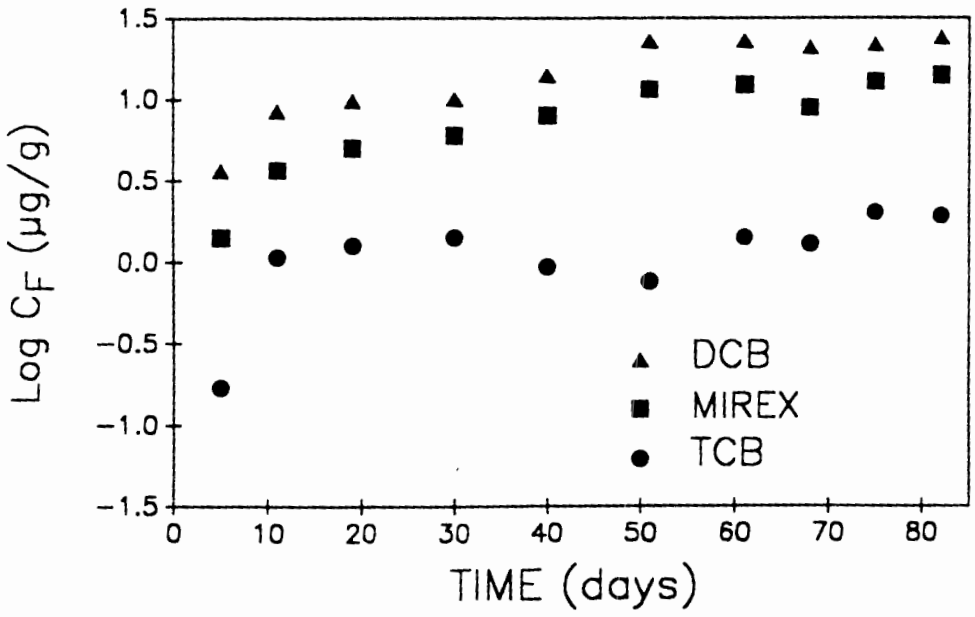


**Figure 1** : The logarithm of the observed concentrations (in µg/g) of 2,4,5-trichlorobiphenyl (TCB), decachlorobiphenyl (DCB), and mirex in the guppy during the dietary uptake experiment.

However, a consistent increase of OCDD and OCDF concentrations over time was not observed. The observed OCDD and OCDF concentrations may thus have been due to the presence of contaminated food in the gastro-intestinal tract at the time of sampling.
During the feeding period, concentrations of TCB, DCB and mirex in the fish increased over time. This behaviour is illustrated in Figure 1 and tends to fit a previously described [18,19] dietary accumulation model:

$$C_F = (E.F/k_T).C_A.(1 - \exp(-k_T.t)) \qquad (1)$$

where $C_F$ is the chemical concentration in the fish ($mol/m^3$), $C_A$ is the chemical concentration in the food ($mol/m^3$), E is the dietary absorption efficiency, F is the feeding rate in g food/g fish/d, $k_T$ is the (total) depuration rate constant ($d^{-1}$) of the chemical by the fish and t is time (d).

Equation 1 shows that with increasing time, the ratio of chemical concentration in the fish and the food i.e. $C_F/C_A$ approaches a constant value, i.e. $E.F/k_T$. Within the 82 d exposure period, TCB reached a constant $C_F/C_A$ ratio of 0.063. For DCB and mirex the 82 d exposure was too short to reach a constant fish/food concentration ratio (i.e. steady-state). It follows from equation 1 that the (steady-state) fish/food concentration ratio can be estimated from the fish/food concentration ratio at 82 d, $(C_F/C_A)_{82d}$, according to

$$(C_F/C_A) = (C_F/C_A)_{82d}/(1 - \exp(-82.k_T)) \qquad (2)$$

Since the $k_T$ was earlier determined to be 0.0050 $d^{-1}$ for DCB and 0.0045 $d^{-1}$ for mirex [16], a steady-state fish/food concentration ratios of 1.3 for DCD and 0.84 for mirex were calculated. The dietary uptake efficiencies, E, of TCB, DCB, and mirex were respectively 0.19, 0.31 and 0.18 or 19%, 31%, and 18% respectively.

The observed fish/food concentration ratios and dietary absorption efficiencies are comparable to those reported by Bruggeman et al. [5]. This demonstrates that the experimental conditions were satisfactory to observe dietary absorption. Since no dietary accumulation of the dioxin congeners was observed, whereas in the same experiment dietary uptake of the PCB congeners and mirex occurred, demonstrates that, relative to PCBs, dietary uptake and bioaccumulation of the dioxin congeners is insignificant.

the reported aqueous solubility. However, the "truly dissolved" concentrations of OCDD and OCDF were approximately 1000 times higher than the reported aqueous solubilities. It thus seems unlikely that the $C_D$ of OCDD and OCDF represents chemical that was truly dissolved. This suggests that Sep-pak filtration was not able to provide a reliable measurement of the "dissolved" solute concentration of OCDD and OCDF. This may be due to the very high $K_{OW}$ of these chemicals or the fact that the aqueous concentration was greatly exceeding the "dissolved" concentration.

During the uptake period, the concentration of TCB and the dioxin congeners in the fish increased with time and then reached a constant level (i.e. steady-state). This is illustrated in Figure 2, which shows the uptake and elimination of 1,2,4-$T_3$CDD. The bioconcentration factors ($K_C$) of the PCDDs were thus determined from the chemical concentration in the fish and the "dissolved" chemical concentration ($C_D$) in the water at steady-state, i.e. $K_C$ equals $C_F/C_D$. The bioconcentration factors are listed in Table 1.

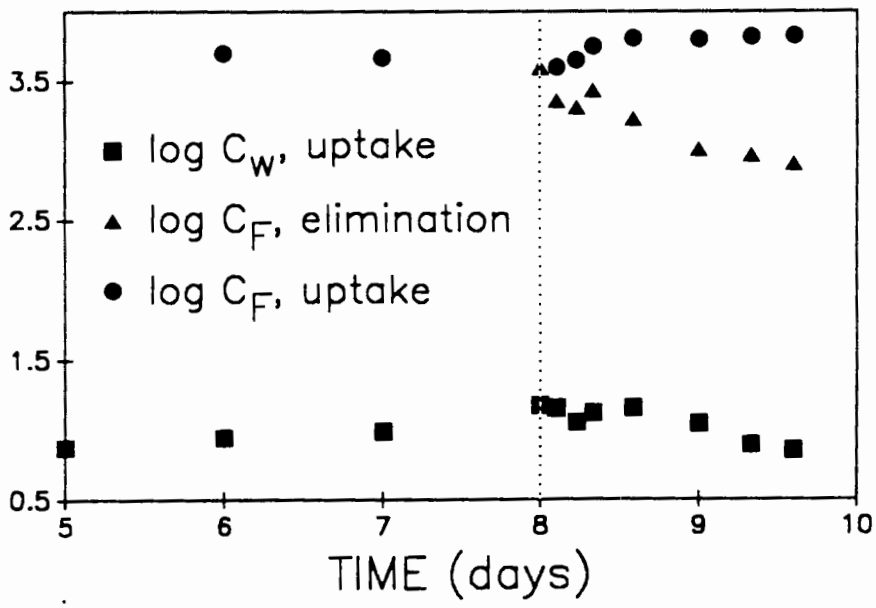


**Figure 2** : The logarithm of the concentration (in µg/kg) of 1,2,4-trichlorodibenzo-p-dioxin in the water ($C_W$) and in the fish ($C_F$) during the uptake and elimination experiment.

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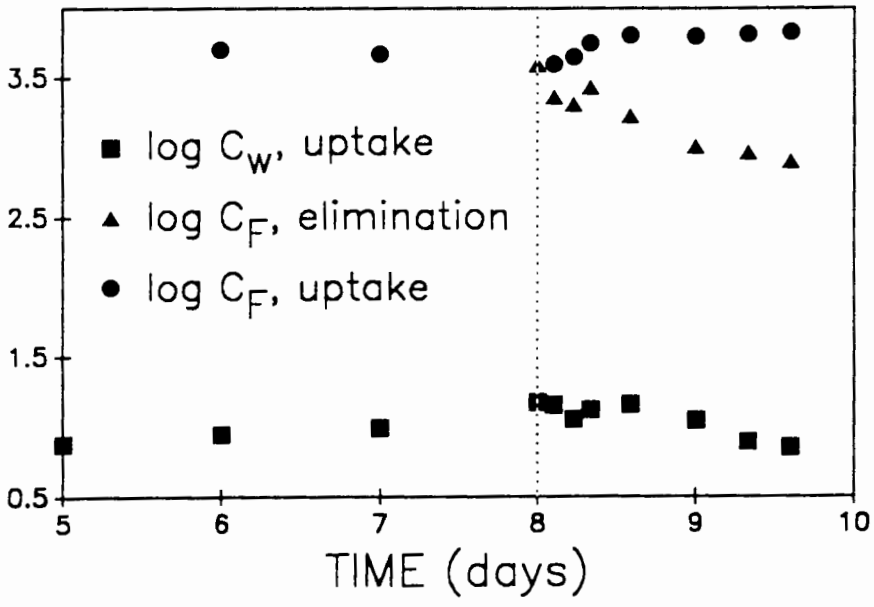


**Figure 2** : The logarithm of the concentration (in µg/kg) of 1,2,4-trichlorodibenzo-p-dioxin in the water ($C_W$) and in the fish ($C_F$) during the uptake and elimination experiment.

Table 2 : Reported 1-octanol-water partition coefficients $K_{OW}$ [1] and wet weight based, $K_C$, and lipid weight based, $K_L$, bioconcentration factors of PCDD congeners in various fish species.

| COMPOUND | log $K_{OW}$ | log $K_C$ | log $K_L$ | FISH SPECIES | REF. |
|---|---|---|---|---|---|
| 2,3,7,8-$T_4$CDD | 6.8 | 4.11 | 5.64 | Poecilia reticulata | [8] |
| 2,3,7,8-$T_4$CDD | 6.8 | 4.70 | 5.70 | Salmo gairderni | [9] |
| 2,3,7,8-$T_4$CDD | 6.8 | 3.97 | 4.97 | Salmo gairderni | [10] |
| 1,2,3,7-$T_4$CDD | 6.9 | 3.44 | 4.44 | Pimephales promelas | [2] |
| 1,2,3,7-$T_4$CDD | 6.9 | 3.17 | 4.17 | Salmo gairderni | [2] |
| 1,3,6,8-$T_4$CDD | 7.1 | 3.83 | 4.83 | Pimephales promelas | [2] |
| 1,3,6,8-$T_4$CDD | 7.1 | 3.39 | 4.39 | Salmo gairderni | [2] |
| 1,2,3,4,7-$P_5$CDD | 7.4 | 3.50 | 4.50 | Pimephales promelas | [2] |
| 1,2,3,7,9-$P_5$CDD | 7.4 | 3.20 | 4.73 | Poecilia reticulata | [8] |
| 1,2,3,7,8-$P_5$CDD | 7.4 | 3.89 | 5.41 | Poecilia reticulata | [8] |
| 1,2,3,4,7-$P_5$CDD | 7.4 | 3.26 | 4.26 | Salmo gairderni | [2] |
| 1,2,3,4,7,8-$H_6$CDD | 7.8 | 4.00 | 5.00 | Pimephales promelas | [2] |
| 1,2,3,7,8,9-$H_6$CDD | 7.8 | 2.95 | 4.48 | Poecilia reticulata | [8] |
| 1,2,3,4,7,8-$H_6$CDD | 7.8 | 3.73 | 4.73 | Salmo gairderni | [2] |
| 1,2,3,4,6,7,8-$H_7$CDD | 8.0 | 3.32 | 4.32 | Pimephales promelas | [2] |
| 1,2,3,4,6,7,8-$H_7$CDD | 8.0 | 3.74 | 4.74 | Salmo gairderni | [2] |
| OCDD | 8.2 | 4.35 | 5.35 | Pimephales promelas | [2] |
| OCDD | 8.2 | 3.93 | 4.93 | Salmo gairderni | [2] |

The bioconcentration factors of OCDD and OCDF are probably an underestimate of the real bioconcentration factors of these substances. The measured "dissolved" concentration ($C_D$) of OCDD and OCDF is likely to represent both dissolved and sorbed chemical. When only the dissolved chemical is able to be absorbed by the fish via the gills, as the study by Black and McCarthy [20] suggests, the use of the total chemical concentration (i.e. dissolved and sorbed) will underestimate the bioconcentration factor.

Figure 3 illustrates the relationship between the lipid-weight based bioconcentration factor ($K_L$) of the PCDDs in the guppies and the 1-octanol-water partition coefficient. Figure 3 also contains the bioconcentration factors of PCDD congeners in several other species of fish, which

are summarized in Table 2, and of a series of chloro- and bromo-benzenes and chloro- and bromo-biphenyls in the guppy [16]. Figure 3 shows that the bioconcentration factors of PCDDs are approximately two orders of magnitude smaller than the bioconcentration factors of halogenated biphenyls and benzenes of equal $K_{OW}$. The only exception is 2,3,7,8-$T_4$CDD, which appears to have a bioconcentration factor comparable to that of a PCB of equal $K_{OW}$.

Figure 4 demonstrates that the PCDDs were quickly depurated, when the contaminated fish were transferred to clean water. During the short elimination period of approximately 2 d, concentrations of the dioxin congeners in the fish dropped by approximately a factor of 10. In contrast, no significant decrease of the TCB concentration in the fish was observed. Since the rate constant for chemical depuration of TCB in the guppy was determined to be 0.063 $d^{-1}$ [16], corresponding to a half-life time of 11 d, a significant reduction of the TCB concentration over the 2 d elimination period was not anticipated.

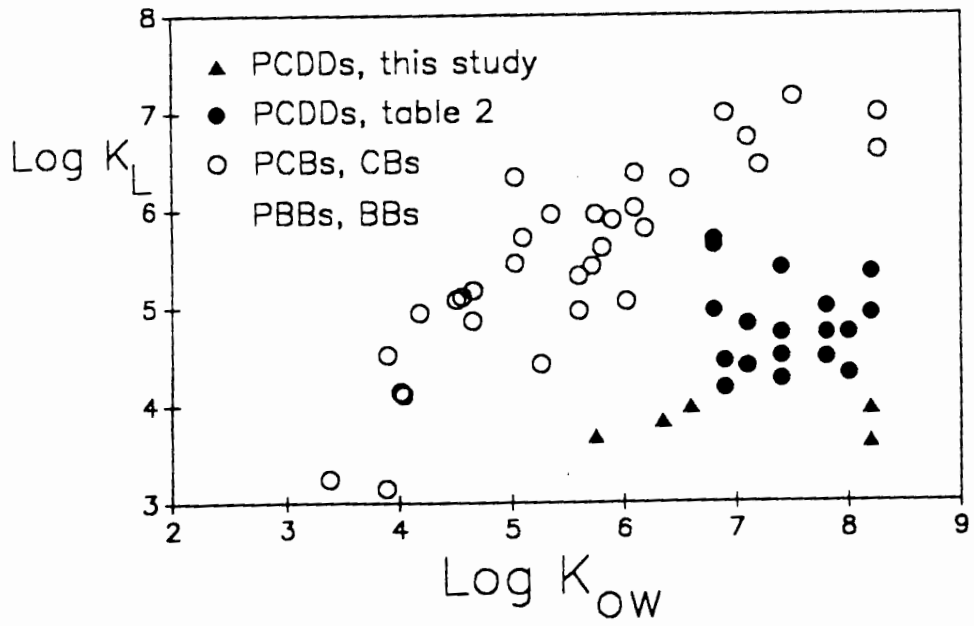


**Figure 3** : The logarithm of the lipid weight based bioconcentration factor ($K_L$) of some chlorinated dibenzo-p-dioxins (PCDDs), polychlorinated biphenyls (PCBs), chlorobenzenes (CBs), polybrominated biphenyls (PBBs) and bromobenzenes (BBs) in the guppy, as a function of the logarithm of the 1-octanol-water partition coefficient ($K_{OW}$).

Figure 4 illustrates that during the elimination period the logarithm of the PCDD concentrations in the fish tend to drop linearly with time. This suggests that, within the experimental detail achieved in this experiment, the uptake and elimination of PCDDs can be described by a fish-water two-compartment model with first-order kinetics [16,18,21] :

$$dC_F/dt = k_1.C_W - k_T.C_F \quad (4)$$

where $C_W$ is the bioavailable chemical concentration in the water, $k_1$ is the uptake rate constant from the water ($d^{-1}$), $k_T$ is the depuration rate constant ($d^{-1}$) and t is time (d). When the concentration in the water is constant, such as during the uptake period, integration of equation 4, results in

$$C_F = C_W.(k_1/k_T).(1 - \exp(-k_T.t)) \quad (5)$$

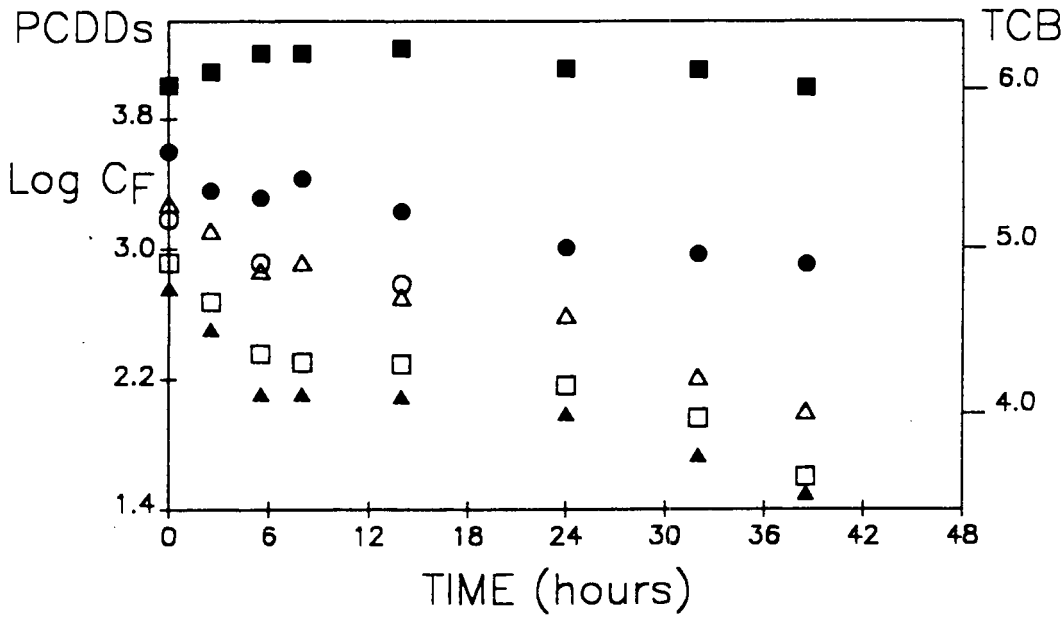


**Figure 4** : The logarithm of the concentration of 2,7-DCDD ( ○ ), 1,2,4-$T_3$CDD ( ● ), 1,2,3,4-$T_4$CDD ( △ ), OCDD ( ▲ ), OCDF ( □ ) and TCB ( ■ ) in the fish (in µg/kg) versus time during the depuration experiment.

When the concentration in the water is zero, such as during the depuration period, integration of equation 4 results in

$$C_F = C_{F,t=0} \cdot \exp(-k_T.t) \quad (6)$$

or

$$\log C_F = \log C_{F,t=0} - (k_T/2.303).t \quad (7)$$

where $C_{F,t=0}$ is the concentration in the fish at the start of the elimination period. Fitting equation 7 to the elimination data, provides an estimate of the depuration rate constant $k_T$ (Table 1). Since at steady-state, $C_F/C_W$ (or $K_C$) equals $k_1/k_T$, the uptake rate constant $k_1$ can be estimated from the bioconcentration factor ($K_C$) and the depuration rate constant (Table 1).

Comparison of the rate constants of PCDDs to those determined, in a similar fashion, for some halogenated benzenes and biphenyls, demonstrates that the uptake rate constants of PCDDs are approximately similar to those of halogenated benzenes and biphenyls of equal $K_{OW}$. For example, the uptake rate constants in the guppy of both PCDDs (this study) and halogenated aromatics [16] with a log $K_{OW}$ between 5.7 and 8.3 vary between 500 and 2000 $d^{-1}$. In contrast, the depuration rate constants of PCDDs are approximately 10 to 300 times larger than those of other halogenated aromatics of equal $K_{OW}$. For example, the depuration rate constant of 1,2,4-$T_3$CDD (log $K_{OW}$ = 6.35, $k_T$ = 0.91 $d^{-1}$) is approximately 100 times larger than that of 2,2',5,5'-tetrabromobiphenyl (log $K_{OW}$ = 6.50, $k_T$ = 0.0098 $d^{-1}$ [16]).

The rapid depuration rates indicate that the 10 d uptake period was sufficiently long for all PCDD congeners, including OCDD and OCDF, to achieve steady-state. The rapid depuration rates of the PCDDs also explain the low bioconcentration factors of the PCDDs. For example, considering the error in the measurements, the observation that the bioconcentration factor of 1,2,4-$T_3$CDD ($K_L = 10^{3.95}$) is approximately 200 fold lower than that of 2,2',5,5'-tetrabromobiphenyl ($K_L = 10^{6.31}$ [16]), tends to correspond to the 100 fold larger depuration rate constant of 1,2,4-$T_3$CDD.

The rapid depuration rates may also explain the lack of significant bioaccumulation of PCDDs from dietary uptake. A similar explanation was proposed by Niimi and Oliver [6], who observed low dietary absorption efficiencies and short half-life times of PCDD congeners in rainbow trout.

Previous studies indicate that the depuration of higher chlorinated and brominated benzenes and biphenyls in the guppy [16] and in other fish species, is mainly due to passive elimination to the water (via the gills) and into faecal matter (via the gastro-intestinal tract). The rates at which these processes occur are largely controlled by the $K_{OW}$ of the chemical and they tend to be considerably slower than the depuration rates of PCDDs. It therefore seems inconceivable that the depuration of the PCDD congeners is due to passive elimination to the water (via the gills) or into faecal matter. The high depuration rate constants of the PCDDs may be explained by the metabolic transformation of the PCDDs in the fish.

**Metabolic Transformation**

To investigate the ability of the guppy to metabolize PCDDs, the presence of metabolic products of PCDDs in fish and water was investigated. GC-MS analysis of the water and fish extract demonstrated the presence of each of the test chemicals in their unaltered form, including 2-MCDD. In addition, a number of other chlorinated products were found. The mass spectra of these chlorinated compounds are summarized in Table 3. Both water and fish contained two mono-chlorinated products with peaks at 234 ($M^+$) and 171 m/e, indicating two different monohydroxy-metabolites of monochlorodibenzo-p-dioxin (i.e. M=218+16). The fish extract also contained a monochlorinated product with peaks at 250 ($M^+$), 230, and 198 m/e, which was identified as a dihydroxy-metabolite of 2-MCDD (M=218+16+16). Two dichlorinated products with peaks at 268 m/e ($M^+$) were found in both the fish and the water extract. The mass spectra of the first metabolic product also showed peaks at 250, 239 and 216 m/e. The other metabolic product showed peaks at 268 ($M^+$) and 205 m/e. Both products were identified as monohydroxymetabolites of 2,7-dichlorodibenzo-p-dioxin. Three trichlorinated products with peaks at 302 m/e ($M^+$) were found in the water and the fish extracts, indicating three monohydroxy-metabolites of $T_3$CDD (M=286+16). Other metabolic products were not observed. Quantification of the actual amounts of the hydroxy-metabolites in the extracts was not possible due to a lack of appropriate standards.

The presence of hydroxy-metabolites of 2-MCDD, 2,7-DCDD and 1,2,4-$T_3$CDD in the fish and in the water suggests that the PCDDs are metabolized by the fish and then eliminated to the water. Over a 7 d period no transformation of PCDDs was observed in a tank containing PCDD saturated water but no fish. Although this experiment did not rule out the possibility of chemical transformation by microbial organisms introduced by the fish to the water, we believe

Table 3 : Summary of mass spectral data of chlorinated compounds observed in fish and water samples.

| $M^+$ | | | | Suggested Product |
|---|---|---|---|---|
| 234 | 171 | | | hydroxy-monochlorodibenzo-p-dioxin |
| 234 | 171 | | | hydroxy-monochlorodibenzo-p-dioxin |
| 234 | 198 | | | hydroxy-monochlorodibenzo-p-dioxin |
| 250 | 230 | 198 | | dihydroxy-monochlorodibenzo-p-dioxin |
| 268 | 250 | 239 | 216 | hydroxy-dichlorodibenzo-p-dioxin |
| 268 | 205 | | | hydroxy-dichlorodibenzo-p-dioxin |
| 302 | 270 | 266 | | hydroxy-trichlorodibenzo-p-dioxin |

that the transformation of the PCDDs was due to metabolic transformation in the fish. The hydroxy-metabolites observed in the fish and the water extracts suggests that the transformation of the PCDDs was mediated by the Mixed Function Oxygenase (MFO) system in the fish. A study by Sijm and Opperhuizen [11] also indicates the role of the MFO-system in the transformation of 2,8-DCDD in the gold fish (Carrassius auratus). The presence of a conjugate of an hydroxylated tetrachlorodibenzo-p-dioxin in the rainbow trout further shows that MFO-systems in fish are able to metabolize PCDDs [3].

## CONCLUSION

Dietary bioaccumulation of the investigated PCDD congeners in the guppy appeared to be insignificant compared to that of PCB congeners of equal $K_{OW}$. With the exception of 2-MCDD, bioconcentration (i.e. chemical accumulation from the water) was observed for all of the PCDD congeners, including those which are substituted at the 1,4-positions and OCDD. In general, however, the bioconcentration factors of the PCDDs were approximately two orders of magnitude smaller than those observed for chlorinated and brominated benzenes and biphenyls of equal $K_{OW}$.

The presence of 1,4-substituted dioxin congeners and their metabolic transformation products in the fish extracts suggests that 1,4-substituted PCDDs, can be absorbed by the guppy from

the water. The uptake rate constants from the water of the PCDD congeners and TCB are approximately the same, suggesting a similar gill uptake mechanism for the PCDD congeners and TCB. Residues of 1,3,6,8-$T_4$CDD, OCDD and a conjugate of an hydroxylated 1,3,6,8-$T_4$CDD congener in the bile of rainbow trout, after aqueous exposure [3], further indicates that 1,4- and 1,6-substituted PCDD congeners can be absorbed by fish. This suggests that transfer of 1,4-substituted PCDDs over the gill membrane occurs. It thus seems unlikely that the low bioconcentration factors of the 1,4- and 1,6-substituted PCDD congeners are due to a lack of, or a particularly low, membrane permeability.

Similar to other very hydrophobic chemicals, PCDDs have a considerable sorption tendency. This is illustrated by the aqueous concentrations of some of the most hydrophobic congeners (e.g. OCDD), which greatly exceed the reported aqueous solubility. This indicates that the majority of these congeners in the water is present in a sorbed state. This may be due to the release of small amounts of organic matter by the fish, which provide sorptive sites for the chemical in the water phase. The chemical concentration in the fish appears to correspond to the concentration of the chemical in true solution [16,20]. It thus follows that a reliable bioconcentration factor can be only be determined when methods are available to measure truly dissolved chemical in the water. The Sep-pak method may give reliable estimates of the truly dissolved chemical concentration for PCDDs with log $K_{OW}$ up to 6.5. However, it largely overestimated the dissolved OCDD and OCDF concentration in the water. As a result, the bioconcentration factors derived for OCDD and OCDF are probably underestimating the true bioconcentration potential of these congeners. More reliable estimates of the bioconcentration factors of OCDD and OCDF may only be attained when methods to measure dissolved and sorbed chemical fractions in the water improve.

The low bioconcentration factors and dietary bioaccumulation factors of the PCDDs in the guppy appear to be due to the rapid depuration rates of the PCDDs. The presence of various metabolic products in the fish and in the water indicates that metabolic transformation is an important factor causing this rapid depuration. The presence of various hydroxylated PCDD congeners suggests that the metabolic transformation of the PCDD congeners is mediated by the mixed function oxidase system.

Since low bioconcentration factors and metabolic transformation of PCDDs have been observed in rainbow trout, fathead minnows, guppies and gold fish, it can be concluded that, in general,

fish tend to have a considerable capability to metabolize dioxin congeners. This metabolic capacity may significantly "reduce" the toxicity of PCDD concentrations in the water and food sources of the fish. When assessing the potential impact of dioxin congeners, it is essential to account for the metabolic transformation in the organism. The sensitivity of aquatic organisms to ambient PCDD concentrations may depend on the presence of a biotransformation capability. The presence of metabolizing enzymes can give the organism a certain tolerance to dioxin congeners, whereas a lack of the biotransformation capability may cause a greater sensitivity to the toxic effects of dioxins.

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