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BIOACCUMULATION OF PCDD'S AND OCDF IN FISH AFTER AQUEOUS AND DIETARY EXPOSURE

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Introduction

The bioaccumulation behaviour of PCDD's and OCDF was studied for three reasons. First, as a result of their hydrophobic nature PCDD's and PCDF's in the aquatic environment have the potential to accumulate in fish and other aquatic organisms. In addition it has been shown that the principal uptake route for humans and other aquatic organisms is by the consumption of fish (1). Since PCDD's and PCDF's are considered to be extremely toxic compounds and appear to be present in water systems such as for example the Great Lakes a potential hazard exists.

Secondly, the prediction of bioaccumulation parameters for PCDD's and PCDF's based on presently available models may be invalid. Since very high 1-octanol-water partition coefficients are suggested for these substances (2) linear correlations relating the bioconcentration factor, BCF, to the 1-octanol-water partition coefficient, K_{OW}, cannot be used. Although non-linear nodels (3) have been proposed to predict the bioconcentration behaviour of compounds with a log K_{OW} larger than 6, these models should be used with great care since they are not based on a fundamental understanding of the mechanism underlying this non-linear behaviour.

Third, it has been suggested that the steric configuration of molecules can have a profound effect on their bioaccumulation behaviour (4). Based on bioaccumulation studies with polychlorinated naphthalenes, hexabromobenzene and OCDD it was observed that molecules with a minimal internal cross section larger than 0.95 nm were not absorbed by guppies whereas molecules with a smaller minimal internal cross section were absorbed and accumulated as expected from their hydrophobic nature (5). It is believed that the inability of molecules with a minimal internal cross section larger than 0.95 nm to bioconcentrate is due to blocked membrane diffusion caused by a high activation energy for membrane interface transfer (4,5). Bioaccumulation studies were performed with a number of dioxin congeners, varying in hydrophobictity and steric configuration. A short summary of the experimental findings is presented below and will be discussed in more detail in a forthcoming paper.

Materials and Methods

The experimental systems used for aqueous and dietary exposure of fish were essentially the

same as used in studies on bioaccumulation of PCB's, PCN's and related compounds (5,6). Individual PCDD's i.e. 2-mono-, 2,7-di-, 1,2,4-tri-, 1,2,3,4-tetra- and octachlorodibenzo-p-dioxins as well as octachlorodibenzofuran, mirex and 2,4,5-tri- and decachlorobiphenyl were introduced to the experimental system as a mixture of compounds in aqueous solution (aqueous exposure) or as contaminants in food (dietary exposure). Bioaccumulation studies were performed with male and female guppies. A detailed description of the experimental procedure will follow later.

Results

Concentrations of all PCDD's and OCDF in the guppies throughout the 31 days of aqueous exposure were very low, resulting in BCF's between 800 and 2500. For 2-monochlorodibenzo-p-dioxin a BCF could not be calculated since a steady state was not reached during the exposure period, probably as a result of an induced metabolic transformation in the fish. For 2,4,5-trichloro- and decachlorobiphenyl, however, BCF's were observed which correspond to values expected and observed by Bruggeman (6). Throughout the 82 days of dietary exposure detectable amounts of PCDD's and OCDF were not observed whereas mirex, 2,4,5 tri- and decachlorobiphenyl were present in high concentrations.

Conclusions

From the experimental results it has been observed that the investigated PCDD's and OCDF are not accumulated to a significant extent in guppies after aqueous and dietary exposure. Based on their hydrophobicity i.e. water solubilities or K_{OW}'s much higher BCF's were expected. Therefore it can be concluded that hydrophobicity parameters such as water solubility and 1-octanol-water partition coefficients cannot be used alone to estimate bioaccumulation parameters for the investigated PCDD's. The inability of these dioxins to accumulate in guppies is believed to be the result of a combined action of a reduced membrane permeation and fat solubility, which will be discussed in more detail elsewhere.

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UPTAKE AND ELIMINATION OF PCDD/PCDF CONGENERS BY FISH AFTER AQUEOUS EXPOSURE TO A FLY-ASH EXTRACT FROM A MUNICIPAL INCINERATOR

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ABSTRACT

After aqueous exposure of fish to a fly-ash extract only a few highly chlorinated dibenzo-p-dioxins and dibenzofurans are accumulated. The estimated uptake rate constants of the accumulated congeners are comparable to those of PCB's or other hydrophobic chemicals. The elimination rate constants however seem to be relatively high. For the 2,3,7,8 TeCDD and 2,3,7,8 TeCDF a slow elimination is found if compared to other PCDD's and PCDF's.

INTRODUCTION

During the last few years several studies report on the bioconcentration by aquatic organisms of chlorinated dibenzo-p-dioxins, as well as on the bioavailability of these chemicals after aqueous exposure.[1-8] Unfortunately the reported data show several discrepancies. In fish sampled in the natural environment and fish exposed to fly-ash extract or fly-ash from a municipal incinerator for instance, only the 2,3,7,8 isomer of 22 TeCDD isomers has been found.[4,5] This in contradiction to what has been found in fish bioconcentration tests with 1,3,6,8 and 1,2,3,7 TCDD and higher chlorinated dibenzo-p-dioxins.[6]

The aim of the present study was to find some evidence for a size or structure specific bioconcentration of highly chlorinated ($\geq$ 4 Cl) dibenzo-p-dioxins and -dibenzofurans, as has been predicted based on previous studies with chlorinated naphthalenes[10], octachlorodibenzo-p-dioxin and hexabromobenzene.[7,8]

In addition it was tried to quantify the uptake and elimination kinetics of bioaccumulated PCDD and PCDF congeners, in order to allow comparison with other hydrophobic chemicals.

Finally by employing fishes, which usually have a low capacity to metabolize chlorinated aromatic hydrocarbons, we tried to find some indication of the influence of metabolism on the structure specific accumulation of PCDD's and PCDF's, as has been found in higher species, such as mammals.[11]

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EXPERIMENTAL

Fly-ash extraction and water saturation: 75 Grams of fly-ash from a municipal incinerator (Zaanstad, the Netherlands) were extracted with toluene after pretreatment with HCl[12]. To the extract solution 1 gram chromosorb WAW (100-120 mesh) was added. This was followed by removal of the organic solvent by evaporation, so that most of the extract was coated onto the surface of the chromosorb. The impregnated chromosorb was added to a water saturation system, comparable to a generator column, in which 500 ml water was contaminated with the components of the extract at concentrations which certainly did not exceed the aqueous solubilities.

Exposure system: In a 6 liter aquarium the 500 ml contaminated water were diluted to 5.5 l, by adding 2.5 l demineralized, and 2.5 l Amsterdam tap water. This was followed by sampling of 500 ml water for the determination of the initial aqueous concentrations of the PCDD's and PCDF's. The water temperature was 22°C and the aqueous oxygen concentration always exceded 90% saturation.

Into the contaminated water 22 one year old male guppies (Poecilia reticulata) were placed. During the exposure period 4 times 3 fishes were sampled, i.e. after 1, 4, 10 and 16 days exposure. In all cases 700 ml water were sampled simultaneously, so that the fish density ($\frac{\text{gram fish}}{\text{liter water}}$) was almost constant throughout the exposure period.

After 16 days exposure the remaining fishes were transferred into clean water to study the elimination process. During this clearance period water was aerated and cleaned by using a aquarium pump and activated carbon.

During the elimination period 2 fishes died spontaneously after 3, 1 after 9 days and 1 after 14 days elimination.

Therefore, only two times 3 living fishes could be sampled, i.e. after 6 and 20 days clearance.

Chemical Analysis. Water samples were extracted with two times 150 ml hexane. After concentration of the sample to 50 µl, 5 µl of the extract were injected in a 5970 Hewlett Packard gaschromatograph which was equipped with a Mass Selective Detector and a Supelco 2340 column.[12]

After killing and homogenization, the fishes were extracted by heating under reflux in a 1 : 1 mixture of hexane and water for 90 minutes.[7,10] The organic solution was concentrated to 2 ml and eluted through silica NaOH and silica H_2SO_4 columns, for the removal of polar contaminants and fish lipids. The clean extract was concentrated to 50 µl, which was analyzed in a similar way as the water samples.

For the determination of the extraction efficiency, as well as for the quantification of the concentrations of the individual PCDD and PCDF congeners in the samples, a standard solution of ^{13}C 2,3,7,8 TeCDD was added to all samples before extraction.

RESULTS AND DISCUSSION

Of all PCDD's and PCDF's with 4 or more chlorine atoms, only 2,3,7,8 TeCDD, 1,2,3,7,8 and 1,2,3,7,9 PeCDD and 1,2,3,7,8,9 HeCDD, 2,3,7,8 TeCDF, 1,2,3,7,8 and 2,3,4,7,8 PeCDF were found in fish after aqueous exposure to a fly-ash extract.

Except for the 1,2,3,7,9 PeCDD, all PCDD and PCDF congeners with 4 and 5 chlorine atoms are similar to the congeners which show relatively high retention in mammalian accumulation studies

with fly-ash extracts[12] or fly-ash[11].

In addition it must be noted the none of the hexa-, hepta- and octachlorodibenzofurans was found in fish, whereas of the chlorinated dibenzo-p-dioxins with more than 5 chlorines, only the 1,2,3,7,8,9 hexa congener was detected. Although the lack of uptake of the OCDF and OCDD is in agreement with other studies[7,8,9], these data of the higher chlorinated CDD's and CDF's are in contradiction to what has been reported for mammals.

The structure specific bioconcentration of TeCDD's, i.e. only the 2,3,7,8 isomer, has been reported previously, both in field[5] and in laboratory studies.[3,4] These results however contradict recently published data on the bioconcentration of 1,3,6,8 and 1,2,3,7 TeCDD and higher chlorinated dibenzo-p-dioxins in Fathead Minnow and Rainbow Trout.[6]

Note that in the present study all accumulated PCDD and PCDF congeners are not simultaneoulsy 1-4 or 1-6 chlorine substituted. This observation is in agreement with a predicted lack of uptake by the gills of hydrophobic chemicals with cross-sections > 0.95 nm, due to a lack of membrane permeation.[8,10] This prediction was based on the lack of uptake of highly chlorinated naphthalenes, hexabromobenzene, highly brominated biphenyls and octachlorodibenzo-p-dioxin in previous experiments.[7,8,10]

However since for instance 2,3,7,8 TeCDD is not the only tetrachlorodibenzo-p-dioxin congener with a cross section smaller than 0.95 nm, membrane permeation cannot be the only factor causing the structure selective accumulation, but it may explain the lack of uptake of for instance some 2,3,7,8 substituted HeCDD congeners. This because the accumulated 1,2,3,7,8,9 HeCDD is the only HeCDD congener which is not simultaneously 1-4 or 1-6 substituted.

It was found that the aqueous concentration of accumulating congeners, such as 2,3,7,8 TeCDD and 1,2,3,7,8,9 HeCDD, decreased significantly during the exposure period, whereas the concentrations of non-accumulated, such as 1,3,6,8 TeCDD and 1,2,3,6,7,8 HeCDD, were almost uninfluenced. This may indicate that the absence of most PCDD and PCDF congeners in fish samples must not be explained by high rates of metabolism of these congeners in biota, which has sometimes been suggested for mammals[13] and other organisms. In addition, hitherto high rates of metabolism of chlorinated aromatic hydrocarbons in fish have not been observed in fish, since these species usually have a very low mixed function oxidase (MFO) capacity.

Furthermore it has been shown elsewhere that the ether bonds between two phenyl rings is very stable in fishes[8], so that influence cleavage of the ether bond of PCDD's and PCDF's, if at all, will be negligible.

TABLE 1. UPTAKE AND ELIMINATION RATE CONSTANTS AND BIOCONCENTRATION FACTORS OF ACCUMULATING PCDD AND PCDF CONGENERS.

		k_1	k_2	K_C
2,3,7,8	TeCDD	600	0.046	$1.3\ 10^4$
2,3,7,8	TeCDF	400	0.062	$6.6\ 10^3$
1,2,3,7,8	PeCDD	700	0.090	$7.7\ 10^3$
1,2,3,7,9	PeCDD	300	0.184	$1.6\ 10^3$
1,2,3,7,8	PeCDF	300	0.124	$2.4\ 10^3$
2,3,4,7,8	PeCDF	400	0.079	$5.0\ 10^3$
1,2,3,7,8,9	HeCDD	200	0.210	$0.9\ 10^3$

In table 1, estimated uptake rate constants of the bioconcentrated PCDD and PCDF congener are listed. The estimated k_1-values ($^{ml}/_{g\ day}$) are in good agreement with uptake rate constants for guppies of chlorinated benzenes, -naphthalenes and -biphenyls.[8,10] In addition the uptake rate constant of 2,3,7,8 TeCDD by guppies, is close to the value of 150 $^{ml}/_{g\ day}$ reported for Rainbow Trout.[3]

Hence it may be concluded that when there is no qualitative selectivity in the uptake process, the uptake rate of PCDD's and PCDF's is quantitatively comparable to the uptake of other hydrophobic chemicals..

This however still leaved the question why not all aqueous PCDD and PCDF congeners with cross-sections < 0.95 nm are found in fish samples. Although data are scarce it may be proposed that the lack of uptake or accumulation of these compounds is caused by a low lipid solubility. It has been proposed previously that hydrophobicity is not equivalent to lipophilicity, since especially for larger non-electrolyte chemicals neither water nor 'lipids' may act as good solvents.[8] Perhaps this is the case for PCDD and PCDF congeners, which probably results in high elimination rates from fish.

Supporting this statement is the observation that estimated elimination rates of the two PeCDD's and 1,2,3,7,8,9 HeCDD as well as the two PeCDF's are relatively high compared to those of other chlorinated aromatic hydrocarbons.[7,8,9,10] That on the other hand some accumulation of these congeners, as well as of the 2,3,7,8 TeCDD and 2,3,7,8 TeCDF, is found must not be explained by the affinity of these chemicals for lipids, but by a high affinity for specific receptors. Recently similar specific accumulation sites have been proposed in mammals for 2,3,4,7,8 PeCDF.[14]

As specific accumulation sites proteins like the Ah receptor[15], or proteins of the endoplasmatic reticulum[14] or other membranes may be proposed.

CONCLUSIONS

1) Bioaccumulation of chlorinated dibenzo-p-dioxins and dibenzofurans is not comparable to that of PCB's, and cannot be predicted by physical-chemical properties like aqueous solubility or octan-1-ol/water partiton coefficients.
2) Only the estimated uptake rate constants of a few PCDD and PCDF congeners are in agreement with the theory of accumulation of hydrophobic chemicals by fish.
3) Metabolism alone cannot explain a lack of accumulation of many PCDD and PCDF congeners.
4) The observation that only some PCDD and PCDF congeners are accumulated by fish after exposure to a crude fly-ash extract can only be explained by a combination of processes.

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