Cytochrome P450 1A induction in the mirror carp (Cyprinus carpio) following exposure to a PCDD/PCDF contaminated sediment.

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ABSTRACT

Three groups of forty mirror carp (Cyprinus carpio) were exposed to three types of sediment during five weeks. Old Ketelmeer sediment (1965-1970, approximately 400 ng TCDD equiv./kg dry weight), recent Ketelmeer sediment (1985-1990, approximately 40 ng TCDD equiv/ kg dry weight) and relatively clean Oostervaardersplassen sediment as reference were used. Both Ketelmeer sediments caused obvious EROD activity and P450 1A protein inductions, approximately 50 to 150 and 10 to 30 times the control values, respectively. These inductions still increased or remained constant during the rest of the experiment. No induction was caused by the reference sediment until the fourth week, in which a 4 and 7 times induction was measured on P450 1A protein and EROD activity, respectively.

INTRODUCTION

The cytochrome P450 superfamily catalyzes the biotransformation of a wide variety of endogenous and exogenous compounds[1]. Induction of the cytochrome P450 1A subfamily occurs after exposure to e.g. polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and some planar biphenyls (PCBs)[2]. These compounds are widespread in the aquatic environment and, due to their lipophilicity, they can accumulate rapidly in fish and other aquatic organisms[3]. In fish, these planar halogenated compounds cause a strong and prolonged induction of the cytochrome P450 1A subfamily. As a result this type of enzyme induction appears to be a very sensitive indicator for the presence of these contaminants in field studies[4,5], but its biological and toxicological significance still have to be determined.
In this study this biological respons, measured as P450 1A induction, in the mirror carp (Cyprinus carpio) was determined during five weeks exposure to three different types of sediment. These sediment samples were selected based on the presence of PCDDs, PCDFs and PCBs, as determined by chemical analysis.
The results of these P450 1A measurements will be discussed in relation to the presence of these compounds in these sediments.

MATERIALS AND METHODS

Experimental
Mirror carp (Cyprinus carpio) with a wet weight of 40.3 ± 12.7 gram were exposed during five weeks to three types of sediment. The following sediments were all sampled in the central part of the Netherlands:
I Sediment from Ketelmeer, which is a sedimentation area of the river Rhine.This sediment originates from the period 1965 to 1970 and is contaminated with PCDDs, PCDFs and PCBs with a concentration of approximately 400 ng TCDD equiv./kg dry weight (Table I).

II Ketelmeer surface sediment, originating from the period 1985 to 1990 and contaminated with approximately 40 ng TCDD equiv./kg dry weight (Table I).
III Oostvaardersplassen sediment, which is considered to be a reference area.

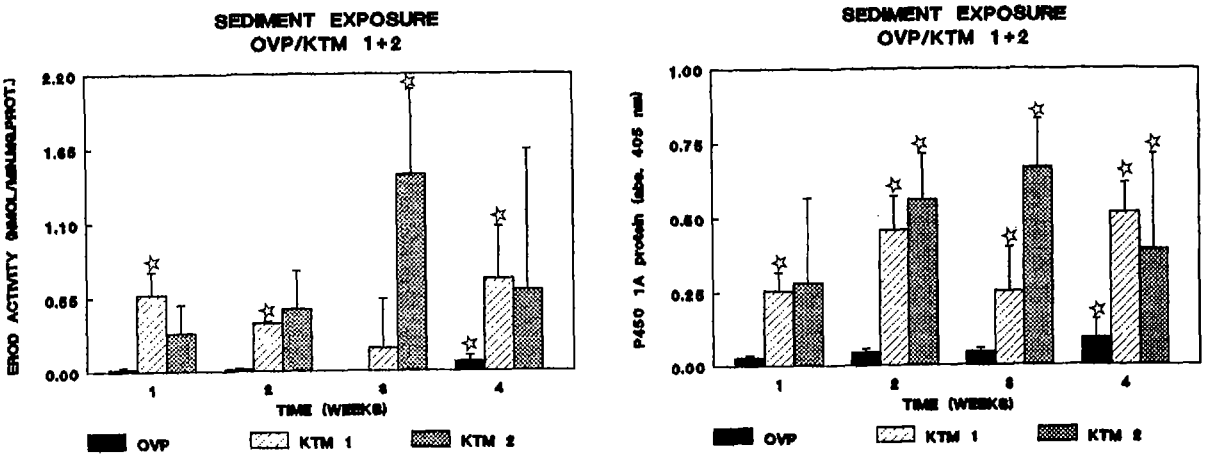
Compound	KTM 2	KTM 1
2,3,7,8-TCDD	300	12
1,2,3,7,8-PCDD	<10	<10
1,2,3,4,7,8-HxCDD	16	<10
1,2,3,6,7,8-HxCDD	26	<10
1,2,3,7,8,9-HxCDD	19	<10
1,2,3,4,6,7,8-HpCDD	550	120
OCDD	5000	1100
2,3,7,8-TCDF	120	17
1,2,3,7,8-PCDF	150	12
2,3,4,7,8-PCDF	85	13
1,2,3,4,7,8-HxCDF	340	50
1,2,3,6,7,8-HxCDF	160	30
1,2,3,7,8,9-HxCDF	19	<10
2,3,4,6,7,8-HxCDF	110	19
1,2,3,4,6,7,8-HpCDF	2000	260
1,2,3,4,7,8,9-HpCDF	220	27
OCDF	9900	1600
PCB 126	<10	<10
PCB 77	700	440
PCB 169	<10	<10
PCB 105	48000	8600
PCB 156	15000	2300
PCB 118	130000	15000
TCDD equiv. (ng/kg d.w.)	530.1	49.4

Table I: PCDD, PCDF and PCB contents (ng/kg dry weight) in old and new Ketelmeer sediment (KTM 2 and KTM 1 respectively)

Two liter of each sediment was diluted in 75 liter of copper-free Utrechts tap water (19 ± 1 °C), and the fish were replaced in 'fresh' sediment every week (semi-static exposure). At the beginning of the experiment a group of forty carps was present in a 100 liter aquarium. Every week four individual fish were sampled at random and killed by a cephalic blow. The livers were excised and prepared for enzyme assays as described earlier[6]. As a marker for P450 1A induction the 7-ethoxyresorufin O-deethylation (EROD) activity was used, according to the methods of Burke et al. [7]. In addition, the P450 1A protein content was measured using specific antibodies, raised against P450 1A protein in the rainbow trout, in a semi-quantitative ELISA technique according to the method of Celander and Förlin[8].
A fourth group of twelve carps was not exposed to any sediment, but used as a temperature control and sampled after 1, 3 and 5 weeks. In addition four different individual fish were sampled after one, three and five weeks for PCDD and PCDF analysis of the liver. Results of these chemical analysis as well as the histopathological evaluation of the liver and spleen are not included in this paper but will be reported later. Differences in enzymes activities of exposed fish, compared to the controls, were tested using the Least Significant Difference Test, LSD ($p<0.01$).

RESULTS AND DISCUSSION

The results of the P450 1A measurements in the liver of fish exposed to recent Ketelmeer (KTM 1), older Ketelmeer (KTM 2) and Oostvaardersplassen (OVP) sediment, are shown in figure 1A (EROD activity) and 1B (P450 1A protein). The enzyme activities of only the first four weeks of the study are shown, due to mortality of all the fish, exposed to KTM 2, five weeks after the start of the experiment.



EROD activity (Fig. 1A) and P450 1A protein content (Fig. 1B) in the mirror carp, exposed to old Ketelmeer (KTM 2), new Ketelmeer (KTM 1) and Oostvaardersplassen sediment (OVP).
The values are means ± SD (n=4) and asterisks indicate a significant difference towards the controls. The control value for EROD is 0.010 nmol/min.mg.prot., and for the P450 1A protein 0.02 (n=8).

The relatively clean Oostvaardersplassen sediment did not induce any P450 1A activity until the fourth week of the exposure study. In the fourth week a 3 times induction was measured in P450 1A protein content, while at that time point EROD was induced 7 times. When considering these data it should be realized, that although this sediment is relative clean, low levels of (polychlorinated) polycyclic aromatics are still present. Thus, the observed induction might still be a result of a low, but continuous uptake of these type of compounds by the carp.
The recent Ketelmeer sediment (KTM 1) caused an induction in EROD activity and P450 1A content, which is approximately 40 to 70 times above the control level. The older sediment from the Ketelmeer area (KTM 2) induced the EROD activity approximately 30 to 150 times (Fig.1A).
Although KTM 2 sediment induced EROD activity to higher levels in most individual fish compared with exposure to KTM 1, in some individuals no EROD activity could be measured. As a result individual variation in the KTM 2 group was large, which has a significant influence on group average and standard deviations. Possible reasons for this large individual variation in EROD activity might be the presence of higher amounts of heavy metals and PCBs in the KTM 2 sediment, compared to KTM 1. Both heavy metals and PCBs have been shown to inhibit the EROD activity in aquatic organisms [9,10].
As far as the P450 1A protein content concerns, both KTM 1 and KTM 2 sediment significantly increased this parameter above control levels. During the experiment the recent and older Ketelmeer sediments induced the P450 1A content 12 to 25 and 14 to 35 times, respectively (Fig. 1B).
In general, it can be observed from the figures 1A and 1B that elevation of the EROD activity and P450 1A protein content followed a similar temporal induction pattern. As a result a highly significant correlation between EROD activity and P450 1A protein content was found (R=0.95; n=60), indicating the P450 1A protein to be the EROD catalyst[8,11].

In spite of the ten times concentration difference in TCDD-equivalents between the older and recent Ketelmeer sediments, both samples caused a comparable and strong P450 1A induction in the liver of the carp. This induction, measured as EROD activity and P450 1A protein content, could already be observed one week after exposure.
This induction of P450 1A is in the same range as reported from a previous study with carps exposed to Rotterdams Harbour sediment containing 500 ng TCDD-equivalents/kg dry weight. In this study approximately 40-50 times induction of EROD activity and 10 to 15 times induction of the P450 1A protein was measured after three to five weeks exposure [5].
The results of our study show, that sediments with concentrations between 40 and 400 ng TCCD equivalents/kg can clearly induce cytochrome P450 1A level in the liver of the carp. Histopathological examination of the liver showed severe lipid accumulation in the hepatocytes of carp exposed to KTM2 sediment. Hepatic lipid accumulation has been observed earlier in yellow perch after a single dose of 2,3,7,8-TCDD [12]. Chemical analysis of the livers might provide more information, related to the role of PCDDs and PCDFs as P450 1A inducers in these experiments.

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