COMPARATIVE TOXICITY AND ENZYME INDUCTION OF 2,3,7,8-TCDD IN THE CARP (CYPRINUS CARPIO) AND THE RAINBOW TROUT (ONCHORHYNCHUS MYKISS).

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ABSTRACT

After a single intraperitonal injection of ≈ 0.01-5.0 μg 2,3,7,8-TCDD/kg body weight, EROD induction, growth inhibition and severe liver and spleen lesions were observed. Carps are more sensitive to 2,3,7,8-TCDD than Rainbow trout, based on lethality (respectively 60 and 20% 12 weeks after a dose of 5 μg/kg) and stronger EROD induction (e.g respectively 40 and 7 times induction 3 weeks after a dose of 0.5 μg/kg).

INTRODUCTION

The global distribution of halogenated polycyclic aromatic hydrocarbons, like PCDDs, PCDFs and PCBs, has been well established. Especially in the aquatic environment large quantities of these compounds have concentrated in the freshwater sediment. From this sediment these low soluble compounds are gradually dissolved in the waterphase, from which they can easily accumulate in aquatic organisms. Although fish and other aquatic organisms are exposed to these chemicals, little is known about the toxicity and mechanisms of action of PCDDs and related compounds in fish in comparision with mammalian species.

From laboratory and environmental studies it is reported, that accumulation of PCDDs, PCDFs and planar PCBs in fish is associated with a strong and prolonged induction of cytochrome P-450I related activities. Although this type of enzymatic activity provides a sensitive parameter for exposure of the fish to these compounds, its possible use and toxicological relevance has not yet been established (1).

At our institute toxicity studies with several fish species are now in progress, to determine a possible (noncausal) relationship between this type of enzyme induction and toxicological effects. In this paper we compare the results from two dose-response studies with Rainbow Trout and Carp using 2,3,7,8-TCDD as toxic agent.

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EXPERIMENTAL

Rainbow trout (45.8 ± 10.9 gram) and Carp (99.8 ± 23.0 gram) received a single i.p. injection of ≈ 0.01, 0.05, 0.1, 0.5, 1.0 and 5.0 µg 2,3,7,8-TCDD/kg body weight with peanut oil used as vehicle. Peanut oil was used as control. The GCMS analyses of the oil in order to determine the exact dose is still in progress. Effects on spleen, liver and body weight gain were monitored for periods of 3, 6 and 12 weeks (n=5). In addition total cytochrome P-450 content and the EROD activity in the liver were determined (2,3). In the Carp liver the EROD activity was determined 1 week after dosage. As molar extinction coefficient for resorufin was used 73 $mMol^{-1}cm^{-1}$ (4).

RESULTS

Based on lethality, the Carp seems to be more sensitive to 2,3,7,8-TCDD than the Rainbow Trout. The lethality for Carp was 60%, starting 6 weeks after dosage of the highest concentration, while only 20 % of the Rainbow trout died in week 12.

Clinical toxicological effects were also more severe for the Carp than for Rainbow Trout. Carp exposed to 0.5 µg/kg exhibited cutaneous hemorrhages in the caudal fin after 3 weeks. Fish dosed with 1.0 µg/kg showed also hemorrhages in the fins as well as the side line system. The group which received 5 µg/kg exhibit the same gross pathology as the 1 µg/kg group, but more severe. Their behaviour was apathetical, but after an external stimulus they got very nervous. This was in contrast with fish exposed to a lower dosage or controls, which were very tame. In addition to the clinical effects stated above fish from this dose group had sunken eyes, swelled gills, no appetite and therefore had lost weight after 4 weeks. These Carp retained this physical bad condition until week 12, but in this last week a slight recovery occurred. Another external observation was a dose related reduction in the healing process of the caudal fin. Caudal fins were cut to mark individual fish in these experiments. Control fish recovered caudal fin size and shape usually after 3 weeks, while this process was almost absent for the highest dose group.

The Rainbow trout is a more nervous animal, the controls as well as the treated organisms. After 9 weeks the highest TCDD dose caused listlessness, head up swimming and the animals changed to a darker colour. Beginning at a dose level of 0.5 µg/kg hemorrhages in the fins and muscles were found after 6 weeks. No lack of appetite was found in this fish species caused by 2,3,7,8-TCDD.

Three weeks after dosage a reduction in body weight gain was observed for Carps dosed with 0.5 µg/kg. After 6 weeks the Carp as well as the Rainbow trout in the groups receiving a dose of 5 µg/kg showed significant growth inhibition. In week 12 no significant growth reduction occurred; an effect which might be contributed partly to the elimination of this compound during this 12 week period.

Histopathological evaluation of the liver and spleen indicated, that both can be considered

as target organs for the effects of these compounds. In the Rainbow trout the relative spleen weight is enlarged at dose levels from 0.01 to 0.1 μg/kg, while in the Carp this effect is less pronounced. In contrast, an increase in the relative liver weight is a more pronounced phenomenon in Carp than in the Rainbow trout. Histopathological examination of the Rainbow trout liver demonstrated an increase in mononuclear inflammatory cells (≥0.01 μg/kg), scattering of hepatocytes (>0.1 μg/kg) and degenerative processes, such as focal coagulation necrosis (≥1 μg/kg) after 6 and 12 weeks. After 3 weeks congestion and accumulation of erythrocytes could clearly be observed in the spleen of the Rainbow trout beginning at a dose level of 0.05 μg/kg after 3 weeks. This congestion is thought to be the most likely cause of the spleen weight increase observed at lower dose levels. Recovery of the spleen was seen at the lower dose levels after 12 weeks. At higher dose levels (≥1 μg/kg) persistent depletion of lymphocytes was observed from 3 weeks. A detailed description of the histopathology will be published separately.

The EROD activity in the Carp was already induced at a dose level of 0.05 μg/kg after one week. In week 3 an induction of approximately 40 times was still found at a dose level of 0.5 μg/kg. In Rainbow trout the EROD activity was induced at a dose level of 0.5 μg/kg, circa 7 times. This higher response of EROD in Carp liver is observed for all dose levels. Significant induction of EROD occurred in the Carp one week after a dosage of 0.05 μg/kg and higher. After 3 weeks EROD activity is significantly induced in Carp treated with 0.1 μg/kg. Based on these results it seems possible that in the Rainbow trout treated with concentrations lower than 0.5 μg/kg the maximum in EROD induction has already been passed after a 3 week period.

DISCUSSION AND CONCLUSIONS

Cytochrome P-4501 related activities, EROD, can easily be induced in the Carp as well as the Rainbow trout. A strong, prolonged and dose related induction of EROD activity was still found at doses of 0.1 μg/kg (Carp) and 0.5 μg/kg (Rainbow trout). At these levels severe pathological lesions were also observed in the liver and spleen.

From these studies it is concluded that some fish species, such as the Carp, can be equally sensitive towards the toxic and biochemical effects of these compounds as some mammalian species, like the guinea pig and some mouse strains. This conclusions are in agreement with the results of Kleeman (5).

The lesions in the liver and spleen of the Rainbow trout are in agreement with those described earlier, as well as the critical dose of circa 1 μg/kg (6); fish dosed with more than 1 μg/kg showed severe clinical toxicity, did not eat and some died.

At dose levels for which significant induction in EROD activity is observed, immunological and hepatotoxic effects can still be observed for the Carp as well as the Rainbow Trout. The coincidence of cytochrome P-4501 induction and the observed toxic effects in both fish

species does not have to be causally related. However, as in some mammalian species it might be possible that this correlation between biochemical and toxicological effects in fish is also caused by an initial binding to a common (Ah-?) receptor. In view of the observed coincidence between cytochrome P-450I induction and hepatotoxic and immunological effects, enzyme induction might be useful in the future as a screening method for aquatic pollution of planar chlorinated aromatic hydrocarbons. However, its specificity should be subject of further research, as some polycyclic aromatic hydrocarbons can induce similar cytochrome P-450I activity in fish at high doses. In view of the higher sensitivity of the Carp for 2,3,7,8-TCDD, based on lethality, the stronger induction of EROD and the Dutch ecological situation, this species appears to be more suitable for screening purposes in the Netherlands.

ACKNOWLEDGEMENT

This work was supported by a grant (DB-475) from the Institute for Inland Water Management and Waste Water Treatment.

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