DISPOSITION AND METABOLISM OF 2,3,7,8-TETRACHLORO-DIBENZOFURAN BY CHANNEL CATFISH (*Ictalurus punctatus*) COMPARED WITH RAINBOW TROUT (*Oncorhynchus mykiss*)

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INTRODUCTION

Polychlorinated dibenzofurans such as 2,3,7,8-tetrachlorodibenzofuran (TCDF) are highly toxic manufacturing biproducts and pyrolysis products of chlorinated aromatic compounds such as the polychlorinated biphenyls. These compounds are widely dispersed in the environment and have been found in the fish and sediments of the Great Lakes (especially Lake Ontario), the Chesapeake Bay and in other industrialized and heavily populated areas[1]. TCDF is taken up and biomagnified by the food web. We have previously reported[2] that rainbow trout concentrate TCDF in edible tissue (muscle filets) to a greater extent than do laboratory rodents, and metabolize and eliminate the compound more slowly (with a half-life of 13 days).

The objective of these studies was to obtain comparative data on the half-life, tissue distribution, and metabolism of TCDF by channel catfish, another economically valuable species extensively used for human food and environmentally exposed to PCDFs and related compounds as a benthic species exposed to sediments and to the benthic food chain.

EXPERIMENTAL AND RESULTS

Tissue distribution and half-life. Twelve catfish (24±5g) were dosed orally (by gelatin capsule) with [^{3}H]TCDF (purity>96%) (per 20 g fish, 1.5 μCi, 20 ng TCDF in 20 μL corn oil:acetone [1:1]). To facilitate the retention of the capsules, the fish were chilled to 5° C. for 30 min before dosing. After dosing, the flowing, dechlorinated water was gradually to warmed to 15°C, and subsequently maintained at that temperature. The fish were fed daily except from 24 hr before treatment until 24 hr after treatment. At 3, 7 and 14 days following treatment, groups of 3-5 fish were sacrificed, dissected, and the tissues were stored at -20° C (-80°C for bile) pending analysis.

Tissues (including carcass) were homogenized; aliquots were measured and dissolved in Soluene 350. The samples were then decolorized with H_2O_2 and (later) mixed with Hionic-Fluor (Packard) and assayed by liquid scintillation counting. Aliquots of bile and blood were also solubilized in Soluene, decolorized and radioassayed. From these data the total radioactivity present in each fish was determined. The half-life of TCDF in these fish was then determined, by linear regression of the logarithmically transformed data, to be 3.1 days.

At 3 days, TCDF-derived radioactivity was found to be highly concentrated in kidney, liver and perivisceral adipose. These concentrations in liver and kidney were the same and 34X the concentration in muscle. The concentration of TCDF equivalents in liver declined rapidly (half-life 1.6 days) until, at 14 days, it was essentially the same as the concentration in muscle. The concentration of TCDF equivalents in kidney and adipose decreased more slowly, with half-lives of 2.6 and 3.6 days, respectively, until these concentrations (at 14 days) were 7X the concentration in muscle.

These data contrast sharply with corresponding data obtained with rainbow trout. In trout the concentration of TCDF equivalents in kidney (at 3 days) was lower than the concentration in muscle. The concentration of TCDF equivalents in trout liver at this time point was only 1.6X the concentration in muscle and was decreasing with a half-life of 7.9 days.

TCDF equivalents were likewise more concentrated in catfish bile than in trout bile (at 3 days, 139 vs 5 ng/g, corresponding to 12 vs 1.0% of the administered dose, respectively). This concentration of TCDF derivatives in catfish bile decreased rapidly, in parallel with the concentration in liver.

Analysis of TCDF-derived radioactivity in bile and liver. To determine conjugated derivatives of TCDF in bile, bile was analyzed following hydrolysis (at pH 7.8) with β-glucuronidase (from *E. coli*) (Sigma type VII-A), 135 U/ml, for 4 hr at 37°C. A control sample was incubated without the enzyme. At the end of the incubations each sample was extracted (at pH 6.5) with 3 x 2 volumes of ethyl acetate. An additional aliquot of bile, similarly diluted with NaOAc buffer at pH 6.5, was immediately extracted with EtOAc without being incubated. Each extract was dried over Na_2SO_4 and evaporated under nitrogen. The resulting residues were exhaustively methylated with diazomethane (in ether). Following evaporation of the ether, each residue was dissolved in methanol (80-100 μL) for analysis by HPLC.

HPLC analysis utilized a Zorbax C18 column eluted at 1.5 ml/min with 75% acetonitrile for 25 min, followed by a 20 min gradient to 92% acetonitrile. Each sample was coinjected with UV-detectable amounts of 4-MeO-2,3,7,8-TCDF and 3-MeO-2,4,7,8-TCDF (4-/3-MeO-TCDF, respectively). Fractions were collected every 20 sec or 1 min and radioassayed. In comparing retention times allowance was made for a 20 sec lag time between the detector and the fraction collector.

A major component of TCDF-derived radioactivity in bile (extracted from the sample incubated with β-glucuronidase and amounting to 24% of total biliary radioactivity) was identified as having been derived from the glucuronic acid conjugate of 4-OH-TCDF, based on the coelution of the methylated derivative of the deconjugated compound with authentic 4-MeO-TCDF (Fig. 1). Methoxylated derivatives of other TCDF metabolites have been shown to be well-resolved from 4-MeO- and 3-MeO-TCDF in this system (data not shown).

Using similar methods, 4-OH-TCDF was identified in (unhydrolyzed) catfish liver as a major TCDF metabolite, amounting to 63% of the ethyl acetate extractable radioactivity. In contrast, unmetabolized TCDF amounted to only 2.7% of the extractable radioactivity.

The same metabolite, 4-OH-TCDF, was likewise identified as the major metabolite in trout liver, amounting to 40% of the extractable radioactivity (39% of the total radioactivity). In trout liver, in contrast to catfish liver, unmetabolized TCDF also amounted to 40% of the extractable radioactivity. The conjugated form of this metabolite, TCDF-4-O-glucuronide was identified as the major TCDF metabolite in rainbow trout bile,[3] and has also been reported as the major TCDF metabolite in rat bile.[4]

CONCLUSIONS

These data suggest that the shorter half-life of TCDF in catfish, compared with rainbow trout (3.1 vs. 13.4 days, respectively), may relate to (at least) two different factors: (1) Catfish *liver* takes up, metabolizes and excretes TCDF equivalents more efficiently than does trout liver (based on the concentration of TCDF equivalents in liver and bile, compared to muscle, at 3 days). (2) Catfish *muscle* retains TCDF less effectively than does trout muscle. The half-life of TCDF in catfish and trout muscle was 4.6 and 14.2 days, respectively. The fraction of the administered dose remaining at 14 days in catfish and trout muscle was 1.5% and 8.5%, respectively. This difference, which may relate to the lipid content of muscle, may depend, not only on the species, but also on the age and nutrition of the fish. A comparison of our data with several published reports[1,2,5,6] suggests that inter-laboratory differences may relate, in part, to differences in the sizes (and consequent muscle lipid content) of the fish and on the nature of the exposure (i.e. single vs. multiple or continuous exposures).

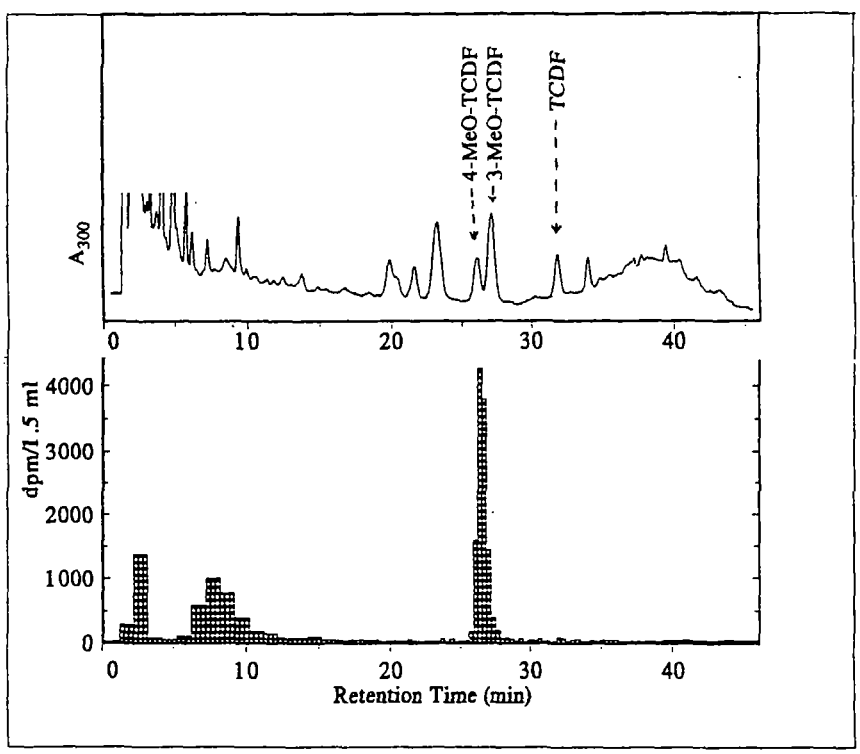

Fig. 1. Reversed phase HPLC analysis of TCDF-derived radioactivity in bile extracted *following hydrolysis with β-glucuronidase* and subsequent methylation with diazomethane. The authentic standards were coinjected with the bile extracts. The other UV peaks are unknown endogenous compounds. The three standard peaks had been initially conclusively identified by spectrum using a diode-array detector.

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