PCDD Metabolites Formed by Mouse Liver Microsomes

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INTRODUCTION

2,3,7,8-Tetrachloro-*p*-dioxin (2,3,7,8-TCDD) and related compounds are metabolized *in vivo* and *in vitro* to give hydroxylated metabolites [1-3]. Moreover, P4501A1, an isozyme induced by 2,3,7,8-TCDD, is also a highly effective enzyme catalyst for this metabolic process. However, the acute toxicity of these metabolites to guinea pigs and rats was at least two orders of magnitude less than that of their parent compound [4,5].
The aim of this investigation is to determine the identities of several PCDD metabolites and delineate pathways for the metabolism of PCDDs. We studied the *in vitro* metabolism of 1,3,6,8-TCDD, 2,3,7,8-TCDD and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (1,2,3,4,7,8-HxCDD) in mouse liver microsomes, and have outlined the metabolic pathways for the three compounds.

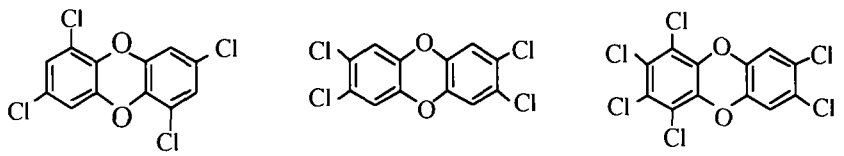

Fig.1 Structures of three PCDD isomers

METHODS

Male C57BL/6 mice weighing about 23 g were divided into six groups with three animals per group. Five groups were treated orally with 2,3,7,8-TCDD dissolved in corn oil at doses of 0.001, 0.01, 0.1, 1 and 10 μg/kg body weight, respectively and another group was given only corn oil as control. Mice were sacrificed 24 hr. after dosing. Hepatic microsomes were prepared as previously described [6], microsomal 7-ethoxyresorufin O-deethylase (EROD) activity was determined as reported previously [7].
The *in vitro* microsomal metabolism of 1,3,6,8-TCDD, 2,3,7,8-TCDD and 1,2,3,4,7,8-HxCDD

were carried out according to a method reported by Isida et al [8], and the resulting extract was purified on an alumina column (0.5 g, Merck neutral, activity I). For analysis of PCDD metabolites, the purified extract was concentrated, dried and adjusted to a volume of 25 μl with n-hexane, and measured on J & W DB-5 capillary column (30 m x 0.32 mm, 0.25 μm thickness) using a Hewlet Packard 5890J gas chromatograph-JEOL SX-102 mass spectrometer (electron impact mode). The column temperature was held for 1 min at 140 °C, and programmed to 220 °C at 10 °C/min and to 310 °C at 4 °C/min.

RESULTS AND DISCUSSIONS

The activity of cytochrome P4501A1-dependent EROD activity was measured in liver microsomes from untreated and treated mice 24 hr. after dosing with 2,3,7,8-TCDD. The activity was obviously increased after treatment with 2,3,7,8-TCDD at doses 0.1 μg/kg and upwards (Fig. 2). At a dose of 10 μg/kg, the activity was induced 24-fold compared to that of untreated mice.

Fig. 3 presents levels of unchanged 1,3,6,8-TCDD and 1,2,3,4,7,8-HxCDD in the suspension medium after incubation for 1hr. with each hepatic microsomes from three mice untreated and treated with 2,3,7,8-TCDD at a single dose of 10 μg/kg. The level of 1,3,6,8-TCDD in suspension decreased to 34.1 to 44.9% compared to the observed average in untreated mice. However, levels of unchanged 1,2,3,4,7,8-HxCDD after incubation with induced microsomes were average 85.8% of the levels observed after incubation with control microsomes. These result indicate that 1,2,3,4,7,8-HxCDD is metabolized more slowly than 1,3,6,8-TCDD.

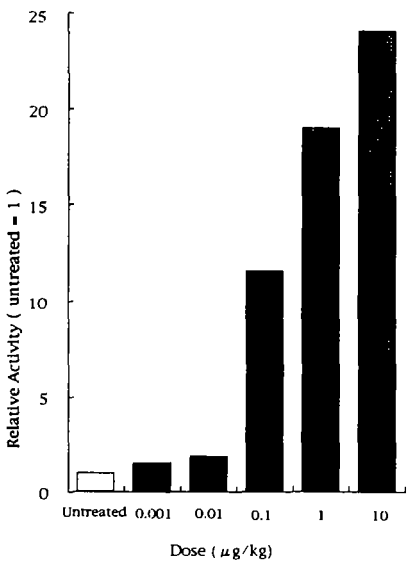

Fig. 2 EROD activities of mouse liver microsomes

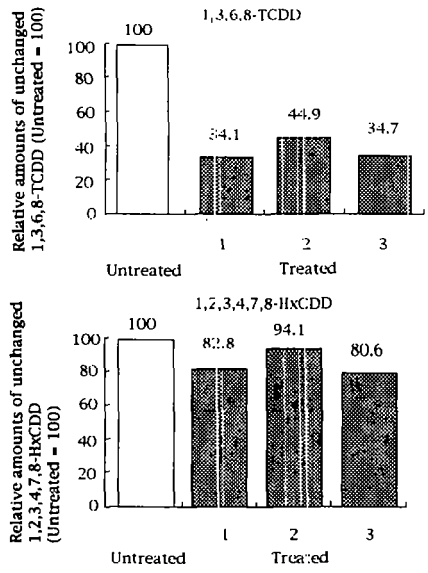

Fig. 3 The amounts of unchanged 1,3,6,8-TCDD and 1,2,3,4,7,8-HxCDD after incubation with each microsomes from three mice treated with 2,3,7,8-TCDD at a single dose close of 10 μg/Kg

Fig. 4 shows the total ion chromatogram and mass spectra of metabolites of 1,3,6,8-TCDD after incubation with microsomes from mice treated with 2,3,7,8-TCDD at a single dose of 10 μg/kg. In contrast, no metabolites were produced after incubation of 1,3,6,8-TCDD with microsomes from control mice. Microsomal enzymes induced by 2,3,7,8-TCDD transformed 1,3,6,8-TCDD to eight metabolites, which included one hydroxytrichlorinated dibenzo-*p*-dioxin (TrCDD), two dihydroxylated TrCDDs, three hydroxylated TCDD isomers and two dihydroxylated TCDD isomers. The major metabolites were two hydroxylated TCDD isomers (peaks No.4 and 5 in the total ion chromatogram). These results indicate that 1,3,6,8-TCDD is easily hydroxylated and dechlorinated by hepatic microsome enzymes induced by 2,3,7,8-TCDD.

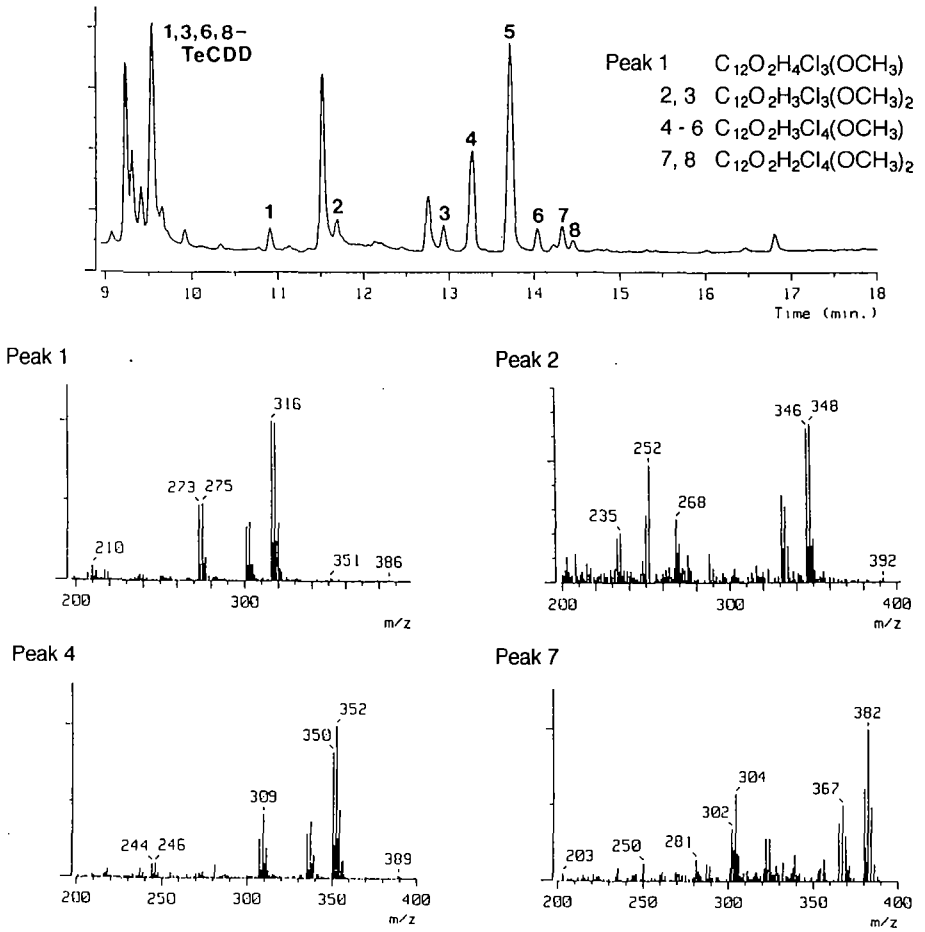

Fig. 4 Total ion chromatogram and mass spectra of 1,3,6,8-TCDD metabolites

Using liver microsome from mice treated with 0.1 μg/kg (data not shown), the 1,3,6,8-TCDD metabolites were similar to those summarized in Fig. 4. In contrast, 2,3,7,8-TCDD metabolites were not detected. In *in vivo* studies [9], six 2,3,7,8-TCDD metabolites were identified in the bile of dogs treated with 2,3,7,8-TCDD at a lethal dose. 1,3,7,8-Tetrachloro-2-hydroxydibenzo-*p*-dioxin was the major metabolite which was formed by way of an NIH-shift of chlorine from C-2 to C-8 in the dibenzo-p-dioxin ring. This suggests that the NIH-shift may also occur in the metabolism of 1,2,3,4,7,8-HxCDD. The results of this study demonstrate that 1,3,6,8-TCDD is more rapidly metabolized that 2,3,7,8-TCDD or 1,2,3,4,7,8-HxCDD by mouse liver microsomes. The rapid metabolism of the former congener is consistent with the low to non-detectable levels of this compound in most environmental and biological samples. Current studies are focused on identifying PCDD metabolites using several different congeners.

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