The Metabolism of 2,3,7,8-Tetrabromodibenzodioxin in the Rat*

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INTRODUCTION

2,3,7,8-tetrabromodibenzodioxin (2,3,7,8-TBDD) belongs to the group of polybrominated *dibenzodioxins (PBDDs). Unintentional formation and subsequent release of PBDDs into* the environment has been observed from a number of sources[1,2,3]. Results from acute and subchronic toxicity studies in rats indicated this compound to be only by a factor 1-10 less toxic than the chlorinated analogue, 2,3,7,8-tetrachlorodibenzodioxin[4,5] (2,3,7,8-TCDD).

Therefore it is of interest to investigate the transformation pathways of the brominated analogue, especially in view of the fact that a bromine-carbon bond is chemically less stable than a chlorine-carbon bond.

The present study was performed to elucidate the structure of 2,3,7,8-TBDD metabolites formed by rat liver enzymes and to examine the preferential metabolic pathways of this compound in comparison with 2,3,7,8-TBDD.

EXPERIMENTAL

Animals. Two female Sprague-Dawley derived ZUR:SIV-Z rats (weight 280 g) were subjected to bile duct cannulation as described before[6].

Dosage. 500 µg 2,3,7,8-TBDD (specified purity 98%, Wellinton Laboratories, Guelph, Ontario, Canada), was dissolved in 3 ml of a toluene/xylene mixture (1:2, v/v). 1 ml of this solution was reduced to a volume of 0.3 ml, mixed with 0.4 ml of corn oil and dosed to each animal by gavage.

Preparation and clean-up of the bile samples. The bile samples were incubated with ß-gluceronidase/arylsulfatase, methylated and submitted to TLC as described before[6].

GC-MS Analysis. Analysis was performed on a VG Tribrid double focusing magnetic sector hybrid mass spectrometer (VG Analytical Ltd., Manchester, Great Britain) operating in the electron ionization (EI, 50 eV, 180°C) mode. EI mass spectra (m/z 35-635) were recorded (1.12 s/scan) at a resolution of m/δm=500. A Carlo Erba model 5360 gas chromatograph equipped with a 25 m SE 54 high resolution (HRGC) fused silica (0.32 mm i.d.) column was used and programmed as follows: 80°C, 2 min isothermal, 20°C/min to 200°C, then at 8°C/min to 280°C followed by an isothermal hold at this temperature. The samples (2 µl in iso-octane) were on column injected at 80°C. Data acquisition and

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retention time measurements were started at 200°C. Brominated compounds were identified from their characteristic EI mass spectra. EI mass spectra of the PBDDs, polybrominated dibenzofurans and diphenylethers were reported earlier[3].

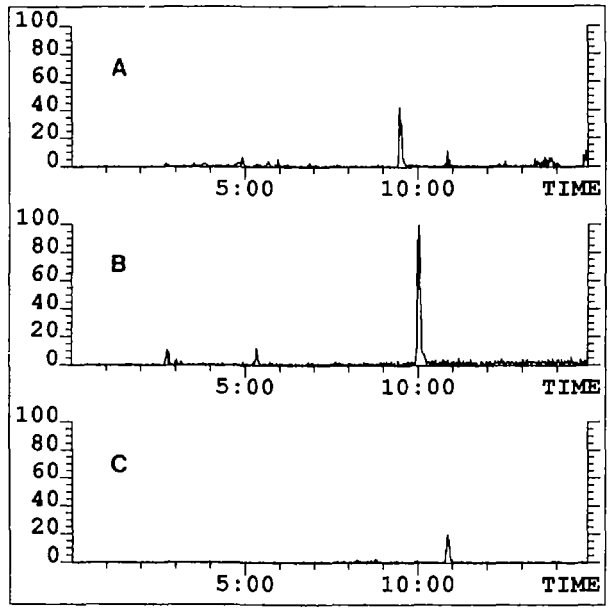

Figure 1a-c: Chromatograms of the three major metabolites of 2,3,7,8-TBDD (A-C: Compound I-III).

RESULTS AND DISCUSSION

Several brominated compounds were detected, including small amounts of 2,3,7,8-TBDD. Metabolites of 2,3,7,8-TBDD, (see table 1) were identified as their methylether derivatives.

In Figure 1a-c, mass chromatograms (m/z: 448, 478 and 542 respectively) of the three major metabolites are shown. Figure 2a-c shows partial EI mass spectra (m/z 285-585) for these components (note the presence of signals from non-halogenated coeluting compounds in Fig. 2c).

Compound I (M^+=448, Br_3) was identified as a methoxytribromodibenzodioxin (MeO-tri-BDD) (see Table 1 and Figures 1a and 2a). The strong M^+-15 fragment relative to the M^+ ion indicates the prescence of a lateral methoxy group[7]. Therefore its structure can be tentatively assigned as 3-methoxy-2,7,8-tribromodibenzodioxin.

Compound II (M^+=478, Br_3) was identified as a dimethoxytribromodibenzodioxin (di-MeO-tri-BDD) (see Table 1 and Figures 1b and 2b). As can be seen in Fig. 2b small signals from 2,3,7,8-TBDD (non-metabolized parent compound) are detectable.

The mass spectrum in Figure 2c (i.e. compound III; M^+=542, Br_4) could be attributed to either a dimethoxytetrabromodiphenylether (di-MeO-tetra-BDPE) or a thiomethyl-tetrabromodibenzodioxin (MeS-tetra-BDD), since both compounds have the same M^+-ion and number of bromine substituents. Retention times of the two compounds are expected

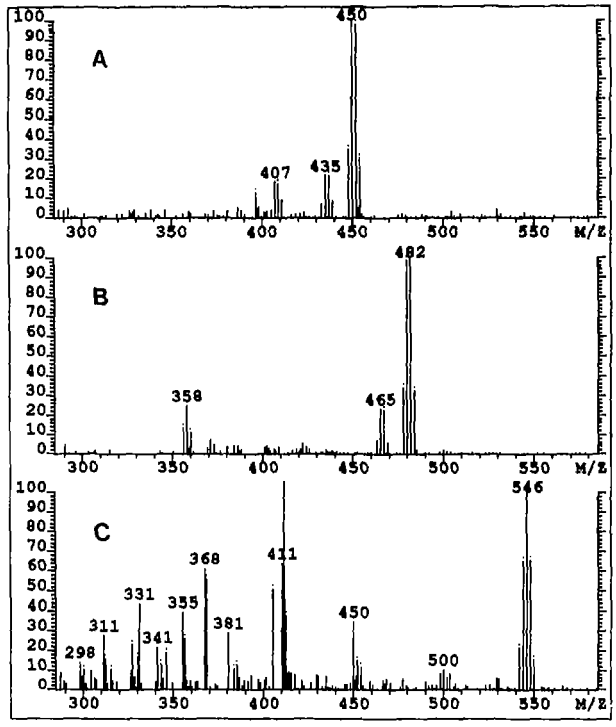

Figure 2a-c: Mass spectra of the three major metabolites of 2,3,7,8-TBDD (A-C: Compound I-III).

to be similar. In case of 2,3,7,8-TCDD, the related metabolite was identified as a dimethoxytetrachlorodiphenylether (di-MeO-tetra-CDPE). Assuming a similar metabolic transformation of 2,3,7,8-TBDD and 2,3,7,8-TCDD, we presume compound III to be the ring-opened rather than the sulfur-containing metabolite. The presence of small signals at m/z 496-502 in spectrum 2c, likely being the M^{+}-46 also observed for the ringopened compound of TCDD, gives a further indication for this[8].

The metabolic pathway of 2,3,7,8-TBDD in the rat can be compared with that of 2,3,7,8-TCDD, since several of the metabolites identified are similar. Nevertheless, some apparent differences between TBDD and TCDD biotransformation routes do exist, some are more quantative, others more qualitative in nature. In the case of 2,3,7,8-TCDD, major metabolites were di-MeO-tri-CDD and di-MeO-tetra-CDPE[8]. Although the relative amounts of metabolites were measured only semi-quantatively, the halogenated dimethoxydiphenyl-ether is clearly more abundant in case of 2,3,7,8-TCDD than in case of 2,3,7,8-TBDD. MeO- and di-MeO-tri-BDDs prevail in the latter case. Thus, dioxin-ring opening seems to play a somewhat greater role in TCDD than in TBDD metabolism.

A more qualitative difference between the two halogenated compounds seems to be the fact that two MeO-tri-CDDs were observed for 2,3,7,8-TCDD, but only one MeO-

tri-BDD for 2,3,7,8-TBDD. A search for a second MeO-tri-BDD was unsuccesfull (Figure 2a).

The excretion half-lives of 2,3,7,8-TBDD and 2,3,7,8-TCDD in the rat are similar, being 17.8 days for 2,3,7,8-TBDD and 31 days for 2,3,7,8-TCDD[9,10]. Thus, it can be concluded that the observed differences in metabolism do not lead to a substantially faster detoxification and elimination of 2,3,7,8-TBDD from the rat when compared to 2,3,7,8-TCDD.

Table 1: GC/MS data of 2,3,7,8-TBDD and its metabolites in the rat.

Compound	Retention*	EI MS Data	Tentative identification
2,3,7,8-TBDD	10.08 min	M^+=498	
I	9.52 min	M^+=448, M^+-CH_3=433, M^+-CH_3-CO=405	MeO-tri-BDD
II	10.04 min	M^+=478, M^+-CH_3=465, M^+-CH_3-CO-Br=356	di-MeO-tri-BDD
III	10.88 min	M^+=542, M^+-CH_3-OCH_3=496	di-MeO-tetra-BDPE or MeS-tetra-BDD

*)For experimental conditions see text.

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