# METABOLISM OF 2,2',4,4'-TETRACHLORODIPHENYL ETHER BY RAT LIVER MICROSOMES

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## Introduction

Polybrominated diphenyl ethers (PBDEs), one of the brominated flame retardants, are world-wide environmental pollutants, and some PBDE congeners have been detected in biota including human samples such as adipose tissue, liver and blood. For example, 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), BDE-71, BDE-99, BDE-100, BDE-153, BDE-197, BDE-207 and BDE-209 have been identified as major components in human blood.[1] Metabolism of PBDEs has been investigated in e.g. rats and hydroxylated/dihydroxylated PBDE metabolites, OH-PBDEs, $(OH)_2$-PBDEs, were detected in feces, bile, liver and blood.[2]

The biotransformation of PBDEs is considered to be initiated by an oxygenation of aromatic ring catalyzed by liver cytochrome P450 (P450), leading to the formation of phenolic compounds. In metabolism of PCBs, the hydroxylating ability of liver microsomes from animals could be explained by the catalytic activity of each P450 isoform. Koga *et al.* reported that rat CYP2B1, phenobarbital (PB)-inducible P450 isoform, catalyze 3-hydroxylation for 2,2',5,5'- and 2,3',4',5-tetrachlorobiphenyl (CB), whereas rat CYP1A1, 3-methylchlanthrene (3-MC)-inducible P450 isoform, catalyze 4-hydroxylation for 2,2',5,5'- and 2,3',4',5-tetraCB.[3] Also CYP2B1 mainly metabolizes non-coplanar PCBs and CYP1A1 hydroxylates selectively the coplanar PCBs such as CB-77.[3] As above, P450 isoforms involved in the metabolism of PCBs are related for the structure of PCB. However, P450 isoforms which is related to metabolism of PBDEs have not yet been elucidated very much. Therefore, we examined the metabolism of 2,2',4,4'-tetrachlorodiphenyl ether (tetraCDE) by rats liver microsomes to determine P450 isoform which is participated in the metabolism of PBDEs and chlorinated diphenyl ethers. Chlorinated diphenyl ethers had been used as flame retardants in Japan and the structure of 2,2',4,4'-tetraCDE used is similar to that of BDE-47, a major congener retainable in human tissues.

## Materials and Methods

***Chemicals***: 2,2',4,4'-tetraCDE was synthesized by chlorination of diphenyl ether with c-HCl and $NaClO_3$. The purity was >95% as determined by GC and GC/MS. The impurities were triCDE, two tetraCDEs and two pentaCDEs. The spectroscopic properties were as follows, mass spectrum; m/z (relative abundance, %): 310 (59, M+4), 308 (100, M+2), 306 (89, $M^+$), 271 (7, $M^+$-Cl), 243 (16, $M^+$-COCl), 236 (94, $M^+$-2Cl); $^1$H-NMR ($CDCl_3$) δ (ppm): 7.48 (2H, d, J=2.4 Hz, 3- and 3'-H), 7.20 (2H, dd, J= 2.5, 8.6 Hz, 5- and 5'-H), 6.80 (2H, d, J=8.8 Hz, 6- and 6'-H). 2'-MeO-2,4,4'-triCDE used for the quantification was prepared from 2'-OH-2,4,4'-triCDE with diazomethane.

***Animal treatment***: Nine male Wistar rats (6 weeks old, body wt. about 160 g) were used and divided into three groups, untreated, PB- and 3-MC-pretreated groups. PB and 3-MC were dissolved in saline and corn oil, and injected intraperitoneally at a dose of 100 and 20 mg/kg/day for 3 days, respectively. Rats were sacrificed 24 hours after the last injection of each P450 inducer and their livers were removed. Liver microsomes were prepared by a conventional centrifugation method.

***Metabolism of 2,2',4,4'-tetraCDE***: 2,2',4,4'-tetraCDE dissolved in DMSO (final conc. 100 μM) was incubated for 20~60 min. at 37℃ with NADPH-generating system and rats liver microsomes (33.0 ~ 38.6 mg protein/mL) in 100 mM HEPES buffer (pH 7.4) under aerobic conditions.

***Extraction and clean up***: After the incubation, 1 % formic acid (4 mL) and MeOH (1 mL) were added to the reaction mixture. Then the solution was applied to cartridge for solid phase extraction (NEXUS, 30 mg, Varian) and the substances (unchanged 2,2',4,4'-tetraCDE and hydroxylated metabolites) were eluted with 2 mL of MeOH after washing the cartridge with 1 mL of $H_2O$. The solvent was reduced to dryness, and then the sample

residue was dissolved in *n*-hexane. An excess of diazomethane was added to the *n*-hexane solution at room temperature to methylate the hydroxyl group and the *n*-hexane solution was treatedt with c-$H_2SO_4$. The samples were analyzed on GC (ECD) and GC/MS for unchanged 2,2',4,4'-tetraCDE and methylated OH-, $(OH)_2$-CDE metabolites.

***Instrument***: Gas chromatography (GC) analyses were performed on a GC-17A (Shimadzu) equipped with a $^{63}Ni$ electron-capture detector (ECD) under the conditions as follows: column, DB-5 fused silica capillary column (30 m×0.25 mm i.d., 0.25μm film thickness); carrier gas, $N_2$; temperature program, 70℃(2 min)-20℃/min-220℃(1 min)-3℃/min-280℃(20 min); injection port temp.,250℃; detector temp., 330℃. Gas chromatography-mass spectrometry (GC/MS) was carried out on a JMS-AX505W (JEOL) apparatus in the EI mode. carrier gas, helium; column conditions and temperature program are analogous to those described for GC. The quantification of metabolites was performed on a GC (ECD) using the calibration curve of 2'-MeO-2,4,4'-triCDE.

**Results and Discussion**

*In vitro* metabolism of 2,2',4,4'-tetraCDE was examined using rats liver microsomes. When 2,2',4,4'-tetraCDE was incubated with NADPH and microsomes at 37℃ for 20~60 min, untreated and 3-MC-treated microsomes formed very small amounts of two OH-tetraCDE metabolites named as M-1 and M-2 (Table 1). On the other hand, PB-treatment showed much higher activity for the hydroxylation than untreated and 3-MC-treated microsomes. At least four $(OH)_2$-tetraCDE metabolites, named as M-3, M-4, M-5 and M-6, in addition to four OH-tetraCDEs including M-1 and M-2, were detected in PB-treated microsomes (Figure 1). Among these OH-, $(OH)_2$-tetraCDEs, M-1 was a major metabolite.

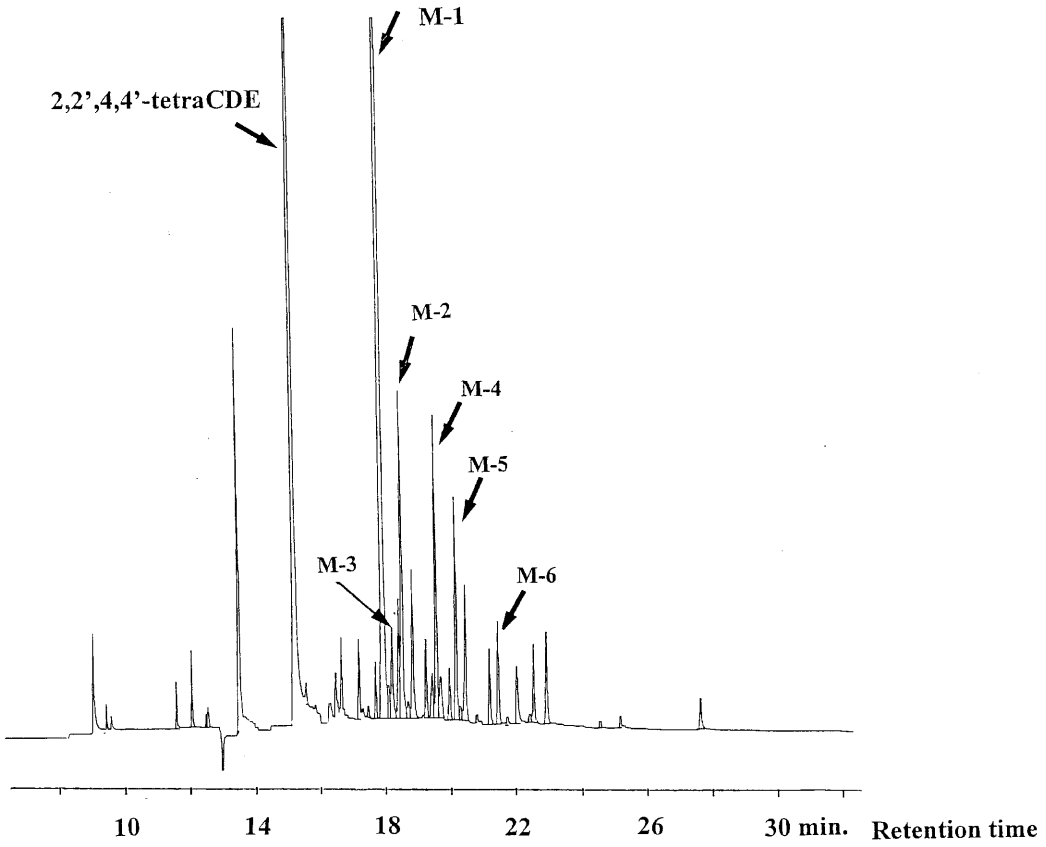


Figure 1. Gas chromatogram (ECD) of methylated derivatives of 2,2',4,4'-tetraCDE metabolites formed by liver microsomes of PB-treated rats. 2,2',4,4'-tetraCDE was incubated with NADPH and microsomes at 37℃ for 60 min.