# Formation and Distribution of Hydroxylated and Methylsulfonyl Metabolites of 2,3',4',5-Tetrachlorobiphenyl in Rats

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## 1. Introduction

PCBs are generally biotransformed to hydroxylated (OH-) metabolites and persistent methylsulfonyl ($MeSO_2$-) metabolites depending on the number and positions of chlorine atoms. Among hydroxylated metabolites of chlorobiphenyls (CBs), several OH-CBs with hydroxy group at *para*- position, e.g. 4-OH-3,3',5,5'-tetraCB, 4-OH-3,3',4',5,5'-pentaCB, 4-OH-2,3,3',4',5-pentaCB, have been shown to be selectively retained in blood from rats[1],mouse[2] and mammals[3]. Some of these OH-CBs are considered to be formed from the parent CBs with a 3,4-dichloro- or 2,3,4-trichloro-substituted phenyl ring, after an arene oxide formation in 4,5-position and subsequent shift of chlorine atom at 4-position to 5-position (NIH shift). In addition, recent studies[4] have indicated that OH-CBs have some biological activities. For example, 4-OH-3,3',4',5-tetraCB, a NIH shift metabolite of 3,3',4,4'-tetraCB, has been suggested to be relevant to the developmental toxicity of 3,3',4,4'-tetraCB[5]. On the other hand, CBs with a 2,5-dichloro- or 2,3,6-trichloro-substituted phenyl ring give rise to 3- and 4-$MeSO_2$-CBs that have been detected in tissues of animals[6,7]. Recently some 3-$MeSO_2$-CBs, e.g. 3-$MeSO_2$-2,2',4',5,5'-pentaCB, have been shown to be a strong phenobarbital-type inducer of liver enzymes[8]. CBs with a 2,5-dichloro-substituted phenyl ring also give rise to OH-CBs as well as $MeO_2$-CBs. For example, 2,2',5,5'-tetraCB is metabolized to 3- and 4-OH-2,2',5,5'-tetraCB[9,10], 3- and 4-$MeSO_2$-2,2',5,5'-tetraCB[11]. However, there is no information on the ratio of OH-CBs and $MeSO_2$-CBs formed simultaneously from a same PCB congener. It is therefore of interest to study the metabolism of PCBs for formation of both OH-CBs and $MeSO_2$-CBs in relation to PCB toxicity.

2,3',4',5-tetraCB is one of the major constituents in commercial PCB mixtures and consists of 2,5-dichloro- and 3,4-dichloro-substituted phenyl ring. Based on the chlorine atoms substituted in 2,3',4',5-tetraCB, we could expect the formation of OH-CBs and $MeSO_2$-CBs from the 2,5-dichloro-substituted phenyl ring and OH-CB with hydroxy group at *para*-position from the 3,4-

dichloro-substituted phenyl ring. Therefore, we chose 2,3',4',5-tetraCB to investigate the formation ratio of OH-CBs and $MeSO_2$-CBs in rats. We also compared the tissue distribution of OH-CBs and $MeSO_2$-CBs in some tissues.

## 2. Materials and Methods

2,3',4',5-tetraCB was synthesized by the method of Cadogan. Male Wistar rats (ca. 190g) were given p.o. 2,3',4',5-tetraCB (50 mg/kg) and were killed 3 days after the injection. Liver, lung, kidney, spleen, adipose tissues and bloodserum were taken out. Feces were collected daily for 3 days. These samples were analyzed for the unchanged 2,3',4',5-tetraCB, OH-CBs (as methylated derivatives) and $MeSO_2$-CBs according to the method described elsewhere[3,6]. Identification and determination were performed by GC/ECD and GC/MS using the authentic standards.

## 3. Results and Discussion

Fecal excretion of metabolites: 3- and 4-OH-2,3',4',5-tetraCB and $(OH)_2$-tetraCB (unidentified) were determined as the hydroxylated metabolites in the feces (Fig.1). Among them, major metabolite was 3-OH-2,3',4',5-tetraCB. Most amounts of 3- and 4-OH-2,3',4',5-tetraCB in the feces were excreted during the first 2 days, so the relative amounts of $(OH)_2$-tetraCB was higher than those of 3- and 4-OH-2,3',4',5-tetraCB on 3 days after the injection (Fig.1). On the other hand, OH-CBs derived from the 3,4-dichloro-substituted phenyl ring such as 4'-OH-2,3',5,5'-tetraCB were not detected. Furthermore, 3- and 4-$MeSO_2$-2,3',4',5-tetraCB were determined at almost same quantities although the amounts of each $MeSO_2$-tetraCBs were about 1/100～4/100 of those of 3- and 4-OH-2,3',4',5-tetraCB. Isomeric pairs of MeSO- and MeS-tetraCB, precursors of $MeSO_2$-CB, were also present at almost 1:1 ratio for the isomers and these metabolites were tentatively assigned as 3- and 4-MeSO-2,3',4',5-tetraCB and 3- and 4-MeS-2,3',4',5-tetraCB. The excreted amounts of total hydroxylated metabolites, S-containing metabolites ($MeSO_2$-CBs and MeSO-CBs) and unchanged 2,3',4',5-tetraCB during 3 days accounted for about 3.8%, 0.5% and 1.1% of the dose, respectively, indicating that hydroxylation is the major pathway of 2,3',4',5-tetraCB.

Tissue distribution of metabolites: OH-CB metabolites gave rise to a different distribution pattern; Interestingly, $(OH)_2$-tetraCB was determined at relatively high concentrations (45～285 ng/g) in all the tissues studied except for adipose tissue. The high concentrations of $(OH)_2$-tetraCB were particularly observed in serum (285 ng/g) and lung (203 ng/g). 3-OH-2,3',4',5-tetraCB, a major fecal metabolite, was determined only in spleen (37 ng/g) and lung (84 ng/g), but 4-OH-2,3',4',5-tetraCB was not found at any detectable amounts in all the tissues. 3- and 4-$MeSO_2$-2,3',4',5-tetraCB were also retained in all the tissues investigated, whereas MeSO-tetraCBs and MeS-tetraCBs were not detected. The adipose tissue contained the highest $MeSO_2$-CBs concentrations (3-$MeSO_2$; 1771 ng/g, 4-$MeSO_2$; 1160 ng/g). Concentrations of total $MeSO_2$-CBs in the liver (3-$MeSO_2$; 216 ng/g, 4-$MeSO_2$; 167 ng/g) and lung (3-$MeSO_2$; 70 ng/g, 4-$MeSO_2$; 805 ng/g) were 8.5 and 3 times higher than those of total OH-CBs in each tissues,

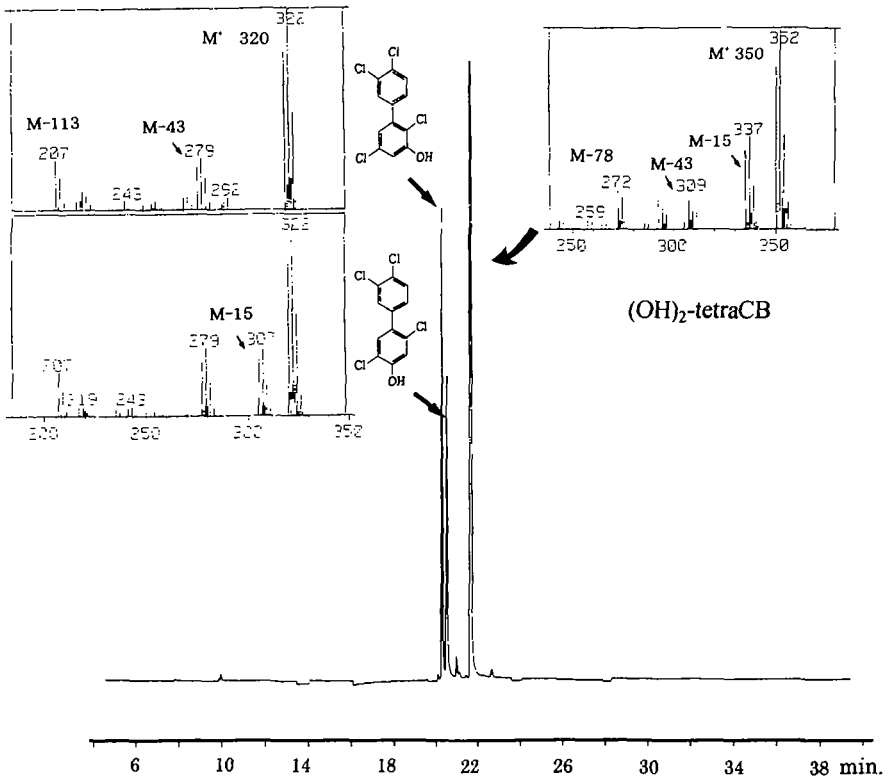


Fig.1. GC-ECD of methylated OH-CBs and $(OH)_2$-CB in the feces of rats 3 days after oral doses of 2,3',4',5-tetraCB

respectively. In the kidney, total $MeSO_2$-CBs concentration (3-$MeSO_2$; 50 ng/g, 4-$MeSO_2$; 88 ng/g) was almost same as total OH-CBs concentration. In contrast, concentrations of total $MeSO_2$-CBs in the serum (3-$MeSO_2$; 14 ng/g, 4-$MeSO_2$; 16 ng/g) and spleen (3-$MeSO_2$; 34 ng/g, 4-$MeSO_2$; 51 ng/g) were 1/10～6/10 of those of total OH-CBs. The ratios of 3-$MeSO_2$-/4-$MeSO_2$-2,3',4',5-tetraCB in the tissues were 0.1 for lung, 0.6 for kidney, 0.7 for spleen, 0.9 for serum, 1.3 for liver and 1.5 for adipose tissue. These results indicate a strong retention of 4-$MeSO_2$-2,3',4',5-tetraCB in the lung. Unchanged 2,3',4',5-tetraCB in the tissues were also determined. The adipose tissue contained the highest 2,3',4',5-tetraCB levels (3889 ng/g) that was higher than total $MeSO_2$-CB level in the adipose tissue. However, levels of 2,3',4',5-tetraCB in the serum, liver, kidney, spleen and lung (6～81 ng/g) were lower than those of $(OH)_2$-CB and $MeSO_2$-CBs in the same tissues.

In conclusion, 3- and 4-OH-2,3',4',5-tetraCB and 3- and 4-$MeSO_2$-tetraCB derived from the 2,5-dichloro-substituted phenyl ring were determined in the feces and several tissues studied,

whereas no metabolites derived from the 3,4-dichloro-substituted phenyl ring were found, indicating that 2,5-dichloro-substituted phenyl ring is more easily metabolized than 3,4-dichloro-substituted phenyl ring. Tissue concentrations of both $(OH)_2$-CB and $MeSO_2$-CBs exceeded that of unchanged 2,3',4',5-tetraCB except adipose tissue. OH-CB and $MeSO_2$-CB metabolites showed a tissue-specific distribution.

4. References


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