HYDROXY AND METHYLSULFONE METABOLITES OF POLYCHLORINATED BIPHENYLS IN THE HUMAN BLOOD AND TISSUES

Yoshito Masuda[1], Koichi Haraguchi[1]

[1]Daiichi College of Pharmaceutical Sciences, Fukuoka

Introduction

Polychlorinated biphenyls (PCBs) are a group of chlorinated compounds which have polluted the global environment, persistently retained in wildlife and humans, and eventually affected the human health. PCBs are biotransformed to mainly hydroxy (HO-) and methylsulfone ($MeSO_2$-) metabolites in the animal and human tissues. About ten thousands of chemical and biological researches on PCBs, HO-PCBs and $MeSO_2$-PCBs have been reported and reviewed so far. Letcher et al.[1] cleverly reviewed the HO-PCBs and $MeSO_2$-PCBs in 2000. We review the contamination of HO-PCBs and $MeSO_2$-PCBs in human tissues and their possible effects to human health. Different positional numberings of Cl-, HO- and $MeSO_2$- on biphenyl rings were used by different authors. Then, nomenclature of PCB metabolite was assessed by Maervoet et al.[2] and they suggested to use the IUPAC chemical name and number of parent PCB congener with the subsequent assignment of the phenyl ring position number of the HO- or $MeSO_2$- substituent number afterward.

Hydroxy PCBs

Presence of HO-PCBs in human tissues was gas chromatographically noticed in Japanese blood. Nine HO-PCB congeners were identified in Swedish blood and the major metabolite, 4-HO-CB187, may be originated from PCB187 and/or PCB183 which are really present in human blood. PCBs are metabolized to HO-PCBs for excretion and some particular HO-PCBs having two chlorine atoms

substituted adjacent to the hydroxy group are long retaining in the blood, as they bind to a thyroxin-transporting protein (transthyretin) in the blood. Table 1 lists the concentrations of HO-PCBs and pentachlorophenol (PCP) in the human blood, which reported in and after 2000. Sandau et al. [3] identified 11 HO-PCB congeners, PCP and PCBs in whole blood from male and female Inuit from northern Quebec and blood from southern Quebec. Geometric mean concentrations of total HO-PCBs for Inuit men and women were much higher than those of southern Quebec. There was a significant correlation between HO-PCBs and PCBs and both correlated significantly with age. Concentrations of 14 HO-PCB congeners, PCP and 4-hydroxy-heptachlorostyrene were determined in umbilical cord plasma samples from three different regions of Quebec, Nunavik (Inuit people), the Lower North Shore of the Gulf of St. Lawrence (fishermen) and a southern Quebec urban center (urbanite)[4]. The main chlorinated phenolic compound in all regions was PCP, and concentrations of PCP were not significantly different among regions. Sum HO-PCBs concentrations were highest in the fishermen and followed in Inuit people and urbanite. Sjödin et al. [5] examined the concentrations of PCBs, HO-PCBs, PCP and others in the plasma of Latvian and Swedish men. Both age and fish consumption was significantly correlated with the concentrations of total PCBs and total HO-PCBs. The Spearman's rank correlation coefficient obtained by comparing the level of the metabolite 4-HO-CB107 to the combined levels of its parent compounds, PCB105 and PCB118, was higher than the median correlation coefficient obtained upon comparing the level of this metabolite to all other possible combinations of two PCB levels. The concentrations of 14 HO-PCB congeners, 24 $MeSO_2$-PCB congeners and 17 PCB congeners were determined in Swedish liver and adipose tissue by the developed method for simultaneous analysis of the three groups of PCB congeners in the same sample[6]. The determined PCB metabolites constituted 3 to 26 % of total PCBs concentration in the liver and 0.3 to 0.8 % of total PCBs in the adipose tissue samples. Fängström et al. determined HO-PCBs and PCBs in serum from pregnant Faroese women whose traditional diet includes pilot whale and blubber and other marine food, which may contain high concentrations of organohalogen substances. The most abundant HO-PCB metabolite was 4-HO-CB187 in all samples analyzed, with four other HO-PCB congeners as dominating metabolites in the serum. From the lipid percent in the serum, the concentrations of total HO-PCBs in the sera of low and high consumption groups, 96 and 560 ng/g lipid, are converted to 749 and 4950 pg/g serum, respectively, corresponding to similar or higher than the levels in the blood of Canadian Inuit. Hovander et al. identified HO-PCB metabolites and other phenolic halogenated pollutants in Swedish blood plasma by gas

chromatography/electron capture detection and gas chromatography/mass spectrometry using methyl derivatives of halogenated phenols and HO-PCB congeners as authentic reference standards. More than 100 phenolic halogenated compounds were identified, and the two major compounds were 2,4,6-tribromophenol and PCP. Thirty-eight HO-PCB congeners were structurally identified on two gas chromatographic columns of different polarity. The major HO-PCB congeners determined in the blood and tissues of Canadian, Latvian, Swedish and Faroese were also identified as big or middle size peaks on the gas chromatograms of the Swedish plasma sample. 4-HO-CB146 and 4-HO-CB187 and PCP were identified in the blood of Yusho patients and control persons (Table 1). Concentrations of the HO-PCBs in Yusho patients were a little higher than the controls, but those of PCP were lower than the controls[7].

In the umbilical cord of neonates from coastal population, the sum of plasma concentration of phenolic compounds, PCP and HO-PCBs, were negatively correlated to free thyroxine plasma levels, suggesting these phenolic compounds can alter thyroid hormone status in newborns[4]. The phenolic compounds could inhibit thyroxin transport by competitive binding to the transthyretin. In the animal experiments using ^{14}C labeled 4-HO-CB107, exposure of pregnant rats to 4-HO-CB107 resulted in drastic reduction in fetal plasma thyroid hormone concentration, and in an accumulation of the compound in fetal liver, brain and plasma. Low dose of HO-PCB (100 pM) suppressed thyroid hormone-induced transcriptional activation in brain-derived cell line [8], suggesting that brain development would be retarded in fetus as the human blood contains HO-PCBs higher than 100 pM. Kester et al. investigated the potential inhibition of human estrogen sulfotransferase by various HO-PCBs and demonstrated that the HO-PCBs identified in human blood were extremely potent inhibitors of human estrogen sulfotransferase, suggesting that they induce estrogenic activity by increasing estradiol bioavailability in target tissues. Shevtsov et al. determined the crystal structure of human estrogen sulfotransferase in the presence of sulfuryl donor product 3'-phosphoadenosine-5'-phosphate and 4,4'-diOH-3,3',5,5'-tetraCB, and the bound crystal structure gives physical evidence that certain HO-PCBs can mimic binding of estrogenic compounds in biological systems.

Table 1. Mean or median concentrations of hydroxy PCB congeners in human blood

	Canadian Inuit blood[3]	Canadian umbilical cord plasma[4]		Latvian and Swedish men blood[5]		Japanese blood[7]
Human Blood	Inuit, northern Quebec males (n = 13)	Nunavik	Lower North Shore	Moderate fish consumotion		Yusho
		Cord blood (n = 10)	Cord blood (n = 10)	Latvian male (n=22)	Swedish male (n=11)	n = 36
Concentration status	pg/g blood	pg/g plasma		ng/g lipid		ng/g blood
	Geometric mean	Geometric mean		Median		Mean
PCP	2740	1870	1430	420	720	0.55
% to PCBs	21	124	53	81	48	20
4-HO-CB107	314	12	49	73	78	< 0.1
4-HO-CB146	219	37	81	44	100	0.2
4-HO-CB187	293	47	95	55	120	0.21
Sum all HO-PCBs	1730	286	553	230	350	0.41
% to PCBs	13	19	20	44	23	15
Sum PCBs	12900	1510	2710	520	1500	2.79

Methylsulfone PCBs

$MeSO_2$-PCBs were identified in the breast milk from a mother who had been formerly employed in a capacitor factory handling PCB products. The fat base concentration was approximately 0.77 ppm, being at 1/20 levels of PCBs. Haraguchi et al. determined two congeners of methylthio PCBs and 16 congeners of $MeSO_2$-PCBs in the tissues of three Yusho patients and two control persons. The concentrations of methylthio PCBs in the liver, lung and adipose tissue of the Yusho patients were 0.1-0.5, 0.2-1.4 and 0.5-1.0 ng/g wet weight, respectively, and those of $MeSO_2$-PCBs were 0.3-0.7, 1.0-2.5 and 0.7-1.0 ng/g wet weight, respectively. The concentration ratios of methylthio and methylsulfone PCBs to unchanged PCBs were 1-2 % in the liver, 4-8 % in the lung and 0.1-0.2 % of in the adipose tissue for Yusho patients, indicating that these metabolite accumulate slightly more in the lung and liver than in the adipose tissue, relative to PCBs. Tissue levels of the PCB metabolites in control persons were one tenth of those in Yusho patients. Distribution in mice of ^{14}C labeled PCB52 was studied by

autoradiography, showing that radioactivity was specifically localized in the bronchial epithelium, the lung parenchyma, the kidney cortex and adipose tissue. And bis(methylsulfonyl)-, 3-$MeSO_2$- and 4-$MeSO_2$-CB52s were identified in lung, kidney and liver of the mice. Lund et al found that drug-metabolizing enzyme was induced by a methylsulfone metabolite to different direction in the liver and lung. After treatment of mice with 4-$MeSO_2$-CB52, a major PCB metabolite in human lung tissue, the pulmonary N-demethylation of aminopyrine in vitro was significantly decreased, while hepatic N-demethylation was concomitantly increased. The results indicate that the methylsulfone PCB inhibits or represses a cytochrome P-450-dependent enzyme activity in the mouse lung. Eighty-six positional isomers of methylsulfonyl PCBs were synthesized. And more than 60 isomers of tri-, tetra-, penta-, and hexachloro-methylsulfone-CBs were detected in the lung of Yusho patient, and the gas chromatographic peaks coincided with those of 40 authentic isomers in retention times by three separate capillary columns[9]. Norén et al. analyzed human milk sampled in Stockholm from 1972 to 1992 for methylsulfone metabolites of PCBs and DDE. During the time course studied, the concentrations of $MeSO_2$-CBs decreased from 9 to 2 ng/g lipids and of $MeSO_2$-DDE from 5 to 0.5 ng/g lipids. 3-$MeSO_2$-DDE was the major isomer of the aryl methylsulfones studied in the milk. Generally, the concentrations of 4-$MeSO_2$-CBs were higher than the corresponding 3-$MeSO_2$-CB compound. Methylsulfonyl metabolites of PCBs and DDE were determined in 7 Swedish adipose and liver tissues. Twenty $MeSO_2$-CBs and two $MeSO_2$-DDEs were found in the analyzed samples. In all samples of adipose tissue, 4'-$MeSO_2$-CB87 and 4-$MeSO_2$-CB149 occurred at higher concentrations than other $MeSO_2$-CBs, while in the liver, 5'-$MeSO_2$-CB132 was by far the most abundant $MeSO_2$-CB, contributing to 61-82 % of the sum of $MeSO_2$-CBs. The ratios of the sum of $MeSO_2$-CBs to the sum of determined PCBs were 1/250 and 1/20 in adipose tissue and the liver, respectively. The concentrations of PCBs, DDE, hexachlorobenzene (HCB) and methylsulfonyl metabolites of PCBs and DDE were determined in human plasma samples and in the fractions obtained by ultracentrifugation of plasma in to very-low-density (VLDL), low-density (LDL), high-density (HDL) lipoprotein and lipoprotein depleted (LPDP) fractions. The organochlorine compounds were associated with all fractions, but predominantly with the LPDP fraction. On an average 44 % of PCBs, 61 % of $MeSO_2$-CBs, 73 % of DDE, 77 % of $MeSO_2$-DDE and 45 % of HCB were distributed in the LPDP fraction. Guvenius et al [6] developed the analytical method based on the strategy for determining both the PCBs and their metabolites, HO-CBs and $MeSO_2$-CBs, in the same sample, and Swedish liver and adipose tissue were analyzed for PCBs, HO-CBs and $MeSO_2$-CBs. The sum of

HO-CB congeners were the same order of magnitude as those of $MeSO_2$-CBs in the same samples. The determined PCB metabolites constituted 3 to 26 % of total PCB concentration in the liver and 0.3 to 0.8 % of total PCBs in the adipose tissue samples. Chu et al.[10] determined methylsulfonyl metabolites of PCBs and DDE in Belgian adipose, liver, brain and lung tissues. Total concentration of $MeSO_2$-CB decreased in the following order: liver 9.30 > lung 2.72 > adipose tissue 1.57 > brain 0.24 ng/g lipid (Table 2). In adipose, brain, and lung tissues, 4'-$MeSO_2$-CB87, 4'-$MeSO_2$-CB101, and 5-$MeSO_2$-CB149 (except brain) occurred at high concentrations than did other $MeSO_2$-CBs. However, 5'-$MeSO_2$-CB132 was by far the most abundant congener in liver, contributing on average to approximately 60 % of the sum of $MeSO_2$-CBs.

Kato et al. investigated the $MeSO_2$-CBs metabolites retained in the human tissues for their effects on enzymatic and/or hormonal mediations in animals. The administration of 3-$MeSO_2$-CB70, 3'-$MeSO_2$-CB87, 3'-$MeSO_2$-CB101, and 3'-$MeSO_2$-CB141 to rats significantly increased the content of cytochromes P-450 and b_5 and the activities of aminopyrine N-demethylase, 7-ethoxycoumarin O-deethylase and benzo[a]pyrene hydroxylase. The 3-$MeSO_2$ derivatives studied are possibly potent phenobarbital(PB)-like inducers of microsomal drug-metabolizing enzymes. On the other hand, the 4-$MeSO_2$ derivatives had almost no effect on both cytochrome contents and these enzyme activities. These enzyme investigations suggest that the 3-$MeSO_2$ metabolites derived from PCBs studied play an important role in the induction of the drug-metabolizing enzymes by the parent PCB congeners. In the additional experiments of administration at various doses of the $MeSO_2$ metabolites and parent PCBs, 3'-$MeSO_2$-CB101 produced nearly dose-related increases in the hepatic concentration of this methylsulfone, in the contents of cytochromes and in activations of the enzymes, indicating that 3-$MeSO_2$ metabolites were strong PB-type inducers of hepatic drug-metabolizing enzymes and 3'-$MeSO_2$-CB101 was an especially strong inducer.. The induction profile of the drug-metabolizing enzymes, CYP2B1 and CYP2B2 in the hepatic microsomes of rats treated with nine 3-$MeSO_2$ derivatives, namely 3-$MeSO_2$-CB31, 3'-$MeSO_2$-CB49, 3-$MeSO_2$-CB52, 3-$MeSO_2$-CB70, 3'-$MeSO_2$-CB87, 3'-$MeSO_2$-CB101, 3'-$MeSO_2$-CB141, 5'-$MeSO_2$-CB132, 5-$MeSO_2$-CB149, was similar to that of rats treated with PB, but was different from that of rats treated with 3-methylcholanthrene (3-MC). These findings indicate that 3-$MeSO_2$ metabolites derived from nine PCBs are PB-type inducers of microsomal drug-metabolizing enzymes. The results of present study show that the structure-CYP2B1/2 induction relationship exists for the 3-$MeSO_2$ derivatives studies. The

inducing abilities of 3'-$MeSO_2$-CB49 and 3'-$MeSO_2$-CB101 on the content of cytochrome P450 were higher than those of coplanar PCBs (PCB118, PCB77 and PCB126). It is noticeable that 3'-$MeSO_2$-CB49 and 3'-$MeSO_2$-CB101 have highly potent PB-type inducing activity on drug-metabolizing enzyme systems. The effects of PCB49, PCB101, PCB149 and their six $MeSO_2$-metabolites on cell communication were investigated in the scrape-loading/dye-transfer assay in IAR 20 rat liver epithelial cells. The results demonstrated that at non-cytotoxic concentrations the PCBs and their 3- and 4-$MeSO_s$ derivatives completely inhibit cell communication within 1 hr. The results show that 3- and 4-$MeSO_2$ derivatives of the PCB congeners tested inhibit gap junction intracellular communication at about the same potency as their parental compounds. When PCB101 was injected intraperitoneally into bile duct-cannulated rats, 3- and 4-$MeSO_2$-CB101 was not detected in liver. In antibiotic-treated rats dosed with PCB101, the concentrations of 3- and 4-$MeSO_2$-CB101s in liver markedly reduced. These finding suggest that the process in which 3- and 4-$MeSO_2$ metabolites of PCB101 are formed involves the biliary secretion of some precursors which will be subjected to metabolism by intestinal microflora. The increasing effects of PCB101 both on the content of cytochrome P450 and on the activity of aminopyrine metabolizing enzyme in hepatic microsomes were not observed in the bile duct-cannulated rats, in which the PB treatment enabled the drug-metabolizing enzymes to be induced. These findings provide the evidence that the induction of some drug-metabolizing enzymes by PCB101 is due not to the action of PCB101 itself but to its 3-methylsulfonyl metabolite, 3-$MeSO_2$-CB101. Male Sprague-Dawley rats received four consecutive intraperitonial doses of five kinds of methylsulfonyl PCB metabolites, 3'-$MeSO_2$-CB49, 3-$MeSO_2$-CB70, 3'-$MeSO_2$-CB87, 3'-$MeSO_2$-CB101 and 4'-MeSO2-CB101, to determine their effects on thyroid hormone levels. All five tested $MeSO_2$-CBs reduced serum total thyroxine levels 16-40 % on days 2, 3, 4, and 7 after the last dosage. The total triiodothyronine level was reduced 37 % by treatment with 3'-$MeSO_2$-CB49 at day 7, but was increased 35 % and 38 % by 3-$MeSO_2$-CB70 and 4'-$MeSO_2$-CB101. A 30 % increase in thyroid weight was produced by 3'-$MeSO_2$-CB101 treatment. Thus it is likely that all five tested $MeSO_2$ metabolite could influence thyroid hormone metabolism and the results suggest that the metabolites may act as endocrine-disruptors. Johansson et al. studied the persistent PCB metabolites with respect to their interaction with the human glucocorticoid receptor (GR). When studying the competitive binding of 24 $MeSO_2$-CBs (relative to dexamethazone) to GR from mouse liver cytosol, seven compounds had a higher affinity to GR than 5-$MeSO_2$-CB149. Structure-activity relationship studies indicated that the presence of three chlorine atoms in the

ortho-position and chlorine and methylsulfone groups on either end of the molecule (4 and 4'- position) increased the affinity to GR. The results stress the need for studying endocrine disruptors that affect hormonal systems other than sex and thyroidogenic hormones.

Table 2. Concentrations of $MeSO_2$-CBs and $MeSO_2$-DDE in the human tissues (ng/g lipid or wet)

	Belgian[10]				Swedish[6]		Japanese, Yusho[9]		
Number	8 men and 3 women				4 men and 1 women		Yusho patient (ng/g wet weight)		
Age (years)	9-62				woman 47, men 66-83		Adult		
	Adipose	Liver	Brain	Lung	Liver	Adipose	Lung	Adipose	Liver
3'-$MeSO_2$-CB87	ND	0.34	ND	0.11	1	0.1	0.13	0.20	ND
4'-$MeSO_2$-CB87	0.33	0.35	0.1	0.55	1	1	1.50	1.47	0.14
3-$MeSO_2$-CB91	ND	0.82	ND	ND	1	0.04			
3'-$MeSO_2$-C8101	0.08	0.15	ND	ND	0.2	0.2	0.36	0.45	0.12
4'-$MeSO_2$-CB101	0.27	0.27	0.1	0.46	0.3	0.3	0.68	1.16	0.11
5'-$MeSO_2$-CB132	ND	5.42	ND	0.37	26	0.1	0.23	0.09	0.03
4'-$MeSO_2$-CB132	ND	ND	ND	0.08	0.02	0.02	0.04	0.03	ND
4'-$MeSO_2$-CB141	ND	0.07	ND	0.08	0.1	0.1	0.25	0.26	0.02
5-$MeSO_2$-CB149	0.18	1.15	ND	0.42	2	0.4			
4-$MeSO_2$-CB149	0.08	0.1	ND	0.08	1	1			
Sum $MeSO_2$-CBs	1.57	9.3	0.24	2.72	34	6	15.18	10.44	3.29
% to Sum PCBs	0.33	2.59	0.40	0.34	4.53	0.64	23.72	0.61	11.34
3-$MeSO_2$-DDE	1.15	4.69	0.22	0.50					
% to DDE	0.24	1.00	0.19	0.05					
Sum PCBs	479.6	358.9	59.7	796.4	751	936	64	1700	29
DDE	484.3	468.9	116.9	1041.8					

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