SPECIES DIFFERENCE IN TISSUE RETENTION OF PCB METABOLITES AFTER *IN VIVO* EXPOSURE TO 2,4,5,2',5'-PENTA- AND 2,3,4,2',3',6'-HEXACHLOROBIPHENYLS

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Introduction

Non-planer PCBs with 2,5- or 2,3,6-substitution are major components of a technical mixture[1] and frequently detected in the environment and biota. These congeners are readily metabolized to hydroxylated (OH-PCB) and methylsulfonyl (MSF-PCB) metabolites. Selective retention of OH-PCBs and MSF-PCBs has previously been reported to occur in blood or liver/adipose tissues of human[2] and mammals exposed environmentally[3], which may be related to the alteration of endocrine system[4] and the induction of hepatic microsomal enzymes[5]. *In vitro* metabolism studies on PCBs revealed that the hydroxylation is mediated by different cytochrome P450 isoforms, depending on the species[6]. However, there is limited information on the spieces-dependent *in vivo* metabolism of persistent PCBs.

In the present study we examined the species difference in the retention of OH- and MSF-PCBs in liver and serum after *in vivo* exposure to 2,4,5,2',5'-pentachlorobiphenyl (CB101) and 2,3,4,2',3',6'-hexachlorobiphenyl (CB132) to rats, mice, hamsters and guinea pigs.

Materials and Methods

Chemicals

CB101 and CB132 were synthesized by using the Cadogan coupling reactions[7]. OH-PCBs were synthesized by the method of Bergman *et al* [8]. MSF-PCBs were synthesized as described previously[9].

Animal treatments

Male Wistar rats (150-200 g), male ddy mice (27-35g), male Syrian hamsters (95-120 g) and male Hartley guinea pigs (400-540 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). All animals were housed three or four per cage with free access to commercial chow and tap water, and maintained on a 12-hr dark/light cycle (8:00 a.m.-8:00 p.m. light) in a room at a controlled temperature (24.5 ± 1 °C) and humidity (55 ± 5 %). The animals received an intraperitoneal injection of CB101 (11 mg/kg) and CB132 (19 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals received an equivalent volume of vehicle. All animals were killed by decapitation on day 4 after the dosing, and the liver and blood were removed, weighed and analyzed for PCBs and their metabolites by our previous methods with GC/ECD and GC/MS[7]. Identification and quantification of PCBs and their metabolites were performed by using three internal standards, 2,3,4,5,6,3',4',5'-octaCB, 4-OH-2,3,5,6,3',4',5'-heptaCB and 4-methyl-3-MSF-5,2',3',4',5'- pentaCB.

Results

After exposure of PCB101 and PCB132 to animals, three OH-PCBs and a $(OH)_2$-PCB metabolite as well as two MSF-PCBs were detected in all species. The concentrations of unchanged PCB and its metabolites in liver and serum are listed in Table 1 and Table 2.

For CB101 metabolism, OH-pentaCB metabolites were present in liver of rats, hamsters and guinea pigs at higher levels than MSF-pentaCBs in all species except for mice, where MSF-pentaCBs were abundant as much as 3-fold in liver. Among hydroxylated metabolites, 3'-OH-2,4,5,2',5'-pentaCB was major in liver rather than $(OH)_2$-pentaCB product in all species except for guinea pigs, where 3-OH-2,4,5,2',5'-pentaCB was a major metabolite in liver. Mice and hamster produced a $(OH)_2$-pentaCB metabolite which was strongly retained in serum, although the level in liver was much lower. The OH-pentaCBs/CB101 ratio was the highest in serum of guinea pigs followed by rats. On the other hand, the MSF-pentaCB levels were the highest in mice, followed by in rats, and present at 1:1 ratio of 3'- and 4'-MSF-pentaCBs. The MSF-pentaCBs/CB101 ratios were 0.03-0.19 in liver and 0.04-0.50 in serum.

For CB132 metabolism, OH-hexaCB metabolites were present in liver of hamsters at higher levels than those of MSF-hexaCBs, whereas in rats, mice and guinea pigs, MSF-hexaCBs were predominant in liver. Among OH-hexaCB metabolites, 5'-OH-2,3,4,2',3',6'-hexaCB was a major metabolite in liver of rats, mice and guinea pigs, whereas a $(OH)_2$-hexaCB metabolite was predominant in liver and serum of hamsters. The OH-hexaCBs/CB132 ratios were 1.1 in liver and 4.9 in serum of hamster. On the other hand, MSF-hexaCBs were predominant in liver of mice and guinea pigs, where the 3'-MSF/4'-MSF-hexaCB ratio was about 3:1. The MSF-pentaCBs/CB101 ratios were 1.5 in liver and 1.0 in serum of guinea pigs.

Discussion

In our previous study, we have found that mono-ortho PCBs such as PCB118 were biotransformed in rats to hydroxylated metabolites, some of which had high affinity to blood[10]. In this study, OH-PCBs and MSF-PCBs derived from CB101 and CB132 were also distributed in blood at levels comparable to those of unchanged PCB. Klasson-Wehler *et al* have reported that CB101 was biotransformed to mono- and dihydroxylated metabolites in mink and mice[11]. Kuroki *et al* have also found that 2,5,3',4'-tetraCB (CB70) was biotransformed to 3- and 4-OH-tetraCBs and a $(OH)_2$-tetraCB in rats, which were distributed in all tissues[12]. The present study confirms that the dihydroxylation occurs commonly in the metabolism of penta- and hexaCBs with 2,5- or 2,3,6-substitution. Interestingly, a dihydroxylation product derived from PCB132 was highly formed in liver of hamsters and selectively retained in serum (Table 2). The potential effects of this accumulation in the blood are still unknown even though some indications of adverse effects have been reported[13]. It is further noted that hydroxylation of CB101 in guinea pigs occurred preferably in the 2,4,5-trichlrinated phenyl ring rather than in the 2',5'-dichlorinated phenyl ring to yield 3-OH-2,4,5,2',5'-pentaCB, although this metabolite was not retained in serum.

In vitro metabolism studies indicated that a marked species difference was observed for CB70 metabolism, in which 3-hydroxylation occurred by guinea pig liver microsomes (CYP2B18)[14], whereas 4-hydroxylation occurred by Golden Syrian hamster liver microsomes (CYP2A8 and CYP1A2)[15]. The species difference in this study would be due to the substrate specificity of the P450 isoforms for both PCBs. Regioselective hydroxylation of CB101 by guinea pigs and dihydroxylation of CB132 by hamsters would suggest a species-specific mechanism including very unique P450 isoforms.

Table 1. Tissue levels of pentaCB and its metabolites in animals after exposure of CB101

Species	*Tissue*	*Concentration (μg/g, wet weight)*				*Ratio***	
		PCB	OH-PCB*	$(OH)_2$-PBC	MSF-PCB**	OH/PCB	MSF/PCB
Rat	liver	0.87 ± 0.12	0.09 ± 0.03	0.07 ± 0.03	0.10 ± 0.03	0.15	0.12
	serum	0.08 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	1.16	0.37
Mouse	liver	1.11 ± 0.25	0.06 ± 0.02	0.01 ± 0.01	0.21 ± 0.07	0.06	0.19
	serum	0.36 ± 0.09	0.10 ± 0.04	0.16 ± 0.06	0.07 ± 0.02	0.71	0.19
Hamster	liver	0.68 ± 0.13	0.06 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.12	0.03
	serum	0.21 ± 0.06	0.04 ± 0.01	0.16 ± 0.05	<0.01	0.96	0.04
Guinea pig	liver	0.19 ± 0.05	0.16 ± 0.04	0.01 ± 0.00	0.03 ± 0.01	0.90	0.17
	serum	0.02 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	3.35	0.50

Values are expressed as mean ± S.E. for 3-6 animals. *OH-PCB;sum of three congeners, **SMSF-PCBs: sum of 3'- and 4'-MSF-pentaCBs, ***OH/PCB; Concentration ratio of SOH-PCBs relative to CB101; MSF/PCB; Concentration ratio of SMSF-PCBs to CB101

Table 2. Tissue levels of hexaCB and its metabolites in animals after exposure of CB132.

pecies	*Tissue*	*Concentration (μg/g, wet weight)*				*Ratio***	
		PCB	OH-PCB*	$(OH)_2$-PBC	MSF-PCB**	OH/PCB	MSF/PCB
Rat	liver	0.27 ± 0.06	0.13 ± 0.04	0.04 ± 0.01	0.20 ± 0.07	0.60	0.76
	serum	0.18 ± 0.08	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.34	0.06
Mouse	liver	1.13 ± 0.28	0.17 ± 0.05	0.01 ± 0.01	0.60 ± 0.21	0.16	0.53
	serum	0.23 ± 0.09	0.06 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.38	0.13
Hamster	liver	0.32 ± 0.11	0.12 ± 0.05	0.22 ± 0.08	0.03 ± 0.01	1.08	0.10
	serum	0.11 ± 004	0.05 ± 0.01	0.48 ± 0.17	<0.01	4.94	0.09
Guinea pig	liver	0.32 ± 0.09	0.08 ± 0.03	0.02 ± 0.01	0.47 ± 0.11	0.27	1.48
	serum	0.06 ± 0.03	0.03 ± 0.01	0.01 ± 0.00	0.06 ± 0.02	0.69	1.00

Values are expressed as mean ± S.E. for 3-6 animals. *OH-PCB: sum of three congeners, **MSF-PCBs: sum of 3'- and 4'-MSF-hexaCBs, ***OH/PCB, Concentration ratio ofSOH-hexaCBs relative to CB132; MSF/PCB, Concentration ratio of MSF-PCBs to CB132

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