2,3,3',4,4'-PENTACHLOROBIPHENYL AND 3,3',4,4'-TETRACHLOROBIPHENYL IN MICE: A COMPARISON OF METABOLISM

Klasson Wehler, E., Brandt, I.*, Jönsson, C.-J.*, Darnerud, P.O.**, Lindberg, C. and Bergman, Å..

Environmental Chemistry, Wallenberg Laboratory, Stockholm University, S-10691 Stockholm, Sweden. *Dept. of Pharmacology and Toxicology, Swedish University of Agricultural Sciences, Biomedicum, Box 573, S-75123 Uppsala, Sweden. **Dept. of Toxicology, Uppsala University, Biomedicum, Box 594, S-75124 Uppsala, Sweden.

ABSTRACT

The metabolism of two PCB congeners, 2,3,3',4,4'-pentachlorobiphenyl (PeCB) and 3,3',4,4'-tetrachlorobiphenyl (TCB), in mouse is compared. The TCB is readily metabolized and as much as 85% of the dose is excreted within five days. Several hydroxylated metabolites were found, both excreted and retained in tissues (liver and adipose tissue). However, only 15% of the dose was excreted within the same time from mice dosed with PeCB. In tissues, high concentrations of unmetabolized PeCB were found. In faeces, hydroxylated metabolites were detected.

KEYWORDS

PCB, 3,3',4,4'-Tetrachlorobiphenyl, 2,3,3',4,4'-pentachlorobiphenyl, metabolism, mouse, distribution.

INTRODUCTION

2,3,3',4,4'-Pentachlorobiphenyl (PeCB), is an abundant PCB congener in commercial PCB mixtures (1) and in environmental samples, eg cod and seal (2). PeCB, containing one chlorine-atom in an *ortho*-position (1-*ortho*-PCB), is structurally quite similar to the noxious 3,3',4,4'-tetrachlorobiphenyl (TCB), which belongs to the group of co-planar PCBs. These PCBs bind to the Ah-receptor and have toxic effects similar to those of TCDD. TCB induces the enzymes aryl hydrocarbon hydroxylase (AHH), ethoxy-resorufin O-deethylase (EROD) (3), has affinity for the Ah-receptor (4) and is teratogenic (5). PeCB can induce AHH and EROD and has a certain affinity for the Ah-receptor, although less effective than TCB (6). In a reproduction study in mink, 1-*ortho* PCBs, where PeCB is the major congener, were shown to have significant effects on the number of pups born (7). These results request further studies to be made on the biological effects and the metabolism of this compound.

The metabolism of TCB has been thoroughly investigated in rats (8,9). We have previously reported on the metabolism on TCB in the mouse (10) and will here report the distribution and metabolism of PeCB in comparison with TCB.

MATERIALS & METHODS

Chemicals: 2,3,3',4,4'-Pentachloro-(^{14}C)-biphenyl (spec. act 1.0 mCi/mmol) and 3,3',4,4'-tetra-(^{14}C)-chlorobiphenyl (1.0 mCi/mmol) were prepared as previously described (11). Solvents used for all analytical work were of pesticide grade (FSA).

Animals: In both studies, ten female mice (C57 Bl, 20 g) were given an oral dose of the ^{14}C-labelled PCB (10 mg/kg bw, PeCB: 0.6 µCi and TCB: 0.5 µCi, respectively) . Urine and faeces were collected daily for five days and the contents were measured for radioactivity. Liver and adipose tissue were removed and analyzed.

Extraction and Clean-up: The analytical procedures of the present study are briefly described below. The method follows in all major parts the procedure used for analysis of TCB metabolites in excreta and tissues in mice (10). Any differences of significance for the clean-up and analysis of PeCB-samples are stated.

The faecal samples from the TCB-dosed mice were pooled to contain faeces from day 1 and 2 (F_{1-2}) and faeces from day 3-5 (F_{3-5}). The corresponding samples from the PeCB dosed mice were pooled cage-wise but each day was treated separately. In both experiments livers and adipose tissue, respectively, were pooled from the ten mice.

All samples were measured for radioactivity before extraction. Homogenized tissues or faeces were extracted in a Soxhlet apparatus with chloroform:ethanol (1:1, 150 ml) for 5 hours. Extracts were measured for radioactivity and subsequently fractionated by GPC, performed on Bio-Beads S-X3 gel (10). The samples were eluted from the column with hexane:dichloromethane (1:1, v/v) and fractions (10 ml) were collected. The radioactivity and lipid contents of the fractions were determined and the fractions pooled to consist of a lipid fraction, a lipidfree metabolite fraction (also containing unmetabolized PCB) and a small intermediate fraction. The metabolite fraction was concentrated, phenolic type metabolites were derivatized with diazomethane and analyzed by GC/EC and GC/MS.

RESULTS AND DISCUSSION

The relative distribution of radiolabelled compounds in tissues and excreta from the mice given PeCB and TCB, respectively are shown in table 1. The distribution was also studied by tapesection autoradiography (data not shown). Urine from the PeCB-mice has not yet been analyzed, but the amount of radioactivity has been determined and is shown in table 1.

Table 1. Concentration (dpm/g fresh weight) of radioactivity in liver and adipose tissue from mice dosed with PeCB and TCB, respectively, and amounts (% of dose) of radioactivity in excreta after five days.

SAMPLE	PeCB	TCB
Liver[a]	24000	2600
Adip. tissue[a]	145000	18000
Urine (total)[b]	1	5
Faeces (total)[b]	14	80

[a] Concentration = dpm/g fresh weight.
[b] Amount = % of dose.

It is obvious from the data shown in table 1, that PeCB is excreted much slower than TCB. While 85 % of the dose was excreted from the TCB-dosed mice, only 15% of dose was excreted from the PeCB-dosed mice. The amount of radiolabelled compound in tissues was also much higher in the PeCB-dosed mice than in the TCB-mice. The high concentration of radioactivity in tissues of PeCB-dosed mice as compared to TCB-dosed mice, indicates that PeCB to a higher extent than TCB is retained in the body. Labelled compounds from adipose tissue from both TCB- and PeCB-treated mice were almost quantitatively extracted. In liver, >95% of the TCB-de ,ed radioactivity and 80% of the PeCB-derived radioactivity were extractable in the Soxhlet-extraction, indicating a certain degree of covalent binding at least in the PeCB-treated mice. Most of the extractable PeCB-derived radioactivity (92% and 98%, in liver and adipose tissue, respectively) eluted in the GPC metabolite fraction. The corresponding values in the metabolite fraction for

the TCB-treated mice were 66% and 84%, respectively, for liver and adipose tissue, while the rest eluted in the lipid fraction. In faeces, an average of 60% of the radioactivity was extractable with the Soxhlet-extraction method. In comparison, more labelled material was extractable from faeces the first day, indicating the presence of unabsorbed PCB. Towards the end of the experiment, an increasing proportion of the extractable radioactivity eluted together with the lipids (Table 2), indicating the presence of lipophilic conjugates formed from PeCB. Similar results in faecal samples, as well as in liver and adipose tissue, have previously been reported for TCB (10).

TABLE 2. The relative distribution of radioactivity in the pooled GPC-fractions from faecal samples from PeCB- and TCB-dosed mice.

SAMPLE	% OF DOSE	GPC FRACTIONS LIPID	INTER-MEDIATE	METABOLITE
Day 1	6	2	1	97
Day 2	3	12	7	81
Day 3	2	13	7	80
Day 4	2	25	27	48
Day 5	1	29	22	49
TCB:				
Day 1-2	61	25	13	62
Day 3-5	12	12	57	25

By comparison with authentic reference compounds (GC/MS), a number of metabolites of the samples from the TCB mice were determined (10). The identified metabolites of TCB were 5-hydroxy-3,3',4,4'-tetrachlorobiphenyl (Urine (U), Adipose tissue (A), Faeces (F)), 4-hydroxy-3,3',4,5'-tetrachlorobiphenyl (F, Liver (L), U, A), 6-hydroxy-3,3',4,4'-tetrachlorobiphenyl (F, U, A) and 2-hydroxy-3,3',4,4'-tetrachlorobiphenyl (U, traces). Also two dihydroxy-tetrachlorobiphenyls, one dihydroxy-trichlorobiphenyl and one hydroxy-trichlorobiphenyl were present as indicated by GC/MS. Unmetabolized TCB was present in all samples (10).

GC/MS analysis of the samples from mice dosed with PeCB indicated several hydroxylated metabolites in the faecal samples. Two of those have been identified, by comparison with authentic reference compounds by GC/MS, as 5-hydroxy-2',3,3',4,4'-pentachlorobiphenyl and 2-hydroxy-2',3,3',4,4'-pentachlorobiphenyl. In adipose tissue only unmetabolized PeCB was found, while in liver the presence of small amounts of two hydroxy-pentachlorobiphenyls was indicated. Additional analysis (GC/MS) is in progress and will be reported at the presentation of this paper.

From the data in table 1, it is obvious that PeCB is metabolized much slower than TCB. This may be due to a higher chemical stability but also to the fact that TCB and PeCB are partly metabolized by different enzymes (6).

ACKNOWLEDGEMENT

Economic support was given by the Swedish Environmental Protection Agency.

REFERENCES

1. Schulz, D.E., Petrick, G. and Duinker, J.C. (1989) Complete characterization of polychlorinated biphenyl congeners in commercial Arochlor and Chlophen mixtures by multidimensional gas chromatography-electron capture detection. Environ. Sci Technol., 23, 852-859.

2. Muir, D.C.G., Norstrom, R.J. and Simon, M. (1988). Organochlorine contaminants in arctic marine food chains: Accumulation of specific polychlorinated biphenyls and Chlordane-related compounds. Environ. Sci. Technol., 22, 1071-1079.

3. Brunström, B. and Andersson, L. (1988). Toxicity and 7-ethoxyresorufin O-deethylase-inducing potency of coplanar polychlorinated biphenyls. Arch. Toxicol., 62, 263-266.

4. Bandiera, S., Safe, S. and Okey, A.B. (1982). Binding of polychlorinated biphenyls classified as either phenobarbitone-, 3-methylcholantrene- or mixed-type inducers to cytosolic *Ah* receptor. Chem. Biol. Interact., 259-277.

5. d'Argy, R., Dencker, L., Klasson Wehler, E., Bergman, Å., Darnerud, P.O. and Brandt, I. (1987). 3,3',*4,4'*-Tetrachlorobiphenyl in pregnant mice: embryotoxicity, teratogenicity, and toxic effects on the cultured embryonic thymus. Pharmacol. Toxicol., 61, 53-57.

6. Safe, S. (1984). Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): Biochemistry, toxicology and mechanisms of action. In CRC Critical reviews in toxicology, 13, 319-385.

7. Olsson, M., Bergman, Å, Jenson, S. and Kihlström, J.-E. (1990). Effects of various fractions of PCB on mink reproduction. Preliminary results from experimental studies within the swedish seal project. Submitted to "Dioxin '90".

8. Yoshimura, H., Yonemoto, Y., Yamada, H., Koga, N., Oguri, K. and Saeki, S. (1987). Metabolism *in vivo* of 3,3',*4,4'*-tetrachlorobiphenyl and toxicological assessment of the metabolites in rats. Xenobiotica, 17, 897-910.

9. Koga, N., Beppu, M., Ishida, C. and Yoshimura, H. (1989). Further studies on metabolism *in vivo* of 3,3',*4,4'*-tetrachlorobiphenyls in rats: identification of minor metabolites in rat faeces. Xenobiotika, 19, 1307-1318.

10. Klasson Wehler, E., Bergman, Å., Brandt, I., Darnerud, P.O., and Wachtmeister, C.A. (1989). 3,3',*4,4'*-Tetrachlorobiphenyl: Excretion and tissue retention of hydroxylated metabolites in the mouse. Drug Metab. Dispos., 17, 441-448.

11. Bergman, Å, Nilsson, A., Riego, J and Öm, U. (1990).Synthesis of ^{14}C-*labelled and unlabelled coplanar* biphenyls (PCBs). Acta Chem. Scand., in press.