Identification of the parent compounds to selectively retained hydroxylated PCB metabolites in rat blood plasma

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Introduction

Certain hydroxylated PCB metabolites have been shown to be retained in blood plasma due to their affinity for transthyretin (TTR), a thyroxin (T4) transporting protein [1,2]. The retained OH-PCB congeners have structural similarities with T4; a hydroxy group in the *para* or *meta* position with chlorine substituents on the adjacent carbons to the hydroxy group. PCB forms hydroxylated metabolites by cytochrome P450 mediated oxidation to arene oxides and subsequent rearrangement to hydroxylated metabolites [3,4] or by direct hydroxylation [5,6]. Since, each parent PCB can be metabolized to several OH-PCBs, metabolism may result in complex mixtures in environmentally exposed species.

The aim of the present study is to determine the parent PCBs to the major OH-PCBs that are present in plasma from humans and wildlife [1]. Based on the knowledge on how hydroxylated metabolites are formed [7] and the structure of the retained OH-PCBs [1], seven PCB congeners were chosen (Table 1). Individual PCB congeners were administered i.v. to rats and blood samples were collected and analyzed at 20 days after exposure.

Experimental methods

Chemicals

n-Hexane pesticide grade was purchased from Fisher scientific (UK), methyl *tert*-butyl ether (MTBE) was purchased from Ratborn (Scotland), dimethylsulfoxide (DMSO) from Sigma Chemical Company (USA) and 2-propanol from LabKemi (Stockholm, Sweden). Sodium chloride, potassium chloride, hydrochloric acid and sulfuric acid was purchased from Merck (Darmstadt, Germany) and potassium hydroxide from Eka Nobel (Bohus, Sweden). Diazomethane, synthesised as described by Fieser and Fieser [8] were used for derivatisation of phenolic compounds. Internal standards used were 2,3,3',4,4',5,5'-heptaCB (CB189) and 4-OH-2,3,3',4',5,5',6-heptaCB (4-OH-CB193).
Reference standards used for identification of OH-PCB metabolites formed were 4'-MeO-2,3,3',4,5'-pentaCB (4'-MeO-CB108), 4'-MeO-2,3',4,5,5'-pentaCB (4'-MeO-CB120), 4'-MeO-2,2',3,3',4,5'-hexaCB (4'-MeO-CB130), 3'-MeO-2,2',3,4,4',5'-hexaCB (3'-MeO-

CB138), 4-MeO-2,2',3,4',5,5'-hexaCB (4-MeO-CB146), 3-MeO-2,2',4,4',5,5'-hexaCB (3-MeO-CB153), 4'-MeO-2,3,3',4,5,5'-hexaCB (4'-MeO-CB159), 4-MeO-2,3,3',4',5,5'-hexaCB (4-MeO-CB162), 4'-MeO-2,2',3,3',4,5,5'-heptaCB (4'-MeO-CB172), 4'-MeO-2,2',3,3',5,5',6-heptaCB (4'-MeO-CB178), 3'-MeO-2,2',3,4,4',5,5'-heptaCB (3'-MeO-CB180), 3'-MeO-2,2',3,4',5,5',6-heptaCB (3'-MeO-CB187), 4-MeO-2,2',3,4',5,5',6-heptaCB (4-MeO-CB187), 3'-MeO-2,2',3,4',5,6,6'-hexaCB (3'-MeO-CB188).

Gas chromatography

Gas chromatography with electron capture detector (GC-ECD) was preformed on a Varian 3400 gas chromatograph using a low-polar DB-5 capillary column (30 m x 0.25 mm i.d., 0.25 μm phase thickness) (J&W Scientific, Folsom, CA, USA) and a polar SB-2331 (60 m x 0.2 mm i.d., 0.2 μm phase thickness) (Supelco, Bellefonte, PA, USA). The temperature program used for the DB-5 column was 80°C for 2 min and then 10°C/min to 300°C that was kept for 10 min, for the SP-2331 column was 80°C for 2 min and then 20°C/min to 150°C, 8°C/min to 280°C that was kept for 5min. The injector temperature was 250°C and detector temperature 360°C.

Animal section

Groups of five Wistar male rats (180-210 g) were dosed i.v. with an individual PCB congener (Table 1) (3.0 μmole/kg b.w.) dissolved in DMSO (100 μl). Blood was sampled 20 days after dosage and the plasma was isolated by centrifugation and stored frozen until analysis (-20°C).

Table 1. Individual PCB congeners used for determination of OH-PCB *in vivo*

Structure	IUPAC	Purity (% w/w)
2,3,3',4,4'-pentaCB	CB105	98
2,3',4,4',5-pentaCB	CB118	97
2,2',3,4,4',5'-hexaCB	CB138	100
2,2',4,4',5,5'-hexaCB	CB153	100
2,3,3',4,4',5'-hexaCB	CB157	99
2,2',3,4,4',5',6-heptaCB	CB183	100
2,2',3,4',5,5',6-heptaCB	CB187	84

Analytical procedure

The method used for extraction and work-up of the plasma samples is described elsewhere [9]. Identification of the methyl ether derivatives of OH-PCB congeners formed were made by comparison of relative retention times (RRT) on DB-5 and SP-2331 GC capillary columns. Difference in RRT of less than 0.0005 on both columns was considered acceptable for identification. Identified OH-PCB metabolites and mother PCBs were then quantified on the DB-5 column.

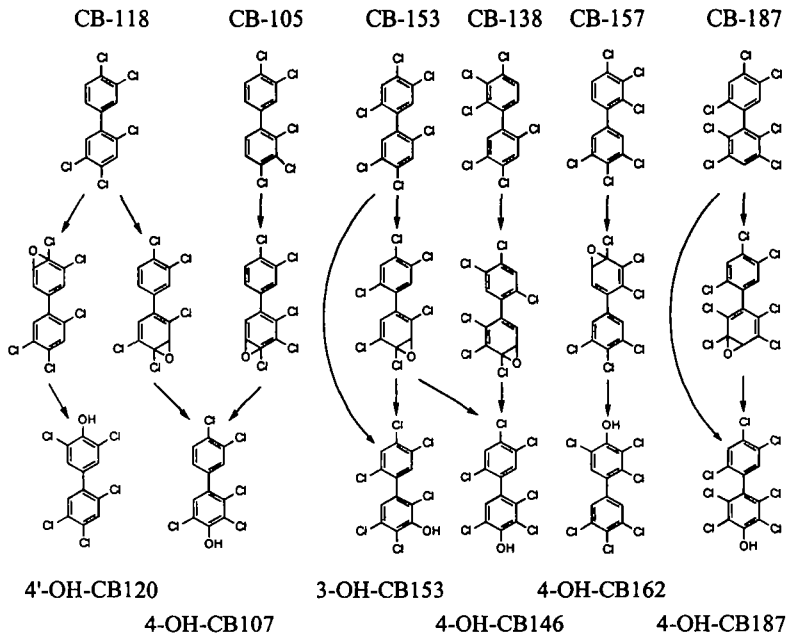
Results and discussion

Six of the seven studied PCB congeners were shown to give metabolites that were present in the plasma and corresponded to the OH-PCBs present in environmental samples [1]. No metabolites were identified for CB183. The relative retention times (RRT) for metabolites formed from each parent CB on the two capillary column used are given in Table 2. The mechanism for the formation of OH-PCB metabolites involves cytochrome P450 mediated formation of arene oxides and subsequent rearrangement to hydroxylated metabolites or direct hydroxylation. The rearrangement of arene oxides often involves a 1,2-shift in which the

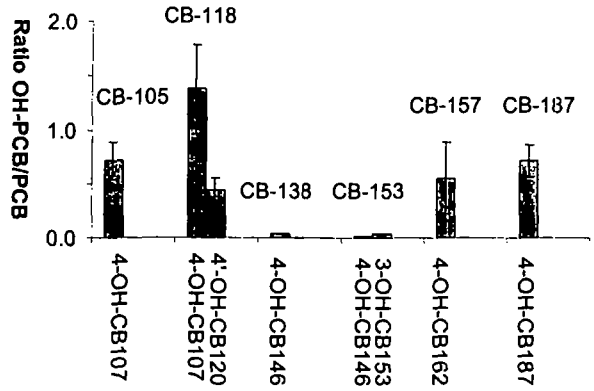
chlorine substitution pattern is changed [7]. A change in chlorine substitution pattern thus indicates formation of an arene oxide intermediate. In figure 1, possible mechanisms for the formation of the identified OH-PCBs are shown. The ratios of formed OH-PCB to parent PCB for the studied PCB congeners are given in figure 2. It is worth noting that, with the same molar dose given, the ratio are higher of OH-PCBs formed from CB105, CB118, CB157 and CB187 than the more persistent CB138 and CB153.

Table 2. Identified OH-PCB metabolites of respective parent PCB and relative retention (RRT) on the columns used

Parent PCB	Metabolite	DB-5 column (RRT)	SP-2331 column (RRT)
CB105	4-OH-CB107	0.9043	0.8847
CB118	4-OH-CB107	0.9043	0.8847
	4'-OH-CB120	0.8814	0.8316
CB-138	4-OH-CB146	0.9170	0.8618
CB153	4-OH-CB146	0.9170	0.8618
	3-OH-CB153	0.9155	0.8591
CB157	4-OH-CB162	0.9650	0.9566
CB187	4-OH-CB187	0.9552	0.8904



Figur 1. OH-PCBs identified in rat plasma after exposure to individual PCB congeners. Possible metabolic routs are indicated.



Figur 2. Ratio of OH-PCB to parent PCB for the metabolites formed, (95% C.I. for 4 to 5 replicate samples). Parent PCBs are indicated in figure

Acknowledgement

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