DISTRIBUTION OF CHIRAL PCBs IN SELECTED TISSUES IN THE LABORATORY RAT

Hans-Joachim Lehmler[1], Izabela Kania-Korwel[1], Jimmy K. Avants[3], Keri C. Hornbuckle[4], Wieslaw W. Sulkowski[2], Larry W. Robertson[1], Wayne A. Garrison[3]

[1]Department of Occupational and Environmental Health, University of Iowa, Iowa City, U.S.A.
[2]Department of Environmental Chemistry and Technology, University of Silesia, Katowice, Poland
[3]U.S. Environmental Protection Agency, National Exposure Research Laboratory, Athens, Georgia, USA
[4]Department of Civil and Environmental Engineering, University of Iowa, Iowa City, U.S.A.

Introduction

Polychlorinated biphenyls (PCBs) were manufactured for a large number of technical applications including for use in transformers and capacitors[1,2]. The widespread commercial utilization of PCBs and their persistence in the environment have resulted in their worldwide distribution. Physicochemical characteristics, such as lipophilicity and stability towards biological and thermal degradation, have resulted in their accumulation in the food chain, raising concerns about human health effects. Animal and epidemiological studies have implicated PCBs in a number of human disease processes, such as carcinogenesis and atherosclerosis. However, mechanisms of PCB toxicity are still poorly understood, partly because technical PCB products contain a complex mixture of the possible 209 PCB derivatives or congeners. One of the most intriguing, but frequently overlooked, aspects of PCB toxicity is related to the existence of chiral PCB congeners, possessing at least three ortho (to the biphenyl bridge) chlorine atoms[3]. Racemic PCBs in this group have been implicated in developmental and neurotoxic effects[1,2]. Two studies with individual congeners have shown that the (+)-enantiomer of PCB 84[4] and 139[5] are selectively enriched in tissues, e.g. the liver, of laboratory animals. However, nothing is currently known about the distribution and enrichment of chiral PCBs after administration of a complex PCB mixture to laboratory animals such as the rat. The present study investigates the enantiomeric fraction of PCBs 91, 95 and 149 in male rats after administration of

(a) Aroclor 1254 and (b) an environmental mixture obtained from soil contaminated with Chlorofen, a Polish PCB mixture[6].

Methods and Materials

Soil and tissues extraction The soil PCB mixture was a hexane-acetone extract from soil collected at the Chlorofen manufacturing site[7]. One-month-old male rats (Sprague Dawley, Harlan) were randomly divided into three groups and injected i.p. with a single dose of the environmental PCB mixture (soil extract, 20 mg/kg b.w., 0.05 mmol/kg b.w.; n = 3) or Aroclor 1254 (16 mg/kg b.w., 0.05 mmol/kg b.w.; n = 4). Control animals received the vehicle alone (corn oil, n = 4). Rats were euthanized on day 7. Blood was collected by cardiac puncture and serum was prepared by centrifugation. Lung, liver, spleen, kidney, brain, skin and adipose tissue were excised *en bloc*. The extraction of PCBs from tissues was performed as described previously by our laboratory[4]. The PCB extraction from blood was described by Gill and co-workers[8]. Internal (PCBs 30 and 204) and recovery (PCBs 14, 65 and 166) standards were used[9].

Enantiomer Fraction determinations Analysis of Aroclor 1254, Chlorofen, soil and tissue extracts for the PCB enantiomers was by chiral capillary gas chromatography, primarily with electron capture detection (ECD) with confirmation by mass spectrometry detection when possible. The GC-ECD system was a Hewlett-Packard 5890; the GC-MS system consisted of a Hewlett-Packard 6890 GC with a 5973 mass selective detector[10]. A standard mixture of 9 chiral PCBs was analyzed before and after every 10 samples.

The column found to provide the best separation of enantiomers for the most chiral PCB congeners was a Chiralsil-Dex column (Chrompack, Raritan, NJ, USA), 25 m x 0.25 mm id x 0.25 μm film thickness with immobilized chiral phase of permethyl 2,3,6-tri-O-methyl β-cyclodextrin on a polysiloxane backbone stationary phase. Conditions used were: column temperature 100° to 150° at a rate of 10°/min, 150 to 200° at 0.5°/min, then hold for 10 min; injection, splitless at 250°; carrier gas, helium at a flow of 1.0 mL/min (for both ECD and MS detectors). Under these conditions, the ECD provided a much more stable background with less noise than the MS, at least for the tissue samples, and became the primary detector. The MS detector was operated in the selected ion mode, monitoring ions 326 and 328 for PCBs 91 and 95 and ions 360 and 362 for PCB 149. Electron voltage was 70 eV. The EF (enantiomer fraction) for standards and samples was calculated as EF = area 1^{st} peak / area (peak 1^{st} + peak 2^{nd}).

Where appropriate the comparison of enantiomeric fractions was performed using analysis of variance (ANOVA with Tukey post-hoc test). The statistical software SYSTAT 8.0 (Systat Software, Inc., Point Richmond, CA, USA) was used for the preliminary statistical analysis in this study.

Results

The EFs of PCBs 91, 95 and 149 were determined using a Chiralsil-Dex column. The EFs of these compounds in Aroclor 1254 and in tissues from Aroclor-treated rats are shown in Table 1. The EFs of Chlorofen, soil extract and soil extract-treated tissues are shown in Table 2. PCBs 95 and 149 are racemic in Aroclor 1254, Chlorofen and the soil extract. PCB 91 is racemic in Aroclor 1254 but was not detectable in Chlorofen. However, PCB 91 was detected in the soil extract and shows a slight enrichment of the second eluting peak. This is probably due to enantioselective microbial degradation in the soil[11].

In the Aroclor treatment group PCB 91 was found in two adipose and one skin samples. All three samples show an enrichment of the second eluting peak. In the soil extract group, PCB 91 was only detected in one adipose sample. Interestingly the enrichment of PCB 91 in this sample is exactly opposite to all three samples in the Aroclor group as well as the EF of the parent mixture. PCB 95 was detected in approximately half of tissue samples in the Aroclor group with skin and adipose tissue showing an enrichment of the first eluting enantiomer. A trend to an enrichment of this enantiomer can also be seen for the other tissues. PCB 95 was only detected in six samples in the soil-extract treatment group. Similar to the Aroclor group, the first eluting congener is enriched in the two adipose tissue samples. No such enrichment is apparent for the other samples.

PCB 149 was detected in most tissues, thus allowing a statistical comparison of the EF of the parent mixture versus the EF of several tissues from the same treatment group. The livers of Aroclor-treated animals show a significant enrichment of the second eluting peak (Table 1), while whole blood shows an enrichment of the first eluting peak. PCB 149 appears to be racemic in all other tissues in this treatment group. PCB 149 appears to be racemic in all tissues from soil extract-treated animals (Table 2).

A statistical comparison of the EF values among some tissues was made for PCB 149 in the Aroclor treatment group. Table 3 summarizes the results of this analysis. The EF of PCB 149 in whole blood is significantly different from kidney, liver, skin, adipose and spleen. As shown in Table 1, the same trend can be observed for heart and brain, whereas the lung and serum sample show an EF similar to the one found in whole blood. There is also a significant difference

between the EF of PCB 149 in the kidney and the liver. In contrast, no differences in the EFs among the tissues of the soil extract treated animals were observed.

Table 1: Enantiomeric fraction of PCB 91, 95 and 149 in tissues and blood from Aroclor 1254-treated animals (data in parentheses are from MS determination, n = 3 if not stated otherwise).

PCB Mixture or Tissue	Enantiomeric Fraction		
	PCB 91	PCB 95	PCB 149
Aroclor 1254	0.52	0.50	0.53
Adipose	0.10, n=2	0.68, n=2 (0.68, n=2)	0.47±0.02 (0.42±0.04)
Brain	n.d.	n.d.	0.55, n=2
Heart	n.d.	0.57, n=1	0.56, n=2
Kidney	n.d.	0.52, n=2	0.53±0.01 (0.52, n=1)
Liver	n.d.	0.58, n=1 (0.51, n=1)	0.45±0.03* (0.47, n=1)
Lung	n.d.	0.58, n=2	0.66, n=1
Skin	0.28, n=1	0.82, n=1 (0.77, n=1)	0.49±0.01
Spleen	n.d.	0.59, n=2	0.50±0.03 (0.46, n=2)
Whole blood	n.d.	n.d.	0.65±0.04* (0.52, n=1)
Serum	n.d.	n.d.	0.62, n=1

* significantly different from Aroclor 1254 ($P < 0.05$)
n.d. = not detected

Table 2: Enantiomeric fraction of PCB 91, 95 and 149 in tissues and blood from soil extract-treated animals (data in parentheses are from MS determination, n = 3 if not stated otherwise).

PCB Mixture or Tissue	Enantiomeric Fraction		
	PCB 91	PCB 95	PCB 149
Chlorofen	n.d.	0.50	0.50
soil extract	0.46	0.50	0.50
Adipose	0.73, n=1 (0.62, n=1)	0.66, n=2 (0.53, n=1)	0.50±0.04 (0.48, n=2)
Brain	n.d.	n.d.	n.d.
Heart	n.d.	n.d.	n.d. (0.53, n=1)
Kidney	n.d.	n.d.	0.52±0.04 (0.55, n=2)
Liver	n.d.	0.47, n=1	0.52±0.04 (0.54, n=2)
Lung	n.d.	n.d.	0.47, n=2
Skin	n.d.	0.54, n=1 (0.41, n=1)	0.49±0.04
Spleen	n.d.	0.51, n=2 (0.56, n=1)	0.54±0.03 (0.54±0.04)
Whole Blood	n.d.	n.d.	0.52, n=1
Serum	n.d.	n.d.	0.06, n=2

n.d. = not detected

Table 3. Differences in the EF of PCB 149 between tissues in the Aroclor 1254 treatment group (* P < 0.05, ** P < 0.01, *** P < 0.001).

Tissue	Aroclor 1254	Adipose	Whole blood	Kidney	Liver	Skin	Spleen
Aroclor 1254	-		***		*		
Adipose		-	***				
Whole blood			-	**	***	***	***
Kidney				-	*		
Liver					-		
Skin						-	
Spleen							-

Discussion

Enantiomeric fractions for several chiral PCB congeners from a variety of environmental matrices including some human samples have been reported. Much less is known about the enrichment of chiral PCBs in laboratory animals. Our laboratory has previously reported that (+)-PCB 84 is enriched in several tissues in mice[4] and that (+)-PCB 139 is enriched in the liver of rats[5]. It is currently unclear which processes are responsible for the enrichment of these two congeners, but enantioselective metabolism and enantioselective binding to proteins have been implicated[3-5]. The present study was designed to answer the question of whether chiral PCB congeners show an enantiomeric enrichment after the administration of a complex technical (Aroclor 1254) or an environmental mixture (soil extract) to laboratory animals. We herein present the preliminary results of our chiral analysis of PCBs 91, 95 and 149.

PCBs 91 and 95 were detected in only a few tissues, but in most cases an enrichment of one enantiomer was observed. Both congeners are subject to enantioselective processes such as metabolism or binding to proteins *in vivo*. However, because these congeners were only detected in few tissues, we can not hypothesize about the chiral processes responsible for the observed enrichment. PCB 149 was detected in most tissues and, therefore, provides more information in understanding the chiral processes involved in its enrichment in certain tissues.

In the Aroclor treatment group PCB 149 shows an enrichment of the second eluting enantiomer in the liver and an enrichment of the first eluting enantiomer in whole blood. In this group the enantiomeric fraction in whole blood is also

significantly different from all other tissues. No enrichment of PCB 149 was observed in the soil extract treatment group. These findings may result from differences in the induction of CYP1A and CYP2B in the liver of rats treated with Aroclor or soil extract (these results will be presented elsewhere during this meeting[12]). We found that in the Aroclor 1254-treated rats, hepatic ethoxyresorufin-*O*-dealkylase (CYP1A) activity was 28.7 times higher compared to that in soil extract treated animals, while in the soil extract-treated animals pentoxyresorufin-*O*-dealkylase (CYP2B) activity was 4.5 times higher than in Aroclor 1254-treated rats. These findings suggest that the second eluting PCB 149 enantiomer may bind selectively to CYP1A, which is drastically induced in the liver of Aroclor treated animals. Because the liver is a highly perfused organ, this enantiomer is selectively removed from the blood stream, thus resulting in an enrichment of the first eluting enantiomer in whole blood. However, other chiral processes such as metabolism and plasma protein binding may also contribute to the observed enrichment of PCB 149.

Our results provide additional evidence that chiral PCB congeners are subject to chiral processes *in vivo* which cause their enrichment in certain tissues. It is currently unknown how the developmental and neurotoxic effects of chiral PCB congeners are altered by such enrichment and further studies are warranted.

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