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Distribution of Chiral PCBs in Selected Tissues in the Laboratory Rat

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Abstract

The enantiomeric enrichment of polychlorinated biphenyl (PCB) atropisomers has been reported in both wildlife and in humans. The biological processes causing this enrichment are only poorly investigated, a fact that limits the use of enantiomeric fractions (EFs) as a tool to study various processes of environmental relevance. To further understand these enantioselective processes, this study investigates the tissue distribution and EFs of some PCB atropisomers after administration of PCB mixtures to immature male Sprague-Dawley rats. The mixtures selected for this study, Aroclor 1254 and an environmental mixture extracted from Chlorofen-contaminated soil, are qualitatively different and are known to induce different groups of hepatic enzymes. Animals were sacrificed six days after dosing, PCBs were extracted, and, whenever possible, the EFs of PCBs 84, 91, 95, 149, 174 and 176 were determined by chiral gas chromatography. The EFs of PCB 95 (adipose tissue, liver and skin) and PCB 149 (adipose tissue, liver, skin and blood) in tissues from Aroclor 1254-treated animals differed significantly from EFs in the Aroclor standard, while only EFs of PCB 95 (blood) and PCB 174 (adipose tissue) in tissues from soil extract-treated animals were different from those of the Chlorofen soil extract. PCB 149 in tissues from soil extract-treated animals underwent no statistically significant enantiomeric enrichment. These differences in the EFs clearly suggest that the enantioselective enrichment of PCB atropisomers may correlate with exposure history, and with the induction of hepatic enzymes, and that EFs may be useful chemical markers of physiologic and biochemical changes following exposure to PCBs.

Keywords

polychlorinated biphenyls; Chlorofen; Aroclor 1254; ortho-substituted PCB; chiral PCB

INTRODUCTION

The large-scale manufacture of polychlorinated biphenyls (PCBs) by the chlorination of biphenyl produced products that were used in a number of technical applications, including in transformers and capacitors (1,2). As a result of the manufacturing process and environmental

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fate processes, commercial and environmental PCB mixtures are complex, consisting of over 150 of the possible 209 PCB congeners. The widespread commercial use 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 epidemiologic studies implicate PCBs in a number of human disease processes, such as carcinogenesis and arteriosclerosis (1). Some ortho-substituted PCB congeners, especially chiral PCB congeners such as PCB 95 and 149, are known to cause neurotoxicity and developmental toxicity (3–5).

Many of the PCB congeners with three or four ortho chlorine substituents are chiral and exist as two stable rotational isomers (or enantiomers) that are non-superimposable mirror images of each other (6). These rotational isomers are called atropisomers. The PCB atropisomers may exhibit different biological effects or undergo enantiomeric enrichment *in vivo*. For example, the atropisomers of PCB 84 demonstrate differential effects on some biological measures associated with neurotoxicity (7). The (+)- and (–)-enantiomers of PCB 84 had a significantly different effect on protein kinase C translocation as determined by [³H]-phorbol ester binding in cerebellar granule cells, with (–)-PCB 84 being slightly more potent; however, (+)- and (–)-PCB 84 did not have a significantly different effect on calcium regulation in microsomes isolated from adult rat cerebellum, another important neurochemical measure. PCB atropisomers have also been shown to have differential potencies in their abilities to induce various liver enzymes in chick embryo hepatocytes (8) and rats (9,10).

Enantiomeric enrichment of several chiral PCB congeners from biological environmental samples in seabirds (11), whales (12,13), dolphins (13,14), porpoises (15) and fish species (16–18) have been reported. From a human health point of view, the enantiomeric enrichment of several chiral PCBs (PCB 91, 95, 135, 149, 174, 176, and 183) found in samples of blubber from bowhead whales is of particular interest (12), because this blubber is a part of the diet of Inuit people in northern Canada. Thus, humans can be exposed to enantiomerically enriched PCBs mixtures via the diet. However, human diet typically contains racemic or near-racemic PCB signatures (enantiomeric fraction ≈ 0.5) (19,20).

Much less is known about the enrichment of chiral PCBs occurring in laboratory animals and humans. Lehmler et al. (21) and Püttmann et al. (10) respectively showed that the (+)-enantiomer of PCB 84 and 139 are selectively enriched in tissues, e.g. the liver, of laboratory animals. Larsson et al. (22) found that chiral methylsulfone PCB metabolites are enantiomerically enriched in liver, lung and adipose tissue of rats exposed to Clophen A50, a technical PCB mixture. Glausch and coworkers reported the enantioselective enrichment of PCB 132 in human breast milk (23,24). A higher abundance of the second eluted peak of PCB 132 was observed, with enantiomeric fractions (EFs) ranging from 0.286 to 0.474. Recently Chu et al. (25) reported an enantiomeric enrichment of PCBs 95, 132 and 149 in liver, but not brain, muscle or kidney samples from eleven human donors. The EFs of PCB 95, 132 and 149 in the liver ranged from 0.51 to 0.75, 0.32 to 0.49 and 0.50 to 0.58, respectively. Harrad and coworkers (20) found that PCB 95 signatures in human feces are typically racemic ($n = 8$) although an enrichment of the second eluting PCB 95 atropisomer was observed in two samples.

This present study is the first investigation of the enantiomeric enrichment of several chiral PCB congeners in the laboratory rat after exposure to complex PCB mixtures (i.e. Aroclor 1254 and a soil extract of Chlorofen). Our previous work has shown that exposure to these two PCB mixtures causes the induction of hepatic enzymes in laboratory animals (26). Specifically, we have shown that Aroclor 1254-treated rats have elevated EROD (CYP 1A) activity and rats treated with a soil extract of Chlorofen have elevated PROD (CYP 2B) activity. The present

study correlates these differences in the PCB-initiated induction of hepatic enzymes with changes in the enantiomeric fractions of PCBs in an attempt to define the origin of the enantiomeric enrichment of PCBs in laboratory animals and, ultimately, in humans.

EXPERIMENTAL SECTION

PCB mixtures

Aroclor 1254 and an environmental PCB mixture were selected for this animal study. The environmental PCB mixture was prepared by the extraction of soil contaminated with Chlorofen as described elsewhere (27,28). The congener profiles of both mixtures was presented in detail elsewhere (26). Individual PCB standards for the chiral PCB analysis were purchased from Accustandard (New Haven, CT).

Animal treatment

One-month-old male Sprague Dawley rats (Harlan, Indianapolis, IN) were randomly divided into three groups. Two groups were 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) as previously described (26). Control animals received an i.p. injection of 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*, wrapped in aluminum foil, and stored at -20°C until they were used for the PCB extraction.

Tissues extraction

We previously reported a detailed description of the extraction procedures (21,26) and the analysis of the PCB congener profiles (26). In short, 0.5 g of a randomly selected tissue sample was homogenized with 11 g sodium sulfate. The recovery standard solution consisting of PCB 14, 65 and 166 (500 μL ; 504 $\mu\text{g/L}$ in hexane) was added and the sample was Soxhlet-extracted for 8 hours using hexane–acetone (1:1 v/v). The extract was concentrated using a rotary evaporator. Lipid clean-up was performed with an aliquot of the concentrated PCB extract by eluting it through a column containing 1 g sodium sulfate, 6 g Florisil (100–200 mesh, activated at 260°C for approximately 16 hours) and 1 g sodium sulfate. An internal standard solution consisting of PCB 30 and 204 (100 μL ; 100.8 $\mu\text{g/L}$ in hexane) was added (29). Sulfur clean-up was performed by shaking a 2 mL aliquot of the PCB extract with 2 mL 2-propanol and 2 mL tetrabutylammonium sulfite, adding 8 mL nano-pure water, and reshaking the sample. The organic extract was removed, mixed with 2 mL concentrated sulfuric acid, and allowed to stand for at least 24 hours before the PCB analysis.

Whole blood and serum samples were extracted three times with hexane-dichloromethane (9:1 v/v), protein was precipitated with acetic acid and the recovery standard was added (50 μL ; PCB 14, 65, 166; 504 $\mu\text{g/L}$ each in hexane) (26). The extracts were concentrated and cleaned as described above. The internal standard solution (PCB 30 and 204; 100 μL ; 100.8 $\mu\text{g/L}$ each in hexane) was added before the PCB analysis.

Quality assurance

Blank samples were extracted with each set of samples. The average PCB content in these sample sets was 44 ± 15 ng for tissues and 4.1 ± 1.2 ng for blood and serum. Total PCB levels in control animals ranged from 0.01 to 0.61 $\mu\text{g/g}$ and were typically 10-fold lower compared to total PCB tissue levels. The method detection limit was 45.7 ng/g and the minimum level of detection was 158 ng/g. Recovery rates from tissue samples were $91 \pm 11\%$ and $89 \pm 10\%$ for PCBs 14 and 166, respectively. The recovery rates for PCB 65 were not determined because its recovery was affected by the Florisil column. Recovery rates from blood samples were 80

$\pm 11\%$, $83 \pm 11\%$ and $75 \pm 12\%$ for PCBs 14, 65 and 166, respectively. Samples with recoveries below 80% were re-extracted. Corrections of total concentration were made for recoveries < 100%.

Enantiomer Fraction Measurements

The PCB atropisomers of Aroclor 1254, Chlorofen, and soil and tissue extracts were analyzed by using a Hewlett-Packard 5890 GC-ECD system (30). The column found to provide the best separation of atropisomers for the most chiral PCB congeners was a ChiralsilDex column (Chrompack, Raritan, NJ, USA), 25 m $\times$ 0.25 mm id $\times$ 0.25 μ m film thickness, with an immobilized chiral phase of permethyl 2,3,6-tri-O-methyl β -cyclodextrin on a polysiloxane backbone stationary phase. A G-TA column (Astec, Whippany, NJ, USA), a B-PA column (Astec), and a BGB172 column (BGB Analytik AG, Anwil, Switzerland) were also explored in an attempt to separate additional chiral PCB congeners present in the tissues (18), but proved to be less proficient than the Chiralsil-Dex column.

For the determination of EF values, tissue extracts were made to 500 μ L volume and 1 μ L was injected into the GC; Aroclor, Chlorofen and soil extracts were made to 1 mL with 1 μ L injected. Analysis by temperature Program B (column temperature 100 to 150°C at 2 °C/min; 150 to 180 °C at 0.2°C/min, then from 180 to 200 °C at 20°C/min) provided almost baseline separation of all chiral PCBs in the standard mixture (resolution values are given in Table 1) and was used to determine EFs for all atropisomers in the standard mixture and, with the exception of PCB 149, in all other samples in which they were detected. Injection was splitless at 250 °C and the carrier gas was helium at a flow of 1.0 mL/min. Due to an interference by other PCB congeners in the Aroclor and soil extracts with one of the atropisomers of PCB 149, all samples except the standard mixture had to be analyzed by Program A (column temperature, 100 to 150 °C at 10°C/min; 150 to 200°C at 0.5°C/min, then held for 10 min.) in addition to Program B in order to measure EFs for PCB 149. A standard mixture of the PCBs (Table 1) was analyzed before and after every 10 tissue samples.

The EF (enantiomer fraction) for standards and samples was calculated as: area 1st peak/area (peak 1 + peak 2). Resolution in Table 1 was calculated as: $R = (RT_2 - RT_1) / 0.5(BW_1 + BW_2)$ where RT_2 and RT_1 are retention times of the second and first eluting atropisomer peaks and BW_1 and BW_2 are the base widths of peaks 1 and 2. The atropisomers of PCB 91, 95, 132 and 149 were completely resolved at the baseline; at the low extreme of resolution, PCB 84 was resolved to about 25% above baseline.

Statistics

The comparison of enantiomeric fractions between parent mixtures and tissues was performed using paired t-test with Bonferroni post-hoc test and confidence level set at 95%. The comparison of enantiomeric fractions between tissues was performed using analysis of variance (ANOVA with Fishers' least-significance-difference LSD post-hoc test). The statistical software SYSTAT 8.0 (Systat Software, Inc., Point Richmond, CA, USA) was used for the statistical analysis in this study.

RESULTS AND DISCUSSION

Enantiomer Fraction Measurements

The Chiralsil-Dex column provided by far the best results for separation of atropisomers of the chiral PCBs listed in Table 1. A combination of two different temperature programs, A and B, was employed to determine the EF values of these PCB congeners in the PCB mixtures and the biological samples. The EF values for the PCBs detected in Aroclor 1254, Chlorofen, and

the Chlorofen-containing soil extract to which the rats were exposed are given in Table 1. The EF values and the resolution for the racemic PCB standards are shown for comparison.

The EF values for Aroclor 1254 indicate that the PCB constituents are not all racemic (Table 1). The EFs of PCB 149 are 0.54; this deviation from 0.50 is probably caused by small amounts of coeluting PCB congeners, not a surprising event considering the complexity of the Aroclor 1254 mixture. A more extreme case is with PCB 174, with an apparent EF of 0.38. The EFs of PCB 174 in the tissue samples were therefore not further analyzed. The EF values for Chlorofen indicate that the PCB constituents are all racemic (Table 1). EF values for the corresponding EFs in soil extracts show a difference for PCB 176 relative to the Chlorofen, and a larger difference for PCB 174. This may indicate some microbial soil degradation of these particular PCBs.

The chiral PCB congeners for Table 1 were analyzed in rat tissue and blood samples. Other chiral PCBs that may have been present in these samples were either below the detection limits, could not be adequately separated from the non-target congeners in the Aroclor or soil extract dosing mixtures, or could not be separated from impurities in the tissue extracts. PCB 176 was not detected in Aroclor 1254 or in any tissue samples from Aroclor-dosed rats, while PCBs 84 and 91 were not detected in the Chlorofen, the soil extract, or the soil extract-dosed rat tissues.

Enantiomeric enrichment of PCBs in tissues from the Aroclor 1254 treatment group

The EFs of PCBs 95 and 149 in selected tissues are shown in Figure 1. PCB 95 was detected in all eight tissues as well as blood and serum (Figure 1A). Compared to Aroclor 1254, a significant enrichment of the first eluting atropisomer was observed in adipose tissue, liver and skin. An enrichment of the first eluting PCB 95 atropisomer was also observed in the two brain samples that contained detectable PCB 95 levels. PCB 95 was not detected in the other brain tissue sample. PCB 95 was not statistically different from Aroclor 1254 in all other tissues (including blood and serum).

We found PCB 149 in most tissues in this treatment group (Figure 1B). Similar to PCB 95, this congener shows a significant enantiomeric enrichment in the adipose, liver and skin. However, with PCB 149 the second eluting atropisomer was enriched and not the first as with PCB 95. On the other hand, the first eluting PCB 149 atropisomer was significantly enriched in blood and showed the same enrichment in lung and serum samples (when present above detection limits). PCB 149 is not statistically enantiomerically enriched in the spleen, kidney, brain and heart.

PCB 84 and PCB 91 were detected in only a few tissues. PCB 84 was detected in two spleen samples and had a near-racemic EF of 0.52. PCB 91 was detected in adipose tissue (n=1), liver (n=2) and skin (n=2) with EF values of 0.56, 0.68 and 0.53, respectively.

Overall, two trends for enantiomeric enrichment were observed in the Aroclor treatment group. First of all, we observed an enrichment for PCB 149 in the blood and serum samples. Second, we found that PCBs 95 and 149, which were detected in most tissues, showed a statistically significant deviation from the EF of the racemic parent mixture in adipose, liver and skin tissue. The observation of an enantiomeric enrichment of PCBs in the liver is similar to previous studies of the distribution of PCB atropisomers in mice (21), rats (10) and humans (15), suggesting that the liver is the organ responsible for the enantiomeric enrichment of PCBs.

There is currently no evidence for significant enantiomeric enrichment of PCBs in human tissues other than the liver (25) and blood (24). The only study analyzing the enantiomeric enrichment of PCBs 95, 132 and 149 in a small set of human brain and other tissue samples only shows an enrichment of these three congeners in the liver but not brain, kidney and muscle

tissue (25). However, in our study PCBs 95 and 149 show an (statistically not significant) enrichment of the first eluting atropisomer in the brain and lung, respectively. In an earlier mouse study the EFs of PCB 84 were also significantly different in the brain, heart, lung, and kidney (21). These laboratory findings imply that there is indeed the potential for enantiomeric enrichment of PCB congeners in the brain of laboratory animals and humans. This is an important finding because the atropisomers of PCB 84 have been shown to differ in their neurotoxicity (7). Therefore, an enantiomeric enrichment of highly neurotoxic PCB congeners, such as PCB 95 and 149 (5), may result either in an increased or decreased toxicity in Aroclor 1254 exposed animals depending on whether the more potent atropisomer is enriched in target tissues such as the brain.

Enantiomeric enrichment of PCBs in tissues from the soil extract treatment group

We show the EFs of PCBs 95, 149 and 174 in selected tissues in Figure 2. PCB 95 and 149 were present in most tissues with a few exceptions such as the brain (Figure 2A and 2B). PCB 174 was detected in all three spleen samples and in a few other tissue samples (Figure 2C). PCB 176 was found in the spleen (n=2) with an EF of 0.49. The extent of enantiomeric enrichment of PCBs 95, 149 and 174 in tissues from this treatment group is drastically different from the ones noted for the Aroclor treatment group (Figure 1).

Compared to the Aroclor treatment group, the EFs of PCBs 95 and 149 were not statistically different from racemic in all samples from the soil extract treatment group. The only exception is a slight enrichment of the second eluting PCB 95 in blood. The enantiomeric enrichment of PCB 149 detected in two of the serum samples appears to be an outlier and may be due to a co-eluting impurity in these two samples. As shown in Figure 2C, for PCB 174 an enrichment of the second eluting atropisomer is observed in PCB storage tissues, i.e. in the adipose tissue. A similar enantiomeric enrichment in the storage tissues is also observed in the Aroclor treatment group; however, in contrast to the Aroclor treatment group the liver does not display significant enantiomeric enrichment of any PCB in this treatment group.

Comparison of the EFs of PCBs in tissues from both treatment groups

We have recently shown that there are differences in the tissue specific disposition of certain PCB congeners, e.g. that higher chlorinated PCBs (for example PCB 174, 180, 193, 194 and 203+196) appear to be selectively retained in the spleen of both treatment groups (26). We analyzed statistically significant differences in the EFs among different tissues (Tables 2 and 3) to determine if the same observation can also be made for the tissue specific disposition of PCB atropisomers. Such differences may be helpful to identify the biological processes that are responsible for the enantiomeric enrichment observed in tissues from PCB-treated animals.

The results of the comparison of the EFs of PCBs in tissues from Aroclor-treated animals are summarized in Table 2. In this treatment group, differences among the tissue EFs were only noted for of PCB 149. Overall, the tissues seem to fall into three groups. The first group is comprised of blood, lung and serum. The EFs of PCB 149 in these three tissues are significantly different from almost all other tissues with the first eluting atropisomer being enriched. The second group consists of tissues such as adipose tissue, liver and skin which show an enrichment of the second eluting PCB 149 atropisomer. The tissue EFs of this group are also significantly different from most other tissues. The third group consists of the remaining four tissues. PCB 149 in these tissues is racemic and the EF in tissues from this group is different from both the first and the second group.

The results of the comparison of the EFs of PCBs in tissues from the soil-extract treatment group are shown in Table 3. In this treatment group, the EFs of PCBs 149 and 174 do not

display any differences between tissues. The EFs of PCB 95 in the adipose tissue and the skin are significantly different from blood, serum and heart.

The differences in the EF of the chiral PCBs between tissues in both treatment groups suggest that the atropisomers of these congeners are distributed differently between tissues, thus resulting in enantiomeric enrichment in certain tissues. One consistent observation is that the EFs of PCBs in storage tissues (adipose tissue, liver and/or skin) are in general significantly different from at least a few non-storage tissues. Otherwise the data presented in Tables 2 and 3 do not reveal any clear trends, an observation which indicates that there are differences in the disposition of chiral PCB congeners. Unfortunately our results do not allow us to hypothesize about the biological processes responsible for the differences in the (enantioselective) disposition of chiral PCB congeners.

Biochemical effects and EFs

The overall extent of enantiomeric enrichment in this study is different between the two treatment groups. A significant enantiomeric enrichment for several chiral PCBs, in particular PCBs 95 and 149, was observed in tissue samples in the Aroclor treatment group (Figure 1). In contrast the soil extract treatment group shows an enantiomeric enrichment in only a few tissue samples for three PCB congeners, i.e. blood (PCB 95) and adipose tissue (PCB 174) (Figure 2). Of particular interest is PCB 149, which, in contrast to the Aroclor treatment group, is clearly racemic in all tissues from this treatment group in which it is detected. As we have reported previously, the two PCB mixtures investigated have different congener profiles which result in the induction of different groups of hepatic enzymes in this study (26), i.e. EROD (CYP 1A) activity in the Aroclor 1254-treated rats is 30 fold higher compared to that in soil extract treated animals, while PROD (CYP 2B) activity in the soil extract-treated animals was about 4.5 times higher than in Aroclor 1254-treated rats.

Structure-activity relationship (SAR) studies of PCB metabolism have shown that multiple ortho-substituted PCB congeners with free, i.e. unchlorinated, meta- and para-positions are readily metabolized in many species such as rats and humans by CYP 2B's but not CYP 1A's (31–33). The relative lack of enantiomeric enrichment in the soil extract treatment group (compared to the Aroclor treatment group) therefore implies that the metabolism of chiral PCBs by hepatic CYP 2B's is not an obvious factor in the enantioselective disposition of PCBs in the rat. Since multiple-ortho-substituted PCBs neither have a high affinity nor are substrates for CYP 1A's, the enantiomeric enrichment observed in the Aroclor treatment group is not likely a result of the relatively higher hepatic levels of CYP 1A's in this treatment group. Thus, the differences in the induction of hepatic cytochrome P-450's between the two treatment groups can not explain the different extent to which enantiomeric enrichment is observed in the two treatment groups.

Overall, our observation that exposure to two different PCB mixtures leads to differences in enantiomeric enrichment is surprising, and the chiral processes responsible for these differences are currently unknown. Once these highly complex processes are better understood, differences in the EFs of certain PCBs, for example PCB 149, and other chiral environmental contaminants may be useful for a better understanding of the disposition of complex mixtures in laboratory animals and in humans. Similar to studies using EFs as an indicator for biotransformation processes in the environment (34), EFs from laboratory animal and human samples may be useful as chemical markers for past exposures and, thus, help us understand and assess risk in exposed populations.

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Figure 1.

Enantiomeric fraction of (A) PCB 95 and (B) PCB 149 in tissues and blood from Aroclor 1254 treated animals. The light blue bars depict tissues where the respective PCB congener was detected in only 1 or 2 tissue samples. The error bars show the standard deviation. The dotted line presents the EF for the PCB in Aroclor 1254. The EF value of the parent mixture is written next to the line. n.d. = not detected. * Statistically different from Aroclor 1254; $P < 0.05$.

Figure 2.

Enantiomeric fraction of (A) PCB 95, (B) PCB 149 and (C) PCB 174 in tissues and blood from soil-extract treated animals. The light blue bars depict tissues where the respective PCB congener was detected in only 1 or 2 tissue samples. The error bars show the standard deviation. The dotted line presents the EF for the PCB in the Chlorofen soil extract. The EF value of the parent mixture is written next to the line. n.d. = not detected. n.d. = not detected; # = possibly co-eluting impurity, * Statistically different from the Chlorofen soil extract; $P < 0.05$.

Table 1
Resolution and EFs of chiral PCBs analyzed in this study using Program B on the Chiralsil-Dex column with electron capture detection in standards, Chlorofen and mixtures used in the study – Aroclor 1254 and soil extract.

PCB	R ^a	EF of PCB Standards	EF of PCBs in Aroclor 1254	EF of PCBs in Chlorofen	EF of PCBs in Soil Extract
84	0.9	0.50 ± 0.00	0.52 ± 0.00	n.d.	n.d.
91	1.4	0.50 ± 0.00	0.48 ± 0.00	n.d.	n.d.
95	1.5	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.01
149 ^b	1.2	0.50 ± 0.00	0.54 ± 0.00	0.50 ± 0.00	0.50 ± 0.01
174	1.0	0.51 ± 0.01	(0.38 ± 0.01)	0.51 ± 0.01	0.56 ± 0.00
176	1.0	0.50 ± 0.00	n.d.	0.50 ± 0.00	0.53 ± 0.01

^aR = Resolution of PCBs in the standard mixture, see text for formula

^bPCB 149 data using GC Program A

n.d. = not detected

Statistically significant differences in the EFs of PCB 149 between tissues in the Aroclor 1254 treatment group. The numbers represent the P values of the statistical analysis.

Table 2

Tissue	Liver	Skin	Spleen	Blood	Lung	Serum	Kidney	Brain	Heart
Adipose				<0.000	<0.000	<0.000		0.007	0.003
Liver	-			<0.000	<0.000	<0.000	0.005	0.002	0.001
Skin		-		<0.000	<0.000	0.001			
Spleen			-	<0.0000	<0.000	0.003			
Blood				-			<0.000	0.001	0.002
Lung					-	0.010	0.001	0.004	0.007
Kidney							-		

Table 3
Differences in the EFs of PCB 95 between tissues in the soil extract treatment group. The numbers represent the P values of the statistical analysis.

Tissue	Liver	Skin	Spleen	Blood	Lung	Serum	Kidney	Brain	Heart
Adipose				0.001		0.001			
Liver	-			0.002		0.002			0.001
Skin		-							0.002
Spleen			-						
Blood				-					
Lung					-				
Kidney							-		