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Clearance of Polychlorinated Biphenyl Atropisomers is Enantioselective in Female C57Bl/6 Mice

Izabela Kania-Korwel^a, Mohammed H.M.E. El-Komy^b, Peter Veng-Pedersen^b, and Hans-Joachim Lehmler^{a,*}

[§]Department of Occupational and Environmental Health, College of Public Health, The University of Iowa, Iowa City, Iowa 52242

^bDivision of Pharmaceutics, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

Abstract

Changes in the enantiomer composition of polychlorinated biphenyls (PCBs) can not only be used to investigate environmental and biological transport processes, but also has human health implications because of enantiospecific adverse health effects. To further understand differences in the disposition of PCB atropisomers *in vivo*, the present study investigates the toxicokinetics of PCB atropisomers in female C57Bl/6 mice after oral administration of a mixture of several PCBs, including racemic PCBs 95, 132, 136, 149, 174 and 176. On the Chirasil-Dex column, an enrichment of the second eluting atropisomers was generally observed, whereas only the first eluting atropisomers E1-PCB 95, (–)-PCB 132 and (–)-PCB 149 had half-lives that were distinctively longer compared to the second eluting atropisomers. The bioavailability normalized clearance of first eluting atropisomers in blood was faster compared to second eluting atropisomers. The opposite trend was observed for the accumulation factors in adipose tissue, which is consistent with the slower clearance of the first eluting atropisomer. The only exception was PCB 174, which showed no differences in the toxicokinetic parameters of both atropisomers. Together, the differences in the toxicokinetics of PCB atropisomers point towards enantioselective biotransformation processes as the origin of PCB's enantiomeric enrichment in mammals and, possibly, humans.

Keywords

Polychlorinated biphenyls; atropisomers; chiral; pharmacokinetics; half-life; clearance; accumulation factor

Introduction

PCBs are a group of persistent organic pollutants that were manufactured as complex mixtures by chlorination of biphenyl. Industrial PCB mixtures were used as coolants and lubricants from the 1930s until 1977, when their production was banned in the United States. However, PCBs are still in use in the United States as dielectric fluids in transformers and capacitors. Although environmental and human PCB levels have dropped significantly between 1970 and 1995,

*To whom correspondence should be addressed: Hans-Joachim Lehmler, University of Iowa, Department of Occupational and Environmental Health, 100 Oakdale Campus #221 IREH, Iowa City, Iowa 52242, Phone: (319) 335-4211, Fax: (319) 335-4290, hans-joachim-lehmler@uiowa.edu.

Brief

The atropisomers of PCBs 95, 132, 136, 149, 174 and 176 eluting second on Chirasil-Dex columns were enriched in mice due to their slower clearance.

recent studies of temporal trends show no or only a slight decrease in environmental PCB levels since the mid 1990s (1). One possible explanation is that PCBs are still released into the environment from sites of secondary PCB contamination, such as building materials and other sites of industrial PCB use (2). These environmental monitoring studies suggest that there is still considerable risk for human exposures to PCBs, which is corroborated by studies demonstrating the persistence of PCB residue levels in foods, particularly fish, and in human tissue samples.

There is increasing evidence that PCB congeners with multiple *ortho* chlorine substituents predominate in environmental (3) and human blood (4) samples. Furthermore, laboratory studies and human epidemiological data suggest a positive association between developmental exposure to these *ortho*-substituted PCBs and behavioral/cognitive deficits in infancy or childhood. Specifically, *in utero* and lactational PCB exposures correlate with decreased intelligence quotient, impaired learning and memory, attentional deficits, and lowered reading comprehension (5). Although several mechanisms for the developmental neurotoxicity of PCBs have been identified (5), the most sensitive is sensitization of ryanodine receptors, an important family of microsomal Ca^{2+} channels expressed throughout the mammalian brain (6,7).

Several neurotoxic PCB congeners that target ryanodine receptors display axial chirality and, due to the hindered rotation around the phenyl-phenyl bond, exist as two non-superimposable mirror images called enantiomers or, in the special case of axis chirality, atropisomers. These two mirror images are not identical molecules, but differ from each other and from the racemic mixture (a mixture with a 1:1 ratio of both enantiomers) in physical and biological properties. Chiral PCBs are important components of technical and environmental PCB mixtures (8,9) and undergo a species-dependent enantiomeric enrichment in wildlife, laboratory animals and human populations (8) probably due to enantioselective binding (10) and/or metabolism (11) by cytochrome P450 (P450) enzymes. Together with our recent observation of enantiomeric specificity of (–)-PCB 136 ((–)-2,2',3,3',6,6'-hexachlorobiphenyl) toward ryanodine receptor types 1 and 2 (12), the enantiomeric enrichment of PCB *in vivo* suggests that differences in the toxicokinetics of PCB atropisomers may influence the risk for adverse neurodevelopmental outcomes following PCB exposure.

There is evidence of enantiomeric enrichment of PCBs in human tissues (13–15) and in rodents (9,16–21). Unfortunately, most animal studies are limited to a few chiral PCB congeners and investigate only a single time point. Detailed toxicokinetic studies of PCB atropisomers, including the atropisomers of the highly neurotoxic PCBs 95, 136 and 149, have not been reported in mammals. The present study addresses this significant gap in our knowledge and determines toxicokinetic parameters of PCB 95, 132, 136, 149, 174 and 176 atropisomers after oral administration of a mixture of these PCBs congeners in the ratio observed in fish from the Fox River (22).

Experimental section

Chemicals

PCBs were obtained from Accustandard (New Haven, CT, US) or synthesized in our laboratory. All other chemicals were obtained from commercial sources (see the Supporting Information).

Animal treatment

The Institutional Animal Care and Use Committee of the University of Iowa approved all animal procedures. Sixty one female C57Bl/6 (7 weeks old; 18.4 ± 1.4 g) were purchased from

Harlan Inc. (Indianapolis, IN, US), allowed to acclimatize for a week and given *ad libitum* access to chow and water during the entire study. The animals were randomly assigned to treatment groups (n=4 or 5) and received 50 mg/kg body weight of a PCB mixture containing racemic PCB 91 (6.1%), PCB 95 (24.5%), PCB 132 (12.7%), PCB 136 (4.7%), PCB 149 (30.5%), PCB 174 (13.4%), PCB 176 (1.6%) and PCB 183 (6.5%) in corn oil (5.0 mg PCB/ml corn oil) by oral gavage. The remaining nine animals were treated as sentinels and received the vehicle alone (10 ml/kg body weight of corn oil). PCB-treated animals were euthanized by asphyxiation with carbon dioxide at different time points, followed by cervical dislocation. One sentinel was euthanized at each time point. Blood was collected by cardiac puncture and stored in glass vial in -20°C . Adipose tissue, brain and liver were excised *en bloc* and were stored in aluminum foil in -20°C .

Tissue extraction

PCBs were extracted from adipose (0.02–0.16 g), brain (0.03–0.17 g) and liver (0.09–0.27 g) using pressurized liquid extraction as described earlier (20). The samples were spiked before extraction with 3,5-dichlorobiphenyl (PCB 14, 200 ng), 2,3,5,6-tetrachlorobiphenyl (PCB 65, 200 ng) and 2,3,4,5,6,4'-hexachlorobiphenyl (PCB 166, 200 ng) as surrogate standards. To allow future analyses of PCB metabolites, 3,5,2',3',4',5'-hexachloro-biphenyl-4-ol (4'-OH-PCB 159) and 2,3,4,5,3'-pentachloro-5'-methylsulfonyl-4'-methyl-biphenyl (4'-Me-5'-MeSO₂-PCB 106) were also added. The extracts were evaporated to dryness and the solvent was exchanged to hexane.

Blood extraction

Aliquots of whole blood (0.07–0.38 g) were spiked with the surrogate standards as described above, denatured with hydrochloric acid (6M, aqueous, 1 ml) and 2-propanol (5 ml), and extracted with hexane-methyl-*tert*-butyl-ether (1:1 v/v, 5 ml) and hexane (3 ml). The extracts were combined, washed with potassium chloride (1% aqueous, 3 ml), evaporated to dryness and the solvent was exchanged to hexane.

Separation of PCBs from its metabolites and clean-up

The separation of PCB and its metabolites was performed as described previously (20,23). In short, the hydroxylated PCB were separated from neutral fraction containing PCBs and methylsulfonyl PCBs by partitioning into potassium hydroxide (0.5M, water:ethanol 1:1 v/v, 2 ml). Subsequently, the methylsulfonyl PCBs were partitioned into anhydrous dimethylsulfoxide (0.5 ml) and sulfur impurities were removed from the PCB fraction by shaking the extract with 2-propanol (2 mL) and tetrabutylammonium sulfite (2 mL), adding nanopure water (5 mL) and reshaking. The organic extract was removed, mixed with 2 mL of concentrated sulfuric acid and stored over sulfuric acid for > 12 hours (20). The PCB metabolite fractions were stored at -20°C and are available for future analyses.

Gas chromatographic analysis

Levels of the PCB atropisomers, adjusted for tissue or blood wet weight, were determined using an Agilent 6890 gas chromatograph equipped with a ⁶³Ni μ -ECD detector and a Chirasil-Dex CP capillary column (β -cyclodextrin chemically bonded to dimethylpolysiloxane, 25 m $\times$ 0.25 mm ID, 0.25 μm film thickness, Varian, Palo Alto, CA, USA). The temperature program was as follows: $2^{\circ}\text{C}/\text{min}$ from 100°C to 150°C , then $0.2^{\circ}\text{C}/\text{min}$ to 185°C , $15^{\circ}\text{C}/\text{min}$ to 200°C , hold for 10 min. The injector and detector temperatures were 250°C . The elution order of the atropisomers was assigned based on published elution orders (24). The resolution ranged from 0.74 (PCB 174) to 1.64 (for PCB 132) (Table S1 and Figure S1). PCB 183 could not be resolved on the Chirasil-Dex column (24). The PCB levels determined using the Chirasil-Dex column were in good agreement with results from gas chromatographic analyses using a SLB5MS

capillary column (60 m × 0.25 mm ID, 0.25 µm film thickness, Supelco, Belfonte, PA, US), with all PCBs having correlation coefficients r^2 typically > 0.9 (range 0.65 to 0.99).

Quality assurance and quality control

PCB concentrations were determined using 2,4,6-trichlorobiphenyl (PCB 30) as internal standard. Concentrations were corrected if the recovery of the appropriate surrogate standard was below 100%. PCB 65 recoveries were used for correction of concentrations of PCB 91 and PCB 95 (average recoveries: 84±12 % for tissue and 85±5 % for blood extractions). PCB 166 recoveries were used for correction of concentrations of remaining congeners (average recoveries: 92±11 % for tissue and 100±6 % for blood extractions). The limit of detection for each congener was determined based on method blanks (17), and varied from not detected to 2.3 ng depending on the congener and matrix (Table S2). The total PCB content in sentinels was 140 ± 230 ng/g tissue (Table S2).

Toxicokinetic analysis

A non-compartmental systems analysis approach was applied to derive the PCB's toxicokinetic parameters (25). Curve-fittings for blood, brain, liver and adipose data were done independently using WINFUNFIT, an interactive Windows (Microsoft) version evolved from the general nonlinear regression program FUNFIT (26). Tri-exponential curve-fitting provided the best-fit approximation to the PCBs levels according to Akaike Information Criteria (AIC) (27) and thus was used in the toxicokinetic analysis (Figures S2–S61).

The maximum PCBs concentrations in the various tissues (C_{max}) were found numerically. The bioavailability normalized clearance (CL/F) was evaluated as the dose/the total area under the curve of the PCB level in blood. The terminal half-life $T_{1/2}$ was calculated as $\ln(2)/(\text{the minimum of the three exponential time coefficients})$ obtained from the exponential model fitting to the PCB level in blood. The accumulation factor (AF) of the PCB in adipose was determined by the ratio of maximum predicted steady state concentration to the maximum estimated concentration from a single dosing assuming two doses/day. The blood-to-tissue distribution coefficient (K_p) was calculated as the ratio of the total area under the curve of the PCB level in tissue to that in blood. The toxicokinetic parameter of PCBs 91, 95, 132, 136, 149, 174, and 176 atropisomers are summarized in Table S3. The toxicokinetic parameters of PCB 183 are presented in Table S3, but this congener was not further investigated because it is not subject to enantioselective metabolism by P450 enzymes (11).

Statistical analysis

The first eluting vs. the second eluting PCB comparisons of population mean values for the toxicokinetic parameters were done by t-test using Microsoft® Excel 2007 (Microsoft Corporation, Redmond, WA, USA). Statistically significant levels were at the 0.05 level for the type I null hypotheses error.

Results and discussion

The toxicokinetics of the chiral PCB congeners investigated in this study showed the same characteristics typical for other PCB congeners, including chiral PCB 136, in rodents (Figure 1 and Figure 2) (28–30): All chiral PCBs were cleared rapidly from the blood (Figure 1 and Figure 2A), which is due to storage in the liver and muscle in the initial distribution phase (29,31,32). Since PCBs 91, 95, 132, 136, 149, 174 and 176 are readily metabolized by P450 enzymes due to the presence of vicinal *ortho-para* H-atoms (11,32,33), it is likely that their metabolism also contributed to their rapid clearance from blood (31). Analogous to other PCBs, chiral PCBs were subsequently redistributed from highly into poorly perfused tissues with high affinity for PCBs (such as adipose tissue; Figure 2B), metabolized and/or eliminated into the

feces. It is important to emphasize that all chiral PCBs, including the highly neurotoxic congeners PCB 95 and PCB 136, were rapidly cleared from the brain and the liver (Figure 2C and 2D), whereas clearance from the adipose tissue was much slower (Figure 2B).

The data shown in Figure 1 and Figure 2 were analyzed using a non-compartmental systems analysis approach to derive the PCB's toxicokinetic parameters (Figure 3 and Table S3) (25). In addition, the toxicokinetic parameters of the first versus the second eluting atropisomer were compared for all congeners to assess if there are general trends (Table 1). Differences in toxicokinetic parameters (maximum PCB concentrations in blood and tissues, bioavailability normalized clearance, terminal half-life and the accumulation factor) displayed atropisomer-specific differences, at least for some congeners, and are discussed below (Table 1 and Figure 3). One possible explanation for the significant differences in the toxicokinetic parameters of the first versus the second eluting atropisomers is that the biological process responsible for the enantiomeric enrichment of PCBs *in vivo* has the same selectivity with regards to the absolute configuration. However, further studies (such as the determination of the absolute configuration of chiral PCBs) are needed to confirm this hypothesis.

Maximum PCB concentrations ($c_{\max}$)

The maximum PCB concentrations in blood and tissues followed the relative composition of the PCB mixture administered to each animal (Table S1 and Figure S1). However, $c_{\max}$ of the second eluting PCB atropisomer was typically higher compared to the first eluting atropisomers, thus providing the first indication of enantiospecific differences in the toxicokinetics of PCBs. This difference was statistically significant in the brain and liver, but not in the blood ($p=0.06$) and adipose tissue ($p=0.09$) when $c_{\max}$ values for all seven congeners were compared.

Terminal half-lives ($T_{1/2}$) in blood

The average terminal half-lives of the racemic PCB congeners decreased in the approximate order PCB 174 > PCB 132 ~ PCB 95 ~ PCB 149 > PCB 136 > PCB 91 ~ PCB 176 (Figure 3). The comparatively long half-lives of PCBs 95, 132 and 149 are not surprising because these congeners are frequently reported in human biomonitoring studies (4) and, as discussed above, are known to undergo enantiomeric enrichment in humans (13,15). A more complex rank order emerged when the half-lives of individual PCB atropisomers were analyzed. (–)-PCB 132 had the longest half-life (380 h) and (–)-PCB 176 had the shortest half-life (13 h). First eluting atropisomers E1-PCB 95, (–)-PCB 132 and (–)-PCB 149 had comparatively long half-lives and were the only atropisomers with half-lives distinctively different from the second eluting atropisomer. For example, the half-life of (–)-PCB 132 was 3-times longer compared to the half-life of (+)-PCB 132. In contrast, both PCB 176 atropisomers had similar half-lives of ~ 270 h. Within the experimental error both PCB 136 atropisomers had comparable half-lives that were slightly shorter compared to the more persistent chiral PCBs. The shortest half-lives were observed for PCB 91 and PCB 176 atropisomers, with half-lives ranging from 13 to 30 h. Overall, the differences in the terminal-half live of PCB atropisomers were surprisingly small and did not reach statistical significance when the half-lives of all atropisomers were compared (Table 1). This is a surprising observation considering the pronounced enantiomeric enrichment of PCB 136 reported in mice in earlier studies (9,17–19).

Despite their environmental and toxicological relevance, surprisingly few half-lives of chiral PCB congeners have been reported. The toxicokinetics of PCB 136 has been studied in several species because of its symmetrical substitution pattern and its comparatively fast clearance due to hepatic metabolism. An earlier study reports a half-life of racemic PCB 136 of 77 h in female mice (28), which is shorter compared to the half-lives observed in this study for (–)- and (+)-PCB 136 (200 and 170 h, respectively). Rats, dogs and Cynomolgus monkey also rapidly

metabolize PCB 136, with half-lives after intravenous administration ranging from 11 to 114 h (29–31,33). Unfortunately, half-lives for PCB 136 in humans have not been reported. PCB 95 is the only other chiral PCB congener for which toxicokinetic parameters have been published. Its half-life in rats is 384 h, which is comparable to the half-lives of E1-PCB 95 of 310 h in this study. In contrast, the estimated half-life of racemic PCB 95 in humans is much longer with 0.3 to 3 years (34,35).

Bioavailability normalized clearance (CL/F)

Clearance describes the elimination of xenobiotics from the body and is expressed in terms of volume of blood cleared per unit of time. In the case of PCBs, primarily their redistribution into storage tissues and their biotransformation contribute to the clearance. In contrast to the terminal half-life, there was a statistically significant difference between the CL/F of the first versus second eluting congeners, with CL/F of the second eluting atropisomers being smaller (Table 1). First eluting atropisomers (–)-PCB 176, E1-PCB 91 and (–)-PCB 132 had the higher clearance of all PCB atropisomers (33, 30 and 28 ng/mL, respectively), whereas second eluting (+)-PCB 136, E2-PCB 95 and (+)-PCB 149 had the lowest clearance of all atropisomers (6.7, 8.3 and 9.2 ng/mL, respectively). The fact that the clearance of first eluting atropisomers was faster compared to second eluting atropisomers points towards enantiomer specific differences in the rates of the biotransformation of PCB atropisomers with a 2,3,6-trichlorophenyl substitution pattern, an interpretation which is supported by differences in binding and metabolism of these congeners by P450 enzymes and the fact that disposition studies have not shown a sequestration of these atropisomers into other organs or excreta. This comparison of the clearances based on bioavailability normalized clearance values (Table 1) assumes the atropisomers have the same bioavailability.

Accumulation factor (AF) in adipose

The AF is a measure of the relative persistence of a xenobiotic in an organism. As shown in Figure 3, the persistence of chiral PCBs increased with increasing degree of chlorination. This trend is consistent with previous observations and results from an increase in lipophilicity and decrease in the rate of metabolism with increasing degree of chlorination (36). The AFs of chiral PCBs displayed a significantly higher tendency of the second eluting PCB atropisomer to accumulate in mice, which is consistent with their longer half-lives and slower clearance (Table 1). The only exceptions were PCBs 136 and 174, which had comparable AFs for both atropisomers. The highest AFs were observed for the atropisomers of both heptachlorobiphenyls (PCBs 174 and 176). The first eluting atropisomers of both pentachlorobiphenyls (E1-PCB 91 and E1-PCB 95) had the smallest AFs. Their AFs were approximately 1.5-times smaller compared to the corresponding second eluting atropisomer, which is the largest difference in the AF observed in this study. Interestingly, the AFs of both PCB 136 atropisomers were smaller compared to the AFs of the other two hexachlorobiphenyls, PCB 132 and 149, and comparable to the AFs of E1-PCB 91 and E1-PCB 95.

Blood-to-tissue distribution coefficients (K_p)

The blood-to-tissue distribution coefficients are a measure of the affinity of a tissue for a xenobiotic and, in the case of PCBs, increases with the lipid content of a tissue (37). The estimated blood-to-adipose distribution coefficients were relatively large and ranged from 78 to 1400 (Table S3), which is in agreement with other studies showing that this compartment is a dominant factor in the distribution of PCBs (38–40). The published human adipose tissue-to-blood distribution coefficient of racemic PCB 95 range from 45 to 62 (41,42) and, thus, are smaller compared to the distribution coefficients of the PCB 95 atropisomers observed in this study (K_p (E1-PCB 95) = 500 and K_p (E2-PCB 95) = 330). The affinity of all PCB atropisomers for the liver and the brain was lower compared to adipose tissue, with blood-to-liver and blood-

to-brain distribution coefficients ranging from 4 to 52 and 0.9 to 23, respectively. No significant difference in the blood-to-tissue distribution coefficients were observed for the first versus the second atropisomers (Table 1). One possible explanation for this observation is that enantioselective binding to proteins, for example hepatic P450 enzymes, is not the mechanism responsible for the enantiomeric enrichment of PCBs in mice. Interestingly, PCB 136 atropisomers had the lowest blood-to-brain distribution coefficient (~0.9 for both atropisomers) of all chiral PCB congener.

Enantiomeric fractions in mice versus humans

In this study, the chiral PCBs investigated typically showed a statistically significant enrichment of the second eluting atropisomers (compared to the racemic mixture), even at early sampling time points. In the case of PCB 136, the extent of the enantiomeric enrichment of PCB 136 was comparable to the enrichment reported for similar studies in C57Bl/6 mice (9, 17–19). In contrast, the direction of the enantiomeric enrichment reported in a limited number of human samples reveals a more complex picture. For example, a significant enrichment of the second eluting atropisomer of PCB 132 and the first eluting atropisomer of PCBs 95 and 149 is observed in liver samples from Belgium (13). In the same study, PCBs 95, 132 and 149 are near racemic in human muscle, brain and kidney tissue. An enantiomeric enrichment of several chiral PCBs has been reported in human breast milk samples (14,15). In agreement with this study, the second eluting atropisomer of PCBs 132 and 176 are enriched in breast milk, whereas PCBs 91, 95 and 149 are typically near racemic. PCB 174 is near racemic in most human breast milk samples, but the first eluting PCB 174 atropisomer is enriched in three (out of 11) samples (15) – which is the opposite of the enrichment observed in mice.

The origin(s) of the different direction of the enantiomeric enrichment in wildlife, rodents and humans is currently unclear, partly because toxicokinetics data for individual PCB congeners in species of interest are not available. Possible explanations include species differences in the enantioselectivity of PCB biotransformation, exposure to enantiomerically enriched PCBs (e.g., via a diet containing trout or blubber), induction of different P450 enzymes due to differences in the diet and/or exposure to xenobiotics as well as genetic polymorphisms of P450 enzymes responsible for the enantioselective metabolism of PCBs in humans. Exposure via the diet is likely to contribute to any unexpected direction of the enantiomeric enrichment in humans. For example, (–)-PCB 136 is enantiomerically enriched in rainbow trout (43,44) and rats (11,20), which is opposite to the enrichment observed in mice (9,17–19). Further fundamental studies of the biotransformation processes contributing to the enantiomeric enrichment in wildlife and mammals as well as in-depth investigations of chiral signatures in foodstuff are, therefore, needed to understand the chiral signatures observed in environmental and laboratory studies and, ultimately, to assess environmental and public health risks.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Levels of (A) PCB 91, (B) PCB 95, (C) PCB 132, (D) PCB 136, (E) PCB 149, (F) PCB 174 and (G) PCB 176 atropisomers in female C57Bl/6 mice showing differences in the toxicokinetics of PCB atropisomers. Each symbol represents the PCB atropisomer level for an individual animal expressed as wet weight. The lines represent the curve-fittings for blood data done independently using WINFUNFIT.

(A) Blood **(B) Adipose tissue** **(C) Brain** **(D) Liver** **Figure 2.**

Levels of the first (E1) and second (E2) eluting PCB 95 atropisomer in (A) blood, (B) adipose tissue, (C) brain and (D) liver showing differences in the toxicokinetics of the PCB 95 atropisomers. Each symbol represents the PCB 95 atropisomer level for an individual animal. The lines represent the curve-fittings for blood or tissue data done independently using WINFUNFIT. Data for all other atropisomers are included in the Supporting Information.

Figure 3.

Comparison of (A) terminal half-lives, (B) bioavailability normalized clearances and (C) accumulation factors indicate a faster clearance and smaller accumulation for the first (red) versus the second (red) eluting PCB atropisomers. Only the terminal half-lives of the first eluting atropisomers of PCB 95, PCB 132 and PCB 149 were clearly different from the second eluting atropisomers. PCBs were administered orally to female C57Bl/6 mice. Error bars indicate the uncertainties of the toxicokinetic parameter determinations.

Table 1

Differential statistical analysis of the toxicokinetic parameters of the first and second eluting PCB atropisomer populations (sample size = 7).

Parameter	Means		Statistical Test*	P-value	Significance**
	1st	2nd			
C _{max} (blood) [ng/mL]	163	245	A	0.06	NS
C _{max} (brain) [ng/mL]	13000	1800	A	0.03	S
C _{max} (liver) [ng/mL]	3650	4690	A	0.01	S
C _{max} (adipose) [ng/mL]	6030	8480	B	0.09	NS
CL/F [mL/h]	22	12	A	0.01	S
Terminal half-life (T _{1/2}) [h]	215	143	B	0.1	NS
Accumulation factor (AF)	19	23	B	0.02	S
K _p (blood-brain)	11	10	B	0.7	NS
K _p (blood-liver)	25	27	B	0.6	NS
K _p (blood-adipose)	439	546	B	0.4	NS

* Statistical Tests used are: A: Paired t-test (One-tail); B: Paired t-test (Two-tail)

** NS = not significant (p > 0.05); S = significant (p < 0.05); CL/F: bioavailability normalized clearance; K_p: tissue-blood distribution coefficient.