2,2',3,5',6-PENTACHLOROBIPHENYL (PCB 95) AND ITS HYDROXYLATED METABOLITES ARE ENANTIOMERICALLY ENRICHED IN FEMALE MICE

SUPPORTING INFORMATION

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Preparation of peanut butter containing PCB 95. A stock solution of PCB 95 (20 mg/mL) in organic peanut oil (Spectrum Organic Products, LLC, Melville, NY, USA) was blended with organic peanut butter (Trader Joe's, Monrovia, CA, USA) using a Bullet Blender (Next Advance, Averill Park, NY, USA) for 3 min at a ball spin speed of 4.

Quality assurance/quality control. The electron capture detector was linear for all analytes in a range of 0.5 ng to 1 μg. The instrument detection limits (IDL) and the method detection limits (MDL), determined based on laboratory blanks containing only the reagents and solvents used in the analysis [1], are presented in Table S4. The background levels in control animals varied from 0.36 to 140 ng/g tissue, depending on analyte and tissue, and these values are provided in Table S4. The recoveries of the surrogate standards were 81 ± 19% and 90 ± 7% for PCBs in tissue and blood samples, respectively, and 80 ± 34% and 63 ± 16% for OH-PCBs in tissue and blood samples, respectively. The concentrations were corrected for surrogate recovery rates below 100%.

Table S1. Cytochrome P450 primer sequences.

Mouse P450 enzyme (human orthologue; rat orthologue)	Primer	
CYP2B10 (CYP2B6; CYP2B1/2)	Forward primer	5'CCAAATCTCCAGGGCTCCAAGGC3'
	Reverse primer	5'TGCGGACTTGGGCTATTGGGAGG3'
CYP3A11 (CYP3A4; CYP3A2)	Forward primer	5'ACAAGCAGGGATGGACCTGGTT3'
	Reverse primer	5'CCCATATCGGTAGAGGAGCACCA3'
CYP1A2 (CYP1A2; CYP1A2)	Forward primer	5'CCAGCCCCTGCCCTTCAGTGGTA3'
	Reverse primer	5'TGGGAACCTGGGTCCTTGAGGC3'
CYP2S1 (CYP2S1; CYP2S1)	Forward primer	5'TCGGGGCTTTTGCGGCTAAGT3'
	Reverse primer	5'CAACCAGGACCACCACGCGG3'

Table S2. Amplification efficiencies[a] of selected cytochrome P450 enzymes in liver and specific brain regions of female C57Bl/6 mice.

		Pgk1	CYP2B10	CYP3A11	CYP1A2	CYP2S1
Liver	R^2	0.9985	0.9888	0.9935	0.9659	0.8971
	Efficiency	96%	90%	79%	99%	112%
Hippocampus	R^2	0.9635				0.9657
	Efficiency	117%	ND[b]	ND	ND	83%
Cortex	R^2	0.997				0.9812
	Efficiency	103%	ND	ND	ND	96%
Cerebellum	R^2	0.9975	0.9975	0.9923		0.9960
	Efficiency	88%	91%	63%	ND	79%

[a] Amplification efficiencies were determined from 6-point dilution curves of liver tissue from saline-treated animals; [b] ND = not detected.

Table S3. Fold-change in hepatic cytochrome P450 enzyme mRNA in PCB 95-exposed female C57Bl/6 mice relative to control animals.

PCB 95 dose	0.1 mg/kg bw/d (n=4)			1.0 mg/kg bw/d (n=5)			6.0 mg/kg bw/d (n=6)		
CYP enzyme	Relative expression	Standard error	95% confidence interval	Relative expression	Standard error	95% confidence interval	Relative expression	Standard error	95% confidence interval
CYP2B10	1.3	0.8 – 2.5	0.6 – 3.4	1.6*	1.1 – 2.2	1.0- 2.9	3.7*	2.1 – 8.5	1.3 - 12
CYP3A11	2.4*	1.6 – 4.2	0.8 – 4.9	2.3*	1.3 – 3.6	0.9 – 4.0	1.8	1.0 – 4.7	0.5 – 5.5
CYP1A2	1.9*	1.1 – 4.5	1.0 – 7.6	2.2*	1.3 – 5.0	1.1 – 7.8	1.6	1.0 – 3.5	0.7 – 6.2
CYP2S1	2.6	1.3 – 9.3	0.4 - 14	3.3	1.0 – 8.5	0.6 - 28	1.9	1.0 – 4.3	0.3 - 10

* Indicates statistically significant change (upregulation) of gene expression in PCB 95 treated animals compared to expression in control animals (n=5) ($p<0.05$). The relative expression is calculated as the ratio of concentration of the gene of interest compared to the concentration of the reference gene (Pgk1). Concentrations, relative expression, standard error and 95% confidence intervals are calculated by REST2009 software (Qiagen, Valencia, CA) based on Ct and efficiency values derived from qPCR analysis of the samples. This software employs randomization and bootstrapping techniques to create a set of permutated expression data based on the actual gene expression values derived from qPCR analysis of the samples. Using the permutated data set, REST2009 calculates relative expression ratios and tests their statistical significance [2,3].

Table S4. Calibration curve parameters, instrument detection limits (IDL), method detection limits (MDL) [1] and background levels for the analysis of PCB 95 and its metabolites in blood and tissue samples from female C57Bl/6 mice. Data are presented as the mean ± SD.

Analyte	PCB 95	3-103	4-95	5-95	4,5-95
Calibration curve (calibration range 0.5 ng/mL to 1 μg/mL for all compounds)					
r^2	0.9990	0.9999	0.9800	0.9700	0.9999
IDL [ng/mL]	11	3	63	68	3
Blood samples					
MDL [ng] (n=3)	1.0	0.34	1.8	0.46	5.0
MDL [ng/g tissue][a]	5.0	1.7	9.0	2.3	25
Tissue samples					
MDL [ng] (n=4)	2.9	1.8	1.2	1.5	1.6
MDL [ng/g tissue][a]					
Adipose [ng/g tissue][a]	48	30	20	25	27
Brain [ng/g tissue][a]	26	16	11	14	14
Liver [ng/g tissue][a]	15	9.5	6.3	7.9	8.4
Control animal levels					
Blood [ng/g tissue] (n=3)	6.4 ± 1.3	1.0 ± 0.8	8.9 ± 1.2	ND[b]	8.1 ± 0.6
Adipose [ng/g tissue] (n=5)	140 ± 133	ND	16 ± 23	76 ± 110	ND
Brain [ng/g tissue] (n=5)	7.2 ± 2.6	1.1 ± 2.5	ND	ND	0.54 ± 1.2
Liver [ng/g tissue] (n=5)	5.5 ± 4.5	0.36 ± 0.81	4.8 ± 10	0.44 ± 0.99	0.75 ± 1.0

[a] Calculated using MDL values in [ng] divided by the average weight of tissue used for extraction (blood 0.20 g; adipose 0.06 g; brain 0.11 g; liver 0.19 g); [b] ND = not detected.

Table S5. PCB 95 levels [ng/g tissue] in blood and tissues of female C57Bl/6 mice after subchronic oral exposure to PCB 95. Data are presented as the mean ± SD.

PCB 95 dose	Adipose	Blood	Brain	Liver
0.1 mg/kg bw/d	1800 ± 500 (n=4)	9.8 ± 2.2 (n=3)	37 ± 12 (n=4)	87 ± 23 (n=4)
1.0 mg/kg bw/d	20000 ± 1600 (n=5)	60 ± 20 (n=4)	100 ± 24 (n=5)	360 ± 48[a] (n=5)
6.0 mg/kg bw/d	47000 ± 2000[a,b] (n=6)	180 ± 50[a,b] (n=5)	360 ± 72[a,b] (n=6)	1200 ± 90[a,b] (n=6)

[a] Significantly higher than 0.1 mg/kg b.w. group, $p<0.05$; [b] significantly higher than 1 mg/kg b.w. group, $p<0.05$.

Table S6. Comparison of enantiomeric fractions of PCB 95 in blood and tissues from PCB 95-exposed female C57Bl/6 mice determined on the BDM and CD columns. Data are presented as the mean ± SD.

PCB 95 dose	Adipose		Blood		Brain		Liver	
	BDM[a]	CD[b]	BDM	CD	BDM	CD	BDM	CD
0.1 mg/kg bw/d	0.18 ± 0.00 (n=2)	0.20 ± 0.09 (n=3)	ND[c]	ND	ND	NA[d]	ND	ND
1.0 mg/kg bw/d	0.21 ± 0.02 (n=5)	0.19 ± 0.08 (n=3)	ND	0.09 ± 0.06 (n=4)	0.17 ± 0.02 (n=5)	NA	0.12 ± 0.01 (n=5)	0.12 ± 0.04 (n=5)
6.0 mg/kg bw/d	0.19 ± 0.01 (n=6)	0.16 ± 0.03 (n=6)	0.13 ± 0.01 (n=5)	0.13 ± 0.08 (n=4)	0.13 ± 0.01 (n=5)	NA	0.09 ± 0.01 (n=6)	0.10 ± 0.04 (n=6)

[a] BDM - ChiralDex B-DM (2,3-di-O-methyl-6-tert-butyl-silyl-β-cyclodextrin); [b] CD - Chirasil-Dex (2,3,6-tri-O-methyl-β-cyclodextrin); [c] ND: The samples were not analyzed on the enantioselective columns due to the low levels of PCB 95; [d] NA: A co-eluting impurity made it impossible to determine the EF values for brain samples on the CD column.

Table S7. PCB 95 levels [ng/g tissue] in blood and tissues of PCB 95-exposed female C57Bl/6 mice as predicted by published pharmacokinetic parameters [4].

PCB 95 dose	Adipose	Blood	Brain	Liver
0.1 mg/kg bw/d	250,000	470	2,800	4,600
1.0 mg/kg bw/d	2,500,000	6,400	41,000	70,000
6.0 mg/kg bw/d	15,000,000	39,000	250,000	430,000

Table S8. Enantiomeric fractions of PCB 95 in blood and tissues in female C57Bl/6 mice as predicted by published pharmacokinetic parameters [4].

PCB 95 dose	Adipose	Blood	Brain	Liver
0.1 mg/kg bw/d	0.39	0.23	0.30	0.32
1.0 mg/kg bw/d	0.40	0.27	0.35	0.35
6.0 mg/kg bw/d	0.40	0.27	0.36	0.36

Table S9. Levels of hydroxylated metabolites of PCB 95 [ng/g tissue] in blood and liver of female C57Bl/6 mice after sub-chronic exposure to PCB 95. Data are presented as the mean ± SD.

Blood					
PCB 95 dose	3-103	4-95	5-95	4,5-95	∑OH-PCBs
0.1 mg/kg bw/d (n=3)	ND[c]	26 ± 10	ND	ND	34 ± 9.8
1.0 mg/kg bw/d (n=4)	4.5 ± 0.6	149 ± 29	2.5 ± 2.9	26 ± 9.2	180 ± 35
6.0 mg/kg bw/d (n=5)	9.7 ± 4.5[a,b]	280 ± 110[a,b]	12.4 ± 2.3[b]	45 ± 20[b]	340 ± 120[a,b]
Liver					
PCB 95 dose	3-103	4-95	5-95	4,5-95	∑OH-PCBs
0.1 mg/kg bw/d (n=2)	ND	ND	3.9 ± 0.9	3.2 ± 1.5	16 ± 14
1.0 mg/kg bw/d (n=5)	5.4 ± 1.3	40 ± 6.4	34 ± 21	5.5 ± 0.7	84 ± 28
6.0 mg/kg bw/d (n=6)	22 ± 15	140 ± 66	72 ± 35	18 ± 7.5[a,b]	250 ± 120[a,b]

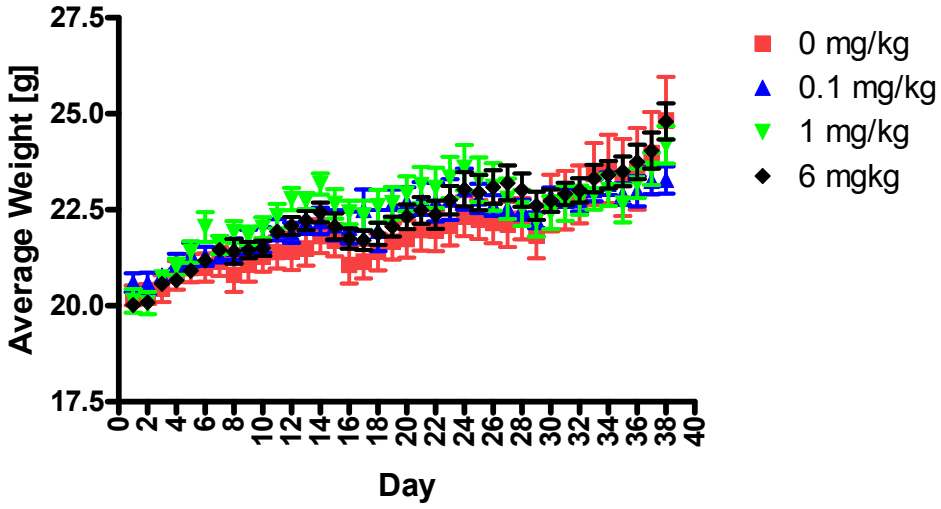
[a] Significantly higher than 0.1 mg/kg b.w. group, $p<0.05$; [b] significantly higher than 1 mg/kg b.w. group, $p<0.05$; [c] ND: Below detection limit (for detection limits and background levels in control animals, see Table S4).

Table S10. Enantiomeric fraction of hydroxylated metabolites of PCB 95 in blood and liver of female C57Bl/6 mice after sub-chronic exposure to PCB 95. Data are presented as the mean ± SD.

PCB 95 dose	4-95[a] (Racemic standard EF=0.47 ± 0.02)		5-95[b] (Racemic standard EF=0.49 ± 0.01)		4,5-95[c] (Racemic standard EF=0.49 ± 0.01)	
	Blood	Liver	Blood	Liver	Blood	Liver
0.1 mg/kg bw/d	ND[d]	ND	ND	ND	ND	ND
1.0 mg/kg bw/d	0.10 ± 0.02 (n=4)	ND	ND	0.60 ± 0.18[e] (n=3)	ND	ND
6.0 mg/kg bw/d	0.07 (n=2)	0.21 ± 0.03 (n=3)	ND	0.69 ± 0.09 (n=5)	0.34 ± 0.02 (n=5)	0.50 ± 0.07[f] (n=4)

[a] Analysis conducted on BGB column (BGB-172, 20% tert-butyldimethyl-silyl-β-cyclodextrin, 30 m x 250 µm x 0.25 µm); [b] analysis conducted on CD column (Chirasil-Dex, 2,3,6-tri-O-methyl-β-cyclodextrin); [c] analysis conducted on BDM column (ChiralDex B-DM, 2,3-di-O-methyl-6-tert-butyl-silyl-β-cyclodextrin); [d] ND: Samples were not analyzed because of low metabolites levels or because the peaks were below the detection limit in the enantioselective analysis; [e] the average implies enrichment of the first eluting enantiomer, but one sample (out of n=3) showed enrichment of the second enantiomer; [f] two samples showed some enrichment of the first, and two samples of the second eluting enantiomer.

A.



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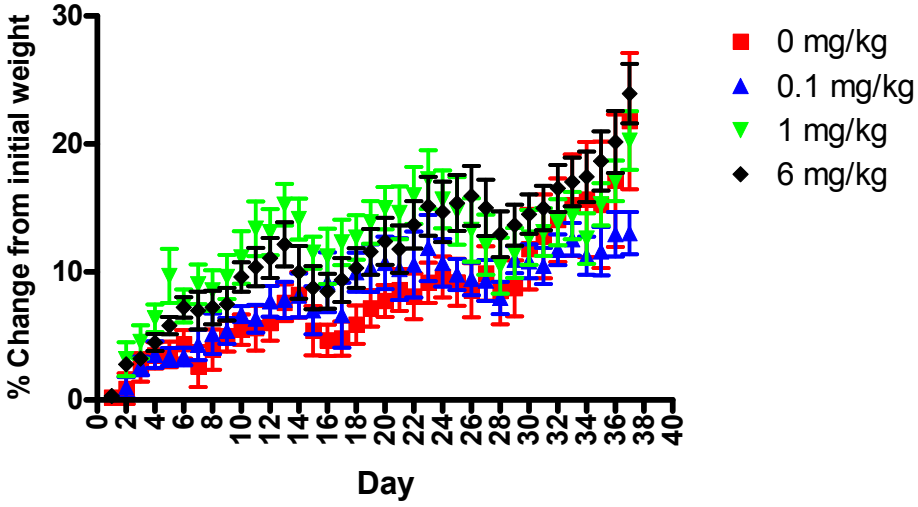

Figure S1. PCB 95 exposure did not alter body weight (A) or growth rate (B) of female C57Bl/6 mice throughout the exposure period. Growth rate was defined as the difference between body weight on the day measured and the initial weight on day 0 divided by the body weight on the day of measurement multiplied by 100.

REFERENCES

1. Kania-Korwel, I.; Shaikh, N.; Hornbuckle, K. C.; Robertson, L. W.; Lehmler, H.-J., Enantioselective disposition of PCB 136 (2,2',3,3',6,6'-hexachlorobiphenyl) in C57BL/6 mice after oral and intraperitoneal administration. *Chirality* **2007,** *19*, 56-66.
2. Pfaffl, M. W., A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **2001,** *29*, (9), e45.
3. Pfaffl, M. W.; Horgan, G. W.; Dempfle, L., Relative expression software tool (REST©) for groupwise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res.* **2002,** *30*, (9), e36.
4. Kania-Korwel, I.; El-Komy, M. H. M. E.; Veng-Pedersen, P.; Lehmler, H. J., Clearance of polychlorinated biphenyl atropisomers is enantioselective in female C57Bl/6 mice. *Environ. Sci. Technol.* **2010,** *44*, (8), 2828-2835.