# HEPATIC METABOLISM AFFECTS THE ATROPSELECTIVE DISPOSITION OF 2,2',3,3',6,6'-HEXACHLOROBIPHENYL (PCB 136) IN MICE

# SUPPORTING INFORMATION

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**Abbreviations:**

3-150, 2,2',3',4,6,6'-hexachlorobiphenyl-3-ol

4,5-136, 2,2',3,3',6,6'-hexachlorobiphenyl-4,5-diol

4-136, 2,2',3,3',6,6'-hexachlorobiphenyl-4-ol

5-136, 2,2',3,3',6,6'-hexachlorobiphenyl-5-ol

ARC, activity-regulated cytoskeleton-associated protein

BDM, ChiralDex BDM

CB, Cyclosil-B

cpr, cytochrome P450 oxidoreductase

EF, enantiomeric fraction

HPRT, hypoxanthine-guanine phosphoribosyltransferase

KO, mice with liver-specific deletion of the NADPH-cytochrome P450 reductase gene

*LOD*, limits of detection

MBP, myelin basic protein

nd, not determined

HO-PCB 136, hydroxylated PCB 136

PCB 136, 2,2',3,3',6,6'-hexachlorobiphenyl

PGK1, phosphoglycerate kinase

PPIA, peptidylprolyl isomerase A

qPCR, quantitative polymerase chain reaction

RC3, neurogranin

SD, standard deviation

SE, standard error

SPN, spinophilin

WT, wild type mice

**Table S1:** Comparison of total P450 content and enzyme activities of 8-week old naïve female mice with liver-specific deletion of the *cpr* gene (KO; n = 6) and congenic wild type mice (WT; n = 5).

| **Liver enzyme measurement**[a] | **KO (n=6)** | **WT (n=5)** |
|---|---|---|
| Total P450 content[b] [nmol/mg protein] | 1.6±0.3* | 0.7±0.2 |
| 7-Ethoxyresorufin-*O*-deethylase (EROD) activity[c] [nmol/mg protein*min] | 0.09±0.01* | 0.13±0.01 |
| 7-Benzyloxyresorufin-*O*-debenzylase (BROD) activity[c] [nmol/mg protein*min] | 0.07±0.04 | 0.10±0.02 |
| Cytochrome P450 reductase activity[d] [nmol/mg protein*min] | 1.9±0.5* | 88±17 |

[a] Enzyme activities were measured in liver microsomes from 8 week old naïve female KO (n = 6) and WT mice (n = 5). Briefly, liver microsomes were prepared by differential centrifugation as described previously.[1] The microsomal pellets were resuspended in 0.25 M sucrose and 0.2 mL aliquots were stored at -80 °C. Microsomal protein concentrations were measured as described by Lowry et al.[2] using bovine serum albumin as the standard.

[b] The total cytochrome P450 content in microsomes was determined from CO difference spectra of dithionite-reduced microsomes using an extinction coefficient of 91 $cm^{-1}$ $mM^{-1}$ between $A_{450}$ and $A_{490}$.[3]

[c] Microsomal 7-ethoxyresorufin-*O*-deethylase (EROD) and 7-benzyloxyresorufin-*O*-debenzylase (BROD) activities were determined using established methods to assess P450 1A and P450 2B activities, respectively.[1]

[d] The activity of NADPH-cytochrome P450 reductase was determined as the rate of the reduction of cytochrome c as quantified spectrophotometrically at 550 nm using the Cytochrome c Reductase (NADPH) Assay Kit (Sigma-Aldrich, Saint Louis, Missouri, USA) according to the manufacturer's instructions.

* Significantly different compared to WT mice (t-test, $p<0.05$).

**Table S2. Liver-specific *cpr* deletion of the cytochrome P450 reductase gene has no significant effect on transcript levels of plasticity-associated genes[a] across different brain regions (i.e., cortex, hippocampus and cerebellum), as determined using quantitative polymerase chain reaction (qPCR).[b]** The observation that the liver-specific deletion of the *cpr* gene does not, in and of itself, interfere with markers of neurodevelopment suggest that this transgenic mouse model is a potentially useful genetic model to study how hepatic metabolism modulates the developmental neurotoxicity of PCBs.

| Gene | Cortex | | | Hippocampus | | | Cerebellum | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fold-Change[c] | 95% CI[d] | p Value[d] | Fold-Change[c] | 95% CI[d] | p Value[d] | Fold-Change[c] | 95% CI[d] | p Value[d] |
| ARC | 1.1 | 0.07-9.4 | 0.9 | 1.1 | 0.4-5.4 | 0.8 | 0.9 | 0.5-1.8 | 0.7 |
| MBP | 0.6 | 0.1-1.3 | 0.07 | 0.9 | 0.4-1.8 | 0.4 | 0.8 | 0.5-1.6 | 0.1 |
| RC3 | 1.2 | 0.2-6.6 | 0.6 | 1.1 | 0.5-2.5 | 0.7 | 0.5 | 0.04-5.9 | 0.3 |
| SPN | 1.1 | 0.3-4.8 | 0.6 | 0.7 | 0.2-2.0 | 0.3 | 0.8 | 0.3-3.2 | 0.3 |

[a] Four plasticity-associated genes were selected based on earlier studies demonstrating their usefulness as biomarkers of synaptic plasticity. ARC is an immediate early gene whose expression is modulated by activity, and it plays a critical role in AMPA receptor trafficking and synaptic plasticity in general.[4] SPN[5, 6] and RC3/neurogranin[7] are selectively localized to postsynaptic densities, and are implicated in regulating synaptic plasticity. Because they are induced by activity, spinophilin and ARC are useful biomarkers of not only synapse density but also synaptic plasticity.[8, 9] RC3/neurogranin is of particular interest in the context of POP neurotoxicity because it is encoded by a thyroid-responsive gene and its transcription is upregulated by POPs.[10, 11]

[b] Transcript levels of activity-regulated cytoskeleton-associated protein (ARC), myelin basic protein (MBP), neurogranin (RC3), and spinophilin (SPN) were measured by qPCR as biomarkers of synaptic plasticity in hippocampi, cortices, and cerebella in untreated KO (n=10) and age-matched congenic WT mice (n=6). Briefly, tissue samples were collected in 600 µL of Buffer RLT (Qiagen, Germantown, MD) with β-mercaptoethanol (Bio-Rad, Hercules, CA) and stored at -20 °C. Two grinding beads (4-mm diameter, stainless steel beads, SpexCertiprep, Metuchen, NJ) were added and the tissues were homogenized in a GenoGrinder 2000 (SpexCertiprep) for 2 min at 1000 strokes per minute. Total RNA was extracted from the tissue lysates using a BioSprint automated nucleic acid workstation (Qiagen) according to manufacturer instructions for the One-For-All Vet Kit (Qiagen). The Quantitect Reverse Transcription Kit (Qiagen) was used for cDNA synthesis following manufacturer instructions with previously described modifications.[12] Each qPCR reaction contained a final concentration of 400 nM of each primer and 80 nM of the TaqMan probe diluted in Taqman Universal PCR Master Mix (TaqMan, Applied Biosystems, Foster City, CA, USA). Five µL of cDNA was added to 384-well plates in duplicate and amplified in an automated fluorometer (ABI PRISM® 7900 HT fast detection system, Carlsbad, CA, USA). Amplification conditions have previously been described.[13] Fluorescent signals were collected during the annealing temperature and quantification cycle (Cq) values extracted with a threshold of 0.2 and baseline values of 3-15. Sequences of primers and probes used for qPCR assessment of synaptic plasticity-associated genes are presented in Table S2a; amplification efficiencies of reference and synaptic plasticity-associated genes are summarized in Table S2b.

[c] Changes in gene expression are expressed as fold-change in expression between target and reference genes in KO relative to WT mice. Hypoxanthine-guanine phosphoribosyltransferase (HPRT), phosphoglycerate kinase (PGK1), and peptidylprolyl isomerase A (PPIA) were measured as reference genes.[14]

[d] 95% confidence intervals (CI) and p values were calculated by REST2009 software.

**Table S2a:** Sequences of primers and probes used for qPCR assessment of synaptic plasticity-associated genes.

| Gene (Full name) | Primer or Probe | Sequence |
|---|---|---|
| ARC (Activity-regulated cytoskeleton-associated protein) | FP | 5'-GATCTGGCTTCCTCATTCTGCT-3' |
| | RP | 5'-GTTCCCTCAGCATCTCTGCTTT-3' |
| | Probe | 5'-/56-FAM/AGTGTCCAGGGCTCTTTGGGTAATCA AGA/3BHQ1/-3' |
| MBP (Myelin basic protein) | FP | 5'-CTACCCATTATGGCTCCCTGC-3' |
| | RP | 5'-GGTGTTCGAGGTGTCACAATGT-3' |
| | Probe | 5'-/56-FAM/CACGGCCGGACCCAAGATGAA /3BHQ1/-3' |
| RC3 (Neurogranin) | FP | 5'-CCAGACGACGATATTCTTGACAT-3' |
| | RP | 5'-TTTATCTTCTTCCTCGCCATGTG-3' |
| | Probe | 5'-/56-FAM/CCCGGAGCCAACGCCGCT/3BHQ1/-3' |
| SPN (Spinophilin) | FP | 5'-AAGGCGGCCCACCATAA-3' |
| | RP | 5'-GCCCATCTGCAGGAACATACTT-3' |
| | Probe | 5'-/56-FAM/TATGGCTCCAACGTCCACCGCATC /3BHQ1/-3' |
| HPRT (Hypoxanthine-guanine phosphoribosyltransferase) | FP | 5'-AGCAGGTCAGCAAAGAACT-3' |
| | RP | 5'-CCTCATGGACTGATTATGGACA-3' |
| | Probe | 5'-/56-FAM/ATTGTGGCC/ZEN/CTCTGTGTGCTCA /3IABkFQ/-3' |
| PGK1 (Phosphoglycerate kinase 1) | FP | 5'-AGCCTTGATCCTTTGGTTGT-3' |
| | RP | 5'-CTGACTTTGGACAAGCTGGA-3' |
| | Probe | 5'-/56-FAM/CGTGATGAG/ZEN/GGTGGACTTCAAC GT/3IABkFQ/-3' |
| PPIA (Peptidylprolyl isomerase A) | FP | 5'-TTCACCTTCCCAAAGACCAC-3' |
| | RP | 5'-CAAACACAAACGGTTCCCAG-3' |
| | Probe | 5'-/56-FAM/TGCTTGCCA/ZEN/TCCAGCCATTCAG /3IABkFQ/-3' |

**Table S2b:** Amplification efficiency[a] of reference and synaptic plasticity-associated genes.

| Gene | Tissue | E[b] | $R^2$ |
|---|---|---|---|
| ARC | Cortex | 100% | 0.996 |
| | Hippocampus | 102.5% | 1.000 |
| | Cerebellum | 94.1% | 0.992 |
| MBP | Cortex | 102.7% | 0.999 |
| | Hippocampus | 101.3% | 0.996 |
| | Cerebellum | 102.8% | 0.999 |
| RC3 | Cortex | 98.6% | 0.998 |
| | Hippocampus | 101.2% | 0.995 |
| | Cerebellum | 95.0% | 0.995 |
| SPN | Cortex | 102.8% | 0.994 |
| | Hippocampus | 103.3% | 0.997 |
| | Cerebellum | 104.5% | 0.997 |
| HPRT | Cortex | 104.9% | 0.992 |
| | Hippocampus | 98.7% | 0.997 |
| | Cerebellum | 97.3% | 0.992 |
| PGK1 | Cortex | 96.7% | 0.996 |
| | Hippocampus | 97.3% | 0.993 |
| | Cerebellum | 99.5% | 0.996 |
| PPIA | Cortex | 101.1% | 0.998 |
| | Hippocampus | 96.9% | 0.999 |
| | Cerebellum | 96.7% | 0.996 |

[a] Efficiency was determined from brain regions of WT animals; [b] E = efficiency.

**Table S3:** Body and organ weights of 8-weeks old female WT (n = 4) and KO mice (n = 7) dosed with PCB136 or vehicle (cookie).[a]

| Dose | Mouse strain | Body weight [g] | | | | Organ weight [g] | | | | | | Organ adjusted by b.w. [%] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Day 1 | Day 2 | Day 3 | Brain | Heart | Kidney | Liver | Lung | Spleen | Brain | heart | Kidney | Liver | Lung | Spleen |
| Vehicle | WT (n=5) | 19±1 | 20±1 | 20±1 | 20±1 | 0.38±0.06 | 0.12±0.02 | 0.25±0.01 | 1.01±0.10 | 0.15±0.02 | 0.07±0.01 | 1.90±0.32 | 0.59±0.09 | 1.28±0.06 | 5.11±0.55 | 0.74±0.12 | 0.37±0.05 |
| | KO (n=5) | 19±2 | 19±1 | 19±1 | 19±1 | 0.41±0.04 | 0.11±0.02 | 0.23±0.04 | 1.36±0.25* | 0.13±0.02 | 0.08±0.03 | 2.16±0.25 | 0.60±0.09 | 1.19±0.15 | 7.22±1.33* | 0.69±0.06 | 0.42±0.12 |
| PCB 136 | WT (n=4) | 20±1 | 20±1 | 20±1 | 20±1 | 0.41±0.03 | 0.11±0.03 | 0.25±0.02 | 1.03±0.09 | 0.14±0.01 | 0.08±0.01 | 2.06±0.13 | 0.56±0.16 | 1.27±0.05 | 5.22±0.34 | 0.69±0.03 | 0.39±0.03 |
| | KO (n=7) | 19±2 | 18±2 | 19±2 | 18±2 | 0.41±0.03 | 0.11±0.01 | 0.20±0.06 | 1.46±0.17* | 0.13±0.01 | 0.06±0.02 | 2.20±0.19 | 0.57±0.05 | 1.09±0.29 | 7.86±0.51* | 0.69±0.03 | 0.34±0.06 |

[a] WT and KO mice received a single oral dose PCB 136 (30 mg/kg b.w.) on a Vanilla Wafer cookie (7.5 g/kg b.w.); WT and KO control groups received the vehicle (Vanilla Wafer cookie; 7.5 g/kg b.w.) alone. * Significantly different compared to WT mice in the respective treatment group (t-test, $p<0.05$); values are means ± SD.

**Table S4:** Extractable lipid content expressed as percent of tissue or feces wet weight (%) in PCB 136 or vehicle-treated WT (n = 4) and KO mice (n = 7).[a]

| Mice | Dose | Adipose | Brain | Liver | Feces (day 1) | Feces (day 2) | Feces (day 3) |
|---|---|---|---|---|---|---|---|
| WT | Vehicle (n=5) | 72±15 | 9.3±0.4 | 8.8±0.6 | 5.8±0.7 | 5.1±0.2 | 6.0±0.3 |
| | PCB 136 (n=4) | 76±18 | 9.0±0.3 | 8.1±0.9 | 5.4±0.2 | 5.5±0.7 | 6.8±0.8 |
| | Combined (n=9) | 74±16 | 9.1±0.4 | 8.4±0.8 | 5.6±0.5 | 5.4±0.9 | 6.2±0.5 |
| KO | Vehicle (n=5) | 64±21 | 9.7±0.3 | 21±4* | 6.4±0.5 | 6.6±1.1 | 7.3±0.8* |
| | PCB 136 (n=7) | 88±7 | 8.9±0.5 | 21±3* | 7.0±0.8* | 6.9±1.8 | 8.0±1.2 |
| | Combined (n=12) | 85±7 | 9.2±0.6 | 21±3* | 6.8±0.8* | 6.8±1.5* | 7.7±1.1* |

[a] Lipids were extracted from tissue and feces samples by pressurized liquid extraction and lipid weights were determined gravimetrically as described under Experimental Procedures. * Significantly different compared to WT mice in the respective treatment group (t-test, $p<0.05$); values are means ± SD.

**Table S5:** Limits of detection (*LODs*), limits of quantification (*LOQs*) and background levels of PCB 136 and its metabolites in tissues from mice dosed with vehicle alone. Background levels are adjusted by wet weight or extracted dry lipid content.[a]

| PCBs | | PCB 136 | 3-150 | 5-136 | 4-136 | 4,5-136 |
|---|---|---|---|---|---|---|
| *LOD [ng]*[b] *(n = 4)* | | 3.0 | 0.6 | 4.8 | 1.6 | 1.1 |
| *LOQ [ng]*[c] *(n = 4)* | | 30 | 6 | 48 | 16 | 11 |
| Background levels adjusted by wet weight [ng/g] (n = 10)[d] | Adipose | 6.6 ±4.9 | 1.4±0.9 | 5.9±5.2 | 1.9±1.1 | 1.3±0.9 |
| | Brain | 0.8±1.3 | nd | 3.4±2.7 | 0.5±0.5 | 0.2±0.2 |
| | Liver | 6.4±4.1 | 0.5±0.4 | 2.6±1.2 | 0.5±0.6 | 1.0±1.0 |
| | Feces (day 1) | 2.1±2.7 | 0.3±0.3 | 3.3±2.4 | 0.6±0.5 | 0.2±0.3 |
| | Feces (day 2) | 1.6±1.8 | 0.1±0.2 | 14.1±23.9 | 1.8±2.4 | 0.2±0.3 |
| | Feces (day 3) | 2.7±2.1 | 0.7±0.7 | 3.2±3.2 | 0.3±0.3 | 0.1±0.2 |
| Background levels adjusted by lipid weight [µg/g] (n = 10)[d] | Adipose | 0.010±0.007 | 0.002±0.002 | 0.009±0.009 | 0.003±0.003 | 0.002±0.002 |
| | Brain | 0.008±0.014 | nd | 0.036±0.028 | 0.005±0.005 | 0.002±0.002 |
| | Liver | 0.058±0.049 | 0.005±0.004 | 0.023±0.011 | 0.005±0.005 | 0.009±0.007 |
| | Feces (day 1) | 0.22±0.21 | 0.07±0.05 | 0.63±0.40 | 0.12±0.10 | 0.02±0.04 |
| | Feces (day 2) | 0.029±0.034 | 0.002±0.003 | 0.26±0.45 | 0.033±0.046 | 0.004±0.006 |
| | Feces (day 3) | 0.040±0.027 | 0.011±0.011 | 0.046±0.044 | 0.004±0.005 | 0.001±0.003 |

[a] PCB 136 and metabolites were extracted by pressurized liquid extraction and analyzed on a gas chromatograph equipped with a $^{63}$Ni-µECD detector, as described under Experimental Procedures. Values are means ± SD. [b] The *LOD*s were calculated based on blank samples containing Florisil and diatomaceous earth only. The blank samples were analyzed in parallel with tissue samples. The *LOD*s were calculated as $LOD = \overline{x_b} + k \cdot s_b$, where $\overline{x_b}$ is mean of all blank samples, $k$ is Student's t-value for n-1 degrees of freedom at 99% confidence level, and $s_b$ is standard deviation of the blank measures.[15] [c] The *LOQ* was conservatively calculated as $LOQ = 10 \cdot LOD$. [d] Average background levels in all control mice treated with vehicle as described under Experimental Procedures. nd, not detected.

**Table S6:** Limits of detection (*LODs*), limits of quantification (*LOQs*) and background levels of PCB 136 and its metabolites in blood and urine from animals dosed with vehicle alone.[a]

| PCBs | | PCB 136 | 3-150 | 5-136 | 4-136 | 4,5-136 |
|---|---|---|---|---|---|---|
| *LOD [ng]*[b] *(n = 6)* | | 8.5 | 1.2 | 7.1 | 2.1 | 0.8 |
| *LOQ [ng]*[c] *(n = 6)* | | 85 | 12 | 71 | 21 | 8 |
| Background levels [ng/g] | Blood | 0.9±0.3 | 0.6±0.2 | 2.0±0.9 | 1.0±0.4 | 0.4±0.2 |
| | Urine | 0.2±0.1 | nd | 2.0±0.2 | 0.4±0.1 | 0.2±0.1 |

[a] PCB 136 and metabolites were extracted with liquid-liquid extraction and analyzed on a gas chromatograph equipped with a $^{63}$Ni-μECD detector, as described under Experimental Procedures. Values are means ± SD. [b] The *LOD*s for blood and urine samples were calculated based on blank samples containing buffer only. The blank samples were analyzed in parallel with the respective blood or urine samples. The *LOD*s were calculated as $LOD = \overline{x_b} + k \cdot s_b$, where $\overline{x_b}$ is mean of all blank samples, $k$ is Student's t-value for n-1 degrees of freedom at 99% confidence level, and $s_b$ is standard deviation of the blank measures.[15] [c] The *LOQ* was conservatively calculated as $LOQ = 10 \cdot LOD$. nd, not determined.

**Table S7:** Concentrations of PCB 136 (ng/g wet weight) in tissues, blood and excreta in WT (n = 4) and KO mice (n = 7) after oral administration of PCB 136.

| Tissue | WT | KO |
|---|---|---|
| Adipose | 46000±15000 | 80000±30000$ |
| Blood | 28±9 | 100±20* |
| Brain | 140±26 | 800±180* |
| Liver | 410±120 | 16000±3900* |
| Feces (day 1) | 4200±1900 | 16000±9500* |
| Feces (day 2) | 140±50 | 800±320* |
| Feces (day 3) | 98±30 | 560±140* |
| Urine (day 1) | 83±67 | 320±220* |
| Urine (day 2) | / | 110±80* |
| Urine (day 3) | / | / |

* Significantly different compared to PCB-treated WT mice (t-test, $p<0.05$); $ different compared to PCB-treated WT mice (t-test, $p=0.06$); / lower than detection limit (see Tables S5 and S6); values are means ± SD.

**Table S8:** Concentrations of HO-PCB 136 metabolites (ng/g wet weight) in tissues and excreta in WT (n = 4) and KO mice (n = 7) after oral administration of PCB 136.

| Tissue | WT | | | | KO | | | |
|---|---|---|---|---|---|---|---|---|
| | 3-150 | 5-136 | 4-136 | 4,5-136 | 3-150 | 5-136 | 4-136 | 4,5-136 |
| Adipose | / | / | / | / | / | / | / | / |
| Blood | / | 13±3 | 31±9 | 20±4 | / | 20±8 | 16±8* | 24±10 |
| Brain | / | / | / | / | / | / | / | / |
| Liver | 2.3±0.5 | 83±27 | 38±8 | 12±0 | 2.9±0.7 | 130±30* | 31±10 | 27±5* |
| Feces (day 1) | 690±220 | 92000±21000 | 19000±5400 | 940±200 | 61±28* | 51000±17000* | 4400±1700* | 500±180* |
| Feces (day 2) | 69±30 | 17000±5100 | 4400±1300 | 390±120 | 43±27 | 24000±5700 | 2500±1000$ | 450±160 |
| Feces (day 3) | 49±30 | 12000±4100 | 3800±1500 | 240±79 | 35±15 | 20000±4300* | 2100±710 | 430±150* |
| Urine (day 1) | / | 78±58 | 31±25 | 23±3 | / | 89±24 | 25±10 | 8±4 |
| Urine (day 1) (**with enzyme**) | / | 330±180# | 25±16 | 210±110# | / | 280±140# | 17±7 | 94±39# |
| Urine (day 2) | / | / | / | / | / | / | / | / |
| Urine (day 2) (**with enzyme**) | / | / | / | 48±29# | / | 84±23# | / | 44±12# |
| Urine (day 3) | / | / | / | / | / | / | / | / |
| Urine (day 3) (**with enzyme**) | / | / | / | / | / | / | / | / |

# Significantly increased HO-PCB 136 urine levels after β-glucuronidase/sulfatase deconjugation compared to urine without β-glucuronidase/sulfatase treatment;
* significantly different compared to PCB-treated WT mice (t-test, $p<0.05$); $ different compared to PCB-treated WT mice (t-test, $p=0.05$); / lower than detection limit (see Tables S5 and S6); values are means ± SD.

**Table S9**: Lipid adjusted concentrations of PCB 136 (µg/g lipid) in tissues and feces in WT (n = 4) and KO mice (n = 7) after oral administration of PCB 136.

| Tissue | WT (µg/g lipid) | KO (µg/g lipid) |
|---|---|---|
| Adipose | 61±8 | 92±40 |
| Brain | 1.6±0.3 | 9.1±2.3* |
| Liver | 5.0±1.2 | 75±17* |
| Feces (day 1) | 770±370 | 2200±1100* |
| Feces (day 2) | 2.5±1.0 | 12±6* |
| Feces (day 3) | 1.2±0.2 | 7.6±2.0* |

* Significantly different compared to PCB-treated WT mice (t-test, $p<0.05$); values are means ± SD.

**Table S10:** Lipid adjusted concentrations of HO-PCB 136 metabolites (µg/g lipid) in tissues and feces in WT (n = 4) and KO mice (n = 7) after oral administration of PCB 136.

| Tissue | WT (µg/g lipid) | | | | KO (µg/g lipid) | | | |
|---|---|---|---|---|---|---|---|---|
| | 3-150 | 5-136 | 4-136 | 4,5-136 | 3-150 | 5-136 | 4-136 | 4,5-136 |
| Adipose | / | / | / | / | / | / | / | / |
| Brain | / | / | / | / | / | / | / | / |
| Liver | 0.03±0.01 | 1.0±0.3 | 0.47±0.07 | 0.15±0.01 | 0.01±0.00* | 0.63±0.15$ | 0.15±0.04* | 0.13±0.03 |
| Feces (day 1) | 130±40 | 17000±3800 | 3600±1000 | 170±40 | 9±5* | 7600±3200* | 660±320* | 74±30* |
| Feces (day 2) | 1.3±0.6 | 320±130 | 82±30 | 7.3±2.9 | 0.7±0.5 | 360±140 | 38±20$ | 6.9±3.5 |
| Feces (day 3) | 0.5±0.1 | 140±30 | 44±12 | 2.8±0.7 | 0.5±0.3 | 250±90$ | 27±13 | 5.6±2.9$ |

* Significantly different compared to PCB-treated WT mice (t-test, $p<0.05$); $ different compared to PCB-treated WT mice (t-test, $0.05 \leq p<0.1$); / lower than detection limit (see Tables S5 and S6); values are means ± SD.

**Table S11:** The amount of PCB 136 and HO-PCB 136 metabolites in tissues, blood and excreta expressed as percent of the total PCB 136 dose in WT (n = 4) and KO mice (n = 7) after oral administration of PCB136.

| Tissue / Excreta | WT (% of total dose) | | | | | | KO (% of total dose) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PCB 136 | 3-150 | 5-136 | 4-136 | 4,5-136 | total | PCB 136 | 3-150 | 5-136 | 4-136 | 4,5-136 | total |
| Adipose[@] | 9.1±2.9 | / | / | / | / | 9.1±2.9 | 16±6 [$] | / | / | / | / | 16±6 [$] |
| Blood[@] | 0.006±0.002 | / | 0.002±0.001 | 0.006±0.002 | 0.003±0.001 | 0.01±0.00 | 0.02±0.01[*] | / | 0.004±0.002 | 0.003±0.002 | 0.004±0.002 | 0.03±0.01[*] |
| Brain | 0.01±0.00 | / | / | / | / | 0.01±0.00 | 0.05±0.01[*] | / | / | / | / | 0.05±0.01[*] |
| Liver | 0.07±0.02 | 0 | 0.01±0.01 | 0.006±0.001 | 0.002±0.000 | 0.09±0.02 | 4.2±1.1[*] | 0.001±0.000[*] | 0.03±0.01[*] | 0.008±0.003 | 0.006±0.001[*] | 4.2±1.1[*] |
| Feces (day 1) | 0.89±0.22 | 0.14±0.03 | 19±3 | 4.0±0.9 | 0.18±0.01 | 24±4 | 4.5±3.6[*] | 0.01±0.01[*] | 12±3[*] | 1.0±0.3[*] | 0.10±0.02[*] | 17±3[*] |
| Feces (day 2) | 0.04±0.01 | 0.02±0.01 | 4.4±1.2 | 1.1±0.2 | 0.09±0.02 | 5.7±1.4 | 0.24±0.13[*] | 0.01±0.01 | 6.5±2.2[$] | 0.66±0.21[*] | 0.11±0.04 | 7.5±2.6 |
| Feces (day 3) | 0.02±0.01 | 0.01±0.01 | 2.6±0.8 | 0.83±0.31 | 0.05±0.02 | 3.5±1.3 | 0.18±0.11[*] | 0.01±0.01 | 5.7±2.9[*] | 0.60±0.32 | 0.11±0.06[*] | 6.6±3.3[$] |
| Feces (total) | 0.95±0.23 | 0.17±0.04 | 26±4 | 5.9±1.3 | 0.32±0.04 | 33±6 | 4.9±3.6[*] | 0.04±0.01[*] | 24±4 | 2.3±0.4[*] | 0.33±0.07 | 31±3 |
| Urine (day 1)[&] | 0.02±0.02 | / | 0.05±0.00 | 0.003±0.000 | 0.03±0.01 | 0.11±0.01 | 0.05±0.03[*] | / | 0.04±0.02 | 0.002±0.001[*] | 0.01±0.01[*] | 0.10±0.05 |
| Urine (day 2)[&] | / | / | / | / | 0.01±0.00 | 0.01±0.00 | 0.01±0.01[*] | / | 0.01±0.00[*] | / | 0.005±0.001[*] | 0.03±0.01[*] |
| Urine (day 3)[&] | / | / | / | / | / | / | / | / | / | / | / | / |
| Urine (total) [&] | 0.02±0.02 | / | 0.05±0.00 | 0.003±0.000 | 0.04±0.01 | 0.12±0.01 | 0.06±0.05 | / | 0.05±0.02 | 0.002±0.001[*] | 0.02±0.01[*] | 0.13±0.06 |
| Total | | | | | | 43±8 | | | | | | 52±7 |

[@] The percentage of body weight was assumed to be 5.9% b.w. for adipose and 5.85% b.w. for blood;[16] [&] calculated based on the total amount of PCB 136 and/or HO-PCB 136 determined after β-glucuronidase/sulfatase deconjugation; * significantly different compared to PCB-treated WT mice (t-test, $p<0.05$); [$] different compared to PCB-treated WT mice (t-test, $0.05 \leq p < 0.1$); / lower than detection limit (see Tables S5 and S6); values are means ± SD.

**Table S12:** Comparison of the enantiomeric fraction (EF) of the PCB 136 and its metabolites, 5-136 and 4-136, in tissues and excreta from WT (n = 4) and KO mice (n = 7) after oral administration of PCB136.[a]

| Tissue/Excreta | | WT mice | | | | KO mice | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PCB136 | | 5-136 | 4-136 | PCB136 | | 5-136 | 4-136 |
| | | BDM | CB | (BDM) | (CB) | BDM | CB | (BDM) | (CB) |
| Adipose | | - | 0.584±0.003 | - | / | - | 0.58±0.01 | - | / |
| Blood | | 0.62[b] | 0.63±0.06 | 0.47[b] | 0.94±0.01 | 0.64[b] | 0.66±0.03 | 0.31[b] | 0.88±0.05[*] |
| Brain | | - | 0.65±0.01 | - | / | - | 0.65±0.02 | - | / |
| Liver | | 0.74±0.03 | 0.74±0.03 | 0.53±0.06[*N] | 0.67±0.02 | 0.70±0.03[*] | 0.70±0.03 | 0.62±0.03[$] | 0.59±0.05[$] |
| Feces (day 1) | | 0.57±0.01 | 0.58±0.02 | 0.43±0.06 | 0.43±0.02 | 0.53±0.02[*] | 0.53±0.02[*] | 0.33±0.01[*] | 0.33±0.01[*] |
| Feces (day 2) | | 0.63±0.08 | 0.63±0.01 | 0.52±0.01[*N] | 0.61±0.02 | 0.65±0.03 | 0.64±0.02 | 0.41±0.01[*] | 0.43±0.04[*] |
| Feces (day 3) | | 0.74±0.02 | 0.67±0.03 | 0.54±0.01 | 0.69±0.01 | 0.70±0.03[$] | 0.68±0.02 | 0.46±0.02[*] | 0.50±0.04[*,N] |
| Urine day 1 | with enzyme[c] | 0.76±0.11[d,N] | 0.71±0.11 | 0.22±0.06[d] | 0.34±0.05 | 0.84±0.01[d] | 0.83±0.02 | 0.25±0.03[d] | 0.35±0.05 |
| | without enzyme[c] | | 0.72±0.13 | | 0.31±0.10 | | 0.79±0.06 | | 0.37±0.05 |
| Urine day 2 | with enzyme[c] | / | / | / | / | / | 0.82±0.10 | / | / |
| | without enzyme[c] | / | / | / | / | / | 0.80±0.13 | / | / |
| Urine day 3 | with enzyme[c] | / | / | / | / | / | / | / | / |
| | without enzyme[c] | / | / | / | / | / | / | / | / |

[a] EF values were determined separately using the formula EF = Area $_{E(2)}$/(Area $_{E(1)}$ + Area $_{E(2)}$) on BDM (ChiralDEX BDM column, 30 m length x 0.25 mm inner diameter, 0.12 µm film thickness) and CB (Cyclosil-B column, 30 m length x 0.25 mm diameter, 0.25 µm film thickness) columns; the EF values for the racemic standards of PCB 136 on the BDM column and CB column, 5-136 on the BDM column and 4-136 on the CB column were 0.50, 0.50, 0.51 and 0.50, respectively; [b] blood samples were pooled by genotype to create a single sample for the enantioselective analysis; [c] with or without β-glucuronidase/sulfatase deconjugation; [d] samples were pooled by genotype to created three pooled samples; - not determined on this column; * significantly different compared to PCB-treated WT mice (t-test, $p<0.05$); [$] different compared to PCB-treated WT mice (t-test, $0.05\leq p<0.1$); [N] EF value is not significantly different from the respective racemic standard (t-test, $p<0.05$); / lower than detection limit (see Tables S5 and S6); values are means ± SD.

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