# EFFECT OF PREGNANCY ON THE DISPOSITION OF 2,2',3,5',6-PENTACHLOROBIPHENYL (PCB 95) ATROPISOMERS AND THEIR HYDROXYLATED METABOLITES IN FEMALE MICE

## Supporting Information

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**ABBREVIATIONS:**

| | |
|---|---|
| 4-95 | 2,2',3,5',6-pentachlorobiphenyl-4-ol |
| 5-95 | 2,2',3,5',6-pentachlorobiphenyl-5-ol |
| X'-95 | unidentified mono-hydroxylated metabolite of PCB 95 |
| 4,5-95 | 2,2',3,5',6-pentachlorobiphenyl-4,5-diol |
| ASD | autism spectrum disorder |
| BDM | ChiralDex B-DM column (2,3-di-O-methyl-6-tert-butyl-silyl-β-cyclodextrin) |
| CD | Chirasil-Dex enantioselective column: (2,3,6-tri-O-methyl-β-cyclodextrin) |
| EF | enantiomeric fraction |
| HS-SPME | head space-solid phase microextraction |
| MDL | method detection limit |
| ND | below detection limit |
| NR | not resolved |

**Table S1.** Quality assurance/quality control data for gas chromatographic quantification of PCB 95 and metabolites in tissues.

| | **PCB 95** | **3-103** | **4-95** | **4'-95** | **5-95** | **4,5-95** |
|---|---|---|---|---|---|---|
| **Adipose** | | | | | | |
| Mean tissue mass [g] | | | 0.27 ± 0.14 | | | |
| MDL[a] [ng] (n=9) | 0.23 | ND | ND | 9.1 | 0.15 | 0.37 |
| MDL[b] [ng/g] | 0.84 | ND | ND | 33 | 0.55 | 1.4 |
| Control animals [ng/g] (n=2) | 1.0 ± 1.0 | ND | ND | ND | ND | ND |
| **Blood** | | | | | | |
| Mean tissue mass [g] | | | 0.32 ± 0.14 | | | |
| MDL[a] [ng] (n=23) | 1.6 | 0.60 | 0.83 | 1.1 | 0.82 | 1.9 |
| MDL[b] [ng/g] | 5.0 | 1.9 | 2.6 | 3.4 | 2.6 | 5.9 |
| Control animals [ng/g] (n=7) | 0.47 ± 0.61 | 0.15 ± 0.30 | 2.0 ± 2.9 | 0.03 ± 0.06 | 4.1 ± 9.7 | 2.7 ± 6.5 |
| **Brain** | | | | | | |
| Mean tissue mass [g] | | | 0.23 ± 0.10 | | | |
| MDL[a] [ng] (n=7) | 1.2 | ND | 0.56 | ND | 0.74 | 1.3 |
| MDL[b] [ng/g] | 5.1 | ND | 2.4 | ND | 3.2 | 5.6 |
| Control animals [ng/g] (n=2) | 2.8 ± 2.8 | ND | ND | ND | 3.0 ± 0.4 | 9.9 ± 6.8 |
| **Liver** | | | | | | |
| Mean tissue mass [g] | | | 1.54 ± 0.64 | | | |
| MDL[a] [ng] (n=10) | 1.5 | 0.27 | 0.96 | 0.77 | 0.43 | 1.2 |
| MDL[b] [ng/g] | 0.96 | 0.17 | 0.63 | 0.50 | 0.28 | 0.77 |
| Control animals [ng/g] (n=4) | 0.3 ± 0.3 | 0.02 ± 0.02 | 0.26 ± 0.14 | 0.14 ± 0.14 | 0.06 ± 0.04 | 0.03 ± 0.02 |

[a] Method Detection Limit (MDL) was determined from blank samples (i.e. samples containing only chemicals used in the analytical procedure and subjected to same extraction and clean-up process as real samples), as described previously by Kania-Korwel *et al.* (2007).[1]

[b] Determined by dividing the MDL by the average mass of tissue used for extraction.[2] Data presented as mean ± standard deviation. For analysis details, see Experimental procedures.

**Table S2.** Final body weights and organ to body weight ratios of dams exposed to racemic PCB 95 throughout gestation and lactation.

| Dose (mg/kg b.w./d, p.o.) | Pregnancy weight gain (g) | Birth weight loss (g) | Weight change during lactation (g) | Overall weight gain (g) | Final weight (g) | Liver to final body weight ratio (%) | Brain to final body weight ratio (%) |
|---|---|---|---|---|---|---|---|
| 0 | 19.2 ± 2.4 (n=8) | 12.2 ± 2.1 (n=8) | 1.1 ± 2.0 (n=8) | 8.1 ± 2.3 (n=8) | 29.2 ± 2.0 (n=8) | 6.5 ± 3.5 (n=4) | 0.52 ± 0.39 (n=2) |
| 0.1 | 20.1 ± 2.2 (n=10) | 12.8 ± 2.4 (n=10) | 1.1 ± 1.8 (n=10) | 8.3 ± 1.7 (n=10) | 28.6 ± 2.0 (n=10) | 5.3 ± 1.6 (n=10) | 0.90 ± 0.27 (n=8) |
| 1 | 20.5 ± 1.1 (n=5) | 12.0 ± 1.4 (n=5) | 0.3 ± 1.4 (n=5) | 8.7 ± 2.3 (n=5) | 28.7 ± 1.5 (n=5) | 5.3 ± 2.2 (n=5) | 1.1 ± 0.38 (n=5) |
| 6 | 18.2 ± 2.7 (n=8) | 10.7 ± 1.8 (n=8) | 1.2 ± 1.4 (n=8) | 8.7 ± 1.2 (n=8) | 28.8 ± 1.2 (n=8) | 5.0 ± 1.9 (n=8) | 0.60 ± 0.05 (n=7) |

Data presented as mean ± standard deviation.

**Table S3A.** Levels of PCB 95 in tissues of dams exposed to racemic PCB 95 during gestation and lactation increased with increasing dose.

| | Tissue level (ng/g wet weight) | | | |
|---|---|---|---|---|
| Dose (mg/kg b.w./d, p.o.) | **Adipose** | **Blood** | **Brain** | **Liver** |
| 0.1 | 170 ± 110 (n=9) | *2.6 ± 1.0*[‡] *(n=8)* | *3.7 ± 0.9*[‡] *(n=8)* | 12 ± 7.0 (n=10) |
| 1 | 2000 ± 590* (n=4) | 14 ± 4.9 (n=5) | 31 ± 11 (n=7) | 100 ± 48 (n=6) |
| 6 | 11600 ± 2200*[#] (n=2) | 52 ± 23[#] (n=7) | 140 ± 43[#] (n=7) | 700 ± 240*[#] (n=8) |

[‡] Levels below detection limit (see Table S1 for QA/QC values).

* Significantly higher than 0.1 mg/kg/d treatment group, $p<0.05$.

[#] Significantly higher than 1 mg/kg/d treatment group.

Data presented as mean ± standard deviation.

**Table S3B.** Comparison of PCB 95 levels in tissues from dams *versus* non-pregnant adult age-matched congenic female mice exposed to racemic PCB 95.

| Tissue | Dose [mg/kg b.w./d, p.o.] | Tissue level (ng/g wet weight) | |
|---|---|---|---|
| | | **Dams**[a] | **Adult females**[b] |
| Adipose | 0.1 | 170 ± 110 | 1800 ± 500[#] |
| | 1 | 2000 ± 590 | 20000 ± 1600 |
| | 6 | 11600 ± 2200 | 47000 ± 2000 |
| Blood | 0.1 | 2.6 ± 1.0 | 9.8 ± 2.2 |
| | 1 | 14 ± 4.9 | 60 ± 20[#] |
| | 6 | 52 ± 23 | 180 ± 50[#] |
| Brain | 0.1 | 3.7 ± 0.87 | 37 ± 12 |
| | 1 | 31 ± 11 | 100 ± 24[#] |
| | 6 | 140 ± 43 | 360 ± 72[#] |
| Liver | 0.1 | 12 ± 7.0 | 87 ± 23[#] |
| | 1 | 100 ± 48 | 360 ± 48[#] |
| | 6 | 700 ± 240 | 1200 ± 90[#] |

[a] Dams were dosed daily throughout gestation and lactation for 56 days as described under Experimental procedures.

[b] Adult female mice were dosed daily with racemic PCB 95 for 39 days as reported earlier by Kania-Korwel *et al.* (2012).[2]

[#] Significantly higher than 1 mg/kg/d treatment group.

Data presented as mean ± standard deviation.

**Table S4A.** Levels of hydroxylated metabolites of PCB 95 in tissues from dams exposed to racemic PCB 95 during gestation and lactation increased with increasing dose.

| Tissue | Dose [mg/kg b.w./d, p.o.] | Tissue level [ng/g wet weight] | | | |
|---|---|---|---|---|---|
| | | **4-95** | **5-95** | **4,5-95** | **ΣOH-PCB** |
| Blood | 0.1 (n=8) | 8.1 ± 3.9 | *0.20 ± 0.28*‡ | *2.2 ± 3.1*‡ | 11 ± 5.4 |
| | 1 (n=5) | 82 ± 17 | *2.5 ± 2.5*‡ | *5.1 ± 4.4*‡ | 90 ± 19 |
| | 6 (n=7) | 270 ± 130 | 6.6 ± 4.2 | 18 ± 6.8 | 290 ± 140*# |
| Liver | 0.1 (n=10) | 3.0 ± 1.7 | 1.5 ± 0.5 | *0.45 ± 0.18*‡ | 4.9 ± 2.2 |
| | 1 (n=6) | 25 ± 9.3 | 13 ± 5.1 | 2.5 ± 1.4 | 40 ± 15* |
| | 6 (n=8) | 130 ± 65 | 75 ± 45 | 12 ± 8.0 | 220 ± 110*# |

‡ Levels below detection limit (see Table S1 for QA/QC values).

* Significantly higher than 0.1 mg/kg/d treatment group, $p<0.05$.

# Significantly higher than 1 mg/kg/d treatment group.

Data presented as mean ± standard deviation.

**Table S4B.** Comparison of tissue levels of hydroxylated metabolites of PCB 95 in dams *versus* age-matched congenic female mice exposed to racemic PCB 95.

| Tissue | Dose [mg/kg b.w./d, p.o.] | Tissue level [ng/g wet weight] | | | |
|---|---|---|---|---|---|
| | | Blood | | Liver | |
| | | **Dams[a]** | **Adult females[b]** | **Dams[a]** | **Adult females[b]** |
| 3-103 | 0.1 | ND | ND | ND | ND |
| | 1 | ND | 4.5 ± 0.6 | ND | 5.4 ± 1.3 |
| | 6 | ND | 9.7 ± 4.5 | ND | 22 ± 15 |
| 4-95 | 0.1 | 8.1 ± 3.9 | 26 ± 10 | 3.0 ± 1.7 | ND |
| | 1 | 82 ± 17 | 149 ± 29 | 25 ± 9.3 | 40 ± 6.4 |
| | 6 | 270 ± 130 | 280 ± 110 | 130 ± 65 | 140 ± 66 |
| 5-95 | 0.1 | 0.20 ± 0.28 | ND | 1.5 ± 0.5 | 3.9 ± 0.9 |
| | 1 | 2.5 ± 2.5 | 2.5 ± 2.9 | 13 ± 5.1 | 34 ± 21 |
| | 6 | 6.6 ± 4.2 | 12.4 ± 2.3 | 75 ± 45 | 72 ± 35 |
| 4,5-95 | 0.1 | 2.2 ± 3.1 | ND | 0.45 ± 0.18‡ | 3.2 ± 1.5 |
| | 1 | 5.1 ± 4.4 | 26 ± 9.2 | 2.5 ± 1.4 | 5.5 ± 0.7 |
| | 6 | 18 ± 6.8 | 45 ± 20 | 12 ± 8.0 | 18 ± 7.5 |
| ΣOH-PCB | 0.1 | 11 ± 5.4 | 34 ± 9.8 | 4.9 ± 2.2 | 16 ± 14 |
| | 1 | 90 ± 19 | 180 ± 35 | 40 ± 15* | 84 ± 28 |
| | 6 | 290 ± 140 | 340 ± 120 | 220 ± 110 | 250 ± 120 |

[a] Dams were dosed daily throughout gestation and lactation for 56 days as described under Experimental procedures.

[b] Adult female mice were dosed daily with racemic PCB 95 for 39 days as reported earlier by Kania-Korwel *et al.* (2012).[1]

[#] Statistically significantly different from adult female mice, t-test, $p<0.01$.

Data presented as mean ± standard deviation.

**Table S5A.** Atropisomeric enrichment of PCB 95 in tissues from dams exposed to racemic PCB 95 during gestation and lactation was independent of dose.

| Dose (mg/kg b.w./d, p.o.) | Enantiomeric fraction (EF) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Adipose** | | **Blood** | | **Brain** | | **Liver** | |
| | BDM[a] | CD[b] | BDM | CD | BDM | CD | BDM | CD |
| 0.1 | 0.19 ± 0.03 (n=6) | 0.16 ± 0.02 (n=9) | ND[c] | ND | 0.23 (n=1) | 0.19 ± 0.03 (n=4) | 0.11 ± 0.02 (n=4) | 0.15 ± 0.04 (n=4) |
| 1 | 0.22 ± 0.01 (n=4) | 0.21 ± 0.02* (n=4) | 0.20 (n=1) | 0.16 ± 0.05 (n=3) | 0.17 ± 0.03 (n=0.03) | 0.14 ± 0.02 (n=6) | 0.11 ± 0.04 (n=5) | 0.11 ± 0.02 (n=9) |
| 6 | 0.23 ± 0.02 (n=3) | 0.26 ± 0.01*[#] (n=3) | 0.17 ± 0.02 (n=4) | 0.18 ± 0.04 (n=10) | 0.15 ± 0.03 (n=4) | 0.18 ± 0.02 (n=7) | 0.11 ± 0.02 (n=6) | 0.11 ± 0.02 (n=7) |

Enantiomeric fraction is defined as $A_1/(A_1+A_2)$, where $A_1$ and $A_2$ are areas of first and second eluting peak, respectively. Data presented as mean ± standard deviation. In several samples, the first eluting atropisomer, $E_1$-95 was below the detection limit, resulting in a calculated EF = 0.0. These EF values were excluded from the calculation of the mean EF values to avoid overestimating the enrichment.

[a] BDM - ChiralDex B-DM column (2,3-di-O-methyl-6-tert-butyl-silyl-β-cyclodextrin).

[b] CD - Chirasil-Dex column (2,3,6-tri-O-methyl-β-cyclodextrin).

[c] ND - below detection limit.

* Significantly higher than 0.1 mg/kg/d treatment group, $p<0.05$.

[#] Significantly higher than 1 mg/kg/d treatment group.

Data presented as mean ± standard deviation.

**Table 5B.** Comparison of atropisomeric enrichment of PCB 95 in tissues from dams *versus* age-matched congenic female mice exposed to racemic PCB 95.

| Tissue | Dose [mg/kg b.w./d, p.o.] | Enantiomeric fraction (EF) | |
|---|---|---|---|
| | | **Dams**[a] | **Adult females**[b] |
| Adipose | 0.1 | 0.19 ± 0.03 | 0.18 ± 0.00 |
| | 1 | 0.22 ± 0.01 | 0.21 ± 0.02) |
| | 6 | 0.23 ± 0.02[#] | 0.19 ± 0.01 |
| Blood | 0.1 | ND[c] | ND |
| | 1 | 0.20 | ND |
| | 6 | 0.17 ± 0.02[#] | 0.13 ± 0.01 |
| Brain | 0.1 | 0.23 | ND |
| | 1 | 0.17 ± 0.03 | 0.17 ± 0.02 |
| | 6 | 0.15 ± 0.03 | 0.13 ± 0.01 |
| Liver | 0.1 | 0.11 ± 0.02 | ND |
| | 1 | 0.11 ± 0.04 | 0.12 ± 0.01 |
| | 6 | 0.11 ± 0.02[#] | 0.09 ± 0.01 |

Enantiomeric fraction is defined as A1/(A1+A2), where A1 and A2 are areas of first and second eluting peak, respectively. Data presented as mean ± standard deviation. Samples were analyzed using a BDM column (ChiralDex B-DM; 2,3-di-O-methyl-6-tert-butyl-silyl-β-cyclodextrin).

[a] Dams were dosed daily throughout gestation and lactation for 56 days as described under Experimental procedures.

[b] Adult female mice were dosed daily with racemic PCB 95 for 39 days as reported earlier by Kania-Korwel *et al.* (2012).[1]

[c] ND – below detection limit.

[#] Statistically significantly different from adult female mice, t-test, $p<0.01$.

Data presented as mean ± standard deviation.

**Table S6A.** Atropisomeric enrichment of hydroxylated metabolites of PCB 95 in tissues from dams exposed to racemic PCB 95 during gestation and lactation was independent of dose.

| Tissue | Dose [mg/kg b.w./d, p.o.] | Enantiomeric fraction (EF) | | |
|---|---|---|---|---|
| | | **4-95**[a] | **4,5-95**[b] | **X'-95**[b] |
| Blood | 0.1 | ND[c] | ND | ND |
| | 1 | 0.13[#] | NR[d] | ND |
| | 6 | 0.15 ± 0.06 (n=5) | 0.47 ± 0.06* (n=7) | 1.0 |
| Liver | 0.1 | ND | ND | 0.78 (n=1) |
| | 1 | ND | 0.26 (n=1) | 0.80 ± 0.11* (n=5) |
| | 6 | 0.26 (n=1) | 0.41 ± 0.05 (n=4) | 0.78 ± 0.03 (n=5) |

[a] Analysis conducted on BGB column (BGB-172, 20% tert-butyldimethyl-silyl-β-cyclodextrin).

[b] Analysis conducted on CD column (Chirasil-Dex, 2,3,6-tri-O-methyl-β-cyclodextrin).

[c] ND – below detection limit.

[d] NR – not resolved.

[#] Samples were pooled for the analysis to improve detection.

* Different from liver in 6 mg/kg/d dose group, $p<0.05$.

Data presented as mean ± standard deviation.

**Table S6B.** Comparison of atropisomeric enrichment of hydroxylated metabolites of PCB 95 in tissues from dams *versus* age-matched congenic female mice exposed to racemic PCB 95.

| Metabolite | Dose [mg/kg b.w./d, p.o.] | Enantiomeric fraction (EF) | | | |
|---|---|---|---|---|---|
| | | Blood | | Liver | |
| | | **Dams**[a] | **Adult females**[b] | **Dams** | **Adult females** |
| 4-95[c] | 0.1 | ND[e] | ND | ND | ND |
| | 1 | 0.13[g] | 0.10 ± 0.02 | ND | ND |
| | 6 | 0.15 ± 0.06 | 0.07 | 0.26 | 0.21 ± 0.03 |
| 4,5-95[d] | 0.1 | ND | ND | ND | ND |
| | 1 | NR[f] | ND | 0.26 (n=1) | ND |
| | 6 | 0.47 ± 0.06[#] | 0.34 ± 0.02 | 0.41 ± 0.05 | 0.50 ± 0.07 |

[a] Dams were dosed daily throughout gestation and lactation for 56 days as described under Experimental procedures.

[b] Adult female mice were dosed daily with racemic PCB 95 for 39 days as reported earlier by Kania-Korwel *et al.* (2012).[1]

[c] Analysis conducted on BGB column (BGB-172, 20% tert-butyldimethyl-silyl-β-cyclodextrin).

[d] Analysis conducted on BDM column (ChiralDex B-DM, 2,3-di-O-methyl-6-tert-butyl-silyl-β-cyclodextrin).

[e] ND – below detection limit.

[f] NR – not resolved.

[g] Samples were pooled for the analysis to improve detection.

[#] Different from adult females, t-test, $p<0.01$.

Data presented as mean ± standard deviation.

**Table S7.** Levels and atropisomeric enrichment of PCB 95 in blood and liver from pups exposed to racemic PCB 95 via the maternal diet; see Experimental procedures for additional details (subset of data published in Barnhart *et al.*, manuscript in preparation).

| Dose (mg/kg b.w./d, p.o.) | **Blood** | | | **Liver** | | |
|---|---|---|---|---|---|---|
| | Level (ng/g wet weight) | EF[a] | | Level (ng/g wet weight) | EF[a] | |
| | | BDM[b] | CD[c] | | BDM | CD |
| 0.1 | *1.5 ± 1.5*[d] (n=5) | ND[e] | ND | 5.0 ± 2.1 (n=12) | 0.00 ± 0.00 (n=6) | 0.00 ± 0.01 (n=8) |
| 1 | 7.4 ± 4.9 (n=7) | ND | ND | 15 ± 4.9 (n=10) | 0.01 ± 0.03 (n=10) | 0.01 ± 0.01 (n=10) |
| 6 | 19 ± 16 (n=5) | 0.00 (n=1) | ND | 75 ± 48 (n=9) | 0.01 ± 0.02 (n=9) | 0.03 ± 0.03 (n=9) |

[a] Samples with EF = 0.00 were included in the calculation of the mean.

[b] Analysis conducted on BDM column (ChiralDex B-DM, 2,3-di-O-methyl-6-tert-butyl-silyl-β-cyclodextrin).

[c] Analysis conducted on CD - Chirasil-Dex column (2,3,6-tri-O-methyl-β-cyclodextrin).

[d] Levels below detection limit (for details and QA/QC see Barnhart *et al.*, manuscript in preparation).

[e] ND – below detection limit.

Data presented as mean ± standard deviation.

**Table S8.** Levels of hydroxylated metabolites of PCB 95 in blood and liver from pups exposed to racemic PCB 95 via the maternal diet.

| Tissue | Dose [mg/kg b.w./d, p.o.] | Tissue level [ng/g wet weight] | | | |
|---|---|---|---|---|---|
| | | **4-95** | **5-95** | **4,5-95** | **ΣOH** |
| Blood | 0.1 | *1.6 ± 0.7*[a] | *0.37 ± 0.43*[a] | *0.74 ± 0.52*[a] | 2.7 ± 1.1[a] |
| | 1 | **7.0 ±3.2** | *0.70 ± 0.45*[a] | *2.5 ± 1.2*[a] | 10 ± 4[a] |
| | 6 | **31 ± 17** | *0.48 ± 0.68*[a] | **6.5 ± 3.4** | 38 ± 20[a] |
| Liver | 0.1 | *0.83 ± 1.9*[a] | *0.18 ± 0.55*[a] | *1.3 ± 1.1*[a] | 2.1 ± 2.2[a] |
| | 1 | *0.43 ± 1.1*[a] | *0.72 ± 1.8*[a] | *0.08 ± 0.1*[a] | 1.0 ± 2.1[a] |
| | 6 | **4.4 ± 4.1** | **5.6 ± 9.9** | *1.5 ± 2.0*[a] | 12 ± 12[a] |

[a] Levels below detection limit (for details and QA/QC see Barnhart *et al.*, manuscript in preparation).

**Table S9.** Atropisomeric enrichment of selected chiral PCBs in extracts from human brain samples. For details on sample extraction and PCB levels see Mitchell *et al.* (2012).[3]

| Sample | Enantiomeric fraction | | |
|---|---|---|---|
| | PCB 95 | PCB 136 | PCB 149 |
| Subject 1<br>IDIC156294 | **0.50** | ND | **0.50** |
| Subject 2<br>IDIC156856 | ND | **0.49** | **0.50** |
| Subject 3<br>IDIC157014 | **0.50** (0.50[b]) | ND (0.50[b]) | **0.50** |
| Subject 4<br>IDIC157041 | **0.50** (0.44[b]) | ND (0.54[b]) | ND |

[a] IDIC denotes samples from subjects with an idiopathic autism spectrum disorder (ASD).

[b] Analysis using CD column (Chirasil-Dex, 2,3,6-tri-O-methyl-β-cyclodextrin) and temperature program as described under "Atropselective analysis of human brain samples" (next page). Samples were introduced using HS-SPME with 100 μm PDMS fiber, extraction of 60 min at 60°C and desorption for 5 min at 250°C. For details on sample extraction and PCB levels see Mitchell *et al.* (2012).[3]

**Atropselective analysis of human brain samples**. The origin of human brain samples from individuals with idiopathic ASD and the extraction and quantification of PCBs was previously published by Mitchell *et al.* (2012).[3] The extracts were further analyzed using an Agilent 7890 chromatograph connected to 5975C mass selective detector and equipped with a Chirasil-Dex enantioselective column: (CD, 2,3,6-tri-O-methyl-β-cyclodextrin, 30 m x 250 μm x 0.39 μm, Agilent, Santa Clara, CA). The injector temperature was 250 ºC. Samples were introduced to gas chromatograph by means of liquid injection or head space-solid phase microextraction (HS-SPME). For HS-SPME, samples were placed in glass vial with 1.5 mL of phosphate buffer (pH 7.4) and the head space was extracted with a 100 μm PDMS fiber (Sigma-Aldrich, St. Louis, MO, USA) for 60 min at 60°C, followed by desorption for 5 min at 250°C. The following temperature program was used for gas chromatographic analysis: 60 ºC for 1 min, 10 ºC/min to 150 ºC, 1 ºC/min to 200 ºC, hold for 30 min. The injector was kept at 250 ºC. The two most intense isotope ions were used for selected ion monitoring (*m/z* 325.9 and 327.9 for PCB 95, and *m/z* 359.8 and 361.8 for PCBs 136 and 149). Enantiomeric fraction was calculated as $A_1/(A_1+A_2)$, where $A_1$ and $A_2$ are areas of first and second eluting peak, respectively. The criteria used to evaluate the quality of the EF value determinations were in line with those used by Harrad *et al.* (2006),[4] except that a signal to noise ratio of S/N > 3 was used.

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