

Early-life exposure to polychlorinated biphenyl 126 in mice leads to metabolic dysfunction and microbiota changes in adulthood

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Table S1. Serum cytokine (32-Plex) assay results.

| Unit: pg/mL | 6 th day | | | 13 th week | | |
|--------------------------------|---------------------|-----------------|---------|-----------------------|-----------------|---------|
| | Vehicle | PCB 126 | P value | Vehicle | PCB 126 | P value |
| Eotaxin | 13646 ± 3784 | 12163 ± 1254 | 0.40 | 13794 ± 3276 | 15966 ± 3008 | 0.26 |
| G-CSF | 326.5 ± 84.8 | 327.3 ± 53.3 | 0.98 | 512.3 ± 192.5 | 618.8 ± 186.5 | 0.35 |
| GM-CSF | 22.7 ± 4.0 | 23.7 ± 5.6 | 0.73 | 25.2 ± 6.1 | 23.5 ± 2.1 | 0.55 |
| IFNγ | 34.7 ± 7.1 | 36.0 ± 4.0 | 0.70 | 34.2 ± 5.3 | 33.2 ± 5.2 | 0.75 |
| IL-1α | 43.8 ± 15.4 | 43.7 ± 17.3 | 0.99 | 61.7 ± 50.4 | 32.3 ± 5.3 | 0.21 |
| IL-1β | 25.5 ± 19.3 | 20.5 ± 5.4 | 0.56 | 18.5 ± 4.1 | 18.3 ± 2.0 | 0.93 |
| IL-2 | 23.0 ± 3.7 | 20.3 ± 4.7 | 0.30 | 20.3 ± 3.6 | 20.5 ± 4.3 | 0.94 |
| IL-3 | 25.5 ± 3.8 | 28.5 ± 3.9 | 0.21 | 34.2 ± 11.9 | 31.3 ± 9.5 | 0.66 |
| IL-4 | 33.4 ± 2.2 | 34.2 ± 7.5 | 0.82 | 37.7 ± 6.9 | 32.8 ± 5.0 | 0.20 |
| IL-5 | 93.5 ± 51.7 | 49.5 ± 8.3 | 0.09 | 94.7 ± 59.1 | 70.8 ± 11.3 | 0.37 |
| IL-6 | 47.2 ± 15.7 | 39.3 ± 3.9 | 0.28 | 62.7 ± 47.0 | 55.8 ± 25.7 | 0.76 |
| IL-7 | 43.8 ± 4.5 | 41.0 ± 3.0 | 0.27 | 48.8 ± 11.5 | 40.8 ± 1.6 | 0.15 |
| IL-9 | 40.5 ± 3.6 | 41.5 ± 10.0 | 0.83 | 50.0 ± 13.0 | 39.0 ± 4.8 | 0.10 |
| IL-10 | 37.8 ± 14.9 | 33.0 ± 5.3 | 0.48 | 33.0 ± 4.7 | 34.8 ± 2.9 | 0.44 |
| IL-12p40 | 31.8 ± 4.4 | 33.7 ± 3.4 | 0.44 | 35.7 ± 7.3 | 34.7 ± 13.8 | 0.88 |
| IL-12p70 | 29.8 ± 3.5 | 25.8 ± 3.4 | 0.09 | 29.5 ± 4.1 | 25.3 ± 4.1 | 0.11 |
| IL-13 | 42.6 ± 5.5 | 40.8 ± 3.9 | 0.56 | 41.3 ± 8.5 | 37.3 ± 3.9 | 0.33 |
| IL-15 | 44.2 ± 3.0 | 42.3 ± 5.8 | 0.51 | 48.3 ± 11.9 | 43.2 ± 8.7 | 0.41 |
| IL-17 | 48.5 ± 19.1 | 38.8 ± 5.4 | 0.28 | 43.0 ± 1.3 | 42.2 ± 10.8 | 0.86 |
| IP-10 | 2745.7 ± 344.7 | 2469.8 ± 207.7 | 0.13 | 2669.3 ± 963.7 | 3075.2 ± 501.9 | 0.39 |
| KC | 125.2 ± 43.5 | 158.5 ± 32.4 | 0.17 | 114.3 ± 49.1 | 94.0 ± 21.9 | 0.39 |
| LIF | 40.4 ± 6.9 | 43.5 ± 4.3 | 0.41 | 40.3 ± 4.0 | 39.5 ± 2.6 | 0.68 |
| LIX | 4829.8 ± 2785.0 | 4763.7 ± 2037.8 | 0.96 | 3516.0 ± 4883.2 | 2371.3 ± 1466.6 | 0.60 |

| | | | | | | |
|---------------------------------|---------------|---------------|------|---------------|---------------|------|
| MCP-1 | 22.0 ± 8.0 | 21.8 ± 3.3 | 0.96 | 24.2 ± 3.0 | 20.0 ± 4.6 | 0.10 |
| M-CSF | 31.2 ± 2.2 | 29.2 ± 3.7 | 0.29 | 33.5 ± 16.1 | 33.3 ± 6.8 | 0.98 |
| MIG | 733.6 ± 131.6 | 688.0 ± 218.1 | 0.68 | 627.0 ± 271.9 | 618.5 ± 174.9 | 0.95 |
| MIP-1α | 30.5 ± 5.4 | 34.7 ± 3.0 | 0.14 | 33.3 ± 7.0 | 33.8 ± 3.6 | 0.88 |
| MIP-1B | 59.3 ± 8.9 | 69.8 ± 9.6 | 0.08 | 68.3 ± 7.1 | 62.0 ± 14.0 | 0.35 |
| MIP-2 | 40.0 ± 5.3 | 45.3 ± 4.0 | 0.08 | 44.0 ± 9.9 | 42.2 ± 5.6 | 0.70 |
| RANTES | 36.6 ± 10.3 | 50.5 ± 8.9 | 0.05 | 57.7 ± 21.7 | 50.5 ± 8.3 | 0.48 |
| TNFα | 28.3 ± 4.4 | 23.3 ± 3.5 | 0.06 | 25.3 ± 2.7 | 25.7 ± 4.3 | 0.87 |
| VEGF | 37.0 ± 8.4 | 42.2 ± 6.1 | 0.25 | 36.2 ± 7.2 | 40.7 ± 5.2 | 0.24 |

G-CSF: granulocyte colony stimulating factor; GM-CSF: Granulocyte/macrophage colony stimulating factor; IFN γ : interferon gamma; IL: interleukin; IP-10: interferon gamma inducible protein 10; KC: keratinocyte chemoattractant; LIF: leukaemia inhibitory factor; LIX: lipopolysaccharide-induced CXC chemokine; MCP-1: monocyte chemoattractant protein-1; M-CSF: macrophage colony-stimulating factor; MIG: monokine induced by IFN- γ ; MIP: macrophage inflammatory protein; RANTES: regulated upon activation, normal T cell expressed and presumably secreted; TNF α : tumor necrosis factor alpha; VEGF: vascular endothelial growth factor.

Table S2. mRNA gene-targeted primers used in this study

| Gene | Abbreviation | Sequence (5'-3') |
|--|--------------|--|
| Cytochrome P450, family 1, member A1 | Cyp1a1 | CTCTCCCTGGATGCCTGAA GGATGTGCCCTCTCAAATG |
| Cytochrome P450, family 1, member A2 | Cyp1a2 | GCCCCTGCCCTCAGTGGTACAG AGGAGTGGAGCCGATGCGGA |
| Stearoyl-CoA desaturase-1 | Scd1 | TTCTTGCATACACTCTGGTGC CGGGATTGAATGTTCTGTCGT |
| Cluster of differentiation 36 | Cd36 | TGGCCTTACTTGGGATTGG CCAGTGTATATGTAGGCTCATCCA |
| Acetyl-CoA carboxylase alpha | Acaca | TAACAGAACATGACACTGGCTGGCT ATGCTGTTCCCTCAGGCTCACATCT |
| Carnitine palmitoyltransferase 1A | Cpt1a | CGTGACGTTGGAATC TCTCGGTTATGCCTATC |
| Diacylglycerol O-Acyltransferase 2 | Dgat2 | CGCAGCGAAAACAAGAATAA GAAGATGTCTGGAGGGCTG |
| Fatty acid synthase | Fasn | GGTGTGGTGGGTTGGTGAATTGT TCACGAGGTCATGCTTAGCACCT |
| Cytochrome P450, family 7, subfamily A, polypeptide 1 (Cholesterol 7 α -hydroxylase) | Cyp7a1 | AGCAACTAAACAACCTGCCAGT ACTAGTCCGGATATTCAAGGATGCA |
| Cytochrome P450, family 7, subfamily B, polypeptide 1 (Oxysterol 7 α -hydroxylase) | Cyp7b1 | TAGCCCTTTCCCTCCACTCATA GAACCGATCGAACCTAAATTCCCT |
| Cytochrome P450, family 8, subfamily B, polypeptide 1 (Sterol 12 α -hydroxylase) | Cyp8b1 | GGCTGGCTCCTGAGCTTATT ACTTCCTGAACAGCTCATCGG |
| Cytochrome P450, family 27, subfamily A, polypeptide 1 (Sterol 27-hydroxylase) | Cyp27a1 | GCCTCACCTATGGGATCTTCA TCAAAGCCTGACGCAGATG |
| Aldo-keto reductase family 1 member D1 | Akr1d1 | TGCACACCACCAAATATCCCT CTTCACTGCCACATAGGTCTTC |
| Cysteine dioxygenase | Cdo | GGGGACGAAGTCAACGTGG ACCCCCAGCACAGAACATCATCAG |
| Cysteine sulfinate decarboxylase | Csad | CCAGGACGTGTTGGGATTGT ACCAGTCTGACACTGTAGTGA |
| Taurine transporter | Taut | GCACACGGCCTGAAGATGA ATTTTGATCAGAGGGTACGGG |
| Bile acid-CoA: amino acid N-acyltransferase | Baat | GGAAACCTGTTAGTCTCAGGC GTGGACCCCCATATAAGTCTCC |
| Bile acid-CoA synthetase | Bacs | ACCTGGATCAGCTCCTGGAT GTTCTCAGCTAGCAGCTTGG |
| Hepatic nuclear factor 4 α 1 | Hnf4a1 | AAATGTGCAGGTGTTGACCA CACGCTCCTCTGAAGAACATC |
| Phosphoenolpyruvate carboxykinase | Pepck | GGCCACAGCTGCTGCAG GGTCGCATGGCAAAGGG |
| Glyceraldehyde-3-phosphate dehydrogenase | Gapdh | CCTCGTCCCGTAGACAAAATG |

TGAAGGGGTCGTTGATGGC

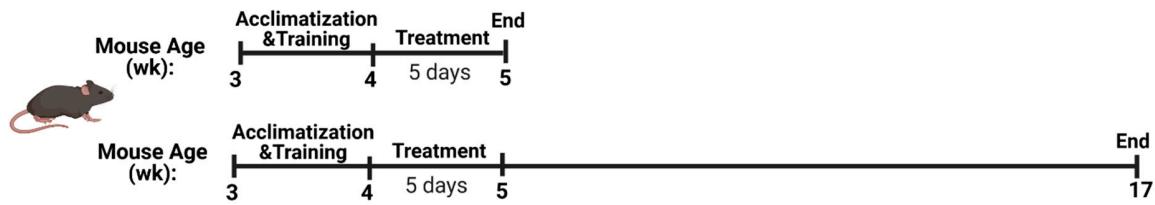


Figure S1. Experimental schedule of the present study.

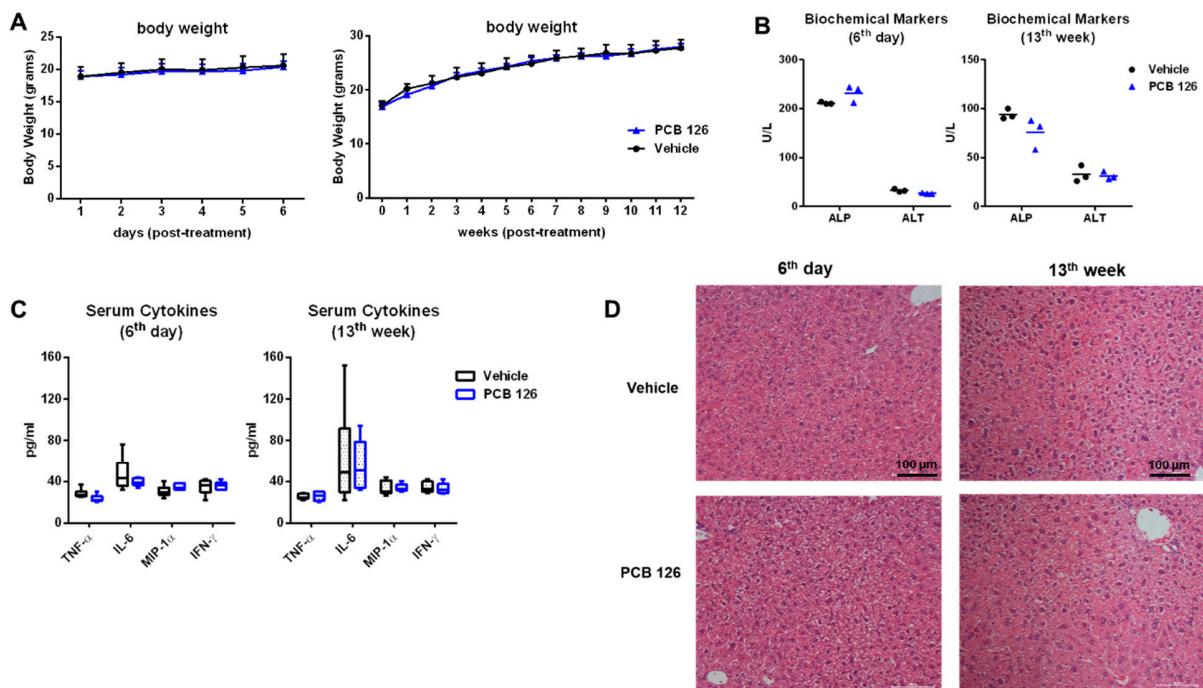


Figure S2. (A) Body weight of mice recorded from mice with vehicle or PCB 126 exposure. (B) Serum concentrations of alanine transaminase (ALT) and alkaline phosphatase (ALP) from mice with vehicle or PCB 126 exposure. (C) Serum cytokines from mice with vehicle or PCB 126 exposure. (D) Light microscopic examination of H&E-stained liver sections from mice with vehicle or PCB 126 exposure. Values are means \pm S.D. or median and interquartile range ($n = 3$ per group, serum biochemical markers; $n = 6$ per group, other analysis).

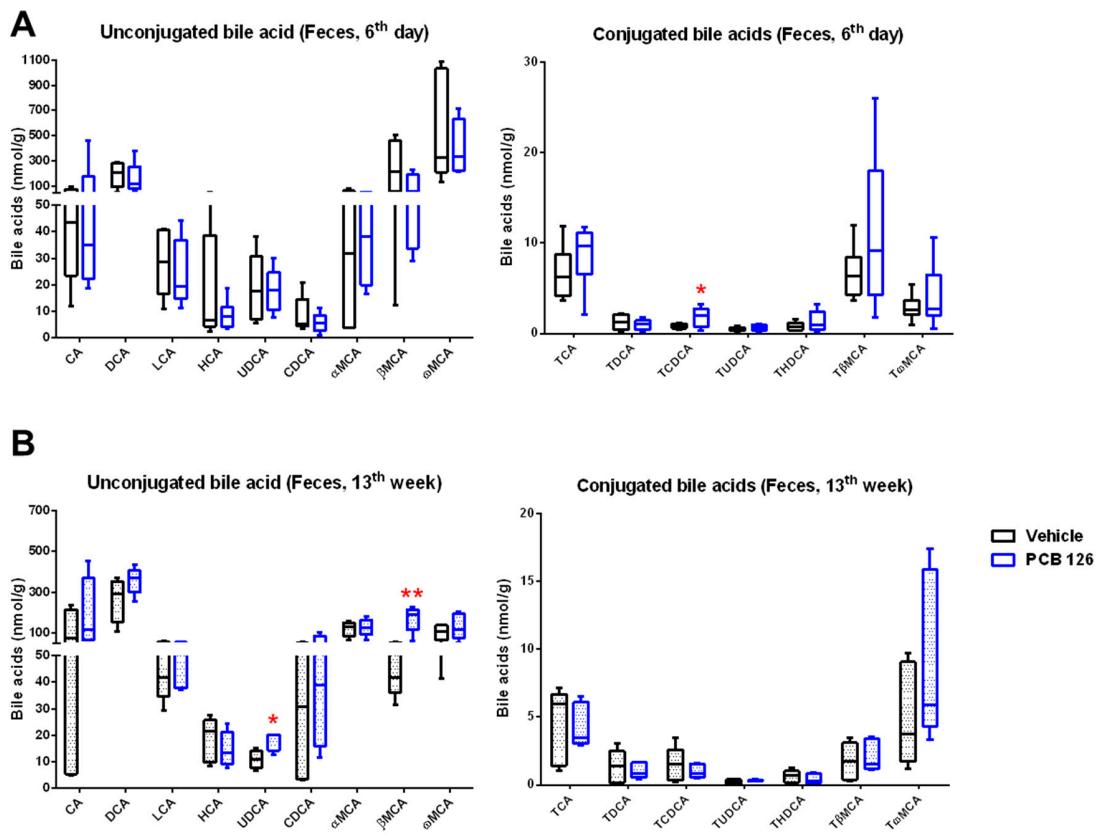


Figure S3. (A,B) Quantitative UHPLC-MS/MS analysis of bile acids in the feces from mice with vehicle or PCB 126 exposure. Values are median and interquartile range ($n = 6$ per group). * $P < 0.05$, ** $P < 0.01$ compared to vehicle.

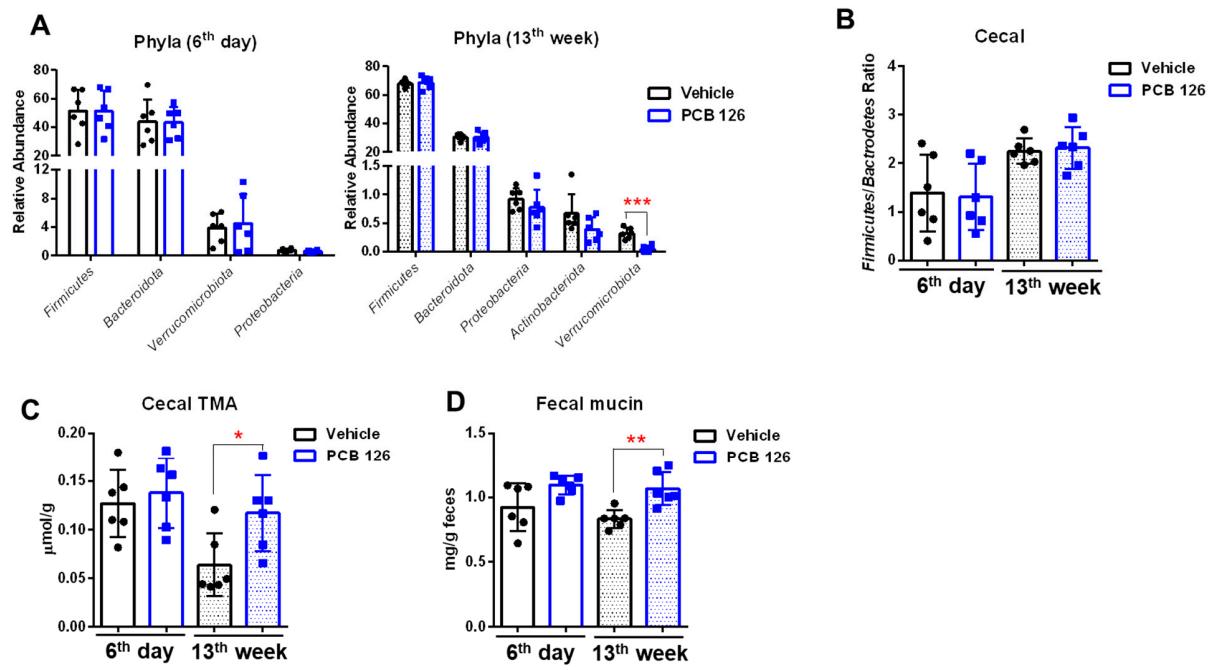


Figure S4. **(A,B)** Relative abundance of cecal bacteria from phylum **(A)** and ratio of *Firmicutes* to *Bacteroidetes* **(B)** in cecal content from mice with vehicle or PCB 126 exposure. **(C)** NMR analysis of cecal trimethylamine (TMA) from mice with vehicle or PCB 126 exposure. **(D)** Fecal mucin levels from mice with vehicle or PCB 126 exposure. Values are means \pm S.D. ($n = 6$ per group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to vehicle.