Fatty Liver and Impaired Hepatic Metabolism Alters the Congener-specific Distribution of Polychlorinated Biphenyls (PCBs) in Mice with A Liver-specific Deletion of Cytochrome P450 Reductase

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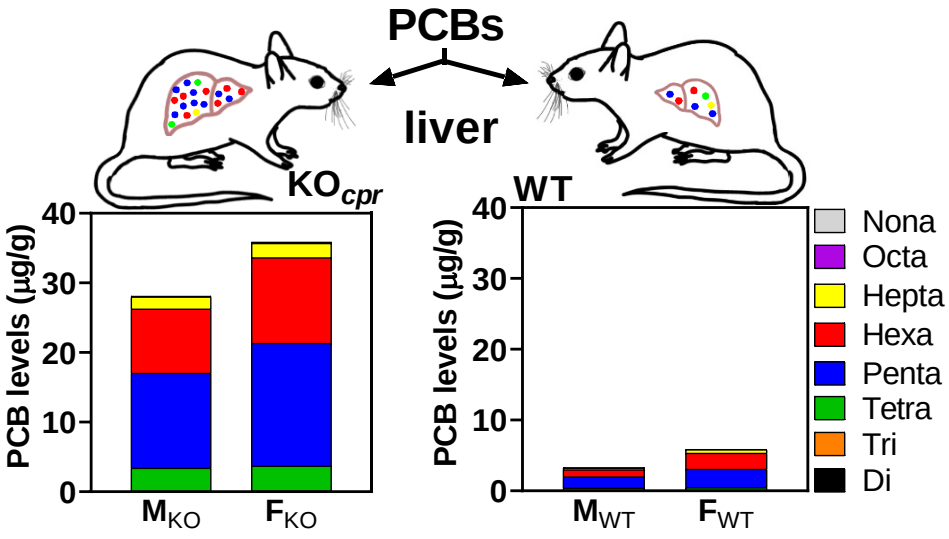
ABSTRACT

Polychlorinated biphenyls (PCBs) are persistent organic pollutants that are linked to adverse health outcomes. PCB tissue levels are determinants of PCB toxicity; however, it is unclear how factors, such as an altered metabolism and/or a fatty liver, affect PCB distribution *in vivo*. We determined the congener-specific disposition of PCBs in mice with a liver specific deletion of cytochrome P450 reductase (KO), a model of fatty liver with impaired hepatic metabolism, and wildtype (WT) mice. Male and female KO and WT mice were exposed orally to Aroclor 1254, a technical PCB mixture. PCBs were quantified in adipose, blood, brain and liver tissues by gas chromatography-mass spectrometry. PCB profiles and levels in tissues were genotype and sex dependent. PCB levels were higher in the liver from KO compared to WT mice. PCB profiles showed clear differences between tissues from the same exposure group. While experimental tissue : blood partition coefficients in KO and WT mice did not follow the trends predicted using a composition-based model, the agreement between experimental and calculated partition coefficients was still reasonable. Thus, a fatty liver and/or an impaired hepatic metabolism alter the distribution of PCBs in mice and the magnitude of the partitioning of PCBs from blood into tissues can be approximated using composition-based models.

39 TOC Art


PCBs
liver
KO cpr
WT
PCB levels (µg/g)
40
30
20
10
0
M KO
F KO
M WT
F WT
Nona
Octa
Hepta
Hexa
Penta
Tetra
Tri
Di

40

INTRODUCTION

Exposure to polychlorinated biphenyls (PCBs), a group of 209 persistent organic pollutants, is associated with adverse human health effects, including cancer, developmental neurotoxicity, and effects on the immune system.[1] Humans are exposed to complex mixtures of >150 PCB congeners via the diet, dermally or, as recent studies demonstrate, by inhalation.[1-4] In contrast, *in vitro* and *in vivo* studies of PCBs typically employ individual congeners or simplified and technical PCB mixtures. The disposition of individual PCBs in standard laboratory animals, especially after oral exposure, is well investigated. Several more recent studies also report the tissue distribution of PCBs after inhalation exposure in rats.[5-7] Toxicokinetic models have been developed for individual PCB congeners or simple PCB mixture in rodent models following oral[8, 9] and intravenous exposure.[10, 11] These studies provide insights into the levels of PCBs in target tissues, such as the adipose tissue, blood, liver, or brain, thus allowing the translation of results from toxicity studies with PCBs to humans. As a rule of thumb, PCB levels are directly proportional to the fat content of a tissue, and, in wildtype rodents, follow the general order adipose > liver > brain > blood.[12-14] However, the PCB disposition can differ from this general trend, depending on the animal model, the experimental design or other factors.

Factors affecting PCB disposition include an altered (hepatic) metabolism and redistribution away from the site of metabolism to tissues with high-fat content.[10, 15] The induction of hepatic cytochrome P450 enzymes will more rapidly eliminate episodic and some persistent PCB congeners.[16] In sub-chronic studies, growth dilution can contribute to the distribution of PCBs.[17] Also, changes in the composition of body compartments can shift the distribution of PCBs. Starvation shifts the partitioning of PCB 153 from adipose to other tissues.[18] Similarly, PCB 153 is mobilized from fat tissues during lactation and redistributed to the breast milk.[19] Fatty liver caused by exposure to PCB 126 produces a redistribution of PCBs from the adipose tissue to the liver.[20, 21] There is also evidence that transgenic animals, which are powerful tools to study the toxicity of PCBs *in vivo*, can have a bodyweight or body composition that differs from wildtype animals and, thus, can affect the disposition of PCBs. Multidrug resistance

transporter 1a/b knockout mice have a significantly higher body weight compared to congenic wildtype animals, which significantly alters PCB tissue levels.[13] Mice with a liver specific deletion of cytochrome P450 reductase (KO mice), the obligate electron donor of cytochrome P450 enzymes, have much higher levels of PCBs in the liver than congenic wildtype mice (WT mice) due to a higher hepatic fat and protein content.[22, 23]

The objective of the present study was to determine how a fatty liver and an impaired hepatic PCB metabolism alters the congener-specific disposition of PCBs following exposure to a complex PCB mixture. PCB congener profiles, levels, and tissue : blood ratios were determined in target tissues (adipose tissue, blood, brain, and liver) after acute oral exposure of male and female KO and congenic WT mice to Aroclor 1254, a technical PCB mixture. PCB profiles and, to some extent, PCB levels in these tissues were genotype and sex-dependent and, at the time point investigated, showed clear differences between tissues from the same exposure group. An acceptable agreement was observed between the experimental tissue : blood partition coefficients of the PCB congeners included in the analysis and partition coefficients calculated using a composition-based model. These results demonstrate that a fatty liver and an impaired hepatic metabolism alter the distribution of PCBs in mice, but that the partitioning of PCBs from blood into tissues can be approximated using composition-based models.

EXPERIMENTAL SECTION

Chemicals and Reagents. Aroclor 1254 (lot #: KB 05-612) was provided by Dr. Larry Hansen. A detailed characterization of this Aroclor 1254 batch has been reported previously.[24] Analytical standard solutions containing all 209 PCB congeners was purchased from AccuStandard (New Haven, CT, USA). $^{13}C_{12}$-2,5-Dichlorbiphenyl (^{13}C-PCB 9, purity 100%, $^{13}C_{12}$, 99%) and $^{13}C_{12}$-2,2',3,3',4,4',5,5'-octachlorobiphenyl (^{13}C-PCB 194, purity 98%, $^{13}C_{12}$, 98%) were obtained from Cambridge Isotope Laboratories (Tewksbury, Massachusetts, USA). 2,4,6-Trichlorobiphenyl-2',3',4',5',6'-d_5 (d-PCB 30), and 2,3,5,6-tetrachlorobiphenyl-2',3',4',5',6'-d_5 (d-PCB 65) were purchased from CDN Isotopes (Pointe Claire, Quebec, Canada). Florisil (60-100 mesh), diatomaceous earth (DE), pesticide grade solvents and stripped

corn oil (lot #: A0234007, product of BE) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The PCB nomenclature used follows the EPA nomenclature.[25]

Mouse model maintenance. Alb-Cre$^{+/-}$/Cpr$^{lox+/+}$ mice with a liver-specific deletion of the cytochrome P450 oxidoreductase (EC 1.6.2.4) gene (knockout, KO) and congenic Alb-Cre$^{-/-}$/Cpr$^{lox+/+}$ mice (wildtype, WT) were obtained from Dr. Xinxin Ding (University of Arizona, AZ, USA) to establish a breeding colony at the University of Iowa.[26, 27] The mouse colony was maintained as described previously.[23, 28] Briefly, animals were housed in standard plastic cages in a temperature- and humidity-controlled environment (21−23 °C) with a 12h light-dark cycle. Water and Basal diet (Harlan 7913 with 18% protein, 6% fat, and 5% fiber) were provided *ad libitum*. Compared with WT mice, the KO mice have a fatty liver and do not display any CPR activity in liver.[23]

Animal exposure. The Institutional Animal Care and Use Committee of the University of Iowa approved all animal procedures (protocol #: 1206120). Daily animal welfare-assessments were performed by laboratory personnel, and no adverse outcomes were observed throughout the study. Eight-week-old mice were randomly divided into control and exposure groups and exposed to corn oil (10 mL/kg body weight) alone or Aroclor 1254 (16 mg/kg body weight in corn oil, 10 mL/kg body weight), respectively. The dose was selected based on a previous animal study.[12] The animals were euthanized 24 h after PCB exposure. Whole blood was collected by cardiac puncture and stored at – 20 °C in glass tubes with 80 µL of ethylenediaminetetraacetic acid solution (EDTA, 7.5% w/w).[12] Tissues (adipose tissue, brain, and liver) were collected and stored at -80 °C.

PCB extraction. PCBs were extracted from adipose (0.05-0.09 g), brain (0.10-0.15), liver (0.06-0.18 g) and blood (0.55-0.73 g) with a pressurized liquid extraction method [23, 29] on the Dionex ASE200 system (Dionex, Sunnyvale, CA, USA). Extraction cells (33 mL) loaded with pre-combusted Florisil (12 g) and diatomaceous earth (DE, 2 g) were extracted with a mixture of hexane and acetone (1:1, v/v) on an ASE200 at 100 °C and 1500 psi (10 MPa) with preheat equilibration for 6 min, 60% of cell flush volume, and 1 static cycle of 5 min. The tissue was homogenized with the top DE layer, and the DE-tissue mixture

was placed back into the extraction cell. Blood samples were loaded directly onto the cellulose filter of the cell. Surrogate standards, d-PCB 30 and d-PCB 65 (80 ng each), were added to every sample before the extraction. The cells were extracted with the same parameters described above. The extracts were concentrated to ~0.5 mL on a TuboVap II (Biotage LLC, NC, USA), transferred to a pre-combusted tube, and treated with 2 mL of concentrated sulfuric acid for lipid removal. The extract was transferred to a new glass tube, evaporated under a gentle stream of nitrogen to ~50 µL, and transferred to a gas chromatography vial. The internal standards (volume correctors), $^{13}C_{12}$-PCB 9 and $^{13}C_{12}$-PCB 194 (50 ng each), were spiked to every sample before analysis.

Gas chromatographic determinations. Samples were analyzed using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C Inert Mass Selective Detector (MSD) operated in electronic ionization mode. An SLB-5MS capillary GC column (30 m length, 250 µm inner diameter, 0.25 µm film thickness; Supelco, Bellefonte, PA, USA) was used in the select ion monitoring (SIM) mode.[30-32] The temperature program was as follows: 80 °C, hold for 1 min, 2 °C/min to 160 °C/min, 1°C/min to 170 °C, hold for 15 min, 1 °C/min to 180 °C, hold for 15 min, 1 °C/min to 245 °C, t10 °C/min to 300 °C, and hold for 15 min. The injector temperature was 280 °C, and the operating temperatures were 280 °C, 230 °C and 150 °C for transfer line, source, and quadrupole, respectively. The flow rate of carrier gas helium was 1.1 mL/min. By this congener specific method, 162 individual or co-eluting peaks of PCBs can be separated and quantified. Twenty-eight channels were set up in GC-MS SIM mode (see details in Table S1). The MSD was linear over the concentration range encountered in this study (Table S2). For Figures with PCB congener profiles, see Figs. S1-S4. The peak corresponding to PCB 138, PCB 158, PCB 163, and PCB 164 is abbreviated as PCB 138 throughout the manuscript.

Model prediction of the tissue-blood partitioning of PCBs. A composition-based model[33] was used for the prediction of the partition coefficients of PCBs between tissues and whole blood. In this model, tissues and blood are assumed to contain five biological components, including albumin, muscle protein, membrane lipid, storage lipid, and water. The volume fractions and lipid content of all biological

components, both in tissues and blood, were taken from published experimental values (Table S3).[23, 33, 34] The partitioning of PCBs between the biological components was determined with published Abraham solvation parameters[35] and polyparameter linear free energy relationships (PP-LFERs) describing the correlations between the chemical descriptors and tissue/water partition coefficients.[36-39] The equation used for the calculations of tissue-blood partitioning coefficients ($K_{tissue/blood}$) were as follows:[33]

$$K_{tissue/blood} = \frac{K_{tissue/water}}{K_{blood/water}}$$

$$= \frac{K_{ap/w}f_{ap}^{tissue}+K_{mp/w}f_{mp}^{tissue}+K_{sl/w}f_{sl}^{tissue}+K_{ml/w}f_{ml}^{tissue}+f_{w}^{tissue}}{K_{ap/w}f_{ap}^{blood}+K_{mp/w}f_{mp}^{blood}+K_{sl/w}f_{sl}^{blood}+K_{ml/w}f_{ml}^{blood}+f_{w}^{tissue}} \quad (1)$$

where $K_{tissue/water}$ and $K_{blood/water}$ are the partition coefficients of PCBs between tissue and water and between blood and water, respectively. $K_{ap/w}$, $K_{mp/w}$, $K_{pl/w}$ and $K_{ml/w}$ are the partition coefficients of PCBs between albumin protein and water, muscle protein and water, storage lipid and water, and membrane lipid and water, respectively. f_{ap}^{tissue}, f_{mp}^{tissue}, f_{sl}^{tissue}, f_{ml}^{tissue} and f_{w}^{tissue} are the volume fractions of albumin protein, muscle protein, storage lipid, membrane lipid, and water in tissue, respectively. f_{ap}^{blood}, f_{mp}^{blood}, f_{sl}^{blood}, f_{ml}^{blood} and f_{w}^{blood} are the volume fractions of albumin protein, muscle protein, storage lipid, membrane lipid, and water in the blood, respectively.

Data visualization and statistical analysis. PCBs congener profiles and PCB levels in tissues from different exposure groups and tissues were analyzed with MetaboAnalyst 4.0.[40] Similarity coefficients cos Θ were calculated are described previously.[24]

RESULTS AND DISCUSSION

Levels of total PCBs (ΣPCB) in tissues. In WT mice, the ΣPCB levels followed the rank order adipose > liver ~ brain > blood (Fig. 1). This rank order is consistent with earlier studies reporting an accumulation of PCBs and related organochlorines in fat-rich organs from different mammalian species.[12-14] In KO mice, the ΣPCB levels in adipose and liver tissue were similar and followed the rank order adipose ~ liver > brain > blood. Similar trends were observed when tissue levels of individual PCB congeners (i.e., PCB 91 and PCB 136) were compared across tissues in WT or KO mice exposed to either Aroclor 1254 (Fig. S5), PCB 91 or PCB 136.[22, 23] We observed minor genotype and sex-dependent differences in the ΣPCB levels in adipose, blood and brain (Fig. 1). For example, ΣPCB levels were 1.8-times higher in the female than the male liver, a difference that reached statistical significance for WT mice (3.3 ± 1.8 μg/g vs. 5.9 ± 1.8 μg/g for M and F_W mice, respectively).

A drastic enrichment of ΣPCB was observed in the liver of KO compared to WT mice, irrespective of the sex. This difference in the distribution of PCBs was also observed in disposition studies of single PCB congener in KO and WT mice.[22, 23] The accumulation of PCBs in the liver of KO mice is a consequence of the higher lipid content and the elevated protein levels in the liver of KO compared to WT mice. In turn, the higher lipid content in the liver of KO mice is caused by an impaired hepatic fat metabolism.[41, 42] In addition to an increased lipid content in the liver, the expression of hepatic proteins that bind PCBs, such as cytochrome P450 enzymes,[43, 44] is increased in the liver to compensate for the liver-specific deletion of *cpr*.[26] For example, dioxin-like PCBs and structurally related compounds are sequestered in the liver because they bind to particular cytochrome P450 isoforms.[45, 46] Analogously, it is likely that cytochrome P450 enzymes in the liver from KO mice bind PCBs in the absence of metabolic conversion, thus contributing to the hepatic accumulation of PCBs in KO mice.

PCB homolog composition in tissues. The homolog composition of the PCB residue in the adipose tissue from WT and KO mice, expressed as a mass percentage, was comparable to the homolog composition of Aroclor 1254 (Fig. 1; see Table S4). However, an increase in the percentage of higher

chlorinated PCBs was apparent in the adipose tissue from WT mice, irrespective of the sex. The homolog composition of the PCB residues from blood, brain, and liver tissues showed differences compared to the homolog composition of Aroclor 1254. Typically, the percentage of lower chlorinated homologs decreased, whereas the percentage of higher chlorinated homologs increased in tissues compared to Aroclor 1254. More pronounced shifts in the homolog composition were observed for WT compared to KO mice. For example, hexachlorinated PCBs were the major constituents in the brain from F_{WT} mice, whereas pentachlorinated PCBs are the major homolog group in Aroclor 1254 (Table S4). These shifts in the PCB homolog composition are consistent with the more rapid clearance of lower chlorinated PCBs in rodents.[47, 48]

The changes in the homolog mass profiles translated into sex and genotype-dependent differences in PCB homolog levels, adjusted by tissue wet weight, in blood, brain, and liver (Fig. 1; see Table S4). Consistent with the higher ΣPCB levels described above, the levels of all homolog groups in the liver were more elevated in KO compared to WT mice because of the higher fat and protein content of the liver of KO mice. Levels of tetra- and pentachlorinated PCBs were lower in blood from F_{WT} mice compared to all other exposure groups. Moreover, levels of tetra- and pentachlorinated PCBs were lower in the brain of F_{WT} but higher in the brain of F_{KO} mice. For example, levels of pentachlorinated PCBs in the brain of F_{KO} mice were 1,110 ± 70 ng/g tissue, which is almost 2-fold higher than the levels in F_{WT} mice (630 ± 70 ng/g tissue).

Distribution of different PCB Classes in tissues. Although lower chlorinated PCBs are generally more rapidly metabolized than higher chlorinated PCBs, the metabolism and elimination of PCBs also depend on the chlorine substitution pattern.[49] Therefore, we compared differences in the mass percentage of PCBs congeners with a 4-, 3,4-, and 3,4,5- substitution pattern and zero or one *ortho* chlorine substituent (Class A); PCB congeners with two or more *ortho* chlorine substituents and a 2,4- or 2,3,4-substitution pattern (Class B); PCBs congeners with a 2,4,5-substitution pattern (Class C); and PCB congeners that are considered to be episodic because they are rapidly eliminated following exposure.[16]

Class D congeners have adjacent, unsubstituted C-atoms and, typically, contain a 2-, 2,3-, 2,5- and 2,3,6-substitution pattern in at least one phenyl ring (Class D).[50, 51]

The mass percentage of PCBs belonging to Class C increased relative to Aroclor 1254 in all tissues from all four exposure groups (Fig. 1; Table S5). This enrichment of Class C congeners is expected because higher chlorinated PCBs with two *para* chlorine, such as PCB 153, are less susceptible to metabolic transformation than PCBs without *para* chlorine substituents. Conversely, the mass percentage of PCBs in Class A decreased in all tissues. The metabolism of (some) Class A congeners is one explanation for the relative decrease in the contribution of this PCB Class to the ΣPCBs. Although PCB congeners with two *para* chlorine substituents are thought to be less susceptible to metabolic conversion, some Class A congeners are readily oxidized by cytochrome P450 enzymes. For example, PCB 77 is readily metabolized in rodents,[52, 53] and hydroxylated metabolites of PCB 28 have been detected in humans.[54] Interestingly, the mass percentage of Class D congeners tended to be higher in the adipose tissue and blood, especially in KO mice, and lower in the brain and liver compared to Aroclor 1254. These differences are potentially due to differences in the initial distribution and redistribution of PCBs to the adipose tissue, as well as their (extrahepatic) metabolism.[52]

We also observed differences in the percentage of PCBs when comparing the mass percentages across genotypes and between sexes (Fig. 1; Table S5). The percentage of more persistent PCBs (i.e., Classes A, B, and C) appeared to be higher in WT compared to KO mice, irrespective of the sex. Importantly, the levels of PCBs by Class, expressed as tissue wet weight, revealed similar sex and genotype-dependent differences. These overall trends are consistent with impaired metabolism of PCBs belonging to Class D in WT mice, which ultimately is caused by the lack of CPR activity in the liver. It is noteworthy that this effect was more pronounced in F_{WT} mice, especially in the liver and brain.

Comparison of PCB profiles in adipose tissue from different exposure groups and Aroclor 1254. PCB congener profiles in adipose tissues were different from the PCB profile of Aroclor 1254 ($\cos \Theta$ = 0.84 to 0.88; Table S6). In contrast, PCB profiles in adipose tissue were quite similar across all four

exposure groups (cos Θ = 0.97 to 1.0; Table S7). Further analysis of individual PCB congeners revealed minor genotype and, for some congeners, sex-dependent differences in the adipose tissue (Fig. 2a). Persistent PCB congeners, for example PCB 118 (Class A) and PCB 180 (Class C), contained a relatively higher percentage in adipose tissue from WT mice. In contrast, PCB congeners belonging to Class D, such as PCB 70, PCB 95, and PCB 136, showed the opposite trend, with lower percentages of these PCB congeners being present in the adipose tissue from WT compared to KO mice. Moreover, the percentage of many Class D congeners were lower in F_{WT} mice compared to the other exposure groups. Importantly, the differences in the PCB congener profiles typically did not result in differences in the PCB levels in the adipose tissue (Fig. S6a). This finding is not surprising because the high PCB content masks small differences in PCB levels in the adipose tissue.

Comparison of PCB profiles in whole blood from different exposure groups and Aroclor 1254. PCB congener profiles in blood were different from the PCB profile of Aroclor 1254 (cos Θ = 0.72 to 0.74; Table S6). The percentage of major PCB congeners present in Aroclor 1254 (e.g., PCB 70) decreased in blood from all four exposure groups (Fig. 2b). Irrespective of the sex, PCB 138 (analyzed as a peak of four co-eluting PCB congeners) was the major PCB congener in WT mice. PCB 101 was the major PCB congener in blood from KO mice. The percentages of other PCB congeners, for example PCB 95 and PCB 180, increased in blood from all exposure groups compared to Aroclor 1254 (Fig. 2b). PCB 52, a PCB congener that is structurally similar to PCB 95, was the third most abundant PCB congener detected in blood (8.1 to 9.0 % in blood vs. 1.7% in Aroclor 1254). The same PCB congeners are among the most abundant PCB congeners detected in serum samples from the MARBLES cohort, a group of pregnant women at risk of having a child with a neurodevelopmental disorder.[55] PCB 52, PCB 95, and PCB 101 are among the twenty most frequently detected PCB congeners in air and in humans.[56]

The congener profiles revealed minor genotype-dependent differences in the blood (cos Θ = 0.98 to 0.99, Table S7). F_{WT} mice were an exception and displayed a PCB congener profile that was different from the profiles observed in the blood from the other exposure groups (cos Θ = 0.92 to 0.97). The

percentage of some congeners (e.g., PCB 70) was higher in KO compared to WT mice (Fig. 2b). Different congeners (e.g., PCB 99 and PCB 180) had a lower percentage in KO compared to WT mice. In the blood from F_{WT} mice, PCB congeners belonging to Class D were present at a lower mass percentage, and more persistent PCB congeners from Classes A, B, and C were present at a higher mass percentage compared to M_{WT}, F_{KO}, and M_{KO}. Similar trends between KO and WT mice were observed for these congeners when comparing PCB levels adjusted for wet weight (Fig. S6b). For example, PCB 95 levels, expressed on a wet weight basis, were lower in the blood from F_{WT} mice (8±1 ng/g wet weight) compared to the other exposure groups (13 to 14 ng/g wet weight, depending on the exposure group).

Comparison of PCB profiles in the brain from different exposure groups and Aroclor 1254. PCB residues in the brain from all exposure groups were distinctively different from the Aroclor 1254 profile (Fig. 2c), with similarity coefficients ranging from 0.80 to 0.86 (Table S6). PCB 138 (analyzed as a peak of four co-eluting PCB congeners) and PCBs 132/153 were the two dominant PCB congeners in the brain from both WT and KO mice. PCB 118, the dominant PCB congener in Aroclor 1254 (12.6 %), contributed a similar percentage to the ΣPCBs in all exposure groups (9.8 to 12.3 %). These PCBs were also prevalent PCB congeners detected in the brain from postnatal day 31 rats exposed to Aroclor 1254 throughout development via the maternal.[57] PCB 138, PCB 153 and PCB 118 were also prevalent PCB congener in postmortem human brain samples.[58, 59] Unlike the present study, these studies analyzed a small set of PCB congeners and, thus, provide limited insights into the congener profiles present in the rodent or human brain.

The PCB congener profiles in the brain showed genotype and sex-dependent differences (Fig. 2c), with similarity coefficients ranging from 0.96 to 0.99 (Table S7). Many congeners belonging to Class D (e.g., PCB 136) were lower in F_{WT} mice compared to the other exposure groups, especially F_{KO} mice. The differences observed in the congener profiles F_{WT} compared to F_{KO} mice translated into differences in the levels of Class D congeners in the brain (Fig. S6c), an observation that is toxicologically relevant. For example, PCB 95 and PCB 136 are potent sensitizers of the ryanodine receptors,[60] cellular targets

implicated in PCB-induced developmental neurotoxicity.[30, 61, 62] PCB mixtures (i.e., Aroclor 1254) and PCB 95 display a non-monotonic dose-response relationship in rodent studies of PCB developmental neurotoxicity.[57, 63] Thus, depending on the experimental design and the dose, relatively small differences in the levels of potent, neurotoxic PCB congeners in the developing brain may significantly affect neurotoxic outcomes. In the present study, levels of PCB 95 and PCB 136 were 1.4- and 3.0-fold higher in the brain from F_{KO} compared to F_{WT} mice, respectively. Further studies are needed to assess if similar differences in the levels of these neurotoxic PCB congeners are present in the fetal and neonatal brain of KO compared to WT mice exposed to PCBs.

Comparison of PCB profiles in the liver from different exposure groups and Aroclor 1254. PCB profiles in the liver were clearly different from the profile observed for Aroclor 1254 (Fig. 2d), with similarity coefficients ranging from 0.77 to 0.88 (Table S6). PCB congener profiles in the liver of M_{WT}, $M_{KO,}$ and F_{KO} mice showed only small differences, with similarity coefficients of 0.97 to 1.0. (Table S7). As with blood and brain tissue, more pronounced differences were observed for F_{WT} mice compared to the other exposure groups (similarity coefficient 0.92 to 0.93; Table S7). Levels of individual PCB congeners were always much higher in KO compared to WT mice, irrespective of the sex, because of the higher fat and protein content of the liver of KO mice (Fig. S6d).[26, 41, 42]

Female mice had higher levels of specific PCB congeners in the liver than male mice of the same genotype; however, this difference typically did not reach statistical significance (t-test, $p > 0.05$). We observed a similar trend when comparing PCB levels on a ΣPCB and PCB homolog basis (Fig. 1). Sex differences in PCB levels of WT mice, especially in the liver, would be consistent with the higher expression of Cyp2b10, a cytochrome P450 isoform implicated in the metabolism on Class D PCB congeners,[64] and other hepatic and extrahepatic cytochrome P450 enzymes in the female compared to the male mouse liver.[65] Indeed, PCB 136 is more rapidly oxidized in precision-cut tissue slices from male compared to female rats.[66] However, no sex-dependent differences in PCB levels have been reported in earlier disposition studies in rodents after developmental PCB exposure.[57, 67-69] These observations suggest

that sex-dependent differences in the hepatic metabolism of PCBs have only a small effect on PCB tissue levels and, possibly, toxic outcomes, at least in rodent models.

Differences in PCB profiles in tissues from the same exposure group. Laboratory and biomonitoring studies generally assume that the profiles and levels of PCB in the blood are a reasonable approximation of tissue profiles and levels. For example, the distribution of PCBs in juvenile rats shows that serum levels are predictive of liver levels;[15] however, studies that investigate the congener-specific distribution of PCBs across tissues are limited, especially for lower chlorinated PCBs. In the present study, PCB profiles displayed modest differences between tissues within each exposure group, as indicated by tissue-to-tissue similarity coefficients ranging from 0.85 to 0.98 (Table S8; Fig. S7). Several other animal studies also reported modest inter-tissue differences in the PCB profiles after oral or inhalation exposure ($\cos \Theta \geq 0.80$).[6, 32, 70]

Sparse partial least squares-discriminant analysis (sPLS-DA) was used to investigate differences in the PCB profiles across tissues within the same exposure group (Fig 3; Figs. S8-S10). sPLS-DA comparing the PCB congener profiles from F_{WT} mice showed clear groupings according to tissue, with three principal components (PCs) accounting for 84.6% of the data variance (Fig. 3). PC1 separated adipose tissue from the brain and liver. Based on their loadings, PCB congeners belonging to Class D (i.e., PCB 52 and PCB 70) were higher in adipose tissue, but lower in brain and liver. PCBs belonging to Class B (i.e., PCB 138) and Class C (i.e., PCB 146 and PCB 183) made a lower contribution to the PCB profile in adipose tissue, but a higher contribution in the brain and liver. PC2 separated blood from adipose tissue, with some Class D congener (i.e., PCB 139/149 and PCB 151) being higher in blood, but lower in adipose tissue and liver. PC3 separated the liver from other tissues because of lower relative levels of Class D congeners (i.e., PCBs 134/143 and PCB 136). Overall, differences in the PCB congener profiles across tissues from W_{WT} were due to differences in the percentage of metabolically more labile PCB congeners (Class D) compared to congeners resistant to metabolites (Class A through C). Similar differences in the PCB congener profiles across tissues were observed for the other exposure groups

333 despite the impaired hepatic metabolism in KO mice (Figs. S8-S10). In general, these comparisons
334 support the assumption that PCB profiles in the blood are a reasonable approximation of PCB tissue
335 profiles in mice and other mammals.

336 ***Tissue-to-blood distribution of PCBs within each exposure group.*** The distribution of individual
337 congener from the blood into tissues has toxicological relevance. Tissue : blood ratios followed the rank
338 order adipose : blood (median: 201; range: 12-841) >> liver : blood (median: 22; range: 7-213) > brain :
339 blood (median: 11; range: 3-34) in WT mice. This rank order is consistent with earlier studies in mice
340 and other mammalian models.[11] The differences between liver : blood vs. brain : blood partition
341 coefficients were more pronounced in KO mice because of the higher lipid content in the liver, with
342 adipose : blood (median: 71; range: 40-470) ~ liver : blood (median: 91; range 47-481) >> brain : blood
343 (median: 5; range 3-28). With the exception of the liver : blood partition coefficients in KO mice, the
344 partition coefficients in this study are within the range of partition coefficients observed in animal studies
345 and in humans.[11, 71] For example, adipose to plasma partition coefficients of 24 PCB congeners in
346 occupationally exposed individuals ranged from 50 to 370.[71]

347 The partitioning of PCBs between tissues and blood changed with increasing degree of chlorination.
348 For example, tetra- to hexachlorinated PCBs appeared to have a much higher affinity for adipose tissue
349 than higher chlorinated homologs in F_{WT} mice at the time point investigated (Fig. 4). The opposite was
350 true for the brain, where the partitioning into the brain appeared to increase with an increase in
351 chlorination. The partitioning of PCBs from the blood into the liver from F_{WT} mice seemed to be
352 comparable across homolog groups. Similar trends were observed for M_{WT}, $M_{KO,}$ and F_{KO} mice (Figs.
353 S11-S13). The decrease in the adipose : blood partitioning by the homolog group is unexpected based on
354 a structure-based model of the tissue-to-blood partitioning of PCBs.[72] Besides, the partitioning of
355 individual congeners from the blood into tissues was congener-specific and displayed considerable
356 variability (Figs. 4a-c). Thus, congener specific differences in the blood-to-tissue partitioning exist at
357 specific time points that may influence toxic outcomes following PCB exposure. These differences are

not captured when, as discussed above, only PCB profiles are compared between tissues. Especially systematic studies of tissue : blood ratios in human postmortem samples remain limited,[73-75] and further congener-specific studies are needed, especially for lower chlorinated PCB congeners that, unlike persistent legacy PCBs, are still inadvertently produced and represent a current public health concern.[56]

Comparison of the experimental and theoretical tissue-to-blood distribution of PCBs. A number of models have been developed to predict the partition coefficients in tissues in the absence of experimental data, including tissue composition-based models.[33, 34] These models use readily available information, including the quantities of the biological components (e.g., lipids, protein, and water) in tissues and the partition coefficients of PCBs (e.g., lipid/water partitioning coefficient), to estimate the distribution of PCBs *in vivo*. For example, a PP-LFER-composition-based model was adopted to determine the liver : blood partitioning coefficients mono- to hexachlorinated PCBs and the adipose : plasma partition coefficients of di- to octa-chlorinated PCBs in rats.[33] This model has several advantages: It can be used to predict the partitioning of PCBs between the biological components of all PCB congeners can be described with published Abraham solvation parameters[35] and PP-LFERs describing the correlations between the chemical descriptors and tissue/water partition coefficients.[36-39] Moreover, this model should be able to estimate the tissue : blood partitioning of PCBs in mouse models with a fatty liver.

The theoretical tissue : blood ratios, determined with this published model,[33] are shown for all 209 PCB congeners in Fig. 4d. The model did not predict the congener-specific variability of the partition coefficients. Moreover, the model predicted an increase, not a decrease in the adipose : blood ratios with increasing degree of chlorination. These differences between the calculated and experimental value are not entirely surprising because the model does not take structural features into account that, for example, influence the rate of metabolism of individual PCB congeners. Moreover, individual PCBs probably had not reached steady-state levels in our study. The model also overestimated the partitioning of PCBs into the brain. Similarly, earlier studies noted that the levels of PCBs in the brain are lower than expected

382 based on the lipid content of the brain, a fact that can be explained with the unique lipid composition of
383 the human brain.[58, 76]

384 Despite these differences, the predicted and experimental partition coefficients were in reasonable
385 agreement with each other (i.e., deviations were within one order of magnitude, Fig. 4e) and matched the
386 calculated adipose : blood partition coefficients in rats.[33] The model correctly predicted the more
387 pronounced partitioning of PCBs into the liver of KO mice. While not perfect, composition-based models
388 are, therefore, an effective tool to approximate tissue levels based on blood PCB levels, which are
389 experimentally more accessible, especially in humans. However, more detailed, PCB congener-specific
390 studies of the PCB levels in human tissues and blood from the same donors are needed to establish
391 whether or not the model used in our research also allows a rough estimate of PCB tissue levels based on
392 experimentally determined blood levels. Additional work is also necessary to better predict the
393 partitioning of PCBs into brain tissues.

394 ## CONFLICT OF INTEREST STATEMENT

395 The authors declare no competing financial interest.

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SUPPORTING INFORMATION

Details regarding GC-MS parameters and the detector response for individual PCB congeners, the biological composition of mouse tissues, PCB composition by homolog group and class, similarity coefficients, PCB congener profiles in tissues, levels of PCB 91 and PCB 136 in tissues from KO and WT mice, comparison of the PCB levels and congener profiles in different tissues, sPLS-DA comparing PCB congener profiles in tissues from M_{WT}, M_{KO} and F_{KO} mice, tissue : blood ratios of different PCB homolog groups in M_{WT}, M_{KO} and F_{KO} mice; and predicted tissue : blood ratios. This material is available free of charge via the Internet at http://pubs.acs.org.

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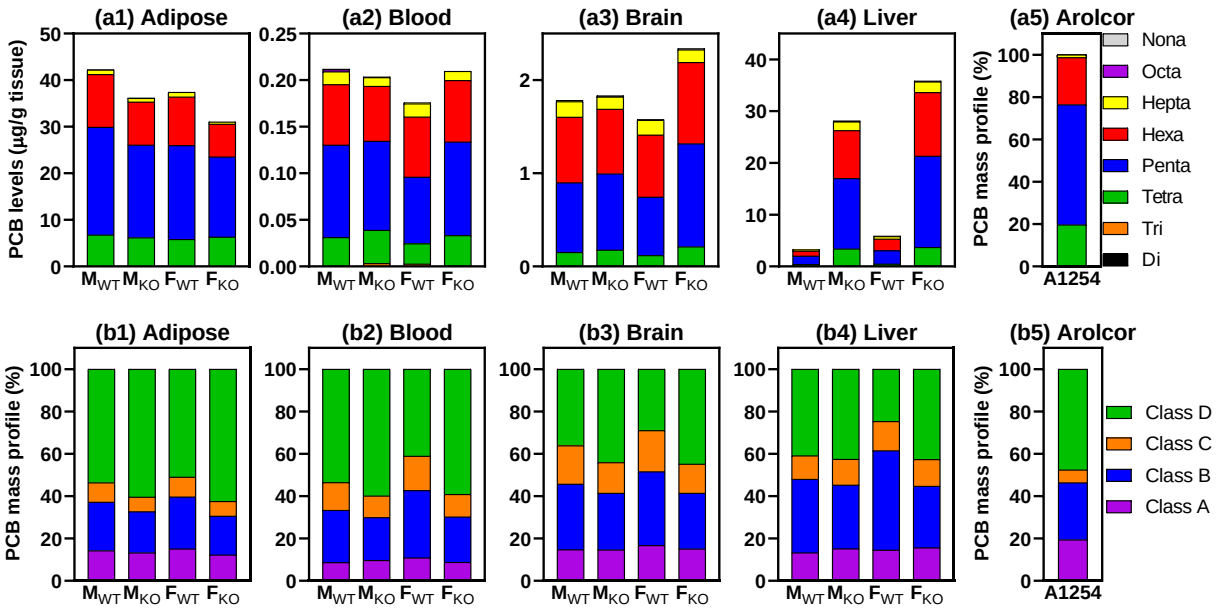


665

666 **Fig 1.** Both the total PCB levels (ΣPCB) with homolog composition in (a1) adipose, (a2) blood, (a3) brain,
667 and (a4) liver and mass percentages based on PCB Class in (b1) adipose, (b2) blood, (b3) brain, (b4) liver
668 show genotype and sex-dependent differences. Levels of PCB congeners were measured by GC-MS in
669 tissues from male wildtype (M_{WT}), male knockout (M_{KO}), female wildtype (F_{WT}), and female knockout
670 (F_{KO}) mice exposed orally to Aroclor 1254. The composition of Aroclor 1254 by homolog group and Class
671 is shown for comparison in panels (a5) and (b5). Class A: PCBs congeners with a 4-, 3,4-, and 3,4,5-
672 substitution pattern and zero or one ortho chlorine substituent. Class B: PCB congeners with two or more
673 ortho chlorine substituents and a 2,4- or 2,3,4- substitution pattern. Class C: PCBs congeners with a 2,4,5-
674 substitution pattern. Class D: PCB congeners that, in contrast to Classes A through C, are readily
675 metabolized and, for example, have a 2-, 2,3-, 2,5- and 2,3,6-substitution pattern in at least one phenyl
676 ring.[50]

677

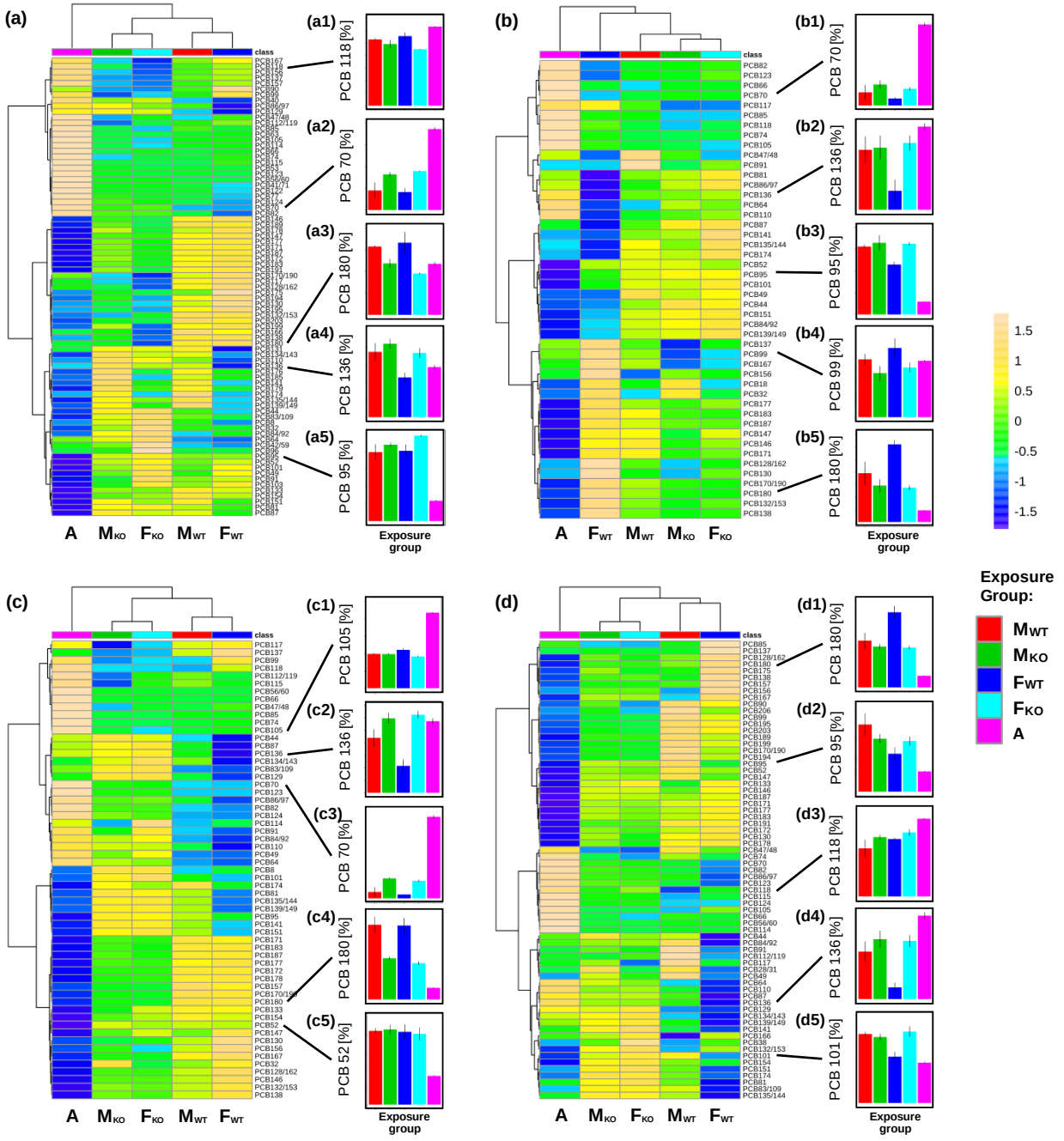
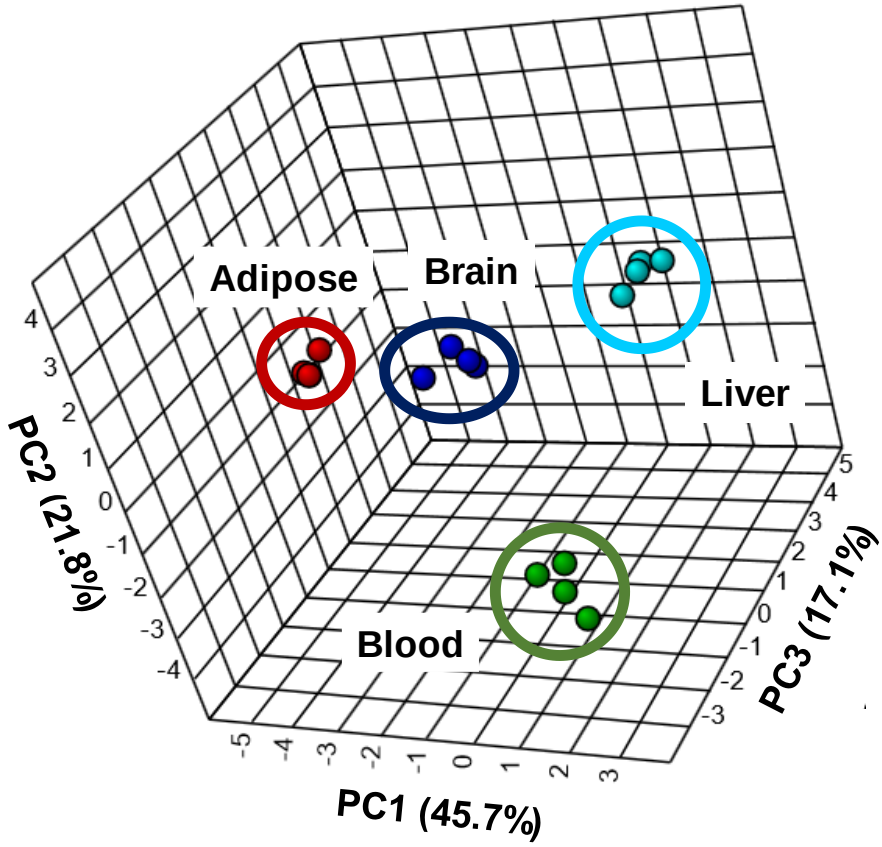


Fig 2. A comparison of the PCB congener profiles in (a) adipose tissue, (b) blood, (c) brain and (d) liver from male wildtype (M_{WT}), male knockout (M_{KO}), female wildtype (F_{WT}), and female knockout (F_{KO}) mice reveals genotype-dependent differences in the distribution of individual PCB congeners in these tissues. The congener profile of Aroclor 1254 is shown for comparison. The small panels illustrate differences in the mass percentages of selected PCB congeners. Heatmaps were generated after removing

684 features with >25% missing values and autoscaling features of the original data using the Heatmap

685 function as implement by MetaboAnalyst 4.0.[40]



686

687 **Fig 3.** Sparse partial least squares - discriminant analysis (sPLS-DA)[77] revealed differences in the PCB
688 congener profiles between tissues from F_{WT} mice (n = 4), with three principal components (PCs)
689 accounting for 84.6% of the data variance. These differences were due to tissue-specific changes in the
690 relative levels of PCB congeners that are more readily metabolized (Class D) vs. PCB congeners that are
691 more resistant to metabolism (Classes A through C). PC1 separated adipose tissue from the brain and
692 liver; PC2 separated blood from adipose tissue; PC3 separated the liver from other tissues. sPLS-DA was
693 performed with PCB congener profiles using MetaboAnalyst 4.0 after removing variables for a threshold
694 of 25%, cubic root transformation, and autoscaling features.[40] For analogous analyses of PCB congener
695 profiles from M_{WT}, M_{KO}, and F_{KO} mice, see Figs. S8-S10 in the Supporting Information.

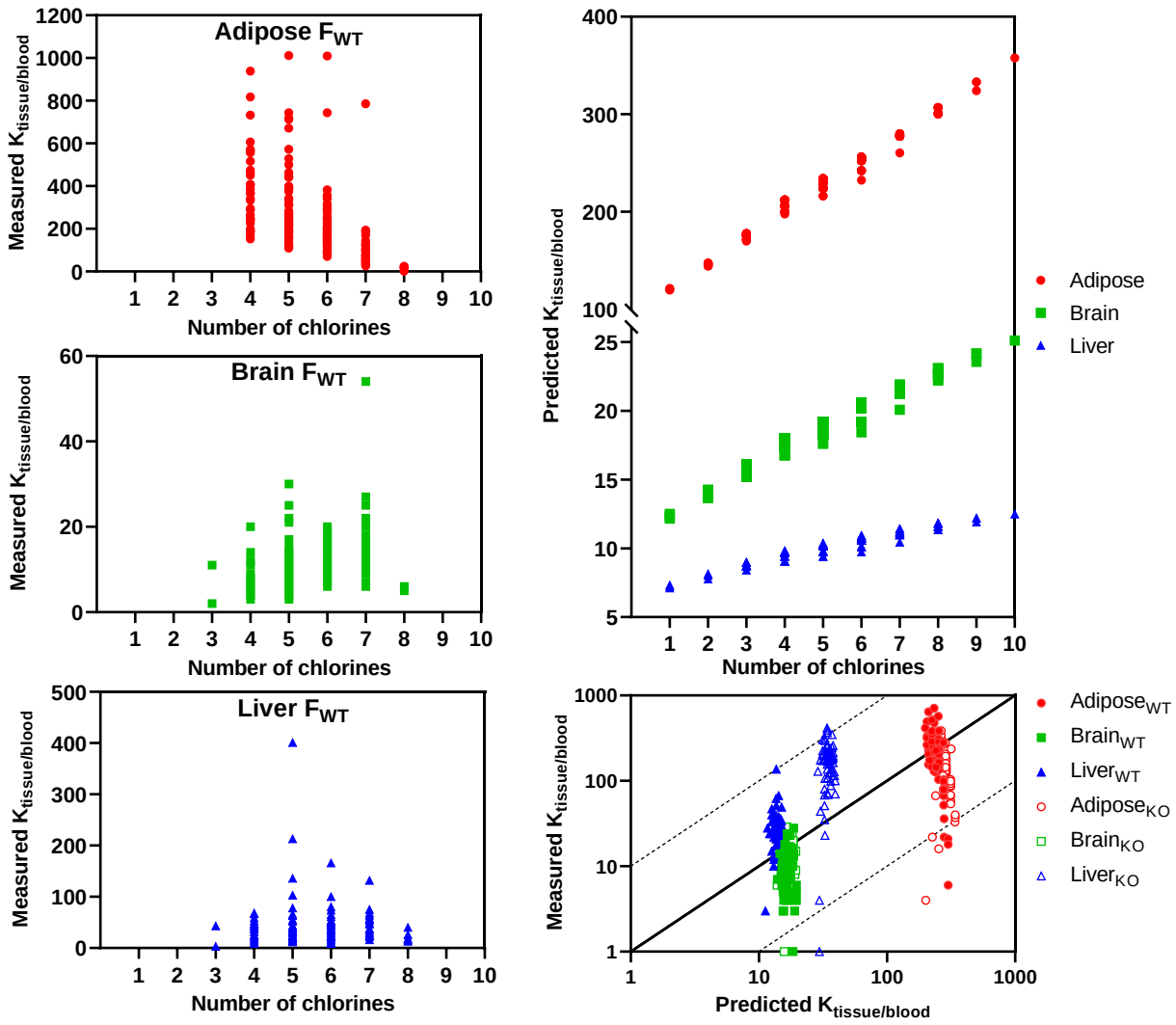


Fig 4. Tissue : blood ratios ($K_{tissue/blood}$, (ng/g)/(ng/g)) of different PCB homolog groups in female wild type mice ($F_{WT,}$ n = 4) show different trends with an increasing degree of chlorination: (a) Adipose : blood ratios decrease, (b) brain : blood ratios increase, and (c) liver : blood ratios of PCBs remain comparable with increasing degree of chlorination. Similar trends were observed with all other exposure groups (Figures S11-S13). (d) In contrast to the experimental findings, predicted tissue : blood ratios (i.e., adipose : blood, brain : blood, and liver : blood ratios) increase with an increasing degree of chlorination. Data for the tissue composition were obtained from the literature.[33] (e) Despite this discrepancy, the predicted tissue : blood ratios are in reasonable agreement with the experimental values (for the

705 underlying tissue composition data, see Table S3). Each symbol represent the tissue : blood ratio of an

706 individual PCB congener.