Supporting Information

Toxicokinetics of Chiral PCB 136 and its Hydroxylated Metabolites in Mice with a Liver-Specific Deletion of Cytochrome P450 Reductase

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Table of Content

Abbreviations

PCB: polychlorinated biphenyl

MDL: method detection limits

LOD: limits of detection

LOQ: limits of quantification

M-KO: male knockout;

M-WT: male wildtype

F-KO: female knockout

F-WT: female wildtype

Table S1: Summary of mice wet tissue weight and body weight [a] (g)

Tissue	M-KO (n = 35)	M-WT (n = 36)	F-KO (n = 29)	F-WT (n = 34)
Brain	0.43 ± 0.03	0.43 ± 0.02	0.42 ± 0.03	0.42 ± 0.03
Gonad	0.15 ± 0.02 *	0.16 ± 0.02 $	0.07 ± 0.04	0.08 ± 0.04
Heart	0.12 ± 0.02 *#	0.13 ± 0.01 $	0.11 ± 0.01	0.10 ± 0.02
Kidney	0.29 ± 0.04 *	0.32 ± 0.04 $	0.23 ± 0.03	0.23 ± 0.03
Liver	1.60 ± 0.30 *#	1.2 ± 0.2 $	1.30 ± 0.20 ^	1.00 ± 0.20
Lung	0.15 ± 0.03	0.16 ± 0.02 $	0.15 ± 0.04	0.14 ± 0.02
Spleen	0.07 ± 0.02 *	0.08 ± 0.01 $	0.09 ± 0.02	0.09 ± 0.01
Body weight	23 ± 3 *#	24 ± 3 $	19 ± 2	19 ± 2

[a] The body and tissue weight values are means ± SD, and animal ages are 8 weeks ± 2 days. * M-KO is significantly different from F-KO ($p < 0.05$); $ M-WT is significantly different from F-WT ($p < 0.05$); # M-KO is significantly different from M-WT ($p < 0.05$); ^ F-KO is significantly different from F-WT ($p < 0.05$).

Table S2: Wet tissue weight adjusted by body weight [a] (g tissue/kg BW) [g/kg]

Tissue	M-KO (n = 35)	M-WT (n = 36)	F-KO (n = 29)	F-WT (n = 34)
Brain	19 ± 2 *#	18 ± 2 $	22 ± 2	22 ± 2
Gonad	6.6 ± 1.9 *	6.6 ± 0.7 $	3.7 ± 1.8	4.2 ± 1.9
Heart	5.2 ± 0.6	5.3 ± 0.5	5.4 ± 0.6	5.2 ± 0.6
Kidney	12.9 ± 1.1 *	13.1 ± 1.1 $	11.7 ± 1.1	12.0 ± 0.9
Liver	69 ± 7 #	51 ± 6	67 ± 8 ^	50 ± 7
Lung	6.8 ± 1.1 *	6.5 ± 0.9 $	7.9 ± 1.9	7.5 ± 0.9
Spleen	3.3 ± 0.5 *	3.1 ± 0.4 $	4.5 ± 0.7	4.5 ± 0.5

[a] The adjusted body and tissue weight values are means ± SD, and animal ages are 8 weeks ± 2 days * M-KO is significantly different from F-KO ($p < 0.05$); $ M-WT is significantly different from F-WT ($p < 0.05$), # M-KO is significantly different from M-WT ($p < 0.05$); ^ F-KO is significantly different from F-WT ($p < 0.05$).

Table S3: Method detection limits (MDL), limits of detection (LOD), and limits of quantification (LOQ) for the gas chromatographic quantification of PCB 136 and its metabolites in whole mouse blood.[a]

PCB and metabolites	**PCB 136**	**3-150**	**5-136**	**4-136**	**4,5-136**
MDL[b] [ng] (n = 10)	0.13	0.11	0.20	0.75	0.08
LOD[c] [ng] (n = 28)	0.50	0.45	0.51	0.71	0.48
LOD[d] [ng/g] (n = 28)	0.66	0.92	0.92	1.25	0.88
LOQ[e] [ng/g] (n = 28)	6.62	9.20	9.17	12.49	8.79

[a] PCB 136 and metabolites were extracted with a liquid-liquid extraction method and analyzed on a gas chromatograph equipped with a ^{63}Ni-μECD detector, as described under Experimental Procedures. All the values are means ± SD.

[b] MDL, method detection limit, was calculated based on the method blank samples containing 1 mL of 1% KCl. The method blank samples were analyzed in parallel with blood samples.

[c] LOD, limit of detection, was calculated based on the background levels of mass (ng) of PCB 136 and its metabolites in whole blood from mice dosed with vehicle (corn oil) alone.

[d] LOD adjusted by whole blood weight (ng/g blood).

[bcd] LOD or MDL was calculated as $MDL\ or\ LOD = \overline{x_b} + k \times s_b$, where $\overline{x_b}$ is mean of all method blank signals (for MDL) or matrix blank (for LOD), k is Student's t-value for n-1 degree of freedom at 99% confidence level, and s_b is standard deviation of the corresponding measures.

[e] The LOQ was conservatively calculated as $LOQ = 10 \times LOD$.

Table S4: Summary of toxicokinetic parameters; see Experimental Procedures for additional details regarding the estimation of toxicokinetic parameters.

PCBs	genotype	C_{max} (ng/g)	T_{max} (h)	$T_{1/2}$ (h)	Cl/F*10^{-6} (ml/h)/(g BW)	AUC24/10 (ng/g)h	AUC24ss/10 (ng/g)h	SSa*100
(±)-PCB 136	M-WT	372(118)	4.07(1.39)	21.8(10.7)	2.40(0.400)	283(44.5)	426(85.1)	51.8(28.3)
	M-KO	210(58.6)	5.03(1.59)	18.4(8.32)	4.01(1.14)*	176(41.6)*	290(52.3)	69.0(30.3)
	F-WT	257(65.6)	4.06(1.42)	16.9(7.18)	3.74(0.904)	186(38.9)	226(40.4)^	22.8(8.69)
	F-KO	163(37.5)	5.37(1.33)	27.5(12.8)	4.32(0.552)	156(19.1)	336(91.1)	117(59.8) #
(+)-136	M-WT	225(67.5)	4.25(1.44)	23.2(11.3)	NA	177(27.1)	286(63.3)	63.3(35.2)
	M-KO	123(34.3)	5.20(1.59)	20.2(9.09)	NA	105(24.6)*	192(36.3)	89.9(41.3)
	F-WT	157(39.0)	4.20(1.46)	17.5(7.41)	NA	118(24.1)	149(25.5)^	28.1(10.9)
	F-KO	96.1(20.5)	5.66(1.24)	29.1(12.9)	NA	94.3(11.0)	229(65.7)	145(70.6) #
(-)-136	M-WT	149(51.6)	3.65(1.33)	18.9(9.12)	NA	103(18.0)	137(23.8)	34.3(17.1)
	M-KO	87.3(24.1)	4.79(1.64)	14.7(6.69)	NA	68.2(17.2)	95.3(18.3)	42.8(17.6)
	F-WT	101(26.4)	3.82(1.39)	16.3(7.13)	NA	65.7(15.2)	75.4(15.2)^	15.7(5.29)
	F-KO	67.8(16.8)	4.97(1.40)	23.6(12.0)	NA	58.3(8.29)	102(25.0)	77.3(43.4)#
4-136	M-WT	88.5(24.9)	12.0(6.44)	24.6(12.0)	NA	58.7(23.7)	202(67.3)	249(32.3)
	M-KO	(a)	(a)	(a)	NA	(a)	(a)	(a)
	F-WT	106(40.2)	8.43(4.67)	28.0(12.4)	NA	72.0(28.4)	203(66.5)	234(55.8)
	F-KO	(a)	(a)	(a)	NA	(a)	(a)	(a)
5-136	M-WT	59.8(13.1)	5.20(1.83)	25.2(11.0)	NA	51.0(8.88)	94.1(16.0)	88.7(34.9)
	M-KO	40.3(11.0)	4.85(1.43)	26.5(9.71)	NA	29.0(5.30)*	58.2(8.79)*	105(36.4)
	F-WT	90.0(22.0)	5.41(1.61)	16.8(6.01)	NA	69.6(21.3)	91.6(22.2)	35.4(17.7)
	F-KO	55.1(13.9)	6.20(1.47)	37.7(9.74)	NA	44.1(9.93)	101(19.7)	137(53.7) #
4,5-136	M-WT	63.3(9.67)	10.7(5.67)	26.7(9.85)	NA	66.3(7.80)	217(55.4)	231(91.7)
	M-KO	28.3(5.82)*	18.5(7.63)	23.9(8.08)	NA	24.7(3.02)*	87.7(19.3)*	260(90.8)
	F-WT	46.4(6.30)	9.26(4.95)	15.0(4.99)	NA	52.1(5.49)	115(23.5)	123(47.5)
	F-KO	35.0(7.96)	13.4(8.02)	13.4(8.02)	NA	29.6(6.01) #	110(50.1)	280(175) #

(a) Not included in the toxicokinetic analysis because of the large number of data below the detection limit.

NA: not applicable.

* Significant difference from M-WT ($p < 0.05$), # Significant difference from F-WT ($p < 0.05$), ^ Significant difference from M-WT ($p < 0.05$).

Table S5: EF values of PCB 136 and hydroxylated metabolites in whole blood from mice collected 5 h after exposure to racemic PCB 136

Animal	PCB and Metabolites	EF value [a]	SD
M-KO (n = 6)	PCB 136	0.59	0.01
M-WT (n = 6)		0.61	0.02
F-KO (n = 5)		0.58 *	0.01
F-WT (n = 5)		0.62	0.01
M-KO (n = 4)	4-136	0.65 ^	0.03
M-WT (n = 6)		0.67	0.06
F-KO (n = 3)		0.59 #	0.03
F-WT (n = 4)		0.72	0.05
M-KO (n = 5)	5-136	0.28	0.06
M-WT (n = 6)		0.25	0.06
F-KO (n = 3)		0.27 $	0.02
F-WT (n = 4)		0.23	0.02

[a] EF values were calculated based on equation, EF = Area E_2/(Area E_1 + Area E_2). For PCB 136, 5-136 and 4-136, all the second peaks in the elution order are the (+)-atropisomer.[1,2] * Significant difference from F-WT ($p < 0.05$); ^ Significant difference from F-KO ($p < 0.05$); # Significant difference from F-WT ($p < 0.05$); $ Significant difference from F-WT ($p < 0.05$).

Table S6. EF values of PCB 136 in whole blood over exposure time of racemic PCB 136

Time (h)	M-KO	M-WT	F-KO	F-WT
1	0.56 ± 0.01 (n = 6)	0.56 ± 0.01 (n = 5)	0.56 ± 0.01 (n = 6)	0.57 ± 0.01 (n = 4)
3	0.57 ± 0.01* (n = 3)	0.60 ± 0.02 (n = 5)	0.58 ± 0.01# (n = 3)	0.61 ± 0.01 (n = 6)
5	0.59 ± 0.01 (n = 6)	0.61 ± 0.02 (n = 6)	0.58 ± 0.02# (n = 6)	0.62 ± 0.01 (n = 5)
7	0.61 ± 0.01* (n = 6)	0.66 ± 0.02 (n = 5)	0.62 ± 0.02# (n = 3)	0.67 ± 0.03 (n = 6)
16	0.66 ± 0.02*$ (n = 5)	0.69 ± 0.02^ (n = 5)	0.69 ± 0.01# (n = 4)	0.74 ± 0.02 (n = 5)
24	0.71 ± 0.02* (n = 4)	0.74 ± 0.01 (n = 5)	0.72 ± 0.01 (n = 4)	0.74 ± 0.04 (n = 4)
48	0.77 ± 0.01 (n = 5)	0.75 ± 0.02^ (n = 4)	0.77 ± 0.02 (n = 5)	0.78 ± 0.01 (n = 6)

* Significant difference from M-WT at same time points group ($p < 0.05$); # Significant difference from F-WT at the same time points groups ($p < 0.05$); $ Significant difference from F-KO at same time point group ($p < 0.05$); ^ Significant difference from F-WT at same time points group ($p < 0.05$).

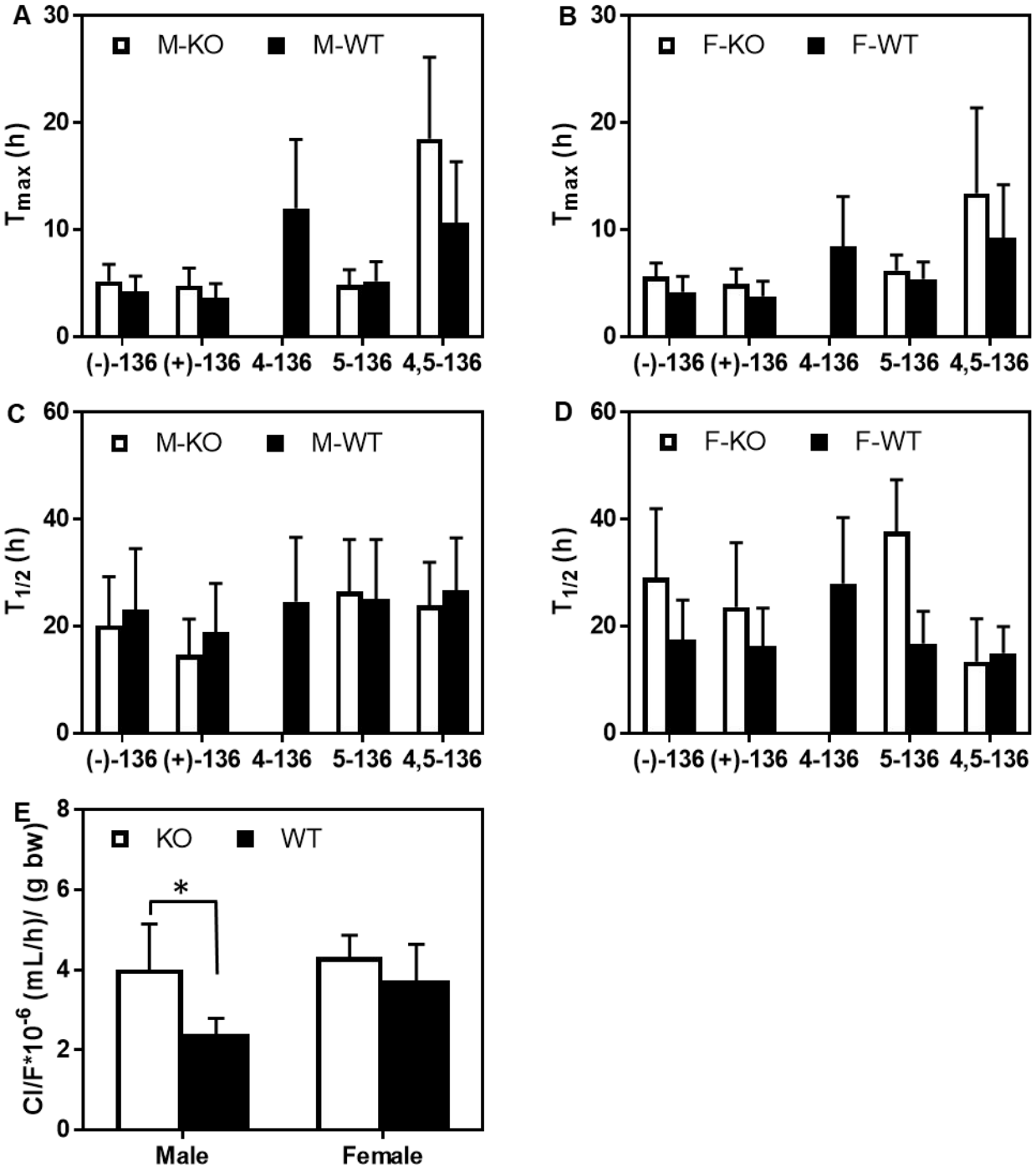

Figure S1. Comparison of toxicokinetic parameters of PCB 136 and its hydroxylated metabolites in whole blood from male and female KO and WT mice exposed orally to racemic PCB 136, including the time when the maximum concentration of PCB 136 and its metabolites are reached, T_{max} in (A) male and (B) female mice; the terminal half-life, $T_{1/2,}$ of PCB 136 and its metabolites in (C) male and (D) female mice; and (E) the bioavailability normalized clearance, Cl/F*10^{-6}, of PCB 136 in male and female mice.

Reference

(1) Wu, X., Barnhart, C., Lein, P. J., and Lehmler, H. J. (2015) Hepatic metabolism affects the atropselective disposition of 2,2',3,3',6,6'-hexachlorobiphenyl (PCB 136) in mice. *Environ. Sci. Technol. 49*, 616-625.

(2) Wu, X., Pramanik, A., Duffel, M. W., Hrycay, E. G., Bandiera, S. M., Lehmler, H. J., and Kania-Korwel, I. (2011) 2,2',3,3',6,6'-Hexachlorobiphenyl (PCB 136) is enantioselectively oxidized to hydroxylated metabolites by rat liver microsomes. *Chem. Res. Toxicol. 24*, 2249-2257.