2,2',3,3',6,6'-Hexachlorobiphenyl (PCB 136) is Enantioselectively Oxidized to Hydroxylated Metabolites by Rat Liver Microsomes

SUPPORTING INFORMATION

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Abbreviations: 4,5-diMeO-PCB 136, 4,5-dimethoxy-2,2',3,3',6,6'-hexachlorobiphenyl (methylated derivative of 4,5-diOH-PCB 136); 4-MeO-PCB 136, 2,2',3,3',6,6'-hexachloro-4-methoxybiphenyl (methylated derivative of 4-OH-PCB 136); 5-MeO-PCB 136, 2,2',3,3',6,6'-hexachloro-5-methoxybiphenyl (methylated derivative of 5-OH-PCB 136); 4,5-diOH-PCB 136, 2,2',3,3',6,6'-hexachlorobiphenyl-4,5-diol; 4-OH-PCB 136, 2,2',3,3',6,6'-hexachlorobiphenyl-4-ol; 5-OH-PCB 136, 2,2',3,3',6,6'-hexachlorobiphenyl-5-ol; BROD, 7-benzyloxyresorufin-O-debenzylase; BQ, 7-benzyloxyquinoline debenzylation; DEX, dexamethasone; EROD, 7-ethoxyresorufin-O-deethylase; ID, inner diameter; LOD, limit of detection; PB, phenobarbital; PCBs, polychlorinated biphenyls; PCB 136, 2,2',3,3',6,6'-hexachlorobiphenyl; VEH, vehicle (corn oil).

TABLE S1. Effect of treatment on body and liver weights of the male rats used for the preparation of liver microsomes.

Treatment	Final weight	Body weight change	Liver weight	Liver/body weight ratio
Corn oil (VEH) (n=6)	232 ± 9	-0.98 ± 0.89	8.6 ± 0.9	3.7 ± 0.4
Phenobarbital (PB) (n=6)	232 ± 4	-0.53 ± 1.8	10.2 ± 0.5	4.4 ± 0.2 $^{\$}$
Dexamethasone (DEX) (n=4)	175 ± 16 ***,###	-28 ± 13 ***,###	11.8 ± 2.2 *	6.7 ± 0.7 ***,###

$^{\$}$ Significantly different from VEH, one-sample, one-sided test, 95% confidence level, p<0.05,*** Significantly different from VEH, one-sample, one-sided test, 95% confidence level, p<0.01; ### Significantly different from PB, one-sample, one-sided test, 95% confidence level, p<0.01. R open-source statistical software (version 2.0.0, http://www.r-project.org/index.html) was used for all statistical analyses.

TABLE S2. Activities of P450 enzymes in male rat liver microsomes. Data are the average of duplicate measurements.

Activity assay	Microsomal preparation	Reaction rate (pmol/min/mg protein) (fold induction over VEH)
7-Ethoxyresorufin-*O*-deethylase (EROD)*	Corn oil	340
	Phenobarbital	1300 (4)
	Dexamethasone	350 (1)
Benzyloxyresorufin-*O*-debenzylase *	Corn oil	140
	Phenobarbital	13100 (100)
	Dexamethasone	930 (7)
7-Benzyloxyquinoline debenzylation (BQ)*	Corn oil	6500
	Phenobarbital	15800 (2)
	Dexamethasone	54600 (8)

* Hepatic microsomal EROD, BROD and BQ activities were measured using established methods.[1,2] EROD was used to detect the activity of P450 1A with ethoxyresorufin as a substrate; BROD was used to detect the activity of P450 2B with benzyloxyresorufin as a substrate; BQ was used to detect the activity of P450 3A with benzyloxyquinoline as substrate.

TABLE S3: Limits of detection [3], resolution and background levels of PCB 136 and hydroxylated its metabolites in control samples in the microsomal incubations.

	PCB 136 [ng]		**5-OH-PCB 136 [ng]**		**4-OH-PCB 136 [ng]**		**4,5-diOH-PCB 136 [ng]**
	(-)	**(+)**	**$E_{(1)}$**	**$E_{(2)}$**	**$E_{(1)}$**	**$E_{(2)}$**	
LOD on Chirasil-Dex column*	22.9 (n = 5)	0.9 (n = 5)	9.9 (n = 16)	16.8 (n = 16)			
LOD on Cyclosil-B column*					4.3 (n=19)	4.7 (n=19)	
Resolution&	0.910		0.714		0.741		
LOD on DB1-MS column*	55.3 (n = 11)		6.0 (n = 11)		12.9 (n = 11)		1.6 (n = 11)
Background	21.0 ± 36.2 (n = 6)		2.9 ± 1.5 (n = 14)		4.2 ± 1.2 (n = 14)		0.5 ± 0.3 (n = 14)

* The *LOD* was calculated from blank samples as $LOD = \overline{x_b} + k \cdot s_b$, where $\overline{x_b}$ is mean of 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; & The resolution (R_s) was calculated using the formula $R_s = (t_{R2} - t_{R1})/0.5(BW_1 + BW_2)$, where t_{R2} and t_{R1} are the retention times of the first and second eluting enantiomer, and BW_1 and BW_2 are the baseline width of the first and second eluting enantiomer.[4]

TABLE S4: Ratios of 5-OH-PCB 136 and 4-OH-PCB 136 formed in different microsomal incubations over time (50 μM PCB 136).

Incubation (min)	5-OH-PCB 136			4-OH-PCB 136		
	PB:VEH	DEX:VEH	PB:DEX	PB:VEH	DEX:VEH	PB:DEX
1	48	5.7	8.5	1.2	0.6	1.9
2	84	7.8	10.7	2.5	0.9	3.0
3	58	6.3	9.3	1.4	0.7	2.0
5	56	5.7	9.9	1.8	0.6	3.0
10	49	5.1	9.6	1.6	0.6	2.6
20	43	4.5	9.4	1.6	0.6	2.6
30	37	3.7	10.1	1.6	0.6	2.8

TABLE S5: Ratios of 5-OH-PCB 136 and 4-OH-PCB 136 formed in different microsomal incubations over time (5 μM PCB 136).

Incubation (min)	5-OH-PCB 136			4-OH-PCB 136		
	PB:VEH	DEX:VEH	PB:DEX	PB:VEH	DEX:VEH	PB:DEX
1	46	7.1	6.6	1.2	0.8	1.5
2	44	7.1	6.2	1.2	0.9	1.3
3	34	6.3	5.4	0.7	0.8	0.9
5	21	6.7	3.1	0.4	1.2	0.3
10	0	5.9	0	0.0	1.1	0.0
20	0	6.5	0	0.0	0.8	0.1
30	0	6.8	0	0.0	0.7	0.1

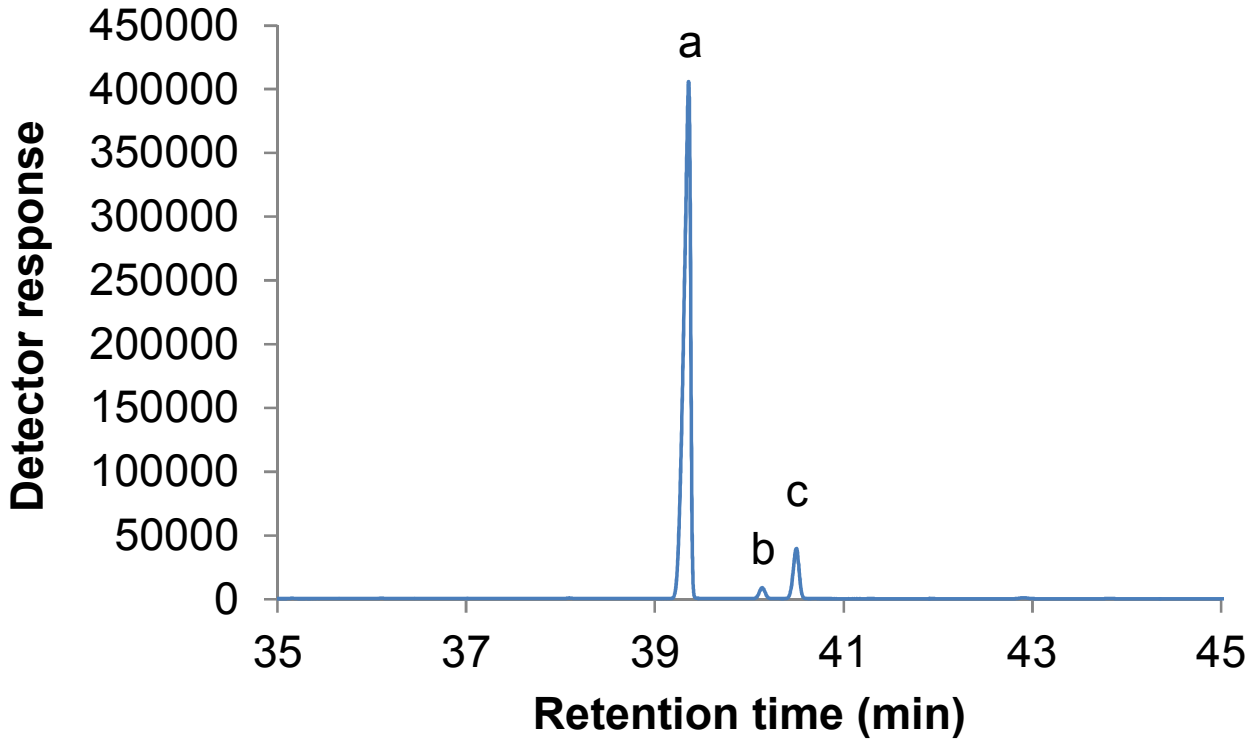

FIGURE S1:PCB 136 is metabolized to three hydroxylated metabolites by liver microsomes prepared from PB-treated rats. PCB 136 (50 µM) was incubated with rat liver microsomes for 30 min, the incubation mixture was extracted as described under Materials and Methods, derivatized with diazomethane and analyzed by GC/MS in the selected ion monitoring mode using *m/z* = 389.9 and 419.8. The analysis of hydroxylated metabolites was carried out using an Agilent 6890N gas chromatograph with 5975 mass selective detector and a DB1-MS capillary column (60 m x 0.25 mm ID x 0.25 µm film thickness; Agilent, Santa Clara, CA, USA). The temperature program was as follows: 100 °C for 1 min, 5 °C/min to 250 °C, hold for 20 min, 5 °C/min to 280 °C, hold for 3 min. Based on the mass spectra and the retention times of the authentic compounds (a) is 5-OH-PCB 136, (b) is 4-OH-PCB 136 and (c) is 4,5-diOH-PCB 136. The same three metabolites were observed in the total ion chromatogram and their mass spectra are shown in Fig S2.

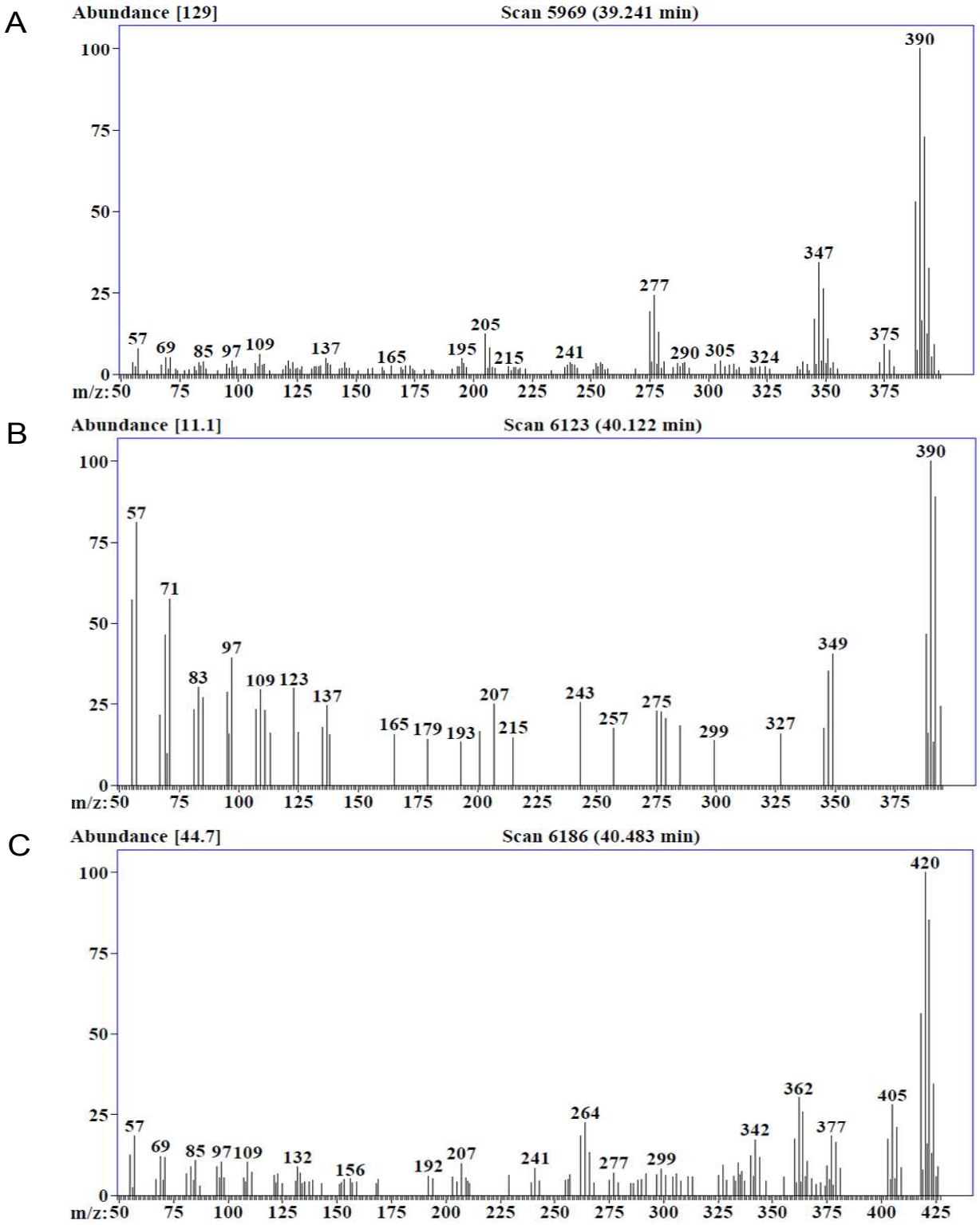

FIGURE S2: Mass spectra of the methylated derivatives of 5-OH-PCB 136 (A), 4-OH-PCB 136 (B) and 4,5-diOH-PCB 136 (C). The anticipated molecular weights (first isotopic peak only) are 390.9, 390.9 and 421.94 for 5-MeO-PCB 136, 4-MeO-PCB 136, and 4,5-diMeO-PCB 136, respectively. The only difference in the mass spectra of 5-MeO-PCB 136 and 4-MeO-PCB 136 is that 5-MeO-PCB136 gave a peak at *m/z* 375 [M-15]. The mass spectra are in good agreement with the published mass spectra of methylated PCB 136 metabolites.[5, 6] The retention times are 39.2, 40.1 and 40.5 min for the methylated derivatives of 5-OH-PCB 136, 4-OH-PCB 136 and 4,5-diOH-PCB 136, respectively.

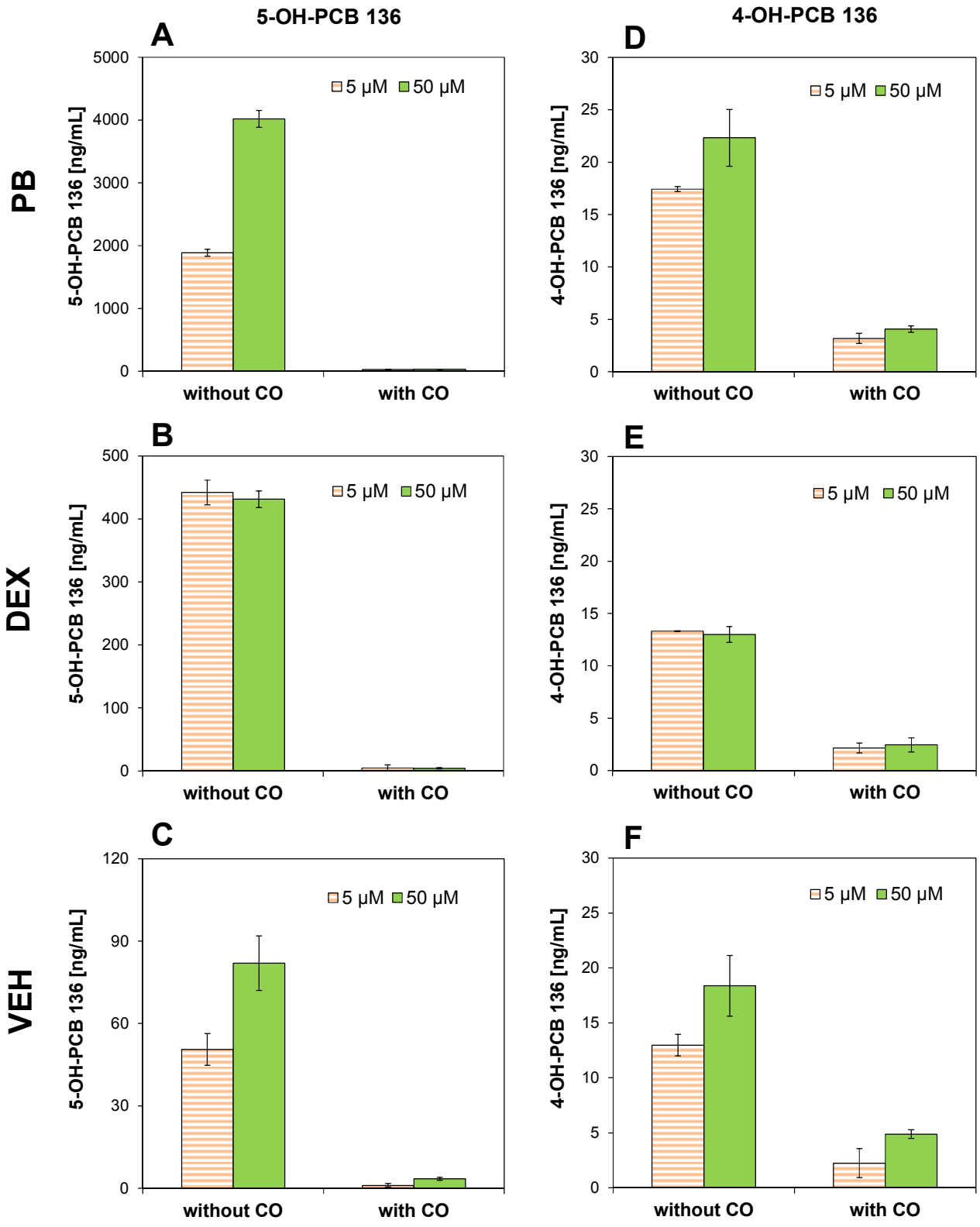

FIGURE S3: Pre-treatment of rat liver microsomes with carbon monoxide (CO) inhibited the metabolism of PCB 136 (5 µM and 50 µM) and reduced the formation of 5-OH and 4-OH PCB 136. The microsomes were obtained from the rats treated with PB (A and D), DEX (B and E), or VEH (C and F). All microsomal incubations were performed at 37 ºC for 5 min. The data represented as mean ± SD (n=3). The level of 4-OH-PCB 136 was below the detection limit in incubations with CO (**Table S3**)

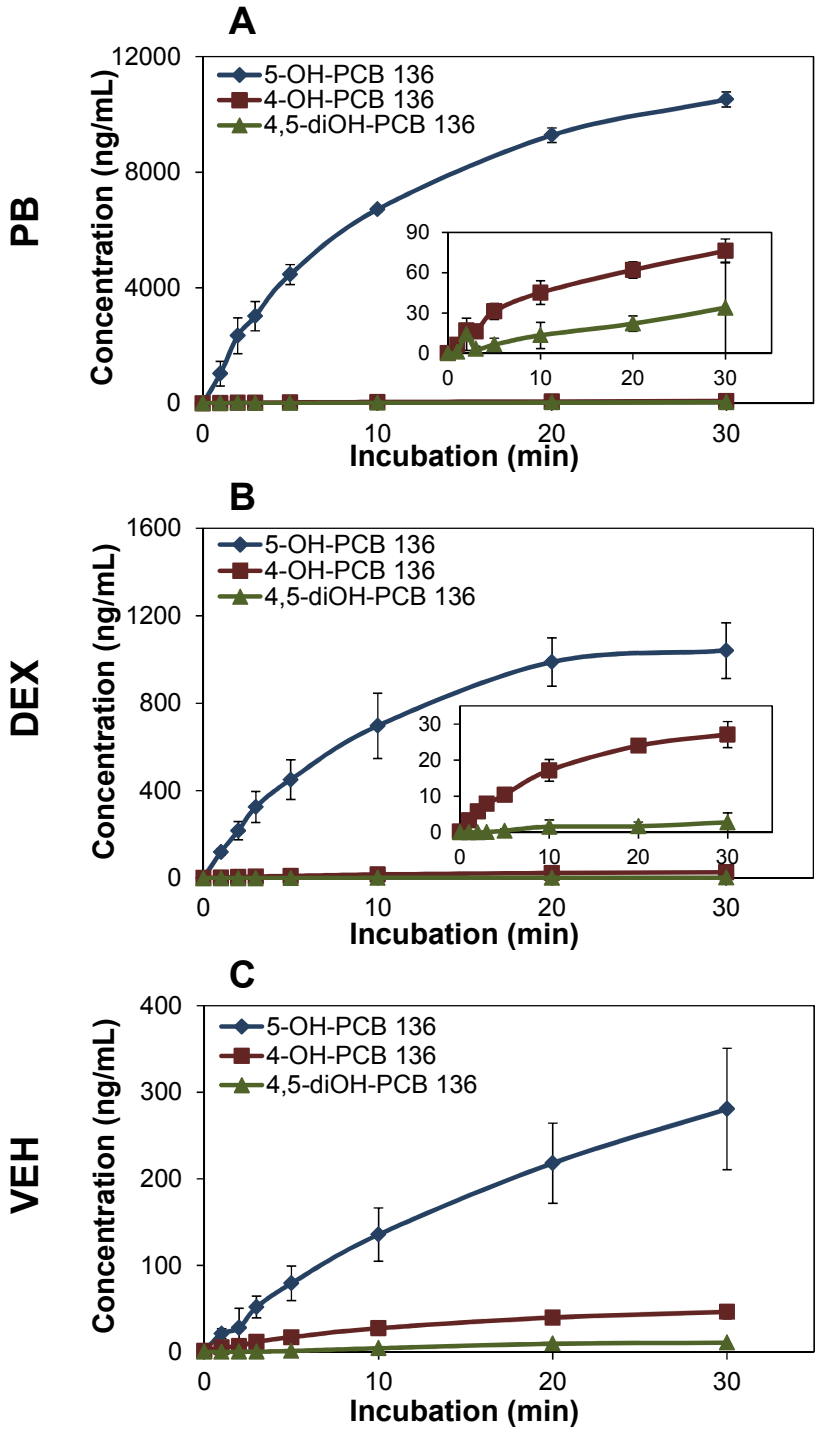

FIGURE S4: Time-dependent formation of hydroxylated PCB 136 metabolites by liver microsomes from (A) PB-, (B) DEX- and (C) VEH-treated rats at a PCB 136 concentration of 50 µM. PCB 136 was incubated for 30 min at 37 ºC and 2 mL aliquots of the incubation mixture were removed at defined time points. The hydroxylated PCB metabolites were extracted as described under Materials and Methods. The gas chromatographic analysis was carried out using an Agilent 6890N gas chromatograph with ^{63}Ni- µECD detector and DB1-MS capillary column (60 m x 0.25 mm ID x 0.25 µm film thickness; Agilent, Santa Clara, CA, USA). The temperature program was as follows: 100 °C for 1 min, 5 °C/min to 250 °C, hold for 20 min, 5 °C/min to 280 °C, hold for 3 min. Inserted figures in (A) and (B) showed the concentrations of 4-OH-PCB 136 and 4,5-diOH-PCB 136 by liver microsomes from PB and DEX-treated rats, respectively.

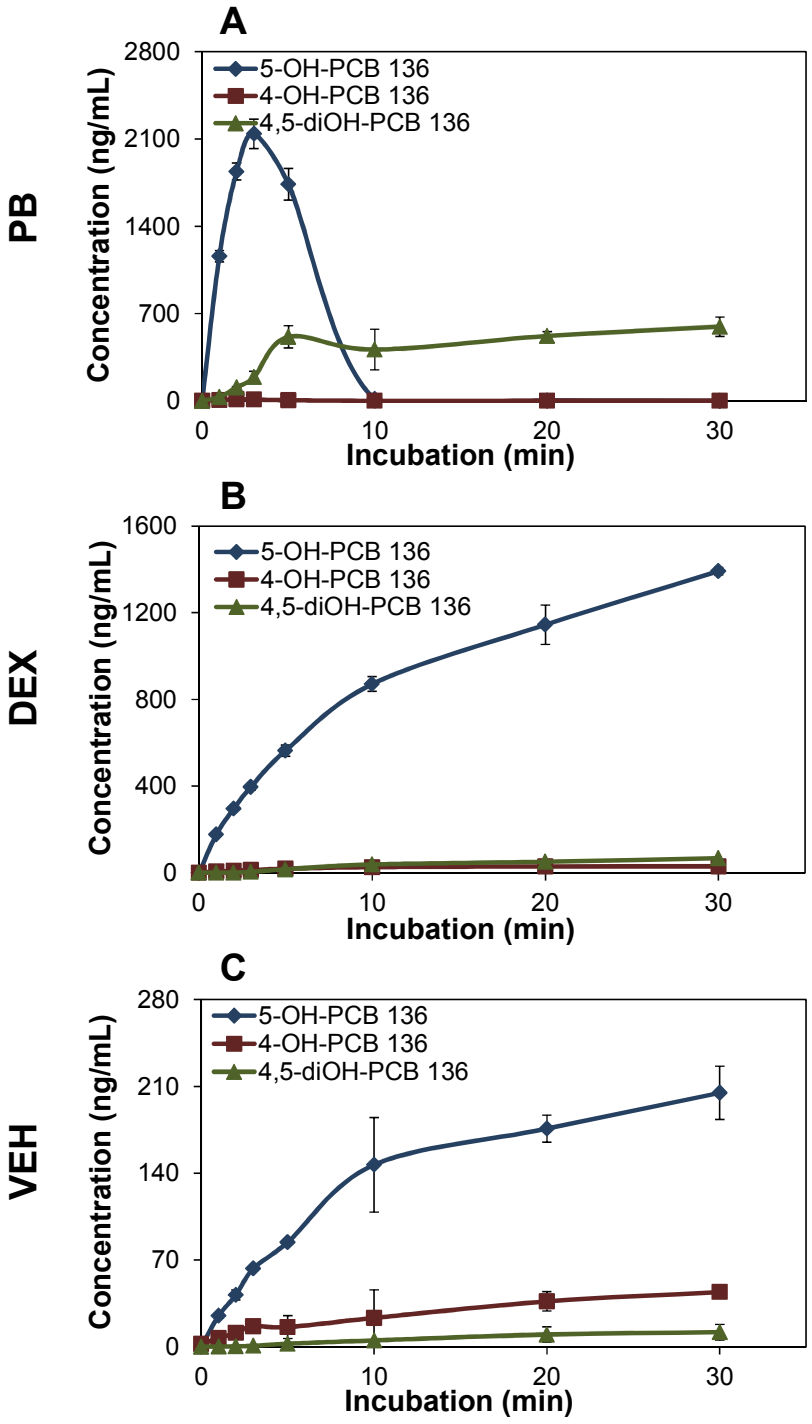

FIGURE S5: Time-dependent formation of hydroxylated PCB 136 metabolites by liver microsomes from (A) PB-, (B) DEX- and (C) VEH-treated rats at a PCB 136 concentration of 5 µM. PCB 136 was incubated for 30 min at 37 ºC and 2 mL aliquots of the incubation mixture were removed at defined time points. The hydroxylated PCB metabolites were extracted as described under Materials and Methods. The gas chromatographic analysis was carried out using an Agilent 6890N gas chromatograph with ^{63}Ni- µECD detector and DB1-MS capillary column (60 m x 0.25 mm ID x 0.25 µm film thickness; Agilent, Santa Clara, CA, USA). The temperature program was as follows: 100 °C for 1 min, 5 °C/min to 250 °C, hold for 20 min, 5 °C/min to 280 °C, hold for 3 min.

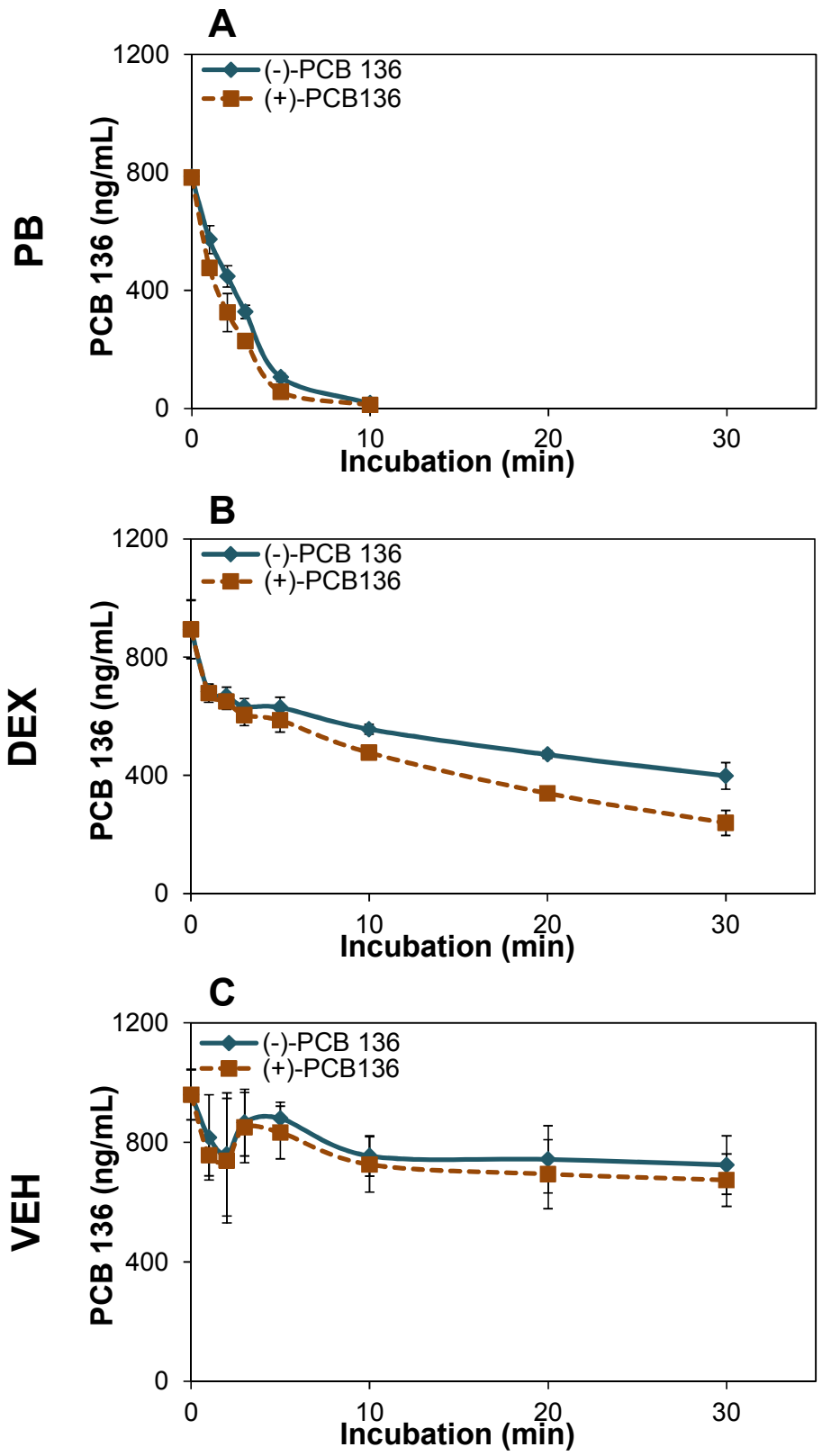

FIGURE S6: Time-dependent metabolism of PCB 136 atropisomers by liver microsomes from (A) PB-, (B) DEX- and (C) VEH-treated rats at a PCB 136 concentration of 5 µM. PCB 136 was incubated for 30 min at 37 ºC and 2 mL aliquots of the incubation mixture were removed at defined time points. See Figure S4 and Materials and Methods for additional details.

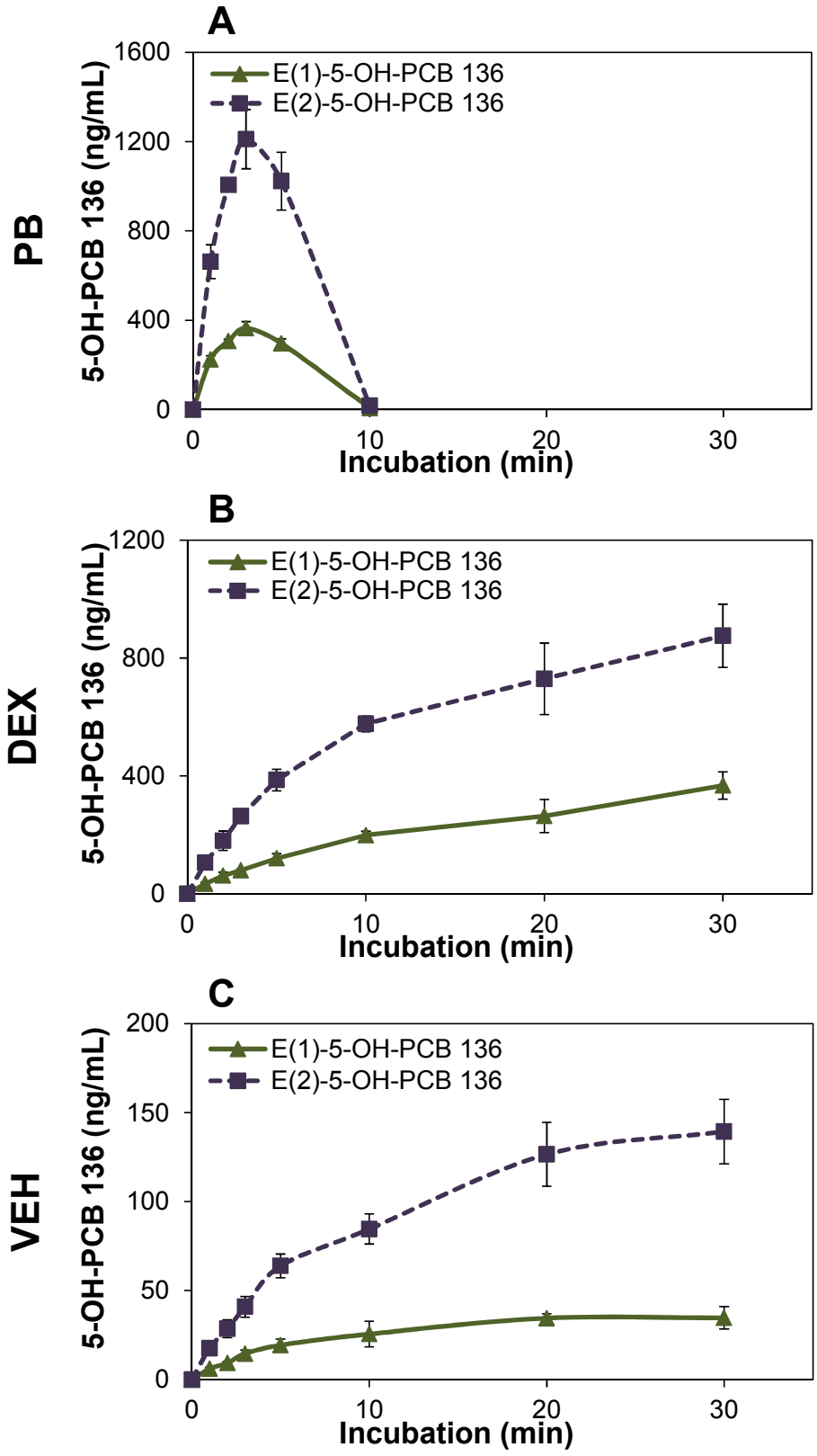

FIGURE S7: Time-dependent formation of 5-OH-PCB 136 atropisomers by liver microsomes from (A) PB-, (B) DEX- and (C) VEH-treated rats at a PCB 136 concentration of 5 µM. PCB 136 was incubated for 30 min at 37 ºC and 2 mL aliquots of the incubation mixture were removed at defined time points. See Figure S4 and Materials and Methods for additional details.

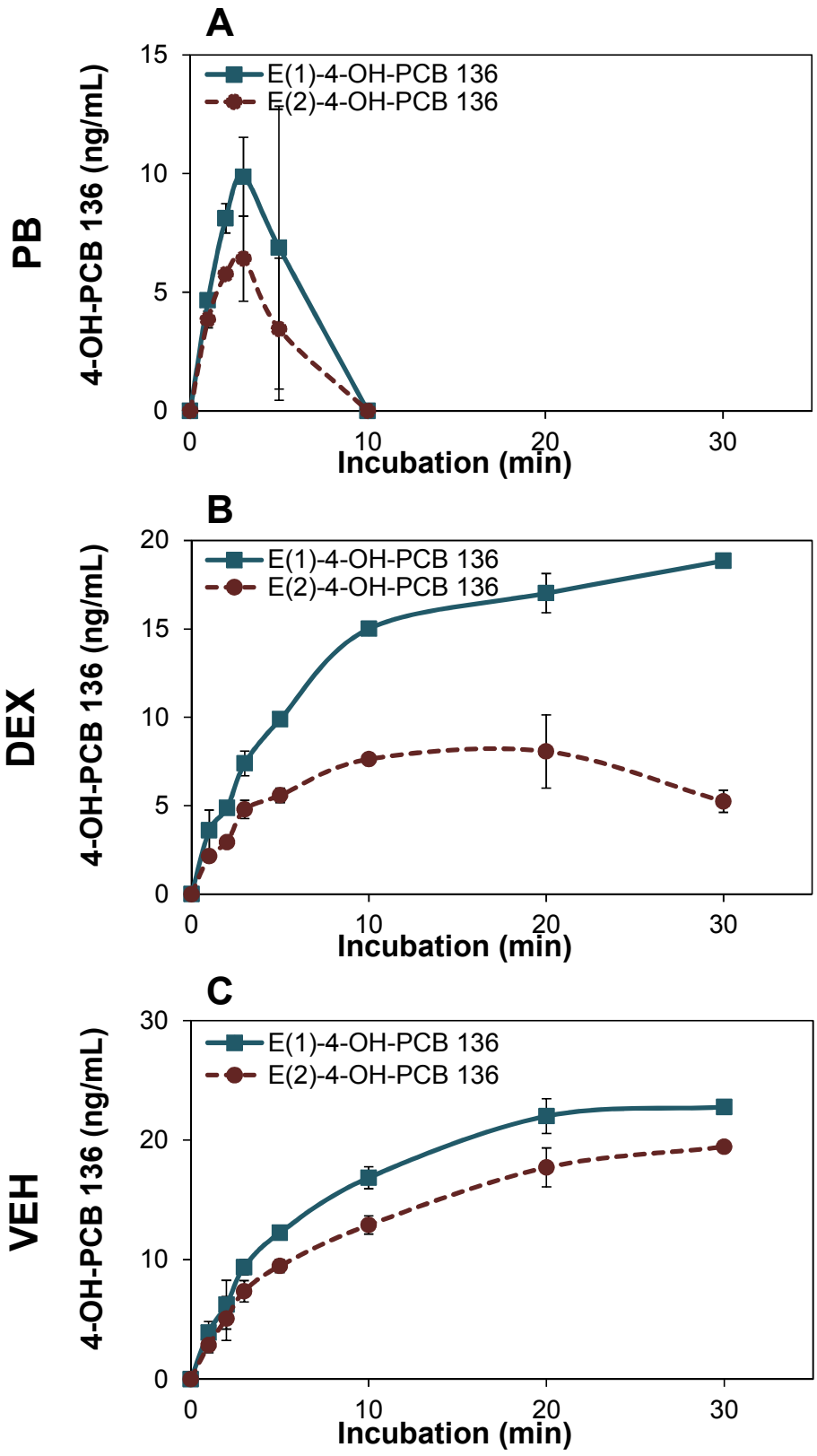

FIGURE S8: Time-dependent formation of 4-OH-PCB 136 atropisomers by liver microsomes from (A) PB-, (B) DEX- and (C) VEH-treated rats at a PCB 136 concentration of 5 µM. PCB 136 was incubated for 30 min at 37 ºC and 2 mL aliquots of the incubation mixture were removed at defined time points. See Figure S4 and Materials and Methods for additional details.

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