Supporting Information

Factors Affecting Phase I Stereoselective Biotransformation of Chiral Polychlorinated Biphenyls (PCBs) by Rat Cytochrome P-450 2B1 Isozyme

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Experimental Section

Chemicals and Regents

Racemic chiral PCBs 45, 95 and 132, recovery standards PCB 30 and 204, and internal standard PCB 159 (all purity>99%) were purchased from Accustandard (West Haven, CT). PCB solutions were prepared in acetone. Individual PCB 132 atropisomer were isolated as previously published (*1*). Rat CYP2B1, insect cell control supersomes (P450 reductase and cytochrome b_5) (stored at -70ºC until use) and NADPH regeneration solutions (solution A: 31 mM NADP+, 66 mM glucose-6-phosphate and 66 mM $MgCl_2$ in water; solution B: 40 U/ml glucose-6-phosphate dehydrogenase in 5 mM sodium citrate) (stored at -20ºC until use) were purchased from BD Biosciences (San Jose, CA). Mass-labeled hydroxylated PCBs (4-hydroxy-[$^{13}C_{12}$]-PCB 29; 4-hydroxy-[$^{13}C_{12}$]-PCB 61; 4-hydroxy-[$^{13}C_{12}$] PCB-120; 4-hydroxy-[$^{13}C_{12}$]-PCB 159; 4-hydroxy-[$^{13}C_{12}$]-PCB 172; 4-hydroxy-[$^{13}C_{12}$]-PCB 189) were purchased from Wellington Laboratories (Guelph, ON, Canada) as recovery standards for hydroxylated PCBs metabolite extraction (chemical purity > 98%, isotopic purity > 99%). Mass-labeled methoxy-PCBs (4-methoxy-[$^{13}C_{12}$]-PCB 29; 4-methoxy-[$^{13}C_{12}$]-PCB 61; 4-methoxy-[$^{13}C_{12}$]-PCB 120; 4-methoxy-[$^{13}C_{12}$]-PCB 159; 4-methoxy-[$^{13}C_{12}$]-PCB 172; 4-methoxy-[$^{13}C_{12}$]-PCB 189) were also purchased from Wellington Laboratories (Guelph, ON, Canada) (chemical purity > 98%, isotopic purity > 99%). Testosterone and its metabolite 16α-testorsterone were purchased from Steraloids (Newport RI, USA) as a positive control to correct biotransformation activities of CYP2B1 in all incubations.

Extraction and separation of PCBs and hydroxylated metabolites

Briefly, PCBs 30, 204 and mass-labeled OH-PCBs were added as recovery standards to the incubation tubes after biotransformation reactions were terminated. The incubations were then acidified with HCl and washed by KCl and extracted with 6 mL of 1:1 methyl-*t*-butyl ether (MTBE)/hexane by separating funnel. The organic phase was collected and partitioned with 6 mL of 1 M KOH to separate the OH-PCBs from the neutral organic phase. Chiral PCBs in the neutral organic fraction was

purified using an acidified silica gel column (3 g, 22% H_2SO_4) and eluted with 20 mL of 15% (v/v) dichloromethane (DCM)/hexane. OH-PCBs in the aqueous fraction were then acidified by H_2SO_4 and back-extracted into MTBE/hexane (1:1). The organic phase containing OH-PCBs was derivatized with diazomethane into their respective methoxy-PCBs (MeO-PCBs). The MeO-PCB fraction was purified using a 5 g column of the acidified silica gel and eluted with 50 mL of 1:1 DCM/hexane. Both PCBs and MeO-PCBs fractions were solvent exchanged to hexane and PCB 159 was used as an internal standard. Testosterone and 16α-testorsterone in the positive control samples were also acidified with HCl and washed by KCl and extracted with 10 mL ethyl acetate (*2,3*). All extraction procedures were repeated three times for each sample.

Recoveries of PCBs 30 and 204 were 85±14% and 90±12%, respectively. PCB concentrations were corrected based on the average recoveries of PCBs 30 and 204. Recoveries of mass-labeled derivatized OH-PCB standards were 39±26% (MeO-PCB 29), 34±19% (MeO-PCB 61), 115±12% (MeOPCB 120), 105±9% (MeOPCB 159), 107±3% (MeOPCB 172) and 99±5% (MeOPCB 189), similar to previous reports using the same extraction method (*4*). Recoveries of testosterone and 16α-testosterone were 78±10% and 80±17%, respectively. PCB concentrations were determined using PeakFit v4.12 (Systat Software, San Jose, CA) to deconvolute chromatograms (*1*). All these recovery data were reported as average number ± standard deviation.

Instrumental Analysis

Chiral PCBs were quantified by using Hewlett Packard 5890 gas chromatograph equipped with a Hewlett Packard 5989 mass spectrometer (GC/MS) under electron impact ionization (70 eV) with selected ion monitoring (*m/z* 256,258,260 for PCB 30; *m/z* 290, 292, 294 for PCB 45; *m/z* 324, 326, 328 for PCB 95; *m/z* 358, 360, 362 for PCB 132 and PCB 159; *m/z* 426, 428, 430 for PCB 204). A Chirasil-Dex column (30 m×0.25 mm×0.25 μm d_f, Varian, Palo Alto, CA) was used for quantification and atropisomer analysis of PCBs 95 and 132 (*5*). Cyclosil-B column (30m×0.25mm×0.25μm, J&W Scientific) was used for quantification and atropisomer

analysis of PCB 45 (*5*). one μL samples were injected in splitless mode at 250 ºC with helium as carrier gas and at a constant flow rate of 36 cm/s. Initial oven temperature was held at 40 ºC for 2 min, then ramped at 15 ºC/min to 160 ºC, ramped at 1 ºC/min to 210ºC, held for 5 min. The MeO-PCB fraction was separated by a DB-XLB column (30 m×0.25 mm×0.5 μm d_f, J&W Scientific) and initial oven temperature was held at 100ºC initial temperature for 2 min, ramped at 20 ºC/min to 240ºC, held for 25 minutes, ramped at 10 ºC/min to 275 ºC followed by a 14 min hold (*4*).

Testosterone and 16α-testorsterone were quantified by high performance liquid chromatography using a Waters 1525 pump(Ontario, CA), and 2487 dual wavelength absorbance detector at 254 nm, and a Symmetry C_{18} (150×4.6 mm i.d., 5µm particle size) column. The injection volume was 20 μL and the column temperature was 25.0 ºC. The mobile phase was isocratic with 0.02 M sodium dihydrogen phosphate, acetonitrile and methanol at 51:47:2, respectively, at 1.0 ml/min (*6*). All the biotransformation activity data were corrected by the testosterone control, which showed CYP2B1 activities of 2.3-4.1pmol product/min/pmol CYP2B1/mL throughout our experiments.

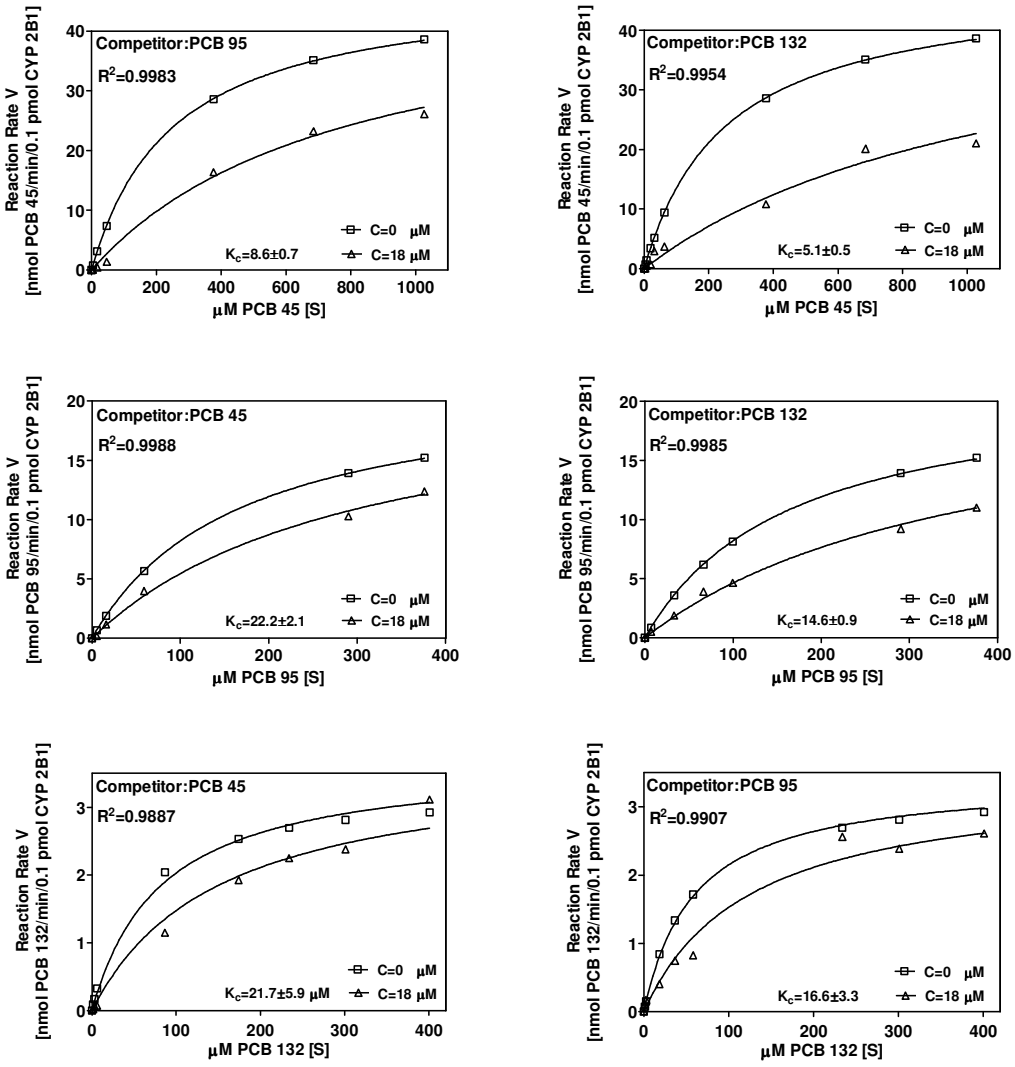

Figure S-1. Inhibition kinetics for PCB 45, PCB 95 and PCB 132. K_c is the equilibrium dissociation constant for the enzyme-competitor complex; C the abbreviation of competitor; R^2 the global goodness of fit of nonlinear kinetic regression.

Figure S2

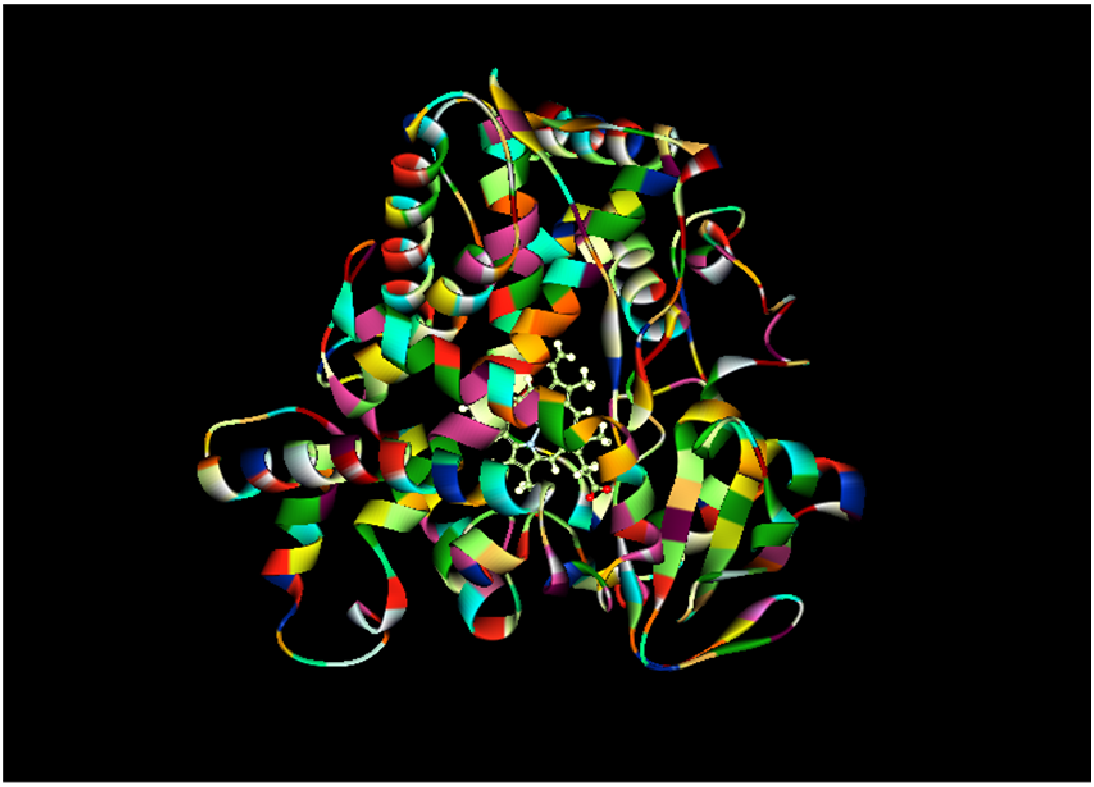

Figure S-2. Three-dimensional structure of rat CYP2B1 from homology modeling

Figure S3

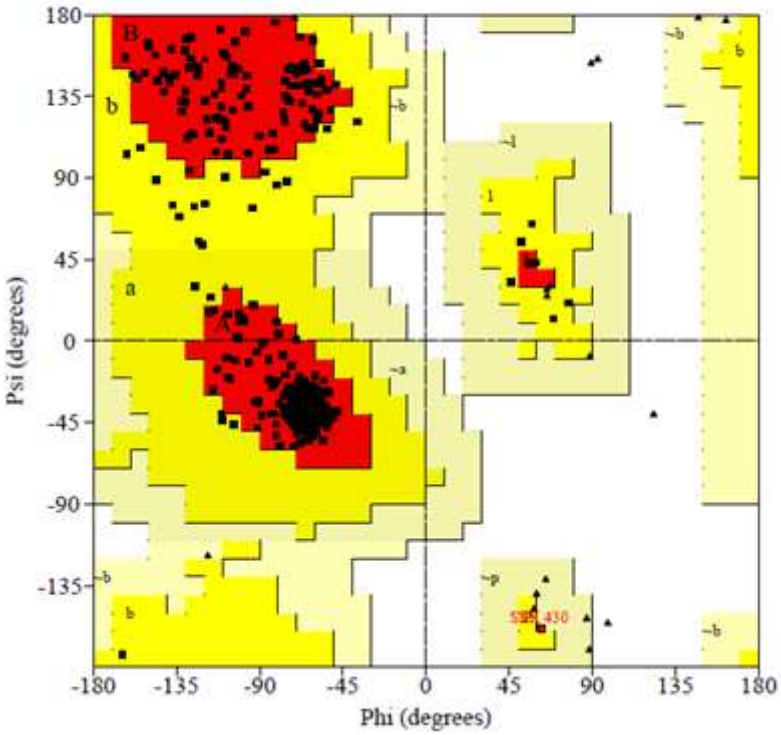

Figure S-3. Ramachandran plots of the rat CYP2B1 model. The different colored areas indicate "disallowed" (white), "generously allowed" (light yellow, ~a, ~b, ~l, ~p), "additional allowed" (yellow, a, b, l, p), and "most favored" (red, A, B, L) regions.

Literature cited

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