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2 *In vitro* and *in silico* analyses for predicting hepatic cytochrome P450-dependent
3 metabolic potencies of polychlorinated biphenyls in the Baikal seal

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23 The supporting information contains 35 pages with 8 Tables and 10 Figures.

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36 congeners examined.

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38 Page S24: **Figure S1.** Concentrations of PCB and OH-PCB congeners detected in the liver of Baikal

39 seals. (A) PCB concentrations. (B) OH-PCB concentrations. Data were cited from Nomiya et al.

40 (2014).

41 Page S25: **Figure S2.** PCBs metabolic pathways in Baikal seal livers predicted from congener profiles

42 of PCBs and OH-PCBs observed in *in vitro* PCB metabolism assay. (A) Highly activated pathways.

43 (B) Weakly activated pathways.

44 Page S26-27: **Figure S3.** Nucleotide and deduced amino acid sequences of bsCYP2s. (A) bsCYP2A

45 (accession no. LC071719), (B) bsCYP2B (accession no. LC071720), and (C) bsCYP2C (accession no.

46 LC071721).

47 Page S28: **Figure S4.** Alignment of amino acid sequences of the Baikal seal and other mammalian

48 CYP2s. (A) CYP2A, (B) CYP2B, and (C) CYP2C. The substrate recognition site (SRS) is marked in

49 red line, and each attached number indicates the SRS number. The heme-binding region is marked in

50 blue line.

51 Page S29: **Figure S5.** Phylogenetic analysis of amino acid sequences of the Baikal seal CYP2A, 2B,

52 2C, and other mammalian CYP2s.

53 Page S30: **Figure S6.** *In silico* homology models constructed by using amino acid sequences of

54 bsCYP1A1, 1A2, 1B1, 2A, 2B, and 2C.

55 Page S31-32: **Figure S7.** Alignment of amino acid sequences of human CYP2C9 and bsCYPs. The

56 amino acid residues marked in yellow are predicted to be located in the lining of the access channel in

57 each CYP.

58 Page S33: **Figure S8.** Predicted substrate access channel 2c in bsCYP proteins. (A) bsCYP1A1

59 (access channel volume: 239 Å³), (B) bsCYP1A2 (206 Å³), (C) bsCYP1B1 (236 Å³), (D) bsCYP2A
60 (209 Å³), (E) bsCYP2B (195 Å³), and (F) bsCYP2C (437 Å³). The access channel is colored in orange,
61 and the heme is in black.

62 Page S34: **Figure S9.** Comparison of the distance from Cl-unsubstituted carbon of docked CB77 to
63 the heme Fe in CYP1A1 homology models of 4 mammalian species. (A) Baikal seal, (B) Rat, (C)
64 Guinea pig, and (D) Hamster. The heme, amino acid residues, and CB77 are shown in orange, grey,
65 and pink, respectively. The shortest distance (Å) between Cl-unsubstituted carbon atom of CB77 and
66 the heme Fe is shown in green line.

67 Page S35: **Figure S10.** Scatter plot of PC1 and PC4 obtained by PC analysis for all 62 PCB congeners
68 examined. PCB congeners were divided into 2 groups (0–20% and >20%) based on the decreased
69 ratio. PCB congeners with 0–20% and >20% decreased ratios are shown in white circles and red
70 circles, respectively.

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72

73 **Materials and methods**

74 **Chemicals**

75 The 62 PCB congeners used for the in vitro metabolism assay included CB1, CB3, CB4, CB8,
76 CB10, CB15, CB18, CB19, CB22, CB28, CB33, CB37, CB44, CB49, CB52, CB54, CB70, CB74,
77 CB77, CB81, CB87, CB95, CB99, CB101, CB104, CB105, CB110, CB114, CB118, CB119, CB123,
78 CB126, CB128, CB138, CB149, CB151, CB153, CB155, CB156, CB157, CB158, CB167, CB168,
79 CB169, CB170, CB171, CB177, CB178, CB180, CB183, CB187, CB188, CB189, CB191, CB194,
80 CB199, CB201, CB202, CB205, CB206, CB208, and CB209. We selected these congeners based on
81 their I-V structural properties, as categorized by Boon et al. (1997)¹, and the PCB congener profiles
82 detected in the livers of Baikal seals². Information on individual congeners is summarized in Table S1.
83 These congeners were purchased from Wellington Laboratories Inc.

84 **Sample collection**

86 The liver tissues of Baikal seals were collected from Lake Baikal in 2005, as has been reported in
87 our earlier studies.^{3,4} Permission was granted by the Lake Baikal Basin Committee for Protection,
88 Reproduction of Fish Resources and Fishing Control under the annual seal culling quota. The livers
89 were removed on board immediately after animal collection, and the sub-samples were frozen and
90 stored in liquid nitrogen until microsomal preparation.

91 **Microsomal preparation and CYP spectral analysis**

93 Liver microsomal fractions were prepared following the method of Guengerich (1982)⁵. About 6 g
94 of liver tissue sample from one adult male Baikal seal (40.5 years old) was homogenized in five-fold
95 volumes of cold homogenization buffer (50 M Tris-HCl, 0.15M KCl, pH 7.4–7.5) with a teflon-glass
96 homogenizer (10 passes) and was centrifuged for 10 min at 750 × g. The supernatant was then
97 centrifuged at 12,000 × g for 10 min. The supernatant was further centrifuged at 105,000 × g for 98
98 min. The supernatant (cytosol) fraction was removed, and the microsomal pellets were resuspended in
99 one volume of TEDG buffer [50 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, 20% (vol/vol)
100 glycerol, pH 7.4–7.5]. Microsomal fractions were immediately frozen in liquid nitrogen, and stored at

-80°C until use.

Protein concentrations in microsomal fractions were determined by the bicinchoninic acid method.⁶ BCA Protein Assay Reagent (Pierce, Rockford, IL) and bovine serum albumin as a standard were used for the protein assay. Absorbance at 560 nm was measured using a multiwell plate reader (SpectraFluor Plus, Tecan Austria GmbH, Groedig, Austria). The content of hepatic microsomal CYP was determined from the dithionite difference spectra of CO-treated samples⁷ with a DU800 spectrophotometer (Beckman Coulter, Inc.).

Measurements of PCBs and OH-PCBs

The measurement of PCBs and OH-PCBs in the reaction mixture was performed according to the method of Nomiyama et al. (2010)⁸. Briefly, the reaction mixture was denatured with 6 M HCl and 2-propanol, and PCBs and OH-PCBs were extracted by 50% methyl t-butyl ether (MTBE)/hexane. The extract was partitioned into neutral and phenolic fractions using 1 M KOH in 50% ethanol/water. The neutral fraction containing PCBs was passed through a 4 g activated silica-gel column and eluted with 80 mL of 5% DCM/hexane.

The KOH solution containing OH-PCBs was acidified (pH 2) with sulfuric acid and was extracted twice with 50% MTBE/hexane. The organic fraction containing OH-PCBs was passed through a column packed with 3 g of hydrated silica gel (Wako-gel S-1, deactivated with 5% H₂O), and OH-PCBs was eluted with 50% dichloromethane (DCM)/hexane (100 mL). The eluted OH-PCB fraction was concentrated and dissolved in 1 mL hexane. The OH-PCBs were derivatized overnight by trimethylsilyldiazomethane. The derivatized (methylated) MeO-PCBs were passed through a 3 g activated silica-gel column, and were then eluted with 140 mL of 10% DCM/hexane. The PCBs and MeO-PCB fractions were concentrated to near dryness. Then, ¹³C₁₂-labeled PCBs dissolved in up to 50 µL of decane were injected as surrogates for the gas chromatograph (GC: 6890 series, Agilent) coupled with high resolution (>10,000) mass spectrometer (HRMS: MS-800D, JEOL) analysis. Identification and quantification of PCB congeners were performed under previously reported GC-HRMS analytical conditions.⁸ Unknown OH-PCB metabolites were quantified as mean values of

relative response factors based on the identifiable $^{12}\text{C}_{12}$ -OH-PCB homologues and the corresponding $^{13}\text{C}_{12}$ -isomer in standard solution, following the method of Kunisue et al. (2007).⁹

Cloning and sequencing of bsCYP2 cDNAs

The cDNA of Baikal seal liver was prepared from poly(A)⁺RNA using a Marathon cDNA Amplification Kit (BD Biosciences Clontech)³. Adaptors of adaptor primer 1 and 2 sequences were added to both ends of each cDNA. Partial cDNA sequences of bsCYP2A, 2B, and 2C were obtained from the cDNA library of the Baikal seal that was prepared for making the custom oligoarray³. To identify the full-length cDNA, primer sequences for RACE were designed based on the partial sequences (Table S2). The bsCYP2A primer for 5'-RACE was not designed because the 5'-sequence containing the start codon has already been obtained from the cDNA library. Amplification of the 5'- and 3'-ends of the cDNA was performed according to the protocol described in the MarathonTM Amplification Kit (Clontech Laboratories, Inc.). PCR reactions were as follows: 94°C for 30 sec, followed by 5 cycles of 94°C for 5 s and 72°C for 4 min, 5 cycles of 94°C for 5 s and 70°C for 4 min, and 25 cycles of 94°C for 5 s and 68°C for 4 min. BLAST homology searches in NCBI nucleotide sequence databases were applied to identify the bsCYP2 cDNAs based on high similarities to deposited sequences of other mammalian CYP2 genes. The molecular weight of bsCYP2 proteins was estimated using GENETYX-MAC version 14.0.11.

Multiple alignments of CYP2A, 2B, and 2C amino acid sequences were performed using the CLUSTAL_W in Mac Vector 11.1.1 program. The aligned amino acid sequences were used to construct a phylogenetic tree with the UPGMA and bootstrap (1000 samplings) method using Mac Vector.¹⁰ Amino acid sequences of mammals except for the Baikal seal were obtained from the DNA Data Bank of Japan (DDBJ). The DDBJ accession numbers of mammalian CYPs used for constructing the phylogenetic tree are shown in Table S3.

***In silico* analysis**

All *in silico* analyses were carried out using the Molecular Operating Environment (MOE) program

(Chemical Computing Group, Montreal, Canada). For constructing homology models of the entire proteins of bsCYPs (1A1, 1A2, 1B1, 2A, 2B, and 2C), the following templates of CYP1 and 2 isoenzymes were taken from the Protein Data Bank (<http://www.rcsb.org>): human CYP1A1 (PDB code: 4I8V), human CYP1A2 (2HI4), human CYP1B1 (3PM0), human CYP2A6 (1Z10), rabbit CYP2B4 (1SUO), and human CYP2C9 (1OG5). All crystallographic water molecules were deleted from each template CYP structure. To adjust for structural defects and the clash of atoms, the Structure Preparation module in MOE was used. The amino acid sequences of bsCYPs and their template structures were aligned and carefully checked for conserved residual structure and gap positioning using the Protein Contacts program. In order to construct the 3D structure of heme-containing CYP proteins, a total of 500 generated structures (10 side chain samples per 50 main chain models) for each bsCYP were obtained by employing the ‘induced fit’ option that allows the heme iron to fit into the template structure. The 3D structures of bsCYPs were optimized by PFROSST force field¹¹ with an energy gradient of 0.05.¹² To generate the final model structure, the generalized Born/volume integral (GB/VI) model parameters¹³ were applied. The overall geometric and stereochemical qualities of the homology models were assessed using Protein Geometry. Ramachandran plots of phi (φ) and psi (ψ) torsion angles for all of the residues and the clash of atoms in each model were checked and adjusted by energy minimization using the PFROSST force field.

Molecular docking simulations were performed to simulate the binding of 62 PCB congeners to bsCYP proteins using ASEDock (Ryoka Systems Inc., Tokyo, Japan) following the method of Goto et al. (2008).¹⁴ Prior to the ASEDock analysis, structures of PCBs were constructed and energy minimized using Rebuild3D with MMFF94x force field in the MOE. A total of 500 confirmations for each PCB congener were generated using the default systematic search parameters by LowMode MD method. The parameters used for the refinement step were as follows: cutoff value of 4.5, RMS gradient of 10, and energy threshold of 500.¹⁵ The energy of the PCB-CYP complex was refined using PFROSST of MOE under the limited conditions for which the side chains of amino acid residues were fixed. Each docking simulation was evaluated in terms of a U-dock score (kcal/mol): [U_ele (electric energy) + U_vdw (Van der Waals energy) + U_solv (solvation energy) + U_strain (strain energy)].

The distance between the Cl-unsubstituted carbon in a given PCB congener and the heme Fe in each CYP was measured. The substrate binding pocket in each CYP was designed using Atom Region in MOE.

Since the structure of substrate access channel may be a critical factor to affect the substrate specificity of each CYP, we attempted to identify the access channel 2c, which is the most probable candidate channel for substrates,¹⁶ in each bsCYP protein, and compare their conformational characteristics. By reference to the access channel 2c in human CYP2C9 model (PDB code: 1OG2) reported in Otyepka et al. (2012),¹⁶ the dummy sites in CYP2C9, which were selected by MOE, were edited to fit the shape of channel 2c, and the channel was determined by using Atom Region in MOE. The amino acid residues lining the access channel in CYP2C9 were identified, and the corresponding amino acids in each bsCYP were assigned by aligning the bsCYP sequence with CYP2C9 sequence. The structure of the channel 2c in each bsCYP, the cavity surrounded by the assigned amino acids, was predicted by using Atom Region in MOE, and the volume was then measured.

Statistical analyses

All statistical analyses were conducted using the IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY). Statistical significance was set at $p < 0.05$. Prior to PC analysis, Spearman's rank correlation test was performed to examine correlations between decreased PCB ratios obtained in *in vitro* metabolism assays and 119 factors (Table S4), which included structural and physicochemical parameters of PCB congeners and *in silico* docking variables. Using these variables, PC analysis with VARIMAX rotation was conducted.

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255 Table S1. IUPAC numbers and structural characteristics of 62 PCB congeners used in *in vitro*
 256 metabolism assay.

IUPAC no.	Cl no.	IUPAC name	No. pairs <i>o</i> , <i>m</i> - vic. H-atoms	No. pairs <i>m</i> , <i>p</i> - vic. H- atoms	No. <i>o</i> -Cl atoms	Metabolic group ^a
1	1	2-Chlorobiphenyl	3	4	1	III, IV
3	1	4-Chlorobiphenyl	4	2	0	III, IV
4	2	2,2'-Dichlorobiphenyl	2	4	2	II, IV
8	2	2,4'-Dichlorobiphenyl	3	2	1	III, IV
10	2	2,6-Dichlorobiphenyl	2	4	2	II, IV
15	2	4,4'-Dichlorobiphenyl	4	0	0	III
18	3	2,2',5'-Trichlorobiphenyl	1	3	2	II, IV
19	3	2,2',6'-Trichlorobiphenyl	1	4	3	II, V
22	3	2,3,4'-Trichlorobiphenyl	3	1	1	III, IV
28	3	2,4,4'-Trichlorobiphenyl	3	0	1	III
33	3	2,3',4'-Trichlorobiphenyl	2	2	1	III, IV
37	3	3,4,4'-Trichlorobiphenyl	3	0	0	III
44	4	2,2',3,5'-Tetrachlorobiphenyl	1	2	2	II, IV
49	4	2,2',4,5'-Tetrachlorobiphenyl	1	1	2	II, IV
52	4	2,2',5,5'-Tetrachlorobiphenyl	0	2	2	IV
54	4	2,2',6,6'-Tetrachlorobiphenyl	0	4	4	IV
70	4	2,3',4',5'-Tetrachlorobiphenyl	1	1	1	III, IV
74	4	2,4,4',5'-Tetrachlorobiphenyl	2	0	1	III
77	4	3,3',4,4'-Tetrachlorobiphenyl	2	0	0	III
81	4	3,4,4',5'-Tetrachlorobiphenyl	2	0	0	III
87	5	2,2',3,4,5'-Pentachlorobiphenyl	1	1	2	II, IV
95	5	2,2',3,5',6'-Pentachlorobiphenyl	0	2	3	V
99	5	2,2',4,4',5'-Pentachlorobiphenyl	1	0	2	II
101	5	2,2',4,5,5'-Pentachlorobiphenyl	0	1	2	IV
104	5	2,2',4,6,6'-Pentachlorobiphenyl	0	2	4	V
105	5	2,3,3',4,4'-Pentachlorobiphenyl	2	0	1	III
110	5	2,3,3',4',6'-Pentachlorobiphenyl	1	1	2	II, IV
114	5	2,3,4,4',5'-Pentachlorobiphenyl	2	0	1	III
118	5	2,3',4,4',5'-Pentachlorobiphenyl	1	0	1	III
119	5	2,3',4,4',6'-Pentachlorobiphenyl	1	0	2	II
123	5	2,3',4,4',5'-Pentachlorobiphenyl	1	0	1	III
126	5	3,3',4,4',5'-Pentachlorobiphenyl	1	0	0	III
128	6	2,2',3,3',4,4'-Hexachlorobiphenyl	2	0	2	II
138	6	2,2',3,4,4',5'-Hexachlorobiphenyl	1	0	2	II
149	6	2,2',3,4,5',6'-Hexachlorobiphenyl	0	1	3	V
151	6	2,2',3,5,5',6'-Hexachlorobiphenyl	0	1	3	V
153	6	2,2',4,4',5,5'-Hexachlorobiphenyl	0	0	2	I
155	6	2,2',4,4',6,6'-Hexachlorobiphenyl	0	0	4	I
156	6	2,3,3',4,4',5'-Hexachlorobiphenyl	1	0	1	III
157	6	2,3,3',4,4',5'-Hexachlorobiphenyl	1	0	1	III

158	6	2,3,3',4,4',6-Hexachlorobiphenyl	1	0	2	II
167	6	2,3',4,4',5,5'-Hexachlorobiphenyl	0	0	1	I
168	6	2,3',4,4',5',6-Hexachlorobiphenyl	0	0	2	I
169	6	3,3',4,4',5,5'-Hexachlorobiphenyl	0	0	0	I
170	7	2,2',3,3',4,4',5-Heptachlorobiphenyl	1	0	2	II
171	7	2,2',3,3',4,4',6-Heptachlorobiphenyl	1	0	3	II
177	7	2,2',3,3',4,5',6'-Heptachlorobiphenyl	1	0	3	II
178	7	2,2',3,3',5,5',6-Heptachlorobiphenyl	0	0	3	I
180	7	2,2',3,4,4',5,5'-Heptachlorobiphenyl	0	0	2	I
183	7	2,2',3,4,4',5',6-Heptachlorobiphenyl	0	0	3	I
187	7	2,2',3,4',5,5',6-Heptachlorobiphenyl	0	0	3	I
188	7	2,2',3,4',5,6,6'-Heptachlorobiphenyl	0	0	4	I
189	7	2,3,3',4,4',5,5'-Heptachlorobiphenyl	0	0	1	I
191	7	2,3,3',4,4',5',6-Heptachlorobiphenyl	0	0	2	I
194	8	2,2',3,3',4,4',5,5'-Octachlorobiphenyl	0	0	2	I
199	8	2,2',3,3',4,5,5',6'-Octachlorobiphenyl	0	0	3	I
201	8	2,2',3,3',4,5',6,6'-Octachlorobiphenyl	0	1	4	V
202	8	2,2',3,3',5,5',6,6'-Octachlorobiphenyl	0	0	4	I
205	8	2,3,3',4,4',5,5',6-Octachlorobiphenyl	0	0	2	I
206	9	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	0	0	3	I
208	9	2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl	0	0	4	I
209	10	Decachlorobiphenyl	0	0	4	I

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258 ^a Classification of PCB congeners based on the predicted potency of CYP-mediated metabolism
259 proposed by Boon et al. (1997) as follows;

260 I: Congeners without any vicinal hydrogen (H)-atoms (persistent)

261 II: Congeners with vicinal H-atoms exclusively in the *ortho*- and *meta*-positions in combination with
262 ≥ 2 *ortho*-Cl substituents (persistent)

263 III: Congeners with vicinal H-atoms in the *ortho*- and *meta*-positions in combination with ≤ 1 *ortho*-
264 Cl (metabolizable at a constant rate or by inducible CYP isozymes)

265 IV: Congeners with vicinal H-atoms in the *meta*- and *para*-positions in combination with ≤ 2 *ortho*-Cl
266 (metabolizable at a constant rate or by inducible CYP isozymes)

267 V: Congeners with vicinal H-atoms in the *meta*- and *para*-positions in combination with ≥ 3 *ortho*-Cl
268 (metabolizable at a constant rate or by inducible CYP isozymes)

269

270 Table S2. Primer sequences used for the RACE of bsCYP2 cDNAs.

Primer name	Sequence
bsCYP2A 3'-RACE	5'-CCAGCACTTCCTGGATGAGAATGGGCAG-3'
bsCYP2B 5'-RACE	5'-AGCCGCAGCAATTCAGGGTCTCTGTAGC-3
bsCYP2B 3'-RACE	5'-CTAGCTTCAGAGGGTACATCATTCCCA-3
bsCYP2C 5'-RACE	5'-CTAAGCAAGCTAGCAGCAGAGAAT-3'
bsCYP2C 3'-RACE	5'-GCAAGACAGGAGCCGCATGCCCTACACG-3'

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273

274 Table S3. Accession numbers of mammalian CYP2 sequences used for constructing the phylogenic
 275 tree shown in Figure S5.

CYP	accession number
CYP2A	dog CYP2A13 (DQ238561), dog CYP2A25 (DQ238562), horse CYP2A13 (EU286274), human CYP2A6 (AF182275), human CYP2A13 (AY513606), monkey CYP2A23 (DQ074790), mouse CYP2A5 (P20852), mouse CYP2A4 (Q91X75), rat CYP2A1 (P11711), rat CYP2A2 (P15149), rat CYP2A3 (P20812), pig CYP2A19 (AB052255), rabbit CYP2A11 (Q05556), rabbit CYP2A10 (Q05555)
CYP2B	rat CYP2B1 (P00176), rat CYP2B2 (P04167), rat CYP2B3 (P13107), cattle CYP2B6 (no accession no.), dog CYP2B11 (P24460), monkey CYP2B6 (DQ074793), monkey CYP2B30 (AY635461), human CYP2B6 (P20813), human CYP2B7 (DQ198366), mouse CYP2B9 (P12790), mouse CYP2B10 (P12791), mouse CYP2B13 (A6H6J2), mouse CYP2B19 (O55071), mouse CYP2B23 (no accession no.), rabbit CYP2B4 (P00178), pig CYP2B22 (AB052256)
CYP2C	dog CYP2C21 (P56594), dog CYP2C41 (AF016248), horse CYP2C92 (EU014893), human CYP2C8 (P10632), human CYP2C9 (P11712), human CYP2C19 (P33261), monkey CYP2C18 (DQ297681), minke whale CYP2C78 (AB290008), mouse CYP2C29 (Q64458), rabbit CYP2C1 (P00180), rabbit CYP2C2 (P00181), rat CYP2C6 (P05178), rat CYP2C7 (Q4QQW7), pig CYP2C33 (AB052257), pig CYP2C49 (AB052258)
CYP3A	human CYP3A4 (P08684)

276
 277 The human CYP3A4 is used as an outlier.

278

279

280 Table S4. Parameters used for principle component analysis.

Group	Parameter
<i>In vitro</i> PCB metabolism assay	PCB decrease ratio%
PCB structural parameters	number of Cl in PCB
	presence of non- <i>ortho</i> -position
	presence of mono- <i>ortho</i> -position
	number of H atom at <i>ortho</i> -position
	number of H atom at <i>meta</i> -position
	number of H atom at <i>para</i> -position
	number of sites with vicinal H atoms at <i>ortho</i> - and <i>meta</i> -positions of PCB
	number of sites with vicinal H atoms at <i>meta</i> - and <i>para</i> -positions of PCB
	number of Cl at <i>ortho</i> -position
	number of Cl at <i>meta</i> -position
	number of Cl at <i>para</i> -position
	number of H at $\sum(ortho+meta)$
	number of H at $\sum(para+meta)$
	presence of vicinal H atoms at 2 and 3 position of PCB
	presence of vicinal H atoms at 2' and 3' position of PCB
	presence of vicinal H atoms at 3 and 4 position of PCB
	presence of vicinal H atoms at 3' and 4' position of PCB
	presence of vicinal H atoms at 4 and 5 position of PCB
	presence of vicinal H atoms at 4' and 5' position of PCB
	presence of vicinal H atoms at 5 and 6 position of PCB
	presence of vicinal H atoms at 5' and 6' position of PCB
	bilateral symmetry
Physicochemical parameters	Log Kow
	molecular weight of PCB
	PEOE_VSA+0_(electricity effect)
	PEOE_VSA+1_(electricity effect)
	PEOE_VSA-1_(electricity effect)
	SlogP_VSA6_(hydrophobicity)
	SlogP_VSA7_(hydrophobicity)
	SlogP_VSA9_(hydrophobicity)
	SMR_VSA3_(molar refractivity)
	SMR_VSA5_(molar refractivity)
	SMR_VSA7_(molar refractivity)

281 (continued)

282

Group	Parameter
<i>In silico</i> results	U_dock value of the interaction of PCB with bsCYP1A1
	presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 2 position of PCB
	presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 2' position of PCB
	presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 3 position of PCB
	presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 3' position of PCB
	presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 4 position of PCB
	presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 4' position of PCB
	presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 5 position of PCB
	presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 5' position of PCB
	presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 6 position of PCB
	presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 6' position of PCB
	shortest measurable distance(Å) from bsCYP1A1 heme Fe to Cl-unsubstituted carbons of PCB
	ranking number of docking simulation in bsCYP1A1
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 5Å distance from the heme Fe in bsCYP1A1
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 6Å distance from the heme Fe in bsCYP1A1
	U_dock value of the interaction of PCB with bsCYP1A2
	presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 2 position of PCB
	presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 2' position of PCB
	presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 3 position of PCB
	presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 3' position of PCB
	presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 4 position of PCB
	presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 4' position of PCB
	presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 5 position of PCB
	presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 5' position of PCB
	presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 6 position of PCB
	presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 6' position of PCB
	shortest measurable distance(Å) from bsCYP1A2 heme Fe to Cl-unsubstituted carbons of PCB
	ranking number of docking simulation in bsCYP1A2
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 5Å distance from the heme Fe in bsCYP1A2
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 6Å distance from the heme Fe in bsCYP1A2
	U_dock value of the interaction of PCB with bsCYP1B1
	presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 2 position of PCB
	presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 2' position of PCB
	presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 3 position of PCB

283 (continued)

284

Group	Parameter
<i>In silico</i> results	presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 3' position of PCB
	presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 4 position of PCB
	presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 4' position of PCB
	presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 5 position of PCB
	presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 5' position of PCB
	presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 6 position of PCB
	presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 6' position of PCB
	shortest measurable distance(Å) from bsCYP1B1 heme Fe to Cl-unsubstituted carbons of PCB
	ranking number of docking simulation in bsCYP1B1
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 5Å distance from the heme Fe in bsCYP1B1
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 6Å distance from the heme Fe in bsCYP1B1
	U_dock value of the interaction of PCB with bsCYP2A
	presence of measurable distance from bsCYP2A heme Fe to Cl-unsubstituted carbon at 2 position of PCB
	presence of measurable distance from bsCYP2A heme Fe to Cl-unsubstituted carbon at 2' position of PCB
	presence of measurable distance from bsCYP2A heme Fe to Cl-unsubstituted carbon at 3 position of PCB
	presence of measurable distance from bsCYP2A heme Fe to Cl-unsubstituted carbon at 3' position of PCB
	presence of measurable distance from bsCYP2A heme Fe to Cl-unsubstituted carbon at 4 position of PCB
	presence of measurable distance from bsCYP2A heme Fe to Cl-unsubstituted carbon at 4' position of PCB
	presence of measurable distance from bsCYP2A heme Fe to Cl-unsubstituted carbon at 5 position of PCB
	presence of measurable distance from bsCYP2A heme Fe to Cl-unsubstituted carbon at 5' position of PCB
	presence of measurable distance from bsCYP2A heme Fe to Cl-unsubstituted carbon at 6 position of PCB
	presence of measurable distance from bsCYP2A heme Fe to Cl-unsubstituted carbon at 6' position of PCB
	shortest measurable distance(Å) from bsCYP2A heme Fe to Cl-unsubstituted carbons of PCB
	ranking number of docking simulation in bsCYP2A
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 5Å distance from the heme Fe in bsCYP2A
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 6Å distance from the heme Fe in bsCYP2A
	U_dock value of the interaction of PCB with bsCYP2B
	presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 2 position of PCB
	presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 2' position of PCB
	presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 3 position of PCB
	presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 3' position of PCB
	presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 4 position of PCB
	presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 4' position of PCB

285 (continued)

286

Group	Parameter
<i>In silico</i> results	presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 5 position of PCB
	presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 5' position of PCB
	presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 6 position of PCB
	presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 6' position of PCB
	shortest measurable distance(Å) from bsCYP2B heme Fe to Cl-unsubstituted carbons of PCB
	ranking number of docking simulation in bsCYP2B
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 5Å distance from the heme Fe in bsCYP2B
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 6Å distance from the heme Fe in bsCYP2B
	U_dock value of the interaction of PCB with bsCYP2C
	presence of measurable distance from bsCYP2C heme Fe to Cl-unsubstituted carbon at 2 position of PCB
	presence of measurable distance from bsCYP2C heme Fe to Cl-unsubstituted carbon at 2' position of PCB
	presence of measurable distance from bsCYP2C heme Fe to Cl-unsubstituted carbon at 3 position of PCB
	presence of measurable distance from bsCYP2C heme Fe to Cl-unsubstituted carbon at 3' position of PCB
	presence of measurable distance from bsCYP2C heme Fe to Cl-unsubstituted carbon at 4 position of PCB
	presence of measurable distance from bsCYP2C heme Fe to Cl-unsubstituted carbon at 4' position of PCB
	presence of measurable distance from bsCYP2C heme Fe to Cl-unsubstituted carbon at 5 position of PCB
	presence of measurable distance from bsCYP2C heme Fe to Cl-unsubstituted carbon at 5' position of PCB
	presence of measurable distance from bsCYP2C heme Fe to Cl-unsubstituted carbon at 6 position of PCB
	presence of measurable distance from bsCYP2C heme Fe to Cl-unsubstituted carbon at 6' position of PCB
	shortest measurable distance(Å) from bsCYP2C heme Fe to Cl-unsubstituted carbons of PCB
	ranking number of docking simulation in bsCYP2C
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 5Å distance from the heme Fe in bsCYP2C
	number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 6Å distance from the heme Fe in bsCYP2C

287

288

289 Table S5. Validation of the homology models of bsCYP1A1, 1A2, 1B1, 2A, 2B, and 2C.

CYP isozyme	PCB congener	Distance (Å) between Cl unsubstituted carbon of PCB and the heme Fe in the top 3 CYP models		
		average	std	cv %
bsCYP1A1	CB180	8.1	0.1	0.6
	CB199	7.6	5.0	65.3
	CB209	5.1	1.2	23.5
bsCYP1A2	CB15	7.0	1.2	17.2
	CB44	5.1	0.6	11.6
	CB110	5.8	1.5	25.4
bsCYP1B1	CB52	6.2	0.8	12.7
	CB183	5.7	1.8	32.1
	CB206	8.9	1.8	20.6
bsCYP2A	CB19	4.1	0.6	14.7
	CB70	3.6	0.8	20.9
	CB205	5.7	0.7	11.6
bsCYP2B	CB3	2.8	0.3	9.5
	CB74	3.6	0.3	7.9
	CB206	6.1	1.0	16.1
bsCYP2C	CB37	9.3	2.5	26.5
	CB44	9.3	0.6	6.9
	CB191	9.8	0.5	5.2

290

291 Top 3 homology models of each CYP with the 1st, 2nd, and 3rd lowest total potential energies were
 292 constructed, and validated by the variation (average, standard variation (std) and CV%) in the distance
 293 between Cl unsubstituted carbon of some PCB congeners and the heme Fe in the top 3 CYP models.
 294 For the validation of the top 3 models for each bsCYP isozyme, we applied 3 PCB congeners which
 295 showed the shortest, longest, and median distance between Cl unsubstituted carbon of the PCB and
 296 the heme in the 1st ranked model.

297

298 Table S6. Estimated shortest distance (Å) between Cl-unsubstituted carbon of each PCB congener and
 299 the heme Fe in bsCYP1 and bsCYP2 protein homology models.

IUPAC No.	CYP1A1	CYP1A2	CYP1B1	CYP2A	CYP2B	CYP2C
1	8.98	4.95	4.75	5.80	3.94	8.15
3	8.11	6.14	7.33	4.92	3.08	9.64
4	7.99	4.34	4.01	4.21	3.55	7.81
8	8.90	6.27	5.31	4.31	3.26	7.06
10	7.99	4.34	4.01	4.21	3.55	7.81
15	8.00	8.43	4.88	2.98	3.13	10.16
18	7.69	7.47	5.35	4.87	4.81	9.69
19	8.07	4.60	4.23	4.70	3.38	9.21
22	7.75	5.14	4.16	3.30	3.55	8.33
28	11.04	6.46	5.99	4.45	3.68	8.06
33	7.39	5.64	5.26	3.89	4.15	8.66
37	8.69	4.74	5.67	3.72	3.17	6.62
44	6.78	5.75	5.62	4.79	4.30	9.50
49	7.39	4.27	4.42	3.27	3.47	7.60
52	7.85	5.37	5.48	4.40	3.65	9.77
54	9.19	5.19	4.16	3.92	3.46	9.60
70	8.23	5.07	4.58	2.73	3.37	10.27
74	8.28	6.26	5.67	4.70	3.80	10.03
77	7.88	5.75	5.88	3.62	3.77	7.68
81	8.05	5.94	5.86	4.21	4.02	8.29
87	8.93	6.27	5.82	5.09	4.37	8.25
95	8.37	6.33	10.70	4.57	3.79	9.99
99	8.43	4.51	4.67	3.13	3.39	8.36
101	8.23	5.34	4.56	4.96	3.90	8.78
104	8.01	4.91	4.13	3.71	3.66	8.18
105	8.29	4.62	4.42	4.25	4.23	9.59
110	6.89	4.14	6.75	4.78	4.10	9.01
114	8.04	6.33	6.65	4.76	3.60	7.98
118	7.77	6.39	5.79	5.36	3.70	9.93
119	7.06	5.95	6.03	4.39	4.00	7.76
123	8.03	4.60	6.74	4.67	3.72	8.25
126	8.86	6.03	4.93	4.99	4.12	8.96
128	9.04	6.29	5.90	4.08	4.18	8.26
138	7.86	4.15	4.00	5.18	3.37	8.22
149	7.04	5.78	7.78	4.47	4.10	8.33
151	9.07	5.17	5.41	3.83	3.79	10.20
153	8.48	5.04	4.98	4.75	3.99	8.65
155	8.08	5.50	4.72	4.12	4.23	9.70
156	8.81	6.44	8.54	5.43	5.55	8.43
157	7.67	6.39	7.82	4.63	5.08	8.71
158	7.19	6.03	6.99	4.68	4.15	8.29
167	8.02	5.22	5.39	4.85	4.90	9.03
168	8.98	6.44	4.77	5.74	3.63	9.26
169	9.81	6.41	6.98	5.69	5.63	8.74
170	9.05	4.40	5.91	5.49	4.98	8.44
171	7.85	7.35	4.23	5.14	4.63	9.35
177	8.22	6.54	5.88	4.67	3.76	9.08
178	8.37	5.06	9.32	3.59	4.88	8.71
180	8.14	6.10	4.56	5.67	3.12	8.47
183	8.36	4.68	3.86	4.27	3.73	10.25
187	8.73	6.14	5.66	4.77	3.53	8.92
188	8.75	6.39	5.45	4.72	3.17	9.76
189	9.63	6.57	6.58	5.35	5.64	8.52
191	7.04	4.69	5.20	5.77	3.43	10.40
194	8.84	5.39	9.12	5.04	5.78	9.56
199	13.04	6.06	5.25	4.42	5.31	8.25
201	7.14	6.65	8.15	4.75	3.74	9.55
202	8.10	5.35	4.69	3.33	6.11	9.78
205	10.85	7.93	10.39	6.42	5.72	10.11

206	10.89	5.26	10.86	4.85	6.38	8.39
208	7.80	6.65	5.95	4.75	3.77	8.02
209	6.10	4.85	4.23	4.83	3.92	9.19

300

301

302 Table S7. PC1-5 scores obtained by PC analysis for all of 62 PCB congeners examined.

Parameter	PC1	PC2	PC3	PC4	PC5
<i>In vitro</i> decreased ratio of PCB (%)	0.55	0.19	0.08	0.51	-0.01
Number of Cl in PCB	-0.95	-0.10	-0.16	-0.02	0.05
Molecular weight of PCB	-0.95	-0.10	-0.16	-0.02	0.05
Log Kow of PCB	-0.89	-0.20	-0.36	-0.02	0.12
Number of H atom at <i>meta</i> -position of PCB	0.85	0.18	0.18	-0.07	-0.16
Number of sites with vicinal H atoms at <i>meta</i> - and <i>para</i> -positions of PCB	0.75	0.29	0.50	-0.04	-0.19
Presence of vicinal H atoms at <i>meta</i> - and <i>para</i> -positions of PCB	0.50	0.11	0.80	-0.11	-0.03
Presence of Cl-substitution at 4' position of PCB	-0.01	-0.13	-0.80	-0.25	0.14
Presence of vicinal H atoms at 2 and 3 positions of PCB	0.45	-0.19	-0.07	0.59	0.08
Presence of vicinal H atoms at 5 and 6 positions of PCB	0.64	-0.08	-0.19	0.21	-0.12
Presence of vicinal H atoms at 4' and 5' positions of PCB	0.32	-0.07	0.82	0.16	-0.01
Presence of measurable distance from bsCYP1A1 heme Fe to Cl-unsubstituted carbon at 3' position of PCB	0.08	0.27	0.26	0.34	0.01
Presence of measurable distance from bsCYP1A2 heme Fe to Cl-unsubstituted carbon at 6 position of PCB	-0.15	-0.20	0.03	-0.07	0.69
Number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 6 Å distance from the heme Fe in bsCYP1A2	0.01	0.63	0.16	-0.26	0.10
Presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 4' position of PCB	0.04	0.27	0.05	0.67	-0.16
Presence of measurable distance from bsCYP1B1 heme Fe to Cl-unsubstituted carbon at 5 position of PCB	-0.09	0.06	-0.18	-0.11	0.74
Shortest measurable distance (Å) from bsCYP2A heme Fe to Cl-unsubstituted carbons of PCB	-0.28	-0.78	0.16	-0.29	0.11
Number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 5 Å distance from the heme Fe in bsCYP2A	0.09	0.81	0.11	0.21	-0.21
Number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 6 Å distance from the heme Fe in bsCYP2A	0.16	0.82	0.05	0.12	-0.05
Presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 4' position of PCB	-0.14	0.01	0.19	0.78	-0.08
Presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 6 position of PCB	-0.14	-0.05	-0.02	0.02	0.89
U_dock value of the interaction of PCB with bsCYP2C (kcal/mol)	0.85	0.13	0.16	0.11	-0.14

303

304

305 Table S8. PC1-4 scores obtained by PC analysis for 1-5 chlorine substituted PCB congeners examined.

Parameter	PC1	PC2	PC3	PC4
<i>In vitro</i> decreased ratio of PCB (%)	-0.41	0.59	0.23	0.37
Number of Cl in PCB	0.94	-0.03	-0.20	-0.17
Molecular weight of PCB	0.94	-0.03	-0.20	-0.17
Log Kow of PCB	0.90	-0.08	0.00	-0.21
Number of H atom at <i>meta</i> -position of PCB	-0.75	0.28	0.00	0.18
Presence of Cl-substitution at 5 position of PCB	0.22	-0.13	-0.01	-0.92
Presence of vicinal H atoms at 2 and 3 positions of PCB	-0.41	0.19	0.64	0.05
Presence of vicinal H atoms at 5 and 6 positions of PCB	-0.43	-0.08	0.14	0.69
Number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 6 Å distance from the heme Fe in bsCYP1A2	0.13	0.40	-0.66	0.18
Shortest measurable distance (Å) from bsCYP1B1 heme Fe to Cl-unsubstituted carbons of PCB	0.43	-0.28	0.62	0.18
Shortest measurable distance (Å) from bsCYP2A heme Fe to Cl-unsubstituted carbons of PCB	0.03	-0.81	0.11	0.09
Number of posing of docked PCBs of which Cl-unsubstituted carbon was allocated within 6 Å distance from the heme Fe in bsCYP2A	0.11	0.79	-0.09	0.14
Presence of measurable distance from bsCYP2B heme Fe to Cl-unsubstituted carbon at 4' position of PCB	-0.20	0.27	0.72	0.20
Shortest measurable distance (Å) from bsCYP2B heme Fe to Cl-unsubstituted carbons of PCB	0.35	-0.67	-0.33	0.10
U _{dock} value of the interaction of PCB with bsCYP2C (kcal/mol)	-0.88	-0.07	0.16	0.15

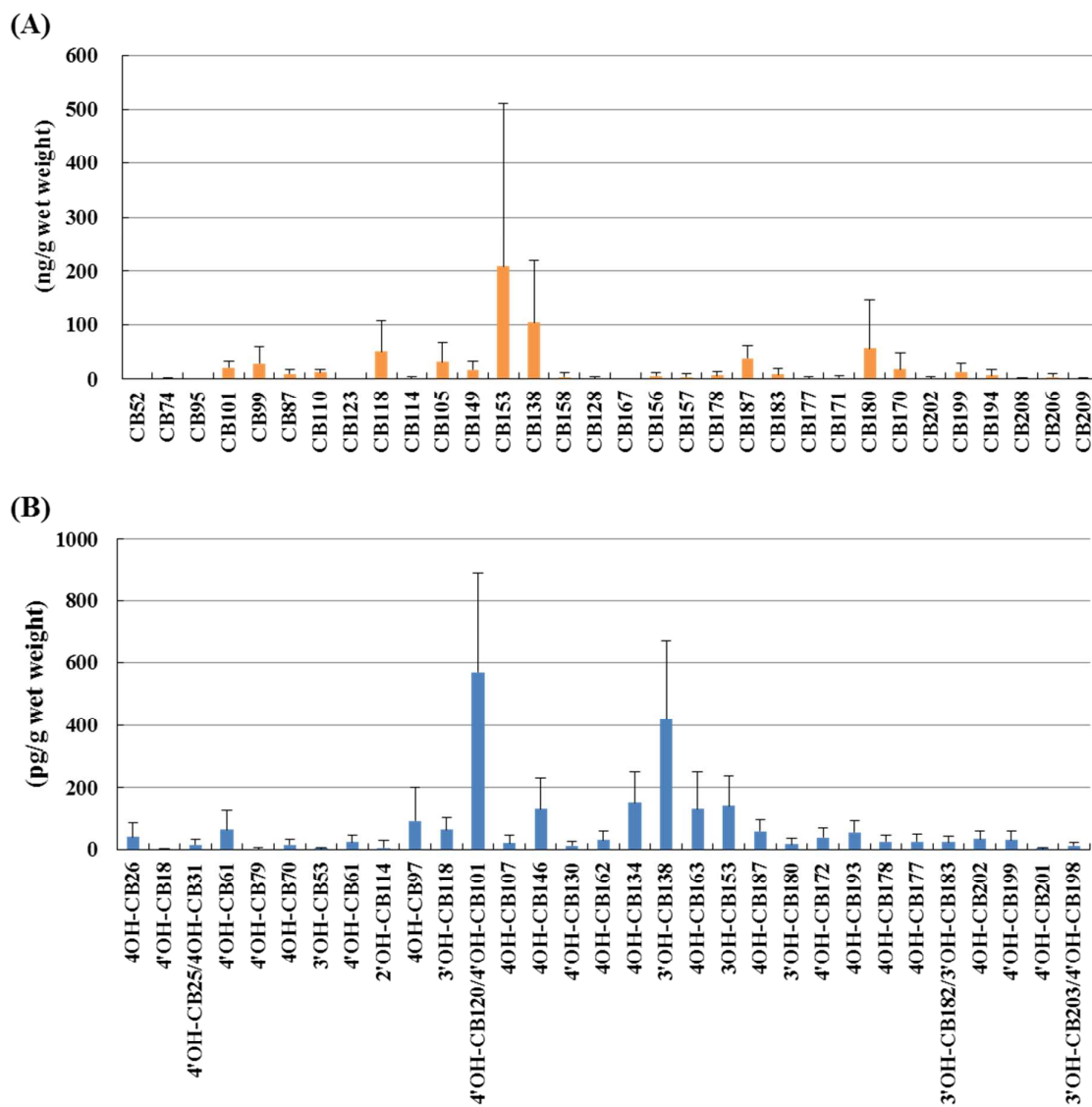
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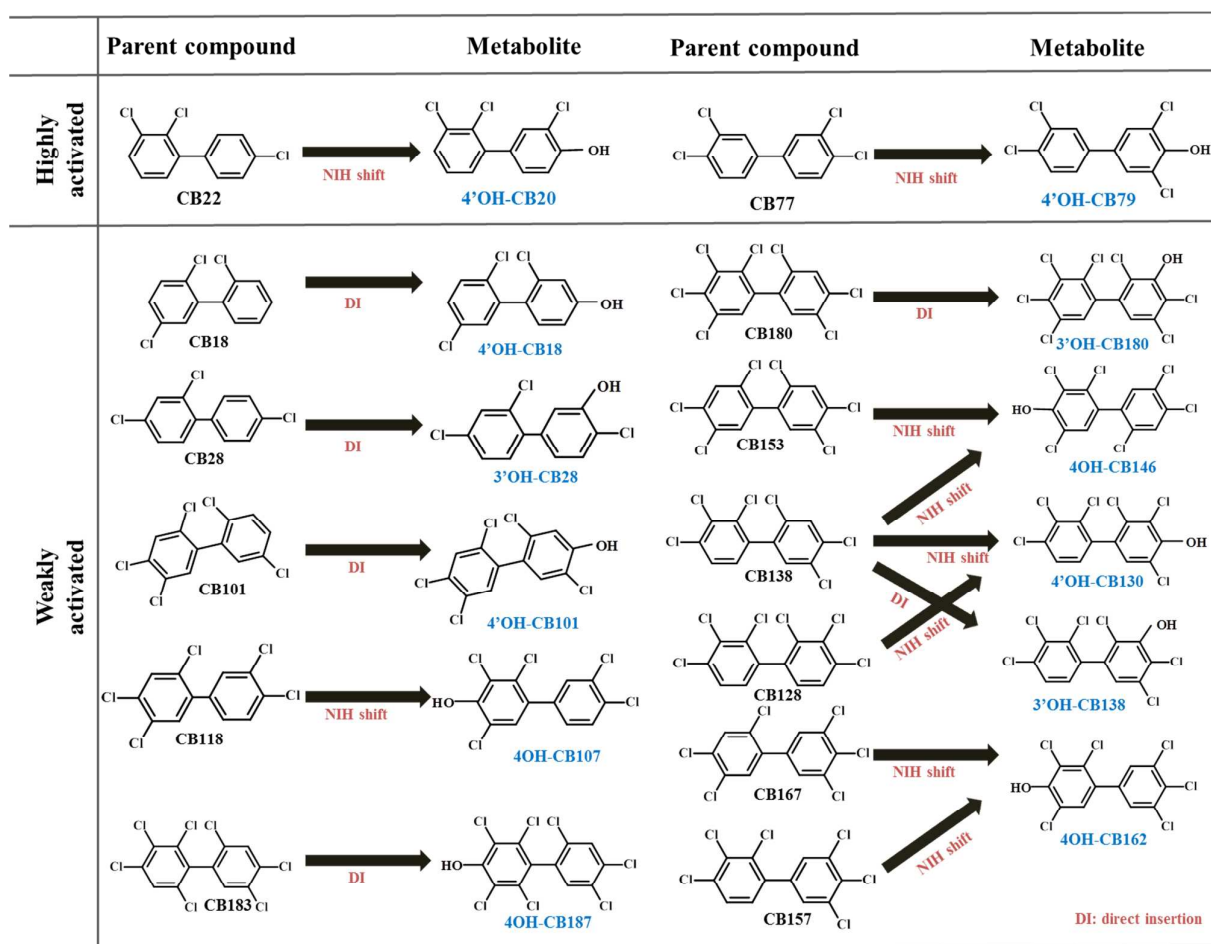
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312 Figure S1.

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314

315 Figure S2.

316

(A) 1 ATGCTGGCCTCAGGTTGCTTCTGGTGGCTTGTCTCACCTGCCTGACCAATGGTCTTGATGTCTGTGGAGGCAGAGGAAGCTCTGGGAGAACTCCCTCAGGACCCACCCATTG 120
M L A S G L L L V A L L T C L T T M V L M S V W R Q R K L W E K L P P G P T P L

121 CCCTTCATTGGGAACCTACTGCAGCTGAACACTCAGCAGATGTCTGATTCTTCATGAAGATCAGCGAGCGATATGGCCCGGTCTTCATGGTCCACCTGGGGCCCCGGCATCGTGGT 240
P F I G N Y L Q L N T Q Q M S D S F M K I S E R Y G P V F M V H L G P R R I V V

241 CTGTGTGACACGAGCGGTGAAGGAGGCTCTGGTGGACAGGCTGAGGAGTTCAGCGGGCAGGCGCACAGGCCACCTTCGACTATCTCTCAAAGGCTATGGGGTACGCTTCAGCAAC 360
L C G H E A V K E A L V D Q A E E F S G R G A Q A T F D Y L F K G Y G V T F S N

361 GGGGAGCGCGCAAGCAGCTCCGGCGCTTCCATCACCAGCTGCGGGACTTTGGAGTGGGCAAGCGCGGCTTGGAGGCGCATCCAGGAGGAGCGGGCTTCCTCATTGAAGCCTTT 480
G E R A K Q L R R F S I T T L R D F G V G K R G I E E R I Q E E A G F L I E A F

481 CGGGGCACACCGTCTTCATCGATCCACCTTCTCTGAGCGCAAGTGTCCAATGTATCAGCTCCATTGTCTTTGGGACCGCTTTGACTATGAGGACAAAGATTCTGTCA 600
R G T H G A F I D P T F F L S R T V S N V I S S I V F G D R F D Y E D K E F L S

601 CTGCTGCTATGATGCTGGGAAGCTTCAGTTCACAGCTACCTCTATGGGGAGCTCTGTGAATGTTCATTGATGATGAAGCACCTGCCAGGGCCACAGCAACAGCGTTTAAGAG 720
L L R M M L G S F Q F T A T S M G Q L C E M F H S V M K H L P G P Q Q Q A F K E

721 CTGCGAGGTCTGGAAGCTTCATAGCCAAGAAGGTGGAGCAGAATCAACGCACCTTGAGCCCAATTCCCCGAGGACTTCATCGACTCTTCTCATTGCTATGCTGAGGAGGAGCAAC 840
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841 AACCCCAACAGGAGTTCATAGAAGAACTGGTCTGACCACTGAACCTCTCTTTGGGCACTGAGCGGTGAGCACAACCTCGCGGTACGGCTTCCTGCTGCTCATGAAGAC 960
N P N T E F Y M K N L V L T T L N L F F A G T E T V S T T L R Y G F L L L M K H

961 CCAGAGGTGGAGGCAAGTCCATGAGGAGATTGACCGGTGATTGGCAAGACCTGAGCCCAAGTTTGGAGCCGGGCAAGATGCCCTACAGAGGCGGTGATCCACGAGATCCAA 1080
P E V E A K V H E E I D R V I G K N R Q P K F E D R A K M P Y T E A V I H E I Q

1081 AGATTGGAGACATAATCCCATGGGCTGGCCCGCAGAGTCACCAAGGACACCAAGTTTCAGAGATTCTCTCTCCCAAGGGCACTGAAGTGTTCCTATGCTGGGCTCGGTGCTGAGA 1200
R F G D I I P M G L A R R V T K D T K F R E F L L P K G T E V F P M L G S V L R

1201 GACCCCAAGTCTTCTCCAAACCCCGAGACTTCCACCCAGCACTTCTGGATGAGAATGGGCAAGTTTAAGAAGAGTGATGCTTTTGTGCCCTTCTCCATTGGAAGCGGTACTGTTTT 1320
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1321 GGAGAAGCGCTGGCTAGAATGGAGCTCTTCTCTCTCACCACCTCTTGCAGAACTTCCGCTTCAAGTCCCCGAGCTGCCCAAGACATCAACGTGTCTCCCAAGCTCGTAGGCTTA 1440
G E G L A R M E L F L F L T T I L Q N F R F K S P Q L P Q D I N V S P K L V G L

1441 GCCACCATCCACGAAATTACACCATGAGCTTCAGCCCCGCTGA 1485
A T I P R N Y T M S F Q P R *

(B) 1 ATGGAGCTCA GCGTCTCTCT CTCTCTTGT CTCTCACGG GACTCTTGT TCTGTGGCC AGGGGCCACC TGAAGGCCTA TGGCTGCTG CGGCCAGGC CCCGCTCTCT GCTCTTCTG 120
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121 GGAACCTTC TTCAATGGA CAGAAGTGG TTAATCAAT CCTTCTCAG TTCCAAGAG AAATACGGG ATGTCTTCA GGTGTACCTG GGGCCAAGGC CTGTGTCAT GCTATGTGG 240
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241 ATAAAGGCCA TACGGAGGC CTGGTGGAC CAGGCTGAGA CCTTCTCCG CCGGGGGAAA ATTGCTATAC TAGAGCGAGT CTTCAGGAA TATGGTGTGG TCTTTGCCAA TGGGGAACGC 360
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W K T L R R F S L A T M R D F G M G K W S M E K R I Q E E A Q C L V E E L R K T

481 CAGGGAGCCC TCCAGACCC CACCTTATTC TTCCACTCCA TGACCACTAA CGTCATCTGT TCCATTGTCT GTGGAAAACG CTTTGGCTAC AGAGACCTTG AATTGCTGG GCTGCTGGAC 600
Q G A L Q D P T L F F H S M T T N V I C S I V C G K R F G Y R D P E L L R L L D

601 CTGTTCTACC AGTCTTTCG GCTCATCAGC TCCTTCTCCA GCCAGGTGTT CGAGCTTTTC CACAGCTTCT TGAAGTACTT CCCTGGTACA CACAGGCAAG TCTACAAAAA CCTGCAGGAA 720
L F Y Q S F A L I S S F S S Q V F E L F H S F L K Y F P G T H R Q V Y K N L Q E

721 ATCACCCGCT TCATTGACCG CGTGTGGAG AAGCACCGTG AAACCTGGA CCCCAGTCC CCCCAGGACT TCATCGACGC CTACCTGATC CGCATGGACA AAGAGAAGGC CGACCCCGGC 840
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841 AGCGAGTTCC ACCAGGGAA CTCTATCTAC ACCGCGTGT CGCTCATCTT CGCCGGCAGC GAGACCAACA GCACCAAGCT CCGCTATGGA TTCCTGCTCC TGCTCAAATA CCCCACATC 960
S E F H Q R N L I Y T A L S L I F A G T E T T S T T L R Y G F L L L L K Y P H I

961 ACAGAGAGAA TCCACAAGA GATTGACCAG GTGATTGGCC CACACCGCT TCCATCCTTT GATGACCGAG CCAAAATGCC ATACACTGAT GCAGTCATCC ATGAGATTCA GAGATTGGG 1080
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1081 GACCTCTCC CGATTGTGT GCCCATATG GTCACCAAG ACATAGCTT CAGAGGGTAC ATCATTCCCA AGGGCACTGA AGTATTTCCT ATCCTGCACT CGGCTCTCAA TGATCCACAT 1200
D L L P I G V P H M V T K D T S F R G Y I I P K G T E V F P I L H S A L N D P H

1201 TACTTTGAAA AACCAGAAG CTTCACCTTC GACCGCTTTC TGGATGCCAA TGGGGCATTG AAGAAGAATG AAGCTTTTAT CCCCTCTCC GTAGGGAACG GCAGTTGTCT TGGTGAAGGC 1320
Y F E K P E V F N P D R F L D A N G A L K K N E A F I P F S V G K R S C L G E G

1321 ATCGCCCGCA TGGAAATTAT CTCTTCTTC ATCACCATCC TCCAGAACTT CTCTGTGGCC AGCCCCGTGG CCCCAGGGA CATTGACCTC ACACCCCGGG AGAGTGGTGT GGGCAAAAGT 1440
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1441 CCCCAGTGT ACCAGATCAG CTTTCTGGCT CATGGAGGAT GCTGA 1485
P P V Y Q I S F L A H G G C *

318

(C) 1 ATGGATCCAG TTGTGGTCT AGTGCTCTGT CTCTCCTTTT GGCTTCTCTT TTCACTCTGG AACAGAGCT CTGGAAGGG GAAGTCCCA CTGGGCCCA CTCTCTCCC TTTCATTGGA 120
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121 AATATCTCC AGGTAGATGT GAAGGACATC GGCAATCCT TAATCAATCT CTCAAAGCC TATGGCCCTG TATTCACCT GTATCTTGGC ATGAAGCCCA CTGTTGTGCT GCATGGGTAT 240
N I L Q V D V K D I G K S L I N L S K A Y G P V F T L Y L G M K P T V V L H G Y

241 GAAGCAGTGA AGGAAGCCCT GATTGATATG GGAGAAGAGT TTTCTGCAAG AGGAAGTTTC CCAATAGCTG AAAAATTAC TGAAGGACAC GGACTCCTTT TCACCACTGG AAAGAGATGG 360
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361 AAGGAGTTAC GGCCTCTCTC CCTCATGACC TGCGGAATT TGGGGATGGG GAAGAGTGAC CTGAGAGCC GAGTTCAAGA GGAAGCCTGC CACCTTGTAG AAGAATTGAG AAAACCAAT 480
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481 GCCTTACCCT GTGATCCAC TTTGTCTG GCGTGTGCTT CCTGCAATGT GATCTGCTCC GTTATTTTCC AGCATCATTT TGATTATACA GATGAGACTT TAATTGGTTT CCTCAAGAGA 600
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601 TTTAATGAAA ACTTCAGGAT TTTGAGCTCC CCAATGATCC ATGTCTACAA TAGCTTCCCC GCTCTCCTTG ATTATCTCCC AGGAAGTCAT AATACAATGT ATAAAAATTC TGTTTTCTA 720
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721 AAAAATTACA TTTTGGAGAA AATAAAGAA CACCAAGAAT CCTGGACAT TAACAATCCT CGGGAATTCA TTGATTATTT TCTGATGAAA ATGGAACAGG AAAAGTACAA TGAGCAGTTG 840
K N Y I L E K I K E H Q E S L D I N N P R D F I D Y F L M K M E Q E K Y N E Q L

841 GAGTTTACTT CTGAAAATTT GATAAACTT GCAGCTGATT TGTTGTCAGC TGGGACAGGG ACAATAAGCA CCACCCTACG ATATGGTCTC CTGATGCTGC TGAAGCACCC AAAAGCACA 960
E F T S E N L I N T A A D L F A A G T G T I S T T L R Y G L L M L L K H P K V T

961 GCTAAAGTCC AGGAAGAGAT TGACTGTGTA ATTGGCAGAC ACCAGACCCC TTGCATGCAA GACAGGAGCC GCATGCCCTA CACGAATGCA GTATTGCATG AGATCCAGAG ATACATTGAC 1080
A K V Q E E I D C V I G R H Q T P C M Q D R S R M P Y T N A V L H E I Q R Y I D

1081 CTGTGCCAA ACAACCTGCT TCATGCAATG ACCTGTGACA TTAAATTCAG AAATATTTT ATCCCAAGG GCACAACCAT ATTAACATCA CTGACTTCTG TGCTCCATGA TGACCAAGAA 1200
L V P N N L L H A V T C D I K F R N Y F I P K G T T I L T S L T S V L H D D Q E

1201 TTCCCAACCC CAGAGATATT TGACCTGCC CACTTCCTGG ATGATAGCGG CAACCTTAAG AAGAGTGACC ATTTGCGGC TTTCTCAGCA GGAAAACGAG TATGCGTAGG AGAAGGACTG 1320
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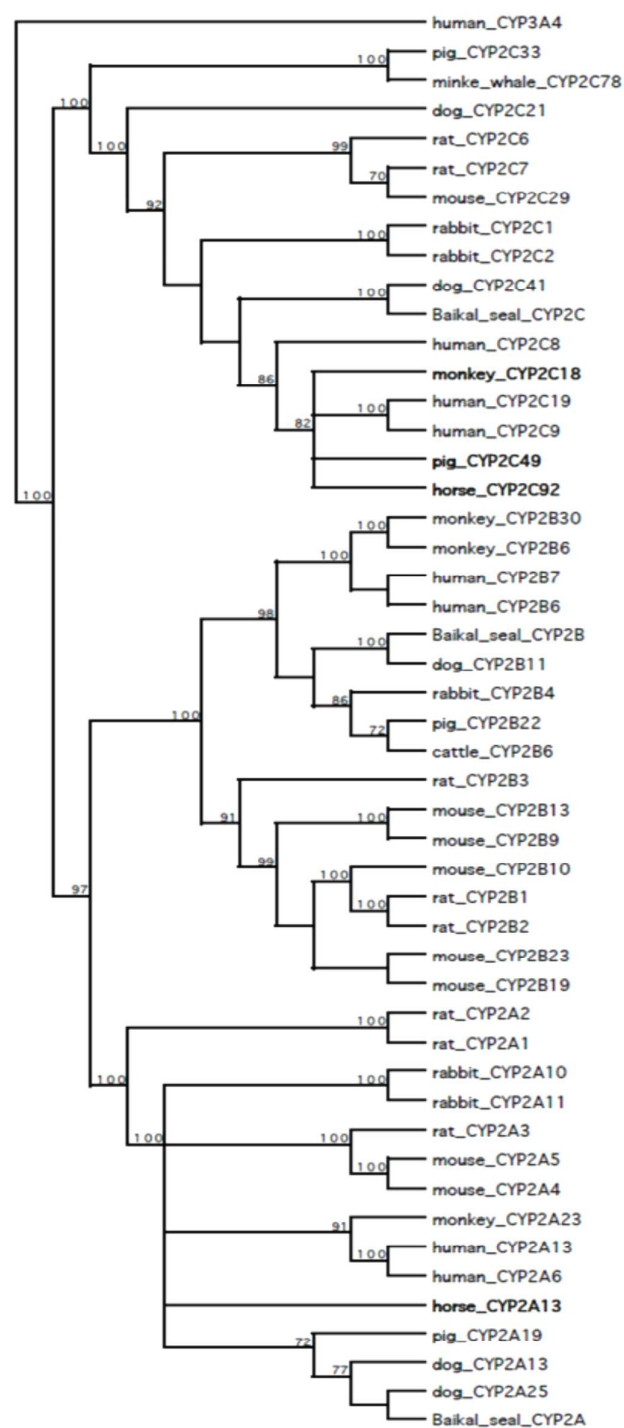
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1441 CCCCCCTACC AGGTGTGCTT CATTCCCATG TGA 1473
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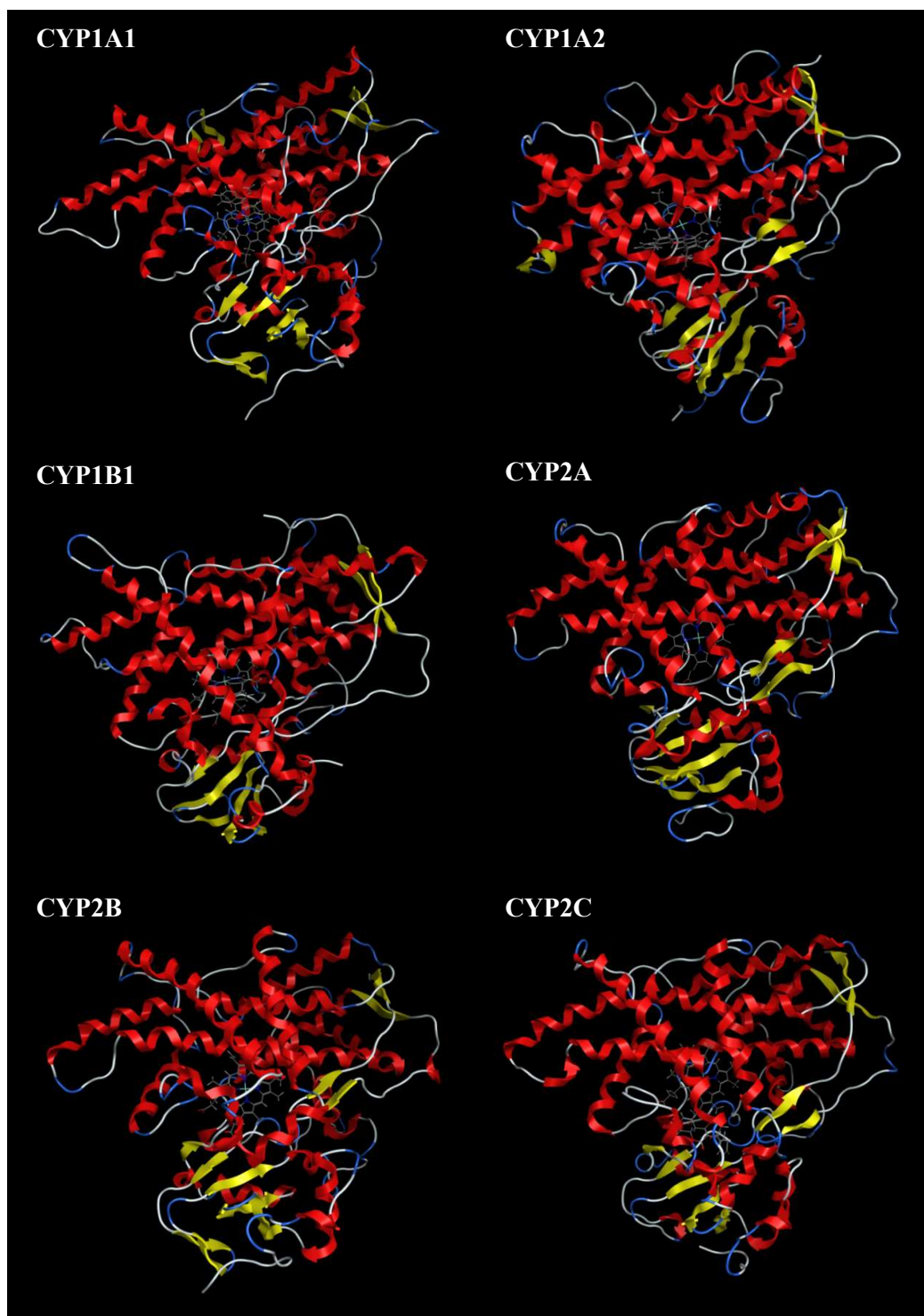
320 Figure S3.

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324 Figure S5.

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hCYP2C9	1	-----MDS----LVVLVLCLSCLLLLSLWRQSSGRGKLP	39
bsCYP1A1	1	-----MFSASRLSIPISATELLLLASAVFCLMLWVVR	53
bsCYP1A2	1	-----MALSQMATELLLLASAVFCLMLWVVR	47
bsCYP1B1	1	MATSLGAEAPLQPSALSSQQTLLLLSVLAHVHGQWLLRQRRRQ	60
bsCYP2A	1	-----MLASGLLLVALLTCLTTMVLMSVWRQKLWEKL	43
bsCYP2B	1	-----MELSVLLLLALLTG---LLLLLARGHLKAYGCL	40
bsCYP2C	1	-----MDP---VVVLVLCLSFLLLLSLWKQSSGKGKLP	39

hCYP2C9	40	GNILQIGIKDISKSLTNLSKVYGPVFTLYFGLKPIVVLHGYEAVKEALIDLGEEFSGRGI	99
bsCYP1A1	54	GNVLT LG-KNPHLALSRLSQRYGDVLQIHIGSTPVLVLSGLD TVRQALVRQGEDFKGRPD	112
bsCYP1A2	48	GNVLT LR-KNPHLALSRLSQRYGDVLQIHIGSTPVLVLSGLD TVRQALVRQGEDFKGRPN	106
bsCYP1B1	61	GNAAMG-PAPHL SFARLARRYGDVFQIRLGNCPPVVLNGERAIRQALVQQGAADFPR	119
bsCYP2A	44	GNYLQ LNTQQMSDSFMKISERYGPVFMVHLGPRRIVVLCGHEAVKEALVDQAEFSGRGA	103
bsCYP2B	41	GNLLQMDRSGLLKSFLRFQEKYGDVFTVYLGPRPVMLCGIKAIREALVDQAEFSGRGK	100
bsCYP2C	40	GNILQVDVKDIGKSLINLSKAYGPVFTLYLGMKPTVVLHGYEAVKEALIDMGEEFSARG	99

hCYP2C9	100	FPLAERANRGFGIVFS--NGKKWKEIRRFSLMTLRNFGMGKRS-----IEDRVQEEAR	150
bsCYP1A1	113	LYSFTLI TNGQSM SFS PDSPGVWAARRRLAQNALKSFSIASDPGSSSSCYLEEHSKEAE	172
bsCYP1A2	107	LYSFTLI TNGQSM SFS PDSPGVWAARRRLAQNALESFSIASDPGSSSSCYLEEHSKEAE	166
bsCYP1B1	120	FASFRVVS GGRSLAFG-PYSQSWKVRRAAHSTMAFSTRQPR---SRRVLEGHVLGEAR	175
bsCYP2A	104	QATFDYL FKG YGVTF S--NGERAKQLRRFSITTLRDFGVGKRG-----IEERI QEEAG	154
bsCYP2B	101	IAILEAV FQEYGVVFA--NGERWKT LRRFSLATMRDFGMGKWS-----MEKRI QEEAQ	151
bsCYP2C	100	FPIAEKL TEGHGL LFT--SGKRWKE LRRFSLMTLRNLGMGKSD-----LESRVQEEAC	150

hCYP2C9	151	CLVEELRKT KASP--CDPTFILGCAPCNVICSIIFHKRFDYKDQQFLNLMEKLNENIKIL	208
bsCYP1A1	173	ALLSRLQE QMAEVGHFDPYRYVVSVANVVCAMCFGKRYDHDQELLSLINLNNE---FG	229
bsCYP1A2	167	ALLSRLQE QMAEVGFDPYNQVLLSVANVIGAMCFGQHFPQSNEEMLSLIKSND---FV	223
bsCYP1B1	176	ELVALLVRGSAGGAFVDPRLTVVAVANVMSAVCFGCYSHDDAEFRELLSHNEEFGRTV	235
bsCYP2A	155	FLIEAFRGTHGAF--IDPTFFLSRTVSNVSIIVFGDRFDYEDKEFLSLLRMMLGSFQFT	212
bsCYP2B	152	CLVEELRKTQ GAL--QDPTLFFHSM TTNVICSVICGKRFGYRDP ELLRLLDLFYQSFALI	209
bsCYP2C	151	HLVEELRKTNALP--CDPTFVLGCASCNVICSVIFQHFDYDDETIGFLKRFNENFRIL	208

hCYP2C9	209	SSPWIQICNNFSPIIDYFP GTHNKLKNV-AFMKSYILEKVKEHQESMDMNN-PQDFIDC	266
bsCYP1A1	230	EAVASGNPVDFFPILRYLPNPALDFFKDLNKR FY SFMQKL VKEHYKTFEKGH-IRDITDS	288
bsCYP1A2	224	ETASSGNPVDFFPILQYMPNPALQRFAFNQKL VQFLQKIVQEHYQDFDESS-IQDITGA	282
bsCYP1B1	236	GAGSLVDVLPWLQRFNPVRTAFREFEQLNRNFSNFVLDKFLRHRESLQPGAGPRDMDA	295
bsCYP2A	213	ATSMGQICFMFHSVMKHI PGPOQOAFKFI-OGI FDF TAKKV FQ NORTI DPNS-PRDFIDS	270
bsCYP2B	210	SSFSSQVFELFHSFLKYFP GTHRQVYKNL-QEITRFIDRVVEKHRETLPSS-PRDFIDA	267
bsCYP2C	209	SSPWIHVNSFPALLDYLPGSHNTMYKNS-VFLKNYILEKIKEHQESLDINN-PRDFIDY	266

hCYP2C9	267	FLMKMEKEKHNPQS---EFTIESLENTAVDLFGAGTETTSTTLRYALLLLKHPEVTAK	322
bsCYP1A1	289	LIKHCQDKRLDENAN--IQLSDEKIVNVVLDLFGAGFDVTTAISWSLLYLVTSPPSVQKK	346
bsCYP1A2	283	LLKHNEKGS RAGGG---HIPHEKIVSLINDIFGAGFEPITMAISWSLIYLVTNPEIQRK	338
bsCYP1B1	296	FIISAGTEAAEGSEDG GARQDLEYVPATVTDIFGASQDTLSTALQWLLILFTRYPEVQAR	355
bsCYP2A	271	FIRMQEEQNPNPT---EFYMKNLVLTLLNLFAGTETVSTTLRYGFLLLMKHPEVEAK	326
bsCYP2B	268	YLIRMDKEKADPRS---EFHQRNLIY TALS LIFAGTETTSTTLRYGFLLLKYPHITER	323
bsCYP2C	267	FLMKMEQEKYNEQL---EFTSEN LINTAADLFAAGTG TISTTLRYGFLMLLKHPKVTA	322

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hCYP2C9	323	VQEEIERVIGRNRSPCMQDRSHMPYTDVVHEVQRYIDL	L	PTS	L	PHAVTCDIKFRNYLIP	382
bsCYP1A1	347	IQEELDTVIGRARQPRLSDRPQLPYLEAFIETFRHASF	V	PFT	I	PHSTTKDTSLSGFYIP	406
bsCYP1A2	339	IQEELDTVIGRARQPRLSDRPQLPYMEAFIETFRHTSF	V	PFT	I	PHSTTRDTTLKGFYIP	398
bsCYP1B1	356	VQAELDQVVGRDRLPCLDDQPNLPYVVAFLYEAMRFSSF	V	PVT	I	PHATTTSTSVLGYHIP	415
bsCYP2A	327	VHEEIDRVIGKNRQPKFEDRAKMPYTEAVIHEIQRFGDI	I	PMGL	ARRVT	KDTKREFLLP	386
bsCYP2B	324	IHKEIDQVIGPHRLPSLDDRAKMPYTDVIHEIQRFGDL	L	PIGV	PHMVT	KDTSFRGYIIP	383
bsCYP2C	323	VQEEIDCVIGRHQTPCMQDRSRMPYTNAVLHEIQRYIDL	V	PNNL	L	HAVTCDIKFRNYFIP	382

hCYP2C9	383	KGTTILISLTSVLHDNKEFPNPEMFDPHHFLDEGGN---	FKKSKYFMPFSAGKRICVGEA	439
bsCYP1A1	407	KGRCVFVNQWQINHDQELWGDPEFRPERFLTLDGT-	INKALSEKVILFGMGKRKCIGET	465
bsCYP1A2	399	KERCVFINQWHVNHQKQVWGDPFEFRPERFLTADGTS	SINKILSEKVMIFGMGKRRCIGEL	458
bsCYP1B1	416	KDTVVFVNQWSVNHDPAKWPNEFDPGRFLDKDGC-	IDKDLASSVMIFSMGKRRCIGEE	474
bsCYP2A	387	KGTEVFPMLGSLVLRDPKFFSNPRDFHPQHFLDENGQ---	FKKSDAFVPFSIGKRYCFGEG	443
bsCYP2B	384	KGTEVFPILHSALNDPHYFEKPEVFNPDRFLDANGA---	LKKNEAFIPFSVGKRSCLEGEG	440
bsCYP2C	383	KGTTILTSVLHDDQEFNPNEIFDPAHFLDDSGN---	FKKSDHFAAFSAGKRVCVGEG	439

hCYP2C9	440	LARMELFLFLTSILQNFNLKSLVDPKNLDTTPVVNGFASVPPFYQLCFIPV-----	490
bsCYP1A1	466	IARLEVFLFLAILLQQVEFSVPRG-TKVDMTPIYGLTMKHARCEHVQVRVRA-----	516
bsCYP1A2	459	LAKWEIFLFLAILLQRLEFSVPDG-VKVDLTPIYGLIMKHTRCEHVQARPRFSTK-----	512
bsCYP1B1	475	LSKMLFLFLFISILAHECNFKANPD--EPSKMDFNYGLTIKPKSFRINVTLRESMELLDSA	532
bsCYP2A	444	LARMELFLFLTTILQNFREFKSPQLPDINVSPKLVGLATIPRNYTMSFQPR-----	494
bsCYP2B	441	IARMELFLFFITILQNFVSPVAPEDIDLTPRESGVGKVPVYQISFLAHGGC-----	494
bsCYP2C	440	LARMELFLFLTTILQKFTLKSLVDPKDIDTTPIASGFGHVPPPYQVCFIPM-----	490

hCYP2C9	491	-----	490
bsCYP1A1	517	-----	516
bsCYP1A2	513	-----	512
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bsCYP2B	495	-----	494
bsCYP2C	491	-----	490

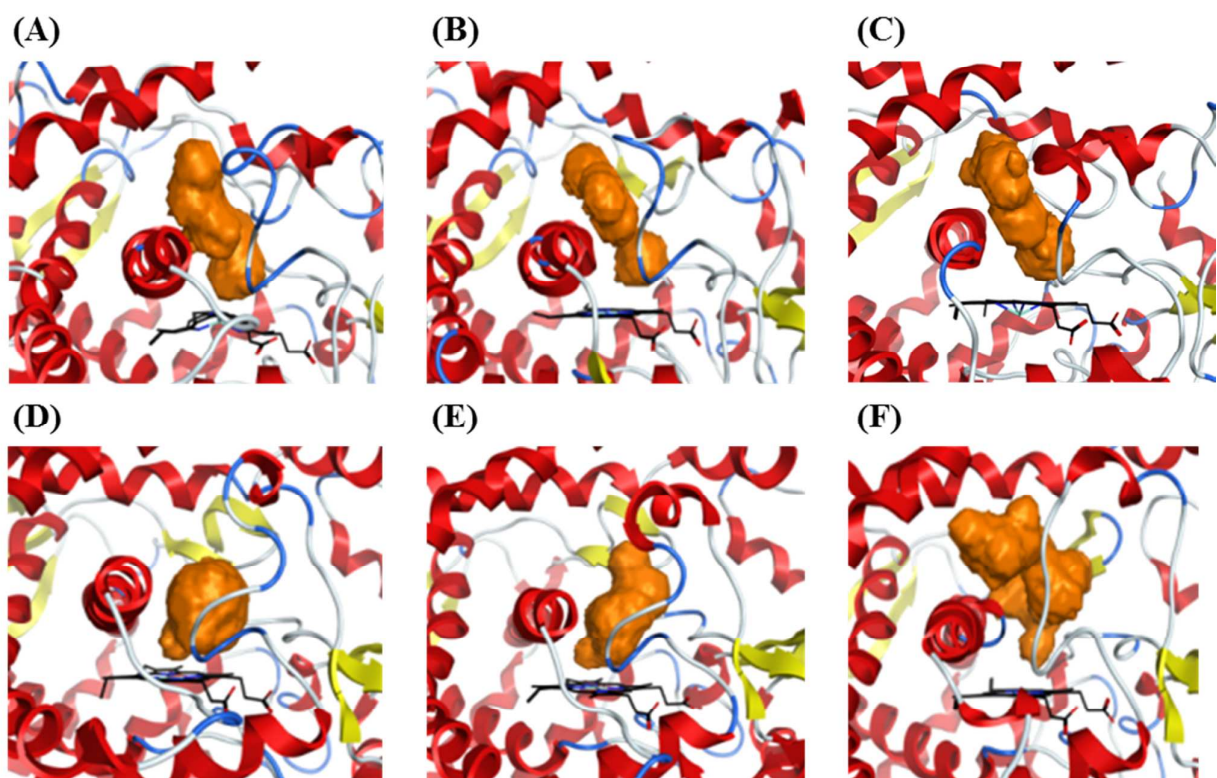
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334 Figure S7.

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341 Figure S8.

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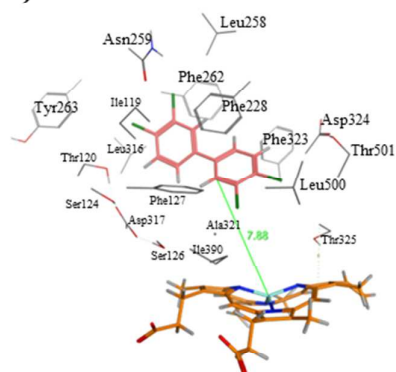
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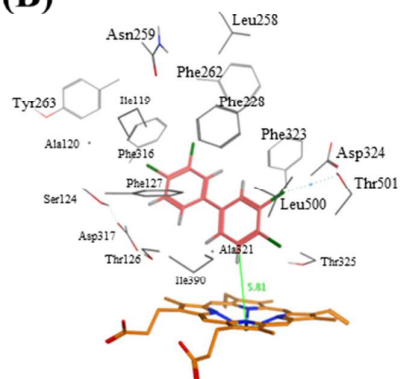
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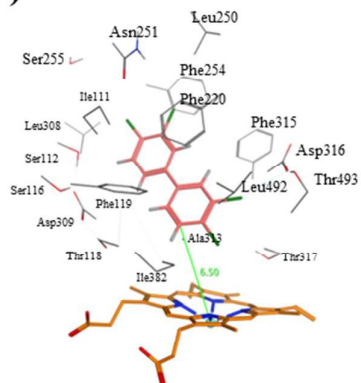
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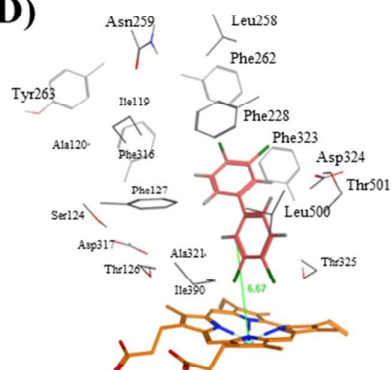
(B)



(C)



(D)



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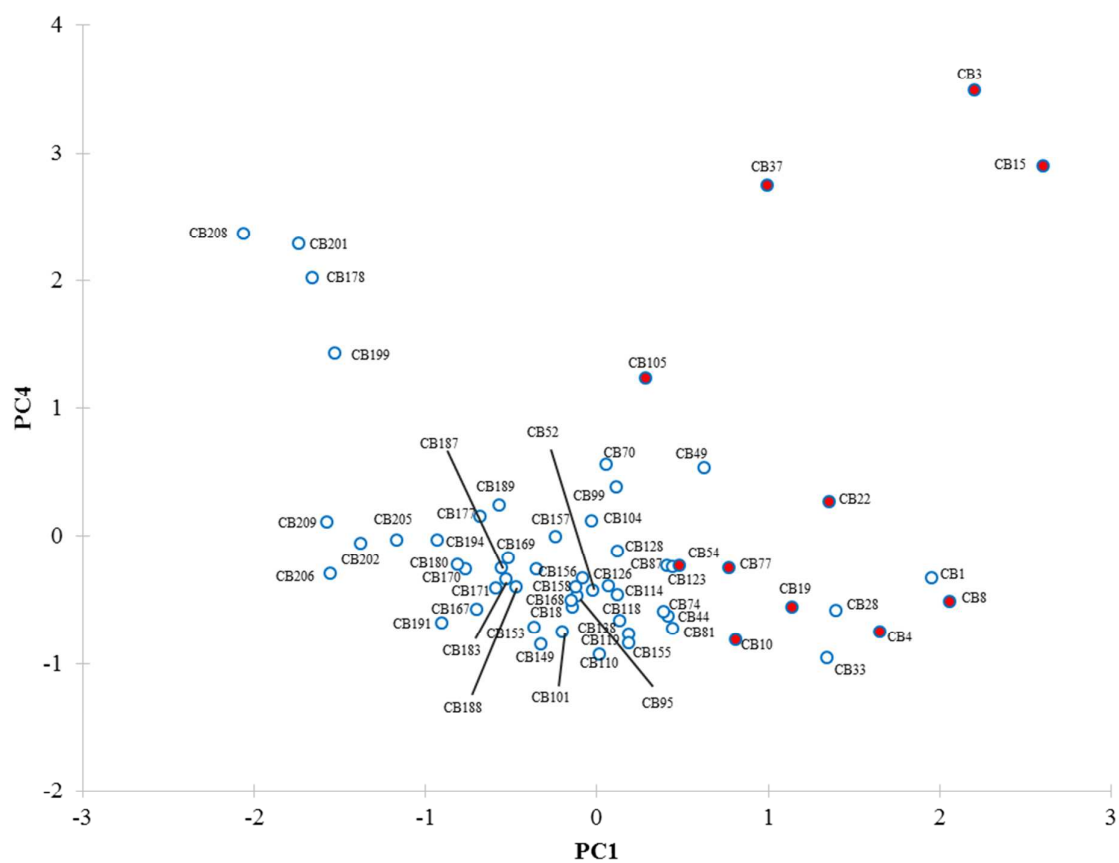
352 Figure S9.

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361 Figure S10.

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