



Enantioselective metabolism of chiral polychlorinated biphenyl 2,2',3,4,4',5',6-Heptachlorobiphenyl (CB183) by human and rat CYP2B subfamilies

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ABSTRACT

Chiral polychlorinated biphenyls (PCBs) have atropisomers that have different axial chiralities and exist as racemic mixtures. However, biochemical processes often result in the unequal accumulation of these atropisomers in organisms. This phenomenon leads to enantiospecific toxicity enhancement or reduction because either of the atropisomers mainly affects toxicity expression. Enantioselective accumulation is caused by cytochrome P450 (CYP, P450) monooxygenases, especially the CYP2B subfamilies. Therefore, this study investigates the metabolism of a chiral PCB *in vitro*. Both atropisomers isolated from racemic 2,2',3,4,4',5',6-heptachlorobiphenyl (CB183) were metabolized by human CYP2B6, but not rat CYP2B1. This may be due to the difference in the size of the substrate-binding cavities of CYP2B6 and CYP2B1. The stable accommodation of (–)-CB183 in the cavity without any steric hindrance explained the preferential metabolism of (–)-CB183 compared to (+)-CB183. Two hydroxylated metabolites, 3'-OH-CB183 and 5-OH-CB183, were identified. The docking study showed that the 3'-position of the trichlorophenyl ring closely approaches the heme of CYP2B6. To our knowledge, this is the first study to elucidate the structural basis of chiral PCB metabolism by P450 isozymes. These results will help promote the precise toxicity evaluation of chiral PCBs and provide an explanation of the structural basis of chiral PCB metabolism.

Keywords: atropisomer, chiral polychlorinated biphenyl, cytochrome P450 monooxygenase, enantioselective metabolism, CB183

1. Introduction

Polychlorinated biphenyls (PCBs) have been widely used in industrial products like insulating oils for electrical machinery, transmitters, and condensers owing to their incombustibility and insulation properties. PCBs are known as legacy persistent organic pollutants (POPs) and continuous efforts are being made to eliminate their production and usage. Yet, environmental contamination (Zhu et al., 2022) due to PCBs and instances of their bioaccumulation in humans and wildlife are frequently reported (Quinete et al., 2014). Multiple adverse effects of PCBs on human health are known, such as carcinogenicity, reproductive impairment, and developmental neurotoxicity. Of the 209 PCB congeners, 19 PCB congeners that have either three or four *ortho* chlorine substituents display axial chirality and are called chiral PCBs. Generally, atropisomers of chiral PCBs that are non-superimposable mirror images of each other exist as racemic mixtures in the environment and have identical physical and chemical properties. However, they show different metabolism, accumulation, and toxicity *in vivo* because of their different three-dimensional structures. 2,2',3,4,4',5',6-Heptachlorobiphenyl (CB183) was reported to accumulate enantioselectively in human breast milk and adipose tissue, suggesting that the concentration of one atropisomer is lower *in vivo* (Bordajandi et al., 2008; Konishi et al., 2016; Toda et al., 2012). Mammalian cytochrome P450 (CYP or P450) monooxygenases are known to metabolize various PCB congeners. Rat CYP1A1 metabolizes the most toxic PCB congener 3,3',4,4',5-pentachlorobiphenyl (CB126) to hydroxylated metabolites (Yamazaki et al., 2011). Docking models of CB126 with CYP1A1 revealed that the 4-OH-3,3',4',5-tetrachlorobiphenyl metabolite is formed because CB126 occupies the active site of CYP1A1 in such a manner that its 4-position is closest to the heme iron. In contrast, 2,3',4,4',5-pentachlorobiphenyl (CB118) is metabolized by human and rat CYP2B subfamilies (Mise et al., 2016). Docking studies

showed that CB118 is positioned closer to the heme iron of human CYP2B6 compared to that of rat CYP2B1. Therefore, human CYP2B6 produces higher amounts of hydroxylated metabolites. These studies suggest that the stable accommodation of PCBs in the substrate-binding cavities of P450s and their vicinity to the heme iron are responsible for the high PCB metabolism. The CYP2B subfamily has also been employed for the enantioselective metabolism of chiral PCBs. While both rat CYP2B1 and human CYP2B6 biotransformed 2,2',3,6-tetrachlorobiphenyl (CB45) to hydroxylated metabolites, they showed a different atropisomer preference that reflected in the opposite enantiomer fractions obtained for the two isozymes (Warner et al., 2009). Furthermore, CYP2B-induced rat liver microsomes preferentially metabolized (+)-CB136 to (+)-5-OH-CB136 (Wu et al., 2011).

Enantioselective binding activities of 2,2',3,5',6-pentachlorobiphenyl (CB95) atropisomers toward a ryanodine receptor (RyR) were also observed (Feng et al., 2017). (–)-CB95 showed a higher binding activity toward RyR than its racemate, leading to enantiomer-specific toxicity. The same phenomenon was observed in the case of (–)-2,2',3,3',6,6'-hexachlorobiphenyl (CB136), which acts as a sensitizer toward RyR and causes neurodevelopmental toxicity (Yang et al., 2014). Therefore, it is important to further explore the enantioselective metabolism of chiral PCBs by P450 isozymes, especially by the CYP2B subfamily, since PCB toxicity is dependent on metabolism. Chiral CB183 is one of the most potent congeners to activate the RyR-Ca²⁺ channel complex type 1 (Holland et al., 2017; Pessah et al., 2006). This receptor is regulating a cellular signaling through calcium release. Therefore, clarification of its metabolic fate is of great interest. It was reported that the serum, feces, and liver microsomes from CYP2B-induced rats showed the production of 3'-OH-CB183 and 5-OH-CB183 (Ohta et al., 2007,

2006). However, the enantioselective metabolism of CB183 and its structural basis have not been explored.

In this study, we show the enantioselective metabolism of CB183 atropisomers by human and rat CYP2B subfamilies *in vitro*. Separated atropisomers (Figure 1) were used as substrates of P450 isozymes in metabolism experiments. This study reveals several important aspects of the enantioselective metabolism of CB183, which will allow an adequate evaluation of its toxicity. Furthermore, docking models of CB183 atropisomers and the human CYP2B6 reveal the structural basis underlying its enantioselective metabolism.

2. Materials and methods

2.1. Chemicals

The two atropisomers were separated from racemic CB183 (AccuStandard, New Haven, CT) by chiral HPLC on a CHIRALCEL® OJ-H column (Daicel Co., Osaka, Japan) under the conditions reported previously (Toda et al., 2012). Each of the atropisomers were dissolved in dimethyl sulfoxide to achieve a final concentration of 0.25 mM. ¹³C-labeled OH-PCBs (MHPCB-MXA: [¹³C₁₂]-4-Hydroxy-3',4'-dichlorobiphenyl, [¹³C₁₂]-4-hydroxy-2',4',5'-trichlorobiphenyl, [¹³C₁₂]-4-hydroxy-2',3',4',5'-tetrachlorobiphenyl, [¹³C₁₂]-4-hydroxy-2',3,4',5,5'-pentachlorobiphenyl, [¹³C₁₂]-4-hydroxy-2',3,3',4',5,5'-hexachlorobiphenyl, [¹³C₁₂]-4-hydroxy-2,2',3,3',4',5,5'-heptachlorobiphenyl, and [¹³C₁₂]-4-hydroxy-2,2',3,4',5,5',6-heptachlorobiphenyl), used as internal standards, and [¹³C₁₂]-2,3',4',5-tetrachlorobiphenyl, used as the syringe spike, were purchased from Wellington Laboratories (Guelph, Canada). The 3'-OH-CB183 and 5-OH-CB183 standards were kindly provided by Dr. T. Okumura (Environmental

Pollution Control Center, Osaka Prefecture, Osaka, Japan). Microsomes containing NADPH-cytochrome P450 oxidoreductase (CPR) and cytochrome *b₅*, along with human CYP2B6 or rat CYP2B1 that are heterologously produced in recombinant insect cells were purchased from BD Biosciences (San Jose, CA, USA).

2.2. Measurement of hydroxylation activity of CYP2B subfamilies toward CB183

The reaction solution contained 2.5 μ M (+)- or (–)-CB183, 80 nM human CYP2B6 or rat CYP2B1, 3.3 mM MgCl₂, 100 mM potassium phosphate buffer (pH 7.4), NADPH regenerating system (5 mM NADPH, 5 mM glucose-6-phosphate, 1 unit glucose-6-phosphate dehydrogenase) in a total volume of 0.5 mL. The reaction was initiated by adding a microsomal fraction and was incubated for 120 min at 37 °C with continuous shaking. Control experiments were performed using the same procedure without the addition of NADPH. The reaction was terminated moving the tubes containing the reaction mixture on ice, and 20 μ L of 50 ng/mL ¹³C-OH-PCB was added as an internal standard. The metabolites were extracted twice with 2 mL of hexane and derivatized by methylation as described previously (Sakiyama et al., 2007). Quantification and identification of the metabolites (Table S1–S3 of Supplementary Information) were performed by high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS: GC, 6890N [Agilent Technologies, Tokyo, Japan]; MS, JMS-800D [JEOL Ltd., Tokyo, Japan]) equipped with an HT8-PCB column (Kanto Chemical Co. Inc., Tokyo, Japan) using SIM modes under the conditions described previously (Goto et al., 2018). The identification of retention times between metabolites and the authentic standards, and isotope ratios ([M+2]⁺: [M+4]⁺) of MeO-heptachlorobiphenyls were employed to identify OH-PCBs. Relative calibration curves that are prepared by the division of the peak areas of different concentrations of 5-MeO-

CB183 by that of [$^{13}\text{C}_{12}$]-4-MeO-2,2',3,4',5,5',6-heptachlorobiphenyl were used to quantify M1 and M2. The recovery rates for metabolites were calculated by using an internal standard and syringe spikes as 57–125% for hydroxylated heptachlorobiphenyls.

2.3. Molecular docking of CB183 in the substrate-binding site of human CYP2B6

Both atropisomers of CB183 were docked in the substrate-binding site of human CYP2B6 using Surflex Dock in SYBYL 8.0 (Tripos, St Louis, MO) (Gay et al., 2010; Ruppert et al., 1997). To determine a reasonable conformation of CB183, 13 crystal structures of human CYP2B6 stored in the Protein Data Bank (PDB IDs: 3IBD, 3QOA, 3QU8, 3UA5, 4I91, 4RQL, 4RRT, 4ZV8, 5EM4, 5UAP, 5UDA, 5UEC, and 5UFG) were superimposed.

3. Results and discussion

3.1. Detection and identification of CB183 metabolites

In microsomes containing human CYP2B6, two different NADPH-dependent hydroxylated heptachloro metabolites (M1 and M2) were detected for both the atropisomers by HRGC/HRMS (Figure 2B–E). In contrast, no metabolites were detected in rat CYP2B1 for both isomers (Figure 2F–I). A possible reason for this could be the different volumes of the substrate-binding cavities of rat CYP2B1 (472 Å) and human CYP2B6 (559 Å) (Mise et al., 2016). This is an important factor when considering the stable accommodation of substrates in the binding site, which is responsible for the varying metabolizing activities of the different P450 isozymes. This suggests that the binding cavity of rat CYP2B1 is too small to accommodate the heptachlorinated CB183. However, rat CYP2B1 has been reported to metabolize hexachlorinated PCB congeners, such as 2,2',3,3',4,6'-hexachlorobiphenyl (CB132) and CB136 to hydroxylated products,

which suggests that the binding site of rat CYP2B1 can only accommodate up to hexachlorinated PCBs (Warner et al., 2009). Yet, in a contradictory report, 3'-OH-CB183 and 5-OH-CB183 were detected in the serum of rats intraperitoneally dosed with phenobarbital as a CYP2B inducer, followed by CB183 (Ohta et al., 2007). This contradiction can be attributed to the different experimental conditions as one study was conducted *in vitro* and the other *in vivo*. CYP2B1 is a frequently used isoform among rat CYP2B subfamilies for *in vitro* experiments. However, there are other CYP2B subfamilies in rats, such as CYP2B2, CYP2B3, CYP2B12, CYP2B15, and CYP2B21. Therefore, it is possible that CB183 is metabolized by other rat CYP2B subfamilies.

As the retention time of methylated M1 was consistent with that of the authentic standard S1 (3'-MeO-CB183), M1 was identified as 3'-OH-CB183 (Table S2). Similarly, M2 was identified as 5-OH-CB183. Their peaks also matched the isotope ratios ($[M+2]^+:[M+4]^+$) of MeO-heptachlorobiphenyl, indicating that the peaks represent hydroxylated heptachloro compounds (Figure S1, Table S3). According to the fragmentation patterns of the CB183 metabolites, M1 and M2 had larger peaks in $[M+2-\text{COCH}_3]^+$ than in $[M+2-\text{CH}_3\text{Cl}]^+$ (Figure S2). The peak of $[M+2-\text{COCH}_3]^+$ indicated that the substituted hydroxyl group was located at the *meta*- or *para*-position (Kunisue and Tanabe, 2009). This result was consistent with the identification of M1 as 3'-OH-CB183 and M2 as 5-OH-CB183. Further, CYP2B subfamilies preferentially hydroxylate at *meta*-position of PCBs whereas CYP1A1 preferentially hydroxylates at *para*-position (Mise et al., 2016). 3'-OH-CB183 and 5-OH-CB183 were also detected in the serum of rats injected with CB183 and the concentration of these metabolites increased due to the presence of phenobarbital (Ohta et al., 2007). These reports support our observation that CB183 can be metabolized to 3'-OH-CB183 and 5-OH-CB183 by the CYP2B subfamily.

3.2. Production rates of hydroxylated CB183 by human CYP2B6

Higher concentrations of metabolites were obtained in case of (–)-CB183 compared to (+)-CB183, with the concentration of 3'-OH-CB183 being 5.5 times higher and that of 5-OH-CB183 being 4.3 times higher (Figure 3). The production rates of 3'-OH-CB183 from (+)-CB183 and (–)-CB183 were 2.7 and 3.5 times higher than those of 5-OH-CB183, respectively. The proposed metabolic pathways of CB183 are shown in Figure 4. It has been reported that (+)-CB183 is more abundant than (–)-CB183 in human breast milk, while a racemic CB183 is present in fish, which is a major source of PCB intake (Konishi et al., 2016). These results suggest that CYP2B6 is related to the metabolism of (–)-CB183 in the human body. In contrast, it was reported that human CYP2B6 did not show enantioselective metabolism of racemic CB183 (Nagayoshi et al., 2018; Warner et al., 2009). This suggests that atropisomers influence each other's metabolism. This hypothesis is supported by the study in which (–)-CB132 inhibited the biotransformation of (+)-CB132 in the racemic mixture owing to the difference in the binding affinity of each atropisomer toward rat CYP2B1 (Lu and Wong, 2011).

3.3. Docking models of CB183 with human CYP2B6

To understand the molecular basis of enantioselective metabolism and different production rates of the two metabolites, both the atropisomers of CB183 were docked in the substrate-binding cavity of human CYP2B6 (PDB ID: 3IBD) (Figure S3A). The 13 crystal structures of human CYP2B6 obtained from PDB were superimposed to identify the substrate-binding cavity of human CYP2B6 (Figure S3B). Binding modes of eight conformations showed that either the 3'- or 5-position of CB183 approaches the heme iron in the substrate-binding cavity (Figure S3A). From these results, a plausible conformation of CB183 (displayed in red) in the cavity was selected (Figure S3A). Our

docking models indicate that the (–)-CB183 conformation experiences no steric hindrance in the substrate-binding cavity (Figure 5A). In contrast, steric hindrance between Cl at 3-position of (+)-CB183 and the cavity was observed (Figure 5B). Bulky side chains of amino acids I101, I209, and V474 were positioned 2.8, 2.9, and 3.3 Å away from the Cl residue at 3-position, respectively (Figure 5C). This adversely affects the conformational stability of (+)-CB183, resulting in its lower metabolizing activity. In the future studies, a binding energy calculated in the docking simulation is compared between human CYP2B6 or rat CYP2B1 and atropisomers of CB183 to support these models.

The selective production of two metabolites could be associated with two factors. Firstly, CB183 has seven chlorines and is composed of a trichlorophenyl and a tetrachlorophenyl ring (Figure 1). The electrostatic potential map of CB183 is shown in Figure S4. The tetrachlorophenyl ring is electron-deficient due to the presence of four electron-withdrawing chlorine groups, whereas the trichlorophenyl ring has three chlorines, making it relatively electron-rich, which facilitates the reaction at 3'-position. The dipole moment of the tetrachloro-substituted phenyl ring was larger than that of the trichloro-substituted ring. Therefore, it is possible that dipole-induced dipole interactions occur in the lipophilic area (Figure 6A). Conversely, when a trichlorophenyl ring occupies the right side of the cavity, the stability is decreased, causing low hydroxylation activity at the 5-position (Figure 6B). Thus, the preferential metabolism of (–)-CB183 atropisomer to the 3'-OH-CB183 metabolite by human CYP2B6 can be explained by these docking models.

4. Conclusions

All chiral PCBs are considered to have no dioxin-like toxicity because the expression of dioxin-like toxicity requires a co-planar structure that does not contain chlorine or has only one chlorine at the *ortho*-position. However, chiral PCBs alter calcium regulation associated with neuronal signaling through RyR (Wong and Pessah, 1996). (–)-CB95 and (–)-CB136 are known to cause enantioselective neurodevelopmental toxicity via RyR (Feng et al., 2017; Yang et al., 2014). Since CB183 also has RyR-binding activity, enantioselective binding activities of CB183 atropisomers have been observed (Pessah et al., 2006). Furthermore, it has been reported that chiral PCBs with a hydroxyl group, especially at the *meta*-position, decrease the binding activity toward RyR. These results suggest that hydroxylation of chiral PCBs by P450 species plays an important role in decreasing toxicity. Atropisomer-specific binding activity of CB183 toward RyR should be examined to understand how human CYP2B6 alters toxicity patterns.

In this study, we have shown that the atropisomers of chiral CB183 are metabolized to 3'- and 5-hydroxylated heptachloro metabolites by human CYP2B6. Furthermore, (–)-CB183 was metabolized to a greater extent than (+)-CB183. These observations were explained with the help of docking models of CB183 atropisomers with human CYP2B6. To our knowledge, this is the first study to explore the structural basis of chiral PCB metabolism by P450 species. Our results will accelerate research on the clarification of the structural basis of chiral PCB metabolism and precise toxicity evaluation of chiral PCBs. In addition to the biotransformation of chiral PCBs, factors like differences in the binding activity of transporter proteins toward chiral PCBs and cell membrane permeability should be taken into consideration for enantiomeric enrichment of chiral PCBs (Nagayoshi et al., 2018).

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244

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248

249 **References**

- 250 Bordajandi, L.R., Abad, E., González, M.J., 2008. Occurrence of PCBs, PCDD/Fs,
251 PBDEs and DDTs in Spanish breast milk: Enantiomeric fraction of chiral PCBs.
252 Chemosphere 70, 567–575. <https://doi.org/10.1016/j.chemosphere.2007.07.019>
- 253 Feng, W., Zheng, J., Robin, G., Dong, Y., Ichikawa, M., Inoue, Y., Mori, T., Nakano,
254 T., Pessah, I.N., 2017. Enantioselectivity of 2,2',3,5',6-Pentachlorobiphenyl (PCB
255 95) Atropisomers toward Ryanodine Receptors (RyRs) and Their Influences on
256 Hippocampal Neuronal Networks. Environ. Sci. Technol. 51, 14406–14416.
257 <https://doi.org/10.1021/acs.est.7b04446>
- 258 Gay, S.C., Shah, M.B., Talakad, J.C., Maekawa, K., Roberts, A.G., Wilderman, P.R.,
259 Sun, L., Yang, J.Y., Huelga, S.C., Hong, W.X., Zhang, Q., Stout, C.D., Halpert,
260 J.R., 2010. Crystal structure of a cytochrome P450 2B6 genetic variant in complex
261 with the inhibitor 4-(4-chlorophenyl)imidazole at 2.0-Å resolution. Mol.
262 Pharmacol. 77, 529–538. <https://doi.org/10.1124/mol.109.062570>

263 Goto, E., Haga, Y., Kubo, M., Itoh, T., Kasai, C., Shoji, O., Yamamoto, K., Matsumura,
 264 C., Nakano, T., Inui, H., 2018. Metabolic enhancement of 2,3",4,4",5-
 265 pentachlorobiphenyl (CB118) using cytochrome P450 monooxygenase isolated
 266 from soil bacterium under the presence of perfluorocarboxylic acids (PFCAs) and
 267 the structural basis of its metabolism. *Chemosphere* 210, 376–383.
 268 <https://doi.org/10.1016/j.chemosphere.2018.07.026>
 269 Holland, E.B., Feng, W., Zheng, J., Dong, Y., Li, X., Lehmler, H.J., Pessah, I.N., 2017.
 270 An extended structure-Activity relationship of nondioxin-like PCBs evaluates and
 271 supports modeling predictions and identifies picomolar potency of PCB 202
 272 towards ryanodine receptors. *Toxicol. Sci.* 155, 170–181.
 273 <https://doi.org/10.1093/toxsci/kfw189>
 274 Konishi, Y., Kakimoto, K., Nagayoshi, H., Nakano, T., 2016. Trends in the
 275 enantiomeric composition of polychlorinated biphenyl atropisomers in human
 276 breast milk. *Environ. Sci. Pollut. Res.* 23, 2027–2032.
 277 <https://doi.org/10.1007/s11356-015-4620-6>
 278 Kunisue, T., Tanabe, S., 2009. Hydroxylated polychlorinated biphenyls (OH-PCBs) in
 279 the blood of mammals and birds from Japan: Lower chlorinated OH-PCBs and
 280 profiles. *Chemosphere* 74, 950–961.
 281 <https://doi.org/10.1016/j.chemosphere.2008.10.038>
 282 Lu, Z., Wong, C.S., 2011. Factors affecting phase i stereoselective biotransformation of
 283 chiral polychlorinated biphenyls by rat cytochrome P-450 2B1 isozyme. *Environ.*
 284 *Sci. Technol.* 45, 8298–8305. <https://doi.org/10.1021/es200673q>
 285 Mise, S., Haga, Y., Itoh, T., Kato, A., Fukuda, I., Goto, E., Yamamoto, K., Yabu, M.,
 286 Matsumura, C., Nakano, T., Sakaki, T., Inui, H., 2016. Structural determinants of

287 the position of 2,3',4,4',5-pentachlorobiphenyl (CB118) hydroxylation by
 288 mammalian cytochrome P450 monooxygenases. *Toxicol. Sci.* 152, 340–348.
 289 <https://doi.org/10.1093/toxsci/kfw086>
 290 Nagayoshi, H., Kakimoto, K., Konishi, Y., Kajimura, K., Nakano, T., 2018.
 291 Determination of the human cytochrome P450 monooxygenase catalyzing the
 292 enantioselective oxidation of 2,2',3,5',6-pentachlorobiphenyl (PCB 95) and
 293 2,2',3,4,4',5',6-heptachlorobiphenyl (PCB 183). *Environ. Sci. Pollut. Res.* 25,
 294 16420–16426. <https://doi.org/10.1007/s11356-017-0434-z>
 295 Ohta, C., Haraguchi, K., Kato, Y., Matsuoka, M., Endo, T., Koga, N., 2007. THE
 296 DISTRIBUTION OF METABOLITES OF 2,2',3,4,4',5',6-
 297 HEPTACHLOROBIPHENYL (CB183) IN RATS AND GUINEA PIGS.
 298 *Organohalogen Compd.* 69, 1761–1764. <https://doi.org/10.13040/IJPSR.0975->
 299 8232.7(6).2573-85
 300 Ohta, C., Haraguchi, K., Kato, Y., Ozaki, M., Koga, N., 2006. Biochemical and
 301 molecular mechanisms (CB183) WITH LIVER MICROSOMES FROM RATS ,
 302 GUINEA PIGS AND HAMSTERS *Organohalogen Compounds Vol 68 (2006)*
 303 A) Rat B) Guinea pig C) Hamster *Organohalogen Compounds Vol 68 (2006).*
 304 *Organohalogen Compd.* 68, 1733–1736.
 305 Pessah, I.N., Hansen, L.G., Albertson, T.E., Garner, C.E., Ta, T.A., Do, Z., Kim, K.H.,
 306 Wong, P.W., 2006. Structure-activity relationship for noncoplanar polychlorinated
 307 biphenyl congeners toward the ryanodine receptor-Ca²⁺ channel complex type 1
 308 (RyR1). *Chem. Res. Toxicol.* 19, 92–101. <https://doi.org/10.1021/tx050196m>
 309 Quinete, N., Schettgen, T., Bertram, J., Kraus, T., 2014. Occurrence and distribution of
 310 PCB metabolites in blood and their potential health effects in humans: a review.

311 Environ. Sci. Pollut. Res. 21, 11951–11972. <https://doi.org/10.1007/s11356-014->
 312 3136-9
 313 Ruppert, J., Welch, W., Jain, A.N., 1997. Automatic identification and representation of
 314 protein binding sites for molecular docking. *Protein Sci.* 6, 524–533.
 315 <https://doi.org/10.1002/pro.5560060302>
 316 Sakiyama, T., Yamamoto, A., Kakutani, N., Fukuyama, J., and Okumura, T., 2007.
 317 Hydroxylated polychlorinated biphenyls (OH-PCBs) in the aquatic environment:
 318 Levels and congener profiles in sediments from Osaka. Japan. *Organohal. Comp.*
 319 69, 1380–1383.
 320 Toda, M., Matsumura, C., Tsurukawa, M., Okuno, T., Nakano, T., Inoue, Y., Mori, T.,
 321 2012. Absolute configuration of atropisomeric polychlorinated biphenyl 183
 322 enantiomerically enriched in human samples. *J. Phys. Chem. A* 116, 9340–9346.
 323 <https://doi.org/10.1021/jp306363n>
 324 Warner, N.A., Martin, J.W., Wong, C.S., 2009. Chiral polychlorinated biphenyls are
 325 biotransformed enantioselectively by mammalian cytochrome P-450 isozymes to
 326 form hydroxylated metabolites. *Environ. Sci. Technol.* 43, 114–121.
 327 <https://doi.org/10.1021/es802237u>
 328 Wong, P., Pessah, I.N., 1996. Ortho-substituted polychlorinated biphenyls alter calcium
 329 regulation by a ryanodine receptor-mediated mechanism: structural specificity
 330 toward skeletal- and cardiac-type microsomal calcium release channels. *Mol.*
 331 *Pharmacol.* 49, 740–751.
 332 Wu, X., Pramanik, A., Duffel, M.W., Hrycay, E.G., Bandiera, S.M., Lehmler, H.,
 333 Kania-korwel, I., 2011. Oxidized to Hydroxylated Metabolites by Rat Liver
 334 Microsomes 2249–2257.

Yamazaki, K., Suzuki, M., Itoh, T., Yamamoto, K., Kanemitsu, M., Matsumura, C.,
 Nakano, T., Sakaki, T., Fukami, Y., Imaishi, H., Inui, H., 2011. Structural basis of
 species differences between human and experimental animal CYP1A1s in
 metabolism of 3,3',4,4',5-pentachlorobiphenyl. *J. Biochem.* 149.
<https://doi.org/10.1093/jb/mvr009>

Yang, D., Kania-Korwel, I., Ghogha, A., Chen, H., Stamou, M., Bose, D.D., Pessah,
 I.N., Lehmler, H.J., Lein, P.J., 2014. PCB 136 atropselectively alters morphometric
 and functional parameters of neuronal connectivity in cultured rat hippocampal
 neurons via ryanodine receptor-dependent mechanisms. *Toxicol. Sci.* 138, 379–
 392. <https://doi.org/10.1093/toxsci/kft334>

Zhu, M., Yuan, Y., Yin, H., Guo, Z., Wei, X., Qi, X., Liu, H., Dang, Z., 2022.
 Environmental contamination and human exposure of polychlorinated biphenyls
 (PCBs) in China: A review. *Sci. Total Environ.* 805, 150270.
<https://doi.org/10.1016/j.scitotenv.2021.150270>

Figures

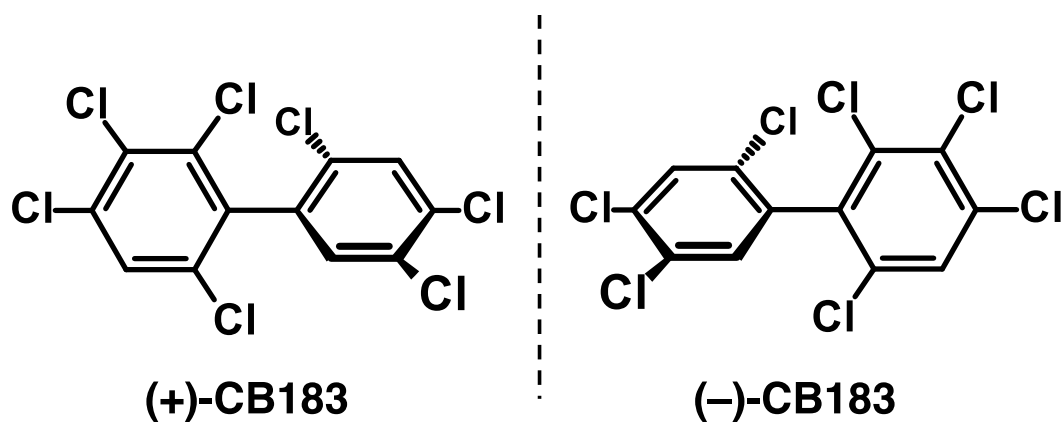


Figure 1 Chemical structures of atropisomers of 2,2',3,4,4',5',6-heptachlorobiphenyl (CB183).

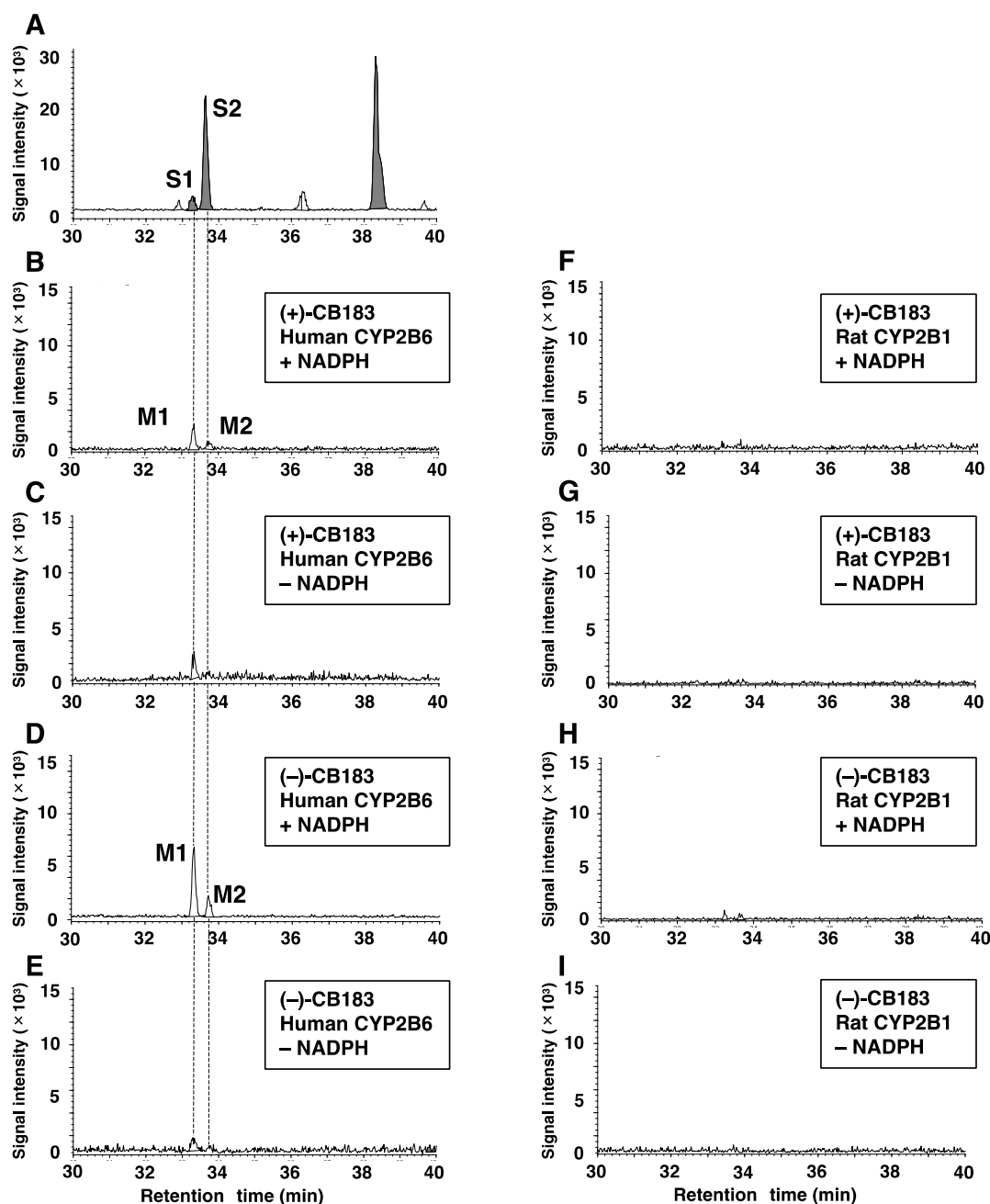


Figure 2 Chromatograms of hydroxylated heptachloro metabolites of CB183 by human CYP2B6 and rat CYP2B1 analyzed by high-resolution gas chromatography/high-resolution mass spectrometry.

(A): S1 and S2 are the authentic standards 3'-MeO-CB183 and 5-MeO-CB183, respectively. (B) and (C): Metabolism of (+)-CB183 by human CYP2B6, with and without NADPH, respectively. (D) and (E): Metabolism of (-)-CB183 by human CYP2B6, with and without NADPH, respectively. (F) and (G): Metabolism of (+)-CB183

402 by rat CYP2B1, with and without NADPH, respectively. (H) and (I): Metabolism of (–)-
403 CB183 by rat CYP2B1, with and without NADPH, respectively. Metabolites are
404 represented as M1 and M2, respectively.
405

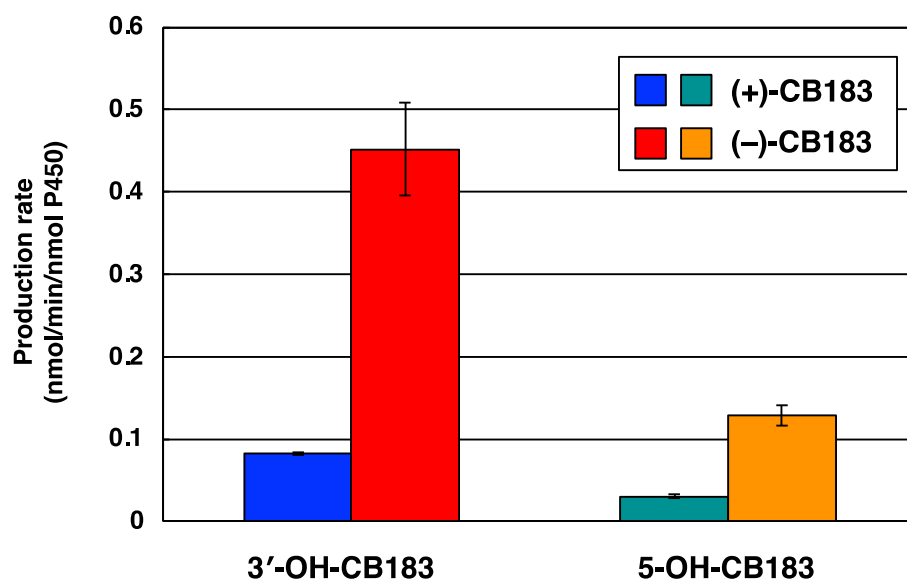


Figure 3 Production rates of CB183 metabolites by human CYP2B6.
Data were presented as mean \pm standard deviation ($n = 4$).

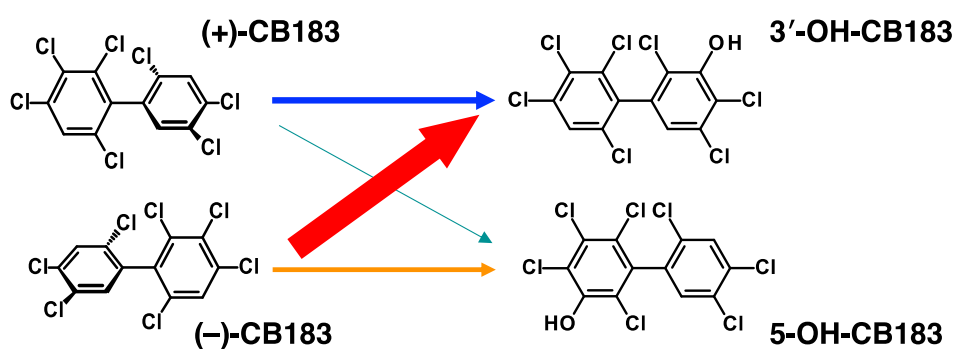


Figure 4 Proposed metabolic pathways of CB183 atropisomers by human CYP2B6. Enantioselective metabolism is shown by arrow thickness, which indicates the level of hydroxylation activity.

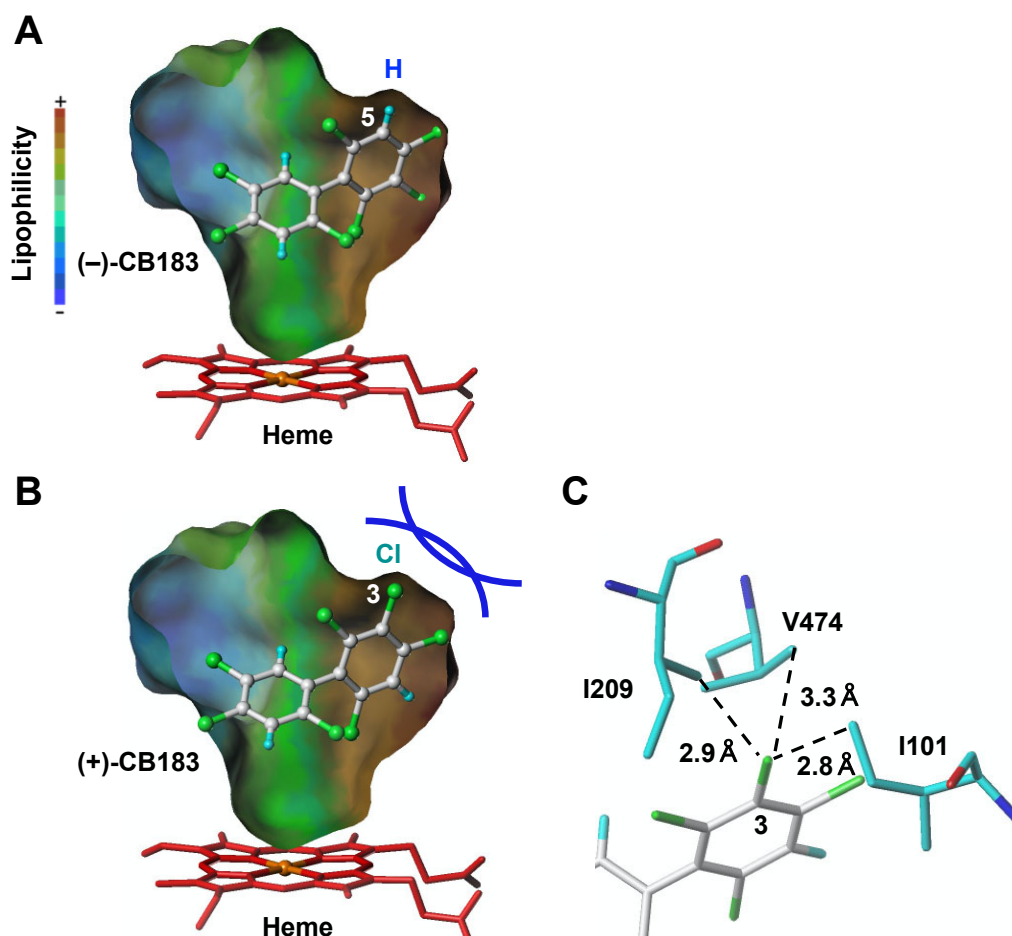


Figure 5 Preferential metabolism of (-)-CB183 by human CYP2B6. Docking models of (-)-CB183 (A) and (+)-CB183 (B) in the binding cavity of human CYP2B6. Blue and green spheres in CB183 indicate the hydrogen and chlorine atoms, respectively. Steric hindrance between the Cl atom at 3-position of (+)-CB183 and bulky side chains of amino acids I101, I209, and V474 that are present in the human CYP2B6 cavity is observed (C).

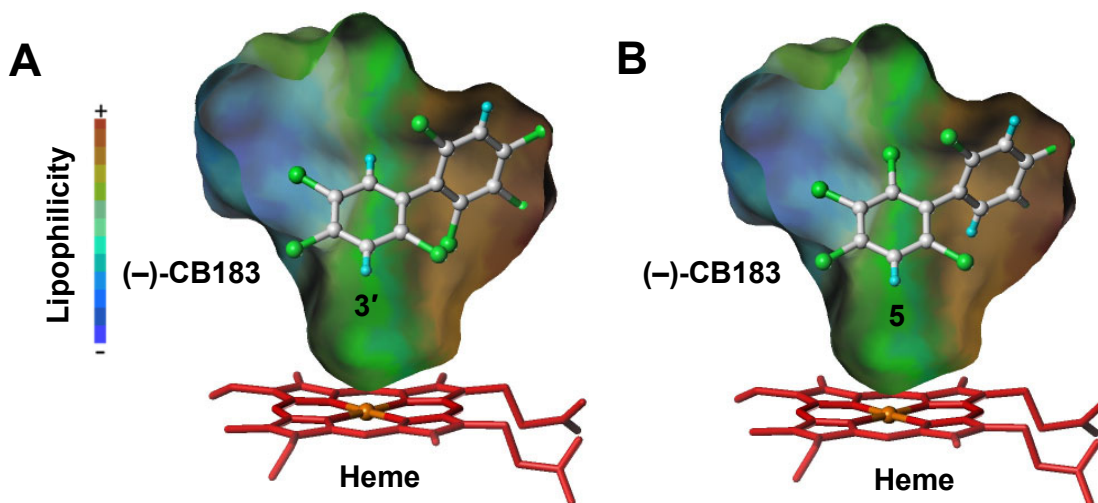


Figure 6 Preferential metabolism at 3'-position of CB183 by human CYP2B6.

(A) and (B): Docking models that 3'- and 5-positions of (-)-CB183 are facing to the heme in the binding cavity, respectively. Blue and green spheres in CB183 indicate the hydrogen and chlorine atoms, respectively. Blue and red circles on CB183 indicate the trichlorophenyl ring and tetrachlorophenyl ring, respectively.