Metabolism of chiral polychlorinated biphenyls by mammalian cytochrome P450 monooxygenases

Ito T[1], Miwa C[2], Haga Y[3], Goto E[1], Itoh T[4], Yamamoto K[4], Mise S[1], Matsumura C[3], Nakano T[5], Inui H[1,6]*
Grad. Sch. of Agr. Sci., Kobe Univ., Japan #1; Facul. of Agr., Kobe Univ., Japan #2 Hyogo Pref. Inst. of Environ. Sci., Japan #3; Lab. of Drug Design. and Medic. Chem., Showa Pharm. Univ., Japan #4; Res. Cent. for Environ. Preserv.,Osaka Univ., Japan #5; Biosignal Res. Cent., Kobe Univ., Japan #6 *hinui@kobe-u.ac.jp

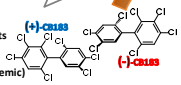
Introduction

Polychlorinated biphenyls (PCBs)
- Low degradability
- Biological concentration
- Long distance mobility
- Toxicity to humans and wildlife

⇨ Persistent organic pollutants (POPs)

Enantioselective accumulation in human breast milk[1]

Chiral PCBs
- Three or four *ortho*-chlorine substituents
- Steric hindrance of C-C bond
- Rotational isomers (atropisomers)
- Existence in the ratio of one to one (racemic)



Mammalian cytochrome P450 monooxygenase

- Monooxygenase reaction
- Metabolism of PCBs[2,3]

Detoxification

Toxicity evaluation toward each atropisomer of chiral PCBs

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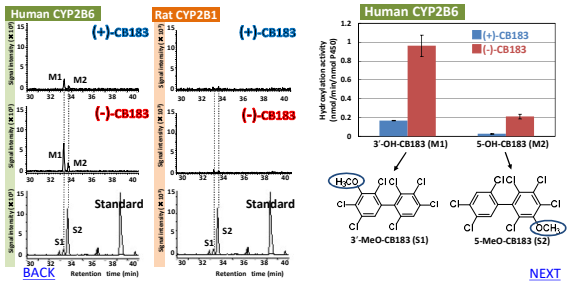
Metabolism of (±)-CB183 by human CYP2B6 and rat CYP2B1



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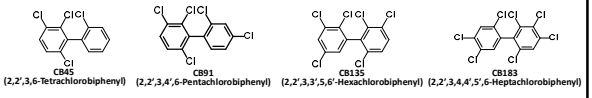
Materials and Methods

Reaction condition

40 pmol	Human CYP2B6, Rat CYP2B1
0.5 mM	NADPH
5 mM	G6P
1 U	G6PDH
3.3 mM	$MgCl_2$
100 mM	Potassium phosphate buffer (pH7.4)
0.8～6.4 μM	(±)-CB45, CB91, CB135, CB183
Total 0.5 ml	Separated each atropisomer

1. Start of the reaction by adding NADPH
2. Incubation for 2 h at 37˚C
3. Addition of the 50 ppb ^{13}C-labeled OH-PCBs as the internal standards — Detection of hydroxylated metabolites
4. Methylation
5. Analysis by high resolution gas chromatography and high resolution mass spectrometry
6. Construction of docking models with P450s and PCBs



Identification of metabolites for each atropisomer
Clarification of the structural basis

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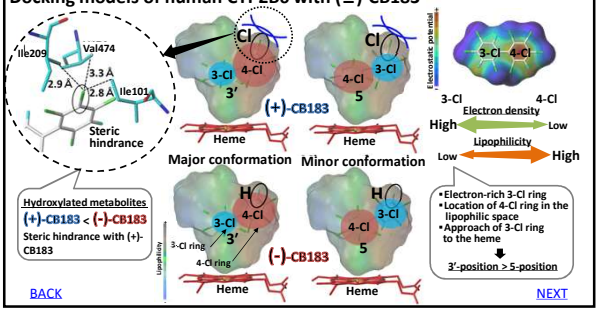
Docking models of human CYP2B6 with (±)-CB183



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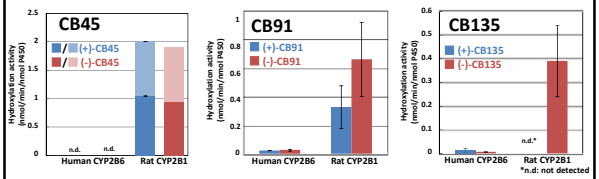
Metabolism of chiral PCBs by human CYP2B6 and rat CYP2B1



Hydroxylation activity	Human CYP2B6 << Rat CYP2B1	Human CYP2B6 < Rat CYP2B1	Human CYP2B6 < Rat CYP2B1
Hydroxylated metabolites	(+)-CB45 > (-)-CB45	(+)-CB91 < (-)-CB91	(+)-CB135 < (-)-CB135

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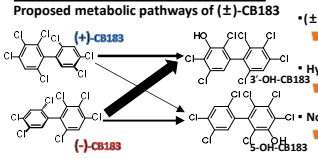
Summary and Conclusions

Proposed metabolic pathways of (±)-CB183



- (±)-CB183 → 3'-OH-CB183 and 5-OH-CB183
  - Detection in rats administered by phenobarbital (CYP2B inducer)[4]
- Hydroxylated metabolites : (-)-CB183 > (+)-CB183
  - Accumulation of (+)-CB183 in human breast milk than (-)-CB183[1]
- No metabolism by rat CYP2B1
  - The smaller cavity of rat CYP2B1 than that of human CYP2B6[5]

Difference of toxicity between each atropisomer of CB183 due to enantioselective metabolism

References

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