# Characteristics of PCB congeners accumulated in Yusho patients and estimation of their cytochrome P450-dependent metabolism by *in silico* docking simulation

Hirakawa S[1], Miyawaki T[1], Hori T[1], Kajiwara J[1], Katsuki S[1], Hirano M[2], Yoshinouchi Y[3], Iwata H[3], Mitoma C[4], Furue M[4]

[1] Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano, Dazaifu, Fukuoka 818-0135, Japan
[2] National Institute of Technology, Kumamoto College, 2627 Hirayamashin-Machi, Yatsushiro, Kumamoto 866-8501, Japan
[3] Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan
[4] Research and Clinical Center for Yusho and Dioxin, Kyusyu University Hospital, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan

## Introduction

In 1968, the Yusho incident occurred in western Japan, in which thousands of people were poisoned by the accidental ingestion of rice bran oil contaminated with polychlorinated biphenyls (PCBs) and various dioxins and dioxin-like compounds [1]. Since the incident, the average concentration of PCBs in the blood of Yusho patients has shown a decreasing trend. However, even more than 40 years later, the concentrations of total PCBs and polychlorinated dibenzofurans (PCDFs) in the blood of Yusho patients are still higher than in that of controls [2, 3]. Furthermore, the composition of PCB congeners in the blood of Yusho patients is different from that of controls, and is characterized by lower concentrations of 2,3',4,4'5-pentaCB (CB118) and higher concentrations of 2,3,3',4,4'5-hexaCB (CB156) [4]. This characteristic difference has been adopted as one of the criteria for the diagnosis of Yusho disease. In addition, the concentration ratios of tetra- and penta-chlorinated congeners to 2,2',4,4',5,5'-hexaCB (CB153) are significantly lower in Yusho patients than in controls, whereas the concentration ratios of hepta- and octa-chlorinated congeners to CB153 are significantly higher in Yusho patients [5]. Thus, we hypothesized that lower-chlorinated PCB congeners were metabolized more efficiently by cytochrome P450 (CYP) isozymes induced by PCDFs and dioxin-like PCBs in Yusho patients compared to controls.

Hydroxylated PCBs (OH-PCBs) are well known as PCB metabolites formed by the CYP monooxygenase enzyme system [6]. Non-dioxin-like PCBs induce transactivation of CYP2 family isozymes mediated by the constitutive androstane receptor, and induced CYP2B metabolizes PCBs to hydroxylated PCBs. CYP1 isozymes induced by dioxin-like PCBs via the aryl hydrocarbon receptor (AHR), also metabolize PCBs. Recent studies have succeeded in assessing CYP-dependent metabolic potencies of PCB congeners by *in silico* docking simulation [7, 8].

In this study, we investigated the characteristics of the accumulation patterns of 69 PCB congeners in the blood of Yusho patients by comparing them to those in the blood of controls. In addition, to examine the CYP-dependent metabolic potential of PCB congeners, we conducted *in silico* docking simulations of 69 PCB congeners with seven CYP isozymes.

## Materials and methods

Blood samples were collected during medical check-ups in 2004 (controls, $n$ = 127) and 2005 (Yusho patients, $n$ = 237), and informed consent was obtained from all participants. The concentrations of PCB congeners in the blood of Yusho patients and controls were quantified by a combination of high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS) [9].

All *in silico* analyses were carried out using the Molecular Operating Environment (MOE) program (Chemical Computing Group, Montreal, Canada). To construct the 3D structure of heme-containing CYP proteins, the following templates of CYP isozymes were taken from the Protein Data Bank (http://www.rcsb.org): human CYP1A1 (PDB code: 4I8V), human CYP1A2 (PDB code: 2HI4), human CYP1B1 (PDB code: 3PM0), human CYP2A6 (PDB code: 1Z10), human CYP2B6 (PDB code: 3QOA), human CYP2C9 (PDB code: 1OG5), and human CYP3A4 (PDB code: 1TQN). Since the structural model of human CYP2B6 (PDB code: 3QOA) (Y226H/K262R) varies, we constructed an original structural model (H226Y/R262K) using

MOE for the docking simulations. All crystallographic water molecules were deleted from the CYP structures. The 3D structures of human CYPs were optimized by a PFROSST force field after hydrogen atoms were added. Molecular docking simulations were performed to simulate the binding of 69 PCB congeners to human CYP proteins using ASEDock (Ryoka Systems, Tokyo, Japan) following the method of Goto et al. [10]. Prior to the ASEDock analysis, PCB structures were constructed and their energies were minimized using Rebuild3D with the MMFF94x force field in the MOE. A total of 500 conformations for each PCB congener were generated by the LowMode MD method. The parameters used for the refinement step were as follows: cutoff value 4.5, RMS gradient 10 and energy threshold 500. The energy of the PCB–CYP complex was refined using PFROSST of MOE under limited conditions in which the backbones of amino acid residues were tethered and the side chains of amino acid residues were unconfined.

**Results and discussion**

The accumulation patterns of 69 PCB congeners in the blood of Yusho patients and controls were investigated. For comparison, we calculated the concentration ratio of each congener relative to CB153, whose concentration level is highest in both Yusho patients and controls. Fig. 1 compares the log2-transformed ratios of Yusho patients to controls, and shows that the Yusho patients had lower proportions of lower-chlorinated congeners than the controls.

We examined the CYP-dependent metabolic potentials of PCB congeners. Using the 3D structures of human CYP isozymes, PCB congeners were simulated *in silico* by the ASEDock program. For each PCB–CYP pair, we measured the distance between the Cl-unsubstituted carbon atom in the biphenyl ring of PCBs, and the heme iron in CYPs. If the target (oxidation) site of the PCB congener is located within 5 Å of the heme iron, the substrate is supposed to be efficiently metabolized by CYP [11, 12]. The docking models between 69 PCB congeners and 7 CYP isozymes showed that human CYP1A1, 2A6, and 2B6 isozymes each had a substrate binding pocket in which the target site in most of the PCB congeners could be positioned within 5 Å of the heme iron (Table 1). These results suggest that these CYP isozymes are extensive catalysts for PCBs.

Furthermore, the docking positions for CB118, which showed a lower concentration in Yusho patients, were compared among the human CYP proteins. The docking positions of CB118 in human CYP isozymes are shown in Fig. 2. On the basis of the distance *in silico* estimated for each human CYP, CYP1A1, 2A6, and 2B6

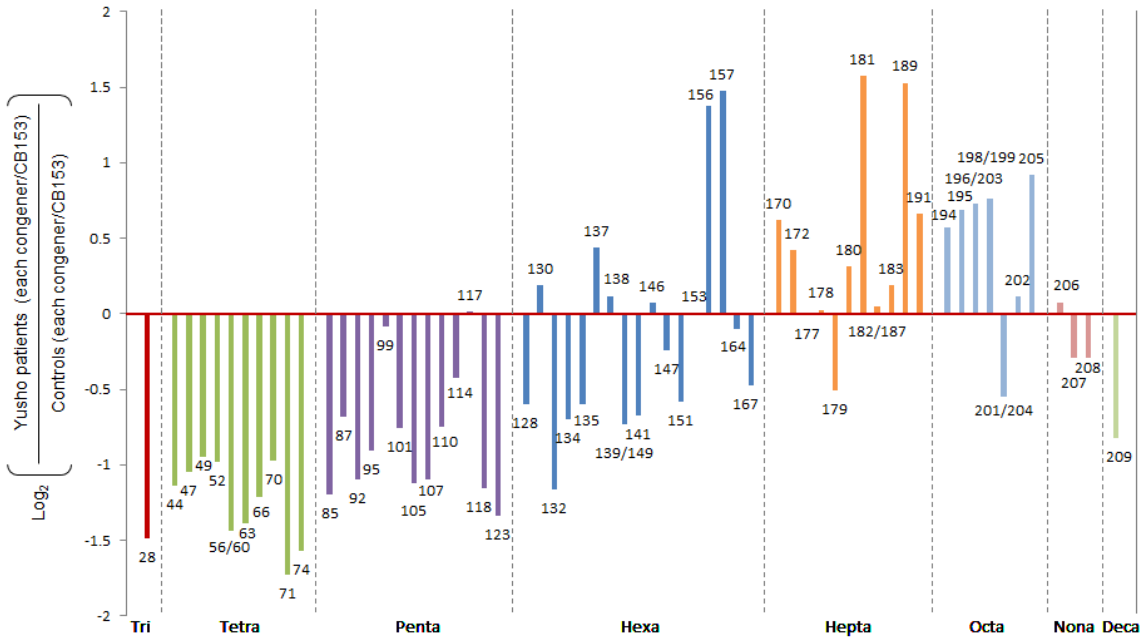


Fig. 1 Comparison of the concentration ratios of PCB congeners relative to CB153 in Yusho patients and controls. The ratios of Yusho patients and controls were log2-transformed. The bars indicate that each congener showed an IUPAC number.

Table 1 The number of PCB congeners that have the Cl-unsubstituted carbon atom positioned within 5 or 6Å of the heme iron in CYP isozymes.

| Distance | CYP1A1 | CYP1A2 | CYP1B1 | CYP2A6 | CYP2B6 | CYP2C9 | CYP3A4 |
|---|---|---|---|---|---|---|---|
| <5Å | 46 | 23 | 37 | 59 | 60 | 5 | 1 |
| <6Å | 60 | 64 | 63 | 65 | 65 | 13 | 3 |

In this study, 69 PCB congeners detected in the blood of Yusho patients were analyzed.

may be efficient catalysts for CB118. Thus, we checked the position of the target site of the PCB structure. The target site by CYP2A6 and 2B6 was the 3-position of the PCB biphenyl ring (Figs. 2D and 2E). The hydroxylation of the meta-position leads to the production of 3-OH-CB118 and 4-OH-CB107 [6]. 4-OH-CB107 is one of the frequently detected OH-PCB congeners in human blood samples, and has also been found in the blood of Yusho patients [13]. Mise et al. reported that human CYP2B6 and rat CYP2B1 metabolized CB118 to 3-OH-CB118, and the docking models revealed short distances between the 3-position of CB118 and these CYP2B isozymes [7]. On the other hand, the target site of CB118 by CYP1A1 was the 2'-position of the PCB biphenyl ring (Fig. 2A). An *in vitro* study reported that rat CYP1A1 metabolized CB118 to 4-OH-CB107 whereas human CYP1A1 was not involved in the metabolism of CB118 to 4-OH-CB107 [7]. However, a review of PCB metabolism reported that 2',4-diOH-CB107 was identified in human plasma [6]. This suggests that there may be a hydroxylation pathway at the 2'-position of a PCB structure by human CYP1A1 isozyme.

The present study suggests that an *in silico* docking simulation is useful for the prediction of CYP-dependent metabolism of PCB congeners, since the results of the docking simulation were consistent with the data of *in vitro* metabolism assays and blood profiles of hydroxylated PCB congeners. We have shown that human CYP1A1, 2A6, and 2B6 have the potential to metabolize PCB congeners. In addition, CYP1A1 and 2A6 are induced by the AHR signaling pathway. Because the concentration of PCDFs is still higher in the blood of the Yusho patients than in that of the controls, these CYP isozymes induced by PCDFs may play critical roles in the specific accumulation profiles of PCB congeners observed in the blood of Yusho patients.

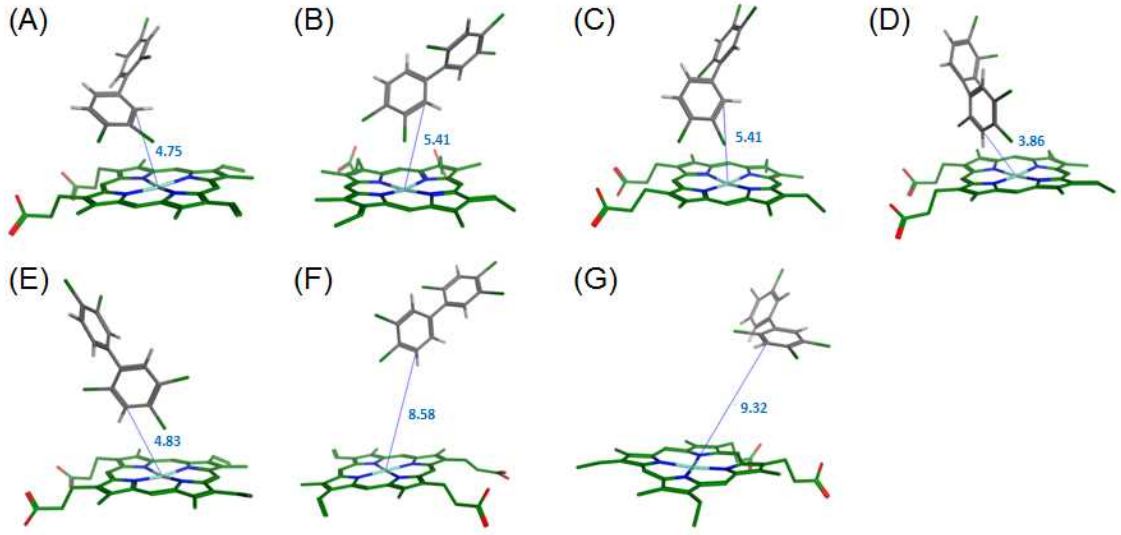


Fig. 2 Comparison of docking positions of CB118 in human CYP isozymes. (A) CYP1A1, (B) CYP1A2, (C) CYP1B1, (D) CYP2A6, (E) CYP2B6, (F) CYP2C9, (G) CYP3A4. The shortest distance (Å) between the Cl-unsubstituted carbon atom of CB118 and the heme Fe is shown by the blue line.

**Acknowledgements**

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare, Japan, and by Joint Usage/Research Center–Leading Academia in Marine and Environment Pollution Research (LaMer), Ehime University from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT).

**References**

1. Kuratsune M, Yoshimura H, Hori Y, Okumura Y and Masuda Y (1996) *Kyushu University Press, Fukuoka*, 1-351
2. Kajiwara J, Todaka T, Hirakawa H, Hori T, Hirata T, Iida T, Uchi H and Furue M (2015) *Fukuoka Igaku Zasshi. Fukuoka Acta Medica*, **106**, 149-153
3. Miyawaki T, Hirakawa S, Todaka T, Hirakawa H, Hori T, Kajiwara J, Hirata T, Uchi H and Furue M (2015) *Fukuoka Igaku Zasshi. Fukuoka Acta Medica*, **106**, 160-168
4. Masuda Y, Kagawa R and Shimamura K (1974) *Fukuoka Igaku Zasshi. Fukuoka Acta Medica*, **65**, 25-27
5. Hirakawa S, Miyawaki T, Hori T, Kajiwara J, Katsuki S, Hirano M, Iwata H and Furue M (2016) *The 9th International Symposium abstract book*, 65
6. Grimm FA, Hu D, Kania-Korwel I, Lehmler HJ, Ludewig G, Hornbuckle KC, Duffel MW, Bergman A and Robertson LW (2015) *Critical Reviews in Toxicology*, **45**, 245-272
7. Mise S, Haga Y, Itoh T, Kato A, Fukuda I, Goto E, Yamamoto K, Yabu M, Matsumura C, Nakano T, Sakaki T and Inui H (2016) *Toxicological Sciences*, **152**, 340-348
8. Yoo J, Hirano M, Mizukawa H, Nomiyama K, Agusa T, Kim EY, Tanabe S and Iwata H (2015) *Environmental Science & Technology*, **49**, 14588-14596
9. Todaka T, Hori T, Yasutake D, Yoshitomi H, Hirakawa H, Onozuka D, Kajiwara J, Iida T, Yoshimura T and Furue M (2009) *Fukuoka Igaku Zasshi. Fukuoka Acta Medica*, **100**, 156-165
10. Goto J, Kataoka R, Muta H and Hirayama N (2008) *Journal of Chemical Information and Modeling*, **48**, 583-590
11. Inui H, Itoh T, Yamamoto K, Ikushiro S and Sakaki T (2014) *International Journal of Molecular Sciences*, **15**, 14044-14057
12. Sykes MJ, McKinnon RA and Miners JO (2008) *Journal of Medicinal Chemistry*, **51**, 780-791
13. Tobiishi K, Suzuki S, Todaka T, Hirakawa H, Hori T, Kajiwara J, Hirata T, Iida T, Uchi H and Furue M (2013) *Fukuoka Igaku Zasshi. Fukuoka Acta Medica*, **104**, 136-142