# METABOLISM OF 2,3',4,4',5-PENTACHLOROBIPHENYL(CB118) BY GUINEA PIG LIVER MICROSOMES

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## Introduction

2,3',4,4',5-Pentachlorobiphenyl(PenCB)(CB118) is one of the major components of PCB preparation, Kanechlor 400, which caused a mass food poisoning called Yusho and are detected in blood and adipose tissues of mammals and human mother's milk at high concentration similarly to di-ortho-PCBs such as 2,2',3',4,4',5-hexachlorobiphenyl(HCB)(CB138) and 2,2',4,4',5,5'-HCB(CB153). Kuroki and Masuda reported that the concentration of CB118 in blood serum and adipose tissues of Yusho patients was much lower than in healthy persons[1)]. Recently, Haraguchi *et al.* found four metabolites in rat feces at a ratio of 12:4:1:1 when CB118 was administered orally to rats, and identified as 4-hydroxy(OH)-2,3',4',5-tetrachlorobiphenyl (TCB), 4-OH-2,3,3',4',5-PenCB, 4'-OH-2,3',4,5,5'-PenCB and 5'-OH-CB118, respectively[2)].

The guinea pig is known to be very sensitive toxicologically to the coplanar compounds such as PCDDs, PCDFs and coplanar PCBs. Our recent study have demonstrated that the guinea pig have high activity to hydrolyze some persistent PCBs such as CB138 and CB153. For example, Ariyoshi *et al.* found three major metabolites, 3-OH-CB153, 2-OH-2',3,4,4',5,5'-HCB and 2-OH-2',4,4',5,5'-PenCB in the feces of guinea pigs administered with CB153[3)] and showed that a guinea pig cytochrome P450(P450) isoform, CYP2B18, can hydrolyze CB153 to form three metabolites mentioned above in a reconstituted system containing purified CYP2B18, NADPH-P450 reductase and NADPH[4)]. More recently, we have reported that CB138 can be metabolized to four metabolites by guinea pig liver microsomes and identified two major metabolites as 2-OH-2',3,3',4,4',5-HCB and 3-OH-CB138[5)].

In this study, we examined the metabolic profile of a mono-ortho-PCB, CB118, in guinea pigs and the involvement of CYP2B18 in CB118 metabolism.

## Materials and Methods

CB118 was synthesized from 2,4,5-trichloroaniline and 3,4-dichlorobenzene as starting materials according to the method of Cadogan[6)]. 3-Methoxy(MeO)-CB118 and 4-MeO-2,3,3',4',5-PenCB were obtained by the method of Cadogan[6)] from 2,3,6-trichloroanisole and 3,4-dichloroaniline. Similarly, 2-OH-3,3',4,4',5-PenCB was synthesized from 3,4-dichloroaniline and 2,3,4-trichlorophenol by the method reported previously[5)]. Nine male Hartley guinea pigs (body wt. about 280 g) were used and divided to three groups, untreated, phenobarbital(PB)- and 3-methylcholanthrene (MC)-pretreated groups. PB and MC were dissolved in saline and corn oil, and injected intraperitoneally at a dose of 80 and 20 mg/kg/day for 3 days, respectively. Guinea pigs were killed the next day after the last injection of each P450 inducer and their livers were removed. Liver microsomes were prepared by a conventional centrifugation method. CB118 was incubated for 1 hr at 37 ºC with NADPH-generating system, $MgCl_2$ and guinea pig liver microsomes in 100 mM HEPES buffer (pH 7.4) under aerobic conditions. After extraction with organic solvents such as chloroform-methanol (2:1) and *n*-hexane and

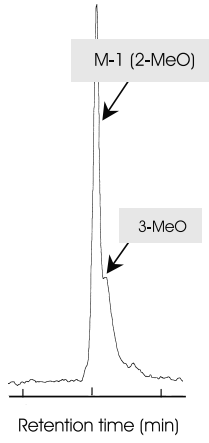


**Figure 1.** Gas chromatogram of CB118 metabolites formed by liver microsomes of PB-treated guinea pigs.

methylation by diazomethane, CB118 and its metabolites were analyzed using a gas chromatograph HP5890 Series II equipped with an electron capture detector (ECD) under the conditions as follows: column, DB-5 capillary column (60 m x 0.25 mm i.d., 0.33 mm thickness); carrier gas, $N_2$ (1 ml/min); column temp., 230 °C; injection port temp., 250 °C; detector temp., 250 °C. Rabbit antiserum against CYP2B18 was prepared as described elsewhere[7].

**Results**

*Effect of P450 inducers*

When CB118 was incubated with guinea pig liver microsomes and NADPH for 60 min at 37 °C, one metabolite (called M-1) was formed by untreated and PB-treated microsomes (data not shown). In addition, the formation of M-1 in PB-treated microsomes was accelerated 5 times faster than that in untreated microsomes. These results suggested that a guinea pig P450, CYP2B18, which is constitutive and PB-inducible is involved in CB118 metabolism.

*Identification of M-1*

To determine the chemical structure of M-1, a large scale incubation was carried out and M-1 was extracted with organic solvent and purified by silica gel column chromatography. The gas chromatogram of the M-1 fraction was shown in Fig. 1. Compared with three authentic compounds, 2-MeO-3,3',4,4',5-PenCB, 3-MeO-CB118 and 4-MeO-2,3,3',4',5-PenCB, the retention time of the methylated M-1 agreed with that of 2-MeO-3,3',4,4',5-PenCB. These three authentic compounds were detected as one peak on DB-1 capillary column (30 m long). However, when DB-5 capillary column (60 m long) was used, they could be separated to three peaks as shown in Table 1. Moreover, small amount of 3-MeO-CB118 was also found very closely to the methylated M-1. From the mass spectral data that the methylated M-1 possesses the molecular ion of 354 and the high intensity of a fragment ion [$M^+$-50], M-1 was assumed to be 2-OH-3,3',4,4',5-PenCB (Table 1).

*Inhibition study*

To clarify the contribution of CYP2B18 on CB118 metabolism in guinea pig liver, antiserum against CYP2B18 was added to the incubation mixture including liver microsomes of PB-treated

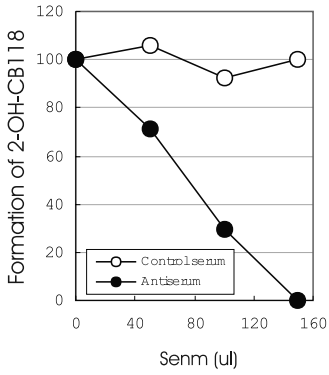


**Figure 2.** Effect of antiserum against CYP2B18 on 2-hydroxylation of CB118 with liver microsomes of PB-treated guinea pigs.

guinea pigs (Fig. 2). Addition of antiserum (150 ml) resulted in almost complete inhibition of the formation of 2-OH-3,3',4,4',5'-PenCB in PB-treated guinea pigs. These results confirmed that CYP2B18 plays a major role in the 2-hydroxylation of CB118 in the liver of PB-treated guinea pigs.

**Table 1.** Mass spectral data and retention times of methylated derivatives of a metabolite M-1 and three synthetic compounds in GC/MS and GC/ECD

| Compound | Molecular weight | Mass spectral data [$M^+$] | [$M^+$-15] | [$M^+$-43] | [$M^+$-50] | Retention time (min) |
|---|---|---|---|---|---|---|
| M-1 | 354 | 100 | - | - | 103 | 28.023 |
| 2-MeO-3,3',4,4',5-PenCB | 354 | 100 | - | - | 154 | 28.024 |
| 3-MeO-2,3',4,4',5-PenCB | 354 | 100 | - | 35 | - | 28.220 |
| 4-MeO-2,3,3',4',5-PenCB | 354 | 100 | 45 | 39 | - | 28.273 |

**Discussion**

Recently, we have reported that two di-ortho-PCBs, CB138 and CB153, can be easily metabolized to three or four metabolites in guinea pigs and a P450 isoform, CYP2B18, is most important in the metabolism of CB138[5] and CB153[4]. In this study, we examined the in vitro metabolism of a mono-ortho-PCB, CB118, by guinea pig liver microsomes. As a result, we found one major metabolite, which was identified as 2-OH-3,3',4,4',5-PenCB by comparison of the retention time in GC and mass spectrum with three synthetic compounds, 2-MeO-3,3',4,4',5-PenCB, 3-MeO-CB118 and 4-MeO-2,3,3',4',5-PenCB.

The postulated metabolic pathways of CB118 in guinea pig liver are shown in Fig. 3. Previously, Haraguchi *et al.* detected two major metabolites, 4-OH-2,3',4',5-TCB and 4-OH-2,3,3',4',5-PenCB, in the 4 days-feces of rats administered with CB118[2]. These results indicated that in rats CB118 metabolism proceeds via the 3,4-arene oxide formation and subsequently an NIH-shift and a dechlorination. With respect to the induction mode of the drug metabolizing enzymes in rat liver, PCBs are categolized into three groups, a PB-type, a MC-type and a mixed-type[8]. Although CB118 shows a mixed-type inducing ability, CB118 metabolism in rats seems to be catalyzed by MC-inducible P450 isoforms, CYP1A1 and CYP1A2[9].

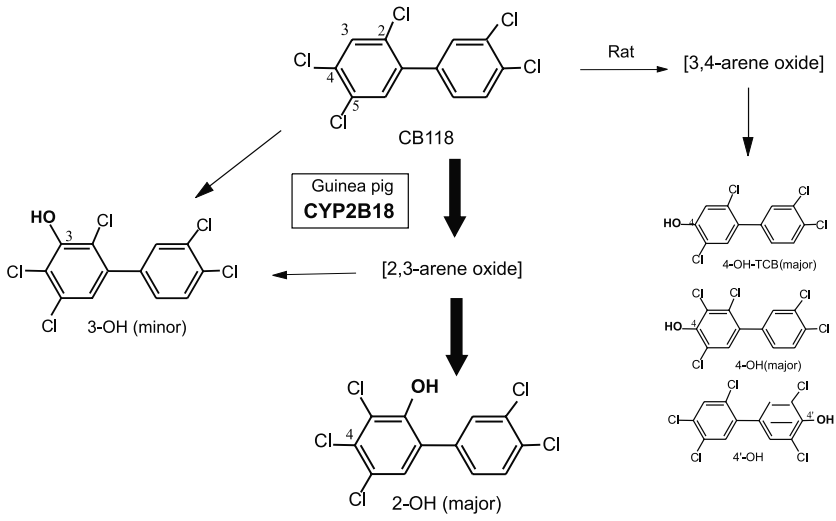


**Figure 3.** Postulated metabolic pathways of CB118 in guinea pigs

On the other hands, guinea pigs showed the different metabolic profile from rats. The formation of 2-OH-3,3',4,4',5-PenCB which is a characteristic metabolite in guinea pigs indicates that CB118 metabolism proceeds via the formation of a 2,3-arene oxide and an NIH-shift of a chlorine at 2-position to 3-position. In addition, immunological study using antiserum against CYP2B18 demonstrated that CYP2B18 is primarily involved in CB118 metabolism. In conclusion, CYP2B18 is the most important isoform catalyzing the formation of a 2,3-arene oxide in the 2,4,5-trichlorinated ring of mono- and di-ortho-PCBs to yield 2-OH-metabolite.

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