Chapter 6

Biochemical Studies of Yusho

6.1. Metabolism of PCBs and Related Compounds, and Their Toxicity

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6.1.1. Elimination of PCBs

In general, PCBs demonstrate extremely high lipophilicity and biological stability, and as a result these substances are not easily eliminated from the body once they are ingested. However, the extent of their biotransformation varies greatly in the congeners and isomers and from species to species. Yoshimura and Oshima (1971), in their earlier study, orally administered Kanechlor-400, a PCB preparation with about a 48% chlorine content, to mice and thereafter examined the tissue distribution and elimination of each congener contained in it. Among the various congeners, TCBs were almost completely eliminated from the body within 3 or 4 weeks, but a small amount of PenCBs and HCBs still remained ten weeks after administration. Similar results were observed in the rats administered Aroclor 1254 (Grant et al., 1971). Hutzinger et al. (1972) reported that 4-chlorobiphenyl, 4,4′-DCB and 2,5,2′,5′-TCB were converted primarily to monohydroxylated metabolite in rats and pigeons, but not in brook trouts. In addition, no metabolite of 2,4,5,2′,4′,5′-HCB was detected in the excreta of rats, pigeons or brook trout. Matthews and Anderson (1975) also reported that in rats, the elimination rate and the amount of metabolites of 1, 2, 5 or 6 chlorines-substituted PCBs decreased as the number of chlorine substituents increased. These results suggested that the metabolism of PCBs depends upon the number of chlorine on the biphenyl ring and the lower chlorinated biphenyls can be metabolized more rapidly than the 5 or more chlorine-substituted PCBs.

The position of chlorine on the biphenyl ring is also one of the important factors affecting the biotransformation. Yoshimura and his colleague compared the amount of metabolites and parent compounds in the feces of rats administered 3,4,3′,4′-, 2,4,3′,4′- or 2,5,2′,5′-TCB at an oral dose of 25 mg/body (Yoshimura and Yamamoto, 1973; Yamamoto and Yoshimura, 1973; Yoshimura et al., 1975). The order of the amount of metabolites in the feces collected over a period of 8 days was 2,5,2′,5′-TCB >> 2,4,3′,4′-TCB > 3,4,3′,4′-TCB. Similarly, Sipes et al. (1979) examined the effect of chlorine position on the excretion of four symmetrical HCBs, namely 2,3,6,2′,3′,6′-, 2,4,6,2′,4′,6′-, 2,4,5,2′,4′,5′- and 2,3,5,2′,3′,5′-HCB, by dogs after intravenous administration. The order of elimination rate was 2,3,6,2′,3′,6′-HCB > 2,4,6,2′,4′,6′-HCB > 2,4,5,2′,4′,5′-HCB > 2,3,5,2′,3′,5′-

HCB, which suggested that meta-chloro-substituted HCBs appear to resist biotransformation, although 2,3,6,2′,3′,6′-HCB, which has one meta-substituted chlorine while it also has adjacent proton-substituted carbons at the meta-para positions, was easily eliminated.

The elimination rate also depends on the animal species. For example, the dog is a unique animal in terms of having an unusual ability to metabolize 2,4,5,2′,4′,5′-HCB which is hardly metabolizable in rats, monkeys and humans (Sipes et al., 1982). Our recent study showed, using 3,4,3′,4′-, 3,5,3′,5′- and 2,5,2′,5′-TCB, that there are species differences in both the basal ability to hydroxylate TCB isomers and in the extent of effect of cytochrome P450 (P450) inducers on the metabolism of these isomers among rats, guinea pigs and hamsters (Koga et al., 1995b). Unlike rats and hamsters, guinea pigs had no hydroxylating activity with liver microsomes for 3,4,3′,4′- and 3,5,3′,5′-TCB. On the other hand, liver microsomes from untreated hamsters and guinea pigs demonstrated a relatively high activity for hydroxylation of 2,5,2′,5′-TCB, but not those from untreated rats.

6.1.2. Metabolism of PCBs in vivo

In 1959, Bloch and Cornish reported for the first time the metabolism of 4-chlorobiphenyl to 4′-hydroxy-4-chlorobiphenyl and its glucuronide in rats. Thereafter, essentially no studies were conducted on the metabolism of PCBs during the next 10 years until the Yusho incident occurred in 1968. Since then, numerous studies on the metabolism of PCBs have been accumulated in animals including rats, rabbits, mice, monkeys, goats, cow, fish and human. Fig. 6.1.1 summarizes the structures of PCB metabolites in the animals. A major metabolite of PCB is a monophenol although minor metabolites such as dihydroxy-PCBs, trans-dihydrodiols, dechlorinated monophenols and sulfur-containing metabolites (i.e. methylsulfide, methylsulfoxide and methylsulfone) have also been found.

In Japan, a few years after the outbreak of Yusho in 1968, Yoshimura and his coworkers started metabolic studies of several TCB isomers including 2,4,3′,4′-, 3,4,3′,4′- and 2,5,2′,5′-TCB, and then later extended their studies to 2,3,4,3′,4′- and 3,4,5,3′,4′-PenCB, and 2,4,5,2′,4′,5′- and 2,4,6,2′,4′,6′-HCB. Fig. 6.1.2 shows the metabolites identified in his laboratory.

The study on 2,4,3′,4′-TCB metabolism demonstrated that 5- and 3-hydroxy-2,4,3′,4′-TCB were excreted to rat feces (Yamamoto and Yoshimura 1973). This was the first report showing the complete structure of the metabolite of PCB congeners substituted with more than 3 chlorines. Recently, Koga et al. (1992) found a small amount of 4-hydroxy-2,5,3′,4′-TCB, an NIH-shifted metabolite, in the feces of rats administered 2,4,3′,4′-TCB.

In the study of 3,4,3′,4′-TCB metabolism, three phenolic metabolites were de-

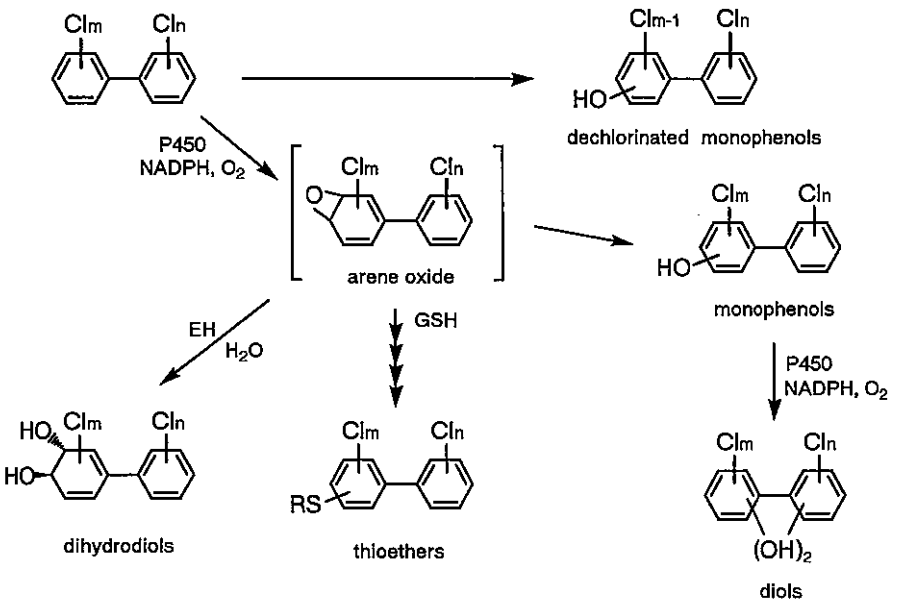

Fig. 6.1.1. Metabolic Pathways of PCBs
P450: cytochrome P450, EH: epoxide hydrolase, GSH: glutathione.

tected in the feces of rats orally administered at a dose of 25 mg/body, although about 64% of the dose was excreted unchanged in the feces during 14 days after administration. A major metabolite was assumed to be a 2- or 5-hydroxy-3,4,3′,4′-TCB. No conjugated metabolites were detected in the feces or urine (Yoshimura and Yamamoto, 1973, 1974). More than ten years later, two major metabolites in rat feces were identified as 5-hydroxy-3,4,3′,4′-TCB and an NIH-shifted metabolite, 4-hydroxy-3,5,3′,4′-TCB (Yoshimura et al., 1987). Furthermore, four minor metabolites, 4-hydroxy-3,3′,4′-triCB, 2,5-dihydroxy-3,4,3′,4′-TCB, 4,4′-dihydroxy-3,5,3′,5′-TCB and 5,6-dihydroxy-3,4,3′,4′-TCB were also identified using rats (Koga et al., 1989). Klasson-Wehler et al. (1989) newly detected 6-hydroxy-3,4,3′,4′-TCB besides two major hydroxylated metabolites in the urine, adipose tissue and fetus of mice. These findings indicate that the metabolite may be specific for mice, because it could not be detected in rats.

In 2,5,2′,5′-TCB metabolism, 3-hydroxy-2,5,2′,5′-TCB was a major metabolite in the feces, when the rats were administered orally or intraperitoneally with 2,5,2′,5′-TCB (Yoshimura et al., 1975; Hanioka et al., 1991). A trace amount of 3- and 4-hydroxy-2,5,2′,5′-TCB was also detected in the rat urine, while unchanged 2,5,2′,5′-TCB was not excreted in the bile of rats when administered intraperitoneally (Yoshimura et al., 1975). As mentioned above, Hutzinger et al. (1972) re-

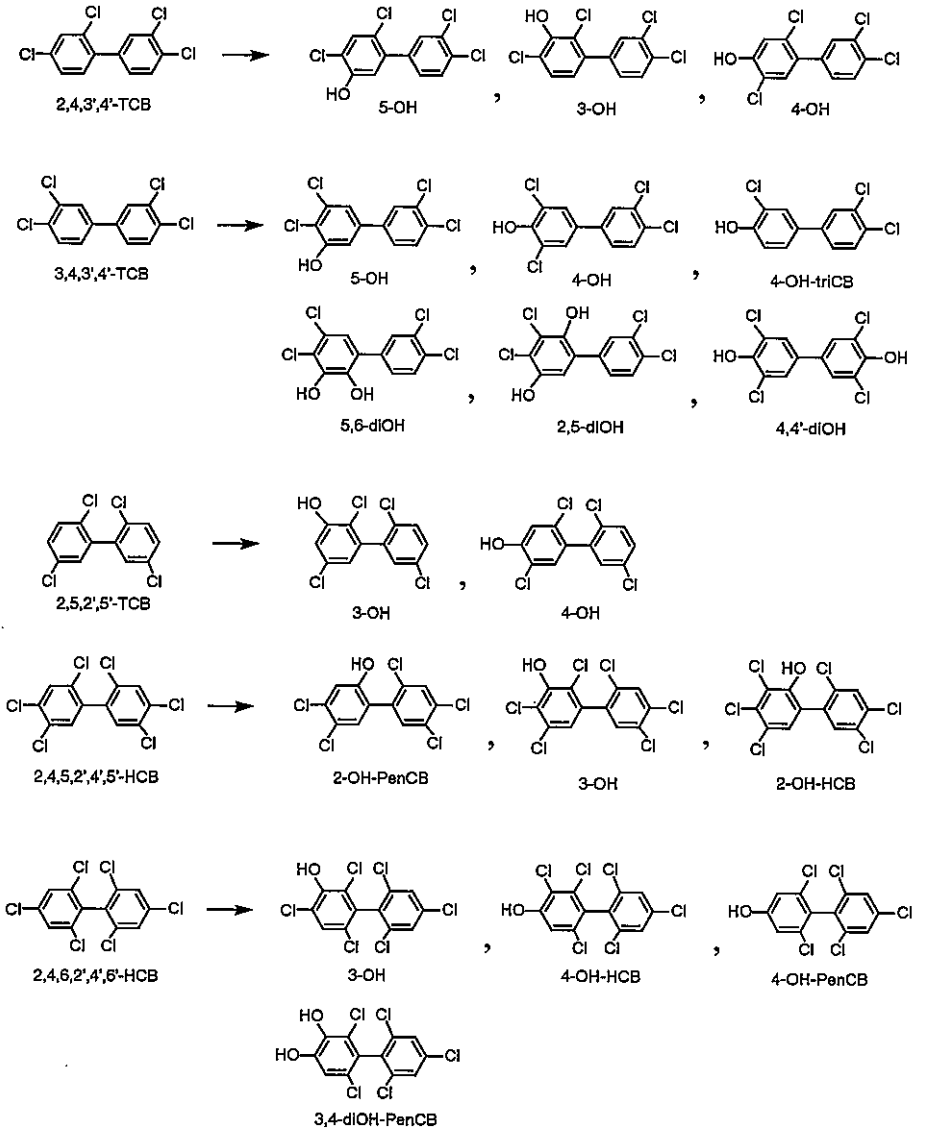

Fig. 6.1.2. PCB Metabolites Identified in Yoshimura's Laboratory

ported that only a monohydroxylated metabolite was observed in rat urine while a large amount of unchanged 2,5,2′,5′-TCB was excreted in rat feces. This study, however, was not conducted properly, because the hydroxy-metabolite could be isolated mostly from the feces, but only in a small amount from the urine of rats (Yoshimura et al., 1975). Thus, a number of studies have since been established in which the major route of phenolic products from PCB congeners is excretion into the feces via the bile. Gardner et al. (1973) nevertheless observed a small amount of trans-3,4-dihydro-3,4-dihydroxy-2,5,2′,5′-TCB as a minor metabolite in rabbit urine.

In addition to TCB isomers, Yamamoto et al. (1976) also investigated the metabolism of 2,3,4,3′,4′-PenCB in rats and reported that no metabolite of this isomer was detected in the feces, urine or tissue of rats during the 8-day-period after administration. 2,3,4,3′,4′-PenCB is a component of Kanechlor-400 and was later detected at a lower level in the serum of Yusho patients than in that of healthy persons (Kuroki and Masuda, 1977). It has recently been reported that 2,3,4,3′,4′-PenCB could be metabolized very slowly and a small amount of five hydroxylated metabolites, 5-hydroxy-2,3,4,3′,4′-PenCB, 5′-hydroxy-2,3,4,3′,4′-PenCB, 4-hydroxy-2,3,5,3′,4′-PenCB, 4′-hydroxy-2,3,4,3′,5′-PenCB and 2′-hydroxy-2,3,4,3′,4′,-PenCB was observed in mink and mice (Klasson-Wehler et al., 1993). The metabolism of 2,4,5,2′,5′-PenCB was reported by Chen et al. (1976). They administered the ^{14}C-labeled isomer intravenously at a dose of 0.6 mg/kg to male rats and observed that 84% of the total dose was excreted within 7 days and 89% of the radioactivity excreted in the feces and urine was in the form of metabolites. Those were 3′-hydroxy-2,4,5,2′,5′-PenCB as a major metabolite and 3′,4′-dihydrodiol-2,4,5,2′,5′-PenCB as a minor metabolite. These results indicated that PenCB with adjacent proton-substituted carbons at the meta-para position can be metabolized very easily. On the other hand, 3,4,5,3′,4′-PenCB, the most toxic isomer among all PCB congeners examined, had been thought to be unmetabolizable in animals, but Koga et al. (1990) found that a trace amount of 4′-hydroxy-3,4,5,3′,5′-PenCB was excreted into rat feces during the 5-day-period after treatment with 3,4,5,3′,4′-PenCB, but accounted for only 1.3% of the dose.

Several studies on HCB metabolism have been also reported in rats, rabbits, mice, monkeys and dogs (Goto et al., 1975; Sundstrom et al., 1976; Kato et al., 1980; Norback et al., 1981; Sipes et al., 1982; Ariyoshi et al., 1992, 1993). As shown in Table 6.1.1, the metabolites were monohydroxylated and monohydroxy-dechlorinated products. Such monohydroxy-dechlorinated metabolite is often observed in the *in vivo* and *in vitro* metabolism of PCBs such as 3,4,3′,4′-TCB (Yoshimura et al., 1987; Koga et al., 1989; Klasson-Wehler et al., 1989), 2,4,5,2′,4′,5′-HCB (Sundstrom et al., 1976; Kato et al., 1980; Ariyoshi et al., 1992)

Table 6.1.1. Metabolites of HCB Isomers in Animals

HCB isomer	Animal	Metabolite	Reference
2,3,5,2′,3′,5′-HCB	Rat	4-OH, 3-OH-2,4,5,2′,3′,5′-HCB 6-OH, 4-OH-2,5,2′,3′,5′-PenCB	Kato et al. (1980)
2,3,6,2′,3′,6′-HCB	Rat	4-OH	Kato et al. (1980)
2,4,5,2′,4′,5′-HCB	Rabbit	3-OH, 4-OH-2,3,5,2′,4′,5′-HCB 3-OH-2,5,2′,4′,5′-PenCB 4-OH-2,5,2′,4′,5′-PenCB	Sundstrom et al. (1976)
	Rat	3-OH	"
	Mouse	3-OH	"
	Dog	metabolite(3-OH?)	Sipes et al. (1982)
	Monkey	3-OH	Norback et al. (1981)
	Dog	3-OH, 2-OH-4,5,2′,4′,5′-PenCB 2-OH-3,4,5,2′,4′,5′-HCB	Ariyoshi et al. (1992)
2,4,6,2′,4′,6′-HCB	Rat	3-OH	Goto et al. (1975)
	Rat	3-OH	Kato et al. (1980)
	Dog	3-OH, 4-OH-2,6,2′,4′,6′-PenCB 3,4-diOH-2,6,2′,4′,6′-PenCB 4-OH-2,3,6,2′,4′,6′-HCB	Ariyoshi et al. (1993)

HCB: hexachlorobiphenyl, PenCB: pentachlorobiphenyl.

and 2,4,6,2′,4′,6′-HCB (Ariyoshi et al., 1993). However, the formation mechanism remains obscure at present.

Novel metabolites were discovered in the feces and liver of 2,5,2′,5′-TCB-treated mice by Mio et al. (1976). These metabolites were identified as sulfur-containing metabolites such as 3- and 4-methylthio-2,5,2′,5′-TCB and also 3- and 4-methylsulfonyl-2,5,2′,5′-TCB. Similar metabolites have also been detected in the blubber of seals from the Baltic Sea (Jensen and Jansson, 1976) and in the lung, liver and adipose tissues of a deceased Yusho patient and a person who died from other than Yusho (Haraguchi et al., 1984, 1986). The formation mechanism of these unique metabolites will be described in a later section.

6.1.3. Metabolism of PCBs in vitro

The *in vitro* studies on PCB metabolism using liver microsomes from animals gave us information that PCB congeners can be metabolized by P450-dependent monooxygenase systems in liver microsomes while the P450 isoform involved in the metabolism varies with the chemical structure of individual PCB congeners. Although a number of P450 isoforms have been purified and characterized in various species, little is known about the P450 isoforms responsible for PCB metabolism. Table 6.1.2 lists the P450 isoforms responsible for hydroxylation of PCB in a reconstituted system containing purified P450, NADPH-P450 reductase, NADPH-generating system and phospholipids.

Table 6.1.2. Cytochrome P450 Isoforms Responsible for the Hydroxylation of PCB

Isozyme	Animal	PCB congener	Reference
1A1	Rat	3,3′-DCB	Kaminsky et al. (1981)
		3,4-DCB	
		3,5-DCB	
		4,4′-DCB	
		3,4,3′,4′-TCB	Ishida et al. (1991)
		3,5,3′,5′-TCB	Koga et al. (1994)
2B1	Rat	2,6-DCB	Kaminsky et al. (1981)
		2,2′-DCB	
		2,5,2′,5′-TCB	Ishida et al. (1991)
2B2	Rat	2,5,2′,5′-TCB	
2B11	Dog	2,4,5,2′,4′,5′-HCB	Duignan et al. (1987)
HPB-1	Hamster	2,5,2′,5′-TCB	Koga et al. (1995a)

DCB: dichlorobipheny, TCB: tetrachlorobiphenyl, HCB: hexachlorobiphenyl.

Kaminsky et al. (1981) showed, using ten individual DCB isomers, that CYP1A1 preferentially metabolizes 3,3′-, 3,5-, 3,4- and 4,4′-DCB with no ortho-chloro substitution whereas CYP2B1 metabolizes 2,2′- and 2,6-DCB which are di-ortho-chloro substituted biphenyls. They also found that single-ortho-chloro substituted biphenyls, 2,4′-, 2,3- and 2,5-DCB were hydroxylated by both P450 isoforms.

Ishida et al. (1991) and Koga et al. (1994) indicated that TCB isomers with no ortho-chloro substituent such as 3,4,3′,4′- and 3,5,3′,5′-TCB are substrates for CYP1A1, whereas 2,5,2′,5′-TCB is an excellent substrate for CYP2B1 and CYP2B2 in a similar manner to DCB isomers. Interestingly, dog P450 isoform, PBD-2 (CYP2B11), can metabolize 2,4,5,2′,4′,5′-HCB at a much greater rate than rat P450 isoforms (Duignan et al., 1987). This isomer seems to be hardly metabolized in humans because it is detected in the tissue of Yusho patients at a higher level (Kuroki and Masuda, 1977). Very recently, Koga et al. (1995a) isolated a new P450 isoform (HPB-1) from hamster liver microsomes, which would be categorized into the CYP2B subfamily by the PB-inducibility and the N-terminal amino acid sequence. This isoform in a reconstituted system catalyzed 3-hydroxylation of 2,5,2′,5′-TCB, but the activity is only one 40th that of CYP2B1. Thus, two groups of the P450 superfamily, namely the CYP1A and CYP2B subfamilies, are involved in the metabolism of PCBs in animals.

6.1.4. Mechanism of PCB Metabolism

Two mechanisms have principally been considered so far for the hydroxylation of aromatic hydrocarbons including PCBs; (1) an arene oxide formation followed

by rearrangement, and (2) an insertion of oxygen between carbon-hydrogen bond (direct hydroxylation). The formation of an arene oxide often results in an NIH-shift of either the hydrogen or chlorine atom. A number of NIH-shifted metabolites have been found in the metabolism of PCB congeners such as 4,4′-DCB, 3,4,3′,4′-TCB, 2,4,3′,4′-TCB, 2,3,4,3′,4′-PenCB, 2,3,5,2′,3′,5′-HCB and 2,4,5,2′,4′,5′-HCB (Safe et al., 1976; Yoshimura et al., 1987; Koga et al., 1992; Klasson-Wehler et al., 1993; Kato et al., 1980). The epoxidation generally occurs at the 3,4-double bond. However, the data suggesting the involvement of 2,3-epoxide in PCB metabolism has been reported (Ariyoshi et al., 1992), and they found 2-hydroxy-3,4,5,2′,4′,5′-HCB to be a metabolite of 2,4,5,2′,4′,5′-HCB in an *in vitro* study using liver microsomes of PB-treated dog.

In some cases, an arene oxide is enzymatically converted to trans-dihydrodiol by epoxide hydrolase, or binds covalently to thiol residue of glutathione (GSH) or macromolecule in the cell. Gardner et al. (1973) found a small amount of trans-3,4-dihydro-3,4-dihydroxy-2,5,2′,5′-TCB in addition to 3- and 4-hydroxy-2,5,2′,5′-TCB in rabbit urine. This was the first report suggesting the formation of 3,4-epoxide as an intermediate in PCB metabolism. The formation of an arene oxide of 2,5,2′,5′-TCB was confirmed by Forgue and Allen (1982). Shimada and his coworkers (1980, 1981, 1983) showed that the binding of 3,4,3′,4′-TCB to a variety of proteins having free sulfhydryl groups including alcohol dehydrogenase (horse liver), hemoglobin (human blood), albumin (bovine serum) and CYP1A1 itself was brought by incubation with CYP1A1, but the binding of 2,4,2′,4′-TCB or 2,4,2′,5′-TCB was increased by incubation with CYP2B1.

On the other hand, Preston et al. (1983) demonstrated a direct hydroxylation mechanism in 2,5,2′,5′-TCB metabolism. They observed that when synthetic 2,5,2′,5′-TCB-3,4-oxide was either incubated with liver microsomes of PB-treated rats or was administrated to rats, 3-hydroxy-2,5,2′,5′-TCB, a major metabolite of 2,5,2′,5′-TCB in the *in vivo* and *in vitro* studies, was formed at a much lesser extent than 4-hydroxy-2,5,2′,5′-TCB. These results thus indicated that 3-hydroxylation of 2,5,2′,5′-TCB proceeds directly, but not via 3,4-oxide.

The postulated mechanisms for the formation of the sulfur-containing metabolites are illustrated in Fig. 6.1.3. As an initial event, PCBs are oxidized to an arene oxide intermediate. Subsequently, the arene oxide is attacked by glutathione to form glutathione conjugate in the liver. This conjugate is then excreted into the gastrointestinal tract via the bile and metabolized to a cysteine conjugate by γ-glutamyl transpeptidase and dipeptidase. The C-S bond of the cysteine conjugate is cleaved to thiol by microbial C-S lyase in the intestine, and thiols are then methylated and oxidized to methylsulfoxide and further to methylsulfone metabolites in the liver. Bakke et al. (1982, 1983a, 1983b) proposed this mechanism through the

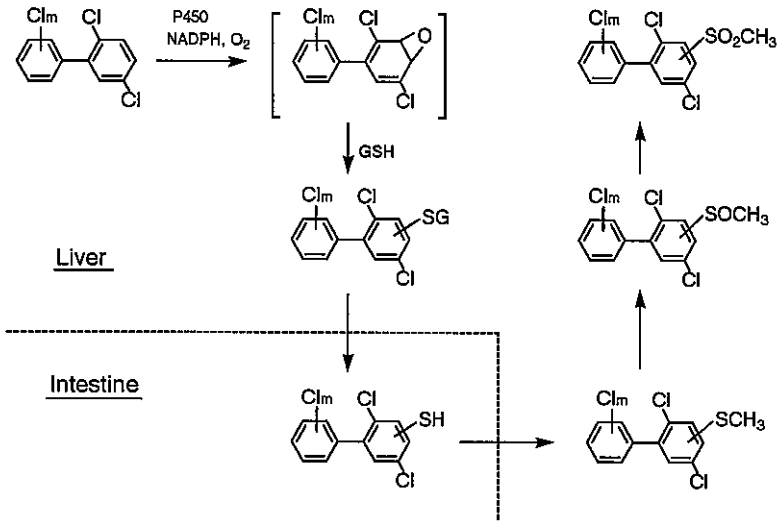

Fig. 6.1.3. Proposed Mechanism for the Formation of Methylsulfone-PCBs

studies on 2,5,4′-triCB metabolism. The evidence that 3-methylsulfone metabolites of 2,5-dichloro substituted PCBs are very often detected in animal tissue together with 4-methylsulfone metabolites thus indicates 3,4-epoxide formation to be an intermediate (Mizutani et al., 1978; Haraguchi et al., 1986).

6.1.5. Metabolism and Toxicity

PCBs exhibit a variety of toxic effects such as body weight loss, atrophy of the thymus and spleen, hyperkeratosis, edema and lethality. Although these toxic effects have been thought to be mediated by cytosolic Ah-receptor, some studies have reported that PCB congeners are metabolically activated to become more toxic metabolites than the parent compounds. The first example for this activation was reported by Yamamoto and Yoshimura (1973). By measuring the LD_{50} value in male CF-1 mice, they found that the 5-hydroxy-2,4,3′,4′-TCB, a major metabolite of 2,4,3′,4′-TCB, had a toxicity that was five times as potent as the parent compound. Six years later, Stadnicki and Allen (1979) showed, using cultured L-929 cells, that 4-hydroxy-2,5,2′,5′-TCB and 2,5,2′,5′-TCB-3,4-oxide inhibited the growth of the cells more strongly than 2,5,2′,5′-TCB. These results indicated the importance of the toxicological assessment of PCB metabolites.

Accordingly, Yoshimura et al. (1987) attempted to assess the acute toxicity of hydroxylated metabolites of PCB congeners on the basis of the effects on the growth and weight of the liver, thymus and spleen, and the activities of liver microsomal enzymes in rats. 4-Hydroxy-3,5,3′,4′- and 5-hydroxy-3,4,3′,4′-TCB which were major metabolites of toxic 3,4,3′,4′-TCB were administered to male Wistar

rats and compared the toxic effects with that of the parent compound. As a result, both metabolites showed either no or much less toxicity than the parent compound, and were evaluated as detoxicated products. Moreover, 4′-hydroxy-3,4,5,3′,5′-PenCB, a metabolite of 3,4,5,3′,4′-PenCB which is the most toxic congener of all PCBs heretofore examined, was also a detoxicated product (Koga et al., 1990). A similar study was performed using 3-hydroxy-2,5,2′,5′-TCB, a major metabolite of 2,5,2′,5′-TCB (Hanioka et al., 1991). 3-Hydroxy-2,5,2′,5′-TCB at a single dose of 0.82 mmol (253 mg/kg) showed no biological changes in the organ weights or liver enzyme activities, although treatment of 2,5,2′,5′-TCB at a similar dose resulted in significant changes including an increase in liver weight, a decrease in the total liver lipids and an increase in the activities of benzo(a)pyrene 3-hydroxylase and benzphetamine N-demethylase. These results indicated that 3-hydroxy-2,5,2′,5′-TCB was an inactive metabolite.

The involvement of PCB metabolites as mimics of thyroids and other steroidal hormones has also been considered. Brouwer and his coworkers (1986, 1990, 1991) showed that among hydroxylated metabolites, 4-hydroxy metabolites had a high affinity to transthyretin, a major plasma thyroid hormone binding protein. This transthyretin forms the plasma transport system of vitamin A together with retinol binding protein. Consequently, the binding of hydroxylated metabolite to transthyretin decreased the blood levels of thyroid hormone and vitamin A in animals. In addition, certain PCB metabolites have been found to be estrogenic (Korach et al., 1988; Jansen et al., 1993), and it was reported that 4-hydroxy-2′,4′,6′-triCB had a higher affinity to estrogen receptor than other hydroxylated compounds such as 4-hydroxy-2′,6′-DCB, 4-hydroxy-3,5,4′-triCB and 4,4′-dihydroxy-3,5,3′,5′-TCB.

Sulfur-containing metabolites have also shown some biological effects including the induction of liver enzymes (Haraguchi et al., 1985) and binding to certain proteins in rat and mouse lung cytosol (Lund et al., 1985) and to α_{2u}-globulin, a major urinary protein which is also present in rat kidney cytosol (Larsen, 1990). Recently, Kato et al. (1995a, 1995b) demonstrated that 3- but not 4-methylsulfonyl 2,5-dichloro substituted PCBs have more potent inducing ability of liver microsomal enzyme than the parent compound. Thus, the toxicological assessment of PCB metabolites has become increasingly important to elucidate the mechanism of PCB toxicity.

6.1.6. Metabolism of PCDFs

In a similar manner to PCBs, PCDFs are metabolized to monohydroxylated and dihydroxylated products as a major metabolite, and to monohydroxy-dechlorinated and sulfur-containing compounds as a minor metabolite (Veerkamp et al.,

1981; Poiger et al., 1984, 1989; Pluess et al., 1987; Kuroki et al., 1989, 1990; Burka et al., 1991). In addition, as a typical metabolite of PCDF, an ether-bond cleavaged product was detected in the bile of rats administered with TCDF (Poiger et al., 1984).

Kuroki and Masuda (1978) detected a high level of 2,3,6,8- and 2,3,7,8-TCDF, 1,2,4,7,8- and 2,3,4,7,8-PenCDF and 1,2,3,4,7,8- and 1,2,3,6,7,8-HCDF in the adipose tissue and liver of Yusho patients, and among them, 2,3,4,7,8-PenCDF, the most toxic PCDF congener, was present at the highest concentration. These findings suggested that the number and the position of chlorine affect the elimination rate of PCDFs and that PCDF congeners with adjacent proton-substituted carbons can be metabolized more easily. Brewster and Birnbaum (1987, 1988) demonstrated that 2,3,4,7,8-PenCDF is metabolized at a much lower rate than 1,2,3,7,8-PenCDF in rats, which indicated that 4- or 6-position of PCDF seems to be a better attacking site for P450 than the 1- or 9-position. These studies provide us with the following additional information on PCDF metabolism: 1) Chlorine-migrated products (e.g. NIH-shift) are often observed, 2) If a PCDF congener has at least 3 chlorines in each ring, its metabolism tends to decrease greatly.

6.1.7. Metabolism of PCDDs

Tulp and Hutzinger (1978) reported that several PCDD isomers and congeners such as 1- and 2-MCDD, 2,3- and 2,7-DCDD, 1,2,4-triCDD and 1,2,3,4-TCDD are converted to monohydroxylated and dihydroxylated metabolites in the rat. Sulfur-containing metabolites were also identified as a minor metabolite. As for 2,3,7,8-TCDD, an extremely toxic PCDD, several studies provided evidence that it is slowly metabolized in animals both *in vivo* and *in vitro* to give more polar metabolites, some of which seem to be glucuronides (Poiger and Schlatter, 1979; Olson et al., 1980; Ramsey et al., 1982; Sawahata et al., 1982; Poiger et al., 1982; Kleeman et al., 1986; Olson, 1986; Wroblewski and Olson, 1985, 1988). For example, Sawahata et al. (1982) succeeded in identifying two metabolites of 2,3,7,8-TCDD. They incubated ^{3}H-labeled 2,3,7,8-TCDD with isolated rat hepatocytes at 37°C for 8 hr, isolated two metabolites from the cell free supernatants using reverse phase HPLC and thus identified 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-triCDD. Moreover, Poiger et al. (1982) found 2-hydroxy-1,3,7,8-TCDD, an NIH-shifted metabolite, to be a major biliary metabolite in the dog. These studies therefore suggest that the metabolism of PCDDs proceeds in a similar manner to that of PCDFs.

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6.2. The Inductive Effects of PCBs and Related Compounds on Hepatic Enzymes and Toxicity in Experimental Animals

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6.2.1. Introduction

One of the most remarkable biochemical responses observed in the animals exposed to polychlorinated biphenyls (PCBs) is that of a potent and long-lasting inductive effect on the hepatic microsomal mixed function oxidase system (MFO) consisting of a hemoprotein cytochrome P-450 (P-450) and a flavoenzyme NADPH P-450 reductase (Fujita et al., 1971; Litterst et al., 1972). MFO catalyzes the metabolism of not only a variety of xenobiotics, including drugs and food additives as well as chemical carcinogens, but also physiologically important endogenous substrates such as steroids, fatty acids and lipid soluble vitamins. As a consequence of the metabolic conversion of these substances, their biological activities are often modified, resulting in inactivation or activation. Based on these facts, it is easy to suppose that a long-lasting induction of MFO might be harmful to humans and could thus potentially become an important factor in the etiology of Yusho. Therefore, one of the major studies on Yusho has been focused on an elucidation of the relationship between the inductive effects of PCBs and polychlorinated dibenzofurans (PCDFs) on several hepatic enzymes including MFO and DT-diaphorase and their toxicity in experimental animals.

6.2.2. The Inductive Effect of PCB Mixtures (Kanechlors) on MFO

The inductive effect of PCBs on the MFO was first reported by Risebrough et al. (1968) who demonstrated the enhanced metabolism of estradiol in a pigeon liver treated with Aroclor 1260, a commercial PCB mixture produced by the Monsanto Chemical Company (U.S.A.). Shortly after the outbreak of Yusho, the property of PCBs as an MFO inducer was reported by Komatsu and Tanaka (1971). They observed that the hexobarbital sleeping time in rats is definitely reduced by pretreatment with Kanechlor 400 (KC-400) and KC-500, Japanese commercial preparations of a PCB mixture with about a 48% and 54% chlorine content, respectively. On the other hand, Fujita et al. (1971) demonstrated that the microsomal metabolizing activities of *p*-nitrophenetole O-deethylation, *p*-chloro-N-methylaniline N-demethylation and aniline hydroxylation all markedly increased, but to a different extent, by the pretreatment of rats with KC-400, KC-500 and KC-600.

At present there are a number of MFO inducers that are known to exist, most of

Table 6.2.1. Inductive Effects of Kanechlor 400 and Individual PCB Congeners on Rat Hepatic MFO

Pretreatment	AM	AN	P-450	b_5	NADPH-cyt *c* reductase
Untreated	100 ± 2	100 ± 1	100 ± 3	100 ± 6	100 ± 1
MC	115 ± 13	174 ± 7[a]	157 ± 8[a]	118 ± 13	107 ± 9
PB	214 ± 11[a]	202 ± 9[a]	242 ± 10[a]	154 ± 9[a]	195 ± 7[a]
MC + PB	230 ± 2[a]	281 ± 8[a]	326 ± 14[a]	159 ± 2[a]	214 ± 0[a]
Untreated	100 ± 2	100 ± 3	100 ± 4	100 ± 6	100 ± 2
4,4′-DCB	210 ± 7[a]	161 ± 7[a]	176 ± 1[a]	152 ± 5[a]	143 ± 4[a]
2,5,2′,5′-TCB	153 ± 4[a]	142 ± 5[a]	160 ± 7[a]	146 ± 1[a]	138 ± 3[a]
2,4,3′,4′-TCB	197 ± 12[a]	198 ± 6[a]	203 ± 9[a]	164 ± 6[a]	179 ± 13[a]
3,4,3′,4′-TCB	99 ± 2	140 ± 4[a]	172 ± 1[a]	94 ± 2	95 ± 0
3,4,5,3′,4′-PenCB	84 ± 14	162 ± 1[a]	182 ± 1[a]	120 ± 10	94 ± 4
3,4,5,3′,4′,5′-HCB	107 ± 9	199 ± 9[a]	163 ± 9[a]	105 ± 3	108 ± 2
DecaCB	186 ± 4[a]	148 ± 2[a]	174 ± 5[a]	122 ± 1[a]	116 ± 2[a]
KC-400	215 ± 11[a]	297 ± 21[a]	277 ± 15[a]	187 ± 19[a]	196 ± 13[a]

PB (90 mg/kg) was injected *sc* once a day for 2 days and MC (40 mg/kg) was injected *ip* at a single dose 2 days before sacrifice.

DCB, 2,5,2′,5′-TCB, DecaCB and KC-400 (100 mg/kg), and 3,4,3′,4′-TCB (50 mg/kg) were administered *ip* once a day for 3 days, and the animals were sacrificed 5 days after the first dose. 2,4,3′,4′-TCB (100 mg/kg), 3,4,5,3′,4′-PenCB (0.5 mg/kg) and 3,4,5,3′,4′,5′-HCB (1 mg/kg) were injected *ip* at a single dose 5 days before the experiment. The value represents the percent of the activity of aminopyrine demethylation (AM) and aniline hydroxylation (AN) and the contents of cytochromes P-450 and b_5 in the untreated rats (mean ± SE from 3–7 animals). [a]: Significantly different from the untreated ($p < 0.05$). These data were summarized from Yoshimura et al. (1978).

which can be categorized as either phenobarbital (PB)-type or 3-methylcholanthrene (MC)-type based on several criteria (Conney, 1976). PB-type inducers enhance the metabolism of a variety of substrates including ethylmorphine and benzphetamine (BZ), while MC-type inducers stimulate the metabolism of more limited substrates including benzo[a]pyrene. These characteristics regarding the enhanced activities solely depend on the distinguished P-450 isozymes, tentatively termed as PB P-450 and MC P-448, which are selectively induced by each type of inducer as described in the following sections.

In 1973, Alvares et al. (1973) reported that the pretreatment of rats with Aroclor 1254 significantly enhances the activities of ethylmorphine N-demethylation and benzo[a]pyrene hydroxylation, both of which are typical metabolic reactions induced with PB and MC, respectively. In addition, similar to the microsomes of MC-treated rats, the microsomes of the Aroclor-treated rats exhibited a peak of the CO-reduced difference spectrum at 448 nm, but not at 450 nm, and the pattern of the ethylisocyanide difference spectrum was also similar to that of the microsomes from the rats pretreated with MC. Based on these data, they suggested that Aroclor

1254-induced P-448 might thus be catalytically different from the MC-induced P-448 or that the hemoprotein(s) induced with Aroclor 1254 might be a mixture of P-448 and P-450, and thereby concluded that PCB is a new type of inducer causing the induction of both PB- and MC-types. However, considering the results demonstrated by Alvares et al. together with the fact that commercial PCB preparations such as Aroclors and Kanechlors are a complex mixture of various PCB congeners, the question has arisen as to whether the inductive effect of the individual PCB congeners might differ from each other.

To answer this question, Yoshimura et al. (1978) studied the inductive effects of KC-400 and several pure PCB congeners in rats in comparison to those of PB and MC. As shown in Table 6.2.1, the induction profile of KC-400 was very similar to that of MC plus PB, and was also consistent with the results obtained by Alvares et al. (1973). Furthermore, the individual congeners tested were divided into two groups; namely 4,4′- dichlorobiphenyl, 2,5,2′,5′- and 2,4,3′,4′-tetrachlorobiphenyls (TCBs) were categorized as PB-type, whereas 3,4,3′,4′-TCB, 3,4,5,3′,4′-pentachlorobiphenyl (PenCB) and 3,4,5,3′,4′,5′- hexachlorobiphenyl (HCB) were considered to be MC-type (Yoshimura et al.,1978). It was thus concluded that the unique nature of commercial PCB preparations as an MFO inducer is attributable to the existence of congeners exhibiting the induction property of each type.

6.2.3. *The Inductive Effects of Individual PCB Congeners on Hepatic MFO and DT-diaphorase, and Their Toxicity*

Yoshimura et al. (1978) and Goldstein et al. (1977) have independently shown that PCB congeners can be divided into two groups, namely PB- and MC-types, with respect to the inducibility of MFO as described in the preceding section.

These investigators also reached the same conclusion that the substitution of chlorines at the 3, 4, 3′ and 4′ positions of the biphenyl ring is a minimum requirement for the exhibition of an MC-type induction. On the other hand, in the course of studies concerning the biochemical background of hepatic porphyria, Poland and Glover (1973) first observed that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) can potently induce aryl hydrocarbon hydroxylase (AHH) in a dose dependent manner as well as δ-aminolevulinic acid synthetase in the chick embryo liver.

Through their detailed efforts to elucidate the structure-activity relationship of AHH induction, they demonstrated that most of the halogenated derivatives of aromatic compounds, such as dibenzo-*p*-dioxins (Poland and Glover, 1973), dibenzofurans (Poland et al., 1976a), azoxybenzenes (Poland et al., 1976b) and biphenyls (Poland and Glover, 1977), which possess the ability to induce AHH, are co-planar and thus bind to hepatic cytosolic protein, which is regarded as the receptor for the induction of P-448, and was subsequently termed the Ah-receptor

(Poland et al., 1979). They further suggested that the toxicity of these chlorinated aromatics is also mediated through the Ah-receptor.

Up to the late 1970s, however, there had been only a limited number of investigations on the toxicity of individual congeners, except for reports by Goldstein and her co-workers (McKinney et al., 1976; Goldstein et al., 1976). They compared the toxicity in chickens of five symmetrical HCB congeners: 2,3,4,2′,3′,4′-, 2,3,6,2′,3′,6′-, 2,4,5,2′,4′,5′-, 2,4,6,2′,4′,6′- and 3,4,5,3′,4′,5′-HCBs, and found the MC-type 3,4,5,3′,4′,5′-HCB to be the only one to produce thymic atrophy and edema in chickens similar to 2,3,7,8-tetrachlorodibenzofuran(TCDF).

In order to find some correlation between the induction abilities of hepatic enzymes and toxicity, Yoshimura et al. (1979) examined the acute toxicity of several individual PCBs which were categorized as either the PB-type or the MC-type inducers in rats. As shown in Fig. 6.2.1 (on the right), BZ demethylase activity in rat liver 9,000 × g supernatant, a marker reaction for PB P-450 (Ryan et al., 1975), considerably increased 5 days after pretreatment with 2,5,2′,5′-, 2,4,3′,4′-TCBs and 2,3,4,3′,4′-PenCB at the single *ip* dose indicated, whereas the AHH activity, which is regarded as a marker for MC P-448 (Ryan et al., 1975), was markedly enhanced by pretreatment with 3,4,5,3′,4′-PenCB and 3,4,5,3′,4′,5′-HCB. The induction of AHH with 3,4,3′,4′-TCB, a prototype of MC-type PCBs, was weak at a dose of 10 mg/kg, but potent at 50 mg/kg. Thus it was reconfirmed that 3,4,3′,4′-TCB, 3,4,5,3′,4′-PenCB and 3,4,5,3′,4′,5′-HCB are MC-type, while 2,5,2′,5′- and 2,4,3′,4′-TCBs are PB-type as reported previously (Yoshimura et al., 1978).

It was very interesting to note that DT-diaphorase [NAD(P)H: (quinone-acceptor) oxidoreductase, EC 1.6.99.2] was greatly induced by only three MC-type PCBs. This cytosolic flavoenzyme is also known to be induced by 2,3,7,8-TCDD (Beaty and Neal, 1976), and MC, but not by PB (Lind and Ernster, 1974). The inducing potencies of MC-type PCBs for DT-diaphorase were quite similar to those for AHH, which thus suggested that the induction mechanism of these two hepatic enzymes might be closely related to each other. Judging from the dosage employed to induce these enzymes, the most potent congener was 3,4,5,3′,4′-PenCB, followed by 3,4,5,3′,4′,5′-HCB, and the least potent was 3,4,3′,4′-TCB. The marked increase of DT-diaphorase caused by the MC-type inducers is particularly worthy of note because this cytosolic enzyme is known to be an activating enzyme of the precarcinogen 4-nitroquinoline N-oxide (4-NQO) (Sugimura et al., 1966). In fact, 3,4,5,3′,4′-PenCB caused a marked increase in the reduction of 4-NQO to 4- hydroxylaminoquinoline N-oxide, a proximate carcinogen, in the liver, lung and skin of rats treated with 1 mg/kg, which suggests the possible promotion of carcinogenicity of 4-NQO in the exposed animals (Yoshimura et al., 1985).

Acute toxicity of six congeners was tested by measuring the gravimetric

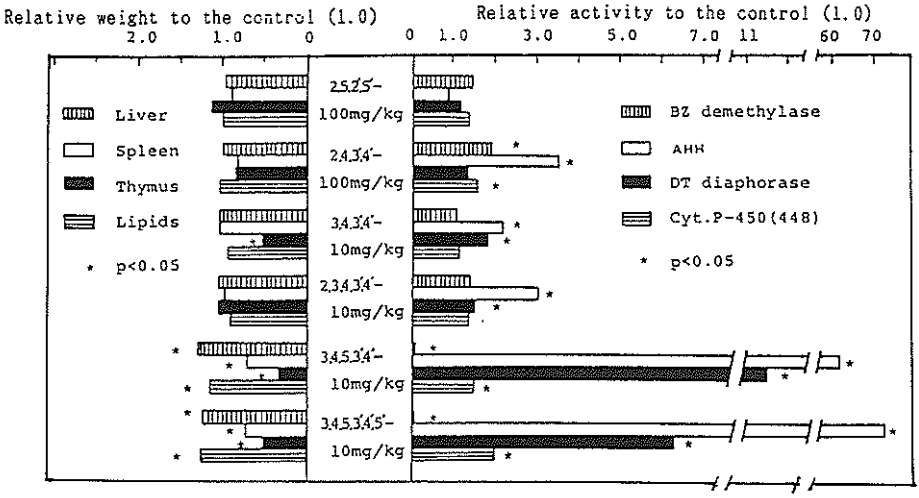

Fig. 6.2.1. The Comparative Effects of Individual PCB Congeners on the Hepatic Enzymes and Organ Weights in Rats

The activities in the control (mean ± SD) were: BZ demethylase 12.93 ± 1.52 nmol HCHO formed/mg protein/15 min, AHH 1.35 ± 0.06 nmol 3-OH benzo[a]pyrene formed/mg protein/5 min, DT-diaphorase 0.583 ± 0.072 μmol DCPIP reduced/mg protein/min, cytochrome P-450 (448) 0.144 ± 0.008 nmol/mg protein. The weight of the organs (g/100 g bw) and liver lipids (mg/g liver) in the control were: liver 3.60 ± 0.25, spleen 0.558 ± 0.091, thymus 0.252 ± 0.058, lipids 44.79 ± 4.98.

Quoted from Yoshimura et al. (1979).

changes of organs and body weights as well as the liver lipid content in the same animals (Fig. 6.2.1, left), in which the inductive effects were examined. The PB-type PCBs, 2,5,2′,5′- and 2,4,3′,4′-TCBs, did not affect the weights of the liver, spleen or thymus as well as the liver lipid content 5 days after treatment. The body weight gain was also unaffected by these PB-type PCBs and an intermediate-type 2,3,4,3′,4′-PenCB. In contrast, the MC-type PCBs showed much higher toxic signs. In line with previous reports which showed the thymus and spleen to be very sensitive to the toxicity induced by PCBs and related compounds (Kimbrough, 1974), severe atrophy of these organs was induced by treatment with 3,4,5,3′,4′-PenCB and 3,4,5,3′,4′,5′-HCB at a dose of 10 mg/kg, and with 3,4,3′,4′-TCB at a dose of 50 mg/kg. Liver hypertrophy and an increased liver lipid content are also the common observations in rats treated with commercial PCB preparations (Kimbrough et al.,1972; Hansell and Ecobichon,1974).

In experiments by Yoshimura et al. (1979), liver enlargement was observed not only by the MC-type PCBs, but also by 2,3,4,3′,4′-PenCB at a dose of 50 mg/kg. It is worth noting, however, that only MC-type PCBs increased the liver lipid con-

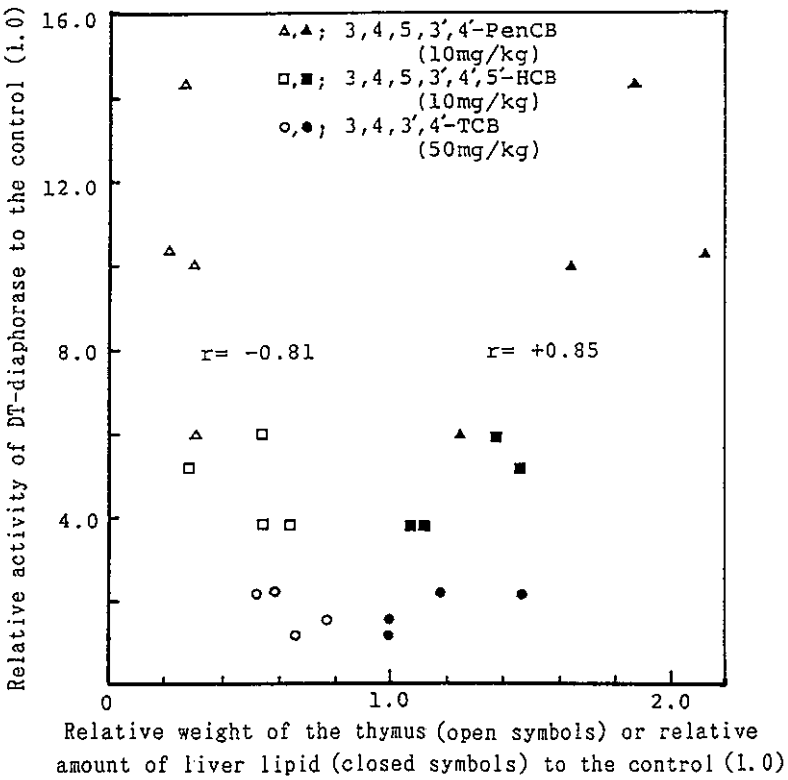

Fig. 6.2.2. The Correlation between DT-diaphorase Activity and Toxicity Induced by MC-type PCBs in Rats
Quoted from Yoshimura et al. (1979).

tent. These results thus suggest that the fatty liver produced by commercial PCB preparations is due to the MC-type PCBs in the preparations. The body weight gains during the first 5 days after injection were suppressed strongly by 3,4,5,3′,4′-PenCB and moderately by 3,4,5,3′,4′,5′-HCB. However, no significant effects were observed with 2,3,4,3′,4′-PenCB and 3,4,3′,4′-TCB at a dose of 50 mg/kg. Fig. 6.2.2 shows the correlation between the MC-type induction ability and the toxic potency of these PCB congeners. When selected as toxic parameters, both the relative weight of the thymus and the amount of liver lipids were closely correlated with the relative activity of DT-diaphorase, a parameter for MC-type inducibility. It should be noted that 3,4,5,3′,4′-PenCB, which was synthesized for the first time by Yoshimura et al. (1978) and Saeki et al. (1979), was the most toxic among all the PCB congeners examined.

In the patients with Yusho, several highly chlorinated PCB congeners, with chlorines at positions 3, 4 and 5 on each phenyl ring, were retained at a higher level

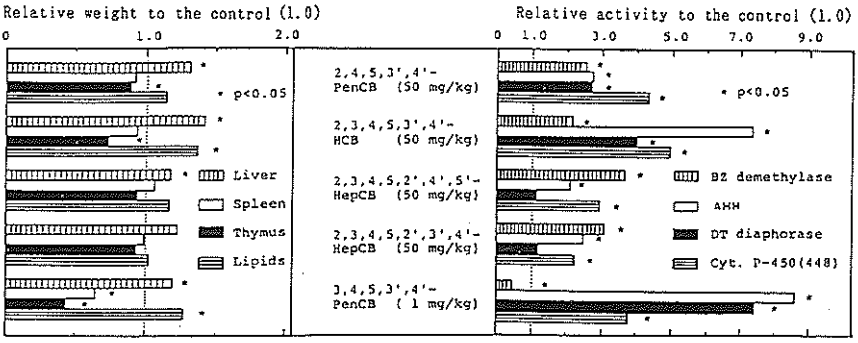

Fig. 6.2.3. The Comparative Effects of PCB Congeners Retained in the Patients with Yusho on Hepatic Enzymes and Organ Weights in Rats

The activities in the control (mean ± SD) were: BZ demethylase 17.77 ± 2.45 nmol HCHO formed/mg protein/15 min, AHH 0.109 ± 0.033 nmol 3-OH benzo[a]pyrene formed/mg protein/5 min, DT-diaphorase 0.134 ± 0.052 μmol DCPIP reduced/mg protein/min, cytochrome P-450 (448) 0.214 ± 0.016 nmol/mg protein. The weights of the organs (g/100 g bw) and liver lipids (mg/g liver) in the control were: liver 4.04 ± 0.1, spleen 0.556 ± 0.044, thymus 0.456 ± 0.019, lipids 50.78 ± 2.77.

Modified from Yoshihara et al. (1979a).

than in normal persons even 9 years after the outbreak of Yusho (Kuroki and Masuda, 1977). To better understanding the etiology of the chronic symptoms of Yusho, it is therefore important to evaluate the toxicity of these highly retained congeners in these patients. Therefore, the acute toxicity and the inducing properties of 2,4,5,3′,4′-PenCB, 2,3,4,5,3′,4′-HCB, and 2,3,4,5,2′,3′,4- and 2,3,4,5,2′,4′,5′-heptachlorobiphenyls (HepCBs) were examined in young male rats (Yoshihara et al., 1979a). As shown in Fig. 6.2.3 (right), pretreatment with all of the retained PCBs enhanced both the activities of AHH and BZ demethylase. Among the four congeners, 2,3,4,5,3′,4′-HCB induced AHH more strongly than BZ demethylase, while 2,3,4,5,2′,3′,4′- and 2,3,4,5,2′,4′,5'-HepCBs enhanced BZ demethylation to a much greater extent than AHH activity. DT-diaphorase was only increased by 2,4,5,3′,4′-PenCB and 2,3,4,5,3′,4′-HCB, although their magnifications were much less than that by 3,4,5,3′,4′-PenCB used as a positive marker in this experiment. This highly toxic PenCB was later detected in either the causal oil or tissue of the patients with Yusho (Kashimoto et al., 1987). These results indicated that 2,3,4,5,2′,3′,4′- and 2,3,4,5,2′,4′,5′-HepCBs are regarded as PB-type while 2,3,4,5,3′,4′-HCB falls into MC-type with a moderate intensity, and 2,4,5,3′,4′-PenCB is an intermediate-type.

As expected from the moderate MC-type induction mode, 2,3,4,5,3′,4′-HCB caused marked liver hypertrophy and thymus atrophy as well as a significant accumulation of liver lipids (Fig. 6.2.3, left). 2,4,5,3′,4′-PenCB caused some slight

Table 6.2.2. Classification of Individual PCB Congeners into PB-type and MC-type Inducers for Hepatic MFO

Cl numbers	PB-type PCB	Intermediate-type PCB	MC-type PCB
2	4,4′- 3,3′-		
4	2,4,3′,4′- 2,5,2′,5′- 2,4,2′,4′-		3,4,3′,4′-
5		2,4,5,3′,4′- 2,3,4,3′,4′-	3,4,5,3′,4′-
6	2,4,5,2′,3′,4′,5′- 2,3,4,2′,3′,4′-		3,4,5,3′,4′,5′- 2,3,4,5,3′,4′-
7	2,3,4,5,2′,4′,5′- 2,3,4,5,2′,3′,4′-		
8 10	2,3,4,5,2′,3′,4′,5′- 2,3,4,5,6,2′,3′,4′,5′,6′-		

Quoted from Yoshimura and Yoshihara (1979).

liver enlargement and thymus atrophy, but did not affect either the weight of the spleen or the liver lipid content. In contrast, the PB-type PCBs such as 2,3,4,5,2′,3′,4′- and 2,3,4,5,2′,4′,5′-HepCBs did not show any toxic signs although the latter caused slight hypertrophy of the liver without any increase in the liver lipid. These data thus indicated that certain retained PCBs, especially 2,3,4,5,3′,4′-HCB, which was detected at a 7 times higher than normal level in these patients even at 9 years after ingestion, might thus act as toxicants in the patients.

These lines of evidence strongly suggest that MC-type PCBs are much more toxic than the PB-type ones and that their toxic potency is well correlated with their MC-type inducing ability (Figs. 6.2.1 and 6.2.2). Although pretreatment of the rats with MC induced both AHH and DT-diaphorase activities, most of the typical toxic signs produced by MC-type PCBs were not observed with MC itself, except for a slight liver enlargement and a lesser atrophy of the thymus. At present it has yet to be elucidated as to why the toxic potency of PCBs so closely correlates with their MC-type inducing ability, while their toxic characteristics differ from that of MC (See the section 6.2.10).

Table 6.2.2 shows the classification of PCB congeners, most of which were also found in the causal oil of Yusho, into the PB-, MC- and intermediate-types based on their induction properties of rat liver MFO (Yoshimura and Yoshihara, 1979). Concerning the inducing ability of PCB congeners on hepatic MFO, the following structure-activity relationship may exist: a) a minimum requirement to exhibit the MC-type induction is to be chlorinated on at least two adjacent lateral positions

of each phenyl ring, that is 3,4,3′,4′-TCB. This TCB is a prototype of these co-planar PCBs. b) additional chlorination of the prototype TCB at the remaining meta-positions (5,5′) enhances its potency as an MC-type, i.e. 3,4,5,3′,4′-PenCB and 3,4,5,3′,4′,5′-HCB, but chlorination at the ortho-positions (2,2′,6,6′) weakens potency and leads to an intermediate-type, i.e. 2,4,5,3′,4′- and 2,3,4,3′,4′-PenCBs. c) PCB congeners substituted with more than two chlorine atoms at the four ortho-positions exhibit PB-type, i.e. 2,5,2′,5′-TCB, 2,4,5,2′,4′,5′- and 2,3,4,2′,3′,4′-HCBs, 2,3,4,5,2′,3′,4′- and 2,3,4,5,2′,4′,5′-HepCBs.

6.2.4. *The Inductive Effects of PCDF Congeners on Hepatic MFO and DT-diaphorase, and Their Toxicity*

Since PCDFs were detected not only in the causal oil (Nagayama et al., 1976) but also in the tissue of patients with Yusho (Nagayama et al., 1977; Kuroki and Masuda, 1978), the toxicological assessment of these contaminants as the causal agent of Yusho is considered to be extremely important. Several studies on the toxicity of PCDF mixture in chickens (Vos et al., 1970), rats (Oishi et al., 1978) and mice (Nishizumi, 1978) have demonstrated that the profile of toxic signs of PCDFs are similar to that of PCBs, but PCDFs are much more toxic than PCBs. Only a limited number, however, of toxicological studies with pure individual PCDF congeners have yet been done, except for 1,4,8-trichlorodibenzofuran (Saeki et al., 1977), 2,3,7,8-TCDF (McKinney et al., 1976; Moore et al., 1979) and 2,3,4,7,8-pentachlorodibenzofuran (PenCDF) (Moore et al., 1979).

On the other hand, Poland et al. (1976) reported that the induction abilities of individual PCDFs on AHH in the chick embryo greatly differed depending on the position as well as the number of chlorines substituted. From this evidence and the toxicity studies of MC-type PCBs, it was strongly suggested that the toxic potency of PCDF congeners might also be correlated with their MC-type induction ability. Therefore, Yoshihara et al. (1981) examined the acute toxicity and the inducing potency of thirteen individual PCDFs (Table 6.2.3) in male rats not only to assess the toxicity of the PCDF congeners retained in the patients, but also to verify the assumption described above.

As shown in Fig. 6.2.4 (right), nine of the thirteen PCDF congeners significantly induced AHH and DT-diaphorase, the indicators for MC-type induction, in the rat liver. It should be noticed that all the nine congeners exhibiting a typical MC-type induction have at least three chlorine atoms in the lateral ring positions such as 1,2,7,8-, 2,3,6,7- and 2,3,7,8-TCDFs, 1,2,3,7,8-, 1,2,6,7,8-, 2,3,4,6,7- and 2,3,4,7,8-PenCDFs, 1,2,3,4,6,7- and 1,2,3,4,7,8-hexachlorodibenzofurans. On the other hand, the congeners with two or fewer chlorine atoms in the lateral positions, such as 2,8-dichlorodibenzofuran, 1,3,6,7- and 1,3,6,8-TCDFs and 1,2,4,6,8-

Table 6.2.3. PCDF Congeners Retained in the Patients with Yusho and Their Structural Characterization

PCDF congener	Occurrence in Yusho[a]		Vicinal hydrogens in the ring[b]	More than three chlorines in the lateral positions[c]
	Oil	Patients		
2,8-	—	—	×	
1,2,7,8-	—	—	×	×
1,3,6,7-	—	—	×	
1,3,6,8-	—	—		
2,3,6,7-	+	nd	×	×
2,3,7,8-	+++	+		×
1,2,3,7,8-	++	+		×
1,2,4,6,8-	+	Trace		
1,2,6,7,8-	++	Trace	×	×
2,3,4,6,7-	++	nd	×	×
2,3,4,7,8-	+++	+++		×
1,2,3,4,6,7-	++	nd	×	×
1,2,3,4,7,8-	++	+++		×

[a]: Quoted from the data by Rappe et al. (1979), —: not identified, +: low, ++: medium, +++: high, nd: not detected. [b]: The congeners with vicinal hydrogens in the ring. [c]: The congeners with at least three atoms in the lateral ring positions.

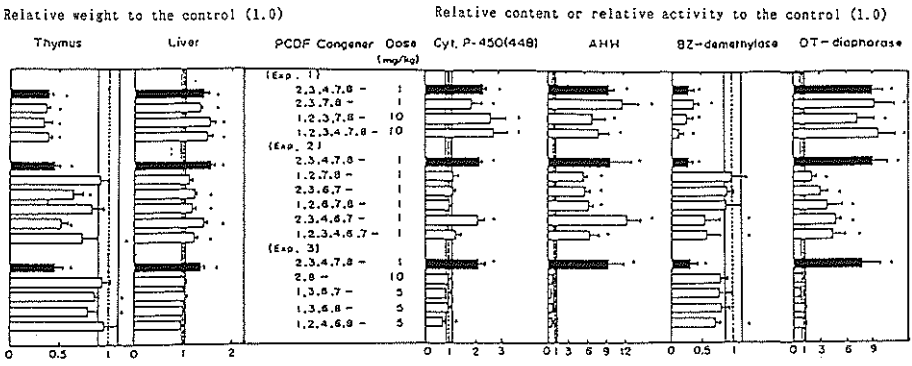

Fig. 6.2.4. The Comparative Effects of Individual PCDF Congeners on the Hepatic Enzymes and Organ Weights in Rats

The vertical lines represent the standard deviations of the means of four rats per group. The shaded area represents the average standard deviation of the means of control groups in three experiments. The average activities in the control (mean ± SD) were: cytochrome P-450 (448) 0.242 ± 0.039 nmol/mg protein, AHH 67.1 ± 9.7 pmol 3-OH benzo[a]pyrene formed/mg protein/min, BZ demethylase 1.37 ± 0.20 nmol HCHO formed/mg protein/min, DT-diaphorase 0.233 ± 0.024 μmol DCPIP reduced/mg protein/min. The average weights of the organs (g/100 g bw) in the controls (mean ± SD) were: thymus 0.313 ± 0.035, liver 3.67 ± 0.10. *Significantly different from the control, $p < 0.05$.

Modified from Yoshihara et al. (1981).

PenCDF did not show any inductive effects on the hepatic enzymes. This structure-activity relationship in rats correlated closely with that found in chick embryos by Poland et al. (1976a).

All the MC-type PCDFs except for 1,2,7,8-TCDF caused a marked atrophy of the thymus and a hypertrophy of the liver in rats 5 days after a single *ip* injection of the congeners, while no toxic signs were observed in the rats treated with the congeners lacking an MC-type inducing ability (Fig. 6.2.4, left). The ranking of the toxic potencies of the MC-type PCDFs again closely correlated with their inducing abilities.

Dose-response studies showed that both 2,3,7,8-TCDF and 2,3,4,7,8-PenCDF are the most potent congeners, inducing AHH and DT-diaphorase even at a single dose of 1 μg/kg. Despite similarities of their structural features and biologic potency, a great difference in the accumulation property of 2,3,7,8-TCDF and 2,3,4,7,8-PenCDF in the liver was observed. At all dose levels, the percentage retention in the liver 5 days after injection was almost constant at about 3% of the dose for the TCDF and at about 60% of the dose for the PenCDF (Yoshihara et al., 1981). This is in consistent with the fact that the concentration of 2,3,4,7,8-PenCDF in the liver of patients with Yusho was much higher than that of 2,3,7,8-TCDF, even though both congeners were found to exist at similar levels in the causal ingested oil (Kuroki and Masuda, 1978). The reason for the great difference in the hepatic disposition between 2,3,7,8-TCDF and 2,3,4,7,8-PenCDF is still not clear at that time. Subsequently, Kuroki et al. (1986) revealed that 2,3,4,7,8-PenCDF bound with a high affinity to P-448 H (P450 1A2), which is one of the P-450 isozymes selectively induced by 3,4,5,3′,4′-PenCB and 2,3,4,7,8-PenCDF (See the following section). Accordingly, the higher retainability of 2,3,4,7,8-PenCDF in the liver is probably attributable, at least in part, to its specific binding to P-448 H induced by the PenCDF itself. In conclusion, they clearly demonstrated that most of the PCDF congeners found in the causal oil or/and retained in the patients exhibit a potent toxicity and MC-type inducibility in rats. Therefore, these PCDFs should not be disregarded when trying to fully understand the etiology of Yusho even though their concentration in the oil was much lower than that of PCBs.

6.2.5. *The Inductive Effects of PCBs and PCDFs on the Hepatic Steroid Metabolism*

Female patients with Yusho had menstrual cycle irregularities, dysmenorrhea and altered serum levels of ketosteroids (Kuratsune et al., 1972). Severe alterations of the menstrual cycles and reproductive dysfunction were also observed in adult female monkeys fed low levels of PCB mixtures (Allen and Barsotti, 1976;

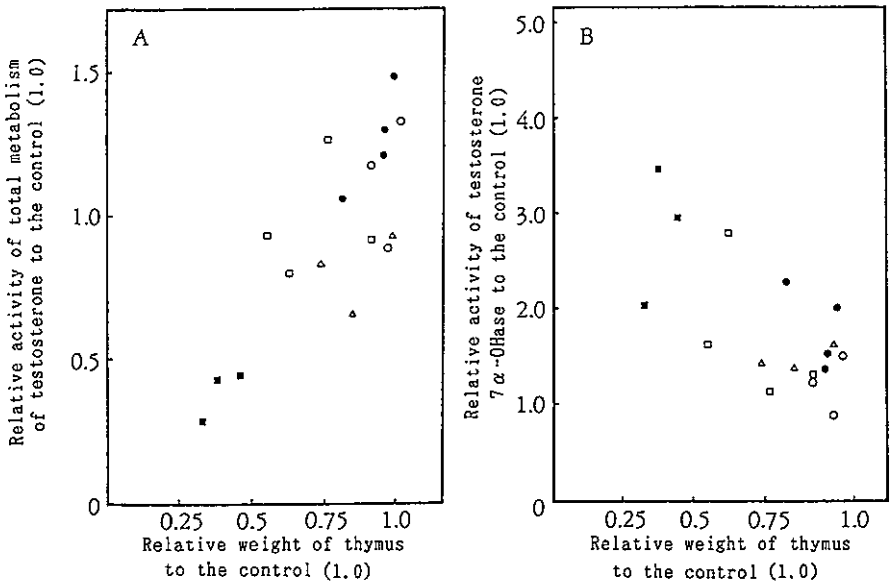

Fig. 6.2.5. The Correlation between Thymus Atrophy and Depressed Total Metabolism (A), and Increased 7α-Hydroxylation (B) of Testosterone Caused by PB, MC and PCBs in Rats

●: PB 100 mg/kg × 3; △: MC 20 mg/kg × 3; □: KC-400 300 mg/kg × 1; ○: 2,4,5,2′,4′,5′-HCB 20 mg/kg × 1; ■: 3,4,5,3′,4′-PenCB 5 mg/kg × 1. (A) $r = 0.817$ ($\alpha < 0.001$), (B) $r = -0.686$ ($\alpha < 0.01$).

Quoted from Yoshihara et al. (1982).

Kimbrough et al., 1978). In rodents, PCBs produced uterine atrophy, ovarian stromal changes and reduced plasma progesterone levels (Örberg and Kihlström, 1973; Jonsson et al., 1976).

However, the mechanism of these endocrine related alterations produced by pretreatment with PCBs and the related compounds has not been fully elucidated. Since steroids are one of the major endogenous substrates for MFO, it is easy to consider that some of these alterations might have been caused through a modification of the steroid metabolism catalyzed by MFO. Indeed, several investigators have shown that steroid metabolism was enhanced by pretreatment with commercial PCBs (Risebrough et al., 1968; Derr, 1978), but only a limited number of reports concerning the effect of individual PCB congeners, as well as PCDF congeners, is presently available.

Thus, Yoshihara et al. (1982) attempted to examine the effects of pretreatment with highly toxic PCB and PCDF congeners on the liver microsomal metabolism of progesterone and testosterone in rats. 2,4,5,2′,4′,5′-HCB and KC-400, which possess a lower toxicity and are classified as PB- and mixed-type inducers, respec-

tively, significantly increased the activities of 16α-, 7α- and 6β-hydroxylations of progesterone and testosterone, and thus resulted in an enhancement of the total metabolism of these steroids, similarly to PB itself. On the other hand, the highly toxic 3,4,5,3′,4′-PenCB, a potent MC-type inducer, selectively enhanced 7α-hydroxylation of both steroids, but strongly suppressed 6β- and 16α-hydroxylations of both steroids, 2α-hydroxylation of progesterone and the formation of androstenedione from testosterone.

Treatment with toxic MC-type PCDF congeners, 2,3,7,8-TCDF and 2,3,4,7,8-PenCDF demonstrated almost the same effects as 3,4,5,3′,4′-PenCB. The 5α-reduction, which is a rate-limiting metabolic reaction of catabolism of Δ^4-steroids, was also decreased by the toxic congeners. Through these effects, the highly toxic PCBs and PCDFs caused a marked depression of the total metabolism of both steroids rather than enhancement. This unique change of the steroid metabolism is mainly responsible for the qualitative and quantitative changes of the microsomal P-450 (448) induced by the highly toxic congeners described in section 6.2.8. Although there is no other direct evidence, the above results suggest that a selective induction of steroid 7α-hydroxylase activity or/and the suppression of the activities of 2α-, 6β- and 16α-hydroxylations as well as 5α-reduction, most of which are predominant catabolic reactions in control animals, by highly toxic PCBs and PCDFs may be responsible for either the endocrine symptoms or atrophy of the thymus (Fig. 6.2.5).

6.2.6. The Inductive Effects of PCBs on Pulmonary MFO

Brandt et al. (1976) have demonstrated that certain selective PCBs including 2,4,5,2′,4′,5′-HCB, a PB-type PCB, showed a tendency to accumulate in the lung of animals, especially the bronchi. On the other hand, the bronchi are also known to be the primary locus of MFO-rich Clara cells (Boyd, 1977). Based on this evidence, it is of interest to study the inductive effects of PCBs on pulmonary MFO to better understand the biochemical background of the respiratory symptom in patients with Yusho. Although the inductive effect of PCB mixture (Aroclor 1254) on pulmonary MFO has been studied in rats (Lake et al., 1979; Ueng et al., 1980), no effort has been made to clarify the effect of pure PCB congeners showing either PB- or MC-type inducibility.

Yoshihara et al. (1983a) comparatively examined the inductive effect of a less toxic PB-type 2,4,5,2′,4′,5′-HCB and a highly toxic MC-type 3,4,5,3′,4′-PenCB as well as KC-400, PB and MC in the liver and lung of rats. Despite the fact that the content of hepatic P-450 (448) increased after pretreatment with all the inducers tested, the pulmonary content was only slightly elevated by the MC-type (MC and the PenCB) and mixed-type KC-400. The pulmonary BZ demethylase activity

was not affected by the MC-type or the mixed-type inducers, while both PB-type inducers (PB and the HCB) decreased the activity. The PB-type inducers, showing a weak inducibility for AHH in the liver, also decreased the AHH activity in the lung. In contrast, a marked enhancement of AHH activity in the lung as well as the liver was caused by pretreatment with the MC-type inducers and KC-400. Thus, the microsomal MFOs in the rat liver and lung were quite different regarding their responsiveness to PB-type induction, while the MFO in both tissues responded to MC-type induction.

6.2.7. *Species Differences in the Responsiveness to the Induction of Hepatic Enzymes and in Toxicity by PCBs and PCDFs*

The halogenated aromatic hydrocarbons, such as polychlorinated dibenzo-*p*-dioxins (PCDDs), PCDFs and PCBs, commonly produce a similar and characteristic toxic spectrum including wasting, thymic atrophy, delayed lethality, chloracne and hyperkeratosis (Poland and Knutson, 1982). In addition, the toxic responses to these chemicals are also highly species- and tissue-specific. For example, the oral LD_{50} of 2,3,7,8-TCDD in hamsters is approximately 5,000-fold greater than in guinea pigs, which is the most highly susceptible species toward this class of chemicals. The liver is a major target organ in rats and mice, exhibiting hypertrophy, lipid accumulation and necrosis, whereas few liver lesions are observed in guinea pigs.On the other hand, at least in rats and mice, both of which show a moderate susceptibility toward the toxicity by these halogenated aromatics, a good correlation between the potencies to induce MFO and toxicity is observed (Yoshimura et al., 1979; Poland et al., 1979). Therefore, it is of interest to clarify the species differences in the responsiveness to the inductive effects on the hepatic enzymes such as MFO and to acute toxicity by PCBs and PCDFs.

Accordingly, in order to confirm whether or not the MC-type induction produced by PCBs is inseparable from their toxic effects, the inductive effects and acute toxicity by 3,4,5,3′,4′-PenCB were examined using two inbred strains of mice. These strains, including the Ah-responsive C57BL/6 mice and the Ah-nonresponsive DBA/2 mice, exhibit genetically different susceptibility toward MC-type induction (Kumaki et al., 1977). As shown in Fig. 6.2.6, the DBA mice exhibited not only a lower responsiveness to the induction of MFO (AHH) and DT-diaphorase but also a lesser sensitivity to the toxicity by 3,4,5,3′,4′-PenCB (Yoshihara et al., 1983b). On the other hand, the C57BL mice showed a higher sensitivity to both induction and toxicity. Dose-response studies demonstrated that DBA mice are roughly 10- to 20-times less sensitive than C57BL mice in their responsiveness to either the inductive or the toxic action by 3,4,5,3′,4′-PenCB. Judging from these results, the responsiveness to the MC-type induction appears to

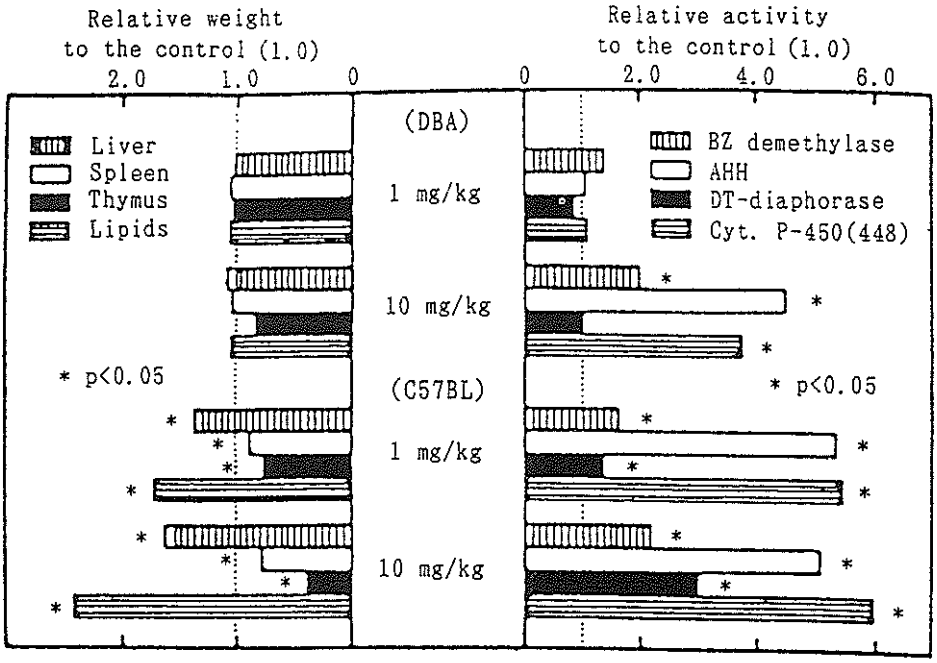

Fig. 6.2.6. The Comparative Effects of 3,4,5,3′,4′-PenCB on the Hepatic Enzymes and Organ Weights in DBA and C57BL Mice

The activities in the control (mean ± SD) were: (DBA) BZ demethylase 9.06 ± 1.76 nmol HCHO formed/mg protein/15 min, AHH 0.044 nmol 3-OH benzo[a]pyrene formed/mg protein/5 min, DT-diaphorase 0.012 μmol DCPIP reduced/mg protein/min, cytochrome P-450 (448) 0.132 ± 0.010 nmol/mg protein, (C57BL) BZ demethylase 8.13 ± 0.51 nmol HCHO formed/mg protein/15 min, AHH 0.165 nmol 3-OH benzo[a]pyrene formed/mg protein/5 min, DT-diaphorase 0.019 μmol DCPIP reduced/mg protein/min, cytochrome P-450 (448) 0.128 ± 0.006 nmol/mg protein. The weight of the organs (g/100 g bw) and liver lipids (mg/g liver) in the control were: (DBA) liver 4.05 ± 0.22, spleen 0.344 ± 0.047, thymus 0.123 ± 0.039, lipids 93.31 ± 5.14, (C57BL) liver 4.28 ± 0.24, spleen 0.279 ± 0.009, thymus 0.223 ± 0.019, lipids 91.83 ± 7.76.

Quoted from Yoshihara et al. (1983b).

be obligatory in the toxic response to 3,4,5,3′,4′-PenCB, at least in these mice. Very similar observations, with regard to 2,3,7,8- TCDD, have also been reported by Poland et al. (1979). Another interesting difference observed in these inbred mice was that the C57BL mice retain 3,4,5,3′,4′-PenCB in the liver at a rate approximately 2-times higher (about 80% of dose) than that observed in the DBA mice 5 days after the injection.

The guinea pig is known as the most sensitive animal species toward the toxicity of 2,3,7,8-TCDD (Poland and Knutson, 1982). Accordingly, the acute toxicity and

Table 6.2.4. Species Differences in the Responses to Toxicity and the Induction of Hepatic Enzymes by 3,4,5,3′,4′-Pentachlorobiphenyl and 2,3,4,7,8-Pentachlorodibenzofuran

Animal species	Chemicals	Toxic response	MC-type induction	References
rat (Wistar)	PenCB[a] PenCDF[b]	fairly toxic, severe to liver functions	responsive (AHH, EROD[c], steroid metabolism), inducible P448 H, P448 L, P452 and DT[d]	Yoshimura et al. (1979) Yoshihara et al. (1982) Nagata et al. (1985a) Nagata et al. (1985b)
mouse (C57BL) (DBA)	PenCB	highly toxic (sensitive) less toxic (resistant)	highly responsive weakly responsive	Yoshihara et al. (1983)
guinea pig	PenCB	highly toxic (body wt. loss), but less damage to liver functions	not so intensive	Yoshimura et al. (1981a) Oguri et al. (1993)
hamster	PenCDF	partially toxic, no lethal effect	responsive (AHH, EROD, DT), but lesser than rat	Koga et al. (1989)
chicken	PenCB	highly toxic, but no effect on liver lipids	responsive (AHH, EROD), but lesser for DT	Hokama et al. (1985)

[a]: 3,4,5,3′,4′-pentachlorobiphenyl, [b]: 2,3,4,7,8-pentachlorodibenzofuran, [c]: 7-ethoxyresorufin 0-deethylase, [d]: DT-diaphorase.

the inductive effects on hepatic enzymes by 3,4,5,3′,4′-PenCB were examined in guinea pigs (Yoshimura et al., 1981a; Oguri et al., 1993; Koga et al., 1994). A remarkable loss of body weight in the guinea pig was observed during the first 4 days after a single *ip* injection of the PenCB even at a dose of 0.1 or 0.5 mg/kg, while in the rat, body weight gain was suppressed, but not lost, at a dose of 5 or 25 mg/kg. An almost similar extent of either liver hypertrophy or thymus atrophy was exhibited in the guinea pig and rat at a dose of 0.5 and 25 mg/kg, respectively. These toxic responses in the two animal species indicate that the guinea pig is much more susceptible than the rat toward the toxicity of 3,4,5,3′,4′-PenCB. On the other hand, the induction of AHH and DT-diaphorase in the guinea pig liver was not as remarkable as that in the rat liver, and was consistent with 2,3,7,8-TCDD treatment (Holcomb et al., 1988; Beaty and Neal, 1978). Thus, no correlation between the toxic potency and the MC-type inducibility by PCBs was observed in the case of the guinea pig. It was interesting, however, to note that ω-hydroxylation of lauric acid, which is predominantly catalyzed by the P450 4A subfamily (Bains et al., 1985), in the guinea pig liver was enhanced 4-fold or more over the control after treatment with toxic co-planar PCBs such as 3,4,5,3′,4′-PenCB, while the same activity in the rat liver treated with these congeners was

observed to significantly decrease (Koga et al., 1994).

Since the hamster is recognized to be the least sensitive animal species against the acute toxicity of 2,3,7,8-TCDD (Poland and Knutson, 1982), the acute toxicity and the inductive effects on the hepatic enzymes by 2,3,4,7,8-PenCDF, a highly toxic PCDF congener, were examined in male Golden Syrian hamsters to further clarify the relationship between the toxic and biochemical responses (Koga et al., 1989). Despite almost no effect on the body weight gain, potent atrophies of thymus, spleen and kidney as well as liver hypertrophy were all observed at a dose of 0.5 mg/kg. In addition, the amount of lipid peroxide in the liver increased to about 3-fold over the control. The activities of AHH and DT-diaphorase also increased about 2-fold and 4-fold, respectively, after PenCDF treatment,which was a somewhat lesser extent than that observed in the rat. These results thus suggest that hamsters exhibit some toxic responses to PCBs and their related compounds, which are similar to those in the relatively sensitive species, such as rats and mice, but these responses may not be lethal in the resistant species.

It is also well known that in birds, PCBs cause weight loss, edema, liver hypertrophy and atrophy of the spleen (Goto et al.,1969; Vos and Koeman, 1970), but their effects on the hepatic enzymes have not yet been fully clarified. Therefore, the inductive effects on the hepatic enzymes and acute toxicity of 3,4,5,3′,4′-PenCB (a highly toxic MC-type) and 2,4,5,2′,4′,5′- HCB (a less toxic PB-type) were studied in chickens (Hokama et al., 1985). As a result, the PenCB caused potent acute toxicity and a marked induction of the hepatic enzymes. For example, hydropericardium, an enlargement of the liver and atrophy of the spleen were all observed, even at a single dose of 5 μg/kg. In the liver, the content of P-450 and AHH activity were potently increased. The inducibility of these enzymes closely correlated with the potency of acute toxicity of the PenCB. On the other hand, the HCB-treated chickens showed neither any significant toxicity nor inductive effects on the hepatic enzymes at a single dose of 10 mg/kg.

In order to develop an experimental model of Yusho, the Study Group for the Therapy of Yusho attempted to chronically administer either KC-400 alone or a mixture of KC-400 and PCDFs (200 : 1), a model causal agent of Yusho, to rhesus and crab eating monkeys (Yoshihara et al., 1979b; Yoshimura et al., 1981b). In general, PCBs and/or PCDFs exhibited higher toxicity in rhesus monkeys than in crab eating monkeys. A remarkable loss of hair on the head, neck and arms was observed within 2 months after the daily administration *po* of a mixture of KC-400 (0.25 mg/kg) and PCDFs (1.25 μg/kg), but no acne developed. The latter finding was, however, not consistent with the report of Barsotti et al. (1976). An electron microscopic study revealed that in the rhesus monkey receiving KC-400 (0.125 mg/kg/day) plus PCDFs (0.625 μg/kg/day) for 4 months, the biopsied he-

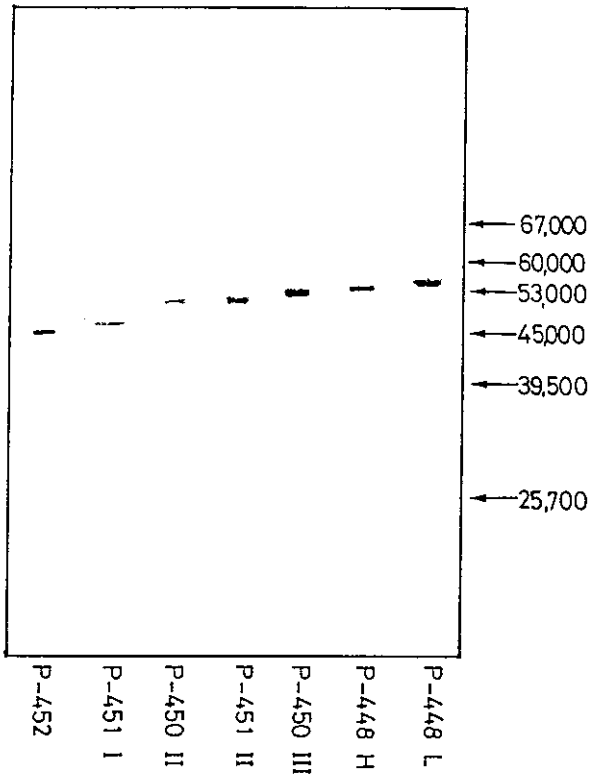

Fig. 6.2.7. The SDS-polyacrylamide Gel Electrophoresis of Seven Forms of Cytochrome P-450 Purified from Male Wistar Rats

Electrophoresis was carried out according to the method of Laemmli using 8% gel containing 0.1% SDS. Each well contained 1.5 μg of purified cytochrome P-450.

Quoted from Nagata et al. (1985a).

patic cells were characterized by a remarkable proliferation of agranular endoplasmic reticulum in either tubular or vesicular form. In the monkeys receiving PCDFs (0.625 μg/kg/day) alone, the change was observed only in the proliferation of agranular endoplasmic reticulum, and it was at a somewhat lesser degree than that observed in the monkeys receiving KC-400 plus PCDFs. The activities of AHH and DT-diaphorase in the liver homogenate, which was prepared from the biopsied sample, increased markedly with continuing administration of PCBs and/or PCDFs. The elevated activities of DT-diaphorase and AHH returned to normal at 6 and 9 months, respectively, after the drug administration was discontinued.

As described above, the toxic response and the inducibility of hepatic MFO and DT-diaphorase by PCBs and PCDFs are quite species-specific, and this is consistent with 2,3,7,8-TCDD as reported by Poland and Knutson (1982). Table 6.2.4

Table 6.2.5. Apparent Molecular Weights and Spectral Properties of Various Forms of Hepatic Cytochrome P-450 Purified from Male Wistar Rats

Form of cytochrome P-450	Treatment	Mr[a]	λ_{max} (nm) of absolute spectrum		
			Oxidized	Reduced	Reduced CO complex
P-451 I	Untreated	49,000	415.0, 532.0, 566.0	409.0, 546.0	451.0, 552.0
P-451 II	Untreated	52,000	416.0, 536.0, 569.0, 660.0[b]	413.0, 547.0	451.0, 554.0
P-450 II	PB-treated	52,000	418.0, 534.0, 570.0	410.5, 543.0	450.0, 550.5
P-450 III	PB-treated	53,500	416.0, 534.5, 568.0	420.0, 550.0[b]	450.0, 421.0[c], 554.0[b]
P-452	PenCB-treated	48,000	416.5, 536.8, 568.0	410.0, 543.0	452.0, 553.0
P-448 L	PenCB-treated	56,000	417.5, 532.0, 569.0	407.0, 542.0	447.5, 550.0
MC-P-448 L	MC-treated	56,000	417.5, 533.0, 568.0	408.0, 542.0	447.5, 550.0
P-448 H	PenCB-treated	54,000	393.0, 644.0	408.0, 542.0	447.5, 548.0
MC-P-448 H	MC-treated	54,000	392.0, 644.0	408.0, 542.0	447.5, 550.0

[a]: The apparent molecular weight was determined by SDS-polyacrylamide gel electrophoresis. [b]: Not accurate due to a broadening of the peak. [c]: Due to the presence of denatured form, cytochrome P-420.
Quoted from Nagata et al. (1985a).

Table 6.2.6. Catalytic Activity of Various Forms of Hepatic Cytochrome P-450 Purified from Male Wistar Rats

Substrate	Untreated[a]		PB-treated[a]		MC-treated[a]		PenCB-treated[a]		
	P-451 I	P-451 II	P-450 II	P-450 III	P-448 L	P-448 H	P-452	P-448 L	P-448 H
Benzphetamine	12.51	51.11	17.57	18.57	10.82	12.71	4.27	16.38	12.51
Aminopyrine	8.04	11.22	8.94	7.55	4.86	13.60	3.28	10.92	12.91
Benzo[a]pyrene	0.23	1.66	0.20	0.33	1.92	0.07	0.08	2.12	0.08
7-Ethoxycoumarin	1.83	2.06	0.11	3.20	56.91	0.80	0.23	109.3	1.03
Biphenyl									
4-OH	2.20	24.14	3.75	6.93	17.59	13.35	0.33	34.72	16.75
2-OH	ND	0.65	ND	0.74	6.49	2.69	ND	7.75	3.40
Estradiol-17β									
2-OH	3.48	23.42	3.48	0.79	1.45	12.05	0.65	1.66	17.05

[a]: Source of cytochrome P-450. The catalytic activity is expressed as nmol of product formed/min/nmol of cytochrome P-450. ND: not detected.
Quoted from Nagata et al. (1985a).

Table 6.2.7. The Activities of Testosterone Hydroxylation Catalyzed by Various Forms of Hepatic Microsomal Cytochrome P-450 Purified from Male Wistar Rats

Reaction	Untreated		PB		MC		PenCB		
	P-451 I	P-451 II	P-450 II	P-450 III	MC-P-448 L	MC-P-448 H	P-452	P-448 L	P-448 H
7α-Hydroxylation	4.27	ND	ND	ND	0.07	ND	10.92	0.09	ND
16α-Hydroxylation	0.63	8.31	0.56	1.49	Trace	Trace	0.43	Trace	Trace
6β-Hydroxylation	1.13	1.64	0.10	Trace	0.28	0.24	0.48	0.72	0.28
2α-Hydroxylation	0.44	8.33	0.40	ND	ND	ND	ND	ND	ND

The catalytic activity (nmol product/min/nmol cytochrome P-450) represents the mean of two determinations. ND: not detected. Quoted from Nagata et al. (1985b)

Table 6.2.8. Immunochemical Quantitation of Four Forms of Cytochrome P-450 in the Hepatic Microsomes from Male Wistar Rats Treated with Various Inducers

Microsomes	Total cytochrome P-450	P-451 II	P-452	P-448 L	P-448 H
Untreated	0.83	0.52 (62.7)	< 0.008 (< 1.0)	BD	BD
PB-treated	2.37	0.46 (19.4)	0.037 (1.6)	BD	BD
MC-treated	1.45	0.43 (29.7)	0.027 (1.9)	0.99 (68.3)	0.47 (32.4)
PenCB-treated	2.69	0.11 (4.1)	0.098 (3.6)	0.85 (31.6)	1.69 (62.8)

Total cytochrome P-450 was determined spectrophotometrically from the CO-reduced difference spectrum. Values (nmol cytochrome P-450/mg protein) represent the mean of two determinations for the pooled microsomes of four rats in each group. The numbers in parentheses represent the percentage of total cytochrome P-450. BD: below limit of accurate determination.
Quoted from Nagata et al. (1985b).

summarizes the species differences in the toxic response and the inducibility of hepatic enzymes caused by PCBs and PCDFs.

6.2.8. P-450 Isozymes Induced with Toxic PCB and PCDF Congeners

Over the past 20 years, the existence of multiple forms of hepatic microsomal P-450 in many animal species, each of which exhibits distinct but overlapping substrate specificity, has been verified by many investigators (Gonzalez, 1989). To date, P-450 are grouped into a gene superfamily including 221 genes of December 14, 1992 and among them 12 gene families which comprise 22 subfamilies are found in all mammals (Nelson et al., 1993).

In the preceding sections, it was demonstrated that the pretreatment of several experimental animals with PCBs and PCDFs causes a marked modification of the metabolisms of xenobiotics such as benzo[a]pyrene and BZ and also endogenous substrates such as testosterone and progesterone. For example, the pretreatment of rats with both highly toxic 3,4,5,3′,4′-PenCB and 2,3,4,7,8-PenCDF markedly enhanced 7α-hydroxylation but strongly suppressed 2α-, 6β- and 16α-hydroxylations of either testosterone or progesterone with rat liver microsomes (Yoshihara et al., 1982). In addition, certain forms of P-450, purified from liver microsomes of untreated and induced rats, have also been shown to catalyze regio- and stereoselective hydroxylations of testosterone (Wood et al., 1983). These lines of evidence strongly suggest that the treatment of animals with highly toxic PCBs and PCDFs might cause a substantial change in the constitutive profile of the liver microsomal P-450s due to a selective induction and/or suppression of certain isoforms of P-450.

To prove this, Nagata et al. (1985a; 1985b) first attempted to purify and characterize the major forms of P-450 from the liver microsomes of 3,4,5,3′,4′-PenCB-treated rats, and then to evaluate their contributions to the microsomal metabolisms of testosterone and some xenobiotics. As shown in Fig. 6.2.7 and Tables 6.2.5 and 6.2.6, nine forms of P-450, including two identical isozymes, were purified from the livers of adult male Wistar rats untreated and treated with 3,4,5,3′,4′-PenCB (5 mg/kg, a single *ip* dose), MC (20 mg/kg, daily *ip* for 3 consecutive days) and PB (100 mg/kg, daily *ip* for 3 consecutive days). They included P-451 I and P-451 II from untreated rats, P-450 II and P-450 III from PB-treated rats, MC-P-448 L and MC-P-448 H from MC-treated rats and P-452, P-448 L and P-448 H from the PenCB-treated rats. Among them, MC-P-448 L and MC-P-448 H were indistinguishable from P-448 L and P-448 H, respectively, with regard to electrophoretic, spectral, catalytic and immunochemical properties. Based on a variety of criteria including the catalytic properties and the responsiveness to inducers, five of them were considered to possibly correspond to the following isozymes according to the newly recommended nomenclature (Nebert et al., 1987): P-451 II to P450 2C11, P-450 III to P450 2B2, P-452 to P450 2A1, P-448 L to P450 1A1 and P-448 H to P450 1A2.

Table 6.2.7 shows the catalytic activity of each form of purified P-450 toward testosterone in a reconstituted system. Furthermore, to assess their contribution to the microsomal metabolism, an immune complex inhibition for the regioselective metabolism of testosterone was examined using the liver microsomes from variously pretreated rats (data not shown) and the individual P-450 in these microsomes was quantitated by the radial immunodiffusion method (Table 6.2.8). As expected, the most dramatic change of the constitution of microsomal P-450

isozymes was exerted by treatment with a highly toxic 3,4,5,3′,4′-PenCB. In these rats, the liver microsomal level of P-450 II, a main constitutive form, was markedly decreased whereas P-452, a minor form in untreated rat liver microsomes, was induced by about 12-fold. Additionally, it should be noticed that almost all of the total P-450 in the PenCB-treated rat liver was occupied by only two isozymes, P-448 L and P-448 H, both of which are typical isozymes induced with MC (Ryan et al., 1979). A similar tendency was shown by treatment with MC, but to a much lesser extent. These characteristic natures of the PenCB, namely to induce not only P-452 to an unprecedentedly high level but also more abundantly P-448 H than P-448 L, was also independently demonstrated by Parkinson et al. (1983).

P-452 showed only a little activity toward the exogenous substrates tested (Table 6.2.6), but predominantly catalyzed the 7α-hydroxylation of testosterone (Table 6.2.7). Judging from the inhibitory effect of anti-P-452, it appears that the enhanced activity of the 7α-hydroxylation in the liver microsomes of the PenCB-treated rats is solely dependent on the P-452 induced. P-448 L, a low spin form induced with an MC-type inducer, showed the highest activity in the deethylation of 7-ethoxycoumarin and the hydroxylations of biphenyl and benzo[a]pyrene(AHH) (Table 6.2.6), which thus suggested that this isozyme must be representative of P-450s to catalyze AHH activity. Another major form with a high spin nature from the PenCB-treated rats, P-448 H, was less effective in the metabolism of 7-ethoxycoumarin and benzo[a]pyrene, but this form effectively catalyzed estradiol 2-hydroxylation and biphenyl 4-hydroxylation (Table 6.2.6). Furthermore, it is noteworthy that this isozyme (P450 1A2) is highly active in the metabolic activation of many carcinogenic arylamines including 2-acetylaminofluorene (Goldstein et al., 1984) and pyrolysates of amino acids such as Trp-P-2 and Glu-P-1 (Kamataki et al., 1983). P-448 L and P-448 H possessed low activities for 6β-hydroxylation of testosterone in the reconstituted system (Table 6.2.7), while the activities in the liver microsomes from either MC- or the PenCB-treated rats, which were still a main metabolic reaction in these microsomes, were almost completely insensitive to either anti-P-448 L or anti-P-448 H. This indicated that both isozymes did not participate in the microsomal 6β-hydroxylation. Another interesting property of P-448 H was its ability to bind tightly but not covalently 2,3,4,7,8-PenCDF with a molar ratio of 1 to 1 (Kuroki et al., 1986). This observation well agreed with the high retainability of the PenCDF in the liver of the PenCDF-treated rats (Kuroki et al., 1980; Yoshihara et al., 1981), which suggested that the induced P-448 H may function as the storage site of highly toxic PenCDF.

Consequently, the unique metabolic profile of testosterone shown by the liver microsomes from the PenCB-treated rats should be attributed to both the induction of P-452 as 7α-hydroxylase and the marked decrease of other constitutive forms

such as P-451 II and the still unisolated form(s) involved in the 2α-, 6β- and 16α-hydroxylations. These dramatic changes in the composition of liver microsomal P-450 in the PenCB-treated rats may also explain, at least in part, the marked increase of the activities of hydroxylations of benzo[a]pyrene and zoxazolamine, and the decrease of the activities of N-demethylations of BZ, aminopyrine and codeine (Ozawa et al., 1979).

It has already been shown that in hamsters, one of the most resistant animal species toward the toxicity of PCDDs and PCDFs, treatment with 2,3,4,7,8-PenCDF induced such liver enzymes as MFO and DT-diaphorase to a much less degree than in rats (Koga et al., 1989). A similar observation that AHH activity in the hamster liver was hardly induced by MC treatment has also been reported by Wroblewski et al. (1988). To obtain further knowledge of the properties of P-450 isozymes in the hamster liver, Koga et al. (1990) purified and characterized two forms of P-450 from the liver microsomes of the PenCDF-treated hamsters. These two forms, namely a high spin form hamster P-450 H and a low spin form hamster P-450 L, had the molecular masses of 52 and 54 KDa, respectively, and showed the absorbance maximum of the CO-reduced difference spectra at 446 nm. In a reconstituted system, both isozymes showed relatively low activities of AHH and O-deethylations of 7-ethoxyresorufin and 7-ethoxycoumarin, while they both effectively catalyzed 7α- and 2α-hydroxylations of testosterone. The immunochemical studies using antisera to each isozyme revealed that hamster P-450 H and P-450 L differ from each other and comprise about 61% and 31% of the total P-450 in the PenCDF-treated microsomes, respectively, and thus indicated that these are the PenCDF-inducible and major forms of P-450 in the PenCDF-treated hamster liver. In addition, MC, 3,4,5,3′,4′-PenCB and isosafrole also preferentially induced hamster P-450 H rather than hamster P-450 L, but β-naphthoflavone preferably increased hamster P-450 L. The results of analyses of NH_2-terminal amino acid sequence demonstrated that hamster P-450 H and hamster P-450 L correspond to rat P-448 H (P450 1A2) and P-452 (P450 2A1), respectively. The determination of the PenCDF level associated with the purified P-450s revealed that hamster P-450 H also binds the PenCDF more preferably than hamster P-450 L, but to a weaker degree than that of rat P-448 H (Kuroki et al., 1986).

It has already been shown that the administration of 3,4,5,3′,4′-PenCB to chickens caused a marked increase of P-450 content and AHH activity in the liver microsomes (Hokama et al., 1985). Two forms of P-450 were then purified from the liver microsomes of the PenCB-treated chickens and characterized (Hokama et al., 1988). Both chicken P-448 L, a low spin form, and chicken P-448 H, a high spin form, showed the absorption maximum of the CO-reduced difference spectra at 448 nm, and gave molecular masses of 56 and 54 KDa, respectively. Chicken P-

448 L exhibited an extremely high activity of 7-ethoxyresorufin O-deethylation and relatively high activities of AHH and testosterone 16α-hydroxylase, but no detectable activities of 7-ethoxycoumarin O-deethylation, *p*-nitroanisole O-demethylation and estradiol 2-hydroxylation. On the other hand, chicken P-448 H effectively catalyzed estradiol 2-hydroxylation and weakly testosterone 6β-hydroxylation. N-Demethylations of aminopyrine, BZ and ethylmorphine were modestly catalyzed by either form, but chicken P-448 L was a little more active than chicken P-448 H. The immunochemical studies revealed that the hemoproteins differ from each other while the amounts of chicken P-448 L and chicken P-448 H in the liver microsomes were negligible in untreated chickens, but comprised 82% and 7% of the total P-450 in the PenCB-treated chickens, respectively. These results suggest that both P-448s are the PenCB-inducible and chicken P-448 L is the major form in the PenCB-treated chicken liver.

Recently, the molecular mechanism of P450 1A1 induction with 2,3,7,8-TCDD, which is mediated by the Ah-receptor, has been progressively elucidated by many investigators. In essence, the postulated sequential mechanism is as follows (Gonzalez et al., 1993; Whitelaw et al., 1993): a) The latent Ah-receptor in cytosol, which is associated with a molecular shaperon heat shock protein (hsp) 90, is activated by liganding of 2,3,7,8-TCDD, and then releases hsp 90. b) The ligand-activated Ah-receptor subsequently interacts with the Arnt coregulator, namely Ah-receptor nuclear translocator, to form the ligand-bound Ah-receptor-Arnt complex. c) This heterodimeric complex translocates into the nucleus and interacts with a specific DNA sequence, termed XRE or DRE (xenobiotic or dioxin responsive element), located upstream of the P450 1A1 gene. d) Thereby, the expression of the P450 1A1 gene is activated and results in the induction of P450 1A1. According to this mechanism, it is highly possible that the MC-type PCBs and PCDFs such as 3,4,5,3′,4′-PenCB and 2,3,4,7,8-PenCDF can induce P450 1A1 (P-448 L) and P450 1A2 (P-448 H) in the rats and mice by acting as the ligand like 2,3,7,8-TCDD.

6.2.9. The Induction of Hepatic Enzymes Other than MFO and DT-diaphorase by PCBs and PCDFs

To fully understand the mechanism of toxicity caused by polyhalogenated aromatics such as PCDDs, PCDFs and PCBs, investigations on the biochemical responses other than the induction of MFO and DT-diaphorase are needed. Although there have been several reports concerning the effects of 2,3,7,8-TCDD on glutathione S-transferase (Baars et al., 1978), aldehyde dehydrogenase (Dietrich et al., 1977) and certain enzymes involved in an intermediary metabolism (Neal et al., 1979), only a few studies have been done with individual PCB and PCDF conge-

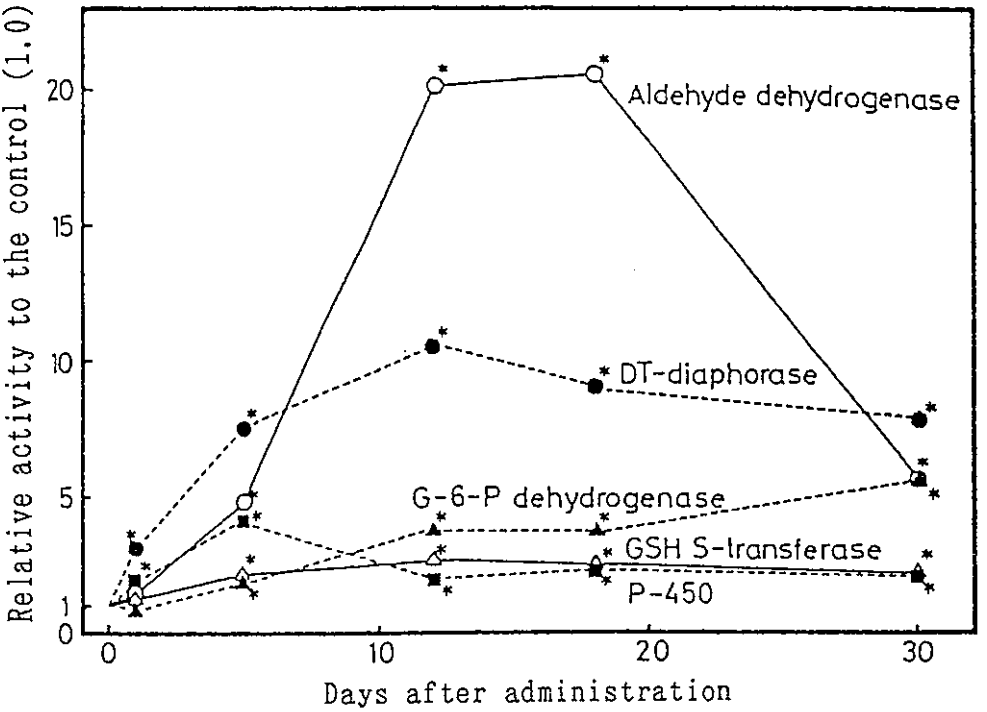

Fig. 6.2.8. The Time Course of Enzyme Induction after a Single Oral Administration of 3,4,5,3′,4′-PenCB in Rats.

3,4,5,3′,4′-PenCB was given orally at a dose of 0.5 mg/kg. Each point represents the relative activity to the control (1.0). *Significantly different from the control, $p < 0.05$.

Quoted from Koga et al. (1985).

ners.

At first, therefore, the inductive effects on some cytosolic enzymes were examined using the liver of rats treated with 3,4,5,3′,4′-PenCB and 2,4,5,2′,4′,5′-HCB, both of which are the representatives of either MC-type and PB-type or highly toxic and less toxic PCB congeners, respectively (Koga et al., 1985). As shown in Fig. 6.2.8, DT-diaphorase, glutathione S-transferase, aldehyde dehydrogenase and glucose-6-phosphate dehydrogenase were increased about 10-, 3- , 18- and 2-fold, respectively, at 12 days after the PenCB treatment at a single oral dose of 0.5 mg/kg. These inductions of the cytosolic enzymes were maximum at 12 days after the administration, in contrast to the microsomal enzymes such as P-450, which were induced maximally at day 5. The HCB treatment also induced these cytosolic enzymes, but the extent was much less than the PenCB.

With regard to the inductions of glutathione S-transferase (Baars et al., 1978) and aldehyde dehydrogenase (Deitrich et al., 1977), it has been demonstrated that

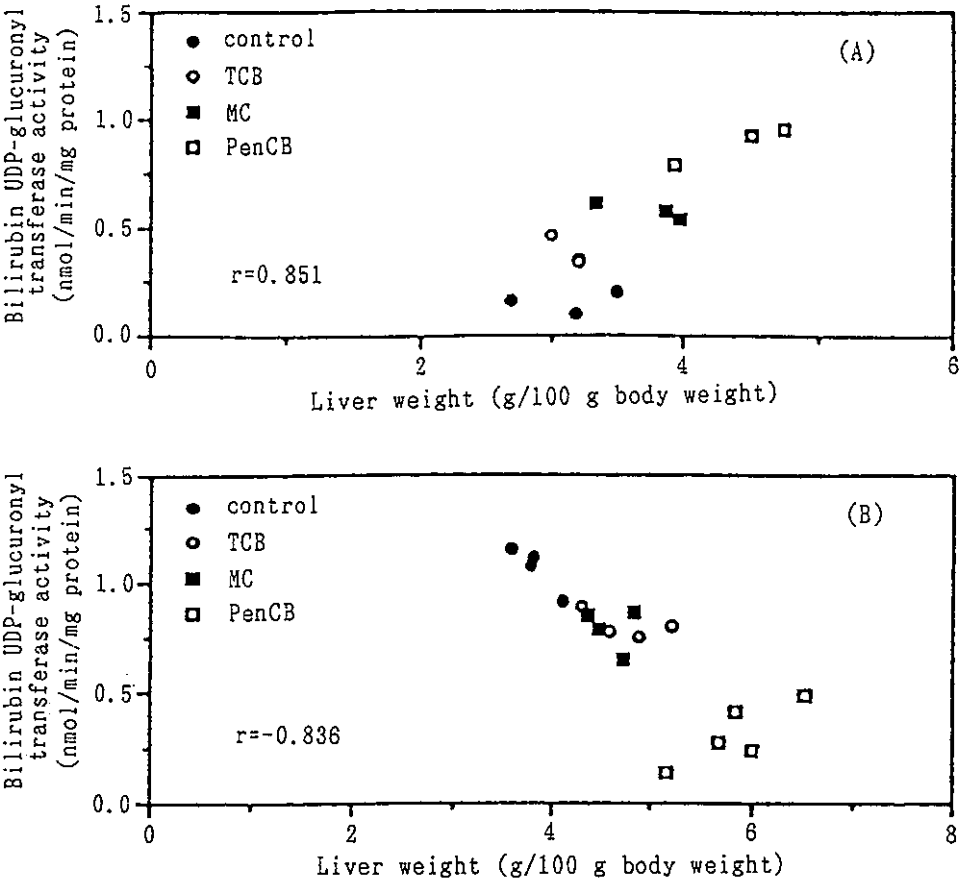

Fig. 6.2.9. The Correlation between Liver Hypertrophy and Bilirubin UDP-glucuronyl Transferase Inducibility Caused by MC, 3,4,3′,4′-TCB and 3,4,5,3′,4′-PenCB in Guinea Pigs (A) and Rats (B)
Quoted from Oguri et al. (1993).

the enzymes induced by PB differ from the enzymes induced by 2,3,7,8-TCDD in either of the respective enzymes. Accordingly, it is possible that the PenCB and the HCB also induce different isozymes of glutathione S-transferase and aldehyde dehydrogenase.

In connection with these isozyme specific induction of glutathione S-transferase, Aoki et al. (1992) reported an interesting observation that toxic co-planar PCB congeners, such as 3,4,5,3′,4′-PenCB and 3,4,5,3′,4′,5′-HCB, induced glutathione S-transferase P-form, a member of the glutathione S-transferase family, in primary cultured rat liver parenchymal cells, but not the less toxic non-co-planar PCBs such as 2,3,4,3′,4′-PenCB, 2,3,4,5,3′,4′- and 2,4,5,2′,4′,5′-HCBs. Glutathione S-transferase P-form is normally expressed in such types of tissue as the

placenta, lung and spleen, but not significantly in the liver (Kano et al., 1987). However, it becomes detectable in preneoplastic hepatic foci and hepatocarcinomas caused by chemical carcinogens (Satoh et al., 1985), and thereby is used as a pathological marker for these lesions (Sato, 1989).

On the other hand, glutathione peroxidase activity, a scavenger of lipid peroxide, was decreased only by 2,4,5,2′,4′,5′-HCB treatment, but the formation of lipid peroxide was not altered by either of the PCB congeners. This finding was not in consistent with the observation by Saito et al. (1981) who demonstrated an increase of lipid peroxide and a decrease of glutathione peroxidase activity in the liver of the rats fed a diet containing 0.05% PCB mixture.

In hamsters, glutathione S-transferase and aldehyde dehydrogenase in the liver cytosol were also moderately induced by 2,3,4,7,8-PenCDF treatment at a single dose of 0.5 mg/kg, but the activity of glucose-6-phosphate dehydrogenase was not changed (Koga et al., 1989). Recently, it was found that MC-type inducers such as MC, 3,4,3′,4′-TCB and 3,4,5,3′,4′-PenCB significantly induced bilirubin UDP-glucuronyl transferase activity in the guinea pig liver microsomes, but afforded any changes of DT-diaphorase activity and a small increase of AHH activity (Oguri et al., 1993; Koga et al., 1994). The highest induction was shown in the PenCB-treated animals (6-fold over the control), followed by MC-treatment (3-fold) and the TCB- treatment (2-fold). Furthermore, the inducibility of this activity appears to be correlated with the potency of toxicity (liver hypertrophy) caused by the MC-type inducers (Fig. 6.2.9). In contrast to the guinea pig, however, this activity in the rat was significantly decreased by these treatments. These results suggest that unlike rats, the inducibility of bilirubin UDP-glucuronyl transferase activity rather than AHH or DT-diaphorase may thus be a good parameter for the toxicity by PCBs and their related compounds in guinea pigs.

6.2.10. Possible Mechanisms of Toxicity Caused by PCBs and Related Compounds

Throughout this chapter, it has been repeatedly demonstrated that all the toxic congeners of PCBs and PCDFs exhibit a potent inducibility for hepatic MFO and DT-diaphorase as an MC-type inducer and *vice versa*. Furthermore, it has also been shown that especially in rats and mice, their potency as the MC-type inducer is well correlated with their toxic potency. Similar results have been reported in the cases of many toxic halogenated aromatics including PCDDs, PCDFs and PCBs by Poland et al. (1979). They also suggested that the first essential event to induce AHH must be a stereospecific interaction of these chemicals to a cytosolic protein, Ah-receptor. In addition, they further demonstrated that the structure-activity relationship for receptor binding is very similar to that for toxicity by these chemicals,

and that some toxic responses such as thymus atrophy segregate with the Ah-locus, the gene which determines the cytosolic receptor, in the Ah-responsive C57BL/6 and the Ah-nonresponsive DBA/2 mice.

Thereby, it was strongly suggested that, at least in these mice and some strains of rats, the toxicity by halogenated aromatic compounds is mediated through binding to the Ah-receptor. Nevertheless, the explanations as to how the induction of MFO or DT-diaphorase, *per se*, is involved in the toxic lesions remain far from clear. However, the following explanations might be accceptable at least in part. The first is a direct toxic effect through the disturbance of certain intermediary metabolism as well as xenobiotic metabolism. For example, the steroid metabolism was certainly disturbed by the extensive alteration of constitutive P450s due to the selective induction of certain isozymes and the concomitant suppression of others (Yoshihara et al., 1982; Nagata et al., 1985). In addition, in recent years P-450 has been recognized as an enzyme to catalyze the oxidation of arachidonic acid to epoxides (EETs) and monohydroxy-derivatives of eicosatetraenoic acids (HETEs) (Capdevila et al., 1981). Accordingly, the altered metabolism of arachidonic acid by P-450s induced or suppressed with 2,3,7,8-TCDD and 3,4,5,3′,4′- PenCB might contribute to the toxicity either directly or by the indirect perturbation of critical cellular processes (Rifkind et al., 1990; Huang and Gibson, 1991; 1992).

A second mechanism for the action of these toxic compounds is an indirect effect through the enhanced metabolic activation of certain xenobiotics to a toxic metabolite, which is catalyzed by particular forms of P-450. For example, P450 1A2 (P-448 H), which is a major isozyme induced by toxic 3,4,5,3′,4′-PenCB (Nagata et al., 1985a; 1985b), efficiently activates carcinogenic arylamines such as 2-acetylaminofluorene (Goldstein et al., 1984) and Trp-P-2 and Glu-P-1 (Kamataki et al., 1983) to the respective proximate forms. 3,4,5,3′,4′-PenCB also enhanced markedly N^4-hydroxylation of sulfanilamide in the kidney microsomes of rats, possibly leading to the kidney damage by this drug (Eyanagi et al., 1982). It should again be noted that DT-diaphorase, an inducible cytosolic enzyme with toxic MC-type PCBs as well as 2,3,7,8-TCDD, is known to catalyze the reduction of procarcinogen 4-NQO to a proximate carcinogen, 4-hydroxylaminoquinoline N-oxide and in fact a marked increase in the reduction of 4-NQO was observed in the liver, lung and skin of rats treated with 3,4,5,3′,4′-PenCB (Yoshimura et al., 1985).

On the other hand, several questions remain regarding the Ah-receptor mediated model for the toxicity by halogenated aromatic compounds (Poland and Knutson, 1982; Matsumura, 1994). Majors of those are as follows: a) The importance of the induction of MFO (AHH) activity, *per se*, in toxicity. Modulations of the metabolism of some vital endogenous substrates such as steroids, fatty acids and vitamins

cannot explain the entire spectrum of toxic responses. In addition, no detectable differences have been observed in the Ah-receptor and the MFO activity after treatment with 2,3,7,8-TCDD in Han-Wistar and Long-Evans rats, which differ markedly in their susceptibility to the lethal effects of the TCDD (Pohjanvirta et al., 1988). Thus, the induction of MFO and DT-diaphorase may be viewed as a signal response, but not implicated directly in the mechanism of toxicity. b) Concentration and affinity of the Ah-receptor. The binding affinity and concentration of the cytosolic receptor in the livers from the guinea pig, rat, responsive mouse (C57BL/6), rabbit and hamster are very similar despite a 5,000-fold difference in the LD_{50} for 2,3,7,8-TCDD between the guinea pig and hamster. c) Polycyclic hydrocarbons versus halogenated aromatic compounds. While both classes of compounds bind to the Ah-receptor and induce AHH activity, the characteristic spectrum of toxic responses are not observed with non-halogenated polycyclic aromatic hydrocarbons such as MC.

Subsequently, an alternative mechanism for the toxicity of co-planar PCBs and related compounds was proposed by Brouwer and van den Berg (1986). They demonstrated that the pretreatment of rats with 3,4,3′,4′-TCB, a prototype of MC-type PCBs, strongly reduces the serum vitamin A concentration, and that a hydroxy-metabolite of the TCB, which structurally resembles thyroxin, is closely associated with transthyretin, a thyroxin binding prealbumin, in the plasma of the rats. A similar reduction of the serum vitamin A level was observed in the mice treated with the toxic 3,4,5,3′,4′,5′-HCB as well as 3,4,3′,4′-TCB, but not with 2,4,3′,4′-TCB and 2,4,5,2′,4′,5′-HCB, non-toxic congeners (their unpublished observation). Considering the functions of transthyretin not only as a member of the plasma retinol transport system, but also as a carrier of thyroid hormones, they thus postulated a model for the mechanism, in which the binding of a TCB metabolite to transthyretin causes interference of the serum vitamin A and thyroxin transports and thus results in a distortion of the physiological function of transthyretin. This may help to explain the existence of a few characteristic toxicological lesions including wasting syndrome by toxic PCBs and related compounds. However, questions regarding the binding properties of transthyretin to other halogenated aromatic compounds and/or metabolites and the subsequent interference in thyroid hormone metabolism, still remain to be solved.

Recently, based on the evidence that 2,3,7,8-TCDD treatment causes a spectacular rise in both cAMP-independent and -dependent protein kinases in the hepatic plasma membrane of rats, an additional mechanism, termed the "protein phosphorylation pathway", was presented by Matsumura (1994). The hypothetical scheme proposed is as follows: The TCDD binding to the Ah-receptor complex in cytosol, consisting of hsp, a protein kinase SRC and Ah-receptor, frees the SRC

and thereby activates its own protein kinase activity. This activation of SRC acts as the trigger for the well-defined "growth factor signal transduction" activities through RAS protein and MAP kinase activation to eventually phosphorylate nuclear transcriptional factors, such as AP-1. The latter factors, in turn, activate "immediate early" genes. The activation of SRC in cytosol could simultaneously result in direct phosphorylation actions on other important proteins in the cytosol, plasma membrane (e.g. growth factor receptors) and other intracellular organelles to cause their functional changes. By this scheme, he pointed out the possibility that at least some part of the action of the TCDD mediated by the interaction with Ah-receptor does not require its direct transcriptional activation step for the inducible enzymes such as P450 1A1, P450 1A2 and DT-diaphorase via XRE.

Finally, it can be said that only plausible and partial mechanism(s) for the toxicity produced by polyhalogenated aromatic hydrocarbons can be suggested at present, while more precise and comprehensive mechanism(s) still remain to be elucidated.

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