[Chem. Pharm. Bull. 26(4)1215—1221(1978)]

UDC 547.622.09 : 577.159.04

Inductive Effect of Polychlorinated Biphenyls Mixture and Individual Isomers on the Hepatic Microsomal Enzymes[1)]

Hidetoshi Yoshimura, Naoki Ozawa, and Seitaro Saeki

Faculty of Pharmaceutical Sciences, Kyushu University[2)]

(Received September 19, 1977)

The inductive effect of Kanechlor 400 (KC-400), the Japanese polychlorinated biphenyl (PCB) preparation containing 48% chlorine, and several individual PCB isomers on the hepatic microsomal enzymes of rats was investigated. Pretreatment with KC-400 increased significantly the activity of microsomal aminopyrine (AM) demethylase, aniline (AN) hydroxylase and NADPH-cytochrome *c* reductase, and the content of cytochromes P-450 and b_5 just like phenobarbital (PB)-pretreatment. However, it afforded the CO-difference spectrum revealing the peak at 448 nm same as pretreatment with 3-methylcholanthrene (MC). The inhibitory effect of SKF 525-A and 7,8-benzoflavone on AM demethylation and AN hydroxylation, respectively, in KC-400-induced microsomes also resembled that in microsomes induced by PB plus MC. Further studies using individual PCBs indicated that these compounds were divided into two groups; namely, 4,4′-dichlorobiphenyl (DCB), 2,5,2′,5′- and 2,4,3′,4′-tetrachlorobiphenyl (TCB) were categorized as PB-type, whereas the other group including 3,4,3′,4′-TCB, 3,4,5,3′,4′-pentachlorobiphenyl (PenCB) and 3,4,5,3′,4′,5′-hexachlorobiphenyl (HCB) was categorized as MC-type inducers. Decachlorobiphenyl, the completely chlorinated biphenyl derivative, was found to belong to PB-type. These conclusions were further supported by a spectral study with hexobarbital, which induced type I spectral changes with microsomes from control and 2,4,3′,4′-TCB-treated rats, and caused modified type II spectral change with microsomes from 3,4,5,3′,4′-PenCB-treated rats. Considering these results with individual PCBs, it can be assumed that chlorination of both of the *para*-(4,4′) and two of the *meta*-positions(3,3′ or 5,5′) of biphenyl is a minimum requirement for the structure to induce cytochrome P-448.

Keywords——Kanechlor-400; individual PCBs; drug-metabolizing enzymes; enzyme induction; induction type; rat liver microsomes

Polychlorinated biphenyls (PCBs) are wide-spread industrial pollutants, and numerous investigations on the biological effects have been made using commercial PCB preparations[3–6)] For example, PCBs induce a variety of hepatic microsomal enzymes.[3,7–16)] Inducers of these

1) Presented to the Third Symposium on Environmental Contaminants and their Toxicology, Chiba, Japan, Oct., 1976; Abstract of papers, p. 15.
2) Location: *3–1–1 Maidashi, Higashi-ku, Fukuoka.*
3) R.W. Riesebrough, P. Rieche, D.B. Peakall, S.G. Herman, and M.N. Kirven, *Nature* (London), **220**, 1098 (1968).
4) L. Fishbein, *J. Chromatogr.*, **68**, 345 (1972).
5) R.D. Kimbrough, *Arch. Environ. Health*, **25**, 125 (1972).
6) K. Higuchi(ed.), "PCB Poisoning and Pollution," Kodansha Ltd., Tokyo and Academic press, New York, 1976.
7) J.A. Goldstein, P. Hickman, and D.L. Jue, *Toxicol. Appl. Pharmacol.*, **27**, 437 (1974).
8) W. Grote, A. Schmoldt, and H.F. Benthe, *Acta Pharmacol. et Toxicol.*, **36**, 215 (1975).
9) J.V. Bruckner, K.L. Khanna, and H.H. Cornish, *Fd. Cosmet. Toxicol.*, **12**, 323 (1974).
10) J.L. Lincer and D.B. Peakall, *Nature* (London), **228**, 783 (1970).
11) S. Fujita, H. Tsuji, K. Kato, S. Saeki, and H. Tsukamoto, *Fukuoka Acta Med.*, **62**, 30 (1971).
12) D.C. Villeneuve, D.L. Grand, W.E.J. Phillip, M.L. Clark, and D.J. Clegg, *Bull. Environ. Contam. Toxicol.*, **6**, 120 (1971).
13) D.R. Bickers, L.C. Harber, A. Kappas, and A.P. Alvares, *Res. Commun. Chem. Path. Pharmacol.*, **3**, 505 (1972).
14) C.L. Litterst, T.M. Farber, A.M. Baker, and E.J. Van Loon, *Toxicol. Appl. Pharmacol.*, **23**, 112 (1972).
15) A.P. Alvares, D.R. Bickers, and A. Kappas, *Proc. Nat. Acad. Sci. USA*, **70**, 1321 (1973).
16) G.J. Johnstone, D.J. Ecobichon, and O. Hutzinger, *Toxicol. Appl. Pharmacol.*, **28**, 66 (1974).

hepatic microsomal enzymes have been divided into two main groups. Phenobarbital (PB) is a prototype of one group and 3-methylcholanthrene (MC) is the representative of another group of inducers. The former stimulates the metabolism of a large variety of compounds and the latter enhances the metabolism of more limited groups of substrates.[17] In addition, PB induces the formation of cytochrome P-450 which differs in spectral and catalytic activities from cytochrome P-448 present in liver microsomes from MC-treated rats.[18] Alvares, *et al.*[15] reported in 1973 that pretreatment of rats with Aroclor 1254, the commercial PCB preparation containing 54% chlorine, increased the rate of metabolism of many drugs and chemicals similarly to PB treatment. However, Aroclor 1254 also shifted the peak of the CO-difference spectrum to a value intermediate between those of MC and PB treated microsomes, and like MC, it altered the shape of the ethylisocyanide difference spectrum and induced benzpyrene metabolism. Based on these data, they suggested that Aroclor 1254-induced cytochrome P-448 might be catalytically different from the MC-induced cytochrome P-448 or that the hemoprotein(s) induced by Aroclor 1254 might be a mixture of cytochrome P-448 and P-450 exhibiting catalytic properties of both cytochromes. Since Aroclor 1254 is a mixture of various PCB isomers, it may consist of two groups of isomers, which exert either PB- or MC-type inductive effects.

Recently many studies have been also made with pure PCB isomers,[11,16,19–21] and several pure PCBs, such as 4,4′-dichlorobiphenyl (DCB), 2,5,2′,5′- and 2,4,2′,4′-tetrachlorobiphenyl (TCB), have been shown to enhance a large variety of drug-metabolizing enzyme activities. On the other hand, pretreatment of rats with 3,4,3′,4′-TCB was reported not to shorten, but to significantly prolong the pentobarbital sleeping time, suggesting that this TCB may have the properties of MC type inducers.[16] Although it was rather contradictory, Alvares and Kappas[22] reported that 2,4,5,2′,4′,5′- and 2,3,4,2′,4′,5′-hexachlorobiphenyl (HCB) had inducing properties similar to those of Aroclor 1254, namely, each of these individual isomers had inductive properties resembling both PB and MC. Quite recently, Goldstein, *et al.*[23] reported that PCB isomers falls into two distinct groups, that is, 2,4,5,2′,4′,5′-, 2,3,4,2′,3′,4′- and 2,4,6,2′,4′,6′-HCB were PB-type inducers, and 3,4,3′,4′-TCB and 3,4,5,3′,4′,5′-HCB were 3,4-benzpyrene-type (namely MC-type).

This report prompted us to publish our own results leading to the same conclusion which has been obtained independently and by different methods, using Kanechlor 400 (KC-400), the Japanese PCB preparation containing 48% chlorine, and several pure PCB isomers. Evidence is presented (1) that KC-400 is also a mixed type inducer same as Aroclor 1254 and (2) that KC-400 consists of two groups of components, each of which is categorized to either PB- or MC-type of the inducers.

Experimental

Materials——KC-400 was supplied by the Ministry of Health and Welfare of Japan. 4,4′-DCB and decachlorobiphenyl (DecaCB) were purchased from Nakarai Chemicals, Ltd., Tokyo and Wako Pure Chemical Ind., Ltd., Tokyo, respectively. 2,5,2′,5′-, 2,4,3′,4′- and 3,4,3′,4′-TCB were prepared according to the methods described in the previous papers.[24] 3,4,5,3′,4′-PenCB (mp 136—137°) was obtained as a by-product

17) A.H. Conney, *Pharmacol. Rev.*, **19**, 317 (1967).
18) N.E. Sladek and J.G. Mannering, *Biochem. Biophys. Res. Commun.*, **24**, 668 (1966).
19) J.R. Allen, L.A. Cartens, L.J. Abrahamson, and R.J. Marlar, *Environ. Res.*, **9**, 265 (1975).
20) J. Örberg, *Acta Pharmacol. et Toxicol.*, **39**, 145 (1976).
21) M.D. Stonard and J.B. Greig, *Chem.-Biol. Interactions* **15**, 365 (1976).
22) A.P. Alvares and K. Kappas, *Hoppe-Seyler's Z. Physiol. Chem.*, **357**, 1019 (1976).
23) J.A. Goldstein, P. Hickman, H. Bergman, J.D. McKinney, and M.P. Walker, *Chem.-Biol. Interactions*, **17**, 69 (1977).
24) S. Saeki, A. Tsutsui, K. Oguri, H. Yoshimura, and H. Hamana, *Fukuoka Acta Med.*, **62**, 20 (1971); H. Yoshimura, H. Yamamoto, and K. Yonezawa, *ibid.*, **66**, 555 (1975).

in the synthesis of 2,3,4,3′,4′-PenCB.[25] 3,4,5,3′,4′,5′-HCB (mp 211—212°) was synthesized from 3,5,3′,5′-tetrachlorobenzidine, mp 226°, stirring with $NaNO_2$ and Cu-powder in about 20% HCl. These individual PCBs were exhaustively purified by alumina column chromatography, sublimation or recrystallization, and their high purity was proved by gas–liquid chromatograph equipped with an electron capture detector (column: 4 mm×1.7 m; column packing: 1.5% OV-1 on Chromosorb W; carrier gas: N_2).

NADPH, NADH, NADP, glucose-6-phosphate (Na salt), glucose-6-phosphate dehydrogenase (E.C. 1,1,1,49; Type XI) and cytochrome *c* were purchased from Sigma Chemical Company. Pregnenolone-16α-carbonitrile (PCN) was a generous gift from Upjon Company.

Pretreatment of Animal——Male Wistar rats weighing 90—142 g were used, and they were given a commercial rat chow, CE-2 (Nippon Crea Co., Ltd., Tokyo), and water *ad libitum*. PB (Na salt), dissolved in physiological saline, was injected subcutaneously into rats once a day for two days at a dose of 90 mg/kg and these rats were killed for preparation of liver microsomes 24 hr after the last dose. MC, dissolved in corn oil, was administered intraperitoneally at a single dose of 40 mg/kg 48 hr prior to sacrifice. PCN, dissolved in water containing Tween 80 (one drop to 10 ml water), was administered orally at a dose of 40 mg/kg, five times at 12 hr intervals.[26] The treated animals were sacrificed 24 hr after the last dose. KC-400 and pure PCBs were dissolved in corn oil and administered intraperitoneally. The dose of PCBs was noted in the legends of figures and tables. A group of 3—7 rats were submitted to each treatment and fasted for 18 hr prior to sacrifice.

Preparation of Microsomes——Rats were sacrificed by decapitation and the livers weighed. They were perfused with 1.15% KCl, minced and then homogenized with three volumes of the KCl solution in a Potter–Elvehjem type glass homogenizer equipped with teflon pestle. The homogenate was then centrifuged at 9000×*g* for 20 min and the resultant supernatant fraction was recentrifuged at 105000×*g* for 1 hr. The pellet surface was washed with 0.1 M phosphate buffer (pH 7.4), suspended in the same buffer at a concentration of about 5 mg protein per ml by gentle homogenization and used for the assays.

Assays for Enzyme Activity——Aminopyrine (AM) demethylation and aniline (AN) hydroxylation activities were assayed incubating the reaction mixture consisted of NADPH-generating system (0.33 mM NADP, 8 mM glucose-6-phosphate, 6 mM $MgCl_2$ and 0.1 unit glucose-6-phosphate dehydrogenase), 0.1 mM EDTA, 0.2 ml microsomal suspension (about 1 mg protein), 1 mM substrate (aniline or aminopyrine) and 80 mM phosphate buffer (pH 7.4) to make a final volume of 1.0 ml, at 37° for 15 min. In some experiments, SKF 525-A (final concentration of 75 μM) or 7,8-benzoflavone (10 μl of 1 mM solution in ethanol) was added. Nothing was added to controls. Activities of AM demethylation, AN hydroxylation and NADPH-cytochrome *c* reduction were measured by determining formaldehyde,[27] *p*-aminophenol[28] and the reduced cytochrome *c*,[29] respectively. Contents of cytochromes P-450 and b_5 were measured by the method of Omura and Sato.[30] The hexobarbital difference spectra were determined in a following manner; each cuvette contained microsomal suspension (1.67 mg protein/ml) and 67 mM phosphate buffer (pH 7.4). Hexobarbital, dissolved in a little excess equivalent of 0.1 N NaOH, was added to the test cuvette in a final concentration of 15 mM. The difference spectra induced by this drug were recorded between 360—440 nm. Protein concentration was determined by the method of Lowry, *et al.*[31]

Results

Prior to examining the effect of KC-400, the effect of pretreatment of rats with MC, PB, MC plus PB, and PCN on the activity of hepatic microsomal AM demethylase, AN hydroxylase and NADPH-cytochrome *c* reductase, and on the content of cytochromes P-450 and b_5 were reconfirmed for the comparison. The results are shown in Table I. As already reported,[17] PB caused significant induction of all of these enzymes, whereas MC induced only AN hydroxylase and cytochrome P-450. An additive effect was observed when rats were treated simultaneously with PB and MC; that is, AN hydroxylase activity and cytochrome P-450 content were strikingly elevated. Although both inducers increased the cytochrome P-450 level, the CO-difference spectra showed absorption maxima at 448 and 450 nm, respectively,

25) H. Yamamoto, H. Yoshimura, M. Fujita, and T. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **24**, 2168 (1976).
26) A.Y.H. Lu, A. Somogyi, S. West, R. Kuntzman, and A.H. Conney, *Arch. Biochem. Biophys.*, **152**, 457 (1972).
27) T. Nash, *Biochem. J.*, **55**, 416 (1975).
28) Y. Imai, A. Ito, and R. Sato, *J. Biochem.*, **60**, 417 (1966).
29) C.H. Williams, Jr. and H. Kamin, *J. Biol. Chem.*, **237**, 587 (1962).
30) T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2379 (1964).
31) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

Table I. Effect of Pretreatment with 3-Methylcholanthrene (MC), Phenobarbital (PB), MC plus PB and Pregnenolone-16α-carbonitrile (PCN) on the Activity of Rat Liver Microsomal Drug-metabolizing Enzymes

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)]
PCN	213± 2[a)]	127±1[a)]	165± 3[a)]	118± 8	144±1[a)]

The value represents percent of the untreated (mean ±SE from 7 animals). The activity of aminopyrine demethylation (AM) and aniline hydroxylation (AN) in untreated rats was 45.1 and 10.3 nmol of the metabolites/mg protein/15 min, respectively. The content of cytochromes P-450 and b_5 in untreated rats was 0.50 and 0.19 nmol/mg protein, respectively. Activity of NADPH-cytochrome *c* reductase in untreated rats was 0.21 unit/mg protein. The other experimental details were described in Experimental.
a) Significantly different from the Untreated ($p < 0.05$).

by pretreatment with MC and PB. Simultaneous treatment of rats with PB and MC afforded the CO-difference spectrum revealing the peak at 448—449 nm. PCN, which was reported as a new type of inducer of drug-metabolizing enzymes in liver,[16] enhanced AM demethylase activity greatly, whereas AN hydroxylation was stimulated only a little (27% over the control value). PCN significantly increased NADPH-cytochrome *c* reductase activity but not cytochrome b_5 content.

As indicated in Fig. 1, KC-400 was found to be a potent inducing agent. This substance enhanced AM demethylase activity and therefore could not be catagorized to the MC-type of inducers. On the other hand, KC-400 induced cytochrome P-448, showing that KC-400 was not a PB-type inducer. Although a single dose of KC-400 (300 mg/kg) has maximum

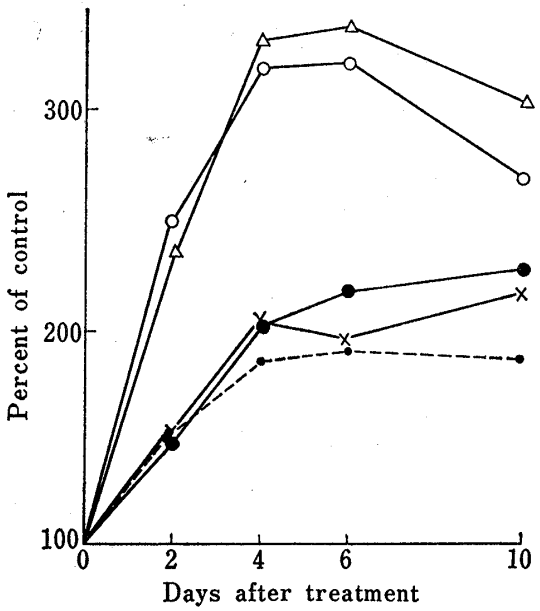

Fig. 1. Time Course of Effect of KC-400 Pretreatment (a single dose of 300 mg/kg) on the Activity of Rat Liver Microsomal Drug-metabolizing Enzymes

The value represents percent of the Untreated (control). The activity of AM demethylation and AN hydroxylation in untreated rats was 71.1±7.8 and 10.5±0.9 nmol of metabolites/mg protein/15 min, respectively. The content of cytochromes P-450 and b_5 in untreated rats was 0.60±0.03 and 0.26±0.01 nmol/mg protein, respectively. The activity of NADPH-cytochrome *c* reductase in untreated rats was 0.17±0.01 unit/mg protein. —△—, AN hydroxylation; —○—, cytochrome P-450; —●—, cytochrome b_5; —×—, AM demethylation; ---●---, NADPH-cytochrome *c* reduction.

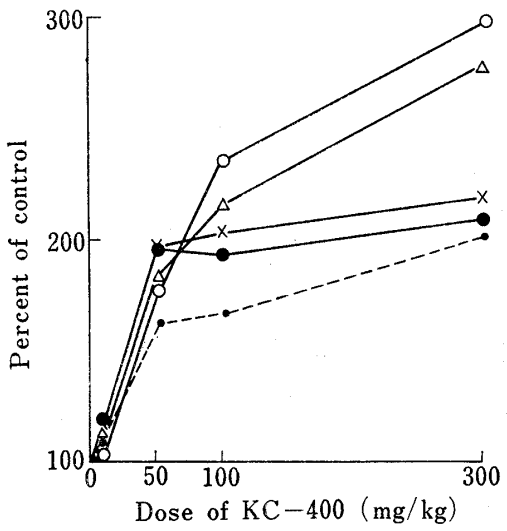

Fig. 2. Dose-Response Relationship of KC-400 Pretreatment on the Activity of Rat Liver Microsomal Drug-metabolizing Enzymes

Experimental details were the same as those described in Fig. 1.

inductive effect at 4—6th days, the increase patterns of these tested enzymes appear somewhat different. Cytochrome P-450 content and AN hydroxylase activity increased more than 3-fold at 4—6 days after the treatment, but these inductive effects decreased considerably at the 10th day. On the contrary, cytochrome b_5 content and the activity of AM demethylase and NADPH-cytochrome *c* reductase increased about 2-fold at 4—6 days after the treatment, and the increases were maintained through the 10th day.

Dose-response relationships of these enzymatic parameters are shown in Fig. 2. The increase patterns of the enzymatic parameters were quite similar to those described in Fig. 1. Both AN hydroxylase activity and cytochrome P-450 content increased dose-dependently up to a dose of 300 mg/kg, whereas activity of AM demethylase and cytochrome b_5 content reached nearly the maximum plateau at a dose of 50—300 mg/kg. These effects of KC-400 resembled that of simultaneous administration of PB and MC mentioned in Table I.

Table II. Inhibition of Aminopyrine Demethylation (AM) by SKF 525-A and of Aniline Hydroxylation (AN) by 7,8-Benzoflavone after Various Pretreatment of Rats

Pretreatment	Percent inhibition	
	AM	AN
Untreated	$80.2\pm0.8^{b)}$	$49.1\pm1.1^{b)}$
MC	$53.7\pm1.0^{a)}$	$83.8\pm0.3^{a)}$
PB	$78.9\pm0.8^{b)}$	$49.3\pm1.5^{b)}$
MC+PB	$61.7\pm1.1^{a,b)}$	$76.9\pm0.6^{a,b)}$
PCN	$85.5\pm0.3^{a,b)}$	$27.6\pm1.1^{a,b)}$
KC-400	$63.3\pm1.2^{a,b)}$	$80.8\pm1.0^{a,b)}$

The value represents percent inhibition (mean ±SE from 7 rats). Activity in control microsomes were the same as those described in Table I. KC-400 (100 mg/kg) was administered *ip* to rats once a day for 3 days, and animals were sacrificed 5 days after the first treatment.
a) Significantly different from the untreated ($p<0.05$).
b) Significantly different from the MC-treated ($p<0.05$).

In order to further characterize the inducing effect of PCBs, the inhibitors of mixed-function oxygenases, SKF 525-A and 7,8-benzoflavone, were utilized, because 7,8-benzoflavone is a very effective inhibitor for monooxygenation in MC-treated rat liver microsomes (MC-microsomes) and less inhibitory in microsomes from control and PB-treated rats (PB-microsomes),[32] whereas the inhibition by SKF 525-A was effective to monooxygenation in PB-microsomes.[33] As summarized in Table II, the percent inhibition of AM demethylation by SKF 525-A in control and PB-microsomes was not significantly different from each other, whereas the percent inhibition in MC-microsomes was significantly less than that in control or PB-microsomes. The percent inhibition in microsomes from rats simultaneously pretreated with PB and MC (PB plus MC-microsomes) situated between the values in PB- and MC-microsomes. AM demethylase activity in PCN-induced microsomes was more inhibited by SKF 525-A than that in control microsomes. 7,8-Benzoflavone inhibited very strongly AN hydroxylation in MC-microsomes and also in PB plus MC-microsomes, but the effect was less inhibitory in control and PB-microsomes and was weakest in PCN-microsomes. Very interestingly, KC-400-induced microsomes behaved same as PB plus MC-microsomes. In this table, the inhibition for AN hydroxylase represents the sum of inhibition by 7,8-benzoflavone and ethanol, since ethanol, which was added as the solvent of 7,8-benzoflavone to the incubation mixture, is known to inhibit microsomal AN hydroxylase to some extent.

32) V. Ullrich, U. Frommer, and P. Weber, *Hoppe-Seyler's Z. Physiol. Chem.*, 345, 514 (1973).
33) G.J. Mannering, "Handbook of Experimental Pharmacology, Vol. 28, Concepts in Biochemical Pharmacology, Part II," ed. by B.B. Brodie and J.R. Gillette, Springer-Verlag, New York, 1971, p. 452.

Table III. Effect of 4,4′-DCB, 2,5,2′,5′-TCB, 2,4,3′,4′-TCB, DecaCB and KC-400 on the Activity of Rat Liver Microsomal Drug-metabolizing Enzymes

Pretreatment	AM	AN	P-450	b_5	NADPH-cyt *c* reductase
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]

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. Value represents percent of the untreated (mean±SE from 3—7 animals). The activity of aminopyrine demethylation (AM) and aniline hydroxylation (AN) and the contents of cytochromes P-450 and b_5 in untreated rats were the same value as described in Table I.
a) Significantly different from the untreated ($p < 0.05$).

Table IV. Inhibition of Aminopyrine Demethylation (AM) by SKF 525-A and of Aniline Hydroxylation (AN) by 7,8-Benzoflavone in Liver Microsomes from Pure PCB Isomers-treated Rats

Pretreatment	No. of rats	Percent inhibition	
		AM	AN
Untreated	7	80.2±0.8[b]	49.1±1.1[b]
4,4′-DCB	4	81.1±0.6[b]	46.6±0.3[b]
2,5,2′,5′-TCB	4	80.2±0.4[b]	45.7±1.0[b]
2,4,3′,4′-TCB	4	77.6±1.1[b]	52.4±1.4[b]
3,4,3′,4′-TCB	3	51.6±2.9[a]	82.0±1.9[a]
3,4,5,3′,4′-PenCB	3	54.1±0.6[a]	83.7±0.9[a]
DecaCB	4	77.0±0.1[b]	48.1±0.5[b]
PB	7	78.9±0.8[b]	49.3±1.5[b]
MC	7	53.7±1.0[a]	83.8±0.3[a]

Experimental details were the same as those described in Table III. Values represent percent inhibitions (means ±SE) by each inhibitor.
a) Significantly different from the Untreated ($p < 0.05$).
b) Significantly different from the MC-treated ($p < 0.05$).

Inductive effect of individual PCBs contained in KC-400 and of 3,4,5,3′,4′-PenCB, 3,4,5,-3′,4′,5′-HCB and decachlorobiphenyl (DecaCB) on hepatic drug-metabolizing enzymes of rats is shown in Table III. 2,4,3′,4′-TCB, 2,5,2′,5′-TCB and 4,4′-DCB increased the activity of AM demethylase, AN hydroxylase and NADPH-cytochrome *c* reductase, and the content of cytochrome P-450 and b_5 as well as KC-400. DecaCB showed the similar effect. Among these four PCBs, 2,4,3′,4′-TCB was the most active inducing agent, followed by 4,4′-DCB, and then DecaCB and 2,5,2′,5′-TCB. On the contrary, pretreatment of rats with 3,4,3′,4′-TCB increased only AN hydroxylase activity and cytochrome P-450 content. The highly toxic 3,4,5,3′,4′-PenCB and 3,4,5,3′,4′,5′-HCB,[34] at a very low single dose, also afforded similar results.

Table IV shows percent inhibition of AM demethylation by SKF 525-A and of AN hydroxylation by 7,8-benzoflavone in microsomes induced by these PCB isomers. Among them,

34) The 103rd Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Nagasaki, July 1977.

the percent inhibitions in microsomes from 4,4′-DCB-, 2,5,2′,5′-TCB-, 2,4,3′,4′-TCB- and DecaCB-treated rats were not different from those in control microsomes and PB-microsomes, suggesting that these PCB isomers are PB-type inducers. On the other hand, the values in microsomes from rats pretreated with 3,4,3′,4′-TCB, 3,4,5,3′,4′-PenCB or 3,4,5,3′,4′,5′-HCB were the same as those in MC-microsomes. These results lead to the conclusion that this group of PCB isomers is MC-type of inducers.

As the representatives of PB- and MC-type components of PCBs, 2,4,3′,4′-TCB and 3,4,5,3′,4′-PenCB were selected and their effect of pretreatment on the microsomal hexobarbital-induced spectral change was examined. Fig. 3 demonstrates the results together with those obtained by microsomes from control, PB- and MC-treated rats. The addition of high concentration of hexobarbital (15 mM) to microsomes from 3,4,5,3′,4′-PenCB-treated rats resulted in the difference spectrum of modified type II as well as those from MC-treated rats, although control, PB-microsomes and 2,4,3′,4′-TCB-treated microsomes showed type I spectral change. These changes also supported that 2,4,3′,4′-TCB was a PB-type of inducers while 3,4,5,3′,4′-PenCB was a MC-type.

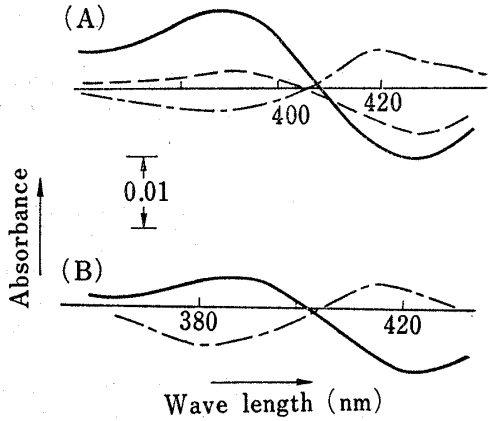

Fig. 3. Hexobarbital-Difference Spectra of Microsomes from Rats Untreated and Treated with 3,4,5,3′,4′-PenCB (2 mg/kg), 2,4,3′,4′-TCB (100 mg/kg), PB or MC

Experimental details were described in Methods.
(A), ——, MC; – – –, Untreated; ——, PB.
(B), ——, 3,4,5,3′,4′-PenCB; ——, 2,4,3′,4′-TCB.

Discussion

Until now several studies have been made on the inductive effect of PCB mixtures[3,7–16] and their individual components[11,16,19–21] on the hepatic drug-metabolizing enzyme systems of rats. We also found by the present investigation that KC-400, one of the commercial PCB preparations in Japan, is a potent inducer of above enzyme systems, and their characteristics as the inducer are categorized to neither PB- nor MC-type, but a mixed type. Further studies using several individual PCB isomers presented evidence supporting the idea that these compounds should be divided into two groups; namely, one group (4,4′-DCB, 2,5,2′,5′- and 2,4,3′,4′-TCB and DecaCB) was categorized to PB-type, inducing all the parameters examined (Table III), whereas the other group (3,4,3′,4′-TCB, 3,4,5,3′,4′-PenCB and 3,4,5,-3′,4′,5′-HCB) was categorized to MC-type, inducing only AN hydroxylase and cytochrome P-448 (Table III). For these characterization, not only the inductive profile of the liver microsomal drug-metabolizing enzyme systems, but also inhibition profile of AM demethylase and AN hydroxylase in the PCBs-induced microsomes by SKF 525-A and 7,8-benzoflavone were effectively utilized. Quite recently, Goldstein, *et al.*[23] independently came to the same conclusion with ours that PCB isomers falled into two distinct groups of inducers and reported that biphenyls chlorinated symmetrically in both the *meta*-and *para*-positions (3,4,3′,4′-TCB and 3,4,5,3′,4′,5′-HCB) increase the formation of cytochrome P-448 and those chlorinated in both the *para*- and *ortho*-positions (2,4,2′,4′-TCB, 2,4,5,2′,4′,5′-, 2,3,4,2′,3′,4′- and 2,4,6,2′,4′,6′-HCB) induce the formation of cytochrome P-450 rather than P-448. Our results further indicated that certain biphenyl chlorinated unsymmetrically (3,4,5,3′,4′-PenCB) was also MC-type inducer. It is very interesting to note that the PCBs possessing inductive property of MC-type such as 3,4,3′,4′-TCB, 3,4,5,3′,4′-PenCB and 3,4,5,3′,4′,5′-HCB exert much higher acute toxicity in rats than the PB-type inducers such as 4,4′-DCB, 2,5,2′,5′-TCB, 2,4,3′,4′-TCB and DecaCB.[34] These findings will be reported elsewhere in detail.