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Induction of Liver Microsomal Drug Metabolism by Polychlorinated Biphenyls Whose Gaschromatographic Profile Having Much in Common with that in Human Milk

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Polychlorinated biphenyls(PCBs),well-known environmental pollutants, are inducers of liver microsomal monooxygenase systems(Fijita et al,1971; Bickers et al,1972; Alvares et al,1973; Shimada et al,1976; Shimada and Sato, 1976). Recent evidence has shown that the enzyme induction following the PCBs treatment is the most sensitive parameter among the biological and toxic responces in mammals. Litterest et al(1972) have reported that in rats fed diets containing PCBs varing in chlorine from 42% to 60% for 4 weeks, nitroreductase activity of liver microsomes is significantly stimulated with increasing chlorine content of the PCBs, and the induction is occured at level as low as 0.5ppm for the all mixtures. Chen and DuBois(1973) have also demonstrated that at a dietary level of 1ppm of Aroclor-1260(an PCB mixture), there is a significant increase in aminopyrine N-demethylase activity of the liver to the 61% increase in rats.

The possible health hazard to human presented by PCBs is as yet obscure, but a number of reports on the presence of the PCBs in human milk raise questions as to the effect of these tissue contaminants on human health.

The purpose of the present study was to characterize the subacute effect of lower oral dosing of PCBs, which is similar in composition to that in human milk of Japanese women, on parameters associated with hepatic microsomal drug metabolism in rats.

MATERIALS AND METHODS

Gaschromatographic(GC) analysis of PCBs was done essentially as described by Jensen and Sundström(1974), and was performed on a Shimadzu 4B Gaschromatography equipped with a ^{63}Ni-electron capture detector. The column used was an Apiezon L(at 2% on chromosorb W,80-100 mesh) packed in 5m x 2mm i.d. glass column. The column operating conditions were: injection temperature, 250°C; column temperature, 240°C; detector temperature, 290°C; Nitrogen carrier gas flow rate at 50ml/min.

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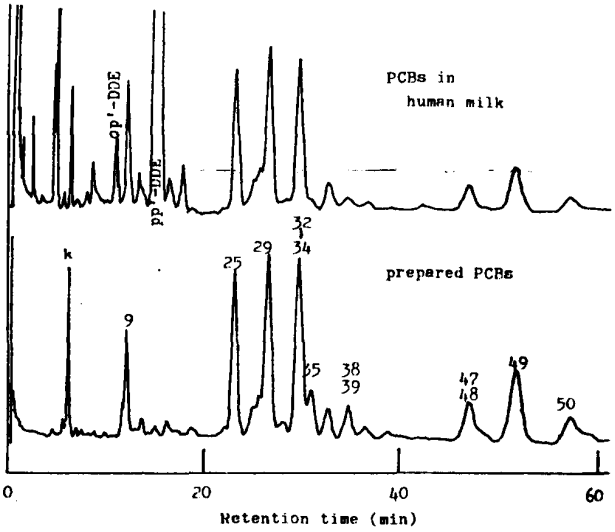

Fig.1. Gaschromatographic pattern of PCBs in human milk and prepared PCBs. Analyzed on Apiezon L column by using ^{63}Ni electron capture detector. Only a limited number of figures are shown due to lack of space.

Number of each peak on chromatogram was made as described by Jensen and Sundström(1974), and Nakamura et al (1977), and the PCBs level in tissue of rats was determined as described by Nakamura et al(1977). PCBs used in this study were Kanechlor-500(KC-500, an PCBs containing 55% chlorine) and prepared-PCBs whose GC profile having much in common with that in human milk of Japanese women(Fig.1). Preparation method of the prepared-

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PCBs was that a number of PCBs components separated from KC-300, KC-400 and KC-500 by using florisil and silica gel column chromatography were mixed throughly as to have in common with PCBs in human milk. Halogenated hydrocarbon pesticide β-BHC, pp'-DDE, and pp'-DDT were obtained from Wako Pure Chemicals Co.

Male Sprague-Dawley rats, weighing about 100g, having free access to water and food were used. The prepared-PCBs were given orally to the rats in olive oil (0.5ml/100g body weight) at levels of 0, 0.00025, 0.0010, 0.0040, 0.0160, 0.0640, 0.256 and 1.025mg/kg, twice a day(except Sunday and holiday, and once on Saturday) for a month(total 38 times administration). The rats were killed under light ether anasthesia after 24h starvation from the last dosing. Livers were quickely remcved, rinsed with 0.15M KCl, and homogenized with 4vol. of 0.25M sucrose. Liver microsomes were prepared as described by Omura and Sato(1964). Microsomal parameters were assayed as described by the following articles:

Table 1. Effects of KC-500, Prepared-PCBs(PCBs), and Pesticides[a] at a Daily Oral Dosing of 10 mg/kg for 3 Days, on Liver Microsomal Parameters in Rats.

	Cytochrome P-450 [b]	Aminopyrine N-demethylase[c]
Control	0.736 ± 0.095	1.31 ± 0.28
KC-500	2.16 ± 0.28 [g]	2.58 ± 0.34[f]
PCBs	2.50 ± 0.31 [h]	3.04 ± 0.60[f]
Pesticides	1.53 ± 0.22 [g]	2.13 ± 0.29[f]

	Benzpyrene hydroxylase[d]	PCBs binding[e]
Control	0.195 ± 0.016	376 ± 105
KC-500	0.341 ± 0.071[f]	1694 ± 230[h]
PCBs	0.430 ± 0.066[g]	2080 ± 62[h]
Pesticides	0.202 ± 0.042	2941 ± 1041

a) containing: β-BHC, 63.5 %; pp'-DDE, 30.9 %; pp'-DDT, 5.6 % b) nm/mg c) nm HCHO formed/mg/min d) nm 3OH-BP formed/mg/min e) dpm/mg/15 min f),g),h); significant differences from coresponding controls, $P<0.05$, $P<0.01$, $P<0.001$, respectively Each value represents the mean for 3 rats and standard deviation.

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cytochrome P-450 and cytochrome b_5 contents, Omura and Sato(1964); aminopyrine N-demethylase, Nazel(1971); benzo(a)pyrene hydroxylase(AHH), Nebert and Gelboin (1968), and Shimada and Sato; covalent binding of ^{14}C-KC-300 metabolites to microsomes(PCBs binding), Shimada (1976); protein content, Lowry et al(1951).

RESULTS AND DISCUSSION

In the initial experiment, enzyme induction of the prepared-PCBs was compared to that induced by KC-500 and pesticide mixture(Table 1). It was obvious that the prepared-PCBs was slightly more effective in inducibility than the KC-500. This finding is acceptable in its higher chlorine content of the prepared-PCBs, since in general highly chlorinated biphenyls were more potent inducer than lowly chlorinated biphenyls(Schmoldt et al, 1974; Fujita et al; Shimada et al,1976). Also the pesticide mixture, whose composition was made as similar as that in human milk was less affective in the induction of microsomal enzymes than KC-500 and prepared-PCBs except that of the PCBs binding.

Table 2 shows the effect on liver microsomal parameters of the prepared-PCBs dosed with various levels for a month. Cytochrome b_5 content in liver microsomes was not changed by all the PCBs administration and liver microsomal protein content was increased only at the highest dose level. However, while increases in cytochrome P-450 content and in PCBs binding were seen at dose level of 0.064 and 0.004 mg/kg respectively, aminopyrine N-demethylase and AHH activities were significantly increased even at a lower PCBs level of 0.0010 mg/kg. In addition, it was observed that the increased ratio of cytochrome P-450 content, aminopyrine N-demethylase activity, AHH activity and amount of PCBs binding at the highest dose level of 1.025mg/kg were 2.1-, 2.9-, 2.5- and 10.7-fold respectively.

The relationship between PCBs concentration in adipose tissue and the liver microsomal enzyme activities is given in Fig.2. It was evident that the induction effect of the prepared-PCBs was closely related with increased levels of the PCBs in adipose tissue. However, the shape of the curve in Fig.2 suggested that the increases in PCBs binding at higher dose levels of the prepared-PCBs was more sensitive than those in aminopyrine N-demethylase and AHH.

Fig.3 shows the PCBs levels in several peaks on gaschromatogram obtained from adipose tissue of rats treated with the prepared-PCBs. Since ratio of amounts of peak 32-34 towards total amounts of PCBs in adipose tissue was almost identical, ordinate in Fig.3 represents

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Table 2. Effect of Prepared-PCBs on Liver Microsomal Parameters in Rats

Dose (mg/kg)	Protein[a]	Cytochrome b_5[b]	Cytochrome P-450[b]
Control	18.8 ± 1.0	0.419 ± 0.028	0.743 ± 0.009
0.00025	19.3 ± 2.1	0.348 ± 0.012[f]	0.771 ± 0.017
0.0010	17.1 ± 0.2[f]	0.366 ± 0.066	0.768 ± 0.026
0.0040	19.4 ± 1.3	0.379 ± 0.016	0.815 ± 0.135
0.016	19.4 ± 3.9	0.356 ± 0.030	0.872 ± 0.095
0.064	18.7 ± 3.6	0.379 ± 0.020	1.056 ± 0.101[g]
0.256	19.5 ± 2.5	0.395 ± 0.023	1.115 ± 0.047[h]
1.025	26.2 ± 2.9[f]	0.460 ± 0.025	1.556 ± 0.105[g]

	Aminopyrine demethylase[c]	Benzpyrene hydroxylase[d]	PCBs binding[e]
Control	2.23 ± 0.15	0.128 ± 0.008	319 ± 46
0.00025	2.30 ± 0.25	0.131 ± 0.001	300 ± 46
0.0010	2.94 ± 0.22[g]	0.169 ± 0.011[g]	331 ± 33
0.0040	3.06 ± 0.11[g]	0.183 ± 0.024[f]	464 ± 75[f]
0.016	3.34 ± 0.25[g]	0.208 ± 0.035[f]	597 ± 30[g]
0.064	4.35 ± 0.99[f]	0.222 ± 0.053[f]	1175 ± 306[g]
0.256	4.98 ± 0.39[h]	0.259 ± 0.019[h]	1845 ± 220[h]
1.025	6.37 ± 1.25[g]	0.313 ± 0.012[h]	3406 ± 225[h]

a) mg/g liver b) nm/mg c) nm HCHO formed/mg/min
d) nm 3-OH-BP formed/mg/min e) dpm/mg/15 min
f),g),h); significant differences from corresponding controls, $P<0.05$, $P<0.01$, $P<0.001$, respectively
Each value represents the mean for 3 rats and S.D..

the percentage of the amount of each peak to that of peak 32-34. The patterns for peaks 35 and 49(and peaks 26,27,30,31,36,37,38,39,40,41,47,48,50 and 51, data not shown) had similar tendency to that for peak 32-34. However, remarkable changes were seen in patterns for peaks k and 9. After the lower dosing of the PCBs, the ratio of peak k(presumably trichlorobiphenyls) and peak 9(presumably tetrachlorobiphenyls) towrds the peak 32-34 were high, while in accordance with the increasing dose levels, these ratios became intensively lower. These results suggested that among the PCB components, there are two or more types of PCB, one is susceptible

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Microsomal Cytochrome P-450[b]	PCBs binding[c]
0.43 ± 0.009	919 ± 46
0.771 ± 0.017	900 ± 46
2.68 ± 0.076	831 ± 38
5.15 ± 0.135	964 ± 75[f]
7.72 ± 0.098	997 ± 36[g]
9.56 ± 0.101[c]	975 ± 306[g]
1.15 ± 0.047[h]	945 ± 220[h]
2.56 ± 0.190[g]	906 ± 275[h]

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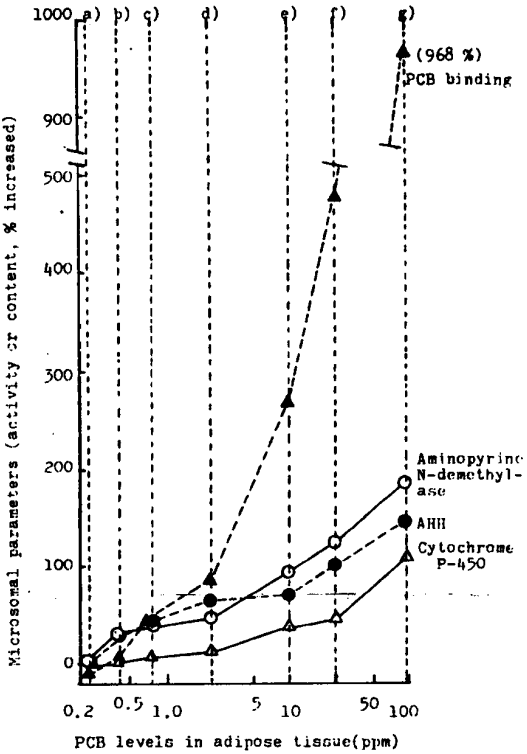

Fig.2 Relationships between microsomal parameters and PCB levels in adipose tissue from rats treated with prepared-PCBs for a month a)-g); PCB levels in adipose tissue from rats treated with prepared-PCBs at a dose level of 0.00025,0.001, 0.004, 0.016, 0.064, 0.256, and 1.025 mg/kg, respectively

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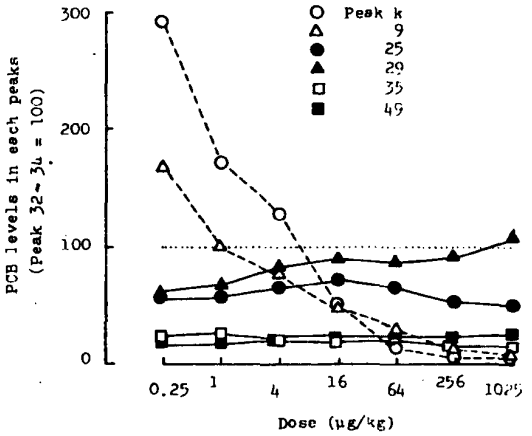

Fig.3 PCB levels in each peaks obtained by gaschromatographic analysis of adipose tissue from rats treated with prepared-PCBs. Ratio of amount of peaks k,9,25,29,(32-34),35, and 49 of the prepared-PCBs were 38:72:78:92:(100):18:31.

to the ability of microsomal enzymes, and others are not.

Recently Yakushiji et al(1976) have reported that the residue level of PCBs in human milk of Japanese women is abouut 0.015ppm. Even if human infants of 6kg of body weight drink the milk of their mothers by 800-900 ml/day, it is possible to caluculate their PCBs intake to 2.4 to 2.7μg/kg/day. In the present study, result was obtained that the treatment of the prepared-PCBs even at a low dose level of 1μg/kg to rats significantly increased the activities of aminopyrine N-demethylase and AHH about 1.3-fold for control rats. Furthermore, since the residue level of pesticide in human milk is about 10-fold of that of the PCBs (Yakushiji et al,1976), it is likely that the enzyme induction appeared in the present study may be more pronounced when PCBs and pesticide are similutaneously administered.

These results indicate that further studies con-

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cering the PCBs induced toxic effect to human infants are necessary.

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