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TRANSFER OF POLYCHLORINATED BIPHENYLS TO THE FOETUSES AND OFFSPRING OF MICE

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Abstract—Mice were fed Kanechlor-500 (KC-500) at a dietary level of 0·01 (control), 0·94, 8·4 or 86 ppm from the day of insemination until day 18 of pregnancy and were then killed. The amounts of polychlorinated biphenyls (PCBs) present in the whole body, liver and foetuses of the mice were respectively, 6·16, 0·83 and 0·1 0·2% of the total PCB intake, regardless of the dietary level of PCB, showing that transplacental transfer of PCBs to foetuses occurs to a relatively small degree. Other pregnant mice were fed a diet containing 0·01 or 0·94 ppm KC-500 and maintained on this until their offspring were 5 wk old. The young (one or two from each litter) were killed at weekly intervals from birth and analysed for PCBs. These offspring contained 100 or more times as much PCB during lactation as was present in the foetuses, indicating a considerable transfer of PCBs via the milk.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are known to have been the cause of a mass outbreak of food poisoning called Yusho, which occurred in western Japan in 1968 (Katsuki, 1969; Kuratsune, Yoshimura, Matsuzaka & Yamaguchi, 1972). Foetuses from mothers with Yusho showed some of the characteristics of the disease (Kikuchi, Hashimoto, Hozumi, Koga Oyoshi & Nagakawa, 1969; Taki, Hisanaga & Amagase, 1969). Several babies who had acquired PCBs from their mothers either transplacentally and/or by breast feeding were also diagnosed as suffering from Yusho (Yoshimura, 1974). Reproductive defects have been reported in animals given PCBs (Dahlgren, Linder & Carlson, 1972; Linder, Gaines & Kimbrough, 1974), and it is evident that PCBs present in mothers can affect their babies. The study described here was undertaken to obtain further qualitative and quantitative data on the transfer of PCBs through the placenta and milk of pregnant and lactating mice.

EXPERIMENTAL

Materials and instrumentation. Ethanol, *n*-hexane, water, sodium hydroxide, anhydrous sodium sulphate and silica gel were purified for PCB analysis as described previously (Masuda, Kagawa & Kuratsune, 1974). Kanechlor-500 and Kanechlor-600 were provided by Kanegafuchi Chemical Industry Co., Osaka. PCB isomers were purchased from Analabs, Inc., North Haven, USA, or synthesized in our laboratory. The gas chromatograph used was a Shimadzu GC-4HM with an electron-capture detector and a glass column (3 mm × 2 m) containing Chromosorb W AW-DMCS (60–80 mesh) coated with 5% SE-30. The column temperature was 210°C and pure nitrogen (99·999%) was used as the carrier gas at a flow rate of 30 ml/min.

Animals and diet. Male and female ddN mice were supplied by the animal centre of Kyushu University. Test diets were prepared by thoroughly mixing 1-kg batches of powdered feed (Oriental M) with ethyl ether solutions containing 0, 1·1, 11 or 110 mg Kanechlor-500 and leaving the mixture exposed to the air overnight to evaporate the solvent. The mixture was then kneaded well with water and with 100 g soluble starch (reagent grade) dissolved in hot water to make a dough, and pieces of the dough weighing 2·3 g were pelleted and dried at 60°C for 15 hr. The dried pellets, calculated to contain 0, 1, 10 or 100 ppm Kanechlor-500, were analysed for PCBs by gas chromatography (see below), the mean levels derived from triplicate determinations being 0·01, 0·94, 8·4 and 86 ppm, respectively.

Experimental procedure. Female mice were divided when 10 wk old into four groups of 7, 12 and mated. Insemination being confirmed by the presence of a vaginal plug. The pregnant mice were caged individually and fed one of the Kanechlor-500 diets until day 18 of pregnancy, when they were killed. The pellets in each cage were weighed every day and their consumption was calculated. For each dam, the foetuses, liver and rest of the body apart from the digestive tract were analysed for PCBs. Two other groups, one of nine and one of 12 mice, were mated and caged similarly and fed the pellets containing 0·01 or 0·94 ppm PCBs from the day of insemination until the end of the experiment. Young born on day 20 or 21 of pregnancy were raised by their dams for 5 wk, one or two offspring from each litter being killed at weekly intervals from 1 wk after birth for whole-body PCB analysis. The dams were killed at wk 5 and in each case the liver and the rest of the body apart from the digestive tract were analysed separately for PCBs.

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Table 1. *Mean concentrations of PCBs in the whole-body homogenate, liver and foetuses of dams fed Kanechlor-500 throughout pregnancy to day 18*

Levels of PCBs in feed (ppm)	No. of dams/group	Basis of calculation*	Concn of PCBs (ppm) in		
			Whole body	Liver	Foetuses
0·01 (control)	8	Total tissue	0·016 ± 0·012	0·056 ± 0·025	0·008 ± 0·011
		Fat	0·27 ± 0·19		0·81 ± 0·96
0·94	12	Total tissue	0·33 ± 0·14	1·4 ± 0·27	0·017 ± 0·007
		Fat	13 ± 6·0		5·4 ± 2·7
8·4	8	Total tissue	5·3 ± 1·1	3·1 ± 0·59	0·20 ± 0·10
		Fat	100 ± 33		26 ± 23
86	7	Total tissue	43 ± 8·9	23 ± 9·1	1·7 ± 0·7
		Fat	990 ± 310		200 ± 88

*Values are means ± SD for concentrations expressed either as a proportion of the whole tissue or of the fat content.

Analytical methods. The official standard analytical method for PCBs established by the Ministry of Health and Welfare (Kawashiro, Ueda, Ueta, Kashimoto, Kitamura, Kuratsune, Tatsukawa, Tanabe, Terashima, Fujiwara, Yoshimura, Masuda & Mizutani, 1972) was used for the extraction, separation and quantitative determination of PCBs. Samples were first homogenized with 100–200 ml *n*-hexane and 20 g sodium sulphate in a Waring blender. The separated *n*-hexane solutions were combined and evaporated to dryness, yielding fatty residues which were saponified with 1 N-NaOH in 50–100 ml ethanol. For liver samples, the saponification was made without extraction. The *n*-hexane extracts of the NaOH solution were combined, concentrated and then chromatographed on a column of silica gel (Wako gel S-1, 2 g) eluted with 150 ml *n*-hexane. The eluate was concentrated and subjected to gas chromatography, the PCBs being determined by comparing the total peak heights of their gas chromatograms with those of a 1:1 mixture of Kanechlor-500 and Kanechlor-600.

RESULTS

All the pregnant mice fed diet containing 0·01, 0·94, 8·4 or 86 ppm PCBs grew well throughout pregnancy, the mean body weights of these four groups on day 18 of pregnancy being 42·3, 42·6, 45·9 and 43·1 g, respectively. There was no significant difference in body-weight gain between these groups. The numbers of foetuses or offspring produced by each dam were normal (7–8) in each group, and there were no abnormalities in appearance.

Tables 1 and 2 summarize the results of the PCB analyses of the tissue samples from the four groups killed on day 18 of pregnancy. The levels of PCBs accumulated in the tissues of dams and foetuses and in the maternal livers increased with increasing PCB content of the diet (Table 1). The liver levels only showed a relatively small increase (×2·2), however, when the dietary concentration was raised from 0·94 to 8·4 ppm. Table 2 shows the total quantities of PCBs retained in the tissues of dams and foetuses. These also increased when the PCB level of the diets was raised, and the amounts present in the whole body, liver and foetuses accounted, respectively, for 6–16, 0·8–3 and 0·1–0·2% of the total PCB intake. Thus, a much smaller proportion was found in the foetuses than in the other tissues. However, the proportions did not vary widely in the different groups in spite of the marked differences in the PCB levels of the different diets.

The PCB content of 1·5-wk-old offspring of dams given PCBs at a dietary level of 0·01 or 0·94 ppm is shown in Table 3, which also lists the total amounts of PCBs per litter estimated from the number of offspring in the litter and their PCB concentration. While both the concentration and the total amount of PCBs present were always very small in the foetuses of the dams fed PCBs, they were much larger in the young killed during the lactation period (about 2 wk after birth). In offspring weaned 2 or 3 wk after birth, the PCB concentration remained roughly constant, even when they consumed the diet containing 0·94 ppm PCBs. Consequently, the total amounts of PCBs in the bodies of offspring aged 3·5 wk increased with time. A dam given pellets containing 0·94 ppm

Table 2. *Mean weights of PCBs in whole-body homogenates, livers and foetuses of dams fed Kanechlor-500 throughout pregnancy to day 18*

Levels of PCBs in feed (ppm)	No. of dams/group	Total intake of PCBs* (μg)	Total weight (μg) of PCBs* in		
			Whole body	Liver	Foetuses
0·01 (control)	8	1·0 ± 0·1	0·42 ± 0·36 (42·0)	0·14 ± 0·06 (14·0)	0·05 ± 0·07 (5·0)
0·94	12	96 ± 7·0	5·7 ± 1·9 (5·9)	3·1 ± 0·7 (3·2)	0·10 ± 0·05 (0·1)
8·4	8	890 ± 100	140 ± 40 (15·6)	9·2 ± 2·0 (1·0)	1·9 ± 1·1 (0·2)
86	7	9300 ± 300	1100 ± 270 (11·6)	73 ± 16 (0·8)	11 ± 6·9 (0·1)

*Values are means ± SD, the figures in parenthesis being the weight of PCBs in the given tissue expressed as a percentage of the total intake.

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Table 3. *Mean concentrations and total weights of PCBs in the offspring of dams fed Kanechlor-500 during and after pregnancy*

Levels of PCBs in feed (ppm)	No. of dams/group	PCB measurement	Age (wk)...	Mean values* for PCBs in offspring of different ages				
				1	2	3	4	5
0·01 (control)	9	Total tissue concn (ppm)		0·006 ± 0·001	0·010 ± 0·006	0·007 ± 0·005	0·005 ± 0·003	0·004 ± 0·00
		Concn based on fat (ppm)		0·12 ± 0·06	0·18 ± 0·07	0·19 ± 0·06	0·17 ± 0·06	0·20 ± 0·1
		Total weight (μg)		0·21 ± 0·03	0·59 ± 0·29	0·59 ± 0·37	0·58 ± 0·36	0·81 ± 0·4
0·94	12	Total tissue concn (ppm)		0·96 ± 0·23	1·1 ± 0·22	0·84 ± 0·35	0·67 ± 0·21	0·73 ± 0·34
		Concn based on fat (ppm)		20 ± 9·4	15 ± 3·4	25 ± 11	26 ± 3·8	25 ± 4·8
		Total weight (μg)		32 ± 7·8	65 ± 13	72 ± 28	89 ± 27	130 ± 45

*Values are means ± SD.

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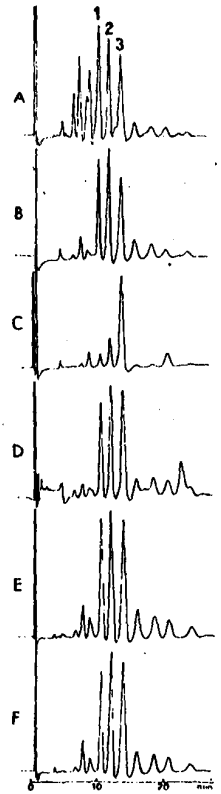

Fig. 1. Gas chromatograms of Kanechlor-500 (A) and of PCBs from the whole-body homogenate (B) the liver (C), a foetus (D), and 2-wk-old (E) and 5-wk-old (F) offspring of mice fed 0·94 ppm PCBs in the diet from the beginning of pregnancy. Peaks 1, 2 and 3 correspond to 2,4,5,3',4'-penta-, 2,4,5,2',4',5'-hexa- and 2,3,4,2',4',5'-hexachlorobiphenyl, respectively.

ingested a total of 147 or 186 μg PCBs on average during 20 days of pregnancy and 1 or 2 wk of lactation. Since the total PCB content of the litter of such a dam averaged 32 and 65 μg at 1 and 2 wk, respectively, after birth (Table 3), the amount of PCBs in the 1- and 2-wk-old offspring accounted for as much as 22 and 35% of the total intake of the dam.

Gas chromatograms of PCBs in the dams, foetuses and offspring of mice fed pellets containing 0·94 ppm PCBs are reproduced in Fig. 1. The peaks recorded show that the 2,4,5,3',4'-penta-, 2,4,5,2',4',5'-hexa and 2,3,4,2',4',5'-hexachlorobiphenyls were the major compounds in the whole-body analyses of all these groups, but the last compound was the main isomer present in the maternal liver.

DISCUSSION

These experiments showed that, in mice, PCBs enter the foetuses through the placenta in relatively small amounts but are transferred to the offspring after birth through the milk in much larger quantities. Similar experimental results were observed by others in studies on rats (Curley, Boise & Grim, 1973; Mizunoya, Taniguchi, Kusumoto, Morita, Yamada, Baba & Ogaki, 1974; Takagi, Otake, Kataoka, Murata, Aburada, Akasaka, Hashimoto, Uda & Kitaura, 1976), although precise comparison of these data is not feasible because of differences in the strain of animal used, in the degree of chlorination of the PCBs and in the feeding conditions. According to our calculations, these studies indicated PCB-transfer ratios from dam to foetuses and to offspring of 0·03–0·2 and 10–20%, respectively, of the amount ingested. The corresponding figures from our experiments were 0·1–0·2 and 20–35%, respectively. Thus, the figures show some level of agreement in spite of the discrepancies in experimental conditions. The experimental conditions used by Mizunoya *et al.* (1974) were very similar to ours, in that PCBs were given to the animals in the diet from the day of insemination, but they used rats and Kanechlor-400 instead of mice and Kanechlor-500 and found PCB concentrations in the foetuses and 2-wk-old offspring to be 0·04 and 2·36 ppm, respectively, when a diet containing 10 ppm Kanechlor-400 was given, while the corresponding figures estimated from our experiment were 0·20 and 10 ppm, respectively, for a dietary level of 8·4 ppm PCBs. (The value of 10 ppm was obtained by a rough extrapolation of the figure obtained when 0·94 ppm PCBs was given.) Thus, the concentrations in mice were about five times as great as those in rats, but the difference may have been due at least partially to the dissimilarity of the two PCB formulations.

Barsotti, Marlar & Allen (1976) obtained some evidence of transplacental and mammary movement of PCBs in monkeys. The concentrations of PCBs in various tissues of the stillborn infant of a monkey that had been fed a diet containing PCBs at a level as low as 5 ppm were much higher than those in the rodents, suggesting a fairly large species variation in the transplacental movement of PCBs. Thus, our present findings may not be applicable to man, and there is an urgent need for qualitative and quantitative data on the transfer and excretion of PCBs in man.

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REFERENCES

Barsotti, D. A., Marlar, R. J. & Allen, J. R. (1976). Reproductive dysfunction in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). *Fd Cosmet. Toxicol.* **14**, 99.

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