Acute, Subacute and Residual Effects of Polychlorinated Biphenyl (PCB) in Rats

I. Biologic Half-Life in Adipose Tissue

R. E. Shapiro 5/25/73

ROBERT C. BRAUNBERG, ROBERT E. DAILEY,

EMILIO A. BROUWER, LOUIS KASZA, and ANDREA M. BLASCHKA

Divisions of Toxicology and Pathology,

Food and Drug Administration,

Washington, D.C. 20204

Please send galley proof to: Miss Helen L. Reynolds

Technical Editor

Food and Drug Administration

Bureau of Foods - HFF-38

200 C Street, S.W.

Washington, D.C. 20204

MONS 064649

Sprague-Dawley rats were fed diets containing Aroclor 1254 at 0, 5, 50, or 500 ppm for 4 weeks. The biologic half-life of Aroclor 1254 in adipose tissue of rats fed 500 ppm, as determined by a gas chromatographic method, was 8 weeks in males and 12 weeks in females. These results are in line with sex-linked differences reported previously for other chlorinated hydrocarbons. It appears that the lower chlorine homologs in the Aroclor mixture are metabolized while those with higher chlorine content are lost more slowly.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are prepared by the chlorination of biphenyl and are complex mixtures containing isomers of chlorobiphenyls with different chlorine contents. They are marketed in the United States under the trade name Aroclor® and have found wide industrial application in a great variety of products.

Increasing awareness of the wide distribution of the PCBs in the environment has led to a concern about their storage in the fat of animals and man. Their high solubility in fat and low solubility in water lead to the kinds of storage phenomena in adipose tissue that previously have been observed in the case of the chlorinated hydrocarbon pesticides. Biros et al. (1970) have reported substantial quantities of PCB in human fat. Because of this observed accumulation, the work reported here was undertaken to determine the biologic half-life of Aroclor 1254 in the adipose tissue of the rat.

METHODS

Aroclor 1254[1] was dissolved in corn oil and mixed with pulverized Purina Rat Chow to give dietary PCB levels of 0 (only corn oil added), 5, 50 and 500 ppm. All of the diet formulations were prepared to contain 3% corn oil. The rat chow containing corn oil only (0 ppm) was fed to control rats throughout the experiment and to all rats after the PCB-containing diets had been fed for 4 weeks. This control diet contained 0.07 ppm PCB,

as shown by gas-liquid chromatographic (GLC) analysis. All diets were stored at -25°C in hexane-cleaned metal cans until use.

Four hundred male Osborne-Mendel rats (FDA colony; average weight, 350 g) were divided into four groups which were fed the diets containing 0, 5, 50 and 500 ppm PCB. In addition, 200 female Osborne-Mendel rats (average weight, 275 g) were fed the diets containing 0 and 500 ppm PCB.

The rats were housed individually in stainless steel cages with wire-mesh bottoms, with a lighting schedule of 12 hr light and 12 hr dark. Feed and water were available ad libitum.

Five rats from each dose level were lightly anesthetized with ether and killed by exsanguination; males were killed at weeks 1-5, 9, 13, 17, 23, and 30 and females at weeks 1-5, 9, 13, 17, and 30. The animals were sacrificed at approximately the same time of day. Whole body and liver weights for each rat were recorded at the time of necropsy. Samples of perirenal fat were collected, placed in hexane-washed sample vials, and frozen immediately in Dry Ice. Samples were stored at -20°C until analysis. Tissue samples were also taken from males only for a histopathology study, which is reported separately (Kasza et al., 1975).

Two-gram samples of adipose tissue from each of the five rats in each test group were pooled and fat was extracted by homogenization with anhydrous sodium sulfate and petroleum ether in a Waring Blendor with a semi-micro disintegrator jar. The

petroleum ether was then evaporated and the weight of extracted fat was determined. The average extractable fat in the adipose tissue was 90.8%. A portion of the extracted fat was partitioned with acetonitrile and eluted from a Florisil column (Food and Drug Administration, 1968), and then saponified with alcoholic KOH (Young and Burke, 1972). PCB concentrations were determined by GLC, using a 6 ft x 4 mm glass column containing 10% DC-200 on 80-100 mesh Chromosorb W(HP). Eighty-six per cent of the standard Aroclor 1254 added to rat adipose tissue was recovered by these procedures.

PCB concentrations were calculated by comparing the total area under the sample curve with the total area under the curve of a measured amount of Aroclor 1254. Total concentrations were calculated on the basis of the weight of extracted fat.

RESULTS AND DISCUSSION

Figures 1 and 2 show the PCB concentrations in the adipose tissue of male and female rats. From these data, the biologic half-life of Aroclor 1254 was calculated to be 8 weeks in males and 12 weeks in females. Grant et al. (1974) reported that Aroclor 1254 fed at 100 ppm to weanling male Wistar rats for 246 days had a half-life of 26 weeks in adipose tissue following the cessation of feeding.

The 4-week feeding period for the diets containing Aroclor was selected on the basis of previous studies (D. L. Grant, Food and Drug Directorate, Department of National Health and Welfare,

Tunney's Pasture, Ottawa, Ontario, personal communication); it was anticipated that the PCB concentration in the fat would approach equilibrium by the fourth week of feeding. Adult rats, rather than weanlings, were chosen to minimize the dilution of stored PCB with increasing fat depots in rapidly growing animals.

Although the PCB concentrations in the fat of animals fed the 500 ppm level were high enough to determine the biologic half-life of Aroclor 1254 in that tissue, the curves had not yet begun to level off, indicating that equilibrium had not been reached (Figs. 1 and 2). The concentration of Aroclor in the fat of male rats fed for 4 weeks at the 50 and the 5 ppm levels was not great enough to draw valid conclusions concerning the half-life (Fig. 1). The PCB concentrations in the control animals did not increase during the experiment; the average PCB concentrations in fat of male and female controls were 0.59 and 1.07 ppm, respectively.

In male rats fed 500 ppm PCB, the liver-to-body weight ratio increased through week 5 of the experiment, and then declined slowly until it reached a value similar to that of the controls at week 30 (Kasza et al. 1975). The ratio in females was highest at week 3 and remained elevated with respect to the controls until the end of the experiment. Upon electron microscopic examination, the treated animals showed proliferation of smooth endoplasmic reticulum (Kasza et al., 1975). Histologic study revealed cellular degeneration and the presence of fat droplets

in the cytoplasm of the liver. These findings are consistent with those of Grant et al. (1971), who reported increases in liver weights and liver lipid concentrations in rats treated with Aroclor 1254.

After the diet containing 500 ppm PCB had been fed for 4 weeks, the PCB concentration in the fat of female rats was about one-third higher than that of the males. As shown by the slopes of the curves in Figs. 1 and 2, the males more quickly divested themselves of the chlorinated hydrocarbon. Similar sex-linked differences in storage occur in rats treated with DDT (Hayes, 1965; Dale et al., 1962), with dieldrin and photodieldrin (Dailey et al., 1970, 1972), and with aldrin (Korte, 1965).

During the first weeks after the cessation of PCB feeding, when the rate of loss from the fat was greatest, there was a disproportionate loss of the Aroclor components that are presumed to be of the lowest chlorine content (Grant et al., 1971; 1974), i.e., those having the shortest retention time in GLC. Representative traces of the GLC chromatograms of pure Aroclor 1254 and of fat of male rats at weeks 4 and 30 are shown in Fig. 3. Similar losses of these early GLC peaks after PCB treatment of Japanese quail have been reported by Koeman et al. (1969), of rats by Grant et al. (1971; 1974) and Curley et al. (1971), and of fish by Hansen et al. (1971). The finding by Biros et al. (1970) that the components of Aroclor 1254 with the shortest GLC retention time do not appear at all in human fat adds credence to

the concept that the lower chlorine homologs of PCB are being metabolized.

The lowest homolog found by Biros et al. (1970) in human fat was pentachlorobiphenyl. Hutzinger et al. (1972), in studies of the metabolism of four pure isomers of PCB, reported that 2,2',5,5'-tetrachlorobiphenyl was hydroxylated by the rat, but found no hydroxylation products of 2,2',4,4',5,5'-hexachlorobiphenyl. Chen and Matthews (1974) reported the hydroxylation of 2,2',4,5,5'-pentachlorobiphenyl in the rat. These facts indicate that upon cessation of feeding PCB in the diet there is a rapid loss of the homologs of biphenyl containing one through five chlorine atoms, followed by a steady loss of one-half of the PCB stored in the fat each 8 weeks in the male rat and each 12 weeks in the female rat.

REFERENCES

BIROS, F.J., WALKER, A.C. AND MEDBERY, A. (1970). Polychlorinated biphenyls in human adipose tissue. Bull. Environ. Contam. Toxicol. 5, 317-323.

CHEN, P.R. AND MATTHEWS, H.B. (1974). Metabolism and excretion of 2,4,5,2',5'-pentachlorobiphenyl (PCB) in the male rat. Toxicol. Appl. Pharmacol. 29, 88.

CURLEY, A., BURSE, V.W., GRIM, M.E., JENNINGS, R.W. AND LINDER, R.E. (1971). Polychlorinated biphenyls: Distribution and storage in body fluids and tissues of Sherman rats. Environ. Res. 4, 481-495.

DAILEY, R.E., WALTON, M.S., BECK, V., LEAVENS, C.L. AND KLEIN, A.K. (1970). Excretion, distribution, and tissue storage of a 14C-labeled photoconversion product of 14C-dieldrin. J. Agr. Food Chem. 18, 443-445.

DAILEY, R.E., KLEIN, A.K., BROUWER, E., LINK, J.D. AND BRAUNBERG, R.C. (1972). Effect of testosterone on metabolism of 14C-photodieldrin in normal, castrated, and oophorectomized rats. J. Agr. Food Chem. 20, 371-376.

DALE, W.E., GAINES, T.B. AND HAYES, W.J., JR. (1962). Storage and excretion of DDT in starved rats. Toxicol. Appl. Pharmacol. 4, 89-106.

FOOD AND DRUG ADMINISTRATION (1968). Pesticide Analytical Manual, eds. H.C. Barry, J.A. Burke, R.E. Duggan, J.A. Gaul, J.C. Hundley and L.Y. Johnson, Vol. I, secs. 211.13f and

211.14d (revised April 1, 1971). Washington: Department of Health, Education, and Welfare.

GRANT, D.L., PHILLIPS, W.E.J. AND VILLENEUVE, D.C. (1971). Metabolism of a polychlorinated biphenyl (AroclorR 1254) mixture in the rat. Bull. Environ. Contam. Toxicol. 6, 102-112.

GRANT, D.L., MOODIE, C.A. AND PHILLIPS, W.E.J. (1974). Toxicodynamics of Aroclor 1254 in the male rat. Environ. Physiol. Biochem. 4, 214-225.

HANSEN, D.J., PARRISH, P.R., LOWE, J.I., WILSON, A.J., JR. AND WILSON, P.D. (1971). Chronic toxicity, uptake and retention of Aroclor 1254 in two estuarine fishes. Bull. Environ. Contam. Toxicol. 6, 113-119.

HAYES, W.J., JR. (1965). Review of the metabolism of chlorinated hydrocarbon insecticides especially in mammals. Annu. Rev. Pharmacol. 5, 27-52.

HUTZINGER, O., NASH, D.M., SAFE, S., DE FREITAS, A.S.W., NORSTROM, R.J., WILDISH, D.J. AND ZITKO, V. (1972). Polychlorinated biphenyls. Metabolic behavior of pure isomers in pigeons, rats, and brook trout. Science 178, 312-314.

KASZA, L., HINTON, D.E., WEINBERGER, M.A., TRUMP, B.F., BROUWER, E.A. AND CARTER C. (1975). Acute, subacute and residual effects of polychlorinated biphenyl (PCB) in rats. II.

Pathology and electron microscopy of liver and serum enzyme study. J. Toxicol. Environ. Health, Submitted.

KOEMAN, J.H., TEN NOEVER DE BRAUW, M.D. AND DE VOS, R.H. (1969). Chlorinated biphenyls in fish, mussels, and birds from the river Rhine and the Netherlands coastal area. Nature 221, 1126-1128.

KORTE, F. (1965). Proceedings of a Panel on Radioisotopes in the Detection of Pesticide Residues, pp. 2-14. Joint FAO/IAEA Division of Atomic Energy in Agriculture. Vienna.

YOUNG, S.J.V. AND BURKE, J.A. (1972). Micro scale alkali treatment for use in pesticide residue confirmation and sample cleanup. Bull. Environ. Contam. Toxicol. 7, 160-167.

Footnotes

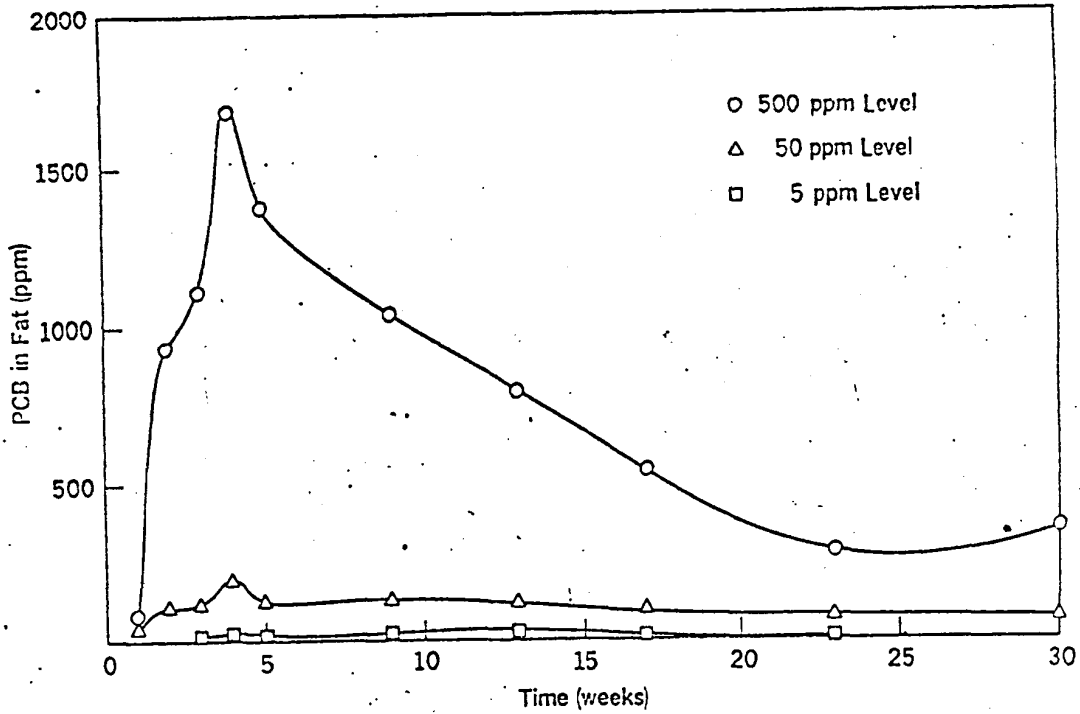
[1]Monsanto Co., Inc., St. Louis, Mo. 63166.

Figure Captions

FIG. 1. Concentration of PCB in adipose tissue of male rats fed Aroclor 1254 at 5, 50, or 500 ppm in the diet for 4 weeks. Each point is the average of three or more replicate analyses of pooled fat samples.

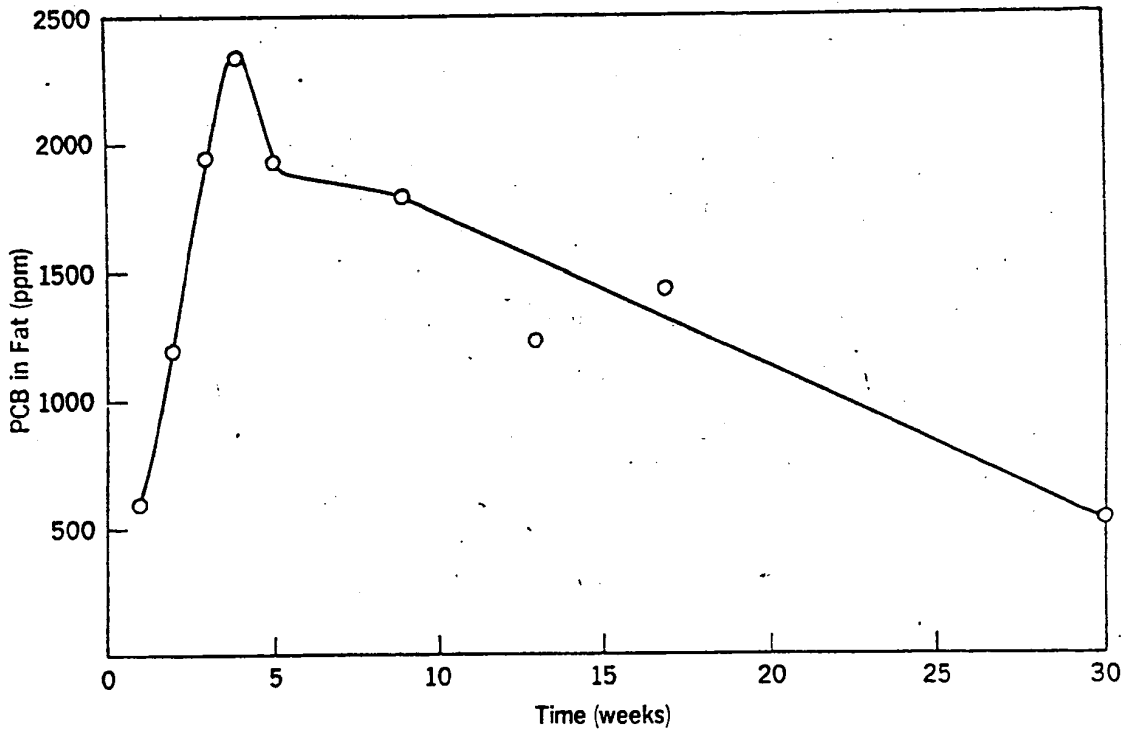
FIG. 2. Concentration of PCB in adipose tissue of female rats fed 500 ppm Aroclor 1254 in the diet for 4 weeks. Each point is the average of three or more replicate analyses of pooled fat samples.

FIG. 3. Representative gas-liquid chromatograms of Aroclor 1254 recovered from adipose tissue. A, Aroclor 1254 standard; B, male rat at week 4; C, male rat at week 30.



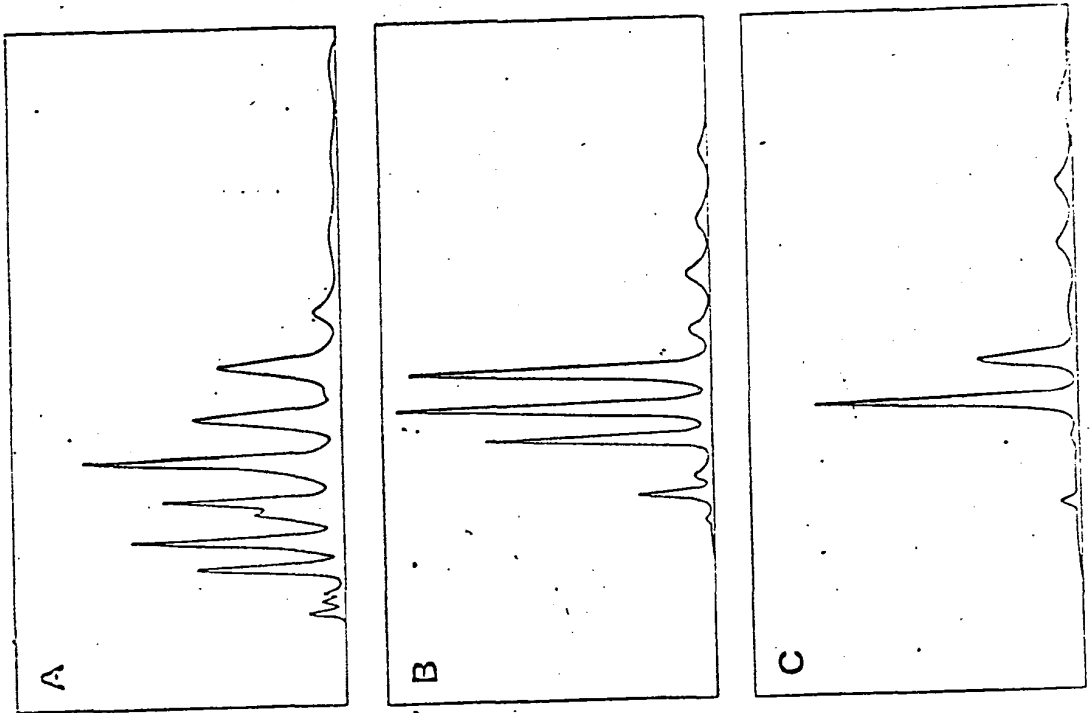
CONCENTRATION OF PCB IN ADIPOSE TISSUE OF MALE RATS FED AROCLOR 1254 AT VARIOUS LEVELS FOR FOUR WEEKS

Figure 1


2500
2000
1500
1000
500
PCB in Fat (ppm)
0
5
10
15
20
25
30
Time (weeks)

MONS 064663

SNOW 064664



REPRESENTATIVE GAS CHROMATOGRAMS FOR AROCLOR 1254 RECOVERED FROM ADIPOSE TISSUE

A. Aroclor 1254 Standard B. Male week 4 C. Male week 30

Figure 3