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EFFECT OF PROLONGED INGESTION OF POLYCHLORINATED BIPHENYLS ON THE RAT

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Abstract—Aroclor 1242, a commercial polychlorinated biphenyl mixture, was fed to rats at dietary levels of 0, 5 and 25 ppm for 2, 4 and 6 months. Significant elevations of urinary coproporphyrin excretion, hepatic lipid and hepatic microsomal-hydroxylase activity were observed in rats ingesting 25 ppm for 2 months. In rats given the 5 or 25 ppm regimens for 4 or 6 months, urinary coproporphyrin excretion and liver weight, lipid content and microsomal-hydroxylase activity were higher than in controls. Histopathological examination revealed lipid vacuolation and proliferation of smooth endoplasmic reticulum in the hepatocytes of treated animals. These findings indicate that long-term, low-level ingestion of Aroclor 1242 may result in changes in a variety of biological parameters.

INTRODUCTION

Polychlorinated biphenyls (PCBs), a series of chlorinated polycyclic hydrocarbons, have become the subject of widespread concern in recent years. Commercial PCB products consist of mixtures of isomers, differing from one another in degree of chlorination. As PCBs are water-insoluble and inert, they have been used extensively as surface coatings, flame retardants, stabilizers, capacitors, heat-transfer agents and components of plasticizers, adhesives and printing inks. Such industrial applications have apparently resulted in world-wide environmental contamination (Nisbet & Sarofim, 1972) and accumulation in the tissues of many biological species (Risebrough & de Lappe, 1972).

Investigations have demonstrated the presence of PCBs in human foodstuffs and adipose tissue. Price & Welch (1972) found that 41–45% of human adipose samples, obtained from the general population of the United States, contained >1·0 ppm PCBs. Potential dietary sources of PCBs include fish, poultry, milk, eggs and cereals (Kolbye, 1972). The FDA interim guideline for PCB contamination of human foodstuffs has been set at 5 ppm, with lower levels recommended for long-term ingestion.

Though current environmental and dietary levels of PCBs are not felt to constitute an immediate health hazard, limited information is available concerning biological manifestations of long-term, low-level exposure in mammals. Kimbrough, Linder & Gaines (1972) reported significant increases in liver to body-weight ratios in rats fed 5 ppm Aroclor 1254 or Aroclor 1260 for 8 months. These same commercial PCB products at dietary levels as low as 0·5 and 5 ppm may induce certain hepatic microsomal-enzyme activities in rats within 4 wk (Litterst, Farber, Baker & Van Loon, 1972). A 1-yr 20 ppm dietary regimen of Aroclors 1242, 1254 or 1260 has been reported to reduce pentobarbitone sleeping time in rats (Villeneuve, Grant & Phillips, 1972).

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The present study was initiated to elucidate biological responses in the rat upon prolonged ingestion of a common PCB product, Aroclor 1242. Dosage levels corresponding to the contemporary FDA guideline and to a level encountered in fish were selected. Emphasis was placed upon biological parameters shown to be altered by acute and subacute dosage regimens in previous investigations (Bruckner, Khanna & Cornish, 1973 & 1974).

EXPERIMENTAL

Animals. Male Sprague–Dawley rats (mean body weight 125 g) were obtained from Spartan Research Farm, Lansing, Mich. The rats were randomly divided into groups of six animals and housed in pairs in stainless-steel cages in air-conditioned quarters, with tap-water and ground Rockland rat and mouse chow available *ad lib.* Only during collection of urine specimens were the rats confined individually in metabolism cages.

Materials and dosage. Aroclor 1242 (lot no. KA 419) was supplied by Monsanto Chemical Company, St. Louis, Mo. Appropriate quantities of Aroclor 1242 were dissolved in acetone and mixed thoroughly with a small amount of ground chow. After the acetone had been allowed to evaporate, the Aroclor-chow mixture was mixed by geometric dilution with increasing amounts of chow until desired Aroclor 1242 concentrations of 5 and 25 ppm were attained. The groups of six rats were fed the diets containing 0, 5 or 25 ppm Aroclor 1242 for 2, 4 or 6 months. Body weights and food consumption were measured at weekly intervals.

Urine analysis. Urinary excretion of coproporphyrin was determined by the method of Schwartz, Zieve & Watson (1951) 1 wk before the rats were killed.

Haematology. At the end of the treatment period, blood samples were taken from tail veins for determinations of haematocrit, erythrocyte counts and haemoglobin levels. Erythrocyte counts, utilizing a Coulter counter, were performed for the 6-month groups only. Haemoglobin levels were measured by a technique involving cyanmethaemoglobin formation (Davidsohn & Henry, 1969). Following anaesthetization with ether, blood was withdrawn by open-chest cardiac puncture. Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxalacetic transaminase (SGOT) activities were determined by standard procedures. The method of Solem & Brinck-Johnsen (1965) was used for analysis of plasma corticosteroids.

Microsomal-enzyme assays. Hydroxylase activity was estimated in the 13,000 *g* supernatant of 5 g liver samples by measurement of *N*-acetyl-*p*-aminophenol formation from acetanilide, and *N*-demethylase activity by measurement of 4-aminoantipyrine formation from aminopyrine. Methods utilized were outlined in a previous investigation (Bruckner *et al.* 1973).

Hepatic studies. Liver weights were recorded and total liver lipid was assayed by the procedure of Bligh & Dyer (1959).

Histopathology. Liver and kidney specimens were processed and stained with haematoxylin and eosin for light microscopic examination. Frozen sections of liver and kidney were stained for fat with Sudan IV. Cubes of liver were minced and fixed in cold 3% phosphate-buffered glutaraldehyde for 2 hr, stained in 2% osmium tetroxide for 2 hr, dehydrated in an ethanol and propylene oxide series and embedded in Epon plastic. Sections were post-stained with uranyl acetate and lead citrate and examined with an AEI Cornith 275 electron microscope.

Statistical analysis. The significance of all results was assessed by Student's *t* test.

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RESULTS

Body weight and food consumption

No significant differences in body-weight gain were seen at any dietary level after 2, 4 or 6 months. Food consumption was comparable at each dietary level throughout the course of the study. With the average food intake of 25 g/rat/day, the 5 ppm groups ingested approximately 0·3 mg Aroclor 1242/kg body weight/day, the 25 ppm groups approximately 1·5 mg Aroclor 1242 kg.day. Total Aroclor 1242 consumption during the course of the studies is expressed in Table 1.

Table 1. *Approximate total consumption of Aroclor 1242 by test groups*

Dose (ppm)	Total Aroclor 1242 consumption* (mg/rat) during test period of (months)		
	2	4	6
5	7	14	21
25	35	70	105

*Values are based on an approximate average consumption of 25 g food/400 g rat/day.

Haematology

Results of blood assays other than SGOT, SGPT and plasma corticosteroid determinations are given in Table 2. No significant alterations in SGOT, SGPT or plasma corticosteroid levels were seen at any dietary level after 2, 4 or 6 months. Only in the group fed 25 ppm Aroclor 1242 for 2 months were significant reductions in haematocrit recorded. A significant reduction in haemoglobin levels occurred in the groups fed 5 or 25 ppm for 2 months. Slight reductions from control values in haematocrit and erythrocyte count were noted in the group given 25 ppm for 6 months, although mean values were not statistically different from those of the controls.

Table 2. *Effect of prolonged, low-level ingestion of Aroclor 1242 on haematocrit, erythrocyte count and haemoglobin level*

Duration of test (months)	Dose (ppm)	Haematocrit† (%)	Erythrocyte count† (10^6/mm^3)	Haemoglobin (g/100 ml)
2	0	51·0 ± 1·0		16·1 ± 0·1
	5	50·6 ± 0·6		15·1 ± 0·1*
	25	48·0 ± 0·6*		14·8 ± 0·2**
4	0	52·6 ± 1·3		17·1 ± 0·3
	5	51·7 ± 0·9		16·2 ± 0·4
	25	53·2 ± 0·9		16·8 ± 0·3
6	0	55·2 ± 1·3	10·3 ± 0·4	16·8 ± 0·4
	5	56·0 ± 0·2	10·1 ± 0·3	16·2 ± 0·4
	25	54·0 ± 1·1	9·7 ± 0·1	15·9 ± 0·5

†Mean values represent an average of two determinations/rat.
Values are means ± S.E.M. for groups of six rats and those marked with asterisks differ significantly (Student's *t* test) from the control values: *$P < 0.01$; **$P < 0.001$.

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Microsomal-enzyme assays

Table 3 presents the effects of the dietary regimens on the activities of hepatic microsomal hydroxylase and *N*-demethylase. A significant induction of hydroxylase activity was manifest in rats that had consumed 25 ppm Aroclor 1242 for 2 months. Hydroxylase activity was induced in some members of the group given 5 ppm for 2 months but not in others. Dose-dependent increases in hydroxylase activity were measured at each sampling period. The 5 ppm diet did not alter *N*-demethylase activity significantly at any sampling period, though the 25 ppm diet induced significant increases in the 4- and 6-month groups.

Table 3. *Effect of prolonged, low-level ingestion of Aroclor 1242 on hepatic microsomal hydroxylase and N-demethylase activities*

		Hydroxylase activity		*N*-Demethylase activity	
Duration of test (months)	Dose (ppm)	*N*-Acetyl-*p*-aminophenol formed (μg/mg protein/20 min)	% of control value	4-Aminoantipyrine formed (μg mg protein 20 min)	% of control value
2	0	0·14 ± 0·01	100	0·13 ± 0·01	100
	5	0·19 ± 0·02*	140	0·13 ± 0·02	100
	25	0·53 ± 0·04***	376	0·17 ± 0·02	131
4	0	0·31 ± 0·03	100	0·30 ± 0·05	100
	5	0·51 ± 0·03***	163	0·36 ± 0·07	123
	25	1·06 ± 0·03****	342	0·59 ± 0·04***	197
6	0	0·34 ± 0·08	100	0·49 ± 0·00	100
	5	0·51 ± 0·03**	147	0·45 ± 0·03	91
	25	1·38 ± 0·06****	401	0·88 ± 0·03****	181

Results are expressed as the mean ± SEM for groups of six rats and as a percentage of the control value. Values marked with asterisks differ significantly (Student's *t* test) from those for controls. *$P < 0.1$; **$P < 0.05$; ***$P < 0.01$; ****$P < 0.001$.

Urine analysis

The effect of repeated low-level exposure to Aroclor 1242 on urinary coproporphyrin excretion is summarized in Table 4. A significant rise in coproporphyrin excretion was

Table 4. *Effect of prolonged low-level ingestion of Aroclor 1242 on urinary coproporphyrin, liver weight and liver lipids*

Duration of test (months)	Dose (ppm)	Urinary coproporphyrin (μg/24 hr)	Liver weight (g/100 g body weight)	Total lipids (mg/g liver wet wt)
2	0	4·7 ± 0·3	3·7 ± 0·2	31·2 ± 0·7
	5	5·8 ± 0·4*	3·6 ± 0·2	35·3 ± 1·1***
	25	9·9 ± 0·6****	3·8 ± 0·1	38·2 ± 1·0****
4	0	5·9 ± 0·9	2·9 ± 0·1	33·4 ± 2·0
	5	8·5 ± 1·4*	3·1 ± 0·1*	40·0 ± 2·1**
	25	16·6 ± 4·4**	3·4 ± 0·1***	41·5 ± 2·0***
6	0	3·9 ± 0·7	3·3 ± 0·2	31·9 ± 0·7
	5	7·9 ± 1·4**	3·9 ± 0·2*	34·1 ± 1·3*
	25	6·3 ± 0·9*	3·9 ± 0·2*	36·0 ± 1·1***

Values are the means ± SEM for groups of six rats and those marked with asterisks differ significantly (Student's *t* test) from the control value: *$P < 0.1$; **$P < 0.05$; ***$P < 0.02$; ****$P < 0.001$.

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Fig. 1. Part of hepatocyte from a rat fed 5 ppm Aroclor 1242 for 4 months, showing a preponderance of smooth endoplasmic reticulum (SER) and paucity of rough endoplasmic reticulum (RER), together with the nucleus (N) mitochondria (M) and space of Disse (S). × 24,000.

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Fig. 2. Part of hepatocyte from a rat fed 25 ppm Aroclor 1242 for 4 months, showing lipid vacuoles (L) similar in size to the mitochondria (M) and cytoplasmic in distribution, smooth endoplasmic reticulum (SER) and a peroxysome (P). × 45,000.

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observed in the group that consumed 25 ppm for 2 months. Although a definitely significant increase was not seen until 6 months of feeding the 5 ppm diet, some animals in both the 2- and 4-month groups fed 5 ppm exhibited high urinary coproporphyrin levels.

Hepatic studies

Although increases in liver weight were not apparent in response to either the 5 or 25 ppm regimens after 2 months, liver lipid was significantly higher than the control level in each group (Table 4). Both liver weight and lipid content were raised in the 5 and 25 ppm groups at 4 and 6 months.

Histopathology

No discernible histopathological changes were present in haematoxylin/eosin-stained liver and kidney specimens of any rat after 2, 4 or 6 months of dosing. The Sudan IV stain, however, revealed increased lipid deposition in the livers of rats fed 5 or 25 ppm Aroclor 1242 for 2, 4 or 6 months. The distribution of vacuolated hepatocytes in the liver lobules was primarily peripheral, with encroachment into the midzonal regions in more severe cases. The sudanophilic vacuoles were quite small and were distributed uniformly within the cytoplasm of afflicted hepatocytes. Limited vacuolation of the renal convoluted tubules was observed only after the feeding of 25 ppm Aroclor 1242 for 4 or 6 months. Minute sudanophilic vacuoles were observed in the peripheral portion of affected tubular epithelial cells.

A proliferation of smooth endoplasmic reticulum and an increase in lipid vacuolation were seen with the electron microscope in hepatocytes of the rats fed 25 ppm Aroclor 1242 for 2 months. Both the 5 and 25 ppm diets produced these effects in the 4- and 6-month groups (Figs 1 & 2). No increases in myelin figures, microbodies or lysosomes were observed.

DISCUSSION

The small reductions in haematocrit and haemoglobin seen in the present study after 2 months are analogous to responses produced in previous studies (Bruckner *et al.* 1973 & 1974) by much higher doses of Aroclor 1242. Reduction in the erythrocyte count and haemoglobin content by PCB exposure has also been reported in severely poisoned humans (Okumura & Katsuki, 1969) and in rhesus monkeys (Abrahamson & Allen, 1973). Abrahamson & Allen (1973) observed hypoplasia of the bone marrow in these anaemic monkeys. Reasons for the lack of anaemic response in the 4- and 6-month groups in the present study are unclear. An adaptive mechanism capable of negating the haematological effects of low levels of Aroclor 1242 may have become operative.

Urinary coproporphyrin excretion proved to be a sensitive indicator of Aroclor 1242 ingestion. Though relatively high doses of PCBs are known to cause porphyria, we are not aware of any previous demonstration that ingestion of low dietary levels may elicit this condition in mammals. The dosage threshold for porphyria induction was 5 ppm Aroclor 1242. Variation of individual responses within groups was pronounced, as evidenced by the rather large standard errors.

Hepatic microsomal-hydroxylase induction also proved to be a sensitive index of Aroclor 1242 ingestion. A threshold effect was observed in response to 2 months' consumption of the 5 ppm diet. As in a previous study (Bruckner *et al.* 1974), hydroxylase activity was

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dose-dependent. A similar phenomenon was reported by Litterst *et al.* (1972) in 4-wk rat-feeding studies, in which Aroclors 1254 and 1260 showed clear dose-response relationships with *N*-demethylase, nitroreductase and hydroxylase activities. *N*-Demethylase activity, however, proved a much less sensitive index of Aroclor 1242 ingestion than hydroxylase activity in the present investigation.

The level of hydroxylase activity in rats fed 25 ppm Aroclor 1242 varied directly with the length of dietary exposure. Such a phenomenon is evidence of a cumulative effect, as would be predicted from the lipid solubility and the persistence of PCBs in biological systems. Other investigations have demonstrated that a single dose of PCBs will induce microsomal enzymes for weeks (Bruckner *et al.* 1974; Fujita, Tsuji, Kato, Saeki & Tsukamoto, 1971). Upon administration of a single oral dose of 25 mg [^{3}H]Kanechlor-400 rat, Yoshimura, Yamamoto, Nagai, Yae, Uzawa, Ito, Notomi, Minakami, Ito, Kato & Tsuji (1971) found that significant amounts of radioactivity remained in some tissues after 8 wk. Dietary levels of 100 ppm Aroclor 1254 have been shown to be cumulative in rats, with a steady build-up in all tissues over an 8-month feeding period (Curley, Burse, Grim, Jennings & Linder, 1971).

The liver hypertrophy with lipid vacuolation and proliferation of smooth endoplasmic reticulum observed in hepatocytes in the present study are typical manifestations of exposure to chlorinated aromatic hydrocarbons. Similar changes have been reported in feeding studies on PCB in mice and monkeys (Nishizumi, 1970) and in rats (Kimbrough *et al.* 1972), though not in response to such low dietary levels. Norback & Allen (1972) proposed that proliferated membranes provided sites for storage, isolation and contact of membrane enzymes with lipophilic aromatic hydrocarbons. Microsomal membranes have been shown to contain the highest amount of radioactivity of any subcellular fraction following oral administration of [^{3}H]Kanechlor-400 to rats (Fujita *et al.* 1971).

Hepatomegaly resulting from exposure to PCBs may be attributed to increases in several hepatic components. Norback & Allen (1972) measured significant increases in hepatic microsomal protein and phospholipid in rat feeding studies. Triglyceride content in the rat liver may also be elevated by ingestion of Aroclors (Litterst *et al.* 1972) as indicated by findings in the present investigation. Fat stains and electron-microscopic studies revealed many small lipid vacuoles in affected hepatocytes, though corresponding haematoxylin–eosin-stained specimens appeared unaltered. The lipid vacuoles were apparently small enough to escape detection by the latter histopathological procedure. Alteration of hepatocytes by Aroclor 1242 was not so extensive that SGOT and SGPT levels were elevated.

Evaluation of the potential hazards posed to man by long-term exposure to PCBs must use, as criteria, incidents of short-term human exposure and animal studies. On the basis of findings in the Yusho incident (Kuratsune, Yoshimura, Matsuzaka & Yamaguchi, 1972), the minimal toxic dose of Kanechlor-400 (chlorine content 48%) in man was estimated to be about 0·5 g patient, or 8 mg kg body weight for a 60-kg adult. The minimal effective dietary level of Aroclor 1242 in the present study was 5 ppm, at which dose level a 400-g rat consumed a total dose of approximately 18 mg kg body weight in 2 months. Although such a comparison suggests a similar bioeffect level for man and rat, one must appreciate the limitations on extrapolation of rodent data to man, the variation in chlorine content and potential contaminants between Kanechlor-400 and Aroclor 1242, the dissimilarity of dosage regimens, the imprecise approximation of total doses and the differences in biological parameters evaluated in the two studies.

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Our findings indicate that prolonged low-level exposure to 5 or 25 ppm Aroclor 1242 may result in changes in a variety of biological parameters. Although these alterations do not constitute an immediate threat to life, they may, in addition to their direct effects, modify biological responses to numerous chemical agents metabolized by the microsomal-enzyme system.

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