

THE EFFECT OF POLYCHLORINATED BIPHENYLS ON RAT REPRODUCTION

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Abstract—Reproduction, pathology and acute toxicity were studied in Sherman rats exposed to the polychlorinated biphenyls, Aroclor 1254 and Aroclor 1260. Rats exposed to Aroclor 1254 at dietary levels of 20 ppm or more had fewer pups per litter than the controls in the F_1 and F_2 generations. The 100 ppm exposure level of Aroclor 1254 increased mortality in the F_1 offspring and markedly decreased mating performance of the F_1 adults. The 500 ppm dietary level of Aroclor 1260 reduced litter size and decreased survival in the F_1 litters. Dietary levels of 5 ppm Aroclor 1254 and 100 ppm Aroclor 1260 had no effect on reproduction in rats exposed through two generations. Liver weights were increased in 21-day-old F_1 male weanlings at the 1 ppm level of Aroclor 1254 and in either sex of F_1 and F_2 weanlings at 5 ppm or higher levels of both Aroclor 1254 and 1260. Histological changes in the liver and increased liver weights were observed in adult rats exposed to the higher levels. Pregnant rats given Aroclor 1254 at the rate of 100 mg/kg/day on days 7-15 of gestation produced grossly normal litters, but only 30-1% of the pups survived to weaning. Reproduction and pup survival were not affected at dosage rates of 50 mg. Aroclor 1254/kg/day or 100 mg Aroclor 1260/kg/day. Oral LD_{50} values in 3-4-wk-old male rats were 1295 and 1315 mg/kg for Aroclors 1254 and 1260, respectively. The iv LD_{50} for Aroclor 1254 in adult females was 358 mg/kg.

INTRODUCTION

Reproductive defects due to commercial mixtures of polychlorinated biphenyls (PCBs) have been reported in experimental birds (Dahlgren, Linder & Carlson, 1972) and mammals (Ringer, Aulerich & Zabik, 1972). Porphyria (Vos & Koeman, 1970), microsomal-enzyme induction (Street, Urry, Wagstaff & Blau, 1969) and liver damage (Kimbrough, Linder & Gaines, 1972; Miller, 1944) have also been reported in experimental animals exposed to PCB mixtures.

The present investigation was initiated in 1970 to study the effects on reproduction and pathology produced by two American-made PCB mixtures sold under the trade-names Aroclor 1254® and Aroclor 1260®. Aroclor 1254 contains 54% (w/w) chlorine and is composed of 11% tetra-, 49% penta-, 34% hexa- and 6% heptachlorobiphenyls; Aroclor 1260 has 60% (w/w) chlorine, with a composition of 12% penta-, 38% hexa-, 41% septa-, 8% octa- and 1% nonochlorobiphenyls (Thurston, 1971). An almost complete identification of isomers in Aroclor 1254 and Aroclor 1260 has been obtained (Sissons & Welti, 1971). Since 1970, the Monsanto Co., the only US manufacturer of PCBs, has voluntarily reduced sales of Aroclor mixtures and has taken steps to limit their use to closed systems (Monsanto Co., 1971).

The present communication is an account of reproduction studies in rats. Also included are acute toxicity values from preliminary studies and comments on pathology and haematology in animals from the reproduction experiments. A detailed account of the liver pathology of the F_0 rats given 20 ppm and higher dietary levels has been published (Kimbrough *et al.* 1972).

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EXPERIMENTAL

Animals and materials. All animals used in the studies were Sherman-strain rats produced under specific-pathogen-free conditions. Aroclor 1254 (Lot AK-38) and Aroclor 1260 (Lot AK-3) were supplied by Monsanto Industrial Chemicals Company, St. Louis, Mo.

Experimental design and conduct

LD₅₀ studies. The single-dose oral LD₅₀ was determined for Aroclor 1254 and 1260 in weanling male rats (3-4 wk old). The ivLD₅₀ was determined in adult females for Aroclor 1254. Between five and eight groups, each of ten rats, were used to obtain each LD₅₀ value. The procedure described by Gaines (1969) was followed for oral dosing; Aroclor 1254 or Aroclor 1260 was dissolved in peanut oil and the solution was administered by oral intubation at the rate of 5 ml/kg. For iv dosing, Aroclor 1254 was first dissolved in peanut oil. One part of this solution was added to nine parts of a 1% lecithin-saline suspension and homogenized. This formulation was administered in a single injection via the tail vein at the rate of 5 ml/kg. A control group of five rats received 10% peanut oil in lecithin-saline in the same volume. Survivors were observed for 10-14 days.

Two-generation reproduction studies. For these studies the rats were fed either untreated ground laboratory chow (control) or ground chow fortified with Aroclor 1254 or Aroclor 1260. The Aroclors were dissolved in ether and mixed with cornstarch. The ether was allowed to evaporate and the Aroclor-cornstarch mixture was mixed into increasing amounts of ground chow. Aroclor 1254 was fed at dietary levels of 0 (control), 1, 5, 20 and 100 ppm, while Aroclor 1260 was fed at levels of 0, 5, 20 and 100 ppm. Because exposure schedules had to be staggered, a specific control group was added each time one or more treated groups were started on a dietary regimen. The F₀ rats were started on the diets at 3-4 wk of age and the F₁ rats at weaning. Exposure was continuous through mating, gestation and lactation until the rats were killed. Ten males and 20 females were fed at each dietary level in both the F₀ and F₁ generations. The F₀ males at each dietary level and half of the females were caged individually. Other F₀ females and all the F₁ rats were caged in groups except during the reproduction cycle. Body weights were recorded weekly on F₀ rats, except during the reproduction phase, and food consumption was measured during wk 2 and 5, the week before each mating and the week after weaning of the F₁ litters. F₁ rats were weighed when started on the diet, during the week before mating and at sacrifice.

The F₀ rats were pair-mated when 3 and 7 months old to produce the F₁ and F_{1b} generations, respectively. Breeding-stock F₁ rats were selected at weaning from all available litters and pair-mated when 3 months old to produce the F₂ generation. F_{1b} rats on dietary levels of 0, 20 and 100 ppm Aroclor 1254 were mated a second time when 6 months old to produce the F_{2b} generation. Viability counts of offspring were made at birth, day 3, day 7 and day 21 (weaning). Litters were inspected daily for condition and the presence of dead pups. After the F_{1b} offspring had been weaned, ten adult F₀ rats of each sex were killed and their livers were weighed and fixed for histological examination. Following the weaning of the F_{2b} generation, for Aroclor 1254, and the F₂ generation, for Aroclor 1260, haematological values (total leucocyte count, haematocrit, haemoglobin and differential leucocyte count) were determined on ten adult F_{1b} rats of each sex at the dietary levels of 0, 20 and 100 ppm. These rats were then killed and weights of the spleen, heart, lungs, brain, kidneys, testes and liver were recorded. Tissues were fixed for microscopic study.

At lower dietary levels, haematology was not done and only the livers were weighed. At dietary levels of 5 ppm or less of both Aroclors, one male and one female weanling from each of ten litters from each generation were killed at 21 days of age and the livers were weighed. F_{2n} weanlings at dietary levels of 0, 20 and 100 ppm Aroclor 1254, and F_{1n} and F_{2n} weanlings at levels of 0, 20 and 100 ppm Aroclor 1260 were killed in the same manner and the livers were fixed for further study.

One-generation reproduction studies. Preliminary studies were conducted on both Aroclors at dietary levels of 0, 100 and 500 ppm. The experimental design was the same as that described for the two-generation studies, except that only ten females were used. This study was terminated after the F_{1n} offspring had been weaned. Rats fed 500 ppm Aroclor 1254 were bred only once. Haematology values and organ weights were determined and tissues were examined microscopically on the F_0 rats.

Post-implantation exposure studies. Stock rats 100 days old were pair-mated. Insemination was verified by microscopic inspection of a daily vaginal smear. Counting the day of insemination as day 0, the females were dosed by oral intubation on days 7-15 of gestation (a total of nine doses). To maintain a constant dose volume on a body-weight basis, Aroclor 1254 or 1260 was dissolved in peanut oil at appropriate concentrations and these formulations were administered at the rate of 5 ml/kg body weight, based on day 7 of pregnancy. Aroclor 1254 was given at dosage levels of 0 (peanut oil only), 10, 50 and 100 mg/kg/day. Aroclor 1260 was given at dosage levels of 0 and 100 mg/kg/day. The females were allowed to deliver and the litters were observed through weaning.

Tissue preparation. Tissues were fixed in buffered 4% formaldehyde solution and stained with haematoxylin and eosin for microscopic examination. An Oil Red O stain on fixed frozen sections was used on occasional livers to demonstrate lipids.

Statistics and calculations

LD_{50} values were calculated by the method of Litchfield & Wilcoxon (1949). A t test was used for comparing the litter sizes, organ weights, body weights and haematological parameters of the treated groups with those of the control groups. Survival percentages of offspring were compared by the Mann-Whitney U test as proposed by Weil (1970). Food consumption values were arrived at by averaging the consumption of both males and females over the five periods in which food was measured.

RESULTS

LD_{50} studies on Aroclor 1254 and 1260

The single-dose oral LD_{50} for Aroclor 1254 in weanling male rats was 1295 mg/kg with 95% confidence limits of 1136-1476 mg/kg. The lowest lethal dose tested was 1200 mg/kg, and 1000 mg/kg produced mild diarrhoea. Signs of toxicity at lethal levels were diarrhoea, depression and salivation, with death occurring in 1-3 days.

For Aroclor 1260, the single-dose oral LD_{50} in weanling males was 1315 mg/kg with 95% confidence limits of 1174-1473 mg/kg. The lowest lethal dose tested was 1000 mg/kg. 800 mg/kg produced no signs of toxicity. Rats given lethal doses developed diarrhoea and depression, with death occurring in 1-7 days.

Under the same test conditions the estimated oral LD_{50} for Aroclor 1254 and 1260 in adult rats was 4-10 g/kg (Kimbrough *et al.* 1972).

The single-dose iv LD_{50} for Aroclor 1254 in adult female rats was 358 mg/kg with 95%

confidence limits of 328–390 mg/kg. The lowest lethal dose tested was 300 mg/kg; 250 mg/kg produced dyspnoea, depression, diarrhoea and salivation soon after dosing. Most rats died within 25–110 min, but at the highest dose (400 mg/kg), death occurred as soon as 5 min after dosing in some animals. Rats that survived appeared normal after 48 hr. In the control group, which received the formulation vehicle only, one rat developed mild diarrhoea.

Preliminary one-generation reproduction studies on Aroclor 1254 and 1260

The reproduction summary for the Aroclor 1254 feeding studies is presented in Table 1. Exposure for 67 days (at the time of pair-mating) to 500 ppm Aroclor 1254 (37.0 mg/kg/day) resulted in fewer litters, smaller litter sizes and 100% mortality by day 3 in the $F_{1\alpha}$ pups. The survival of offspring of the rats fed 100 ppm (7.2 mg/kg/day) for 67 and 186 days before mating was reduced, only 85.9 and 68.1% of the pups surviving to weaning in the $F_{1\alpha}$ and $F_{1\beta}$ generations, respectively. Litter sizes from both matings were smaller, but the difference was not statistically significant. The mean body weight of the 100 ppm pups at weaning was 7.8 and 10 g less than that of the controls for the $F_{1\alpha}$ and $F_{1\beta}$ generations, respectively. This difference was probably dose-related, as the treated litters were smaller, but a statistical evaluation was not done. In general the pups appeared small, but otherwise were normal at weaning.

Aroclor 1260 fed at a dietary level of 500 ppm (35.4 mg/kg/day) for 67 and 186 days prior to mating markedly reduced live-litter size and survival-to-weaning in the $F_{1\alpha}$ and $F_{1\beta}$ generations (Table 2). No effect on reproduction was observed in rats fed 100 ppm (6.9 mg/kg/day) in either the $F_{1\alpha}$ or $F_{1\beta}$ generations.

The F_0 rats in the one-generation studies were sacrificed after exposure for 8 months. Organ-weight analysis indicated that the liver was the primary organ affected. As reported in the previous paper, the livers in male rats receiving either Aroclor 1254 or 1260 at dietary levels of 100 and 500 ppm were heavier than in controls; this was also observed in females fed Aroclor 1254 at levels of 100 and 500 ppm or Aroclor 1260 at 500 ppm (Kimbrough *et al.* 1972). The testes-to-body weight ratios of F_0 rats fed Aroclor 1254 or 1260 at the 500 ppm level were greater than those of the controls ($P < 0.025$). The difference in actual weight of the testes, however, was not significant. This observation may reflect the reduced body-weight gain observed in all F_0 rats fed the 500 ppm level of both Aroclors. No difference in other organ weights was found.

After 8 months exposure, terminal haematological values in F_0 rats indicated a reduction in haemoglobin and haematocrit ($P < 0.005$) in both sexes fed 500 ppm Aroclor 1254 or 1260. The haematocrit was reduced in males ($P < 0.05$) on 100 ppm Aroclor 1260, but haemoglobin values were normal in this group. The total leucocyte count was normal in rats fed Aroclor 1260, but there was a shift in the differential count. The number of lymphocytes was increased in females ($P < 0.005$) fed 100 and 500 ppm Aroclor 1260 and in males ($P < 0.05$) fed 500 ppm. A corresponding decrease ($P < 0.05$) in polymorphonuclear leucocytes was observed. Differential values for rats fed Aroclor 1254 were normal, but an increase ($P < 0.001$) in total leucocytes was observed in the 500 ppm females.

Two-generation reproduction studies on Aroclor 1254

F_0 rats fed 100 ppm Aroclor 1254 (7.6 mg/kg/day) for 62 or 188 days before mating produced smaller litters than the controls in both the $F_{1\alpha}$ and $F_{1\beta}$ generations (Table 1). Survival-to-weaning was not affected in the $F_{1\alpha}$ offspring. Only 73.6% of the 100 ppm $F_{1\beta}$ pups

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Table 1. Reproduction and pup survival in groups of rats fed Aroclor 1254.

Generation	Dietary level (ppm)	Parental exposure (days) [†]	No. of females mated	No. of litters		Litter sizes [‡]		Total pups/group				Survival (%)	Mean body weight at weaning (g)
				Born [‡]	Weaned	At birth	At weaning	Born (found)		Alive at			
								Dead	Alive	Day 3	Weaning		
F ₁	01	67	10	8	7	11-1	10-6	3	89	86	85	95.5	39.2
	100	67	10	9	8	9-4	8-1	6	85	78	73	85.9	31.4
	500	67	10	4 (2)	0	4-0*	—	5	8	0	0	0	—
	0	62	20	17	17	12-4	11-8	0	211	205	201	95.3	38.7
	20	62	20	19	19	11-7	11-5	3	222	220	219	98.6	38.3
	100	62	20	19	19	10-7*	10-3	10	204	199	196	96.1	37.9
	0	67	20	18	18	11-2	11-1	2	202	200	200	99.0	39.5
	1	67	20	16	15	9-2	9-1	3	147	145	145	98.6	42.3
	5	67	20	17	17	10-8	10-8	1	184	184	183	99.5	42.1
	0	186	10	7	7	11-6	11-6	1	81	81	81	100	37.9
F ₂	100	186	10	8	6	11-8	8-0	0	94	74	64	68.1**	27.9
	0	188	20	17	17	12-3	11-1	2	209	194	189	90.4	37.1
	20	188	20	18	18	10-4*	10-1	1	188	187	181	96.3	39.0
	100	188	20	20	19	9-5**	7-0	6	189	164	139	73.6	32.1
	0	201	20	18	18	12-3	11-0	6	222	211	198	89.2	40.7
	1	201	20	18	17	11-1	9-8	3	200	194	176	88.0	38.0
	5	201	20	20	19	11-1	10-2	2	222	218	203	91.4	38.9
	0	129	20	18	18	12-5	12-2	0	225	221	220	97.8	35.5
	20	129	20	17	17	10-6*	10-1	5	181	174	171	94.5	36.8
	100	129	20	7 (2)	4	7-2**	5-6	7	36	29	28	77.6**	35.2
F ₃	0	125	20	19	19	11-7	11-5	3	223	220	218	97.8	37.4
	1	125	20	15	15	11-7	11-3	0	175	174	173	98.9	37.2
	5	125	19	17	17	12-1	11-7	1	205	199	198	96.6	35.7
	0	274	20	17	16	12-7	11-3	4	216	201	191	88.4	31.9
	20	274	20	12	12	9-6**	8-5	6	115	103	102	88.7	36.1
	100	274	20	4 (2)	2	3-5**	3-5	7	7	7	7	100.0	28.3

[†] Conception to mating for the parents of the F₁ and F₂ generations.

[‡] Numbers in parentheses indicate numbers of litters in which no live offspring were found.

[§] Number of live offspring, live litter born.

^{||} One-generation study.

Values marked with asterisks differ significantly from the control value: *P < 0.05; **P < 0.005.

survived to weaning, but this was not statistically significant ($P = 0.058$). Because of the poor condition of a number of the 100 ppm F_{11} pups, all were held for 30-days post-weaning observation. Fifteen of these pups died within 30 days of weaning. The mean body weights of the 100 ppm F_1 generation pups from either mating was about 6 g less than the controls at weaning. This is probably significant, since the treated litters contained fewer pups. A reduction in litter size was also observed in the F_{11} generation at 20 ppm (1.5 mg/kg/day).

F_{11} rats bred after exposure for 129 or 274 days (conception to mating) to 20 or 100 ppm had smaller litters than the controls (F_{11} and F_{21} generations). A marked decrease in mating performance was observed in the 100 ppm group, in which only seven and four litters were born in the F_{21} and F_{22} generations, respectively. In each case two litters contained no viable offspring when first observed. The data also suggest a decrease in mating performance at 20 ppm, at which level only 12 F_{21} litters were produced. Survival-to-weaning was reduced in the 100 ppm F_{21} offspring. All 100 ppm F_{21} offspring survived to weaning, but this is of little significance since only seven pups were found alive. No effect on reproduction was observed in rats fed 5 ppm (0.32 mg/kg/day) or 1 ppm (0.06 mg/kg/day) through two generations.

Although reproduction was not affected at lower dietary levels of PCBs, an increase in liver weight in 21-day-old weanlings was found at all levels tested (Table 2). At 5 ppm or higher, the liver-to-body weight ratios of weanlings were increased in both sexes in both

Table 2. Mean liver weights of 21-day-old rats from parents fed Aroclor 1254 or 1260

Aroclor	Generation	Dietary level (ppm)	Liver weight			
			Males		Females	
			g	% of body weight	g	% of body weight
1254	F_{10}	0	1.40	3.69	1.38	3.83
		1	1.81*	4.01*	1.63	3.91
		5	1.92*	4.34*	1.86*	4.43*
	F_{11}	0	1.58	3.69	1.58	3.84
		1	1.54	3.94*	1.49	3.92
		5	1.77	4.63*	1.72	4.75*
	F_{21}	0	1.37	3.81	1.28	3.74
		20	2.26*	5.78*	2.23*	6.08*
		100	2.63*	6.97*	2.67*	7.10*
	F_{22}	0	1.63	3.78	1.42	3.63
		1	1.49	3.74	1.36	3.69
		5	1.56	4.23*	1.38	4.09*
1260	F_{11}	0	1.54	3.66	1.57	3.92
		5	1.73	4.18*	1.70	4.39*
		20	1.59	3.73	1.55	3.93
	F_{12}	0	2.06*	5.04*	2.14*	5.18*
		20	2.59*	6.33*	2.59*	6.48*
		100	2.59*	6.33*	2.59*	6.48*
	F_{13}	0	1.35	3.59	1.29	3.70
		5	1.58	4.01*	1.52*	4.07*
		20	1.65	3.82	1.44	4.02
	F_{21}	0	2.06*	5.21*	1.94	5.16*
		100	2.50*	6.12*	2.49*	6.85*
		5	1.41	3.85	1.36	3.87
	F_{22}	5	1.66*	4.21*	1.60*	4.25*

Values marked with an asterisk differ significantly from the control value: * $P < 0.05$.

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the F_1 and F_2 generations, but at 1 ppm the liver enlargement was observed only in the F_1 and F_{10} male weanlings. No liver-weight increase was found in either sex of the 1 ppm F_{22} weanlings.

In 10-month-old F_{10} rats, an increase in liver-to-body weight ratios was observed in both sexes at 100 ppm, but at 20 ppm it was seen only in the males (Table 3). At 5 ppm or less no increase in liver weight was found in 6-month-old F_{10} rats or in F_0 rats exposed for 10 months. Both sexes of F_0 rats fed 20 ppm or more Aroclor 1254 for 8 months had enlarged livers (Kimbrough *et al.* 1972). The testes-to-body weight ratios were increased ($P < 0.001$) in 100 ppm F_{10} adult males. Body-weight gains of adults in all the test groups were comparable to those of the controls except in the F_{10} females at 100 ppm, which gained less weight than the controls (Table 3). Reduced haemoglobin ($P < 0.005$) and haematocrit ($P < 0.025$) were observed in female F_{10} adults at 100 ppm, while F_{10} males at this level had reduced ($P < 0.05$) haemoglobin only. Haematological values at lower levels were normal. No dose-related signs of toxicity were observed in adult F_0 or F_{10} rats fed 100 ppm or less.

Two-generation reproduction study on Aroclor 1260

No effect on reproduction was observed in rats fed Aroclor 1260 at levels of 100, 20 or 5 ppm (7.4, 1.5 and 0.39 mg/kg/day) through two generations (Table 4). The liver-to-body weight ratios of 21-day-old pups were increased at all dietary levels (Table 2). This effect was observed at the lowest level tested (5 ppm) in all generations.

An increase in liver-to-body weight ratios was observed at all dietary levels in F_{10} males between 5 and 7 months old, but only at 100 ppm in the F_{10} females (Table 3). After 8 months exposure, an increase in liver weight was observed at 20 and 100 ppm in F_0 males but not in females (Kimbrough *et al.* 1972). No effect on liver weight was found in either sex of F_0 rats fed 5 ppm for 8 months. The testes-to-body weight ratios of the 100 ppm F_{10} males were increased ($P < 0.05$); at 20 ppm the increase was not significant. Body-weight gain in all test groups was comparable to the control groups. Haematological values were normal in the 20 ppm F_0 adults and in F_{10} adults at 100 ppm or less. No signs of toxicity were observed in any of the test animals.

Post-implantation exposure studies on Aroclor 1254 and 1260

The data on reproduction and survival of offspring from females dosed during organogenesis is summarized in Table 5. Nine oral doses of Aroclor 1254 (100 mg/kg/day) given on days 7-15 of gestation resulted in a decrease in the survival of the pups. At this dose only 30.1% of the offspring of the treated group survived to weaning, compared with 98.2% in the control group, and the mean body weight of the test pups was 7.1 g less than that of the controls at weaning. No effect was observed in the groups receiving 50 mg/kg/day or less.

No effect on reproduction or survival was observed with Aroclor 1260 given at a rate of 100 mg/kg/day.

Summary of pathology after treatment with Aroclor 1254 and 1260

A detailed account of the liver pathology in the F_0 rats fed Aroclor 1254 or 1260 at 20 ppm or higher dietary levels has been published (Kimbrough *et al.* 1972). Of the rats considered in the current report, similar changes were observed in the liver of adult rats, particularly in the F_{10} generations at the higher dietary levels. The incidence of the various

Table 3. Body weight, liver weight and liver pathology of F_0 and F_{10} adult rats fed Aroclor 1254 or 1260.

Generation	Sex	Exposure (days)	Dietary level (ppm)	Terminal body weight (g)	Liver weight		No. of livers examined	Liver pathology (no. of livers affected)						
					g	% of body weight		Enlarged hepatocytes	Inclusions	Foamy cytoplasm	Pigment	Fibrous strands	Adenofibrosis	Nodules
Aroclor 1254														
F ₀	M	310	0	601	15.22	2.53	10	0	0	0	0	0	0	0
			1	602	15.88	2.60	10	0	0	0	0	0	0	0
			5	614	15.37	2.51	10	0	0	1	2	0	0	0
F	313	0	370	10.34	2.80	10	0	0	0	1	0	0	0	
		1	358	10.14	2.84	10	1	0	0	4	0	0	0	
		5	362	10.66	2.94	10	4	1	0	3	0	0	0	
F ₁₀	M	328	0	588	14.51	2.47	10	0	0	0	0	0	0	0
			20	602	16.11*	2.67*	8	8	3	7	1	0	0	0
			100	554	19.43*	3.54*	10	10	5	7	4	2	3	1
F	328	0	349	9.92	2.85	10	0	0	0	0	0	0	0	0
		20	340	9.41	2.78	7	7	2	5	7	0	0	3	
		100	300*	10.53	3.60*	10†	10	2	8	9	3	5	7	
F ₁₀	M	190	0	475	11.13	2.34	10	0	0	0	0	0	0	0
			1	453	11.14	2.44	10	0	0	0	0	1	0	0
			5	495	12.19	2.46	10	2	0	2	0	1	0	0
F	190	0	287	8.27	2.87	10	0	0	0	0	0	0	0	0
		1	294	8.44	2.86	10	1	1	0	0	4	0	0	
		5	299	8.92	2.98	10	1	0	0	0	1	0	0	
Aroclor 1260														
F ₀	M	250	0	563	14.45	2.57	10	1	0	0	0	0	0	0
			5	567	15.06	2.65	10	3	0	2	0	0	0	0
			0	322	10.09	3.13	10	0	0	0	0	0	0	0
F ₁₀	M	179	0	331	10.80	3.25	10	1	0	0	0	0	0	0
			0	472	12.68	2.63	10	0	0	0	0	0	0	0
			20	494	14.72*	2.99*	10	8	5	4	0	0	0	0
F	179	0	509	16.63*	3.27*	10	10	10	3	0	0	0	1	
		100	297	9.66	3.27	10	0	0	0	0	0	0	0	
		20	304	10.30	3.40	10	6	0	0	0	0	0	0	
F ₁₀	M	217	0	304	11.14*	3.70*	10	9	0	1	5	2	1	2
			0	492	11.97	2.43	10	0	0	0	0	0	0	0
			5	505	13.16	2.60*	10	7	2	2	1	0	0	0
F	217	0	279	7.93	2.84	10	0	0	0	0	0	0	0	0
		0	279	7.93	2.84	10	0	0	0	0	0	0	0	0
		5	286	8.49	2.96	10	2	0	0	0	0	0	0	0

† Necrosis in two livers.

Values marked with an asterisk differ significantly from control group: * $P < 0.025$.

† Necrosis in two livers.

Values marked with an asterisk differ significantly from control group: * $P < 0.025$.

Table 4. Reproduction and pup survival in groups of rats fed Aroclor 1260

Generation	Dietary level (ppm)	Parental exposure (days)†	No. of females mated	No. of litters		Litter size‡		Total pups/group				Survival (%)	Mean body weight at weaning (g)
				Born	Weaned	At birth	At weaning	Born (found)		Alive at			
								Dead	Alive	Day 3	Weaning		
F ₁	0‡	67	10	9	9	12.3	12.2	2	111	111	110	99.1	37.0
	100§	67	10	8	8	11.6	11.6	0	93	93	93	100.0	35.6
	500§	67	8	8	3	8.5**	3.3	10	64	55	26	34.2*	34.3
	0	63	20	19	19	11.7	11.2	2	223	217	213	95.5	40.9
	20	68	20	17	17	11.8	11.3	0	201	193	191	95.0	39.5
	100	63	20	17	17	11.6	11.2	1	197	192	191	97.0	38.0
	0	71	20	19	19	11.7	11.4	0	222	218	216	97.3	33.8
	5	71	20	18	18	12.1	11.8	1	217	213	212	97.7	38.5
F ₁	0‡	186	10	9	8	12.1	10.3	1	109	96	93	85.3	40.9
	100§	186	9	5	5	11.0	10.4	0	55	54	54	98.2	36.2
	500§	186	8	6	2	6.7**	2.3	1	40	38	14	35.0	40.7
	0	187	20	17	17	11.1	11.0	6	189	159	187	98.9	40.8
	20	187	23	16	16	11.9	11.4	1	191	191	183	95.8	41.0
	100	187	20	13	13	10.4	10.1	5	135	131	131	97.0	39.2
	0	188	20	17	16	10.3	9.2	10	175	160	156	89.1	36.1
	5	188	19	16	15	11.2	10.6	2	179	171	170	95.0	39.3
F ₂	0	128	20	18	18	10.9	10.3	1	197	187	185	93.9	40.3
	20	128	20	19	19	11.3	11.1	1	215	213	211	98.1	39.7
	100	128	20	20	20	10.1	9.9	1	201	200	197	98.0	38.6
	0	127	20	13	13	11.8	11.6	1	153	152	151	98.7	34.8
	5	127	20	18	18	11.8	11.6	0	213	212	209	98.1	38.5

‡ Conception to mating for parents of the F₂ generation.

§ Numbers of live offspring/live litter born.

¶ One-generation study.

Values marked with asterisks differ significantly from the control value: * $P < 0.025$; ** $P < 0.001$.

Effects of PCBs on rat reproduction

Table 5. *Reproduction and survival of pups from dams dosed orally with Aroclor 1254 or 1260 on days 7-15 of pregnancy*

Dose (mg/kg/day)	No. of females treated	No. of litters		Litter size†		Total pups/group				Survival (%)	Mean body weight at weaning (g)
		Born	Weaned	At birth	At weaning	Born (found)		Alive at			
						Dead	Alive	Day 3	Weaning		
Aroclor 1254											
0	9	9	9	12.3	12.1	0	111	110	109	98.2	37.9
100	9	7	5	11.9	3.6	8	83	64	25	30.1*	30.8
0	10	10	10	11.9	11.8	0	119	119	118	99.1	40.6
10	9	9	9	12.1	12.0	0	109	109	108	99.1	37.6
50	10	10	10	13.4	12.7	0	134	131	127	94.8	30.5
Aroclor 1260											
0	12	12	12	12.0	11.5	4	144	140	138	95.8	33.9
100	12	12	11	11.7	10.3	10	140	135	124	88.6	33.9

† No. of live offspring/litter born.

The value marked with an asterisk differs significantly from the control value: * $P < 0.001$.

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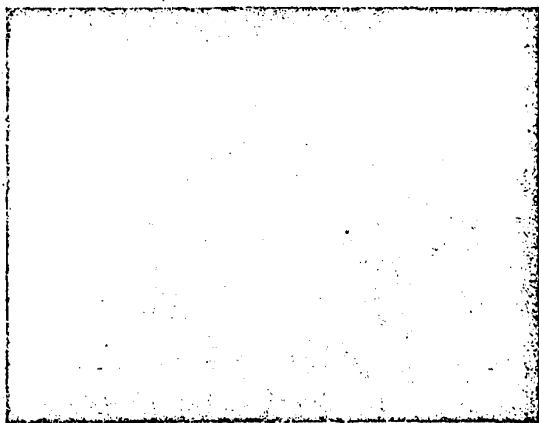


Fig. 1. Section of rat liver, showing a hepatic nodule measuring 0.2 cm in diameter surrounded by normal liver cells (arrow at margin of hepatic nodule). The hepatocytes within the nodule are larger than those of the surrounding tissue and stain less well. Haematoxylin and eosin $\times 100$.

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DISCUSSION

The results of our studies confirm the capability of some commercial PCB mixtures to alter reproductive processes in mammals. Our data are in general agreement with those of Keplinger, Fancher & Calandra (1971), who observed effects on mating performance and/or pup survival in rats fed 100 ppm Aroclor 1242 or 1254, but found no effect at the same dietary level of Aroclor 1260. In our study, 20 ppm Aroclor 1254 affected litter size and probably decreased mating performance in the second mating of the F_1 rats. The trend suggested the possibility that even lower levels might affect reproduction in subsequent generations, but M. L. Keplinger (personal communication 1973) found no effect through three generations in rats fed 10 ppm Aroclor 1254. Reproductive failure in mink exposed to PCBs has also been reported (Ringer *et al.* 1972). Although actual PCB consumption on a mg/kg basis was not reported, the mink is apparently more sensitive to PCB exposure than the rat, since levels of 1 and 5 ppm Aroclor 1254 affected reproduction after only 4 months' exposure. In the present study in rats, fewer pups per litter were observed at dietary levels of 500 ppm Aroclor 1260 and of 20 ppm or more of Aroclor 1254. Reported litter sizes are based on the pups found, but since it is common for the dam to eat dead or defective offspring, the reduced litter size probably reflects decreased fertility and/or increased perinatal and foetal mortality.

Aroclor 1254 (100 mg/kg/day) given to pregnant rats during gestation produced no effect on the viability or morphology of foetuses examined on day 22 of gestation (Villeneuve, Grant, Khara, Clegg, Baer & Phillips, 1971). In the present study under a similar dosing regimen, dams dosed orally during gestation with Aroclor 1254 (100 mg/kg/day) gave birth to litters that were grossly normal, but only 30-1% of the pups survived to weaning. Since PCBs are excreted in milk (Curley, Burse & Grim, 1973; Fries, 1972), it is possible that PCB ingestion via the milk contributed to the increased mortality of pups observed in both the post-implantation and dietary-exposure studies. The milk of rats 11 days after the last of nine daily doses of Aroclor 1254 (50 mg/kg/day) contained 66 ppm of PCB-derived material (Curley *et al.* 1973). Kimbrough *et al.* (1972) estimated the oral LD_{50} for Aroclor 1254 and 1260 at 4-10 g/kg in adult rats. From the oral LD_{50} values in weanlings in the present study (about 1300 mg/kg), it is evident that the Aroclor mixtures tested are considerably more toxic to immature rats than to adults. Although we have no acute toxicity data for PCBs in neonates, nor figures on their milk consumption, it is possible that lacteal exposure could approach a toxic level in suckling rats at the higher exposure levels.

In both the dietary and post-implantation studies, direct PCB exposure occurred *in utero*, since PCBs have been shown to cross the placenta (Curley *et al.* 1973; Grant, Villeneuve, McCully & Phillips, 1971). PCBs have been reported in eggs (Peakall, 1971) and embryonic mortality has been observed in birds treated experimentally with commercial PCB mixtures (Dahlgren *et al.* 1972; Peakall, Lincer & Bloom, 1972). Dahlgren *et al.* (1972) also reported a decrease in survival and a depression of weight gain in 6-wk-old pheasant chicks from hens treated with Aroclor 1254. In the present studies, similar effects were observed in young rats at the higher exposure levels of Aroclor 1254. Besides a reduction in observed litter size, there was additional evidence of late foetal mortality in the group given 100 ppm Aroclor 1254, no live offspring being found in four of the F_1 litters. Thus it is apparent that some PCB mixtures affect the embryo in both mammalian and avian systems, although the mechanism of this effect remains undefined.

Although the suckling rats ate some of the Aroclor-treated diet for 4-5 days before weaning, it is probable that the observed increase in liver weight in 21-day-old rats was

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due to PCB exposure via the mothers' milk. Curley *et al.* (1973) also observed a liver-weight increase in weanlings from dams dosed only during gestation. When Aroclor 1254 was given during pregnancy to rabbits, maternal liver weight was increased but not foetal liver weight on day 29 of gestation (Villeneuve *et al.* 1971), thus eliminating the possibility that placental transfer of PCB was the cause of the liver-weight increase seen in weanlings. When adult rats of the F_{10} generation were killed after dietary exposure for 5-6 months, liver weights at the lower dietary levels were comparable to those in the controls except in males given 5 ppm Aroclor 1260. Presumably once exposure via the milk ceased, the livers returned to normal in spite of a continued low dietary intake of PCB. At a dietary level of 1 ppm Aroclor 1254, only the male weanlings of the F_{10} and F_{11} generations had enlarged livers. The absence of this effect in the F_{12} weanlings is inconsistent. One explanation could be the credibility of the 1 ppm formulation. Problems inherent in consistently reproducing a 1 ppm formulation, as well as contamination of the basal diet with PCBs, are possible sources of error. Over the last 18 months this laboratory has analysed the basal diet for PCB contamination at 6-wk intervals. Most values have been less than 0.1 ppm, but occasionally values of up to 0.5 ppm have been found. The latter level of contamination would introduce a 50% error in a 1 ppm formulation. It is likely that 1 ppm in the parental diet is very near a threshold effect level for the observed liver-weight increase in weanling rats.

At the lower dietary levels, adverse effects were not observed in weanling rats, which exhibited only an increase in liver weight. However, Aroclor 1254 fed to 1-month-old rats at the 100 ppm dietary level (approximately 7.9 mg/kg/day) increased cytochrome P-450 and liver weight after 2 days and microsomal protein after 3 days, while a single oral dose of 10 mg/kg increased cytochrome P-450 after 24 hr (Goldstein, Hickman & Jue, 1973). A 50-70% increase in nitroreductase activity was observed with Aroclor 1254 and 1260 fed to rats at a dietary level of 0.5 ppm for 4 wk (Litterst, Farber, Baker & Van Loon, 1972). In view of these reports it is very probable that offspring in the reproduction studies, even those involving the lowest levels of PCBs, were subject to inductive effects.

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L'effet des biphényles polychlorurés sur la reproduction du rat

Résumé—On a étudié la reproduction, la pathologie et les indices d'intoxication aiguë chez des rats auxquels on administrait l'un ou l'autre des biphényles polychlorurés Aroclor 1254 et Aroclor 1260. Les rats qui recevaient l'Aroclor 1254 à raison de 20 ppm ou plus du régime avaient des nichées moins nombreuses que les animaux témoins aux générations F_1 et F_2 . La dose de 100 ppm d'Aroclor 1254 a fait augmenter la mortalité dans la génération F_1 , et fait nettement diminuer les performances d'accouplement des adultes de cette génération. Administré à raison de 500 ppm du régime, l'Aroclor 1260 a fait diminuer l'importance des nichées et le taux de survie des nichées F_1 .

Des taux de 5 ppm d'Aroclor 1254 et de 100 ppm d'Aroclor 1260 sont restés sans effet sur la reproduction des rats soumis à ces régimes pendant deux générations. Le poids du foie a augmenté chez les mâles F_1 âgés de 21 jours au régime à 1 ppm d'Aroclor 1254 et chez les mâles et femelles F_1 et F_2 sévères des groupes à 5 ppm ou plus d'Aroclor 1254 ou 1260. Des modifications histologiques du foie et des augmentations du poids de cet organe ont été observées chez les rats adultes qui recevaient les plus fortes doses. Des femelles gravides qui avaient reçu 100 mg d'Aroclor 1254/kg de poids vil et par jour du 7ème au 15ème jour de gestation ont mis bas des nichées à peu près normales, mais seulement 30,1% de ces jeunes ont survécu au sevrage. La reproduction et le taux de survie des jeunes n'ont pas été influencés par les doses de 50 mg d'Aroclor 1254/kg/jour et de 100 mg d'Aroclor 1260/kg/jour. Les valeurs LD_{50} orales chez les rats mâles âgés de 3-4 semaines étaient de 1295 mg/kg d'Aroclor 1254 et de 1315 mg/kg d'Aroclor 1260. La LD_{50} intraveineuse d'Aroclor 1254 était de 358 mg/kg chez les femelles adultes.

Adenofibrosis in the Rat Liver

With Persistence of Polychlorinated Biphenyls in Adipose Tissue

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Fifty male Sherman strain rats were fed 500 ppm of a polychlorinated biphenyl (PCB) (Aroclor 1254) for six months. Five each were killed zero, one, two, three, four, six, eight, and ten months after exposure to Aroclor had ceased. The livers of these rats were examined by light and electron microscopy. Liver lesions persisted although exposure to PCBs ceased.

Ten months after exposure ceased, 1,192 ppm PCBs were still present in the rats' adipose tissue and 22.65 ppm in the rat livers.

Aroclor patterns found in the tissues by electron capture gas chromatography differed from patterns of dietary Aroclors. Mass spectral analysis of liver and adipose tissue revealed three major Aroclor components with masses of 324, 356, and 392. These contained isotopic clusters indicative of the presence of C_{12} , C_{13} , and C_{14} , respectively.

In a previously reported study¹ Sherman strain rats were fed polychlorinated biphenyls (PCBs) (Aroclor 1254 and Aroclor 1260). The rats developed characteristic morphological changes in the liver. These changes consisted of hypertrophy of the liver cells, a brown pigment in Kupffer cells, lipid accumulation in the cytoplasm of hepatocytes and, at the higher dietary levels, adenofibrosis. Because of the wide distribution of PCBs in the environment² and

their persistence, the hepatic lesion was studied further. The present study was undertaken to see whether, once exposure to PCBs was stopped, the morphological changes produced in the liver might disappear.

Materials and Methods

A total of 50 male weanling, specific-pathogen-free, random-bred Sherman strain rats were tagged individually and group-caged, ten rats per cage. They were given Aroclor 1254 in their diet for six months. The dose of 500 ppm, which is equivalent to an average daily intake of 35.4 mg/kg body weight,¹ was chosen because in the previous study all ten exposed male rats developed adenofibrosis within a period of eight months. The Aroclor was mixed into ground laboratory chow as previously described.¹ After six months of

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Fig 1.—Area of adenofibrosis in liver section consisting primarily of ducts surrounded by flattened epithelium. Ducts are surrounded by little fibrosis (hematoxylin-eosin, original magnification $\times 150$).

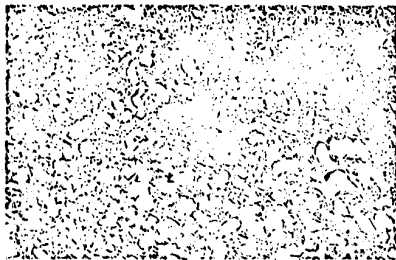
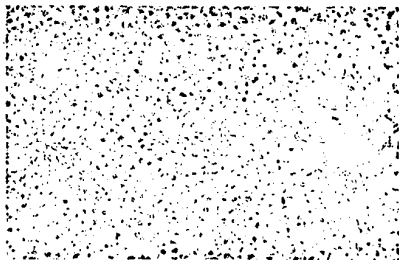


Fig 2.—Rat liver section showing cluster of small columnar cells resembling pancreatic tissue adjacent to blood vessel. Hepatocytes surrounding lesion are vacuolated (hematoxylin-eosin, original magnification $\times 175$).



Use of the present method produces an average slope around 1. For nickel, the slope changes from 0.222 in acid, to 0.666 in this organic base.

Since copper and zinc concentrations are determined after dilution, and therefore subject to a certain amount of dilution error, a National Bureau of Standards sample (No. 15777) of bovine liver was subjected to the same procedure and analyzed. The copper recovery was $113\% \pm 5.0\%$, and zinc, $112\% \pm 6\%$ in three separate analyses.

The precision of the method in reported analyses of control and exposed lung tissue is presented in Table 2. All lungs were divided into approximately five equal portions and each portion analyzed for cadmium, nickel, and zinc. The standard deviations for each lung varied from a low of 0.02 ppm to 1.56 ppm, depending somewhat on the exposure to the inhaled metal. Within measured ranges of cadmium and nickel concentrations, 200 to 300 mg of wet tissue from an exposed animal are necessary for precision. If the animal is unexposed, 300 to 400 mg are required for the cadmium analysis.

Although one would not expect any difference in the nickel concentration before or after exposure to cadmium, the nickel concentration was below the level of detection in the control animals. Sample weights can be reduced by as much as 90% to 95% for the analysis of zinc and copper.⁷

Using the data given in Table 3, the total amount of cadmium recovered from the lung was calculated and compared with the total amount of cadmium present in the inhalation chamber during that period, as well as with the estimated amount of cadmium inhaled by the rats. It can be seen that the percent retained (6% to 20%) of that "inhaled," is larger by a factor of 50 than the amount retained from the total exposure. These figures are somewhat comparable to those reported in the literature,¹² which for short acute exposures varied from 6% to 13% for rats, and under chronic conditions, lasting eight months, are estimated at 2% for rabbits. Both of these studies were performed at higher exposure levels than those in the present experiments. Under the conditions of the present study, all animals are exposed to a

relatively small particle size, (89% are less than 0.53 μ m in diameter)¹³ which for cadmium oxide would be relatively soluble. The animals were killed 16 hours after removal from the chamber, and the total exposures were not large. Experiments performed in these laboratories after five- to six-week exposure periods, to be reported in the future, do not show much increase in lung retention. Under the conditions of these experiments then, it would appear that of the cadmium oxide aerosol absorbed, a substantial portion remains in the lung.

In summary, data have been presented to illustrate a fast and relatively precise method for the analysis of cadmium and nickel in animal tissues.¹³ The technique has been used to measure cadmium concentrations in the rat lung after exposure to cadmium oxide aerosols.

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Emil Pfister, Ph.D., assisted in the construction of inhalation experiments.

William Barkley ran the inhalation chambers. Tony Horstman, Ph.D., performed the particle sizing.

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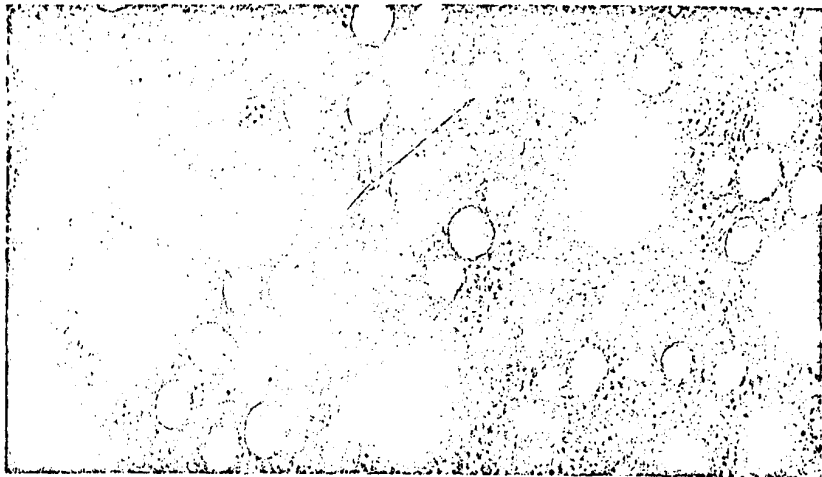
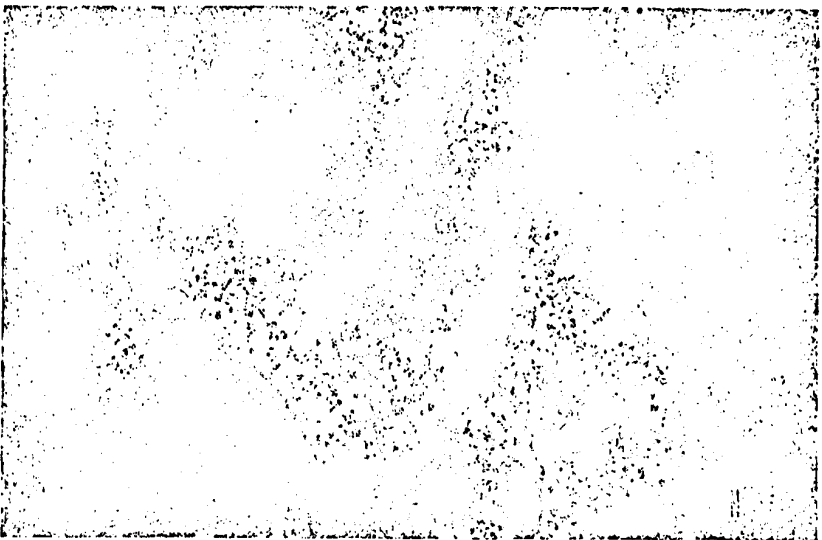


Fig 3.—Section of hepatic tissue in latter part of recovery phase illustrating numerous small lipid vacuoles (LV) (lead citrate-uranyl acetate, original magnification $\times 51,300$).

Fig 4.—Portion of hepatocyte with tubules of endoplasmic reticulum (ER) arranged in disorderly fashion. N represents nucleus; M, mitochondria (lead citrate-uranyl acetate, original magnification $\times 20,295$).



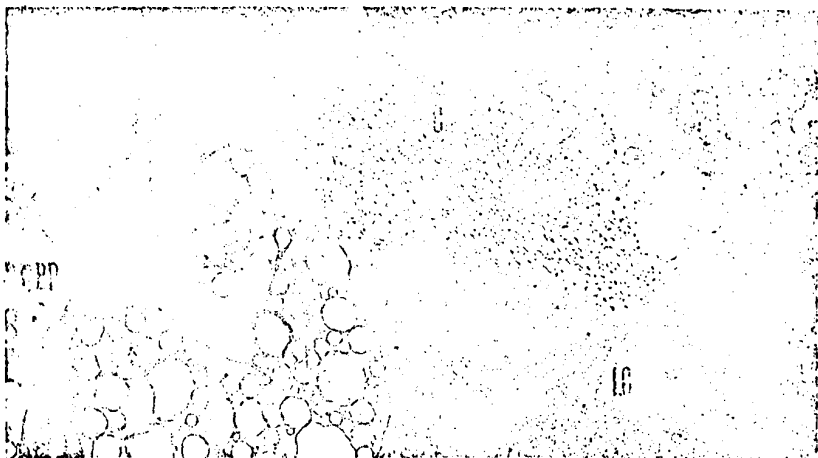


Fig 5.—Border of area of adenofibrosis in liver. Note round clusters of dark granules intermixed with web-like clusters of vacuoles. These formations illustrate appearance of brown pigment (BP). C represents collagen; LC, liver cell (lead citrate-uranyl acetate, original magnification $\times 20,235$).

receiving the experimental diet, the rats received no more Aroclor and were fed plain laboratory chow. At this time five rats were killed. Additional killings of five randomly selected rats were made one, two, three, four, six, eight, and ten months after exposure to PCB had been discontinued. At autopsy the livers were removed, weighed, and the tissue fixed in phosphate-buffered 5% glutaraldehyde for three hours for electron microscopic examination, post-fixed in 1% osmium tetroxide, embedded in maraglass, sectioned with a glass knife, and stained with lead citrate and uranyl acetate. Tissue for the light microscope was fixed in 4% buffered formaldehyde solution and stained with hematoxylin and eosin. Frozen tissue of selected formalin-fixed hepatic tissue was cut on the cryostat and stained with oil red O to demonstrate lipids. At the time of the last sacrifice, ten months after exposure to PCB-containing diets had been discontinued, adipose tissue and liver from seven previously exposed rats and the adipose tissue and liver from two rats of the same age that had been fed only plain chow were analyzed for the presence of PCBs by electron capture (EC) gas chromatography according to methods described by Curley et al.²

In order to determine which Aroclor constituents were of such chemical stability that they remained at a measurable

residual level ten months after dietary intake had ceased, adipose and hepatic composites were analyzed by gas chromatography-mass spectrometry. The conditions for mass spectral analysis were as follows: the mass spectrometer was interfaced with a gas chromatograph and equipped with a mass marker accurate within ± 0.3 mass units. The gas chromatographic column temperature was 220°C; flash heater, 235°C; glass-coiled column, 3.05 m $\times$ 0.64 mm; 1.5% OV-17/1.95% QF-1 on 60/80 mesh chromosorb "W" (high performance, acid washed, dimethyldichlorosilane treated), helium pressure, 0.84 kg/sq cm; flow rate (rotometer setting 31), 45 ml/min; separator temperature, 225°C; source energy, 70 eV; accelerating voltage 3.5 kv; trap current 60 namp; box current 50 namp, and leak current 5 namp.

Results

During the course of the experiment three rats died. The livers of the rats that were killed at the time exposure to Aroclor 1254 was discontinued were enlarged, with average weights of 6.32% of the body weight. When the experimental diets had been discontinued for four months, the liver made up only 3.6% of the body weight, and after eight months recovery the average weight

amounted to 3.34% of the average body weight. The fraction of the body weight for the liver in control adult male rats ranges from 2.44% to 2.59%.¹

Forty livers were studied microscopically. The hepatocytes were enlarged in 39 livers and all 40 livers showed either vacuolated or foamy cytoplasm because of increased lipid accumulation. Cytoplasmic inclusions similar to those described previously¹ were observed in the livers of 24 rats. Adenofibrosis, which in many instances was very extensive, was present in 36 of the 40 livers, and a brown pigment was noted in the Kupffer cells and other macrophages in 25 livers. Of the four rats that did not develop adenofibrosis, one each was killed two and six months after the poisoned diets were discontinued. The other two rats without adenofibrosis were killed ten months after onset of exposure. The adenofibrosis ranged in size from grayish white lesions measuring 0.5 to 1 cm to areas of 5 cm with a pitted surface. Quite often the lesion was multicentric. The adenofibrosis is illustrated in Fig 1 and 2. In this study the appearance of

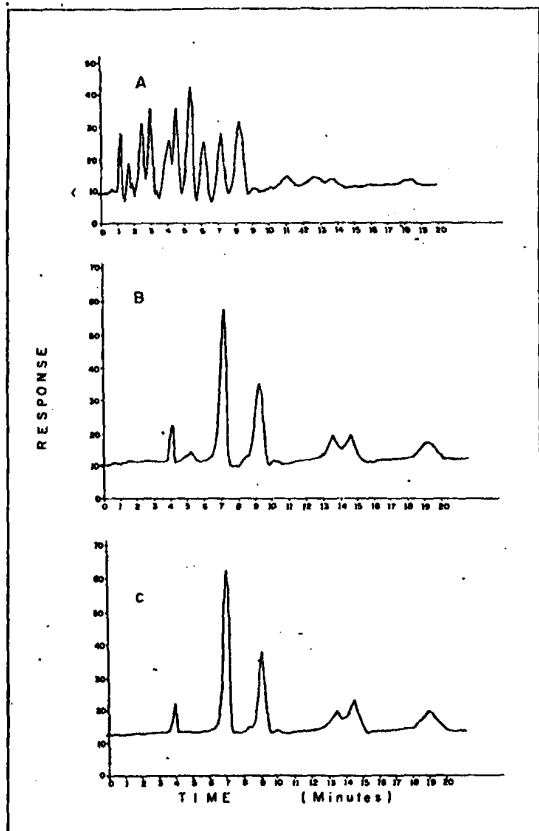


Fig 6. - Gas chromatographic traces of Aroclor 1254 standard (A), hepatic tissue extract (B), and extract of adipose tissue (C). Response expressed as percentages, with 100% = 1.0 inv.

the lesion, particularly when it was extensive, varied a great deal. Small lesions consisted predominantly of glandular pale-staining epithelial cells that formed ducts and were surrounded by very little fibrosis. The larger lesions had extensive fibrosis and contained collagen, and often the ducts which were formed by the epithelial pale-staining cells were

markedly dilated. The dilated ducts contained mucus or necrotic debris, and at times formed small cysts measuring up to 0.5 cm in diameter. Small dilated ducts were lined by columnar epithelium, and larger ones by flattened epithelium (Fig 1).

In areas of extensive fibrosis, and also adjacent to blood vessels in areas of normal hepatic parenchyma (Fig 2),

clusters of small glandular cells were seen in 15 of the livers which resembled the epithelium observed normally in the pancreas. These small clusters of pancreatic-like tissue have not previously been described in the livers of rats or of other species. Special stains for esterase, esterase with sodium taurocholate, and tryptophan showed a positive reaction indicative of salivary gland tissue. The significance of these cells is presently not understood. Normally tissue of a pancreatic type is not observed in the livers of our rats. Clusters of small columnar epithelial cells resembling pancreatic tissue also occurred in portal tracts without being surrounded by typical adenofibrosis. In the present study, one to two rats at each killing showed these small columnar cells with highly acidophilic cytoplasm and a small, round nucleus.

In the older animals, the fibrosis was quite extensive in some instances and the glandular proliferation was present mainly at the periphery of the lesion. Signs of regression of the lesion were not noted, except for the disappearance of epithelial cells from the center of the lesion. Furthermore the lipid accumulation and the hypertrophy of the hepatic cells remained constant throughout the study. Extensive areas of necrosis were observed in three of the 40 livers.

In addition to the change described previously,¹ electron microscopic examinations of sections of liver tissue showed numerous small lipid vacuoles in the cytoplasm of many hepatocytes, particularly in the latter part of the recovery phase (Fig 3). The smooth and rough endoplasmic reticulum was arranged in a disordered fashion (Fig 4). Many of the mitochondria were atypical and surrounded by double membranes.

The electron microscopic appearance of the brown pigment present in the macrophages and Kupfer cells is illustrated in Fig 5. Large clusters of dark granular material, intermixed with lipid vacuoles, were observed, as well as round clusters of granular material about the size of mitochondria. In our previous studies it was found that at least some of this pig-

ment contained iron¹ but in addition high concentrations of uroporphyrin (75% 8-carboxyprophyrins and 25% 7-carboxyprophyrins) were found in the livers,² which could account for some of the pigment. In addition to these findings, oil red O stain demonstrated granules of red-staining lipid material in the area of the brown pigment.

Since the morphological changes in the liver did not disappear, the remaining seven rats were killed after a ten-month recovery phase and their adipose tissue and livers analyzed for Aroclor. In adipose tissue, the concentrations of PCBs ranged from 924 ppm to 1,638 ppm, with an arithmetic mean of 1,192 ppm. The concentrations in the liver ranged from 17.30 ppm to 26.24 ppm, with an arithmetic mean of 22.65 ppm. The PCB levels in control adipose and liver tissues were <1.0 ppm.

The gas chromatogram obtained from the EC analysis of adipose tissue did not differ from that of liver, but was significantly different from standard Aroclor 1254. An average of seven peaks was observed as a residue in the tissues. Under these gas chromatographic conditions, at least 18 peaks have been observed in the standard material (Fig 6). The difference in gas chromatographic patterns between stored and ingested Aroclor has been observed before in this laboratory and by other researchers in the field.^{10,11}

The total ion current (TIC) chromatogram for the liver tissue composite is shown in Fig 7. Mass spectral analysis revealed the presence of only three major Aroclor components with masses of 324, 358, and 392. These components contained isotopic clusters indicative of the presence of Cl₁, Cl₂, and Cl₃, respectively. Adipose tissue analysis by mass spectrometry revealed the same patterns. Although only three different molecular components were present, examination of the EC and TIC chromatograms coupled with measured intensities from multiple scanning of eluting peaks indicates that stereoisomers of these three molecules are obviously present. Peaks 1 to 3 (Fig 7) did not contain any

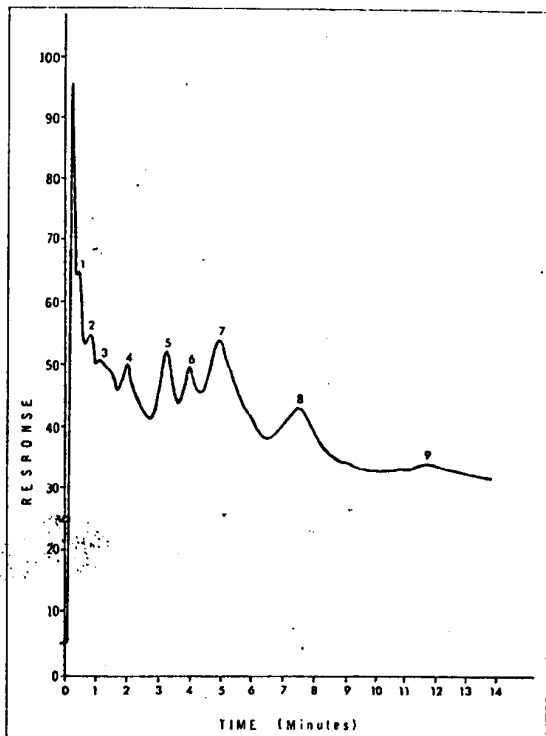


Fig 7.—Total ion current chromatogram of sample of composite liver. Response expressed as percentages, with 100% = 10.0 mv.

chlorinated moieties, while other peaks observed in the liver TIC contained the following molecular ions: peak 4 (324); peaks 5 to 7 (392 and 358); and peaks 8 to 9 (392).

Comment

The adenofibrosis of the liver produced in this study and the one previously reported¹ is the result of Aroclor consumption. Whether the Aroclor itself, or a contaminant such as a chlorinated dibenzofuran, is responsible still needs to be resolved.

A study of the lesion shows that

epithelial proliferation occurs in the periphery, while the central portion of the lesions contains primarily fibrous tissue. The present study does not completely resolve the question of reversibility of this lesion since the storage levels of PCBs in adipose tissue and liver may have been sufficiently high to stimulate continued growth of the altered hepatic tissue. The levels in the liver suggest that a constant low-level turnover of PCBs takes place in the rats. In a previous study, Curley et al¹ found levels of 10,000 ppm Aroclor 1254 in adipose tissue of rats that had been fed 500

ppm of the mixture in their diet for eight months. This finding indicates that a great deal of the Aroclors had been mobilized from the adipose tissue and excreted in the present study. The storage levels found ten months after onset of recovery are in the same range of those of rats fed 100 ppm PCBs in their diet for eight months.² The dietary level of 100 ppm produces a much lower incidence of adenofibrosis,¹ and it is possible that adenofibrosis represents a permanent lesion, particularly since the percent of body weight of the livers had decreased.

The Aroclors found in the tissues differed from those fed in the diet. Metabolism of lower chlorinated biphenyls is considered as a possible explanation for the differences observed analytically between consumed and stored PCB. Earlier work of Block and Cornish³ has shown that biphenyls and chlorobiphenyls can be hydroxylated and excreted in the urine of rabbits. A more recent study by Hutzinger et al⁴ indicates that the rat is capable of hydroxylating mono-, di-, and tetrachlorobiphenyls, but not hexachlorobiphenyls. Since Aroclor 1254 averages five chlorines per molecule, it is conceivable that the heavier chlorinated moieties could remain as a residue.

p-dimethylaminonazobenzene also produces adenofibrosis. Edwards and White¹⁰ and Bennett et al¹¹ described the lesion in rats when a mixture of chlorinated naphthalenes and chlorinated diphenyls were fed. Unfortunately, the finding was not well described in Bennett et al's report, but Edwards and White mentioned Bennett et al's finding as another example of the induction of adenofibrosis by chemicals in an excellent description of the lesion. A critical review of the histopathogenesis of this lesion was made by Stewart and Snell¹².

The presence of the brown pigment in Kupffer cells and macrophages is of interest and was to a lesser extent

also observed in the earlier study.¹ Electron microscopic examination of the pigment shows that particularly the large clusters of it resemble very closely illustrations of ceroid pigment.¹³ Experimentally ceroid pigment can be produced by hemorrhage into fatty tissues rich in unsaturated fatty acids, particularly in the absence of an antioxidant (vitamin E deficiency). Ceroid pigment is commonly referred to as lipofuscin. These lipid pigments are at times associated with hemosiderin and may contain iron-positive granules as in the present case.¹ Ceroid granules were also described by Edwards and White in the livers of rats fed butter yellow. Bennett et al also observed a brown pigment in rats fed a mixture of chlorinated naphthalenes and chlorinated biphenyls. These authors claimed that it failed to stain in a manner characteristic of either hemosiderin or hemofuscin. The porphyrins demonstrated in the livers of our rats fed Aroclor may also contribute to the overall pigment deposits. Ceroid pigment increases in people with age and has therefore been called "Abnutzungspigment" by the Germans. Attempts have been made to relate it to senility. It has been associated with neuronal ceroid-lipofuscinosis (Batten disease) in man. In minks, a disease called yellow fat disease¹⁴ is associated with the accumulation of excessive amounts of ceroid in adipose tissue. The accumulation of ceroid in mink can be prevented by addition of vitamin E to the diet. It occurs when the food rations contain high percentages of fish scrap. However, Gorham et al suggest that other factors may also be involved, since high dietary percentages of fish scrap did not promote growth as well as the stock ration, even when vitamin E was added to the diet. These observations raise the question whether high doses of vitamin E, and possibly the addition of choline to the diet, could prevent or

minimize the observed effects of PCBs on the liver of our rats.

Richard I. Moore assisted with the animal tests.

Linda W. Anderson assisted with preparation and interpretation of the electromicrographs.

Annie R. Allford performed the tissue preparations.

H. L. Stewart, MD, and the tissue laboratory of the National Cancer Institute, Bethesda, MD, performed evaluations of liver slides.

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