The Effect of Age and Sex on the Toxicity of Aroclor® 1254, a Polychlorinated Biphenyl, in the Rat

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It has been reported that PCBs have a low acute toxicity when administered orally, to laboratory male and female rats (11.9 g per kg body weight for Aroclor 1254, Personal Communication, ELMER P. WHEELER, Monsanto Company). However, in a preliminary study we found that the LD_{50} for a single oral dose of Aroclor 1254 in the mature female rat was approximately 2 g per kg body weight. Age has been shown to have an affect on LD_{50} for a number of drugs and pesticides in rats (GOLDENTHAL, 1971).

A number of reports have shown that animals given PCB's have an enlarged liver (TANAKA et al. 1969, GRANT et al. 1971, VILLENEUVE et al. 1971, GRANT et al. 1972 and KIMBROUGH et al. 1972), a marked increase of smooth endoplasmic reticulum (NISHIZUMI 1970 and KIMBROUGH et al. 1972), increased microsomal mixed function oxidase activity (STREET et al. 1969, VILLENEUVE et al. 1971, BICKERS et al. 1972 and GRANT et al. 1972), and fatty infiltration of the liver (NISHIZUMI 1970, GRANT et al. 1971 and GRANT et al. 1972). FURNER et al. (1969) have shown that age and sex influence the effect of drug treatment on the induction of drug metabolism enzymes.

Because of the differences in the LD_{50} values for Aroclar 1254 and of the paucity of data in the literature on the toxicity of PCB's in relation to sex and age in the rat, studies were undertaken to determine the acute and subacute toxicity of the commercial PCB, Aroclor 1254.

METHODS AND PROCEDURES

Toxicity of a single oral dose

Male and female Wistar rats were divided into 42 groups of 6 rats per group. Seven groups of females and males were dosed orally at 30, 60 or 120 days of age. Aroclor 1254 was mixed with corn oil and the rats given 1.6 ml per kg body weight at 30 or 60 days of age and 1.0 ml per kg body weight at 120 days of age. The

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quantities of Aroclor 1254 administered were 0, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 g per kg body weight. Food was removed 16 hours prior to dosing; thereafter food and water were given ad libitum. The animals were observed for 7 days and deaths recorded. LD_{50}'s were calculated according to WEIL (1952).

The 7 day oral dose effect level

Two experiments were carried out. In the first emphasis was placed on organ weight changes, and on fat accumulation in the liver. In the second experiment a more specific indicator of biochemical response, induction of mixed function oxidases (hepatic aniline hydroxylase activity) was measured and the effect of sex and age studied. The rats were given standard laboratory cubes (Maple Leaf Mills, Master Feeds Division, Toronto, Canada) and water ad libitum.

First experiment

Forty female Wistar rats, approximately one year old, were divided into 8 groups of 5 rats per group. Aroclor 1254 was mixed with corn oil and the rats given 1.6 ml per kg body weight. The quantities of Aroclor 1254 administered were 0, 12.5, 25.0, 50.0, 100, 400 and 800 mg per kg body weight, for 7 consecutive days. The rats were killed by decapitation 24 hours after receiving the 7th dose, and liver, kidneys, spleen and heart removed and weighed. Liver samples were analyzed for fat by the method of BLIGH and DYER (1959).

Second experiment

Male and female Wistar rats were divided into 24 groups of 6 rats per group. Four groups of females and males were given oral doses for 7 consecutive days at 30, 60, or 120 days of age. Aroclor 1254 was mixed with corn oil and the rats given 1.6 ml per kg body weight at 30 or 60 days of age and 1.0 ml per kg body weight at 120 days of age. The doses of Aroclor 1254 administered were 0, 5, 10 and 20 mg per kg body weight. The rats were killed by decapitation 24 hours after receiving the 7th dose, and liver, spleen and kidneys removed and weighed. Two g liver samples were placed on ice, prior to being homogenized with 2.5 volumes of buffer (0.05 M Tris, pH 7.4 containing 1.15% KCl). The 17,500 g supernatants were used to measure the hepatic aniline hydroxylase activities (BECKING, 1973).

RESULTS AND DISCUSSION

Toxicity of a single oral dose

The LD_{50}'s (g per kg body weight) were 1.4 and 1.3 for 30 day old male and female rats, respectively; 1.4 for 60 day old male and female rats and 2.0 and 2.5 for 120 day old male and female rats, respectively. The results show that sex and age had little affect on the acute toxicity, except in the case of the 120 day old male and female rats. Because the doses administered usually killed all or none of the rats, 95% confidence limits were not calculated. The results agree with our preliminary finding that the LD_{50} for mature female rats was approximately 2 g per kg body weight. The majority of rats died within 3 days but some survived 4, 5 and 6 days. The reason for the great difference in LD_{50} reported here and that reported by Monsanto can not be explained. Strain difference, Sprague-Dawley compared to Wistar could account for some of the difference in results, but not a six fold difference. In Monsanto's study Aroclor 1254 was administered undiluted and this might affect its absorption. However, LEWIN et al. (1972) found that the 5 day LD_{50}'s of DDT and Aroclor 1254, in corn oil, increased with decreased solute concentration when administered intraperitonealy to mice. TANAKA et al. (1969) reported that the LD_{50} of a commercial polychlorinated biphenyl mixture, administered orally as a single dose in oil, was approximately 2 g per kg body weight for mice and there was no significant difference due to sex.

7 day oral dose effect level

First experiment

All levels tested (Table 1) significantly increased the weight of the liver of the year old female Wistar rats, while no effect was observed on the weight of the heart. The weights of the kidneys and spleen were increased and decreased respectively, compared to controls but the changes were not dose related. Aroclor 1254 had previously been shown to decrease the spleen weight of rats (GRANT et al. 1971). Pheasants responded similarily, due to lymphocyte depletion (DAHLGREN et al. 1972). The fat content of the liver was significantly increased by the 100 mg per kg body weight treatment. Using the most sensitive parameter measured, liver enlargement, the no-effect level would be less than 12.5 mg per kg body weight.

TABLE 1

Effect of 7 consecutive oral doses of Aroclor 1254 in corn oil on the liver weight and % liver fat of year old female rats.

Dose[a] (mg per kg)	Number of rats dead	Liver weight (g)	Liver fat (%)
0	0	9.12 ± 0.58[b]	4.4 ± 0.2[b]
12.5	0	11.11 ± 0.75[c]	4.7 ± 0.1
25	0	12.86 ± 0.96[c]	4.2 ± 0.4
50	0	14.19 ± 1.24[c]	5.2 ± 0.7
100	0	15.07 ± 0.97[c]	5.1 ± 0.3[c]
200	0	17.54 ± 1.38[c]	6.4 ± 0.9[c]
400	1	17.16[e] ± 2.08[c,d]	9.2 ± 0.8[c,d]
800	4	17.76[e]	

[a]Each grown comprised 5 rats and were dosed orally for 7 consecutive days.

[b]Mean of 5 determinations and standard error of the mean.

[c]Significantly different from controls ($P < 0.05$, Student's "t" test).

[d]Mean of 4 determinations and standard error of the mean.

[e]Single value.

WEIL and McCOLLISTER (1963) reported that 87% of all the subacute and 2-year tests were critically delinated by the examination of only body weight and/or relative liver and kidney weights. WEIL et al. (1969) have suggested using the results of 7 day studies to predict the effect dosages for 90 day and 2 year studies. This conversion would give effect levels (mg per kg body weight, 95th percentile) of less than 2 and less than 0.36 for 90 day and 2 year studies respectively.

Second experiment

Treatment had no effect on body weight, and an inconsistent effect on the weight of the spleen and kidneys, except that the kidney weights of the 120 day old rats which were dosed with 10 or 20 mg per kg body weight were significantly increased ($p < 0.05$, Student's "t" test). In the male rats the weight of the liver was consistently increased whether expressed on an absolute basis or as a per cent of body weight (Table 2). The no-effect level, (liver weight expressed as percent of body weight) would be less

TABLE 2

Effect of 7 consecutive oral doses of Aroclor 1254 to male rats.

Dose[a] (mg per kg)	Age (days)	Liver weight (g)	Liver weight (% body weight)	μ moles of PAP formed[b] A	μ moles of PAP formed[b] B
0	30	5.35 ± 0.31[c]	4.53 ± 0.18[c]	0.91 ± 0.11[d]	5.08 ± 1.10[d]
5	30	6.91 ± 0.22[e]	5.80 ± 0.16[e]	1.89 ± 0.36[e]	12.95 ± 1.88[e]
10	30	7.67 ± 0.66[e]	6.97 ± 0.30[e]	2.28 ± 0.46[e]	19.34 ± 6.40[e]
20	30	7.30 ± 0.42[e]	5.37 ± 0.42[e]	2.09 ± 0.48[e]	14.86 ± 3.65[e]
0	60	11.59 ± 0.52	3.98 ± 0.10	1.35 ± 0.29	15.54 ± 2.62
5	60	13.64 ± 0.73[e]	4.37 ± 0.09[e]	2.00 ± 0.50	25.33 ± 5.80
10	60	14.00 ± 0.50[e]	4.85 ± 0.11[e]	1.53 ± 0.30	21.99 ± 4.10
20	60	14.46 ± 0.73[e]	5.49 ± 0.26[e]	1.71 ± 0.05	26.30 ± 1.50[e]
0	120	15.18 ± 0.86	3.49 ± 0.09	1.65 ± 0.28	25.76 ± 4.40
5	120	18.17 ± 0.69[e]	4.08 ± 0.11[e]	5.11 ± 0.24[e]	96.90 ± 5.14[e]
10	120	20.93 ± 1.44[e]	4.41 ± 0.17[e]	5.84 ± 0.21[e]	110.99 ± 7.28[e]
20	120	22.15 ± 1.14[e]	6.07 ± 0.09[e]	7.25 ± 0.41[e]	155.61 ± 8.62[e]

[a]Each group comprised 6 rats and were dosed orally for 7 consecutive days

[b]μ moles of p-aminophenol formed (A) per g liver per hr. and (B) per whole liver per hr.

[c]Mean of 6 determinations and standard error of the mean.

[d]Mean of 4 determinations and standard error of the mean.

[e]Significantly different from controls ($p < 0.05$ Student's "t" test).

than 5 mg per kg for males and females (Tables 2 & 3). The hepatic aniline hydroxylase activities were significantly increased by the 5, 10 or 20 mg per kg treatments in the 30 and 120 day old male and female rats. According to WEIL et al. (1969) the 90 day and 2 year effect levels (95th percentile) could be calculated by dividing by 6.2 and 35.3 respectively. A number of factors (sex, age, endocrine and nutritional status) have an influence on the microsomal mixed function oxidases (CONNEY and BURNS, 1972). The control male rats had higher aniline hydroxylase activity than the female rats. However, the treatment resulted in greater induction in the female rats.

Although only aniline hydroxylase activities were measured in this study, BICKERS et al. (1972) found that; 6 consecutive daily intraperitoneal administrations of 25 mg per kg body weight of Aroclor 1254 significantly increased microsomal protein, aniline hydroxylase, ethylmorphine N-demethylase, cytochrome P-450, cytochrome b5, and decreased zoxazolamine paralysis time and hexobarbital sleeping time.

TABLE 3

Effect of 7 consecutive oral doses of Aroclor 1254 to female rats.

Dose[a] (mg per kg)	Age (days)	Liver weight (g)	Liver weight (% body weight)	μ moles of PAP formed[b] A	μ moles of PAP formed[b] B
0	30	5.14 ± 0.33[c]	4.90 ± 0.22[c]	0.69 ± 0.12[d]	3.36 ± 0.35[d]
5	30	5.82 ± 0.42[e]	5.52 ± 0.21[e]	1.48 ± 0.17[e]	8.25 ± 1.58[e]
10	30	5.98 ± 0.20[e]	5.63 ± 0.16[e]	1.63 ± 0.14[e]	9.78 ± 0.68[e]
20	30	6.37 ± 0.28[e]	6.23 ± 0.20[e]	2.12 ± 0.49[e]	13.76 ± 3.76[e]
0	60	8.35 ± 0.57	4.06 ± 0.19	0.74 ± 0.18	6.55 ± 1.63
5	60	8.85 ± 0.27	4.52 ± 0.11[e]	0.93 ± 0.18	8.57 ± 1.79
10	60	9.22 ± 0.31	4.75 ± 0.13[e]	0.94 ± 0.16	8.92 ± 1.36
20	60	10.80 ± 0.61[e]	5.19 ± 0.15[e]	0.99 ± 0.13	11.30 ± 1.89[e]
0	120	8.87 ± 0.46	3.39 ± 0.13	0.73 ± 0.02	6.49 ± 0.69
5	120	10.41 ± 0.62[e]	3.81 ± 0.06[e]	3.74 ± 0.37[e]	35.37 ± 2.45[e]
10	120	10.38 ± 0.24[e]	4.03 ± 0.10[e]	4.20[f]	45.0[f]
20	120	11.86 ± 0.66[e]	4.34 ± 0.08[e]	7.07 ± 0.51[e]	82.13 ± 3.86[e]

[a]Each group comprised 6 rats and were dosed orally for 7 consecutive days.

[b]μ moles of p-aminophenol formed (A) per g liver per hr. and (B) per whole liver per hr.

[c]Mean of 6 determinations and standard error of the mean.

[d]Mean of 4 determinations and standard error of the mean.

[e]Significantly different from controls ($P < 0.05$, Student's "t" test).

[f]Mean of 2 determinations.

The enlargement of the liver with increased production of microsomal enzyme may be regarded as an adaptive response. In a long term study we have shown that the dominant pathological lesion was micro-droplet fatty change and was reversible (GRANT et al. 1972).

The oral single dose LD_{50} of Aroclor 1254 in the rat was less than 2 g per kg body weight while the 7 day effect level (liver enlargement and increased aniline hydroxylase activity) was less than 5 mg per kg body weight per day. Sex and age had very little affect on the toxicity of Aroclor 1254 to rats. Although in some cases the spleen and kidney weights were affected by treatment, only the increased liver weight was dose related.

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