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Pair-feeding Study of PCB (Aroclor 1254) Toxicity in Rats

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Polychlorinated biphenyls (PCBs) have been acknowledged as significant environmental pollutants since the mid-1960's (JENSEN, 1966) although they have been produced commercially for half a century (PENNING, 1930). The literature describing PCB toxicity contains numerous reports describing the deleterious effects of PCBs on the body weight of monkeys (HORI et al., 1982), racoons (MONTZ et al., 1982), ferrets (BLEAVINS et al., 1982), mink (AULERICH and RINGER, 1977), rats (BRUCKNER et al., 1973, 1974, KIRIYAMA et al., 1974, and KOMIVES, 1979), and mice (SILKWORTH and BRAGSTEIN, 1982). In addition to diminished body weights, dietary PCBs have produced depressed food consumption in weanling rats (CARTER and MERCER, 1983) and sexually-mature rats (KOMIVES, 1979).

PCBs have been shown to adversely affect relative organ weights such as the liver, kidneys, spleen, and heart (CARTER and MERCER, 1983). Since the consumption of dietary PCBs has been associated with decreased body weights and food intakes, it is possible that altered relative organ weights are due to those decreases rather than to the direct toxic effects of PCBs. The purpose of this investigation was to examine the toxic effects of dietary PCBs and depressed food consumption on body weights and relative organ weights in male weanling rats.

MATERIALS AND METHODS

Male Fischer rats 37 days old (Charles River COBS/CDF), were divided into five groups of 10 animals each. The rats were individually housed in wire-bottom cages with food and deionized water available ad libitum, and acclimated to controlled lighting (from 0700 to 1900) and temperature (26°C ± 2°C) for five days. All groups were fed diets which contained either 0, 150, or 350 PPM of Aroclor 1254 (Table 1). The rats were divided according to a randomized block design into the following groups: control (0 PPM), low dose pair-fed (150-PF), low dose (150 PPM), high dose pair-fed (350-PF), and high dose (350 PPM). Food intakes and body weight gains were recorded daily for 10 days, then the animals were sacrificed by diethyl ether anesthesia. The liver, kidneys, and spleen were removed and their wet weights determined. Results were analyzed by one-way analysis of variance with multiple comparisons of group means using Duncan's New Multiple Range Test.

Table 1. Composition of Diet

	% in Diet
[1]Vitamin Mix (A.I.N. Vitamin Mix 76)	1.00
[2]Alphacel (Non-nutritive Bulk)	5.72
[3]Salt (A.I.N. Mineral Mix 76)	3.50
[4]Cornstarch	57.00
[5]Vitamin-Free Casein	25.00
[6]Choline Chloride	0.20
[7]d,l-Methionine	0.08
Fat (Mazola Corn Oil)	7.50

[1-7]I.C.N. Nutritional Biochemicals, Cleveland, Ohio 44128

RESULTS AND DISCUSSION

The data representing the relative wet organ weights are summarized in Table 2. The increases in relative liver weight seen in the 150 PPM and 350 PPM groups are similar to the changes in organ weight predicted in a previous publication (CARTER and MERCER, 1983). There is no indication from the statistical analysis that pair-feeding affected the ability of the liver to increase in mass when exposed to increased dietary concentrations of PCBs.

Table 2. Wet Organ Weights Expressed As Percent Body Weight*

Treatment	Liver	Kidneys	Spleen
0 PPM	$5.050 \pm .056^{a}$	$.798 \pm .104^{ab}$	$.294 \pm .006^{a}$
150-PF	$3.907 \pm .140^{b}$	$.772 \pm .008^{b}$	$.267 \pm .005^{b}$
150 PPM	$8.392 \pm .232^{c}$	$.823 \pm .016^{a}$	$.295 \pm .004^{a}$
350-PF	$6.377 \pm .122^{d}$	$.822 \pm .011^{a}$	$.251 \pm .004^{b}$
350 PPM	$9.355 \pm .170^{e}$	$.836 \pm .014^{a}$	$.259 \pm .007^{b}$

*Mean ± SEM of 10 rats per group. Values in the same column not sharing a common superscript are significantly different ($p < 0.05$).

There were no statistically significant changes in relative kidney weights, although kidney weight (Table 2) tended to increase with increasing dietary PCBs. However, the relative spleen weights were depressed in the high dose group (350 PPM) and its pair-fed group (350-PF). This phenomenon appears to be due to a change in food consumption, for a similar organ weight value is seen in its pair-fed group.

Table 3 depicts the data regarding average daily food consumption. As anticipated, the animals consuming the 150 PPM diet did not exhibit a significant decrease (except on day 5) in average daily food intake throughout the 10-day period (CARTER, unpublished data). However, daily food intake was decreased in the 350-PF and 350 PPM groups beginning on the third day and continuing throughout the duration of the investigation.

Table 3. Daily Diet Consumption* During the Ten-Day Experiment

Dietary	Days after Dietary Treatment			
Treatment	3	5	7	10
0 PPM	11.3 ± .3[a]	12.1 ± .4[a]	12.4 ± .4[a]	13.2 ± .4[a]
150-PF	10.8 ± .2[ab]	11.6 ± .2[ab]	12.2 ± .3[a]	11.9 ± .4[a]
150 PPM	10.6 ± .2[ab]	11.5 ± .2[b]	12.0 ± .3[ab]	12.1 ± .5[a]
350-PF	10.4 ± .2[b]	11.0 ± .3[c]	11.0 ± .4[bc]	10.5 ± .5[b]
350 PPM	10.2 ± .1[b]	10.8 ± .3[d]	10.8 ± .4[c]	10.3 ± .5[b]

*Mean ± SEM of 10 rats per group. Values in the same column not sharing a common superscript are significantly different ($p < 0.05$).

When the food consumption data are expressed on a cumulative basis, no significant differences are observed until day five (Table 4.). At that time, the 350-PF and 350 PPM groups are significantly lower than the control animals. Although the daily

Table 4. Cumulative Diet Consumption* During the Ten-Day Experiment

Dietary	Days after Dietary Treatment			
Treatment	3	5	7	10
0 PPM	22.3±.5[a]	45.7±1.1[a]	70.7±2.0[a]	110.5±3.7[a]
150-PF	19.3±.7[b]	41.7±.8[b]	66.0±1.1[a]	102.0±1.5[ab]
150 PPM	21.3±.3[a]	43.5± .7[ab]	67.6±1.0[a]	102.9±1.9[ab]
350-PF	20.6±.4[ab]	42.3± .8[b]	64.1±1.2[b]	95.3±2.2[b]
350 PPM	21.7±.7[a]	42.9± .8[b]	64.5±1.2[b]	95.1±2.2[b]

*Mean ± SEM of 10 rats per group. Values in the same column not sharing a common superscript are significantly different ($p < 0.05$).

diet consumption of the 150 PPM and 150-PF rats were slightly less than the 0 PPM rats throughout the study (Table 3.), no significant differences were observed for these groups for cumulative food consumption.

When the food consumption data are expressed on a relative basis (daily food consumed per gram body weight) significant decreases appear after the fifth day in the 350-PF and 350 PPM groups (Table 5.). No other differences were observed among the other groups.

Table 5. Diet Consumption Expressed Per Gram Body Weight* During the Ten-Day Experiment

Dietary	Days after Dietary Treatment			
Treatment	3	5	7	10
0 PPM	.107±.003^{a}	.106±.002^{a}	.100±.003^{a}	.095±.001^{a}
150-PF	.105±.002^{a}	.108±.002^{a}	.105±.003^{a}	.094±.002^{a}
150 PPM	.103±.002^{a}	.102±.002^{a}	.098±.003^{ab}	.088±.003^{ab}
350-PF	.104±.002^{a}	.105±.002^{a}	.097±.003^{bc}	.088±.003^{ab}
350 PPM	.100±.002^{a}	.099±.002^{a}	.085±.008^{b}	.082±.003^{b}

*Mean ± SEM of 10 rats per group. Values in the same column not sharing a common superscript are significantly different ($p < 0.05$).

Fig. 1 illustrates the changes in both the final body weight and cumulative body weight gain. The 0 PPM and 150 PPM groups are

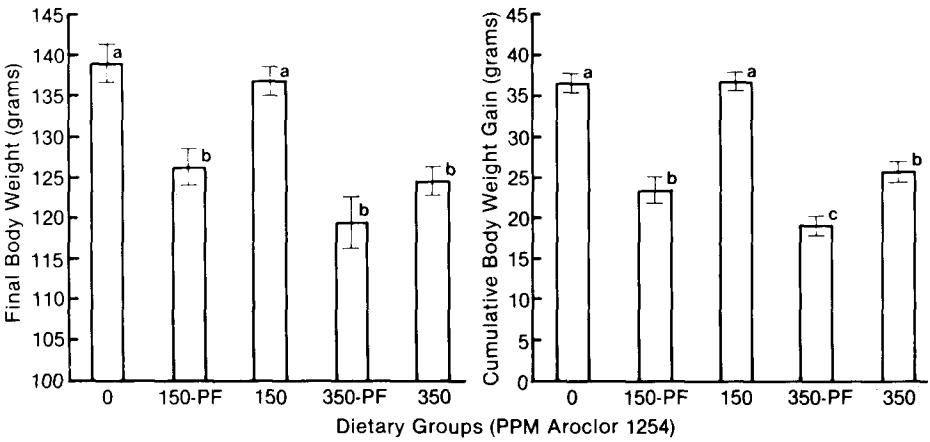

Figure 1. Final Body Weights and Cumulative Body Weight Gains-
Mean ± S.E. Different lower case letters indicate significantly different group means ($p = 0.05$).

significantly higher than all other groups for both parameters. Both the 350 PPM and the 150 PPM groups gained more weight than their respective pair-fed groups.

Relative wet weights of the liver and kidneys are very sensitive criteria in toxicological experiments (DEGROOT and TILL, 1965, SMYTH et al., 1952, and WEIL and MCCOLLISTER, 1963). In a study evaluating the criterion "organ weight" under conditions of growth retardation, it has been reported that growth retardation had a significant effect on the relative organ weights of the kidneys (decreased), liver (decreased), spleen (decreased), thymus (decreased), adrenals (increased), and brain (increased) in male rats fed diets containing 55% cellulose for four weeks (FERON et al., 1973). These authors concluded "that in cases of growth reduction, an increase in the relative weights of the liver or the kidneys must be considered to be an effect of the compound."

The animals in our study also exhibited a retardation in growth (Fig. 1) which was associated with decreased food consumption (Tables 3-5.). Because of a reduction in growth, the relative wet weights of the liver (150-PF) and the spleen (150-PF, and 350 PPM) were significantly decreased (Table 2.). However, in all groups which consumed PCB-containing diets (150 PPM, 350-PF, and 350 PPM), the relative wet liver weights were elevated. This hepatomegaly has been explained earlier as the toxic effects of PCBs on centrilobular hepatocytes (CARTER and CAMERON, 1977).

The differences in body weight in paired groups illustrated in Fig. 1 are explained almost entirely by increases in absolute wet liver weight. When comparing the 150 PPM and 150-PF groups, approximately 65% of the difference in final body weight in these animals is accounted for by higher absolute liver weight. And when comparing the 350 PPM and 350-PF rats, approximately 80% of the difference in final body weight is accounted for by greater absolute liver weight.

The onset of weight loss and decreased food intake in PCB-treated rats varies apparently with the genotype of the animals. In seven-week-old male Sprague Dawley rats given daily doses of 0.05 g Aroclor 1254/Kg body weight by oral intubation, depressed food intake and weight loss commenced on the ninth day and continued until the twenty-first (last) day (KOMIVES, 1979). The dose of PCBs given to those animals is approximately 40% greater than the dietary PCB concentration given to the 350 PPM group in the present study. The five-week-old male Fischer rats used in our study grow at a faster rate (percent body weight increase over time) than seven-week-old male Sprague Dawley rats (ANONYMOUS, 1982). Since decreased diet consumption and weight loss was observed on the fifth day in this study, it is apparent that growth rate affects the onset of symptoms of PCB toxicity (i.e., decreased diet consumption and weight loss).

In summary, daily and cumulative food intakes were depressed in the 350 PPM and 350-PF animals. Final body weights were decreased in the 150-PF, 350-PF, and 350 PPM groups. This reduction in growth resulted in depressed relative wet liver weight (150-PF) and relative wet spleen weight (150-PF, 350-PF, and 350-PPM). Increases in relative wet liver weights (150-PPM, 350-PF, and 350 PPM) were accounted for by PCB toxicity. There were no significant changes in relative kidney weights.

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