

Effect of PCB Ingestion on Sleeping Times, Organ Weights, Food Consumption, Serum Corticosterone and Survival of Albino Mice

by O. T. SANDERS, R. L. ZEPP, and R. L. KIRKPATRICK
*Department of Fisheries and Wildlife Sciences
Virginia Polytechnic Institute and State University*

Polychlorinated biphenyls (PCBs), known environmental contaminants (HOLDEN and MARSDEN 1967; KOEMAN et al. 1969; JENSEN et al. 1969; ANDERSON et al. 1969; PRESST et al. 1970; SASCHENBRECKER 1973), have shown numerous sublethal effects such as hepatic microsomal enzyme induction (VILLENEUVE et al. 1971; LITTERST et al. 1972; STREET et al. 1969; BITMAN et al. 1972), estrogenic activity (BITMAN and CECIL 1970; BITMAN et al. 1972), increased steroid metabolism (RISEBROUGH et al. 1968; LINCER and PEAKALL 1970; NOWICKI and NORMAN 1972; PLATONOW et al. 1972), reproductive inhibition (RINGER et al. 1972; PLATONOW and KARSTAD 1973; immunosuppression (FLICK et al. 1965; VOS and KOEMAN 1970; VOS and BEEMS 1971) and increased susceptibility to an infectious agent (FRIEND and TRAINER 1970).

These effects are suggestive of abnormal endocrine activity; however, there is little information on changes in endocrine and/or reproductive organ weights and steroid hormone levels in PCB-treated animals. FLICK et al. (1965) noted enlarged adrenals and small spleens in PCB treated White Leghorn cockerels. PLATONOW and FUNNEL (1971) reported reduced testes weights in cockerels exposed to dietary PCBs. Recently, JEFFERIES and PARSLow (1972), found increased follicle size, colloid content and weights of the thyroid glands of PCB treated black-backed gulls (*Larus fuscus*).

This paper reports the results of a preliminary experiment designed to evaluate the effects of PCB ingestion on food consumption, pentobarbital-induced sleep time, select endocrine and reproductive organ weights, serum corticosterone levels and survival of laboratory mice.

MATERIALS AND METHODS

Adult albino mice (ICR strain) were allotted to groups of 5 mice each and were fed ad libitum diets containing 0, 62.5, 250, 1000 or 4000 ppm of Aroclor 1254 incorporated into ground Purina Rat Chow. The diluted PCB was dissolved in acetone and added to dry feed while mixing. Body weights

were determined initially and again before the animals were sacrificed. Food consumption was measured daily for each treatment group. After the mice had been fed the experimental diets for 2 weeks, they were injected intraperitoneally with sodium pentobarbital (Nembutal^R) at a dose of 70 mg/kg and sleeping times were recorded. Duration of sleeping was determined as the elapsed time from the loss of the righting ability to the regaining of the righting ability. Forty-eight hours after the pentobarbital injection the animals were sacrificed at random and fresh weights of adrenals, livers, testes, vesicular glands and preputials were recorded. Trunk blood was collected from individual mice by decapitation. Serum was frozen and later assayed for corticosterone by the competitive protein binding method (MURPHY 1967; 1969).

RESULTS

All five of the mice fed 4000 ppm died within seven days after onset of the experiment - one on day 5, two on day 6, and two on day 7. Three of the five mice fed 1000 ppm died - two on day 12 and one on day 15. None of the mice fed 250 ppm died. One animal fed 62.5 ppm died after the pentobarbital injection, and one of the control animals died on day 11 of an unknown cause. Food consumption at 1000 and 250 ppm was 70.0 and 81.6 percent respectively, of consumption by controls. Mean total PCB intake per mouse was calculated to be 178, 552, and 1965 mg/kg body weight for the 62.5, 250 and 1000 ppm groups, respectively.

The effects of the dietary PCB treatments on pentobarbital-induced sleeping time and relative liver weight (% initial body weight) are shown in Table 1.

TABLE 1

Mean ($\pm$ SE) pentobarbital-induced sleeping time and relative liver weight in male albino mice fed Aroclor 1254 in the diet for 2 weeks.

Dietary Level (ppm)	Liver Weight (% Initial Body Wt.)	Sleeping Time (minutes)
0 (4) ^a	5.7 $\pm$.10 ^b	71.7 $\pm$ 2.5 ^b
62.5(4)	5.0 $\pm$.35 ^b	97.2 $\pm$ 13.0 ^b
250 (5)	7.3 $\pm$.49 ^c	32.6 $\pm$ 11.6 ^c
1000 (3)	10.8 $\pm$ 2.0 ^d	39.6 $\pm$ 3.12 ^c

^aNumber of animals in parentheses.

^{b,c,d}Means bearing different subscripts are significantly different from each other at the 5% level (Duncan's Multiple Range Test).

Sleeping time was significantly reduced and liver weight was significantly increased in the groups fed 250 and 1000 ppm ($P < .05$). Neither sleeping time nor liver weight were significantly affected at the 62.5 ppm dietary level.

Table 2 presents the effects of Aroclor 1254 on body weight change, relative adrenal weight and serum corticosterone levels.

TABLE 2

Mean ($\pm$ SE) body weight change, relative adrenal weights and serum corticosterone in male ICR albino mice fed Aroclor 1254 in the diet for 2 weeks.

Dietary Level (ppm)	Body Weight Change (grams)	Serum Corticosterone (μ g/100 ml)	Adrenal Weights (% Initial Body Wt.)
0 (4) ^a	3.8 $\pm$ 1.75 ^b	3.88 $\pm$ 1.6 ^b	0.013 $\pm$.002 ^b
62.5(4)	-1.5 $\pm$.95 ^c	18.10 $\pm$ 5.0 ^c	0.016 $\pm$.003 ^b
250.0(5)	2.4 $\pm$.67 ^b	14.40 $\pm$ 2.9 ^c	0.016 $\pm$.002 ^b
1000.0(2)	-5.0 $\pm$.63 ^c	38.0 $\pm$ 2.0 ^d	0.027 $\pm$.006 ^c

^aNumber of animals in parentheses.

^{b,c,d}Means bearing different subscripts are significantly different from each other at the 5% level (Duncan's Multiple Range Test).

The control group and the group receiving 250 ppm had an average body weight gain of 3.8 and 2.4 g, respectively; whereas, the 1000 ppm and 62.5 ppm groups had a significant average body weight loss of 1.5 and 5.0 g, respectively. Adrenal weights expressed as percent initial body weight were not affected in the 62.5 ppm and 250 ppm groups but were significantly increased at the 1000 ppm level ($P < .05$). Serum corticosterone titers at all PCB levels were significantly higher than those of the control group ($P < .05$). Mice exposed to 1000 ppm PCB had significantly higher corticosterone levels than those exposed to 62.5 ppm ($P < .05$). Relative testes, preputial and vesicular gland weights (Table 3) were not affected by the PCB treatment.

TABLE 3

Mean ($\pm$ SE) relative testes, preputial and vesicular gland weights (expressed as percent of initial body weight) in male ICR albino mice fed Aroclor 1254 in the diet for 2 weeks.

Dietary Level (ppm)	Testes (%)	Preputials (%)	Vesicular glands (%)
0 (4) ^a	0.57 $\pm$.02	0.17 $\pm$.067	0.54 $\pm$.63
62.5(4)	0.52 $\pm$.04	0.16 $\pm$.003	0.43 $\pm$.17
250.0(5)	0.59 $\pm$.03	0.19 $\pm$.003	0.53 $\pm$.05
1000.0(2)	0.64 $\pm$.11	0.09 $\pm$.002	0.29 $\pm$.09

^aNumber of animals in parentheses

DISCUSSION

The 60% mortality reported in this study for male white mice fed 1000 ppm Aroclor for 2 weeks is in the same range of toxicity reported by TUCKER and CRABTREE (1970). These authors reported 100% mortality in male rats after 53 days on a diet containing 1000 ppm Aroclor 1254. In contrast KIMBROUGH et al. (1972) observed only 50% mortality in male rats fed 1000 ppm Aroclor 1254 for 8 months. RINGER et al. (1972) and PLATONOW and KARSTAD (1973) have demonstrated that the ranch mink is extremely sensitive to the toxic effects of PCBs. A dietary concentration of 3.57 ppm Aroclor 1254 produced 100% mortality within 105 days (PLATONOW and KARSTAD 1973).

Data on the effects of PCB ingestion on food consumption is limited. In this study 18 and 30% reductions in food intake were observed for the 250 and 1000 ppm groups, respectively. STREET et al. (1969) found that 25, 100, or 200 ppm of Aroclors given to female rats in the diet for 15 days had no effect on food consumption. TUCKER and CRABTREE (1970) reported that male rats fed 1000 ppm Aroclor 1254 reduced food consumption by 21 percent. CURLEY et al. (1971) observed a 35 and 36% reduction in male and female rats fed 1000 ppm Aroclor 1254.

Barbiturate-induced sleeping times as used in this experiment are commonly employed as indirect measures of hepatic microsomal enzyme induction (VILLENEUVE et al. 1972; GABLICKS 1970; DIXON et al. 1960). Recently VILLENEUVE et al. (1972) found that Aroclor 1254, 1260 and Chlordane reduced pentobarbital-induced sleeping times in male and female rats when fed at a dietary level of 20 ppm. In the experiment reported here Aroclor 1254 fed in the diet of the mouse at 62.5 ppm for 2 weeks did not significantly affect sleeping time or liver weight. However, the reduced sleeping times and the concomitant increase in liver weights observed in the mice fed 250 and 1000 ppm Aroclor 1254 indicate that the hepatic microsomal enzymes were induced at these levels. Induction of the microsomal enzymes of the rat and rabbit by exposure to PCBs has been documented in vitro by VILLENEUVE (1971), and LITTERST (1972).

The enlarged adrenal glands in the 2 surviving mice exposed to 1000 ppm PCB and the elevated serum corticosterone at all PCB levels is indicative of severe stress and could account for the immunosuppressive effect observed by VOS and KOEMAN (1970), and VOS and BEEMS (1971); and the increased susceptibility to disease reported by FRIEND and TRAINER (1970). Although the number of animals used in this study was relatively small, the results strongly indicate a marked effect of PCB ingestion on endocrine activity of the adrenal glands and additional investigations seem warranted in this area.

SUMMARY

The physiological effects of feeding Aroclor 1254 to mature male white mice at dietary levels of 62.5, 250, and 1000 ppm for 2 weeks are reported. Pentobarbital-induced sleeping time and food consumption were reduced and liver weight was increased in the 250 ppm and 1000 ppm groups. Serum corticosterone was elevated at all levels of PCB treatment; whereas, only exposure to 1000 ppm resulted in heavier adrenal glands. Weights of the testes, preputials and vesicular glands were not significantly affected by PCB ingestion.

REFERENCES

- ANDERSON, D. W., J. J. HICKEY, R. W. RISEBROUGH, D. F. HUGHES, and R. E. CHRISTENSEN: Can. Field Natur. 83, 89(1969).
- BITMAN, J. and H. C. CECIL: J. Agric. Food Chem. 18, 1108(1970).
- BITMAN, J., H. C. CECIL, and S. J. HARRIS: Environ. Health Perspect. 1, 145(1972).
- CURLEY, A, V. B. BURSE, M. E. GRIM, R. W. JENNINGS, and R. E. LINDER: Environ. Res. 4, 481(1971).
- DIXON, R. L., R. W. SHULTICE, and J. R. FOUTS: Expt. Biol. Med. 103, 333(1960).
- FLICK, D. F., R. G. O'DELL, and V. A. CHILDS: Poult. Sci. 44, 1460(1965).
- FRIEND, M. and D. O. TRAINER: Science 170, 1314(1970).
- GABLICKS, J. and E. MALTBY-ASBARI: p. 27-31. In W. B. Deichmann (Ed.), Pesticides Symposia, Miami: Halos and Associates, Inc. (1970).
- HOLDEN, A. V. and K. MARSDEN: Nature 216, 1274(1967).
- JEFFERIES, D. J. and J. L. F. PARSLow: Bull. Environ. Contam. & Toxicol. 8, 306(1972).

- JENSEN, S., A. G. JOHNELS, M. OLSSON, and OTTERLIND: *Nature* 224, 247(1969).
- KIMBROUGH, R. D., R. E. LINDER and T. G. GAINES: *Arch. Environ. Health* 25, 354(1972).
- KOEMAN, J. H., M. C. TEN NOWER de BRAUW, and R. H. deVOS: *Nature* 221, 1126(1969).
- LINCER, J. L. and D. B. PEAKALL: *Nature* 228, 783(1970).
- LITTERST, C. L., T. M. FARBER, A. M. BAKER and E. J. VANLOON: *Toxicol. Appl. Pharmacol.* 23, 112(1972).
- MURPHY, B. E. P.: *J. Clin. Endocrinol. Metab.* 27, 973(1967).
- MURPHY, B. E. P.: *Rec. Progr. Horm. Res.* 25, 563(1969).
- NOWICKI, H. G. and A. W. NORMAN: *Steroids* 19, 85(1972).
- PLATONOW, N. S. and H. S. FUNNEL: *Vet. Rec.* 88, 109(1971).
- PLATONOW, N. S., R. M. LIPTRAP, and H. D. GEISSINGER: *Bull. Environ. Contam. & Toxicol.* 7, 358(1972).
- PLATONOW, N. S. and L. H. KARSTAD: *Can. J. Comp. Med.* 37, 391(1973).
- RINGER, R. K., R. J. AULERICH, M. ZABIK: in preprint of papers presented at the 164th national meeting of the American Chemical Society, New York, pp. 149-154(1972).
- RISEBROUGH, R. W., P. RIECHE, D. B. PEAKALL, S. G. HERMAN and M. N. KIRVEN: *Nature* 220, 1098(1968).
- SASCHENBRECKER, P. W.: *Can. J. Comp. Med.* 37, 203(1973).
- STREET, J. C., F. M. URRY, D. J. WAGSTAFF and A. D. BLAU: 158th Am. Chem. Soc. Natl. Meeting Abstracts of Papers, Pest. 17(1969).
- TUCKER, R. V. and D. C. CRABTREE: *Handbook of toxicity of pesticides to wildlife*. U.S.D.I. Resource Pub. No. 84, 131 p. (1970).
- VILLENEUVE, D. C., D. L. GRANT, W. E. J. PHILLIPS, M. L. CLARK and D. J. CLEGG: *Bull. Environ. Contam. & Toxicol.* 6, 120 (1971).
- VILLENEUVE, D. C., D. L. GRANT and W. E. J. PHILLIPS: *Bull. Environ. Contam. & Toxicol.* 7, 264(1972).
- VOS, J. G. and J. H. KOEMAN: *Toxicol. Appl. Pharmacol.* 17, 656(1970).
- VOS, J. G. and R. B. BEEMS: *Toxicol. Appl. Pharmacol.* 19, 617(1971).