Reduction of Pentobarbital-induced Sleeping Times in PCB-treated Cottontail Rabbits

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Many scientists have expressed concern about the effects environmental pollutants may have on wild animal populations, although few studies have demonstrated that small levels of contaminants can have marked influences on the physiology of wild mammalian species. Polychlorinated biphenyls (PCBs) are well known pollutants whose significance within various ecosystems is just beginning to be understood. One property of PCBs, as with other chlorinated hydrocarbons, is their ability to stimulate hepatic microsomal enzymes. Ingestion of 10 ppm PCB for 28 days by domestic rabbits significantly increased the activity of microsomal enzymes in liver homogenates (VILLENEUVE et al. 1971a). Wistar rats fed 20 ppm of the PCB Aroclor 1254, for 30-350 days exhibited shorter sleeping times, indirectly indicating induced hepatic enzyme activity (VILLENEUVE et al. 1972). Barbituate-induced sleeping time is a commonly employed technique used for indirectly measuring hepatic microsomal enzyme induction (PUYEAR and PAULSON 1972, SANDERS et al. 1973, VILLENEUVE et al. 1972). We have employed this method to demonstrate hepatic microsomal enzyme induction in a captive wild mammal, the cottontail rabbit (Sylvilagus floridanus), fed a ration containing 10 ppm Aroclor 1254 for ten to eleven weeks.

METHODS

Wild cottontail rabbits were live-trapped during December, 1972, and feeding of the experimental diet began on January 22, 1973. The experimental design was a 2 x 2 factorial arrangement with two non-PCB-treated groups receiving a commercial rabbit ration (Purina Rabbit Chow, 16% protein) ad libitum or 75% ad libitum. The two remaining groups (PCB-treated) received the same two levels of the ration treated with Aroclor 1254[a]. The PCB was dissolved in acetone and sprayed onto the chow while mixing it on a large feed mixer. The ad libitum ration contained 10 mg Aroclor 1254 per kg of chow (10 ppm). The restricted ration was adjusted in order to provide the same total PCB intake as the ad libitum group. Therefore, 13.3 mg Aroclor 1254 was added to each kg of chow. The rabbits were maintained on these diets for 12 weeks.

During the tenth week all males (17) were injected with sodium pentobarbital (Nembutal®) at a level of 45 mg/kg body weight

[a] Aroclor 1254 generously supplied by Monsanto Company, St. Louis Mo., 63166. (Lot No. KB-01-604).

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(measured to the nearest 100 gm). All females (49) were injected the following week with 40 mg/kg. Sleeping times were measured as the interval from injection of the barbituate to the regaining of the righting ability.

RESULTS

Rabbits treated with PCB at a level of 10 ppm, exhibited significantly shorter periods of sleep (Tables 1 and 2). Sleeping times were shorter both in PCB-treated males ($P<.01$) and PCB-treated females ($P<.05$). Level of nutrition had no significant effect on sleeping times in either sex and there were no significant interactions of PCB and dietary level.

Table 1

Sleeping times for male cottontail rabbits[a].

Treatment	Diet	No. of Animals	Sleeping Times (minutes ± S.E.M.)
PCB[b] (Aroclor 1254)	Ad libitum	4	116.3 ± 12.2[c]
	75% Ad lib.	5	118.0 ± 16.9[c]
Non-PCB	Ad libitum	4	162.8 ± 21.8[c]
	75% Ad lib.	3	231.0 ± 30.2[d]

a Sodium pentobarbital (Nembutal[R]) injected at 45 mg/kg body weight.

b Significantly different from the non-PCB treated group ($P<.01$).

c,d Means bearing different superscripts are significantly different from each other at the 5% Level (Duncan's Multiple Range Test)

Table 2

Sleeping times for female cottontail rabbits[a].

Treatment	Diet	No. of Animals	Sleeping Times (minutes ± S.E.M.)
PCB[b] (Aroclor 1254)	Ad libitum	10	141.9 ± 16.5[cd]
	75% Ad lib.	13	129.7 ± 11.4[c]
Non-PCB	Ad libitum	10	157.8 ± 17.7[cd]
	75% Ad lib.	11	174.1 ± 8.9[d]

a Sodium pentobarbital (Nembutal[R]) injected at 40 mg/kg body weight.

b Significantly different from the non-PCB treated groups ($P<.05$).

c,d Means bearing different superscripts are significantly different from each other at the 5% level (Duncan's Multiple Range Test)

The lengths of time for rabbits to succumb to the sedative effects of the pentobarbital were quite variable. However, the differences were not related to treatment. Five females, two from the ad libitum control group, one from the restricted control, and two from the ad libitum PCB group, failed to sleep. One male from the ad libitum PCB group also failed to sleep. These six animals were not included in the calculations.

DISCUSSION

Although some information has been published concerning the ability of PCBs to induce hepatic microsomal enzymes in laboratory animals or birds, little information is available pertaining to wild mammals. These results suggest that the cottontail rabbit, like the domestic rabbit, responds to relatively low levels of these compounds. Further studies are necessary to determine if enzyme induction is related to lowered reproductive function. VILLENEUVE et al.(1971a) have reported increased carboxylesterase activity in liver homogenates taken from pregnant female laboratory rabbits fed with 10 ppm Aroclor 1254 during the 28 days of gestation. Carboxylesterase activity was used as an indicator of microsomal enzyme activity. Aniline hydroxylase and aminopyrine N-demethylase, both drug-metabolizing enzymes, were also significantly induced. These same workers have reported placental transfer of PCBs and have shown PCBs to be fetopathic at doses of 12.5 and 50 mg/kg/day (GRANT et al. 1971, VILLENEUVE et al. 1971b). However, ICR strain albino mice exhibited no apparent enzyme induction at dietary levels of 62.5 ppm Aroclor 1254. Levels of 250 and 1000 ppm were necessary to induce liver enzymes, demonstrating species-specific sensitivity to the PCB (SANDERS et al. 1973).

Although level of nutrition had no significant effect on sleeping times as shown by analysis of variance, there was a trend when the means were analyzed by Duncan's multiple range test for non-PCB restricted animals to sleep longer. DIXON et al. (1960) have shown that starvation decreases detoxication of drugs; however KATO (1967) and WEATHERHOLTZ and WEBB (1971) have reported an increase in enzyme induction due to starvation or protein restriction.

The fact that we have demonstrated induction of liver enzyme systems in a wild animal is in itself, not surprising, but the fact that such induction was caused by a relatively low level of PCB ingestion should certainly stimulate further study of the effects this increased activity may have on reproduction and the animals' physiology.

SUMMARY

Pentobarbital sleeping time was used to examine the effects of Aroclor 1254 on hepatic microsomal enzyme activity in cottontail rabbits. Ten ppm fed for 10-11 weeks significantly reduced sleeping times in both sexes. Restriction of food intake had no consistant effect on sleeping times.

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