Reproductive Performance of Captive White-Footed Mice Fed a PCB

M. H. Merson and R. L. Kirkpatrick
Department of Fisheries and Wildlife Sciences
Virginia Polytechnic Institute and State University
Blacksburg, Va. 24061

Polychlorinated biphenyls (PCBs) are industrial chemicals which have become widespread contaminants of the global ecosystem (RISEBROUGH et al. 1968). They are powerful inducers of the hepatic microsomal enzyme system in rats (STREET et al. 1969; LITTERST et al. 1972; CHEN and DU BOIS 1973; BRUCKNER et al. 1974a, b; SCHMOLDT et al. 1974) and in rabbits (VILLENEUVE et al. 1971b). High PCB residue levels in the tissues of mothers and young have been implicated in the premature births of sea lions (DELONG et al. 1973). PCB feeding decreased litter sizes at birth in pigs (HANSEN et al. 1975) and adversely affected reproduction in the rat (LINDER et al. 1974). Dietary levels of PCB as low as 0.64 ppm almost completely inhibited the reproduction of mink (PLATONOW and KARSTAD 1973). Injection of a PCB has been shown to significantly increase the length of the estrous cycle in white mice (OERBERG et al. 1972) and PCB feeding significantly increased the length of the estrous cycle and decreased the number of implanted ova observed in treated white mice (OERBERG and KIHLSTROHM 1973). This paper reports observations made on the reproductive performance of captive pairs of white-footed mice (Peromyscus leucopus) which received a ration containing a PCB.

MATERIALS AND METHODS

Male and female white-footed mice from a colony maintained at the Department of Fisheries and Wildlife Sciences, V.P.I. and S.U., Blacksburg, were randomly paired and assigned to one of two treatment groups. The groups received ground Purina Lab Chow ad libitum containing either 0 (control) or 200 ppm PCB (Aroclor 1254). The PCB was added by dripping a PCB-acetone solution into the feed while mixing in a feed mixer. The control feed was mixed with a similar volume of acetone containing no PCB. Both feeds were mixed for 10 minutes after the addition of the acetone or the PCB-acetone solution.

A total of 26 pairs were in the 0 ppm group and 27 pairs were in the 200 ppm group. The pairs were placed on their experimental diets immediately upon pairing and continued on them for a period of 60 days. During the 60 day period the pairs were observed daily for litters and mortality of either member of the pair. Also recorded were the number of young per litter and survival of the young to 21 days of age. Young were left in the cages with the parents until weaning at 21 days of age.

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The experiment was conducted in a series of four replications. The first replication was begun on 13 June 1974 and consisted of 10 pairs per group. The second, third, and fourth replications were begun on 7 January, 14 January, and 18 February 1975, respectively. The second replication consisted of nine pairs (five in the 0 ppm group and four in the 200 ppm group). The third replication consisted of six pairs (three in each group). The fourth replication consisted of 18 pairs (eight in the 0 ppm group and 10 in the 200 ppm group). Animals ranged from 44 to 222 days of age at the time of pairing.

RESULTS

Of the 27 pairs fed PCBs, nine lost one member during the course of the experiment (Table 1). The 26 control pairs experienced no mortality. The time of death of the nine PCB-fed animals ranged from 39-57 days into the experiment (mean ± S.E. = 49.6 ± 1.6 days). The time on experiment before mortality occurred in the PCB-fed pairs was sufficient for those pairs which experienced mortality to produce at least one litter. Since the mean gestation length for non-lactating Peromyscus leucopus females is 23.2 days (ASDELL 1964: 264).

TABLE 1

Survival and reproductive performance of captive white-footed mouse pairs fed 0 or 200 ppm aroclor 1254 for 60 days.

	Level of PCB	
	0	200
Number of pairs begun on experiment	26	27
Percent pairs surviving 60 days	100.0	66.7
Percent of total pairs having at least one litter	84.6	29.6
Percent of total pairs having two litters	42.3	3.7
Total litters produced in 60 days	33	9
Mean number of litters for all pairs begun on experiment (±S.E.)	1.31±0.14	0.33±0.06
Mean number of litters for pairs surviving 60 days (±S.E.)	1.31±0.14	0.28±0.14
Average number of young per litter of known size (±S.E.)	3.82±0.21(20)[a]	3.33±0.33(6)
Mean number of days each pair was on experiment (±S.E.)	60.0 ±0.0	56.5 ±1.1

[a]Numbers in parentheses denote number of litters of known size

The entire control group (26 pairs) produced a total of 33 litters during the experimental period and the 27 PCB-fed pairs produced nine. The mean (± S.E.) number of litters per pair, computed using all litters produced by all pairs regardless of mortality, was 1.13 ± 0.14 litters per pair for the controls and 0.33 ± 0.06 litters per pair for the PCB-fed pairs. When those pairs which experienced mortality during the experiment and the litters which they produced were eliminated from the computation of mean number of litters per pair, the mean (± S.E.) for the 200 ppm group dropped to 0.28 ± 0.14.

Of the control pairs, 84.6 percent had at least one litter and 42.3 percent had two litters. In comparison, only 29.6 percent of all the PCB-fed pairs (including those pairs which experienced mortality) had at least one litter and only 3.7 percent had two litters. Litter size was not significantly affected by PCB feeding. The 20 first litters from control pairs for which litter size was accurately known (cannibalism sometimes made determination of litter size difficult or impossible) had a mean (± S.E.) size of 3.82 ± 0.21 young at birth. The six first litters of PCB-fed pairs for which litter size was accurately known had a mean (± S.E.) size of 3.30 ± 0.33 young at birth. Of the seven first litters which were produced during the experimental period by the PCB-fed pairs, none had any offspring which survived to 21 days of age. The control pairs produced 22 first litters of which 15 had offspring which survived to 21 days of age. The mean (± S.E.) litter size of those first control litters which had surviving young was 3.1 ± 0.3 at 21 days.

Of the nine animals which died during the experiment, eight were females and one was a male. Of the eight females which died six showed evidence of either having been pregnant or were pregnant at the time of their death. Two of the six had dark bands on their uteri. Neither of these females had had a previous litter. These dark bands were thought to be resorption sites from a previous pregnancy which did not go to term. Two other females, one of which had had a previous litter, each had three implantation sites on the horns of their uteri. A fifth female had visible embryos in her uterus at the time of death but there was evidence of resorption. The final female was found dead with bleeding about the urogenital region. Upon necropsy a single large fetus was found, its size indicating that it was probably full term. The head was deformed and the facial features indistinguishable.

DISCUSSION

The results of this experiment indicate that a dietary exposure to Aroclor 1254 in concentrations of 200 ppm inhibits reproduction in captive pairs of white-footed mice. The large difference between groups in the mean number of litters produced per pair in 60 days (1.31 for the controls versus 0.33 for the PCB-fed pairs), the large difference in total litters produced by these groups of similar size, and the large difference in the

percent of pairs in each group having litters (8.6 percent versus 29.6 percent) support this conclusion.

The rate of reproduction exhibited by the control pairs was not thought to be extraordinary. The ratio of litters:days of opportunity seen in the control animals in this exerpiment was similar to that reported by ROOD (1966) after a two year observation of 70 captive female Peromyscus leucopus. A greater percentage of the control females produced young in this experiment (84.6 percent) than those observed by ROOD (60 percent) but the average litter size of the present controls was less than that reported by ROOD (3.82 versus 4.67).

Drastic effects of PCB-feeding before, during, and after gestation upon survival of offspring, such as those observed in this experiment, were reported in a similar study by LINDER et al. (1974) for rats. Exposure of F_0 rats to 500 ppm dietary Aroclor 1254 for 67 days prior to pair-mating and then during pregnancy resulted in 100 percent mortality in the young 3 days post-partum. Survival of young was also reduced, only not as drastically, in the litters of rats fed 100 ppm. LINDER et al. (1974) also reported that litter sizes of parents receiving 500 or 100 ppm Aroclor 1254 for 67 days prior to pair-mating were significantly lower than those of controls. There was a slight decrease in mean litter size due to PCB feeding in this experiment but it was not significant.

SANDERS and KIRKPATRICK (1975) found a significant decrease in the total spermatozoa per testis from that observed in controls after a 2 week feeding of 400 ppm Aroclor 1254. An apparent reduction in total spermatozoa per testis was also observed after a 200 ppm exposure of white-footed mice for 4 weeks to Aroclor 1254 (MERSON 1975). It is not known whether such reductions in spermatozoa per testis represented an actual loss of fertility in the PCB-fed males. Also, if these decreases did represent a loss in fertility, it is not known how soon this loss occurred after the initiation of treatment. It could have been possible for a fertile mating to have taken place in those pairs which had no litters soon after the initiation of treatment. If fertile matings took place routinely among the PCB-treated pairs, it is possible that PCB toxicity to the blastocyst contributed to the low reproductive rate of this group. Another contributing factor could have been the failure of blastocysts to implant in the uterus. OERBERG and KIHLSTROHM (1973) reported a decrease in the number of implanted ova in pregnant white mice which were orally dosed with a PCB (Clophen A60) for 62 days prior to breeding.

Implantation in the white mouse requires the presence of both progesterone and estrogen (JAITLY et al. 1966; SMITH 1968; BINDON and LAMOND 1969; FINN and MARTIN 1972). Aside from the mere presence of these hormones they must be secreted in the proper sequence, for the proper duration,and at a proper level to insure that the uterus is in an optimum condition for implantation when the blastocyst arrives (SMITH 1968; FINN and MARTIN 1972). Any PCB interference

with implantation in the present study could have been at the ovarian level (desensitization to gonadotrophins or inhibition of the secretation of steroids), at the hypothalamic level (desensitization to circulating steroids or inhibition of releasing factor synthesis), or at the pituitary level (desensitization to releasing factors or inhibition of gonadotrophin secretion). If PCB interference with implantation was operating in this experiment, it would have had to have been an all or none phenomenon since litter sizes were not significantly different between groups.

The evidence of resorption in those six PCB-fed females which died during the experiment suggests that at least some implantations successfully took place but the pregnancies failed to continue to term. Circulating levels of polychlorinated biphenyls in the maternal blood stream could have crossed the placenta and exerted a toxic effect upon the fetus. Transplacental transfer of Aroclor 1254 has been reported for rats (CURLEY et al. 1973). The fetotoxicity of Aroclor 1254 has been demonstrated in rabbits when pregnant females were dosed orally during pregnancy (VILLENEUVE et al. 1971a) but this effect was not seen in rats orally dosed with up to 50 mg/kg Aroclor 1254 during organogensis (CURLEY et al. 1973; VILLENEUVE et al. 1971a). Rats which received Aroclor 1254 in the diet 62 days prior to breeding had smaller litters (LINDER et al. 1974) than controls suggesting the possibility of in utero losses due to fetotoxicity and that perhaps a more sustained exposure of the parent to PCB is necessary for a maximal effect upon reproduction. However, decreased ovulation rates, failure to implant, or failure to maintain pregnancy due to PCB exposure also could have accounted for the smaller litters observed in PCB-fed rats.

The other possible explanation as to why pregnancies did not go to term in the PCB-fed white-footed mice in this experiment is that the females could not meet the hormonal requirements for the maintenance of pregnancy after implantation. Work with mice hypophysectomized after implantation and before the second half of pregnancy has shown that both progesterone and estrogen in sufficient amounts are necessary for maintenance of pregnancy in most animals but progesterone alone is enough in some (JAITLY et al. 1966; CHOUDARY and GREENWALD 1969b). If hypophysectomy is done during the first half of pregnancy, progesterone and estrogen must be supplied exogenously to maintain pregnancy (JAITLY et al. 1966; CHOUDARY and GREENWALD 1969b) but degenerative changes in the corpora lutea are noted (CHOUDARY and GREENWALD 1969b). Hypophysectomy in the second half of pregnancy has no effect upon the maintenance of pregnancy in white mice (SELYE et. al. 1933; CHOUDARY and GREENWALD 1969a).

PCBs could have been affecting pregnancy maintenance in this experiment by decreasing circulating levels of progesterone and estrogen because of increased rates of steroid metabolism. PEAKALL (1970) found a decrease in estradiol in the blood of p,p'-DDT-treated ring doves and attributed it to an increased rate of steroid metabolism due to induction of hepatic microsomal

enzymes by the DDT. However, evidence for similar effects of hepatic microsomal enzyme inducers such as DDT and PCBs on endogenous steroid levels is lacking. More work is needed to determine the mechanisms involved in the PCB-induced depression of reproduction found in the white-footed mouse of this study.

SUMMARY

Feeding a diet containing 200 ppm of the polychlorinated biphenyl, Aroclor 1254 (PCB), to pairs of captive white-footed mice adversely affected their reproduction. During the 60 day period in which the experimental diets were fed, 26 pairs receiving no PCB produced 33 litters while 27 pairs receiving feed containing 200 ppm PCB produced only nine. Of the pairs receiving no PCB, 84.6 percent produced litters, whereas only 29.6 percent of the PCB-fed pairs produced litters. No offspring of the PCB-fed pairs survived to an age of 21 days.

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