Chronic Polychlorinated Biphenyls Exposure on Three Generations of Oldfield Mice (Peromyscus polionotus): Effects on Reproduction, Growth, and Body Residues

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Abstract. Effects of chronic dietary exposure to low levels of PCBs (polychlorinated biphenyls) on reproduction, growth and whole body burdens were investigated in three generations of Peromyscus polionotus. Mated pairs were maintained on a diet containing 5 mg/kg PCBs (Aroclor 1254®) for 12 months, beginning exposure as young adults; matched controls received a similar diet without PCBs. Offspring were maintained on the parental regime and paired at maturity with non-siblings in the same group. In first and second generation offspring, birth and weaning weights were significantly lower in PCB-exposed animals; in the second generation, there were also significantly fewer mice born/month, longer intervals prior to birth of the first litter, and decreased survival to weaning (25 days) among exposed mice. Whole body residue of PCBs increased significantly with each generation of exposure. This study clearly shows that chronic exposure to PCBs at a dosage of 5 mg/kg depressed fertility, growth and survival in Peromyscus, and that these effects were amplified through multigenerational exposure.

Polychlorinated biphenyls (PCBs) are a group of industrial chemicals of which Aroclor® 1254 was among the most widely used. Due to widespread environmental contamination, the use of PCBs has been limited in the United States since 1971 and widely restricted globally. However, the persistence of these compounds causes them to remain potentially serious hazards to human and natural mammal populations. Although many PCB commercial products and individual congeners are considered relatively nontoxic in acute exposure, “the full extent of chronic health and ecological effects of PCBs is still not completely understood” (Hansen 1994 p. 207). PCBs are regulated by federal agencies, which have proposed or adopted standards for PCBs based on their activity or possible carcinogenicity (Silbathorn et al. 1990).

A primary concern regarding PCB exposure in mammals is the deleterious effects on many phases of reproduction in both sexes (George and George 1990). In males, fertility is reduced by decreased sperm counts and lower accessory gland weights. In females, plasma concentrations of estradiol and progesterone are reduced, embryo implantation impaired, and maintenance of the placenta diminished. Induction of hepatic microsomal enzyme systems is considered the primary effect of PCB exposure on fertility in both sexes (Litterst et al. 1972; Shull et al. 1982).

Developmental effects attributed to PCB exposure include low birth weight and retarded growth. Prenatal exposure to PCBs has been linked to teratological effects such as cleft palate in the mouse and pig (Fuller and Hobson 1987). Adverse human reproductive effects and developmental deficits have been linked to PCB exposure from consumption of contaminated fish or by direct agricultural contact (Hansen 1994).

The genus Peromyscus is particularly valuable as a biomonitor because it is common, widely distributed and readily collected from the wild (McBee and Bickham 1990). It plays a major role in terrestrial food chains as Peromyscus species are typically omnivorous and are important dietary components of many carnivores. Peromyscus can be readily maintained in the laboratory and breeds well in captivity so the effects of environmental pollutants can be assessed under controlled conditions. Known exposure in the laboratory can be correlated with differences in fertility, growth, and survival as well as with body burdens.

Chronic exposure to 10 mg/kg Aroclor® 1254 of adult Peromyscus leucopus from age 16 weeks resulted in fewer offspring weaned per litter, although other indicators of reproductive success were unaffected; however, similar exposure from 12 weeks produced longer intervals between births, fewer neonates per litter and fewer juveniles at weaning (Linzey 1987). In addition, second generation-exposed animals weighed less at 4, 8 and 12 weeks in comparison to the parental generation of exposed animals or to controls (Linzey 1988). These findings suggest that exposure to PCBs in developing animals has a greater effect than adult exposure, that effects may be cumulative, and that long-term chronic exposure at a relatively low dose may affect population size in wild mammal populations.
The present study, employing half the levels of Aroclor® 1254 used by Linsey (1987, 1988), was undertaken to determine the effects on fertility, growth and survival of chronic, low level (5 mg/kg) exposure to PCBs in the diet over three generations in Peromyscus polionotus (oldfield mouse). Effects of such exposure on body burdens of PCBs were also evaluated.

Materials and Methods

All Peromyscus polionotus were from the Benedict College colony, derived solely from the closed colony maintained at the University of South Carolina as a NSF Genetic Stock Center. This colony is descended from animals captured in Ocala National Forest, FL in 1951 and has been maintained without deliberate selection and with avoidance of sibling mating. These animals are considered representative of the wild ancestors, being normal in color, morphology and behavior (Dawson et al. 1982).

Animals were kept in standard plastic mouse cages within ventilated cage racks. Light was regulated to 18 h light:6 h dark. Temperature was maintained at 22°C, and humidity was at 40-45%. Water was available ad libitum to all animals.

Rodent blocks (Harlan-Teklad) were ground to a powder and a solution of Aroclor® 1254 in 95% ethanol was mixed with the powder (5 mg Aroclor® 1254/kg ground blocks); the resultant slurry was dried in an oven at 37°C for 48 h, producing a diet of 5 mg/kg PCBs. The diet was maintained in the same manner.

A preliminary study was conducted to assess if the ground diet affected weight gain, growth or general health. Juvenile animals at 25 days of age were placed on ground blocks or on a standard diet for 60 days. Males and females were maintained separately; 6 animals were in each group for a total of 12 on standard blocks and 12 on ground blocks.

Young adult male and female Peromyscus polionotus, 6–10 weeks of age, were paired and the pairs were randomly assigned to the PCB-exposed or control group. These pairs or “matings” comprised the F1 generation and 15 pairs were established in each group. Mated pairs were maintained for 12 months; the male was then removed from the cage and the female observed for a final pregnancy. Any young delivered in this period were included in the results.

Pairs were checked daily between 4 and 5 p.m. for general health and births. Litters were weighed on the day of birth as a group. Twenty-five days after birth, juvenile mice were weaned, i.e. removed from parents, weighed individually and separated by sex. These mice were maintained on the parental dietary regime and termed the F1 generation. At maturity, the F1 generation were paired with mates from the same treatment regime (control or exposed), with avoidance of sibling matings, and were maintained as mated pairs for 12 months in the same manner as the parent generation. A total of 15 exposed and 14 control matings were established in the F1 generation. The offspring were termed the F2 generation. If one of a pair died or escaped within the first 4 months, data from the mating were not used. If a mouse died or was lost after 4 months, the observations were included.

Indicators of reproductive success were recorded and compared among PCBs-exposed and control animals in both P1 and F1 generations. Productivity of the mating was measured by the ratio of total number of offspring born to a pair to total number of months the pair was maintained. Litter production was the ratio of litters born to a pair to months the pair was maintained.

Fertility was assessed by the ratio of matings with one or more live litters to total number of matings and by number of offspring born per mating month. A mating month was defined as the total number of months that the pair was together. Mean birth weights, neonates per litter, litter size at weaning, and juvenile weight at weaning were calculated and assessed for normal distribution. As normal distribution was observed, these means were compared by a two-tailed student's t-test ($p < 0.05$ significant, $p < 0.01$ highly significant). The interval from establishment of the pair to birth of the first litter was evaluated as an indicator of fertility. The ratios of juveniles weaned to neonates born per litter were compared as indicators of post-natal reproductive success.

At the conclusion of the investigation, pairs from control and exposed groups were euthanized by cervical dislocation after anesthesia and the carcasses frozen at $<20°C$. Whole body residues were determined for animals from two generations of controls, as well as for the P1, F1 and F2 PCB-exposed animals. Analyses of carcasses were conducted without identifiers as to treatment regime of the animals. PCB concentrations in diet samples were tested in the same manner. These analyses were conducted at The Institute of Wildlife and Environmental Toxicology.

Whole body burdens were assessed for five individual congeners: B228, B2105, B2118, BZ156 and BZ180. Concentrations of each congener were used to calculate the amount of Aroclor® 1254 in the animal. Individual whole carcasses were homogenized and a 5 g aliquot spiked with $o,p'$-DDE. The aliquot was extracted with 50% methanol/25% propanol/25% acetone for 1 h. Extracts were diluted twofold with water and passed through a 1 g C$_18$ Solid Phase Extraction (SPE) cartridge. PCBs were eluted from the SPE cartridge with 2 ml 50% ether/50% hexane. The solution was passed through a 500 mg alumina SPE column, the PCB components were eluted with 3 ml hexane, and the solvent was concentrated to 2 ml. The resulting solution was analyzed with a Hewlett-Packard 5890A gas chromatograph with electron capture detection.

Separations were achieved in a 60 m × 0.25 mm DB-5 column (J and W Scientific). Hydrogen was used as the carrier at 1.3 ml/min, and 5% argon/methane was used at 60 ml/min for make up gas. Each congener used in quantification was calibrated with a three point calibration curve that resulted from injection of dilute solutions of Aroclor® 1254 in hexane. The injector was set at 235°C, and the detector was set at 325°C. Initial temperature was 140°C for 2 min. Rate 1 was 10°C/min; temperature 1 was 190°C; and time 1 was 0 min. Rate A was 5°C/min; temperature A was 265°C; and time A was 5 min. Rate B was 15°C/min; temperature B was 280°C; and time B was 4 min.

Results

In the preliminary dietary study, weight gain was not significantly different between the animals receiving ground diet and those receiving standard blocks. No unusual features or indications of ill health were observed in animals on the ground diet.

Indicators of fertility did not differ significantly between PCB exposed mice and controls in the first generation of chronic exposure to 5 mg/kg PCBs (Table 1). The ratio of offspring born per month was 0.62 in the controls and 0.71 in the exposed for all mated pairs; for pairs that were fertile, ratios were 1.07 and 1.11, respectively. Likewise, mean litter size and number of litters born per month, among all pairs and among fertile pairs, were similar between first generation controls and exposed. The percentage of pairs fertile was higher for the PCB group. The mean interval from establishment of the pair to birth of the first litter for fertile pairs was not significantly different between P1 controls and exposed (71.8 and 76.2 days, respectively).

Mean birth weight in offspring of first generation PCB-exposed animals was significantly lower ($p < 0.05$) than that of the P1 control group (Table 2). In first generation offspring, weaning weight was significantly lower ($p < 0.01$) in PCB-exposed animals. In the P1 generation, the ratios of juveniles weaned per month were not significantly different between
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Table 1. Indicators of fertility in *Peromyscus polionotus*, first and second generation of dietary exposure to 5 mg/kg polychlorinated biphenyls (PCBs)

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Offspring born per month of all pairs, fertile pairs</th>
<th>Mean litter size ± se</th>
<th>Percentage fertile</th>
<th>Litters born per month of all pairs, fertile pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI Control (13)</td>
<td>0.62, 1.07</td>
<td>3.62 ± 0.32</td>
<td>62%</td>
<td>0.17, 0.31</td>
</tr>
<tr>
<td>PI PCB-exposed (14)</td>
<td>0.71, 1.11</td>
<td>3.80 ± 0.31</td>
<td>71%</td>
<td>0.19, 0.29</td>
</tr>
<tr>
<td>F1 Control (10)</td>
<td>0.67, 1.33</td>
<td>3.85 ± 0.36</td>
<td>50%</td>
<td>0.13, 0.31</td>
</tr>
<tr>
<td>F1 PCB-exposed (11)</td>
<td>0.20, 0.47</td>
<td>3.78 ± 0.37</td>
<td>64%</td>
<td>0.09, 0.15</td>
</tr>
</tbody>
</table>

*Number of pairs
†Total number born/total number of months all pairs mated
‡Pairs with 1 or more neonates/total number of pairs
§Number of litters/total number of months all pairs mated

Table 2. Indicators of reproductive success of *Peromyscus polionotus* from birth to weaning, first and second generation of dietary exposure to 5 mg/kg polychlorinated biphenyls (PCBs)

<table>
<thead>
<tr>
<th>Group</th>
<th>Birth weight (g ± se) number litters (range)</th>
<th>Weaning weight (g ± se) number litters (range)</th>
<th>Weaned per month of all pairs, fertile pairs</th>
<th>Percentage survival to weaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI Control</td>
<td>1.85 ± 0.08</td>
<td>10.27 ± 0.96</td>
<td>0.36, 0.59</td>
<td>60%</td>
</tr>
<tr>
<td>13 (1.3-2)</td>
<td>12 (8.8-11.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI PCB-exposed</td>
<td>1.61 ± 0.07</td>
<td>8.94 ± 0.30</td>
<td>0.37, 0.57</td>
<td>55%</td>
</tr>
<tr>
<td>13 (1.1-2.1)</td>
<td>14 (7.1-10.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 Control</td>
<td>1.96 ± 0.08</td>
<td>9.76 ± 0.29</td>
<td>0.49, 1.11</td>
<td>94%</td>
</tr>
<tr>
<td>7 (1.8-2.4)</td>
<td>11 (7.7-10.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 PCB-exposed</td>
<td>1.67 ± 0.11</td>
<td>8.29 ± 0.44</td>
<td>0.19, 0.30</td>
<td>23%</td>
</tr>
<tr>
<td>14 (1.1-2.3)</td>
<td>7 (6.9-9.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Whole body residues of PCBs expressed as µg/g mass (± se) of adult *Peromyscus polionotus* chronically exposed to 5 mg/kg polychlorinated biphenyls (PCBs)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean whole residue number (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI and F1 Controls</td>
<td>0.31 ± 0.13</td>
</tr>
<tr>
<td>PI PCB-exposed</td>
<td>3.28 ± 2.00</td>
</tr>
<tr>
<td>F1 PCB-exposed</td>
<td>7.51 ± 3.36</td>
</tr>
<tr>
<td>F2 PCB-exposed</td>
<td>14.69 ± 7.64</td>
</tr>
</tbody>
</table>

exposed and control groups (Table 2), nor was the percentage of offspring surviving to weaning markedly different: 60% controls, 55% exposed.

In the F1 generation, in which animals had been continually exposed to PCBs since conception, greater differences between PCB-exposed and control animals were evident. There was little difference between PI control, PI exposed and F1 control groups with regard to the number of offspring born per month, which in the range of 0.62-0.71 for all pairs and 1.07-1.33 for fertile pairs; however, in F1 PCB-exposed animals, the ratio of offspring born per month was 0.20 for all pairs and 0.47 in fertile pairs, a marked reduction (Table 1).

The smaller number born per month for F1 exposed animals was not attributable to a reduction in litter size, which did not differ significantly among groups, or to a decrease in total number of pairs fertile. In F1 exposed pairs, however, there were fewer litters born each month. This decrease in number of litters in the second generation exposed is reflected in the significantly longer mean interval to the first litter, which was 143 days in the F1 exposed; the mean for other groups ranged from 72 to 80 days with a collective mean of 75.9 days. The interval from the first to second litter among F1 PCB-exposed animals was not reduced in comparison with other groups; however, the sample was limited, as only three pairs produced a second litter. Mean birth weight was significantly lower (p < 0.01) for F1 exposed animals relative to F1 controls (Table 2). Mean weaning weights were also lower in the F1 exposed relative to controls, although due to a large variance in the exposed group, comparison by the t-test was not possible.

In F1 PCB-exposed pairs, the ratio of offspring weaned per month was approximately half that of the first generation exposed (Table 2). The percentage survival to weaning for the second generation of PCB exposure was less than half that of the first generation exposed.

Whole body residues of Aroclor® 1254 increased with each generation of exposure. PI animals fed the PCBs diet as young adults and maintained on the diet for 12 months had significantly (p < 0.01) elevated levels of PCB residues relative to controls (Table 3). Offspring of these PI exposed animals, the F1 exposed, had more than twice the residue level of the PI generation, a highly significant difference at p < 0.01. Their offspring, the F2 exposed animals, had a burden of PCBs double that of the F1 generation, significant at p < 0.05. Ranges of the three exposed generations overlapped and standard errors of the mean were rather large, indicating considerable individual variation in the whole body residues within groups. Within each group, burdens in males were compared to females, and in no case was there a significant difference. Analysis of diet
Discussion

These observations indicate that, even at this low level, chronic exposure to PCBs has pronounced reproductive effects on mammals and that these effects are amplified through multi-generation exposure. In *Peromyscus polionotus*, chronic dietary exposure to PCBs at a level of 5 mg/kg initiated in young adults depresses birth weight and weight at weaning in their offspring, although litter size is unaffected relative to controls. The number of offspring born per month to these pairs in which exposure was begun as adults did not differ significantly from controls. In the offspring of these PCB-treated animals, however, the number of offspring born per month was markedly reduced. In addition, birth weight and weaning weight are depressed in the second generation of exposure in comparison to the controls, the interval between litters is increased in the second generation of exposure, and survival of neonates is significantly reduced.

Of 52 *P. polionotus* matings established in the laboratory, Dawson (1965) reported 73% fertile, with a mean litter size of 3.65, 1.83 neonates per mating month, and 0.65 weaned mice produced per mating month. In a subsequent study (Dawson et al. 1982), the percentage fertile was 77% based on 35 matings, with a mean litter size of 3.35. In a previous study in our laboratory (Finlay et al. 1979), 60% of the pairs were fertile. These observations accord with those for control mice in the present investigation, indicating no significant effect of the protocol on the control mice. The absence of significant differences in size between experimental and control litters indicates that differences in growth and survival were not due to differences in amount of nutrition or maternal resources available to each neonate.

The trends in these investigations are comparable to the observations of Linzey (1987) with *Peromyscus leucopus*, although there were differences in exposure regimes. Using laboratory-raised *P. leucopus* paired and exposed to 10 mg/kg Aroclor® 1254 in the diet at 16 weeks of age, Linzey (1987) found that litter size, birth interval and other parameters of reproductive success were unaffected relative to controls but survival to weaning was significantly lower. Maternal exposure of nursing pups through milk is considered a significant factor in reproductive success of the mammalian female in many circumstances (Fuller and Hobson 1987). In the present investigation, offspring/month, birth intervals, birth and weaning weights and survival to weaning were significantly depressed in the offspring of F1 generation females which had been exposed to 5 mg/kg PCBs *in utero*, while nursing, and all through life in comparison to the offspring of animals exposed only as adults (F1 generation).

In addition, body burdens increased significantly between each generation of exposure and the progressively more severe reproductive impairment observed in each generation correlates to the increased body burden of PCBs. It is apparent that continued exposure at a low level results in amplified body burdens over three generations. For wild populations that remain in the same area for many generations, cumulative effects may have serious consequences. *Peromyscus leucopus* inhabiting an area contaminated with PCBs were found to have mean whole body burdens of 2.3 mg/kg PCBs with a range of 0.2–4.17 (Batty et al. 1990), which is comparable to the F1 PCB-exposed animals in the present study. At this PCB contaminated site, there was a significantly smaller proportion of juvenile and young animals in the population relative to a similar uncontaminated site, suggesting an adverse effect of PCB exposure on reproduction.

Maternal exposure *in utero* and during lactation, as well as increased body residues in multi-generational exposure, contributes to reduced growth and fertility of *Peromyscus polionotus*. The effects of cumulative exposure appear to amplify over generations so that chronic exposure to low levels has increasingly significant effects on reproductive success. In studies of wild populations, it is evident that the roles of maternal exposure and increasing body burdens must be considered in assessing long term effects of PCB exposure.

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