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Feeding Growing Mink *(Musfela vison)* PCB Aroclor® 1254 Does Not Affect Baculum (Os-penis) Development

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Recent studies reported in the literature have shown a negative correlation between the concentrations ofenvironmental contaminants and baculum (os-penis) mass and/or length in wild-trapped mustelids. Henny *et al.* (1996) found that organochlorinated contaminants, including polychlorinated biphenyls (PCBs), were significantly correlated with smaller testes and bacula in juvenile wild-trapped river otter from Oregon. Harding *et al.* (1999) reported a significant negative correlation between PCB concentrations (as Aroclor 1260) and baculum length in juvenile mink, but not in river otter trapped in British Columbia.

The objective of this study was to examine the effects of dietary exposure to a known concentration of the PCB mixture, Aroclor 1254, on baculum development in growing male mink under laboratory conditions.

MATERIALS AND METHODS

Thirty four, 12-week-old natural dark male mink kits were individually housed in suspended wire-mesh cages (76 cm L x 61 cm W x 46 cm H) in an animal room at the Michigan State University Experimental Fur Farm. The natural photoperiod was simulated by use of a time-clock. Ventilation was provided by ceiling vents and an exhaust fan equipped with a HEPA-filter.

The mink were randomly allocated into a control (15 kits) and a PCB-treated (19 kits) group, except that siblings were not placed in the same group in an attempt to minimize any genetic predisposition to PCBs. The mink were fed a standard mink diet supplemented with 0 (control) or 2 mg Aroclor 1254 (Monsanto Co., St. Louis, MO) per kg feed, wet weight (PCB-treated diet). The diet consisted of 28.13% duck by-products, 28.1% fortified mink cereal, 21.9% water, 9.38% eggs, 6.25% Menhaden fish meal, 6.25% turkey liver, and 0.67 mg d-biotin/kg eggs. Samples, consisting of six blended subsamples, of each diet were collected for nutrient and PCB analyses.

The targeted dietary concentration of 2 mg Aroclor 1254/kg feed was selected as a non-lethal dose for this study based on the results of previous investigations that showed a dietary concentration of 2 mg/kg of Aroclor 1254 impaired mink

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reproduction and was fetotoxic at less than 5 mg/kg. The LC50 for adult mink was calculated to be 6.65 mg Aroclor 1254/kg (Ringer *et al.,* 1981).

Feed and drinking water were provided *ad libitum* throughout the trial. Mink body masses were recorded at the start of the study and monthly during the 20-week exposure period. At the termination of the trial, the mink were euthanized (CO_2) and the bacula collected. The bacula were cleaned of all soft tissue by Dermestid beetles. They were then air-dried for 1 week and the length and mass measured.

Differences in mean body mass gain and baculum length and mass between the control and PCB-treated mink were analyzed by the Student's t-test. Statistical differences were based on $p < 0.05$.

RESULTS AND DISCUSSION

Proximate analysis (Litchfield Analytical Services, Litchfield, MI) of the sample of control feed showed the diet, as fed, contained 54.1% moisture, 17.9% crude protein, 9.22% fat, 3.90% ash, 1.40% crude fiber, 0.88% calcium, and 0.78% phosphorus. PCB analyses (CT & E Environmental Services, Ludington, MI) of the mink diet samples yielded no detectable PCBs, as Aroclors 1016, 1221, 1232, 1242, 1248, 1254, 1260, 1262, or 1268, (detection limit 67 μg/kg) in the control feed. The PCB-supplemented diet contained 3.19 mg Aroclor 1254/kg feed, wet weight (detection limit 67 ug/kg). No other PCBs, as Aroclors, were detected in the PCB-supplemented diet.

There were no clinical signs of toxicity observed in the mink during the trial. Body mass gains of the control and PCB-treated males during the trial were not statistically different (819.8 ± 60.1 g [× ±S.E.], controls vs. 742.8 ± 60.1 g, PCB-treated, $p = 0.20$). No gross abnormalities were found in the bacula. Bacula lengths and masses of the control and PCB-treated mink were not significantly different (44.4 ± 0.6 mm, control vs. 43.4 ± 0.5 mm, PCB-treated, $p = 0.22$; 216 ± 8 g, control vs. 204 ± 7 g, PCB-treated, $p = 0.23$, respectively). Baculum length and mass were strongly correlated with each other in the control mink ($r = 0.705$) but not in the PCB-treated males ($r = 0.074$; see Figure 1). Correlation coefficients for baculum length and body mass for the control and PCB-treated mink were $r = 0.523$ and $r = 0.641$, respectively, and for baculum mass and body mass were $r = 0.279$ (control) and $r = 0.576$ (PCB-treated).

The results of this study indicate that dietary exposure of male, farm-bred mink to 3.19 mg Aroclor 1254/kg feed (2.0 mg/kg targeted concentration) during the late growth period had no significant effect on baculum length or mass or gross effects on baculum morphology. It could be that the concentration of Aroclor 1254 was too low to significantly effect baculum development or that the mink were not exposed to the PCBs early enough in life or long enough to elicit an observable affect on the bacula. Mably *et al.* (1992) reported that spermatogenesis in rats is much more susceptable

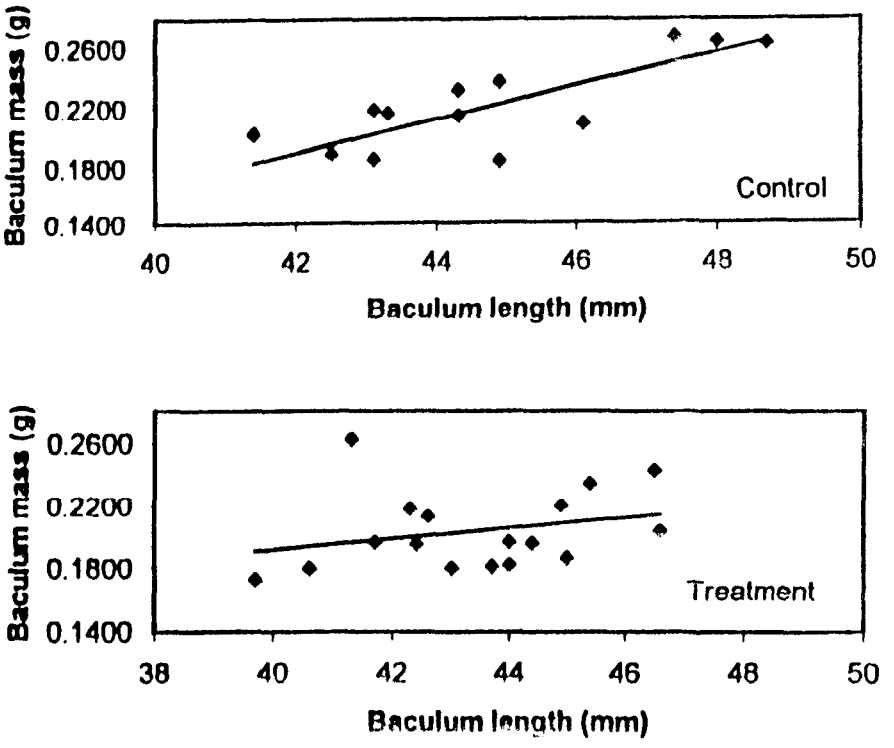

Figure 1. Relationship between baculum length and mass of control (top) and PCB-treated (bottom) mink.

to 2,3,7,8-tetrachlorodibenzo-p-dioxin when exposure occurs perinatally than when it occurs following weaning. Perhaps baculum development is similarly affected by *in utero* and/or lactational exposure to PCBs. It is also possible that the PCB congeners present in Aroclor 1254 do not elicit an affect on baculum development.

Mink attain maximum body mass during the fall of the year. However, the baculum continues to grow at least throughout the first year of life (Parley 1980; Aulerich *et al.*, 1999). Thus, the PCB-treated mink in this study would have been exposed to Aroclor 1254 for 5 months during the middle of the period of baculum growth. Data presented by Paul (1968) for wild-trapped mink taken at various times during the trapping season show the continued growth of the baculum preceding the breeding season (March). The mean masses of mink bacula collected during the trapping season were: 190 mg -November; 210 mg - December l-l 5; 250 mg - December 16-31; 290 mg - January l- 15; 340 mg - January 16-3 1; and 350 mg - February. The mean baculum masses for wild mink taken in December are comparable to the mean baculum masses of 216 and 204 mg for the control and PCB-treated males, respectively, collected on December 17 in our study.

Although Aroclor 1254 is a relatively common contaminant of the environment and consists of a complex mixture of PCB congeners, wild mink and river otter would undoubtedly be exposed to a greater array ofpotential environmental toxins, including pesticides, dioxins, furans, heavy metals, as well as other PCBs. One or more of these chemicals or elements, acting alone or synergistically, could be responsible for the observed effects on the bacula reported for wild mustelids. For example, previous studies conducted at our experimental fur farm have shown that dietary exposure of mink to PCB congeners 169 (3,3',4,4',5,5'-hexachlorobiphenyl; Aulerich *et al.,* 1987) or 126 (3,3',4,4',5-pentachlorobiphenyl; unpublished data), or to 2,3,7,8-tetrachloro-dibenzo-p-dioxin (unpublished data) caused elongated, thickened, and deformed nails that were not observed with other PCB congeners or commercial PCB mixtures (Aroclors). Deformed nails have also been reported in European ferrets *(Mustela putorius furo)* fed a diet supplemented with Aroclor 1242, but were not observed in ferrets fed the same concentration of Aroclor 1016, or in mink fed diets containing either Aroclor 1242 or 1016 (Bleavins *et al.,* 1982).

The strong positive correlation between baculum length and mass for the control males (r = 0.705) was similar to that reported by Harding *et al.* (1999) for wild mink (r=0.785) However, the weak correlation between baculum length and mass (r = 0.074) for the PCB-treated males suggests that possible subtle developmental alterations could be occurring. The composition and density of the bacula of the mink in this study were not determined. However, it could be that mineral metabolism, especially calcium metabolism, was disrupted by exposure to Aroclor 1254. Andrews (1989) reported an increase in femur density and a decrease in the medullary and cortical areas of the femurs of rats dosed with Aroclor 1254 (Andrews 1989).

Baculum development may also be influenced by the nutritional (Korhonen 1985) and/or hormonal (Wright 1947,1950) status of an animal. Korhonen (1985) reported that undernurished farm-raised raccoon dogs *(Nyctereutes procyonides)* had significantly lighter bacula than controls. However, under-nutrition did not have a significant affect on baculum length or width. In studies by Wright (1947, 1950) changes in baculum size and conformation in the long-tailed weasel *(Mustela frenata)* were reported to be regulated by male sex hormone. Since many environmental contaminants are known to be endocrine disruptors and/or to cause reduced food consumption in animals, these factors may be involved in or responsible for the negative correlations between the concentration of environmental contaminants and baculum size reported for wild mink and river otter.

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