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Parathion Effects on Reproductive Characteristics and Vital Organ Weights of Female Cottontail Rabbits (*Sylvilagus floridanus*)

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Organophosphate insecticides (OPs) are used in agricultural and forestry operations, homes, greenhouses, and for a variety of other purposes. Because of the widespread use of these compounds, many wild mammals may be exposed to their toxic effects. OP insecticides prevent breakdown of the neurotransmitter acetylcholine by binding to cholinesterases thus inhibiting their ability to degrade acetylcholine (McEwen and Stephenson 1979). Following severe exposure, intoxicated mammals exhibit symptoms such as tremors, paralysis, and, ultimately, death.

Because some wildlife species rely on high net reproductive rates to maintain populations, decreases in any aspect of reproduction may more adversely affect population levels than losses of individual adults. The added demands of growth, pregnancy, and lactation attendant to reproduction may decrease tolerance of adults to OPs. The present studies were initiated to determine the effects of parathion (O,O-diethyl O-nitrophenylphosphorothionate) on a prolific wildlife species, the cottontail rabbit, *Sylvilagus floridanus*. Previous studies using white rats and mice have demonstrated adverse impacts on reproduction (Fish 1966; Staples et al. 1976; Talens and Wooley 1973); however, our studies were conducted because cottontail rabbits are likely to encounter OPs in the environment and because wild mammals are often more sensitive to OPs than their laboratory counterparts (Tucker and Crabtree 1970). In addition, organ weights which might be altered by OP treatment, e.g., livers (Cecil et al. 1974); and adrenals (Roffi and Ramade 1981), as the

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result of enhanced or diminished organ function were also measured.

MATERIALS AND METHODS

Cottontail rabbits were live-trapped from 5 November 1981 to 9 January 1982 using box traps similar to those described by Mosby (1955) and released in 4 one-tenth ha outdoor pens. All rabbits were tagged with numbered 9.5 mm diameter circular ear tags using colored plastic backup washers (National Band and Tag Co., Newport, Kentucky) and randomly released into one of four adjacent 0.10-ha pens located in a mature mixed-oak woodlot on the Virginia Polytechnic Institute and State University campus. All animals were provided a pelleted diet, water, and cover until the experiment was begun. Adjustments were made immediately prior to the start of the experiment that resulted in sex ratios of (females:males) 2:1, 2.25:1, 4.3:1, and 3:1 in pens 1, 2, 3, and 4, respectively. Because of inability to capture sufficient male rabbits, and losses due to handling and predation, the above sex ratios were not identical. Densities in the pens ranged from 12 to 16 animals per decare at the completion of the experiment. On 15 February 1982, all animals in pens 1 and 2 were recaptured and weighed to the nearest 25 g. Females were randomly selected to receive orally (via stomach intubation) either 8 mg/kg body weight of technical parathion in corn oil (treatment) or corn oil alone (controls) and released. This dose was intended to cause approximately 30% inhibition of brain cholinesterase (ChE) within 24 h based on results of an earlier dose-response experiment conducted in the laboratory. Total volume administered did not exceed 1.5 ml. Males were not treated but were weighed and released. On 17 February 1982, this procedure was repeated in pens 3 and 4. A second dose was administered in an identical manner on 16 March 1982 (pens 1 and 2) and 18 March 1982 (pens 3 and 4).

On 23 March, all animals in pens 1 and 2 were recaptured and weighed. Females were bled via cardiac puncture using heparin coated Monovettes (Sarstedt, West Germany), sacrificed, and their brains immediately removed and placed in labeled Whirl-pac (Nasco, Fort Atkinson, Wisconsin) bags on ice. Carcasses were also kept on ice or in a refrigerator until necropsy. Brains were chilled on ice and placed in a freezer within 1 h of sacrifice and held at -10 C until ChE activity was measured. On 25 March 1983, rabbits in pens 3 and 4 were recaptured and handled identically to those in pens 1 and 2.

Blood samples were drawn into capillary tubes and centrifuged for 5 min and packed cell volumes (PCV) read. Individual animals were necropsied the day of sacrifice, and the following organs and tissues were extracted and weighed to the nearest 0.1 g: liver, adrenals, kidneys, perirenal fat pads, spleens, and reproductive tracts. Following weighing of the reproductive tracts, fetuses were counted, weighed, measured, and their ages estimated to within 3 days using the method described by Rongstad (1969). All organs were preserved in 10% formalin for future histological examination. Kidney fat indices were computed using the method of Riney (1955). ChE determinations basically followed the method of Ellman et al. (1961) with slight increases in substrate concentration (Montz 1983).

Subsequent statistical analysis revealed that treatment with parathion caused reductions in perirenal fat pad weights. Therefore, an experiment was designed to determine if depletion of body fat stores was due to reduction in food intake caused by dosing with parathion. Nine male cottontail rabbits surviving the pen study were singly caged in partitioned stainless steel cages (61 x 56 x 43 cm) and allowed to acclimate to laboratory conditions for at least 30 days. On day 0, five randomly selected animals were weighed and given 8 mg/kg body weight of parathion in corn oil orally via stomach tube. Four controls received an equivalent dose of corn oil. Food consumption was measured for each animal for 9 days following treatment (days 1 to 9). Change in body weight was computed as the difference between weight on day 0 and weight 9 days post-treatment (day 9).

Data were analyzed using analysis of variance procedures (ANOVA) available in the Statistical Analysis System (SAS) computer package (Helwig and Council 1979). Because data on fetuses were not independent observations, log-linear model analysis (Bishop et al. 1978) was employed to test differences in frequency of dead vs. healthy embryos within individual females and across pens and treatments. The computer program used was developed by S. Keith Lee, Department of Statistics, VPI and SU. Differences in weights of only live, healthy fetuses for which weights could be determined were analyzed by a nested ANOVA design.

RESULTS AND DISCUSSION

Although we planned to kill animals near the end of their first pregnancy, 8 of 35 (23%) were pregnant with second litters at the time of necropsy. Attempts to

locate the offspring of these individuals for purposes of estimating litter size and survival were not successful.

Two animals sustained injuries and were not pregnant but all injury-free animals (N = 35) were pregnant. Perirenal fat weight and kidney fat indices were significantly lowered (P = 0.01 and 0.007, respectively) by parathion treatment (Table 1). No differences (P > 0.05) between treatment and control groups were observed in any of the reproductive organ weights following treatment with parathion. *In utero* mortality was not significantly different between control and treatment groups. No differences were observed between animals pregnant with first litters and those pregnant with second litters.

Table 1. Mean values of reproductive characteristics, vital organ weights, and other measurements on female cottontail rabbits treated with parathion.

Variable	N	Parathion Dose: 0 mg/kg $\bar{x}$	0 mg/kg SE	8 mg/kg $\bar{x}$	8 mg/kg SE
Reproductive organ weights					
Paired ovary weight (mg)	34	13.2[1]	2.5a	11.4	2.4a
Mean no. corpora lutea/ female	34	4.0	0.1a	3.7	0.1a
Uterus weight (g)	34	33.8	1.8a	28.3	1.4a
Mean no. embryos/female	34	4.0	0.1a	3.8	0.1a
Estimated stage of pregnancy (days)	31	13.6	0.4a	8.8	0.6b
Vital organ weights					
Spleen weight (g)	31	0.9	0.0a	0.9	0.0a
Paired adrenal weight (mg)	35	26.0	4.0a	25.0	4.0a
Liver weight (g)	35	43.9	0.7a	45.1	0.7a
Other measurements					
Change in body weight (g)	35	10.3	6.6a	-13.8	5.8a
Packed cell volume (%)	34	44.0	0.3a	42.1	0.3a
Kidney fat index (%)	35	44.3	1.4a	23.0	1.0b
Total perirenal fat (g)	35	10.6	0.5a	4.2	0.2b
ChE activity (U/g)	35	6.4	0.1a	6.2	0.0a

[1]Measurements within a row followed by the same letter are not significantly different (P < .05).

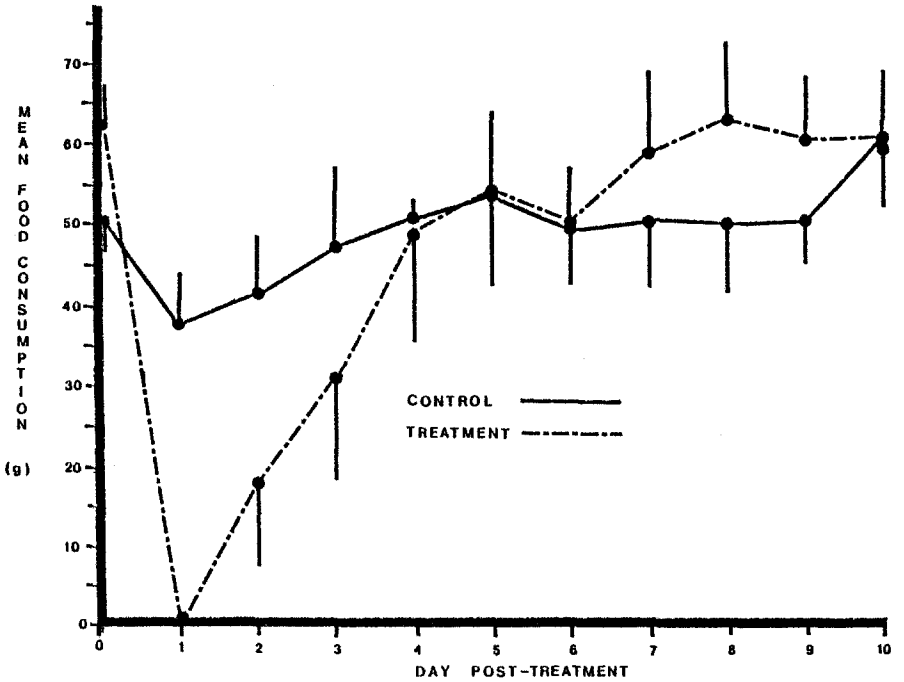

Fig. 1. Food consumption of laboratory housed cottontail rabbits dosed with parathion. Vertical bars represent 1 standard error.

Analyses of results of the laboratory study showed that parathion caused a significant reduction in food consumption on days 1 and 2 after treatment (Figure 1). Minimum mean food consumption (0.84 g) occurred on day 1. Consumption then increased steadily until mean consumption of treated groups was not significantly different from that of controls on day 3. The data show that food consumption of control animals also was reduced slightly on day 1 suggesting that handling may have contributed to decreased food consumption. Body weights of treated animals did not decline significantly compared with controls.

These data clearly show that administration of two 8 mg/kg of body weight doses of parathion 30 days apart did not (1) prevent conception or (2) significantly decrease litter size. This dose was sufficient to cause a substantial reduction in ChE activity (30%) in laboratory trials with cottontails, and the toxicity of parathion to mammals is high, e.g., LD_{50} = 6 mg/kg for the male rat (Alary and Brodeur 1970). It appears unlikely that there exists an oral dosage administered every 30 days that would prevent breeding or reduce

litter sizes without killing the female. Estimated age of cottontail litters was highly variable.

Vital organ weights did not differ significantly between treated and control groups. This is not surprising because of the infrequency with which the subacute dosages were administered. Although OP insecticides are generally hepatic microsomal enzyme inhibitors (Donovan et al. 1978; Mihara et al. 1981), more prolonged exposure to OPs is probably required before reduction in enzyme activity would manifest itself through reduced liver weights.

Parathion-induced reductions in food intake lasted approximately two days. Reductions in food consumption have also been found in rodents following exposure to OPs (Glow et al. 1966; Mehrotra et al. 1967; Staples et al. 1976). Our data also suggest that compensatory increased feeding occurred between 6 and 10 days after treatment. Such increases in intake presumably offset the initial depressant effect of parathion on food intake.

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