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Chronic Dose Effects of Methyl Parathion on Nuthatches: Cholinesterase and Ptilochronology

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The widespread use of organophosphorus (OP) insecticides in agriculture has caused increased concern for the potential exposure to wildlife. Some effects of OPs on wildlife include loss of body weight (Grue 1982), reproductive effects (Stromborg 1977, 1981; Spyker and Avery 1977), susceptibility to environmental stress (Pope and Ward 1972), reductions in visual acuity, vigilance, and food-seeking behavior (Grue et al. 1983), hypothermia (Chattopadhyay et al. 1982) and death.

The mode of action of OPs is to inhibit the enzyme cholinesterase (ChE), causing the accumulation of acetylcholine at nerve synapses and subsequent loss of nerve function. The ability of OPs to inhibit ChE varies among chemicals, animal species, age, sex, and level of exposure. Birds appear to be more sensitive to OP exposure than other vertebrates (Grue et al. 1983).

As a feather grows, growth bars are laid down (Riddle 1908). Each growth bar consists of a dark and light band and represents a 24 hr period (Michener and Michener 1938). Recently, a method called ptilochronology has been developed which uses the daily growth bars to assess the net energy status of a bird (Grubb in press). The potential of this technique in monitoring pesticide exposure to wild populations of birds and for field experimentation is promising. Initial laboratory studies must be done to assess the capabilities of the technique when conditions are controlled and dosages of the chemical are known.

The objectives of the study were to assess the effects of a chronic, subacute dose of methyl parathion on the daily feather growth of white-breasted nuthatches (Sitta carolinensis) using ptilochronology to measure daily growth and to develop a method of accurately dosing captive birds with a chronic, subacute dose of an OP insecticide.

MATERIALS AND METHODS

Eight adult white-breasted nuthatches (4 males and 4 females) were live-trapped and brought into the laboratory between

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February and early March of 1988. The birds were held individually in 56 cm X 56 cm X 56 cm hardware cloth cages. Perches and pieces of bark were placed in the cages. The birds were allowed to acclimate for at least 10 days. Ambient temperatures ranged from 22°-24° C. Photoperiod was kept at a light:dark cycle of 8:16 hr. Birds were fed sunflower (Helianthus sp.) seeds and mealworms (Tenebrio sp.) ad libitum prior to treatment. A vitamin supplement (Vita-Sol, 8 in 1 Pet Products, Inc., Brentwood, N.Y. 11717) was added to the water.

The experimental and control groups consisted of 2 males and 2 females each. On 28 March 1988 each bird was weighed and the outer right rectrix (tail feather) was plucked and stored for future analysis. All birds were then fed ad libitum as before for a period of 2 weeks. Treatments for the experimental and control groups began on 14 April 1988 and ran through 29 April 1988 at which time the birds were weighed, the replacement rectrix plucked, and the birds sacrificed for brain ChE analysis.

Treated birds were dosed with 3.5 mg/kg of methyl parathion in corn oil each day while controls were given an equal amount of corn oil. The dosage was administered via mealworms. Mealworms approximately 20-25 mm in length were euthanized with CO_2 and injected with either corn oil or methyl parathion in corn oil using a Hamilton 25 μL syringe. The birds were fed at approximately the same time every day. All uneaten mealworms were removed from the food dish and the injected mealworm was placed in the dish. The birds were allowed time to eat the mealworm after which time they were given more mealworms. Water and sunflower seeds were available at all times.

Rectrix measurements and data analysis resembled those described in Grubb (in press). In this experiment the birds had insufficient time to grow in fully the replacement feather. Therefore, the length of the feather grown by each bird during the experimental period was divided by the length of the original rectrix. This procedure provided a standard index which controlled for differences in size among birds.

Brains were immediately removed from sacrificed birds following the methods used by the Wildlife Toxicology Research Group, EPA-Corvallis Environmental Research Laboratory (CERL). Dissected brains were double wrapped in aluminum foil and snap frozen in liquid nitrogen. Samples were packed in dry ice and shipped to EPA-CERL for ChE analysis.

A t-test was used to compare treated and controls for feather growth and body weight changes. A linear regression was run between the length of the replacement feather and the total length of the original feather.

RESULTS AND DISCUSSION

No significant difference in total feather growth was found between treated and control birds ($p > 0.05$). The mean value for the index of feather growth in treated birds was 0.46 ± 0.05 and was 0.48 ± 0.02 for controls (Table 1). The mean value (IU/g brain) for brain ChE levels in control and treated birds was 12.14 ± 0.44 and 7.74 ± 0.85 respectively (Table 1). ChE levels were inhibited 36% in treated birds compared to control birds. There was no difference in body weight change between treated and control birds ($p > 0.05$) with the treated and control birds having a mean weight change of $- 0.15 \pm 0.97$ g and $- 0.59 \pm 0.22$ g respectively (Table 1). Regressions were run between length of the replacement feather and the length of the original feather. A significant negative correlation was found for the treated birds ($y = 46.3 - 0.44x$, $r = - 0.96$, d.f. = 2, $P < 0.05$), however the correlation for control birds was not significant ($y = 1.21 + 0.46x$, $r = 0.56$, d.f. = 2, $P > 0.05$).

Table 1. Brain ChE levels, weight change, and the ratio of rectrix feather growth in white-breasted nuthatches given a chronic, subacute dose of methyl parathion over a two-week period.

	Sex	Brain ChE levels (IU/g brain)	Weight change (g)	Feather growth[1]
	M	12.05	- 0.4	0.51
Control	M	11.55	- 0.5	0.50
	F	12.40	- 0.9	0.47
	F	12.55	- 0.5	0.46
X ± S.D.		12.14 ± 0.44	- 0.57 ± 0.22	0.48 ± 0.02
	M	8.80	+ 0.7	0.48
Treated	M	6.95	+ 0.4	0.40
	F	7.15	- 1.5	0.44
	F	8.05	- 0.2	0.52
X ± S.D.		7.74 ± 0.85	- 0.15 ± 0.97	0.46 ± 0.05

[1] The final measurement of growth of the replacement rectrix feather (mm) divided by the length of the original rectrix feather (mm). This ratio allows feather growth comparisons of feathers which were pulled before reaching their original length.

Although there was no significant difference in feather growth between control and treated groups (Table 1) there is still potential for applying ptilochronology to the field of avian toxicology. Growth bars

were not detectable on the replacement rectrices and there was also a reduction of barbules noted on the replacements. The problem of unreadable growth bars has never been encountered prior to this experiment and the reason for this occurrence is not known. Therefore, daily growth could not be measured for these birds. Total feather growth of the replacement feathers was used to measure overall growth instead. The replacement feathers were not fully regrown when removed. Therefore a ratio of total length of the replacement feather : total length of the original feather was used to standardize feather growth and allow for comparisons. The fact that no significant difference was found between treated and control groups could have been a result of the small sample sizes. If daily growth could have been measured using growth bars a difference between control and treated birds might have been observed.

Brain cholinesterase levels were determined at the end of the experiment to see whether the chronic dose of methyl parathion administered produced inhibition. After 3 days of receiving the treated mealworms one of the four experimental birds began rejecting the treated mealworm. After 7 days all four birds in the treated group rejected the mealworms. This avoidance behavior by the nuthatches supports work done by Hill (1972). He found that when house sparrows (Passer domesticus) were given a choice between contaminated and uncontaminated food they chose the uncontaminated food. When only contaminated food was offered the birds did not eat at first but were eventually forced to accept the food to avoid starvation.

For our experiments the birds were given mealworms ad libitum after they ate the treated mealworm. Once rejection started to occur mealworms had to be given at the same quantity as controls to avoid any food type effect. When avoidance began to occur the treated mealworm was given with 3-4 uncontaminated mealworms to try to induce the birds to eat but they appeared to be able to detect the treated mealworm and avoid eating it.

Despite the rejection of the mealworms after only 3-7 days, brain ChE levels were inhibited an average of 36% in the treated birds (Table 1). Ludke et al. (1975) found that ChE inhibition of > 20% is indicative of OP exposure in birds. A chronic, subacute dose (3.5 mg/kg) of methyl parathion caused brain ChE inhibition in white-breasted nuthatches after only 3-7 days of exposure.

The technique of using injected mealworms to administer the toxicant appeared to work well. As long as the birds continued to eat the worms an accurate record of the daily dose received could be obtained. To our knowledge this is one of the few studies in which a chronic, subacute level of an OP was administered to birds as a dosage instead of as a concentration in the food in which case the actual amount of OP ingested is never known.

There were no differences in weight change between controls and treated birds (Table 1) suggesting that there were no effects of pesticide induced anorexia. Grue (1982) showed that mortality of common grackles (Quiscalus quiscula) fed different dietary concentrations (10-400 ppm) of

OPs was largely due to pesticide induced anorexia. Perhaps no effect was noted because birds still had access to uncontaminated food.

A significant negative correlation ($r = -0.96$) was found between feather growth of the replacement feather and the total length of the original feather for the treated birds but no correlation was found in controls ($r = 0.56$). Perhaps a better correlation would have been found in control birds if the sample size was increased. This would then suggest some OP effect on feather growth.

From the results obtained in this preliminary study it is obvious that more work needs to be done to assess the potentiality for the use of ptilochronology in avian toxicology.

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