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Physiological and Behavioral Effects of Guthion on Pine Voles, *Microtus pinetorum*

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Guthion (azinphos-methyl; O O-dimethyl S-[(4-oxo-1,2,3-benzotriazin -3(4H)-yl) methyl] phosphordithioate) is an organophosphate (OP) insecticide used extensively on apple trees in North Carolina and on numerous crops nationwide. Goodman et.al. (1983) estimated that 25% or more of Guthion loss from tree leaves occurs as movement to the soil and ground vegetation causing Guthion exposure to ground-dwelling mammals.

Guthion inhibits brain acetylcholinesterase (AChE) in rodents (Short et.al. 1980) and affects locomotion, reproductive physiology and learning, yet little is known of the effects intermittent and acute exposures have on the physiological and behavioral ecology of wild small mammals. Pine voles (*Microtus pinetorum*), semifossorial residents of orchards throughout eastern United States, present a conflict for growers and conservationists because their semifossorial habits often result in root damage and girdling of trees. Because of this, toxic effects in pine voles following Guthion spraying might be welcomed by growers, yet these effects may suggest toxicity to mammals and birds that interact in complex, ecologically beneficial ways. Further, survivors of Guthion exposure often exhibit abnormal behaviors that could increase rather than decrease pine vole population growth.

We conducted 3 experiments on pine voles: (1) to measure Guthion's inhibition of brain AChE activity in field-exposed voles; (2) to evaluate dose-related reductions of AChE in the laboratory; and (3) to measure Guthion-induced behavioral changes.

MATERIALS AND METHODS

Experiment 1 assayed for Guthion-induced AChE inhibition in field-exposed pine voles. Three 10-vole groups (sex ratio 1:1) were trapped in an apple orchard in Henderson Co., NC. Control subjects were trapped 1 d before a 2.2 kg/ha (2 lbs/A) application of Guthion. A second group was captured 24-48 hr, and a third group

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120-124 hr, after the application. A laboratory control group, exposed to ambient temperature and light for 2 wk, had no history of exposure to pesticides. Voles were weighed, sacrificed by cervical dislocation, placed in plastic bags, and then stored at -5 °C (Hill and Flemming 1982). Thawed brains were homogenized in 0.05 M Tris buffer (pH 8.0, 1:10 w/v) and brain AChE activity (uM acetylthiocholine iodide hydrolized/ml of brain homogenate/min) was determined spectrophotometrically (Ellman et.al. 1961).

Experiment 2, which examined the effects of serial dietary doses of Guthion, began with a pilot study that revealed an abrupt, significant difference in AChE activity between voles fed 0, 50, 100 and 200 ppm dietary Guthion and voles fed 400 and 800 ppm (2 voles at each dose). To refine these dose-related effects, we examined the relationship of Guthion consumption to weight loss, to exposure duration as it affected toxicity, and to the shape of the Guthion-AChE dose response curve.

First through 4th generation captive voles (N=36) were fed ground chow (Wayne F-6 Lab Blox) for 8 d to monitor rates of food consumption and body weight, and then were assigned randomly to one of six Guthion doses (0, 150, 250, 350, 400 and 700 ppm) provided *ad lib* within the chow for the next 5 d. Grams of chow consumed and body weights of voles were monitored daily. Doses, expressed as mg technical grade (91% pure, Mobay Chem. Corp., Kansas City) Guthion/kg food or vole, were prepared by dissolving Guthion in acetone and adding corn oil. This solution was heated for 20-40 min at 65-75 °C to evaporate the acetone. Corn oil was then added and the solution was blended with ground rodent chow to achieve a ratio of 2% w/w vehicle to chow (0-0.16% acetone w/w). Controls received chow that lacked Guthion. On Day 2, 2 voles from each treatment were sacrificed; on Day 5 surviving voles were sacrificed. Brains were weighed, placed in Tris buffer and stored at -5 °C for subsequent AChE assay identical to Experiment 1.

A dose response curve for voles surviving 5 d (including pilot data) was generated using a 4-parameter Weibull dose response model with upper and lower asymptotes (Joyner 1985). The model was based on dosage (mg Guthion ingested/kg vole/g chow) rather than on dietary concentration because of individual variations in body weight and food consumption. Two voles were excluded from analyses because of food spillage and death unrelated to treatment.

Experiment 3 explored the effects of Guthion on social behavior. Wild pine voles live in extended family units containing a single monogamous breeding pair (Fitzgerald and Madison 1983). Home ranges of family units do not overlap and established pairs are aggressive towards other pine voles (Durda 1986). Because changes in aggressiveness may affect pine vole social groups and entire populations, we studied the impact dietary Guthion may have on the aggressive responses of monogomous pine vole pairs toward intruders.

At least one month after establishing mating pairs, mates were separated by a 1.3 cm wire mesh cage divider that prevented mating

but allowed visual, olfactory, and limited physical communication. After 18 d the divider was removed to allow pairs 3 d to become familiar with the chow containers.

A dietary concentration of 195 ppm Guthion (40 mg/kg body wt) was used to depress brain AChE activity to levels found in the wild pine voles of Experiment 1 after 5 d exposure to Guthion in an apple orchard. Twelve pairs of voles were placed into control or treatment groups. Twenty-four hr prior to treatment each pair was tested for aggression against an adult female introduced into the home cage for 15 min. Aggressive interactions between the pair and the intruder were observed and the number of attacks, fights and chases occurring, and the latency to first aggressive response, were recorded (see Durda 1986). On Day 5, all pairs were tested identically with different female intruders and then were given clean food for 10 d. All pairs were tested again on Day 15 with new female intruders.

RESULTS AND DISCUSSION

In Experiment 1, day of capture significantly affected AChE activity ($p<0.0001$, F-test adjusted for body weight, Joyner 1985). The greatest variation in AChE activity was between Day 1 and Day 5 field-exposed voles (orthogonal contrasts) though all comparisons were significant (Tab. 1). Brain AChE activity of voles captured on Day 5 also differed significantly from both laboratory and field controls ($p<0.005$, non-orthogonal contrasts). Mean brain activity of Day 5 voles was reduced 42% relative to laboratory controls. Thus, field application of Guthion significantly depressed brain AChE activity of pine voles trapped 5 d later, supporting observations of the effects of other OP insecticides on wild small mammals. Zinkl et.al. (1980) found maximal enzyme depression in other small mammals 3 d after field application of OP insecticides. Results below from Experiment 2 suggest that 2 d of exposure to dietary Guthion may be sufficient for maximal enzyme depression in pine voles.

Table 1. Brain AChE activity (±SD), percent inhibition of control groups and voles exposed to field application of Guthion, and analyses of variance of orthogonal contrasts between treatment groups in Experiment 1 (degrees of freedom = 1 for all contrasts).

Group	AChE activity*	Percent inhibition	Orthogonal contrast	F	p
Laboratory control	0.71 ± 0.08	-	vs FC, 1D, 5D	21.74	<0.005
Field control	0.66 ± 0.06	8	vs 1D, 5D	15.24	<0.005
1 day post-spray	0.65 ± 0.12	8	vs 5D	24.78	<0.005
5 day post-spray	0.42 ± 0.10	42			

* AChE activity in uM acetylthiocholine iodide hydrolyzed per ml brain homogenate/min.

Voles trapped for our field assay of AChE inhibition had been exposed to 3 previous sprayings of OP insecticides, the most recent of which was 10 d before this study. Nonetheless, enzyme activity between field controls and laboratory controls did not differ, suggesting rapid recovery of AChE activity after subacute exposure. Rattner and Hoffman (1984) found AChE recovery after 14 d in other small mammals that received 400 ppm dietary acephate. The pine vole's rapid recovery may be related to its high basal level of liver cytochrome p-450 (Hartgrove et.al. 1977).

Experiment 2 indicated that neither body weight nor daily food consumption was affected in voles receiving 0 to 350 ppm Guthion (t-test). Voles receiving 400 and 700 ppm experienced daily weight loss ($p<0.02$ and $p<0.0001$, t-test) and reduced food consumption (700 ppm only, $p<0.0001$; t-test). Brain AChE activity for voles surviving 5 d was reduced in a dose dependent manner ($p<0.0001$; t-test). AChE activity decreased between 0 and 250 ppm but remained relatively stable with further increases in Guthion concentration (Tab. 2). AChE activity began decreasing at 14 mg/kg body wt and stabilized at 56 mg/kg (Fig. 1).

Table 2. Mean dosage and AChE activity for pine voles receiving graded dietary concentrations of Guthion.

Dietary concentration (ppm)	Dosage ± SD (mg/kg/d)	AChE activity ± SD**
0	0	0.71 ± 0.06
50	9.80 ± 0.08	0.68 ± 0.01
100	18.68 ± 0.06	0.69 ± 0.20
150	30.39 ± 4.65	0.55 ± 0.13
200	38.97 ± 7.21	0.70 ± 0.05
250	43.81 ± 2.17	0.21 ± 0.03
350	65.23 ± 7.36	0.24 ± 0.03
400	71.32 ± 13.79	0.19 ± 0.01
700*	54.42	0.24

* N=1

** AChE activity in uM acetylthiocholine iodide hydrolyzed per ml brain homogenate/min.

No obvious toxicosis was observed in voles surviving 5 d despite highly depressed AChE levels at high doses. Five voles died: one each at 350 and 400 ppm, and three at 700 ppm (mean AChE activity 0.18 ± 0.09). Duration of exposure to dietary Guthion affected AChE depression only at the 350 ppm dose. Mean AChE activity for the 350 ppm group was 0.50 ± 0.12 on Day 2 but was 0.24 ± 0.03 on Day 5 ($p<0.03$).

Pine voles withstood high dietary levels of Guthion and severe AChE depression without exhibiting overt toxicosis. Although exposures between 150 and 350 ppm Guthion for 5 d resulted in AChE inhibition, no obvious locomotor dysfunctions, convulsions, food avoidance or weight loss were noted. Other small mammals respond similarly (Rattner and Hoffman 1984). Voles that died exhibited toxic effects typical of OP poisoning, and voles receiving 400 and 700 ppm Guthion suffered significant weight loss. Only the 700 ppm

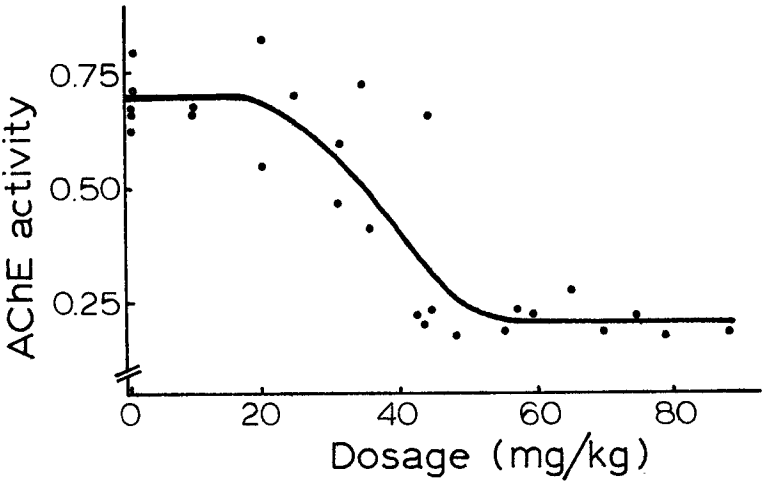

Figure 1. Guthion-AChE dose response curve. AChE activity is expressed as umoles acetylthiocholine iodide hydrolized/ml brain homogenate/min.

group significantly decreased consumption, contradicting Grue's (1982) suggestion that weight loss accompanying OP poisoning is due to pesticide-induced anorexia. The differences in food consumption were not consistent with levels of AChE inhibition and probably resulted from the aversive taste of high Guthion concentration in the food. Loss of body weight without a concomitant decrease in consumption in the 400 ppm group is possibly related to Guthion-induced alterations of gastrointestinal function (Wali et.al. 1984).

The Guthion-AChE dose response curve (Fig. 1) declines sharply between 20 and 50 mg/kg body wt. The upper asymptote apparently reflects the voles' ability to detoxify small quantities of Guthion. The lower asymptote is more difficult to explain. Perhaps, repeated exposure to Guthion stimulates microsomal metabolism allowing pine voles to continue to detoxify the insecticide, or, low doses of AChE may induce *de novo* AChE synthesis necessary to maintain a baseline level of AChE. It is also possible that there is a pool of AChE in the brain that is not accessible to Guthion.

In experiment 3, dietary Guthion significantly affected the number of chases ($p<0.027$, F test on log differences) on Day 5 verses Day 0 and marginally affected chases ($p<0.07$) on Day 15 verses Day 5 (Tab. 3). Control animals exhibited more chases throughout the experiment whereas the number of chases increased slightly on Day 5 and decreased on Day 15 in the treatment group. Adults readily attacked non-resident voles, but voles exposed to dietary Guthion were less aggressive than controls even after 10 d of recovery.

Reduced aggressiveness is positively correlated with reproductive success in laboratory mice (Horn 1974) and in *M. pennsylvanicus* (Madison 1980). All models of microtine population regulation indicate that aggressiveness is important (Madison and McShea 1987, Tamarin and Sheridan 1987). Lack of aggressiveness may allow high winter populations (Madison and McShea 1987) and aggressiveness may lead to population declines (Tamarin and Sheridan 1987). Hansson (1986) concluded that non-aggressive populations of *Clethrionomys glareolus* were not cyclic and could maintain stable high local densities. If spacing behavior and aggression affect population structure and growth, then Guthion-induced alterations of these behaviors could increase social tolerance and allow higher population densities than would occur naturally. *M. ochrogaster*'s population density was observed to increase five-fold after application of dimethoate, an OP insecticide (Barrett and Darnell 1967).

Table 3. Mean number of chases for control and dose groups on the 3 d of testing for aggression against intruders.

Group	Pre-dose	Day 5	Day 15
Control	1.75 ± 2.87	4.50 ± 7.68	7.25 ± 5.25
Dose	2.20 ± 2.39	3.00 ± 4.53	0.20 ± 0.45

We conclude that Guthion depressed the AChE activity in pine voles exposed in apple orchards and in the laboratory, a decrease that is correlated with lowered food consumption and body weight in the laboratory animals studied. Mortality in the laboratory experiments occurred only after severe depression of AChE activity. Finally, Guthion exposures reduced forms of aggression that are known to limit population growth and density in rodents. Such effects could result in unexpected increases in populations of exposed pine voles. Considering that pine voles inflict damage estimated in the millions of dollars annually in North Carolina apple orchards, such secondary impacts on this non-target species could have obvious economic consequences.

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