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Robert L. Carey for the degree of Master of Science in Wildlife Science, presented on August 10, 1993.

Title: Effects of Guthion® (azinphos-methyl) on Individual Fitness Correlates of Gray-tailed Voles in Field Enclosures

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Unpredicted wildlife mortality has been associated with the use of organophosphorus pesticides. Predicting the hazards of pesticides to wildlife is difficult because exposure and susceptibility to toxicants vary with habitat and species. The U.S. Environmental Protection Agency uses the quotient method for predicting the risk to wildlife where "Q", or the quotient, is equal to the expected environmental concentration divided by the hazard or toxicity of the chemical to a particular animal species. Problems arise when predictions based on laboratory studies fail to adequately represent actual responses of wildlife from exposure to agrichemicals. My objective was to compare individual fitness endpoints from field enclosures with the hazard predicted by a risk assessment based on a quotient derived from laboratory toxicity studies. Gray-tailed voles (Microtus canicaudus) were trapped biweekly from June through August 1992 in 20 0.2-ha enclosures planted with alfalfa (Medicago sativa). I monitored several fitness correlates of gray-tailed voles in the enclosures in response to five application rates (four replicates/treatment) of the insecticide Guthion® (azinphos-methyl). The proportion of adult ($\geq$30 g) female voles in reproductive condition ranged from 0.23 to 0.51 and did not vary significantly among treatment and control grids. Recruitment rates of new animals into the population declined significantly through the summer, but did not vary with treatment. Growth rates of males whose weight at first capture was $<$30 g declined during late summer, but not in response to the pesticide application. Vole activity was examined by calculating the probability of capture and mean maximum distance moved (MMDM). The probability of capture for both sexes and MMDM for males did not differ over time or among treatments. MMDM for females decreased as the study progressed but did not differ among treatments. Thus, the insecticide had no measurable effects on fitness correlates of voles. The dense alfalfa canopy may have prevented contamination at the ground level. Laboratory toxicity values overestimated hazard to voles in the field. The decline in recruitment, male growth rates, and MMDM for females in the enclosures may have been caused by seasonal or density dependent factors.

Effects of Guthion® (azinphos-methyl) on Individual Fitness Correlates of Gray-tailed Voles in Field Enclosures

by

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Typed by Robert L. Carey

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This project was undertaken by several groups of researchers. The portion of the project for which I was responsible represents only a fraction of the effort put forth. Many people were involved with data collection, field work, and experimental design. However, for simplicity, I use first-person singular voice throughout this thesis.

My deepest appreciation goes to my wife Dana for patience and support, and to my father who sparked my interest in science and wildlife at an early age.

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INTRODUCTION

Organophosphorus (OP) pesticides have caused wildlife mortality when the risk assessment methods used by the U.S. Environmental Protection Agency (EPA) did not predict a hazard to wildlife (Grue et al. 1983, Blus et al. 1989). Consequently, the EPA is interested in examining the responses of nontarget organisms to exposure in controlled field experiments to improve risk assessment. Currently, ecological risk assessment methods rely on toxicity data for laboratory species, together with exposure information, to predict the risk to wildlife from pesticides. Toxicity data are generated from standard laboratory toxicity testing such as LD_{50} or LC_{50} tests (the dose or dietary concentration necessary to kill half the test population) with mortality being the endpoint most frequently investigated (Peterle 1991:135). Exposure is estimated using the expected environmental concentration (EEC), which is predicted from a model based on the substrate and application rate for the pesticide (Hoerger and Kenaga 1972). The risk represented by a pesticide is calculated from the "quotient method" (QM) (U.S. EPA 1986) where the EEC is divided by the toxicity. Quotients of <1 are thought to pose little hazard to wildlife and quotients ≥ 1 are considered threatening. The quotient is multiplied by a safety factor of 0.1 or 0.2 to allow for variation among species and individuals. Toxicity and exposure information is used in a tiered approach with initial testing on individuals, usually surrogate species such as laboratory rats or mice. Subsequent laboratory tests examine other end-points such as chronic effects on reproduction or physiology. The final field tests attempt to approximate actual pesticide applications more closely than do laboratory tests (Peterle 1991:150). Field testing is done only if extreme toxicity is detected in laboratory tests. The data generated from these tests are used by the EPA to register pesticides for use in this country.

Making assumptions about wildlife response to pesticides is necessary when designing generalized predictive models. The risk assessment methodology (i.e., QM) used by the EPA is based on several assumptions about the response of nontarget organisms to chemical exposure. Three of the main assumptions are: laboratory toxicity tests adequately represent field responses, laboratory species such as rats and mice are appropriate surrogates for wildlife species, and the bioavailability of chemicals and responses of nontarget organisms are linearly related to application rates. Predictive models based on generalizations for toxicity, exposure, and hazard concentrations are necessary because testing every known pesticide on many different species of wildlife is unrealistic.

Models fail to predict the risk to wildlife species in some cases because the underlying assumptions are not valid. Inferring field toxicity from lab tests is difficult because wildlife responses vary from species to species even within the same genus, and responses of surrogate laboratory species and wild animals may be very different (Tucker and Crabtree 1970, Cholakis et al. 1981, Roberts 1988). Also, natural ecosystems are more complex than laboratory situations and laboratory results may not accurately predict the hazard to wildlife in the field. In a series of experiments examining the effects of the insecticide endrin on deer mice (Peromyscus maniculatus), Morris (1968, 1972) found that in the laboratory, mortality was dose-dependent, but he could not duplicate these results in the field.

In laboratory tests, dose-response relationships are established by determining at what dose a particular result or endpoint occurs. In field situations the application rate is known but the actual exposure to the test organisms is not. Exposure can be reduced through food aversion or interception of the chemical before it reaches the substrate inhabited by the organism. Furthermore, the amount of the toxicant with which the organism could possibly come into contact (the bioavailability) may vary with habitat structure and biology of the test species. Few field studies have examined the relationship between application rate, bioavailability, and responses of nontarget organisms to pesticides.

My experiment was designed to test the response of gray-tailed voles in field enclosures by gathering data from four replicates of five application rates of Guthion® (azinphos-methyl), and comparing the results to the toxicity data and risk predictions that were generated in the laboratory. The laboratory LD_{50} and LC_{50} for gray-tailed voles were 33 mg/kg and 297 ppm, respectively (M. Meyers, ManTech Environmental Technologies Inc. [METI], Corvallis, Oreg., and J. Wolff, Oregon State Univ. [OSU], Dept. of Fish. and Wildl., Corvallis, Oreg., unpubl. data), and using these values, EPA's risk assessment paradigm (QM) predicted a hazard to animals exposed above 1.2 kg/ha.

My objective was to determine the effects of the OP insecticide Guthion® on the reproductive performance, activity, and male weight change of gray-tailed voles. I hypothesized that these fitness correlates would be negatively affected by the chemical, and that the level of adverse effects would increase with application rate. Specifically, I predicted that reproductive and recruitment rates would be lower and mean weight change for males would be reduced on treated grids compared to control grids and that vole activity would be affected by the chemical. I predicted activity would either increase as animals attempted to avoid the chemical or decrease as they became intoxicated due to exposure. Furthermore, Guthion® degrades rapidly in the environment with <50% of the chemical present in the soil 12 days following application (Schultz et al.

1970). Because of this rapid breakdown and high productivity of gray-tailed voles, I predicted that all effects of the pesticide would be short-term and treated populations would return to control levels within a few weeks. I used gray-tailed voles in this experiment because they are abundant in agricultural fields in the Willamette Valley. The genus Microtus has a world wide distribution and my results may be applicable to species in different geographic areas.

STUDY AREA AND METHODS

I conducted this experiment at OSU's Hyslop Crop Science Field Laboratory, approximately 10 km north of Corvallis, Oregon. The elevation was approximately 70 m, and the site had a well-drained silty-clay loam soil and level topography. The average annual precipitation was 108 cm, but only 18.8 cm fell during the study, with 10.36 cm occurring in April. The study area received only 6.05 cm of precipitation from June through August.

Field Enclosures

Contractors constructed 24 0.2-ha (45 x 45 m) enclosures made of galvanized sheet metal extending approximately 1 m above the ground and buried 0.6 to 1 m below ground. Each enclosure was planted with alfalfa in spring of 1991. Weeds representing most of the annuals present in the region were a minor component. A strip 1 m wide was mowed along the inside of each fence to minimize small mammal activity near the fence and to prevent contact with abnormally high concentrations of insecticide dripping down the fence following application. I introduced six pairs of voles into each enclosure during 9 through 14 April 1992, when the vegetation had reached a height of approximately 30 cm. Two nulliparous females were added to each enclosure on 23 April 1992. All voles released into the enclosures had a numbered aluminum ear tag in their right ear.

Trapping

I established 100 trap stations in each enclosure, set in a 10 x 10 array with 5 m between stations. One pitfall trap, 45 cm deep and 15 cm in diameter, was placed at all odd-number trap stations (i.e., 1-1, 3-3,) and Sherman live traps (7.5 x 9.5 x 25.5 cm) were placed at the remaining stations. This arrangement allowed for 75 Sherman and 25 pitfall traps/enclosure. Pitfall traps were covered and Sherman traps were locked open when they were not in use. I trapped the enclosures for four days (trap period) every two weeks from 9 June to 21 August 1992. Sherman traps were baited with oats and pitfall traps were unbaited throughout the study. I set traps in the evening and checked them the following morning. I trapped on days two through five and 14 through 17 following the pesticide application and then resumed the prespray trapping regime. I ear-tagged all voles for identification, and recorded weight, age, sex, reproductive condition, and trap location.

Test Chemical

I used Guthion® 2S (azinphos-methyl; O,O-dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)- yl) methyl] phosphorodithioate) for this experiment (Mobay Corp. Ag. Chem. Div., Kansas City, MO 64120). Twenty of the 24 enclosures were randomly assigned to one of five application concentrations: 0, 0.77, 1.55, 3.11, and 4.67 kg/ha with four replicates each. These concentrations were used because they approximated multiples of the 0.84 kg/ha label rate of this pesticide for alfalfa. I used multiples of the label rate to determine if a linear relationship existed between the application concentration and the response to pesticide exposure. The insecticide was applied by a licensed applicator using a four-wheel all terrain vehicle and trailer tank with 7.6-m spray booms. Control enclosures received the same volume of water. The control enclosures were sprayed first and the chemical was applied to each of the four application rates in ascending order. The pesticide application began on 7 July 1992, when the control enclosures were sprayed; however, during the spraying of the first 0.77 kg/ha enclosure, an equipment failure occurred and spraying was completed 9 July 1992. Two each of the remaining four enclosures were treated with 1.55 and 4.67 kg/ha and were designated as removal enclosures for another experiment on biomarkers of exposure.

Fitness Parameters

I used three measures of reproductive performance: proportion of adult ($\geq$30 g) females that were in reproductive condition, the proportion of the total voles that were recruits, and the number of recruits per adult female. Females were considered to be in reproductive condition if they were lactating, pregnant, or had widely open pubic symphyses. I defined recruits as any newly tagged vole; I assumed they were born in the enclosure. The number of recruits per adult female was calculated using the number of newly tagged voles each trap period divided by the total number of adult females in that enclosure four weeks earlier. Adult females were used because I was confident in my ability to detect pregnancy in this size class; determining if smaller females were in the early stages of pregnancy was difficult. The four week time-lag allowed offspring to reach trappable size. I used the average weight change for males, whose weight at first capture was ≤30 g and who were captured during consecutive trap periods, to determine differences among growth rates for each treatment and week. Females were excluded from weight change analysis because of weight change associated with pregnancy. I used the ≤30 g criterion because younger and lighter voles were not captured frequently enough to provide sufficient data for analysis. I measured activity by using the mean maximum distance moved (MMDM), and probability of capture generated by program CAPTURE (White et al. 1978). The MMDM value is calculated as the maximum straight line distance between trap locations averaged over all captures within a trap period (Wilson and Anderson 1985). The probability of capture value refers to the probability of any animal being caught in any trap on each trapping occasion (White et al. 1982:47). The MMDM and capture probability values are relative indices of activity only; measures of absolute activity were impossible to determine with my study methods. Population sizes were estimated using the M_t Chao estimator (Chao 1988) from program CAPTURE.

Data Analysis

I tested the fitness correlate parameters to determine if a pesticide response occurred within the vole populations for any treatment during any week of the study. Fitness correlate values were generated for each enclosure and each trap period of the study. Response values were then analyzed by averaging the four replicate enclosures into treatment values. I used arcsine square-root transformation to adjust for the nonnormal distribution and nonconstant variance of proportional data. However, I report means and 95% confidence intervals for back-transformed data.

I performed two analyses to test for differences between means from one week to another within treatments and to compare means among treatments. First, I examined the responses from each treatment using repeated measures analysis of variance. Second, a univariate analysis treated application rate as a whole plot factor and time as a split plot factor (Huynh and Feldt 1970). This analysis incorporated vole population densities as a covariate to adjust for density dependent effects among enclosures. Density was a significant covariate in all analyses; I present density-adjusted $\underline{F}$ values. For the univariate analysis, attention was paid to the values of the Greenhouse-Geisser epsilon and Huynh-Feldt epsilon. Values of these parameters that are not close to 1 indicate that the univariate analysis is inappropriate because the covariance of the responses do not satisfy the assumptions of the analysis. In all cases the epsilon values were close to 1. The general linear model procedure (SAS Inst. Inc. 1989:891) in the Statistical Analysis System (SAS Inst. Inc. 1987) program and an alpha level of 0.05 were used for each comparison. The above methods allowed me to test the hypothesis that the fitness correlates did not differ pre- and postspray and among treatments.

RESULTS

I captured 1,864 voles a total of 9,956 times in 48,000 trap nights between 10 June and 20 August 1992. The average population density was 60.75 voles/enclosure (SE = 2.45), and ranged from 14 to 137 voles/enclosure during the study (Fig. 1).

Reproductive Performance

The proportion of captures that were recruits and the number of recruits per adult female in each enclosure declined over time; the proportion of adult females in reproductive condition did not. The proportion of adult females in reproductive condition ranged from 0.22 to 0.52 during the study, was highest between 11 and 26 July 1992, but did not differ among treatments ($F = 0.58$; 20,74 df; $P = 0.91$; Fig. 2). The proportion of captures that were recruits in each enclosure declined from 0.75 to 0.09 between 10 June and 20 August 1992 ($F = 45.12$; 5,74 df; $P < 0.001$), but did not differ among treatments ($F = 0.97$; 20,74 df; $P = 0.51$; Fig. 3). The mean number of recruits per adult female in each enclosure ranged from 0.30 to 2.30, and declined during the study ($F = 14.00$; 4,14 df; $P < 0.001$) but did not differ among treatments ($F = 0.87$; 20,74 df; $P = 0.70$; Fig. 4).

Activity

Activity patterns varied depending on the parameter tested and sex. Males moved 8.2 to 14.5 m between captures within a trap period, and no difference was detected in MMDM over time (F = 1.20; 5,74 df; P = 0.32) or treatment (F = 0.82; 4,14 df; P = 0.53; Fig. 5). Females moved 5.5 to 11.3 m between captures within a trap period; MMDM decreased during the study (F = 4.70; 5,74 df; P = 0.001) but this decrease was not associated with treatment (F = 0.60; 20,74 df; P = 0.90; Fig. 6). The mean probability of capture for males ranged from 0.35 to 0.72, and did not change over time (F = 1.83; 5,74 df; P = 0.12), nor did it differ among treatments (F = 0.65; 4,14 df; P = 0.64; Fig. 7). The mean probability of capture for females did not differ among treatments (F = 0.21; 4,14 df; P = 0.13), and did not differ over time (F = 1.72; 5,74 df; P = 0.14; Fig. 8).

Male Weight Change

The rate of male weight gain declined from 7.27 g/two weeks to 1.15 g/two weeks as the experiment progressed (F = 54.62; 4,61 df; $P \leq 0.001$), but did not differ among treatments (F = 1.23; 16,61 df; P = 0.27; Fig. 9).

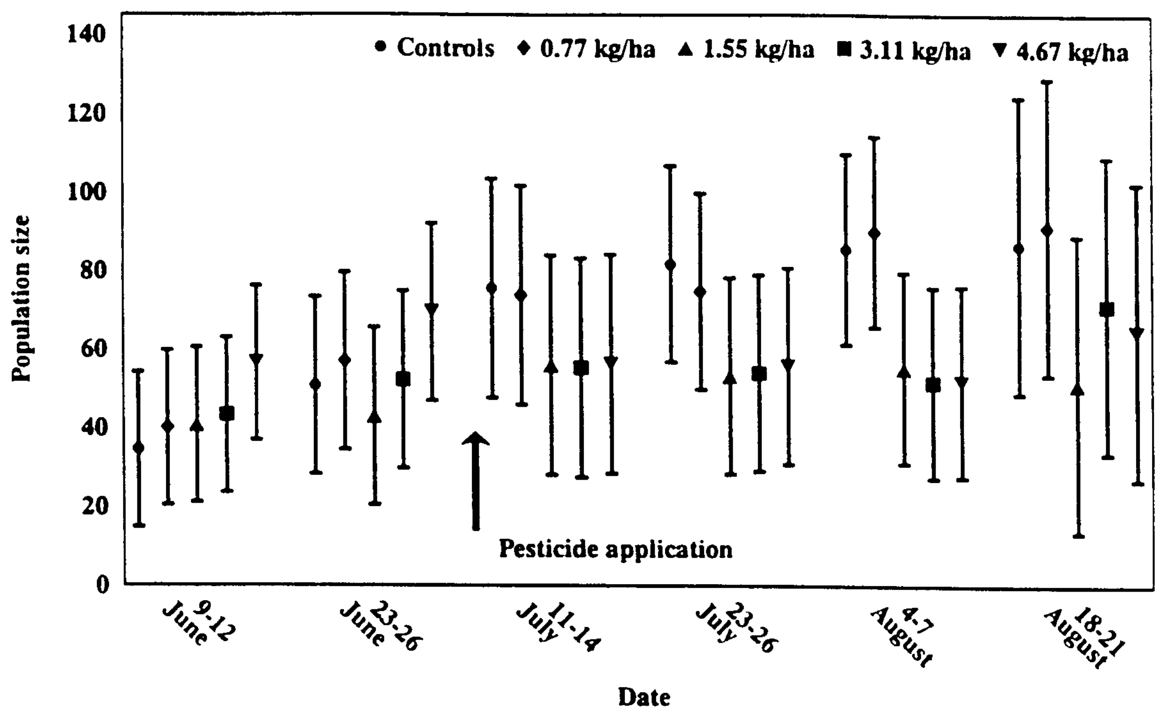

Figure 1. Mean population size and 95% confidence intervals for gray-tailed voles by date and application rate in field enclosures exposed to Guthion[a] at the Hyslop Crop Science Field Laboratory, Benton County, Oregon, 1992.

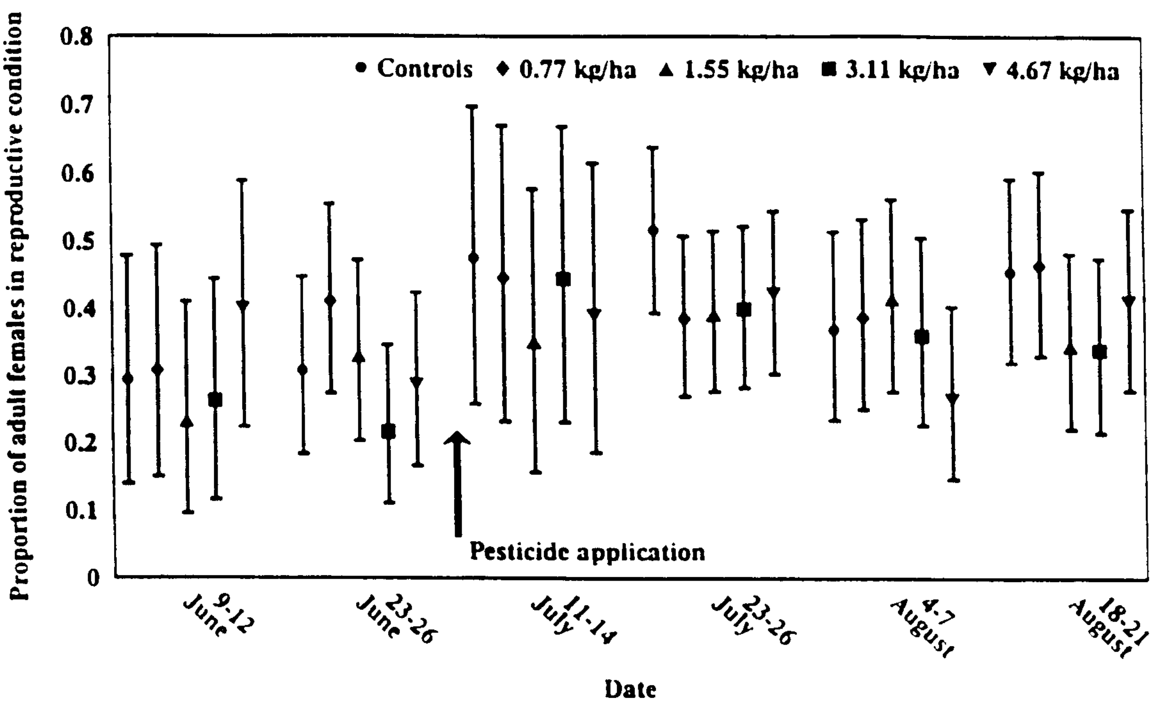

Figure 2. Mean and 95% confidence intervals for the proportion of adult female gray-tailed voles that were in reproductive condition by date and application rate in field enclosures exposed to Guthion® at the Hyslop Crop Science Field Laboratory, Benton County, Oregon, 1992.

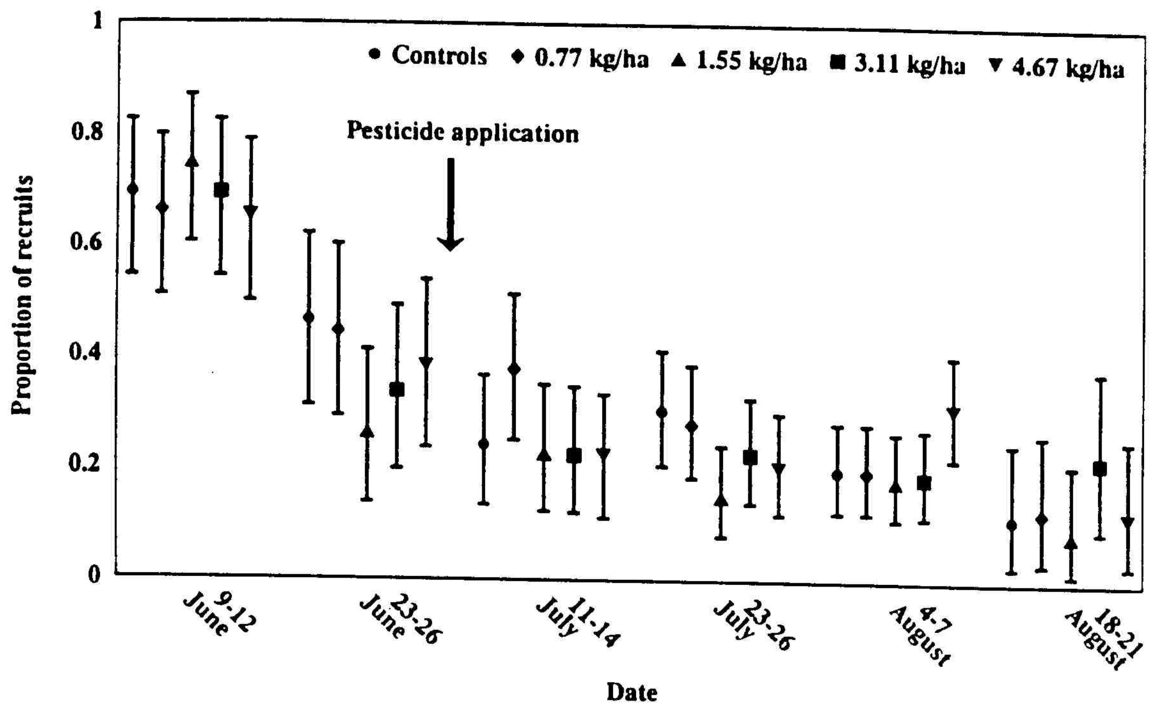

Figure 3. Mean and 95% confidence intervals for the proportion of gray-tailed vole recruits by date and application rate in field enclosures exposed to Guthion® at the Hyslop Crop Science Field Laboratory, Benton County, Oregon, 1992.

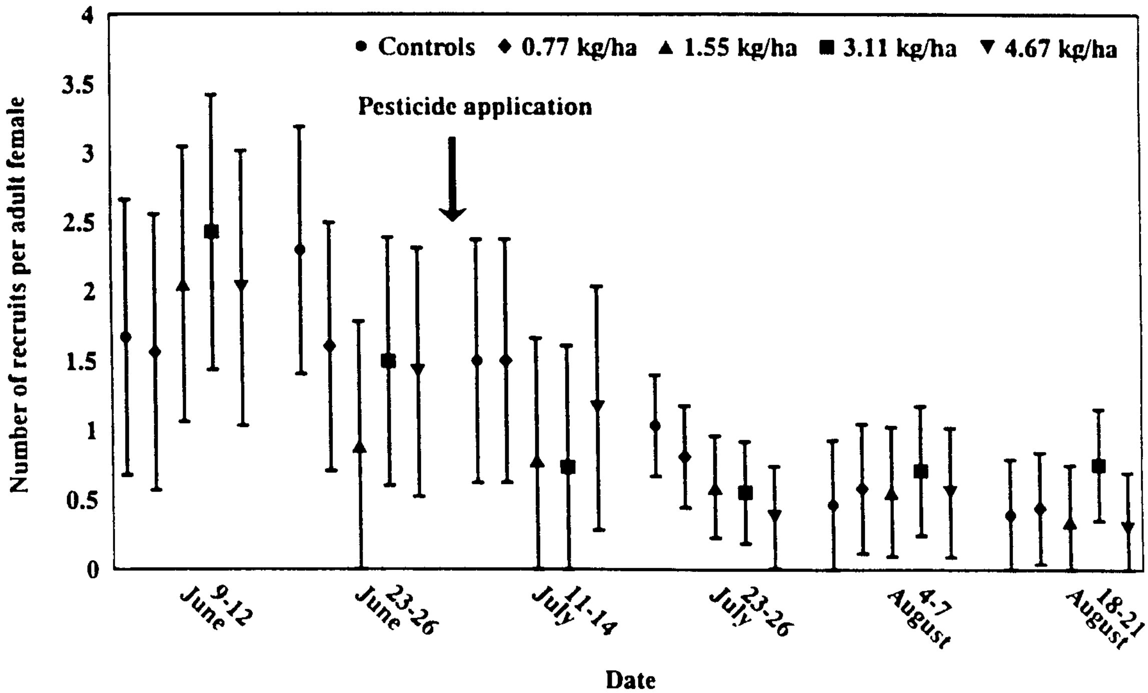

Figure 4. Mean and 95% confidence intervals for the number of gray-tailed vole recruits per adult female by date and application rate in field enclosures exposed to Guthion® at the Hyslop Crop Science Field Laboratory, Benton County, Oregon, 1992.

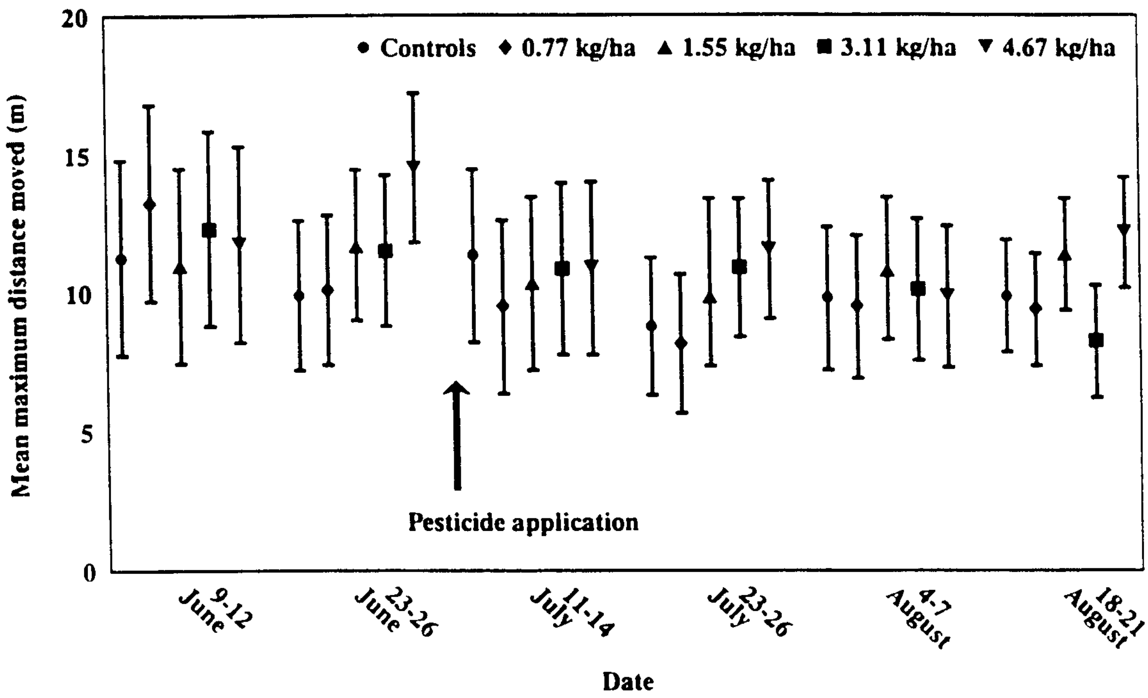

Figure 5. Mean maximum distance moved and 95% confidence intervals for male gray-tailed voles by date and application rate in field enclosures exposed to Guthion® at the Hyslop Crop Science Field Laboratory, Benton County, Oregon, 1992.

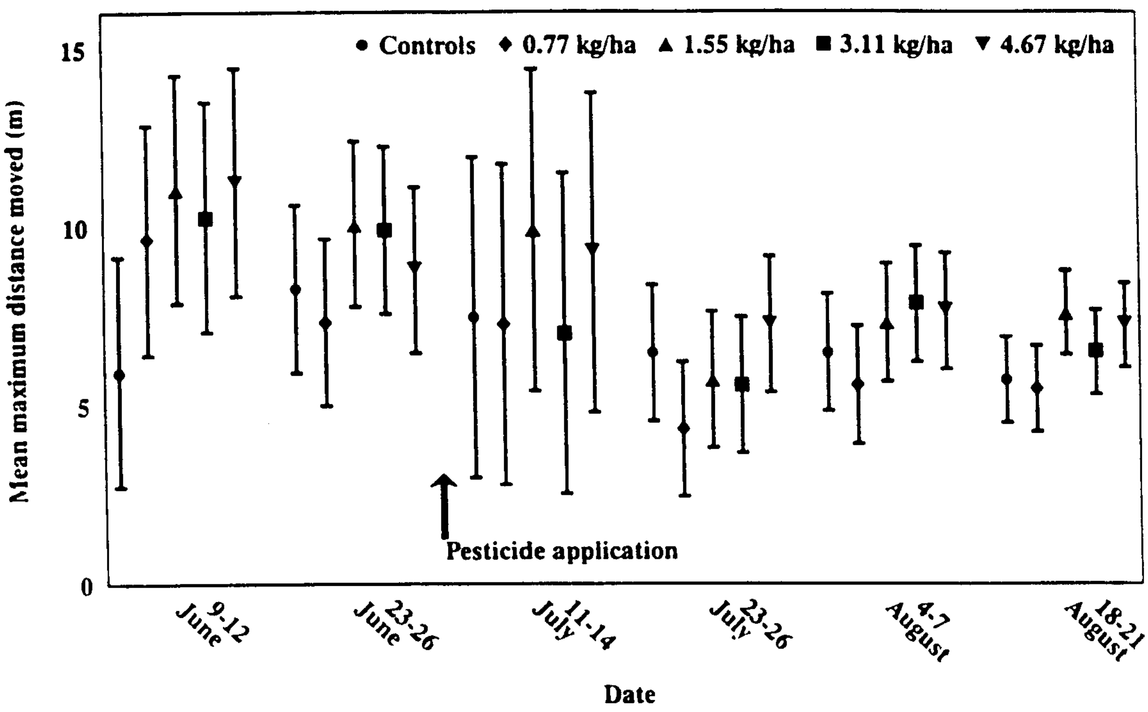

Figure 6. Mean maximum distance moved and 95% confidence intervals for female gray-tailed voles by date and application rate in field enclosures exposed to Guthion® at the Hyslop Crop Science Field Laboratory, Benton County, Oregon, 1992.

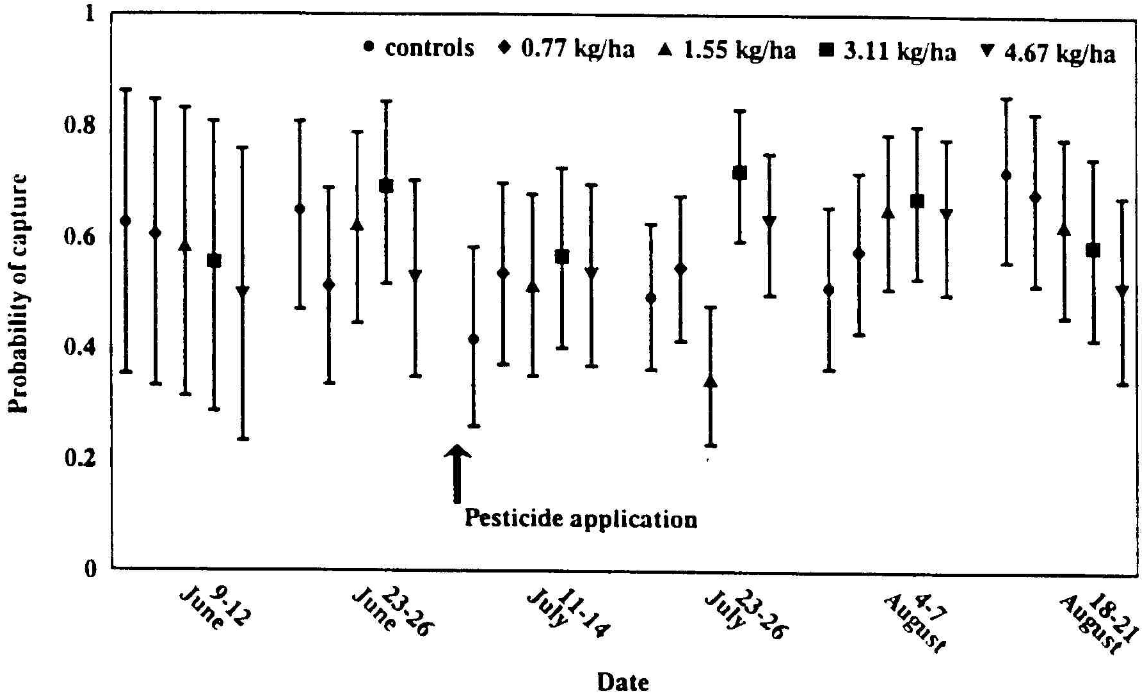

Figure 7. Mean and 95% confidence intervals for the probability of capture for male gray-tailed voles by date and application rate in field enclosures exposed to Guthion® at the Hyslop Crop Science Field Laboratory, Benton County, Oregon, 1992.

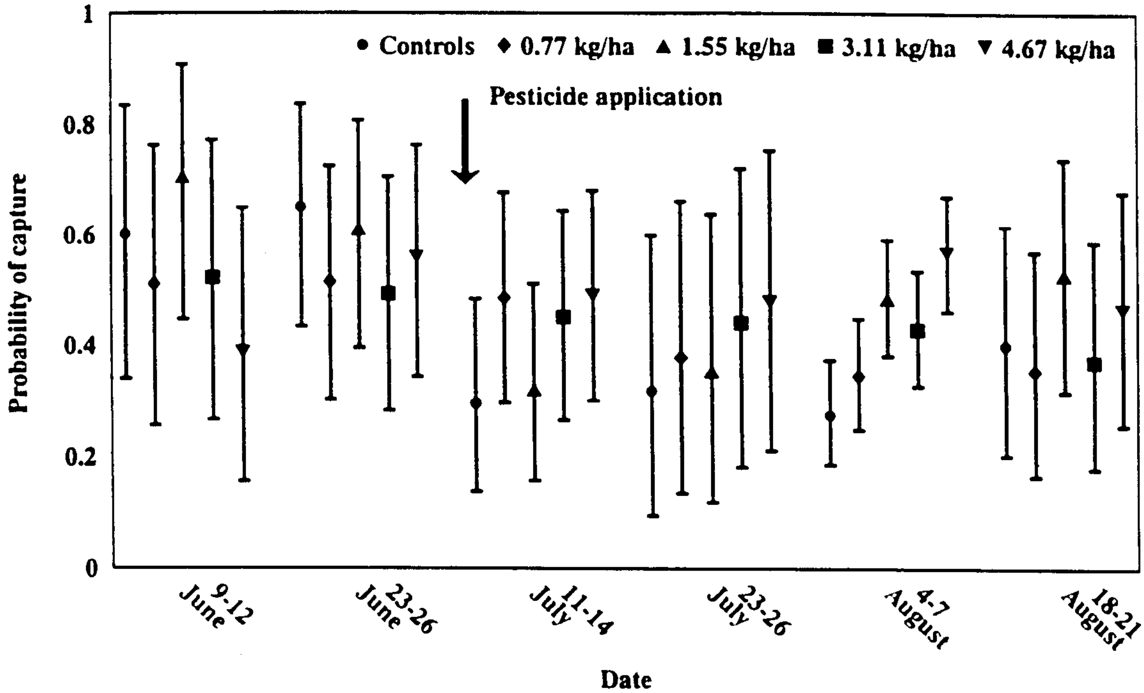

Figure 8. Mean and 95% confidence intervals for the probability of capture for female gray-tailed voles by date and application rate in field enclosures exposed to Guthion® at the Hyslop Crop Science Field Laboratory, Benton County, Oregon, 1992.

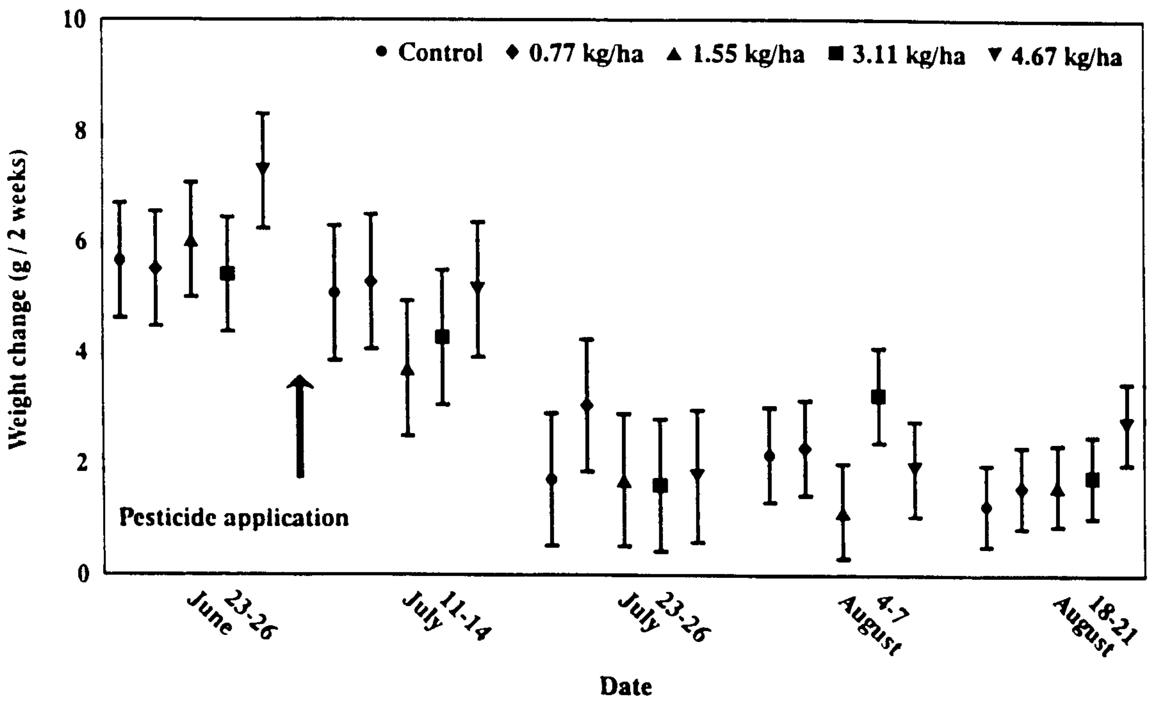

Figure 9. Mean weight change (g per two weeks) and 95% confidence intervals for male gray-tailed voles by date and application rate in field enclosures exposed to Guthion® at the Hyslop Crop Science Field Laboratory, Benton County, Oregon, 1992.

DISCUSSION

I used a replicated field experiment to determine if fitness correlates for gray-tailed voles varied in a dose-dependent manner in response to the application of Guthion®. I was unable to detect measurable changes in reproductive parameters, activity patterns, and male weight change of gray-tailed voles because of exposure to Guthion®.

Reproduction and Recruitment

The endpoints of reproduction that I measured did not respond as I hypothesized. Reproduction and recruitment in small mammals have been measured in response to pesticide applications in other studies. Researchers, both in the laboratory and in the field, have examined effects on small mammal reproduction caused by pesticides. However, only a few have replicated treatments. In a laboratory study testing the effects of Guthion® on rats and mice, doses of 0, 1.25, 2.5, and 5.0 mg/kg did not adversely affect reproduction, and caused reduced maternal care and weight gain of adult females only in rats receiving 5.0 mg/kg (Short et al. 1980). An unreplicated aerial application of malathion to a forested area reduced white-footed mouse (P. leucopus) populations by 20-45%, and was suggested to have been the result of reduced productivity and survival (Giles 1970). In another unreplicated field experiment, Pomeroy and Barrett (1975) determined that the application of 2.25 kg/ha of the insecticide Sevin® caused a delay in the reproduction of cotton rats (Sigmodon hispidus), based on the number of juveniles entering an enclosed population. However, in the same experiment, feral house mouse (Mus musculus) populations increased following the pesticide application. My results suggest that pesticide applications may not directly effect reproduction rates of small mammals in the field.

Activity

Pesticide exposure may alter behavior patterns of animals, but results vary depending on the species, chemical, and behavioral response examined. Pine voles (M. pinetorum) observed in arenas following exposure to 235, 275, and 310 ppm of Guthion® spent less time grooming (at the low dose only) than controls (Durda et al. 1989). In a separate experiment, pine voles had reduced levels of aggression at an exposure of 195 ppm compared to controls (Durda et al. 1989). Common grackles (Quiscalus quiscula) had reduced activity as one of the first signs of intoxication when they were exposed to four different OP pesticides at different concentrations (Grue 1982). However, not all studies investigating the response of small mammals to insecticide stress have found differences in activity following chemical exposure. Trapping efficiency for prairie voles (M. ochrogaster) differed between old-field and agricultural communities, but not in response to the application 2.27 kg/ha of the insecticide Sevin® (Barrett 1988). I found no detectable differences in MMDM or probability of capture for either males or females in response to the pesticide application. A decline in MMDM occurred for females as my experiment progressed but this decline was probably in response to increasing density and competition for space among females. The same decline was not seen for males, possibly because males compete for mates, rather than space to raise young (Ostfeld 1985, Wolff 1993). Again, my results did not support my hypothesis that vole activity would be affected with increasing application concentrations of Guthion®.

Male Weight Change

OP pesticides may cause reduced rates of weight gain because of chemically induced anorexia, food aversion, and direct intoxication (Grue 1982, Bennett 1989). In the laboratory, Guthion® caused a decrease in weight gain in pine voles (Durda et al. 1989). However, results from field studies have been contradictory. Cotton rats exposed to 2.25 kg/ha of Sevin® lost weight during the winter and did not gain weight as rapidly the following spring when compared to an unexposed population, but the difference may have been due to competitive or habitat differences between the treated and untreated plots (Pomeroy and Barrett 1975). Sullivan (1990) detected no difference in growth rates for Oregon voles (M. oregoni) exposed to 3.0 kg/ha of the herbicide Roundup® (glyphosate) applied aerially to a forest ecosystem. Although mean weight change decreased through time in my experiment, there were no differences among treatments. It is possible that any effect on male weight change was not of sufficient duration to be detected by the two-week interval between trap periods in this experiment. I recommend weighing individuals repeatedly within a trap period to measure weight change of shorter duration in response to a pesticide.

I was unable to reject my null hypotheses of no treatment effects on individual fitness correlates of gray-tailed voles in response to increasing application concentrations of Guthion®. However, the statistical power of these tests was low for each of the parameters tested (<30-60%) because of the large variation in the data. Despite this low statistical power, I have confidence in my conclusion of no treatment effect because of three separate lines of evidence provided by other researchers involved with this project: analysis of chemical residues on vegetation and soil within each enclosure, plasma and brain acetylcholinesterase (AChE) levels for gray-tailed voles removed from enclosures that received the 1.55 kg/ha and 4.67 kg/ha concentrations, and food discrimination trials to determine this species' ability to detect and avoid the chemical at sublethal concentrations.

Analyses of Guthion® residues on alfalfa leaves within the top 15 cm and bottom 15 cm of plants and soil samples from each enclosure, (R. Bennett et al., EPA, Corvallis, Oreg., unpubl. data) indicate that the alfalfa canopy intercepted most of the chemical. Residue levels at or near the soil surface even in the 4.67 kg/ha enclosures were less than the LC_{50} value of 297 ppm for gray-tailed voles in the laboratory toxicity tests (M. Meyers, METI, Corvallis, Oreg., and J. Wolff, OSU, Dept. of Fish. and Wildl., Corvallis Oreg., unpubl. data). Chemical residues in the soil samples at the 4.67 kg/ha rate were approximately the same as the residues present at the vegetative canopy in the 0.77 kg/ha enclosures. Thus, Guthion® concentrations in the habitat strata used by voles were below levels predicted to cause adverse affects.

The mode of action for OPs is through the inhibition of AChE, an enzyme responsible for the breakdown of the neurotransmitter acetylcholine; AChE levels in the brain and plasma have been used as indicators of exposure to OP pesticides (Fleming and Grue 1981). AChE inhibition was found to be dose-dependent for pine voles, and inhibition was greatest between 1 and 5 days following field exposure to 2.2 kg/ha of Guthion® (Durda et al. 1989). In a separate but concurrent experiment conducted by researchers participating in my study, four enclosures were used to supply animals for biomarkers of exposure, including brain and plasma AChE activity. AChE activity levels from these tests indicated that exposure to animals in the field was less than that which caused adverse effects in the laboratory. Voles removed from the experimental enclosures had brain AChE levels that were reduced by 18% in the 1.55 kg/ha application rate and 20% in the 4.67 kg/ha application rate (S. Dominguez et al., EPA, Corvallis, Oreg., unpubl. data). Brain AChE activity was reduced by 45-50% for animals that died during laboratory toxicity studies and by approximately 30% for animals that survived laboratory tests (M. Meyers, METI, Corvallis, Oreg., and J. Wolff, OSU, Dept. of Fish. and Wildl., Corvallis, Oreg., unpubl. data). Thus, animals in the field had exposure levels that, even at the highest application rate, were insufficient to cause AChE inhibition of more than 18-20%. A reduction in brain AChE activity of 20% usually indicates exposure in rodents and a reduction of 50% suggests that exposure was the proximate cause of mortality (Johnson and Wallace 1987). Furthermore, behavioral changes in rodents may not occur until AChE inhibition reaches 50% (Peakall 1992:33). Other studies have demonstrated that inhibition of AChE is a short-term effect and recovery may be rapid (Zinkl et al. 1980, Fleming and Grue 1981, Jett et al. 1986). Recovery is possible in some cases via spontaneous reactivation of inhibited cholinesterase (ChE) and synthesis of new ChE (O'Brien [1967] in Grue 1982). Therefore, even if voles were exposed and suffered some adverse effects, it is possible that intoxicated animals recovered before they were captured. Furthermore, AChE inhibition of 18-20% may not have resulted in measurable changes in reproduction and activity in voles.

The lack of a pesticide-related response in my experiment may also have been a function of the species' ability to detect Guthion® on its food. In laboratory tests, gray-tailed voles can detect and avoid Guthion® in their forage at concentrations of about 100 ppm (T. Manning, OSU, Dept. of Fish. and Wildl., Corvallis, Oreg., unpubl. data), which approximates the 1.55 kg/ha application rate used in my experiment. Because voles are able to detect and avoid the chemical at 100 ppm, exposure to amounts that would cause physiological impairment may be minimized through avoidance. Avoidance of contaminated food has been documented for both birds (Grue 1982, Bennett 1989) and mammals (Linder and Richmond 1990).

The EPA's risk assessment paradigm predicted a hazard to gray-tailed voles at an application concentration of Guthion® of approximately 1.2 kg/ha, and thus overestimated the risk to gray-tailed voles. The amount of chemical that reached the substrate inhabited by voles was less than the EEC value used in calculating the risk, or "Q" by current EPA risk assessment methods. In addition, voles are able to detect contaminated food. Consequently, the amount of Guthion® the voles were exposed to probably was insufficient to cause measurable changes in reproduction, activity, or male weight change. Exposure to sublethal concentrations was also indicated by the 18-20% AChE inhibition levels in the voles removed for bioassay (S. Dominguez et al., EPA, Corvallis, Oreg., unpubl. data).

The temporal changes in the parameters that I measured may have been the result of seasonal patterns in reproduction and activity, or a response to the increasing density in many of the enclosures. Changes in population parameters can be the result of environmental factors, physiological and behavioral responses, and individual reproductive performance at various densities (reviewed by Batzli 1992). In areas that provide adequate habitat, small mammal populations can reach high densities (reviewed by Batzli 1992). When high densities are reached, increased predation, intraspecific aggression (Abramsky and Tracy 1980), and reduced food supplies may lead to poor survival even though reproduction continues (Batzli 1992:836).

I found significant changes over time in several of the parameters that I measured. The proportion of captures that were recruits in each enclosure, the number of recruits per adult female, the MMDM for females, and weight change for males all decreased as the study progressed. The probability of capture for both sexes, and MMDM for males did not show seasonal effects. These seasonal changes were possibly because of density-dependent factors. Specifically, as the density within the populations increased, competition for available space and resources may have increased, thereby causing female home range size to decrease and females to move less distance between captures. This finding is consistent with evidence from other Microtus species (Abramsky and Tracy 1980, Boonstra 1984). Female home range size decreased but that of males did not, possibly because females competed for space to rear their young whereas males may have used large home ranges to increase access to females (Ostfeld 1990, Wolff 1993). Infanticide was not uncommon in small mammals, and adult female density was negatively and inversely correlated with juvenile survival (Boonstra 1984, Madison and McShea 1987, Rodd and Boonstra 1988, Heske and Bondrup-Nielsen 1990). It is possible that as competition for food and space became more intense, adult females were killing litters of neighboring females in an attempt to displace them (Wolff and Cicirello 1989, Wolff 1993). Therefore, the decreases in the recruitment, number of recruits per adult female, and female activity are possibly because of density-dependent changes in the population dynamics of this species.

SUMMARY

My experiment examined the effects of Guthion® on individual fitness correlates of gray-tailed voles in field enclosures and compared the results to those expected from the EPA's risk assessment paradigm and QM. I was unable to detect differences caused by the pesticide in the parameters that I examined. The risk assessment methods used by the EPA overestimated the hazard to gray-tailed voles in this study, and thus provided a conservative estimate of risk. I suggest that the life history and biology of the species in question, along with the specific habitat structure, should be addressed when developing predictive tools for pesticide risk assessment. My study provided some information about the difference in the way that small mammals respond to pesticides, and the predictions made by current EPA risk assessment techniques for calculating the hazard to small mammals. Although many unanswered questions remain regarding discrepancies between laboratory predictions and responses actually observed in the field, this experiment will enable other researchers to design and conduct future experiments to address the issue of wildlife response to pesticides.

LITERATURE CITED

Abramsky, Z., and C. R. Tracy. 1980. Relation between home range size and regulation of population size in Microtus ochrogaster. Oikos 34:347-355.

Barrett, G. W. 1988. Effects of Sevin® on small-mammal populations in agricultural and old-field ecosystems. J. Mammal. 69:731-739.

Batzli, G. O. 1992. Dynamics of small mammal populations: a review. Pages 831-850 in D. R. McCullough and R. H. Barrett, eds. Wildlife 2001: populations. Elsevier Applied Science, New York, N.Y.

Bennett, R. S. 1989. Factors influencing discrimination between insecticide-treated and untreated foods by Northern Bobwhite. Arch. Environ. Toxicol. 18:697-705.

Blus, L. J., C. S. Staley, C. J. Henny, G. W. Pendleton, T. H. Craig, E. H. Craig, and D. K. Halford. 1989. Effects of organophosphate insecticides on sage grouse in southeastern Idaho. J. Wildl. Manage. 53:1139-1146.

Boonstra, R. 1984. Aggressive behavior of adult meadow voles (Microtus pennsylvanicus) towards young. Oecologia 62:126-131.

Chao, A. 1988. Estimating animal abundance with capture frequency data. J. Wildl. Manage. 52:295-300.

Cholakis, J. M., M. J. McKee, L. C. K. Wong, and J. D. Gile. 1981. Acute and subacute toxicity of pesticides in microtine rodents. Pages 143-154 in D. W. Lamb and E. E. Kenaga, eds. Avian and mammalian wildlife toxicology: Second conference. Am. Soc for Testing Materials. Philadelphia, Pa. 168pp.

Durda, J. L., R. A. Powell, and G. T. Barthalmus. 1989. Physiological and behavioral effects of Guthion on pine voles, Microtus pinetorum. Bull. Environ. Contam. Toxicol. 43:80-86.

Fleming, W. J., and C. E. Grue. 1981. Recovery of cholinesterase activity in five avian species exposed to dicrotphos, an organophosphorus pesticide. Pestic. Biochem. Physiol. 16:129-135.

Giles, R. H., Jr. 1970. The ecology of a small forested watershed treated with the insecticide malathion-S^{35}. Wildl. Monogr. 24. 81pp.

Grue, C. E. 1982. Response of common grackles to dietary concentrations of four organophosphate pesticides. Arch. Environ. Contam. Toxicol. 11:617-626.

_____, W. J. Fleming, D. J. Busby, and E. F. Hill. 1983. Assessing the hazards of organophosphate pesticides to wildlife. N. Am. Wildl. and Nat. Resour. Conf. 48:200-219.

Heske, E. J., and S. Bondrup-Nielsen. 1990. Why spacing behavior does not stabilize density in cyclic populations of microtine rodents. Oecologica 83:91-98

Hoerger, F. D., and E. E. Kenaga. 1972. Pesticide residues on plants: correlation of representative data as a basis for estimation of their magnitude in the environment. Pages 9-28 in F. Coulston and F. Korte, eds. Environmental Quality and Safety. Chemistry, toxicology, and technology. Vol. 1. Academic Press, New York, N.Y. 267pp.

Huynh, H., and L. S. Feldt. 1970. Conditions under which mean square ratios in repeated measurements designs have exact F-distributions. J. Am. Stat. Assoc. 65:1582-1589.

Jett, D. A., J. D. Nichols, and J. E. Hines. 1986. Effect of orthene on an unconfined population of the meadow vole (Microtus pennsylvanicus). Can. J. Zool. 64:243-250.

Johnson, J. A., and K. B. Wallace. 1987. Species related differences in the inhibition of brain acetylcholinesterase by paraoxon and malaoxon. Toxicol. and Appl. Pharmacol. 88:234-241.

Linder, G., and M. E. Richmond. 1990. Feed aversion in small mammals as a potential source of hazard reduction for environmental chemicals: agrichemical case studies. Environ. Toxicol. Chem. 9:95-105.

Madison, D. M., and W. J. McShea. 1987. Seasonal changes in reproductive tolerance, spacing and social organization in meadow voles: a microtine model. Am. Zool. 27:899-908.

Morris, R. D. 1972. The effects of endrin on Microtus and Peromyscus II. Can. J. Zool. 50:885-896.

Morris, R. D. 1968. Effects of endrin feeding on survival and reproduction in the deer mouse, Peromyscus maniculatus. Can. J. Zool. 46:951-958.

O'Brien, R. D. 1967. Insecticides: action and metabolism. Acad. Press, New York, N.Y. 332pp.

Ostfeld, R. S. 1990. The ecology of territoriality in small mammals. Trends in Ecol. and Evol. 5:411-415.

______. 1985. Experimental analysis of aggression and spacing behavior in California voles. Can. J. Zool. 63:2277-2282.

Peakall, D. B. 1992. Animal biomarkers as pollution indicators. Chapman and Hall. London, UK. 291pp.

Peterle, T. J. 1991. Wildlife toxicology. Van Nostrand Reinhold. New York, N.Y. 322pp.

Pomeroy, S. E., and G. W. Barrett. 1975. Dynamics of enclosed small mammal populations in relation to an experimental pesticide application. Am. Midl. Nat. 93:91-105.

Roberts, D. K. 1988. Brain acetylcholinesterase recovery following acute methyl parathion intoxication in two feral rodent species: a comparison to laboratory rodents. Bull. Environ. Contam. Toxicol. 41:26-35.

Rodd, F. H., and R. Boonstra. 1988. Effects of adult meadow voles (Microtus pennsylvanicus) on young conspecifics in field populations. J. Anim. Ecol. 57:755-770.

SAS Institute Inc. 1987. SAS/GRAPH guide for personal computers, version 6 ed. SAS Inst. Inc. Cary, N.C. 534pp.

SAS Institute Inc. 1989. SAS/STAT User's guide, version 6, fourth ed., volume 2, SAS Inst. Inc. Cary, N.C. 1,686pp.

Schultz, K. R., E. P. Lichtenstein, T. T. Liang, and T. W. Fuhremann. 1970. Persistence and degradation of azinphosmethyl in soils, as affected by formulation and mode of application. J. Econ. Entomol. 62:432-438.

Short, R. D., J. L. Minor, C. Lee, N. Chernoff, and R. L. Baron. 1980. Developmental toxicity of Guthion in rats and mice. Arch. Toxicol. 43:177-186.

Sullivan, T. P. 1990. Influence of forest herbicide on deer mouse and Oregon vole population dynamics. J. Wildl. Manage. 54:566-576.

Tucker, R. K., and D. G. Crabtree. 1970. Handbook of toxicity of pesticides to wildlife. U.S. Fish and Wildl. Serv. Res. Publ. 84. 131pp.

United States Environmental Protection Agency Office of Pesticides Program. 1986. Hazard evaluation division standard evaluation procedure. U.S. Gov. Printing Office. Washington, D.C. 96pp.

White, G. C., D. R. Anderson, K. P. Burnham, D. L. Otis. 1982. Capture-recature and removal methods for sampling closed populations. Los Alamos Nat. Lab., Los Alamos, N.M. 235pp.

______, K. P. Burnham, D. L. Otis, and D. R. Anderson. 1978. User's manual for program CAPTURE. Utah State Univ. Press. Logan. 40pp.

Wilson, K. R., and D. R. Anderson. 1985. Evaluation of two density estimators of small mammal population size. J. Mammal. 66:13-21.

Wolff, J. O. 1993. Why are female small mammals territorial? Oikos (In Press).

______, and D. M. Cicirello. 1989. Field evidence for sexual selection and resource competition infanticide in white-footed mice. Anim. Behav. 36:456-465.

Zinkl, J. G., R. B. Roberts, C. J. Henny, and D. L. Lenhart. 1980. Inhibition of brain cholinesterase activity in forest birds and squirrels exposed to aerially applied acephate. Bull. Environ. Contam. Toxicol. 24:667-683.