EFFECTS OF FIELD EXPOSURE TO DIAZINON ON SMALL MAMMALS INHABITING A SEMIENCLOSED PRAIRIE GRASSLAND ECOSYSTEM. I. ECOLOGICAL AND REPRODUCTIVE EFFECTS

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Abstract—The widespread use of cholinesterase-inhibiting pesticides in the environment presents increasing concerns about their effects on human, wildlife, and ecosystem health. As a group, these pesticides are generally highly toxic and have great potential for negatively affecting nontarget organisms. Small mammals have proven to be ideal biomonitor of environmental contaminants, and were used here to test for possible effects of a widely used cholinesterase-inhibiting insecticide, diazinon, in a natural field setting. Using 12 0.1-ha terrestrial mesocosms, we examined the effects of low-level diazinon exposure on the small mammal communities inhabiting semienclosed grassland ecosystems. Our primary objective was to test the hypothesis that diazinon, applied at two different recommended label application rates, would not cause any observable adverse ecological or reproductive effects on small mammal populations and communities. Experimental small mammal communities consisting of Sigmodon hispidus, Microtus ochrogaster, Reithrodontomys fulvescens, and Mus musculus were stocked at natural densities and sex ratios inside empty mesocosms. Diazinon 4E was applied at two different maximum recommended label application rates, 0.56 kg a.i./ha (1×) and 4.5 kg a.i./ha (8×), and controls remained unsprayed, with four enclosures (replicates) per treatment. Two 30-d trials were run during peak rodent breeding seasons and enclosures were sampled on days 2, 16, and 30 of each trial. Recovery of small mammals was not significantly different among treatments, although fewer animals were recovered from the diazinon-exposed enclosures in both trials. Analysis of trapping data suggested that the normally strong competitive relationship between Sigmodon and Microtus may be altered by the pesticide, favoring Microtus in the diazinon-exposed enclosures. Incidence of reproductive condition was found to be reduced 20 to 80% and 33 to 100% in diazinon-exposed males and females, respectively. Reproductive productivity, including percentage of pregnant females and of females giving birth, was significantly reduced in diazinon-exposed animals. Percentage of pregnant females ranged from 13.6 to 43.5% in diazinon-exposed males and females, respectively. Reproductive productivity, including percentage of pregnant females and of females giving birth, was significantly reduced in diazinon-exposed animals. Percentage of pregnant females ranged from 13.6 to 43.5% in diazinon-exposed animals compared to 40 to 80% for control animals, and percentage of females giving birth ranged from 0 to 17% in diazinon-exposed animals compared to 22 to 50% for control animals. Generally, the effects found in this study suggest that diazinon was relatively persistent in the sprayed enclosures and that oral routes of exposure (consumption of dead and dying arthropods, grooming) may have been important. Ecological relationships and reproduction in both herbivorous and omnivorous mammals were negatively impacted by diazinon exposure. Overall, ecological relationships in the enclosed prairie grassland ecosystem were disrupted by diazinon, probably through a combination of sublethal effects, particularly reproductive effects, impacting individuals and their populations. This suggests that negative impacts on populations and community structure and function may persist longer than diazinon persists in the environment.

Keywords—Diazinon Small mammals Mesocosm Sublethal effects Terrestrial ecotoxicology

INTRODUCTION
Cholinesterase-inhibiting (anti-ChE) pesticides, including organophosphate (OP) and carbamate insecticides, have been the most widely used class of insecticide in North America for over 30 years, which has resulted in much concern over their possible effects on humans and wildlife species and their ecosystems [1–3]. Currently, more than 100 different anti-ChE chemicals are registered as the active ingredient in thousands of different pesticide products in the United States. Total pesticide usage in the United States was estimated at about 2.2 billion pounds of active ingredient in 1993 [4,5]. A relatively small percentage (as little as 1%) of this amount actually reaches target organisms, thereby resulting in movement to surrounding areas and exposure of nontarget organisms to these toxic pesticides [6]. Although OP and carbamate pesticides are relatively less persistent in the environment than organochlorine pesticides and tend not to bioaccumulate in food chains, they generally are more acutely toxic and lack target specificity, tending to exert a potentially more widespread effect on nontarget organisms [1–3,6].

In addition to direct mortality of wildlife, exposure to OP pesticides has been reported to cause various sublethal physiological, biochemical, immunological, behavioral, and endocrine alterations that are critical for survival and reproduction in a number of wildlife species [2]. Both direct mortality and sublethal effects from anti-ChE pesticides have the potential to impact the abundance and distribution of wildlife species. The extent to which these effects may impact recruitment and subsequent population or community dynamics is not known [2,7,8].

Mammals have largely been ignored in favor of birds in studies relating to the regulation of pesticides. Several studies have shown significant impacts of anti-ChE insecticide applications on small mammal populations [9]. The anti-ChE pesticides carbaryl [10–12], dimethoate [13], malathion [14], and azinphos-methyl [15,16] have been documented as causing mild to severe reductions in population size, inhibited repro-
Diazinon in mesocosms: Effects on small mammals

Ronald E. Zabinski and William D. Huhta

Diazinon® \((O,O\text{-diethyl}O-(2\text{-isopropyl-6-methyl-4-pyrimidinyl})\) phosphorothioate; Monsanto, St. Louis, MO, USA) is a broad-spectrum OP insecticide that is widely used in agricultural, range, commercial, and home and garden settings for the control of a wide variety of insect, acarine, and nematode pests [7]. Diazinon has been registered for use since 1952, and since at least 1985, an average of >10 million pounds of active ingredient has been applied annually in the United States, making it among the most widely used insecticides [4,5]. Diazinon was chosen for the study because of its widespread usage, which has resulted in an alarming increase in environmental diazinon concentrations in surface waters, groundwater, treated sewage effluents, and precipitation [17], and because of its high toxicity to a wide variety of organisms, potential for persistence in the environment, and the relative lack of data regarding the effects of diazinon on wildlife and humans. Presently, no data exist regarding the effects of diazinon on natural populations or communities of mammalian species under controlled field conditions, and a recent review of diazinon toxicity to wildlife species designated this as a top priority for research [7].

Relatively few field experimental studies have examined short- or long-term effects of pesticides or other contaminants on natural ecosystems [6]. Controlled field and mesocosm studies are necessary to more precisely assess the impact of pesticide exposures on mammalian populations and communities [18]. Mesocosms have been shown to be useful in examining potential effects of pesticides or other environmental contaminants on experimental organisms [19–21]. The primary advantages of an experimental mesocosm system are that it provides realism that is not possible in the lab, allowing control of certain crucial parameters (e.g., movements and predation), replication, and simultaneous investigation of populations, communities, and ecosystems. Further, mesocosms allow the accurate analysis of reproductive effects, something that cannot be done in open study areas. Little mesocosm work has been done in terrestrial systems. Therefore, the design approach of this study was to conduct replicated field trials under the controlled conditions of a mesocosm to test the effects of a widely used anti-ChE insecticide on experimental small mammal communities.

The objectives of this study were to measure potential ecological and reproductive effects of subchronic field exposure to the OP insecticide diazinon in wild small mammals living in a prairie grassland ecosystem. Specifically, this study evaluated potential interspecific responses of small mammals to diazinon exposure at the population and community levels (density, recovery, recapture rates, trappability, reproductive activity, and reproductive productivity).

MATERIALS AND METHODS

Study site

The study was conducted in a tallgrass prairie ecosystem located about 6.5 km west of Stillwater, Payne County, Oklahoma, USA. Vegetation inside enclosures consisted mainly of grasses (little bluestem \([\text{Schizachyrium scoparium}]\) and Indian grass \([\text{Sorghastrum nutans}]\), forbs (coralberry \([\text{Symphoricarpos orbiculatus}]\), and woody shrubs (smooth sumac \([\text{Rhus glabra}]\) and sandplum \([\text{Prunus angustifolia}]\)). Small mammals that occur locally in this habitat include least shrews \((\text{Cryptotis parva})\), short-tailed shrews \((\text{Blarina hylphaga})\), hispid cotton rats \((\text{Sigmodon hispidus})\), prairie voles \((\text{Microtus ochrogaster})\), pine voles \((\text{Microtus pinetorum})\), fulvous harvest mice \((\text{Reithrodontomys fulvescens})\), plains harvest mice \((\text{Reithrodontomys montanus})\), deer mice \((\text{Peromyscus maniculatus})\), white-footed mice \((\text{Peromyscus leucopus})\), eastern woodrats \((\text{Neotoma floridana})\), and house mice \((\text{Mus musculus})\). The study area has no known history of pesticide application but was subjected to a controlled burn in the spring of 1990.

Enclosure design and construction

A series of 12 0.1-ha enclosures \((32 \times 32 \text{ m})\) was constructed of galvanized sheet metal \((1.27 \text{ m above ground, 0.36 m below ground})\). Mowed strips inside \((0.75 \text{ m})\) and outside \((1.5 \text{ m})\) all enclosure walls were maintained throughout the study in order to deter predators from climbing walls as well as discouraging experimental animals from spending time (i.e., digging or climbing) along the walls.

Field methodology

A split-plot design was employed for the group of 12 enclosures, which allowed for four replicates of three experimental treatments \((0 \times = \text{control}, 1 \times = 0.56 \text{ kg active ingredient [a.i./ha]}, \text{ and } 8 \times = 4.5 \text{ kg a.i./ha})\). Treatments were applied randomly to enclosures, and enclosures received the same treatments during each of the two trials in the study. Each 0.1-ha enclosure contained a trapping grid consisting of 20 trapping stations arranged in a five by four matrix with two Sherman live traps \((\text{H. B. Sherman Traps, Tallahassee, FL, USA})\) at each station \((40 \text{ traps/enclosure})\). Traps were baited with rolled oats, set near dusk, and checked early the following morning. All trap doors were kept shut during nontrapping days. In addition, six pieces of sheet metal, approximately \(1.0 \times 1.3 \text{ m}\) in size, were placed in each enclosure to provide cover for nesting sites. Before each trial, we conducted removal trapping in each enclosure for 14 d to ensure that enclosures were free of nonexperimental animals. Experimental animals consisted of wild-caught individuals of the desired species, sex, and age from surrounding grasslands. All small mammals were held in captivity for varying periods of time before each trial. Before release into enclosures, animals were toe-clipped for identification, and we recorded weight, reproductive condition, and general condition (e.g., parasites and overall health). Toe-clipping was conducted at least 7 d ahead of release to avoid creating another route of exposure for the pesticide. Small mammals were released into enclosures 3 to 5 h before spraying at densities equal to those of local natural populations. During trial 1 \((\text{July 31–September 1, 1993})\), each enclosure contained 12 \(\text{S. hispidus (120/ha)}\), 5 \(\text{M. musculus (50/ha)}\), and 3 \(\text{R. fulvescens (30/ha)}\) at the start \((\text{day 0})\). During trial 2 \((\text{June 9–July 10, 1994})\), each enclosure contained 12 \(\text{S. hispidus (120/ha)}\), 12 \(\text{M. ochrogaster (120/ha)}\), and 5 \(\text{R. fulvescens (50/ha)}\) at the start. At least one pregnant female \(\text{S. hispidus}\) was included in each enclosure in both trials. Both experimental trials were conducted during peak small mammal breeding seasons. All animals in this study were captured, handled, and maintained following an approved animal care and use protocol \((\text{Oklahoma State University Institutional Animal Care and Use Committee Protocol 399})\).

\text{Sigmodon hispidus}\ generally is the most numerous rodent in the grasslands of the southern Great Plains, and although predominantly herbivorous, is known to consume arthropods, seeds, and soil on occasion \([22]\). \text{Reithrodontomys fulvescens}
is omnivorous, eating plant material, especially seeds, as well as insects and other invertebrates. *Microtus ochrogaster* is mainly herbivorous, but will consume arthropods when available. These three rodent species naturally coexist together and comprise the major component of the small mammal community in prairie grassland habitats in northern Oklahoma [23,24; S.R. Sheffield, personal observation]. Although an exotic, *Mus musculus* coexists with native rodents in areas where they have become feral. *Mus musculus* is omnivorous, although they tend to eat more insects and other animal matter than most omnivores. The strong competitive relationship between *S. hispidus* and *M. ochrogaster* is well documented [22,25], but ecological relationships between *S. hispidus* and both *R. fulvescens* and *Mus musculus* are less understood. Gestation periods for these species range from 21 to 27 d [22,26] and placental scars normally remain in the uterus six to seven weeks [27].

Diazinon 4E (4 lb/gal), an emulsifiable liquid formulation consisting of 47.5% active ingredient (Estes Chemical, Oklahoma City, OK, USA), was mixed with water and applied to the experimental mesocosms at a low (0.56 kg a.i./ha = 56 ×) or high (4.5 kg a.i./ha = 8 ×) maximum recommended field application rate of diazinon and these were compared to controls (no spraying = 0 ×). The two different maximum recommended label application rates used here represent rates recommended for different pests under different uses. Diazinon 4E was applied at day 0 of each trial using a CO₂-powered backpack unit with 1.83-m boom at a constant rate under 40 lb of pressure using 20 gal H₂O/acre, and applied relatively close to the ground (<0.5 m). The exposure scheme using diazinon was that of a pulse, or one-time, exposure applied at the start of each trial and terminating on day 30 after diazinon application, the approximate half-life of diazinon in the environment [28,29].

During each trial, field sampling of small mammals took place on days 2, 16, and 30 after diazinon application using Sherman traps that were set and baited the previous night. Data recorded for each animal captured included identification number, capture location, general condition (parasites, injuries), reproductive condition, and body weight (nearest 1 g for *S. hispidus* and nearest 0.5 g for all other species). For each species, capture days were recorded, and recapture rate (the percent of marked animals captured/the total number of animals captured) and trappability (percent of animals captured on any trapping day/total number of animals known to be present at the time) were calculated. Reproductive activity, as measured by incidence of reproductive condition, was defined in males by scrotal condition and in females by a combination of vaginal (perforate or imperforate), pregnancy (pregnant or not pregnant), and lactational (lactating or nonlactating) status [30]. After the day 30 sampling, we trapped for an additional 14 d to record the presence of individuals that avoided capture during previous sampling periods. At day 30, virtually all animals were trapped within 48 h and few were found beyond this point. Animals caught within 48 h were included in the analysis. All animals caught on day 30 were returned to the laboratory, were euthanized through a heavy inhalation dose of Metofane (Pitman-Moore, Mundelein, IL, USA) followed by cervical dislocation, and were necropsied. Uteri were examined for enumeration of placental scars and embryos. Reproductive productivity in females was determined through analysis of a number of reproductive parameters, including the percentage of females with embryos, the number of embryos and placental scars found in uteri, and the percentage of pregnant females and females giving birth. Pregnant animals used at the beginning of each trial were noted. Previously existing placental scars were found only in *S. hispidus* and were differentiated from scars resulting during the experimental trials by size (e.g., in some female *S. hispidus*, one set of large scars and one set of small scars were found).

**Data analysis**

The statistical package SAS® [31] was used to run analyses of variance testing for differences between replicated treatments (0 ×, 1 ×, 8 ×) for all species for both trials. Trials were not temporal replicates of each other and were analyzed independently. Analysis of variance (PROC ANOVA) was used to analyze trapping data, including captures, recaptures, and trappability, for each species by treatment level. An analysis of variance for incidence of reproductive condition and reproductive productivity was computed for each species in each trial using the general linear model (PROC GLM) program. Replicates were treated as statistically independent and not pooled before analyses in order to derive an estimate of variation between replications. Bonferroni tests were used to test for pair-wise differences between treatment means. Means and standard errors (SEs) for treatment effects are presented for most parameters; a significance level of *p* < 0.05 was used for all comparisons.

**RESULTS**

**Small mammal recovery**

No evidence was found of acute mortality in any species after diazinon application in either trials 1 or 2. In trial 1, a total of 164 individuals were captured 306 times, consisting of 115 *S. hispidus*, 20 *R. fulvescens*, and 29 *M. musculus* captured 254, 23, and 29 times, respectively. At day 30, a total of 45, 39, and 30 animals were recovered from 0 ×, 1 ×, and 8 × enclosures, respectively, for a total recovery of 114 animals out of 224, or 50.9% (Fig. 1). Over all three trapping days for all species, 108, 104, and 94 animals were trapped from 0 ×, 1 ×, and 8 × enclosures, respectively. Fewer *S. hispidus* and *R. fulvescens* and more *M. musculus* were trapped from diazinon-exposed enclosures, although this difference was not statistically significant for any species (*p* < 0.250). Final recovery totals for trial 1, and percentage of all animals recovered, included 100 *S. hispidus* (75.8%), 12 *R. fulvescens* (36.4%), and 2 *M. musculus* (3.4%). In trial 2, a total of 311 individuals were captured 505 times, including 130 *S. hispidus*, 129 *M. ochrogaster*, and 52 *R. fulvescens* captured 259, 177, and 71 times, respectively. At day 30, a total of 91, 79, and 77 animals were recovered from 0 ×, 1 ×, and 8 × enclosures, respectively, for a total recovery of 247 animals out of 346, or 71.4% (Fig. 1). Over all three trapping days for all species, 177, 170, and 161 animals were captured from 0 ×, 1 ×, and 8 × enclosures, respectively. The trend of trapping fewer *S. hispidus* from diazinon-exposed enclosures was nearly significant overall (*p* < 0.087), significant between 0 × and 1 × enclosures (*p* < 0.049), and nearly significant between 1 × and 8 × enclosures (*p* < 0.063; Table 1). The trend of trapping more *M. ochrogaster* from diazinon-exposed enclosures was significant overall (*p* < 0.008) and between 0 × and 8 × enclosures (*p* = 0.003) but was not significant between 1 × and 8 × enclosures (*p* = 0.323; Table 1). The trend of trapping more *R. fulvescens* from diazinon-exposed enclosures was not
significant overall ($p < 0.600$) or between control and 1× enclosures ($p < 0.432$) and control and 8× enclosures ($p < 0.362$; Table 1). Final recovery totals for trial 2, and percentage of all animals recovered, included 101 $S$. hispidus (70.1%), 111 $M$. ochrogaster (76.4%), and 35 $R$. fulvescens (58.6%).

**Ecological effects**

In trial 1, significantly more $S$. hispidus were trapped than were $R$. fulvescens or $M$. musculus for all sampling days ($p < 0.001$; Fig. 1). Recapture frequencies for $S$. hispidus were highest in 8× animals and decreased slightly in 1× and again in 0× animals at both days 16 and 30 (Tables 1 and 2). Trapping success for $M$. musculus or $R$. fulvescens was not sufficient to calculate recapture frequencies. Trappability generally decreased in diazinon-exposed $S$. hispidus (Tables 1 and 2). Differences in trappability between control and diazinon-exposed ($8\times$) animals ranged from 12–23%. No $R$. fulvescens were recovered from 8× enclosures after day 2. No significant differences in trapping sex ratios between 0×, 1×, and 8× enclosures were seen. For $S$. hispidus, females outnumbered males in 1× and 8× enclosures and males outnumbered females in 0× enclosures. For both $R$. fulvescens and $M$. musculus, the opposite trend was seen.

In trial 2, significantly more $S$. hispidus were trapped than were $M$. ochrogaster or $R$. fulvescens on days 2 and 16 ($p < 0.001$; Fig. 2). For sampling day 30, the same significant trend was seen; however, approximately equal proportions of each species were recovered within 48 h of day 30 because the first night of trapping removed many of the more dominant $S$. hispidus (Table 3). Overall, the ratio of $S$. hispidus to $M$. ochrogaster taken was increased significantly in the diazinon-exposed 1× and 8× enclosures for all sampling days ($p < 0.001$; Fig. 1). In control enclosures, ratios of trapped $S$. hispidus to $M$. ochrogaster ranged from 3.5:1 to 4.4:1, whereas in 1× and 8× diazinon-treated enclosures, ratios ranged from 1.7:1 to 2.9:1 and 1.3:1 to 1.6:1, respectively. Recapture frequencies for $S$. hispidus were lowest in 8× animals and increased from 1× to 0× animals at both days 16 and 30 (Tables 1 and 3). Differences in recapture frequencies between control and diazinon-exposed ($8\times$) animals ranged from 11 to 39%. Recapture frequencies for $M$. ochrogaster and $R$. fulvescens were higher in diazinon-exposed animals than in control animals at both days 16 and 30. Differences in recapture frequencies between control and diazinon-exposed ($8\times$) animals ranged from 89 to 430% for $M$. ochrogaster and 291 to 332% for $R$. fulvescens. Trappability of $S$. hispidus generally decreased sharply in diazinon-exposed animals, with differences ranging from 11 to 35%. Alternatively, trappability of $M$. ochrogaster and $R$. fulvescens generally increased in diazinon-exposed animals at days 2 and 16, but decreased at day 30, with differences ranging from 78 to 81% and 103 to 104% for $M$. ochrogaster and $R$. fulvescens, respectively, at days 2 and 16, and 15% and 29% for $M$. ochrogaster and $R$. fulvescens, respectively, at day 30.

**Reproductive effects**

Reproductive activity. Over both trials, reproductive activity, as measured by incidence of reproductive condition, of

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**Table 1. Capture (CAPT), recapture (RECAP), and trappability (TRAP) replicate means for enclosed small mammal species over all days. Significant differences ($p < 0.05$) are indicated by a difference in superscript uppercase letters using Bonferroni tests for pair-wise differences between treatment means**

<table>
<thead>
<tr>
<th>Species</th>
<th>Diazinon application rate</th>
<th>CAPT</th>
<th>RECAP</th>
<th>TRAP</th>
<th>CAPT</th>
<th>RECAP</th>
<th>TRAP</th>
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<tbody>
<tr>
<td></td>
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<td>Trial 1</td>
<td></td>
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<td>Trial 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigmodon hispidus</td>
<td>0×</td>
<td>7.750A</td>
<td>0.702A</td>
<td>0.606A</td>
<td>8.125A</td>
<td>0.824A</td>
<td>0.688A</td>
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<tr>
<td></td>
<td>1×</td>
<td>7.167A</td>
<td>0.725A</td>
<td>0.661A</td>
<td>6.312A</td>
<td>0.784A</td>
<td>0.593A</td>
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<td></td>
<td>8×</td>
<td>6.250A</td>
<td>0.768A</td>
<td>0.571A</td>
<td>6.437A</td>
<td>0.622A</td>
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<td>Mus musculus</td>
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<td>0.417A</td>
<td></td>
<td></td>
<td>3.937A</td>
<td>0.207A</td>
<td>0.396A</td>
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<tr>
<td></td>
<td>1×</td>
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<td></td>
<td></td>
<td>4.250A</td>
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<td>0.410A</td>
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<td>Microtus ochrogaster</td>
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<td>1.167A</td>
<td></td>
<td></td>
<td>5.125A</td>
<td>0.518A</td>
<td>0.424A</td>
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<tr>
<td>Reithrodontomys fulvescens</td>
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<td>0.833A</td>
<td></td>
<td></td>
<td>1.625A</td>
<td>0.106A</td>
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<tr>
<td></td>
<td>1×</td>
<td>0.667A</td>
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<td></td>
<td>2.000A</td>
<td>0.250A</td>
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<tr>
<td></td>
<td>8×</td>
<td>0.417A</td>
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<td></td>
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</tbody>
</table>

*0× = control; 1× = 0.56 kg a.i./ha; 8× = 4.5 kg a.i./ha.

*NT = not tested.
Table 2. Capture (CAPT), recapture (RECAP), and trappability (TRAP) replicate means for enclosed small mammal species for each trapping and sampling day in trial 1. Significant differences (*p* < 0.05) are indicated by a difference in superscript numbers (by day) and uppercase letters (by treatment) using Bonferroni tests for pair-wise differences between treatment means.

<table>
<thead>
<tr>
<th>Species</th>
<th>Diazinon application rate</th>
<th>CAPT</th>
<th>RECAP</th>
<th>TRAP</th>
</tr>
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<tr>
<td></td>
<td>2</td>
<td>16</td>
<td>30</td>
<td>16</td>
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<td>Sigmodon hispidus</td>
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<td>5.75&lt;sup&gt;A&lt;/sup&gt;</td>
<td>8.25&lt;sup&gt;2A&lt;/sup&gt;</td>
<td>9.25&lt;sup&gt;2A&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>1×</td>
<td>6.00&lt;sup&gt;A&lt;/sup&gt;</td>
<td>6.75&lt;sup&gt;2A&lt;/sup&gt;</td>
<td>8.75&lt;sup&gt;2A&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>8×</td>
<td>4.75&lt;sup&gt;A&lt;/sup&gt;</td>
<td>7.00&lt;sup&gt;2A&lt;/sup&gt;</td>
<td>7.00&lt;sup&gt;2B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mus musculus</td>
<td>0×</td>
<td>1.00&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.25&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;A&lt;/sup&gt;</td>
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<td>2.50&lt;sup&gt;A&lt;/sup&gt;</td>
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<td>8×</td>
<td>2.50&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Reithrodontomys fulvescens</td>
<td>0×</td>
<td>0.00&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.25&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1×</td>
<td>0.75&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.00&lt;sup&gt;A&lt;/sup&gt;</td>
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<td></td>
<td>8×</td>
<td>1.25&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;A&lt;/sup&gt;</td>
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</table>

*0× = control; 1× = 0.56 kg a.i./ha; 8× = 4.5 kg a.i./ha.*
Table 3. Capture (CAPT), recapture (RECAP), and trappability (TRAP) replicate means for enclosed small mammal species for each trapping and sampling day in trial 2. Significant differences ($p < 0.05$) are indicated by a difference in superscript numbers (by day) and uppercase letters (by treatment) using Bonferroni tests for pair-wise differences between treatment means.

<table>
<thead>
<tr>
<th>Species</th>
<th>Diazinon application rate $^a$</th>
<th>CAPT</th>
<th>RECAP</th>
<th>TRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0×</td>
<td>2</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td><em>Signodon hispidus</em></td>
<td>8.50 $^A$</td>
<td>7.00 $^A$</td>
<td>7.75 $^A$</td>
<td>9.25 $^A$</td>
</tr>
<tr>
<td></td>
<td>1×</td>
<td>8.25 $^A$</td>
<td>5.00 $^{2A}$</td>
<td>4.25 $^{3B}$</td>
</tr>
<tr>
<td></td>
<td>8×</td>
<td>5.50 $^{1B}$</td>
<td>5.25 $^{1A}$</td>
<td>6.75 $^{1A}$</td>
</tr>
<tr>
<td><em>Microtus ochrogaster</em></td>
<td>0×</td>
<td>2.25 $^A$</td>
<td>2.00 $^A$</td>
<td>1.50 $^A$</td>
</tr>
<tr>
<td></td>
<td>1×</td>
<td>2.75 $^A$</td>
<td>2.75 $^{2A}$</td>
<td>2.25 $^{3A}$</td>
</tr>
<tr>
<td></td>
<td>8×</td>
<td>3.50 $^{1A}$</td>
<td>3.25 $^{1A}$</td>
<td>5.25 $^{3A}$</td>
</tr>
<tr>
<td><em>Reithrodontomys fulvescens</em></td>
<td>0×</td>
<td>0.75 $^A$</td>
<td>1.00 $^{1A}$</td>
<td>1.25 $^{1A}$</td>
</tr>
<tr>
<td></td>
<td>1×</td>
<td>1.50 $^A$</td>
<td>2.50 $^{1B}$</td>
<td>2.55 $^{1A}$</td>
</tr>
<tr>
<td></td>
<td>8×</td>
<td>1.50 $^A$</td>
<td>2.00 $^{1A}$</td>
<td>2.25 $^{1A}$</td>
</tr>
</tbody>
</table>

* $0×$ = control; 1× = 0.56 kg a.i./ha; 8× = 4.5 kg a.i./ha.

$^a$ Includes animals trapped on day 30 only.

$^b$ Includes animals trapped within 48 h of day 30.

**DISCUSSION**

Ecological effects on small mammal populations and communities

During trial 1, most *S. hispidus* were recovered, but only slightly more than one third of *R. fulvescens* were recovered, and recovery of *M. musculus* was minimal. Because of relatively low recovery of *R. fulvescens* and *M. musculus*, few clear effects can be concluded regarding the impacts of diazinon on their individual populations or on small mammal communities from this trial. Overall, generally fewer *S. hispidus* and *R. fulvescens* and more *M. musculus* were taken in diazinon-exposed enclosures than in control enclosures, and the effects on reproduction of *S. hispidus* and *R. fulvescens* may be significant. In addition to the significantly lower percentage of females giving birth during the trial, a significantly lower percentage of females was found with embryos (15.8% in control to 4.5% in 8× enclosures). For *M. ochrogaster*, the number of females giving birth during the trial was significantly lower (0% in 1× to 4.5% in 8× enclosures) than in control animals (17.4% in 1× and 13.0% in 8× enclosures), respectively. The number of females giving birth (1×) was significantly lower (0% in 1× to 6% in 8× enclosures) than in control animals (6.4% in 1× and 4.5% in 8× enclosures), respectively. The number of females giving birth (1×) was significantly lower (0% in 1× to 6% in 8× enclosures) than in control animals (6.4% in 1× and 4.5% in 8× enclosures), respectively.

**Fig. 2.** Trapping results of *Signodon hispidus* and *Microtus ochrogaster* exposed to diazinon (trial 2). Experimental treatments: 0× control (no spray); 1× 0.56 kg a.i./ha; 8× 4.5 kg a.i./ha.

$$\text{Number of individuals captured}$$

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>2</th>
<th>16</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>0×</td>
<td>0 ×</td>
<td>1×</td>
<td>8×</td>
<td>1×</td>
</tr>
<tr>
<td>1×</td>
<td>0×</td>
<td>1×</td>
<td>8×</td>
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<td>0×</td>
<td>1×</td>
<td>8×</td>
<td>1×</td>
</tr>
</tbody>
</table>

$0×$ = control; 1× = 0.56 kg a.i./ha; 8× = 4.5 kg a.i./ha.
Fig. 3. Incidence of reproductive condition in male small mammals exposed to diazinon (trials 1 and 2). Experimental treatments: 0X = control (no spray); 1X = 0.56 kg a.i./ha; 8X = 4.5 kg a.i./ha. Values with superscript letters differing from each other are significantly different (p < 0.05). Error bars represent standard error.

the diazinon-exposed 1X and 8X enclosures. Analysis of trapping data suggested that the larger S. hispidus exerted its dominance in enclosures and controlled movements and trapping success of the smaller R. fulvescens and M. musculus. Small mammal movements were noticeable, and some escapes by R. fulvescens and M. musculus occurred, moving mainly from sprayed enclosures to control enclosures. Movements and trapping success are thought to have been influenced by the presence of the larger S. hispidus. Recapture frequencies for S. hispidus were higher in diazinon-exposed animals, and increased from day 16 to day 30 in all three treatments. This may indicate increased movements, and therefore increased contact with traps, of animals exposed to diazinon. Overall, small mammals appeared to be less trappable in the diazinon-exposed enclosures. Trappability was consistently lower in diazinon-exposed animals but increased from day 2 to day 30.

In trial 2, most S. hispidus and M. ochrogaster were recovered, and a much improved recovery of R. fulvescens was seen from trial 1. With high recovery of each species and no recorded escapes, this trial offered a more reliable look at possible effects of diazinon on small mammal populations and communities than the first trial. Generally, fewer animals were recovered from 1X and 8X than from 0X enclosures. However, more M. ochrogaster and R. fulvescens were trapped from 1X and 8X enclosures at day 2 and 16. Significantly more M. ochrogaster were trapped in 8X than in 0X enclosures. Fewer S. hispidus were trapped in 1X and 8X enclosures throughout the trial. The difference between 0X and 8X was nearly sig-
significant, whereas the difference between 0× and 1× was significant. This probably is a result of decreased trapping success for *S. hispidus* in enclosures 1 and 2 (both 1× treatments), most likely due to some avian predation in those two enclosures. As in trial 1, the larger *S. hispidus* appeared to exert its dominance in the enclosures and controlled movements and trapping success of the smaller *M. ochrogaster* and *R. fulvescens*. Recapture frequencies were decreased for *S. hispidus* and increased for *M. ochrogaster* and *R. fulvescens* in 1× and 8× enclosures. Overall, for all species, recapture frequencies increased from day 16 to day 30. Trappability decreased for *S. hispidus* and increased for *M. ochrogaster* and *R. fulvescens* in 1× and 8× enclosures. Trappability was significantly decreased for *S. hispidus* in the 8× enclosures. Overall, for all species, trappability generally increased from day 2 to day 30. Other studies have found that small mammal trappability has remained relatively constant following anti-ChE insecticide application. Barrett [12] found that trappability was not significantly altered in small mammals exposed to the carbamate insecticide carbaryl for agricultural and old-field communities, although trappability was consistently lower in the treated enclosures, and *M. musculus* was recaptured nearly twice as frequently as meadow voles (*Microtus pennsylvanicus*) within both community types. Shore and Dell’Omo [32] found that exposure to the OP insecticide dimethoate caused no changes in trappability of wood mice (*Apodemus sylvaticus*) or bank
Pesticides on communities and ecosystems

The effects of anti-ChE pesticides on ecosystem structure and function would probably be quite similar to those of organochlorine pesticides as outlined by Woodwell [33]. These include having an effect on every trophic level, reducing reproductive capacity, altering behavior patterns, and disrupting competitive relationships between species favoring generalist (broad-niched) species. These effects result in decreased species diversity, nutrient cycling efficiency, and stability, especially with regard to sizes of populations of small, rapidly reproducing organisms such as insects and rodents. Effects of diazinon at the ecosystem level have been found to include reduced species diversity, net primary productivity, total density of vegetation, and species diversity and density of soil microarthropods. These negative impacts affect rates of succession, decomposition, and nutrient cycling, ultimately affecting the whole ecosystem [34,35].

Currently, little evidence exists for anti-ChE pesticides causing alterations to community and ecosystem structure and function. Barrett and Darnell [13] found that a field application of the OP insecticide dimethoate had no overall effect on small mammal density, but a shift occurred in species composition from omnivores to herbivores that was attributed to a decrease in insect availability. Both Baker [36] and Clark and Bunck [37] found evidence, through analyses of barn owl diets, that small mammal communities have changed favoring herbivorous over insectivorous small mammals in the United States over the later half of the 20th century, possibly through the widespread application of pesticides.

Morris [38] found that endrin caused more than 50% mortality in experimental M. pennsylvanicus populations immediately after treatment. Microtus that survived the spray subsequently survived as well as control Microtus. Recruits entering the experimental population during postspay periods survived significantly better than young entering the more crowded control population. This increased survival, combined with active postspay breeding, yielded a final experimental population that significantly exceeded the control. Edge et al. [15] found that enclosed populations of gray-tailed voles (Microtus canicaudus) responded in a dose-dependent manner to a single application of the OP insecticide azinphos-methyl. Population responses increased with application rate, especially at or above the 1.55-kg/ha concentration and a decline in cumulative number of recruits was found in azinphos-methyl-exposed animals. Schauber et al. [16] found that azinphos-methyl applied at 3.61 kg/ha caused reduced recruitment, survival and body growth in M. canicaudus, resulting in vole densities <40% of control densities. They also found that population recruitment and growth rates of P. maniculatus in mowed enclosures was significantly reduced. These conditions persisted over a six-week postspay period. Barrett [12] found that densities of M. musculus in agricultural plots were 50 to 75% higher than controls, whereas densities of M. pennsylvanicus were 50 to 140% smaller than controls after carbaryl application. The significantly lowered densities of voles was attributed to the effect of carbaryl on pregnant females that exhibited a five- to six-week delay in an increase in reproductive rate after application of the pesticide. The application of carbaryl in the agricultural community resulted in a long-term dominance by M. musculus due to the subtle but significant delayed reproductive response of carbaryl-exposed M. pennsylvanicus. Although trapping efficiencies did not differ
significantly between treatments for either community type, trapping efficiency was consistently lower in carbaryl-exposed enclosures, and *M. musculus* was recaptured nearly twice as much as *M. pennsylvanicus* within both community types. Pomeroy and Barrett [11] found that carbaryl caused a pronounced decrease in body weight of newborn *S. hispidus* and inhibited *Sigmodon* reproduction, resulting in a lower peak population density compared to the control area. As a result, coexisting *M. musculus* population densities increased and sex ratios were altered in carbaryl-treated areas. Overall, altered sex ratios, increased interspecific competition, weight changes, and changes in population growth rates were seen in the three small mammal species exposed to carbaryl. Although ecosystem resources were similar in both enclosed areas, small mammal population composition remained altered for several months after carbaryl treatment [11], demonstrating that a short-term decrease in the population size of a single species may have ramifications in the responses of other members of the small mammal community.

Reproductive effects

Incidence of reproductive condition (reproductive activity) in all small mammal species of both sexes generally was significantly less or increased slower from initial or control activity levels in diazinon-exposed animals during the two trials. Incidence of reproductive condition in control animals tended to increase or remain high for all species during both trials. The negative impact of diazinon on incidence of reproductive condition was most striking for *M. ochrogaster*. Incidence of reproductive condition for males and females reached 100% by day 16 in control populations, but remained low in diazinon-exposed populations throughout trial 2. The percentages of the small mammal populations (males and females) that were found to be reproductively active were found to either decrease or increase slower during a time of normal peak rodent breeding activity.

Reproductive productivity in females of all small mammal species was significantly impacted by diazinon exposure. A significant reduction occurred in the number of females giving birth from diazinon-exposed populations during both trials. This was reflected both by the number of females with live young in enclosures as well as in the number of females with recent placental scars. The number of female *S. hispidus* and *M. ochrogaster* with embryos also decreased significantly in diazinon-exposed animals in trial 2. In addition, diazinon-exposed female *M. ochrogaster* were found to have significantly smaller mean numbers of embryos per female. The general decrease in female reproductive productivity for all species is not surprising in light of the reduced reproductive activity seen in diazinon-exposed animals.

These results suggest that reproductive activity (as measured as incidence of reproductive condition) in females appeared to be impacted more than males by diazinon exposure. In the laboratory, female rats and dogs have been demonstrated to be more sensitive to diazinon than are males [39–41]. The reason for this is not clear; the presence of high testosterone levels in males may convey a detoxification advantage to males over females that have only low levels of testosterone. Negative effects on reproduction can impact recruitment, population density, and long-term population stability, ultimately affecting other coexisting populations in the community.

Significant reproductive effects have been demonstrated in enclosed *S. hispidus* [10,11] and *M. pennsylvanicus* [12] populations exposed to the carbaryl. These effects resulted in significant delays in reproduction in these populations, which was thought to have been due to the relatively high embryotoxicity of carbaryl in mammals [12]. Reproductive activity or the proportion of adults that were pregnant or lactating was found not to be significantly affected in either *M. canicaustrus* or *P. maniuculatus* exposed to azinphos-methyl, even at the high application rate [15,16]. However, a decline was seen in the cumulative number of recruits in *M. canicaustrus* [15,16] and *P. maniuculatus* [16] exposed to azinphos-methyl. It should be pointed out that these studies did not analyze male reproductive condition and used a different, less reliable method than the present study for assessing female reproductive condition in the field.

Several recent laboratory studies have clearly demonstrated that OP insecticides can potentially negatively impact reproduction in mammals. In male rodents, OP insecticides have been shown to cause numerous alterations in the testes, including sperm shape abnormalities [42], altered sperm capacitation and inhibition of fertilization [43], alterations of the seminiferous epithelium and Leydig cells [44], decreased testicular weight, increased spermatid degeneration and reduced total sperm counts [45], and decreased testicular sperm density, steroidogenesis, and enzyme activity, along with damage to the spermatogenic cells [46]. Diazinon and other OP insecticides have also been shown to cause severe negative effects on the female reproductive system and developing young, including maternal weight loss and toxicity, decreased birth and weaning weights [47], embryonic ChE inhibition, an increase in stillbirths and neonatal deaths accompanied by a reduction in juvenile weight gain [48], slightly decreased uterine and ovary weights and mean number of embryos per female and significant decrease in stage of pregnancy [49], and significant mortality of pups before weaning [50]. In addition, placental transfer of OPs, bioconcentration in fetal tissues [51,52], and lactational transfer of OPs have been documented [53]. Reproductive hormones have been found to be altered by exposure to OP insecticides, including reduced plasma follicle-stimulating hormone, luteinizing hormone, and testosterone titers and reduced cholesterol esterification, which is an important step in steroid hormone production [45,54,55]. Interference with the production or metabolism of reproductive hormones may considerably impact mating success, fertility, and neonatal survival. This may, in turn, negatively impact exposed populations of small mammals, possibly leading to alterations at the community and ecosystem levels.

Exposure

That significant exposure to diazinon occurred in many of the animals in sprayed enclosures was apparent. The results of this study, supported by soil residue analysis and plasma and brain ChE activity data, suggest that diazinon persisted in sprayed enclosures throughout the 30-d trials. Soil samples were found to contain up to 5.3 (1×) and 7.7 ppm (8×) diazinon at day 2, 2.8 (1×) and 4.2 ppm diazinon at day 16, and 0.6 (1×) and 2.3 ppm (8×) diazinon at day 30 (S.R. Sheffield, unpublished data). Plasma and brain ChE activities were significantly inhibited in all small mammal species in both trials, and although activities tended towards recovery by day 30, they were still significantly below those of control animals for all species [56; S.R. Sheffield, unpublished data]. Arthropod communities in the sprayed enclosures were severely impacted
by diazinon (S.R. Sheffield, unpublished data). Dead and dying arthropods were routinely seen in enclosures sprayed with diazinon. Little rainfall occurred during either 30-d trial (5.3 and 1.2 cm, respectively), allowing continued persistence of diazinon in enclosures. Potential routes of exposure for small mammals included oral, inhalation, and dermal. Oral exposure appears to be the major route of exposure, probably occurring mainly through the opportunistic consumption of dead and dying arthropods or vegetation, soil consumption, or through grooming. The opportunistic consumption of arthropods is not surprising considering the fact that all species used in this study are known to take relatively large numbers of arthropods when available [22,25,26]. Both trials were conducted during the peak rodent breeding season when additional protein is necessary for reproduction. Stehn et al. [57] found a 70, 300, and 400% increase in weakened or dead arthropods in the diets of Blarina brevicauda, P. leucopus, and Clethrionomys gapperi, respectively, over control animals after an aerial application of the OP insecticide acephate. Other possible means of oral intake of diazinon by small mammals included ingestion of soil containing diazinon residues [58], and through grooming of fur that contained diazinon residues picked up from plants and soil. Additional exposures may have occurred through the dermal and inhalation routes during the trials as well. The dermal LD50 and inhalation LC50 in rabbits for diazinon 4E are the lowest values for any diazinon formulation [7]. The suggestion has been made that a greater hazard exists from inhalation than from ingestion of equivalent amounts of the OP insecticide malathion in rabbits and quail [59].

The Prairie grassland habitat in the enclosures provided some protection to small mammals from diazinon; however, diazinon was applied relatively close to the ground in order to ensure exposure and avoid drift between enclosures. Wang et al. [60] found no apparent effects of azinphos-methyl (1.55 kg/ha) on M. canicaudus in enclosures planted with a mixture of pasture grasses. Previous studies in this same enclosure system with azinphos-methyl (1.55 kg/ha) planted with alfalfa revealed several negative effects on M. canicaudus. Wang et al. [60] speculated that much of the pesticide in the mixed pasture-grass system accumulated in the upper strata of the grasses and never reached the ground.

The relatively high water solubility (40–60 mg/L at 20°C) and high estimated \( K_c \) (1,000) of diazinon result in a relatively longer environmental persistence than many other anti-ChE insecticides [7,28,29]. Diazinon does not bind tightly to soil particles and seldom penetrates below the top 5 cm of soil [61,62]; therefore, diazinon would be readily bioavailable on the litter layer or soil surface. This results in a greater chance of uptake by organisms as well as indicating a propensity for storage and, hence, a longer persistence in the body [62,63]. The 50% persistence rate of diazinon in soil is estimated to be from two to four weeks [28] to 40 d [29]. Diazinon may remain biologically active in soils for up to one year or more under certain environmental conditions [7]. The major degradation product of diazinon in soils is oxypyrimidine, which is more persistent than diazinon under most environmental conditions [7]. In plants, diazinon generally persists for up to 7 d [28,61]; however, diazinon and its degradation products may have persisted in and on plants for longer periods in the enclosures. The bioconcentration factor for diazinon has been estimated at 35 to 77, a relatively high number for an OP insecticide [64]. McEwen et al. [65] found that white-footed mice (P. leucopus) captured 6 to 8 d after a diazinon application (5.0–8.0 oz/acre) to shortgrass prairie contained 0.10 to 0.17 ppm of diazinon. Diazinon and its metabolites may be deposited in lipid and other tissues and a relatively slow breakdown would allow for a continuous exposure through the blood. Mendelsohn and Paz [66] showed that the OP insecticide monocrotophos, applied at two times the recommended label rate, can bioaccumulate in rodents at levels high enough to cause significant secondary poisoning of avian predators.

**Toxicity**

Several factors possibly contributed to the toxicity and subsequent effects of diazinon in this study. The acute toxicity (oral LD50) of diazinon ranges from 34 to 900 mg/kg body weight in lab rats, but only 65 to 96 mg/kg in lab mice, ranking it as one of the more acutely toxic OP insecticides [67,68]. Some diazinon metabolites and degradation products have greater toxicity than the parent compound. Some formulations of diazinon, particularly emulsifiable liquids, can be converted to much more toxic compounds on contact with air [67] and ultraviolet irradiation [69]. Other components of the diazinon 4E formulation (e.g., xylene and ethylbenzene) may have been a factor in the toxicity found. Both inert ingredients individually are potentially toxic, and are on a list of inert ingredients that the U.S. Environmental Protection Agency strongly encourages pesticide registrants to remove or substitute from their products.

Little is known about the subchronic or chronic toxicity of diazinon [7]. The few chronic toxicity tests conducted with mammals suggest that daily intake exceeding 5 to 10 mg/kg body weight diazinon is probably fatal over time to pigs (Sus scrofa) and dogs (Canis familiaris) [41]. A chronic no-effect level of 0.1 mg/kg body weight in the diet has been calculated for lab rats [68].

Diazinon exposure has been demonstrated in the laboratory [7] and in the field [56; S.R. Sheffield, unpublished data] to result in a wide variety of sublethal effects using several species of mammals. Sublethal effects in mammals have been seen at exposures as low as 0.18 mg diazinon/kg body weight daily through gestation in pregnant lab mice, 0.5 mg diazinon/kg body weight for five weeks in lab rats, and at single doses of 1.8 mg diazinon/kg body weight for lab rats and 2.3 mg diazinon/kg body weight for P. leucopus [7,47,50,70,71]. Young mice exposed in utero and through lactation experienced both endocrine [70] and immune [71] system alterations. Sublethal effects can be significant because they increase the likelihood of mortality of the exposed organism [72]. Small mammals exposed sublethally to diazinon may face increased predation and experience aberrant behavior, learning disabilities, decreased endurance, motor coordination, and immunocompetence, anorexia, and vision and hearing impairment in addition to hypothermia, ChE inhibition, pathologic changes, and reproductive impairments [7,47,50,70,71; this study].

**Conclusions**

Exposure to the OP insecticide diazinon negatively affected small mammal populations and communities in our field experiments. Fewer animals were recovered from diazinon-exposed enclosures, and evidence was found to indicate that the ecological relationship between S. hispidus and M. ochrogaster may have been altered by exposure to diazinon. In addition, a marked decline in reproductive activity and productivity was evident in diazinon-exposed animals in this study. Detecting effects such as these would not have been...
Diazinon in mesocosms: Effects on small mammals

possible with laboratory studies only, and we believe that field experimental ecosystems such as the one constructed for this study are useful and necessary for adequately assessing possible real-world exposure and effects of pesticides to nontarget organisms. Further, trapping data such as this would be expected to give a realistic representation of the toxic effects of a pesticide on small mammals, both in terms of effects on population dynamics and biomarker responses in individuals.

The findings of this study must be considered in the context of the single application of diazinon used in the study. The responses of small mammal communities to multiple applications of diazinon or a combination of diazinon and other pesticides remains to be studied in a replicated field experiment. We hypothesize that under conditions of multiple applications of diazinon or a combination of diazinon and other pesticides, negative impacts on small mammals would be compounded.

From these and the findings of other studies, rodents apparently are the most sensitive group of mammals yet tested with diazinon, and they have proven to be sensitive to other OP and carbamate insecticides as well. However, differences in behavior, foraging habits, habitat, and experimental design could affect routes and degree of exposure, and thus render some species of small mammals more vulnerable to OP insecticide exposure in the field [73]. With few, yet conflicting data available, it is not at all clear as to whether wild rodent populations are more susceptible or resistant to pesticide exposure than are lab rodents [73–76]. However, considerable limitations exist in the use of lab rodents in toxicologic studies that attempt to predict toxicant-induced effects on ecological systems [8,77].

Based on the findings of this study, it seems essential to human, wildlife, and ecosystem health to reexamine recommended label application rates and pesticide registration and reregistration procedures for diazinon and other similarly toxic, widely used OP pesticides because of their high toxicity and potential for debilitating sublethal effects and subsequent effects on populations, and perhaps communities, of nontarget organisms. The U.S. Environmental Protection Agency’s quotient method, widely used during pesticide registration evaluations for assessing risk of contaminant exposure to wildlife [78], has proven unreliable in many cases [2,16] and a re-evaluation of expected environmental concentration and overall hazard quotient for diazinon from laboratory to field should be considered for diazinon. We urgently need to examine further the effects of chronic exposure to low levels of widely used anti-ChE pesticides, particularly when a single application at recommended label application rates is found to negatively impact experimental small mammal populations and communities. Field studies under natural conditions, such as mesocosms, where population-, community-, and ecosystem-level studies can be carried out simultaneously, are crucial to fully understanding the subtle sublethal and reproductive effects that species can experience when exposed to pesticides as well as the longer-term ecological effects of pesticide applications that occur at higher levels of ecological organization.

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