EFFECTS OF FIELD EXPOSURE TO DIAZINON ON SMALL MAMMALS INHABITING A SEMI-ENCLOSED PRAIRIE GRASSLAND ECOSYSTEM.

I. ECOLOGICAL AND REPRODUCTIVE EFFECTS

II. SUBLETHAL EFFECTS

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PREFACE

The study of anticholinesterase (anti-ChE) pesticide exposure and effects on wild animal populations and communities in the field has been severely limited. Further, our knowledge of ecological and sublethal effects following chronic or sub-chronic exposure is nearly non-existent. I built a 5 acre enclosure facility to provide a controlled, replicated experimental means of assessing ecological and sublethal effects of a subchronic anticholinesterase pesticide exposure on wild small mammal communities placed into the enclosures. The use of a terrestrial mesocosm allowed me to have control over certain important variables (movements, predation, community structure, densities, food resourses) while at the same time maintaining realistic conditions for exposure to the organophosphate insecticide diazinon. In addition, the mesocosm allowed a comprehensive analysis of potential reproductive effects from anticholinesterase pesticide exposure. This dissertation is comprised of three manuscripts formatted for submission to peer-reviewed journals. The manuscripts are complete as written and require no supporting material.

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EXPOSURE, TOXICITY, FATE, AND PERSISTENCE OF THE ORGANOPHOSPHATE INSECTICIDE DIAZINON IN THE TERRESTRIAL ENVIRONMENT: A REVIEW

Abstract-For almost 40 years, cholinesterase-inhibiting (anti-ChE) pesticides, including organophosphate (OP) and carbamate insecticides, have been the most widely used group of insecticides throughout the world. Human reliance on these generally highly toxic compounds has resulted in concern over their possible effects on humans, wildlife species, and ecosystems. The OP insecticide diazinon is one of the most widely used insecticides for control of pests in both agricultural and non-agricultural settings. The widespread use of diazinon has resulted in significant increases in diazinon concentrations in all environmental media. As a result of its wide usage, high toxicity to a wide variety of organisms, potential for persistence in the environment, and the relative lack of data regarding its effects on wildlife and humans, increases of environmental diazinon concentrations are cause for concern. This paper reviews the current state of our knowledge on the OP insecticide diazinon dealing with exposure, toxicity, fate and persistence in the terrestrial environment. Data from lab and field studies with mammals and birds are included here, as there is no data available for reptiles. The more subtle sublethal effects of diazinon exposure and the use of terrestrial wildlife species as biomonitors of diazinon exposure are emphasized. More work identifying diazinon usage and its transport into various environmental media is badly needed. In addition,

examining diazinon exposure and effects in wild animal populations, particularly with reptiles and mammals, is critical in order to more accurately characterize the risks involved with widespread diazinon use and subsequent exposure in terrestrial animal species.

Keywords- Diazinon, OP insecticide, exposure, toxicity, sublethal effects, fate, persistence, terrestrial environment

INTRODUCTION

The effects of pesticides on human health and the environment have been of concern for over three decades now (Carson, 1962). For nearly 40 years, anti-ChE pesticides, including organophosphate (OP) and carbamate pesticides, have been the most widely used groups of insecticides in North America, and the continued reliance on these compounds has resulted in concern over their possible effects on wildlife species and ecosystems (Brown, 1978; Grue et al., 1983; Smith, 1987). Although OP and carbamate pesticides are relatively less persistent in the environment and tend not to bioaccumulate in food chains, they generally are much more acutely toxic than organochlorine (OC) pesticides and have a relative lack of target specificity, tending to exert a potentially more widespread effect on the many non-target organisms. Currently, there are more than 100 different OP and carbamate chemicals registered as the active ingredient in thousands of different pesticide products in the United States. Total pesticide usage in the U.S. was estimated at about 2.2 billion lbs. of active ingredient in 1993 (Aspelin, 1994). Among the most widely used is the OP insecticide diazinon. In 1993, it was estimated that approximately 11 - 16 million pounds of the active ingredient diazinon was applied in the United States in both agricultural and non-agricultural uses (Aspelin, 1994). Diazinon is also widely used in many other countries throughout the world (ATSDR, 1994).

Over the past several years, the widespread usage of diazinon has resulted in an alarming increase in environmental diazinon concentrations in ambient air, indoor air,

surface waters, groundwater, treated sewage effluents, sediment, and precipitation (Amato et al., 1992; ATSDR, 1994; Carey and Kutz, 1985; Crocker et al., 1992; Norberg-King et al., 1989). A recent national survey conducted by the EPA found diazinon in 7,230 of 23,227 surface water samples and 115 of 3,339 groundwater samples in 46 states (USEPA, 1987). Diazinon was found up to 33.4 mg/l in surface waters and 0.084 mg/l in groundwater. A national survey conducted by the National Effluent Toxicity Assessment Center (NETAC) in 1988 documented the presence of diazinon at potentially hazardous levels in municipal sewage treatment plant effluents across the United States (Norberg-King et al., 1989). In addition, relatively high amounts of diazinon have been found in fog and rain in California (Glotfelty et al., 1990; Schomburg et al., 1991; F. Knopf and M. Marsh, pers. comm.). Residues of diazinon exist on food for human consumption in the United States, Canada, and other countries (Davies and Holub, 1980a). Careless use, storage, disposal, and overuse are prevalent, and may result in significant exposures of humans and other organisms to diazinon, particularly in urban areas (ATSDR, 1994). As a result of its wide usage, high toxicity to a wide variety of organisms, potential for persistence in the environment, and our relative lack of data regarding its effects on wildlife and humans, these occurrences of diazinon in the environment are cause for serious concern.

Due to a lack of data, no generalizations can presently be made about the effects of diazinon on wildlife populations. Present data suggests that mortality of wildlife occurs following OP pesticide application. Exposure to OP insecticides is known to produce many different sublethal effects which act to render the exposed organism more likely to

die as a result of its exposure. These sublethal effects include physiological, biochemical, immunological, reproductive, and behavioral alterations critical for survival and reproduction and they have been seen in a variety of organisms, although mainly in laboratory animals. In addition, indirect effects of OP pesticide applications have the potential to alter the distribution and abundance of wildlife species, but the extent to which these effects may alter recruitment and population size is virtually unknown (Eisler, 1986; Grue et al., 1983). Several field studies have reported that OP and carbamate insecticide applications have had little or no impact on small mammal populations, whereas some studies have shown significant impacts of pesticide applications on small mammal populations (Mineau, 1991). Studies using the anti-ChE insecticides carbaryl (Barrett, 1968; Pomeroy and Barrett, 1975), dimethoate (Barrett and Darnell, 1967), malathion (Giles, 1970), and azinphosmethyl (Edge et al. in press, Schauber et al. in press) have documented mild to severe population reductions, inhibited reproduction, and increased population turnover rates, leading to altered small mammal community structure. Currently, there is a lack of field experimental studies examining short- or long-term effects of pesticides or other contaminants on natural ecosystems. Presently, no data exist regarding the effects of diazinon on natural populations or communities of mammalian species under field conditions, and a recent review of diazinon toxicity to wildlife species designated this as a top priority research area (Eisler, 1986). In addition, we know very little about acute or chronic effects of low-level exposure of mammals or other taxa to diazinon.

The objective of this chapter is to summarize the published literature on the OP

insecticide diazinon in the terrestrial environment, including the potential exposure, toxicity (including lethal and sublethal effects), environmental fate, and persistence of diazinon.

Diazinon Use

The compound diazinon (O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate) is a broad spectrum organophosphate (OP) insecticide that is widely used in agriculture, range, commercial, and home and garden settings for the control of a wide variety of insect, acarine, and nematode pests (Eisler, 1986). In agriculture, diazinon is widely used on such crops as corn, alfalfa, rice, onions, and sweet potatoes, and is used in controlling pests in agricultural soils, on livestock, in animal holding facilities, and on ornamental plant species (Eisler, 1986). Diazinon is one of the most popular home and garden insecticides on the market, has label clearance for almost all arthropod and nematode pests, and is used extensively by pest control operators, and home owners. Currently, all formulations of diazinon are restricted for use on sod (turf) farms and golf courses, and the granular formulation is a restricted use pesticide for all uses due to its avian and aquatic toxicity (USEPA, 1988, 1989). Diazinon has a wide spectrum of insect-killing power and can control various soil insects, DDT-resistant flies, household pests, and various vegetable and forage crop insects. Diazinon has been registered for use since 1952, and since 1985, more than 10 million pounds of active ingredient, on average, has been applied annually in the United States, making it among the most widely used insecticides (Aspelin, 1994; Gianessi and Anderson, 1995;

Matsumura, 1985). In 1993, it was estimated that approximately 11 - 16 million pounds of the active ingredient diazinon was used in the United States, including 3 - 6 million pounds in agricultural uses and 8 - 10 million pounds in non-agricultural uses (Aspelin, 1994). It is estimated that up to 43% of diazinon currently applied in the United States is for non-agricultural purposes (ATSDR, 1994). Many different formulations of diazinon are produced for insecticidal use, including granular, emulsifiable liquid, wettable powder, dust, seed dressings, and microencapsulated among others (Eisler, 1986). Diazinon is classified as highly toxic or very highly toxic to warm water fishes, invertebrates, birds, and mammals (USEPA, 1989).

Exposure

Potential routes of exposure for terrestrial vertebrates include dietary, inhalation, and dermal. However, dietary exposure appears to be the most significant route of exposure in many cases. Other possible means of oral intake of diazinon by terrestrial vertebrates include consumption of plants, seeds or prey containing diazinon residues, ingestion of soil or water containing diazinon residues, and grooming of fur or preening of feathers that contain diazinon residues picked up from the environment (Beyer et al., 1994; Eisler, 1986; Garten, 1980). In birds, particularly waterfowl, dermal exposure through the feet and legs may be significant (Eisler, 1986). Inhalation may pose a significant route of exposure for diazinon. In mammals, 27.2 mg diazinon/l air killed 50% of test rabbits after exposure for only 4 h (Eisler, 1986). Weeks et al. (1977) suggest that a greater hazard exists from inhalation than from ingestion of equivalent amounts of malathion in

rabbits and quail.

Marked changes in diet (representing opportunistic feeding) following pesticide application may result in significant pesticide exposure. Stehn et al. (1976) found that there was a marked increased consumption of nontarget arthropods weakened or killed following an aerial application of the OP insecticide acephate. They found a 70%, 300%, and 400% increase in arthropods in the diets of short-tailed shrew (Blarina brevicauda), white-footed mice (Peromyscus leucopus), and red-backed vole (Clethrionomys gapperi), respectively, over control animals at 7-11 days post-spray. Small mammal diets were found to have returned to normal by five weeks post-spray. The dietary level of protein has been shown to be a factor in the toxicity of diazinon by affecting amounts of detoxifying enzymes in the body. Xenobiotics are less rapidly removed from the body in an organism fed a diet deficient in protein. Boyd et al. (1969) found that diazinon was as much as seven times more toxic to rodents when dietary protein levels were low. Serum ChE, serum and liver triacetinesterase, and brain ChE increased with increasing dose of the OP insecticide parathion and with decreasing casein content of the diet (Casterline and Williams, 1971). Food restriction through continued exposure to the anorexia-causing diazinon may ultimately lead to decreased food consumption. Animals subject to water deprivation and food restriction generally are much more susceptible to the effects of anti-cholinesterase pesticides (Adams, 1977; Baetjer, 1983; Glow et al, 1966). Feed aversion can be seen when animals are given diets containing relatively high acute or subacute levels of pesticides, and is thought to arise from the unacceptable taste of the food. Feed aversion was reported in Microtus ochrogaster for the carbamate insecticide

carbofuran, but involved relatively high (subacute) concentrations in food (Linder and Richmond, 1990).

Fata and Persistence in the environment

Diazinon has several chemical properties that serve to make it prone to persist in the environment. It is relatively highly soluble in water (40-60 mg/l at 20^{o}C) and has an estimated soil sorption coefficient (K_{oc}) of between 570 - 1000, indicating that it does not bind tightly to soil particles (Kenaga, 1980; Wauchope et al., 1992). Diazinon is moderately to highly mobile in some soil types (Kenaga, 1980). It seldom penetrates below the top 5 cm of the soil (Kuhr and Tashiro, 1978; Malone et al., 1967), and therefore may be readily bioavailable on the litter layer or soil surface. The 50% persistence rate of diazinon in soil is estimated to be from 2-4 weeks (Bartsch, 1974) to 40 days (Wauchope et al., 1992), and its 75-100% degradation rate in soil is estimated to be 12 weeks (Matsumura, 1985). Diazinon can be degraded on the soil surface by photolysis (Burkhard and Guth, 1979) and in soils and sediments by hydrolysis (Chapman and Cole, 1982) and by microbial degradation (Barik and Munnecke, 1982). Degradation rate of diazinon is strongly influenced by pH (Chapman and Cole, 1982). The major degradation product of diazinon in soils is oxypyrimidine, which is more persistent than diazinon under most environmental conditions (USEPA, 1988). Diazinon applied at a rate of 14 lbs active ingredient (a.i.)/acre remained at high concentrations in the 0-5 cm soil fraction for several weeks, remaining in this layer until day 68 (Malone et al., 1967). Diazinon applied at a rate of 12 lbs a.i./acre also remained at high

concentrations in the upper 5 cm of soil for several weeks, but decreased rapidly to less than 1.0 ppm by 60 days (Shure, 1971). However, diazinon was still detected in trace amounts after 6 months post-treatment. Diazinon may remain biologically active in soils for up to 1 year or more under conditions of low temperature, low moisture, high alkalinity, and lack of microbes necessary for degradation (Eisler, 1986). Soil moisture variation produced up to a 100-fold difference in the persistence (toxicity) of diazinon in soils (Harris, 1967). Diazinon and its degradation products may have persisted in and on the grasses and forbs for at least a week or longer in the enclosures. In plants, diazinon generally persists for up to 7 days in turf grass, less than 7 days for certain vegetables and cereal grains, and less than 2 days for leafy vegetables and forage crops (Bartsch, 1974; Kuhr and Tashiro, 1978). Watering increases the amount of diazinon residues in the soil for both liquid and granular applications; however, amounts of diazinon recovered from grass is unchanged by watering following liquid application and decreases following granular application (Kuhr and Tashiro, 1978). Plants grown in sandy soils absorb higher amounts of pesticides than ones grown in soils with high organic matter, thereby making the pesticides more available for biological intake (Matsumura, 1985). In the atmosphere, diazinon is subject to degradation due to photolysis (Gore et al., 1971), and in water, it is subject to hydrolysis, photolysis and microbial biodegradation (ATSDR, 1994).

Diazinon has a surprisingly high partition coefficient, suggesting an increased chance of uptake by organisms as well as indicating a propensity for storage and hence, a longer persistence in the body (Freed et al., 1979). The aquatic bioconcentration factor (BCF)

for diazinon has been estimated at 35 - 77, a relatively high number for an OP insecticide but relatively low overall (Kenaga, 1980). No data on uptake or bioconcentration from food chains in terrestrial systems are available. In terrestrial systems, diazinon most likely would not bioaccumulate or bioconcentrate in animal tissues unless relatively frequent multiple applications of diazinon were applied in a given area.

Acute toxicity

The acute toxicity of diazinon has been established for several of the standard laboratory animals and wildlife species used in toxicity studies. Acute oral LD_{50}'s for lab rats and mice range from 150 - 220 mg/kg body weight (BW) and 85 - 135 mg/kg BW, respectively (Matsumura, 1985). Acute oral LD_{50}'s for lab rats were also calculated as 231 - 270 mg/kg BW for males and 259 - 314 mg/kg BW for females (Gaines, 1969). Acute dermal LD_{50}'s for lab mice was calculated as 2750 mg/kg BW, and onset of symptoms from dermal exposure took 10 hours (Skinner and Kilgore, 1982). Acute dermal LD_{50}'s for lab rats was calculated as 455 - 900 mg/kg BW (Gaines, 1969). The acute inhalation LD_{50} for lab rabbits was 27.2 mg/l air after 4 h of exposure (Eisler, 1986).

For avian species, acute oral LD_{50}'s for almost all species tested range from 2.0 - 10.0 mg/kg BW (Eisler, 1986; Smith, 1987). Diazinon has great potential for causing acute avian poisoning events (see mass mortality section below). The granular form of diazinon is occasionally mistaken by birds, particularly seed-eating species, for food or grit. Ingestion of fewer than 5 granules of diazinon 14G (14.3% a.i., each containing

about 215 μg diazinon) could be lethal to sparrow-sized birds (15 to 35 g BW; Hill and Camardese, 1984). Ingestion of 5 granules of diazinon 14G killed 80% of house sparrows (Passer domesticus) and 100% of red-winged blackbirds (Agelaius phoeniceus) to which they were administered (Balcomb et al., 1984). In 5-day feeding trials with 2 wk old Japanese quail (Coturnix japonica), followed by 3 days on untreated food, the LD_{50} was 167 mg/kg BW. No deaths were observed at dietary levels of 85 mg/kg BW, but there was 53% mortality at 170 mg/kg BW and 87% mortality at 240 mg/kg (Hill and Camardese, 1986). At present, there is no data on the acute toxicity of diazinon on reptile or amphibian species, but available evidence indicates that these taxa would be at least as sensitive to diazinon exposure as avian species (Hall and Clark, 1982).

Diazinon exerts its toxic effects through its binding to the neuronal enzyme acetylcholinesterase (AChE) for relatively long periods after exposure (Eisler, 1986; Grue et al., 1983). Through oxidation by the MFO system, the oxygen analog diazoxon is formed, which is one of the most potent AChE inhibitors known (Eisler, 1986). Thus, the metabolite has greater toxicity than the parent compound. Accompanying the inhibition of AChE is the concommitant rise in acetylcholine levels at muscarinic and nicotinic receptors, leading to their excessive activation, ultimately preventing muscular movement and causing death by inhibiting respiration (Eisler, 1986; Grue et al., 1983; Sultatos, 1994). There is some evidence to show that some OP insecticides such as diazinon can produce in birds and mammals a second lesion quite unrelated to the inhibition of AChE known as delayed neuropathy (Baron, 1981; Johnson, 1975).

Mass mortality

Diazinon is highly toxic to wildlife for usually short periods after its application and has been documented to cause mass mortalities in birds and mammals (Eisler, 1986; Grue et al., 1983). Mammals appear to be less sensitive than birds to acute diazinon poisoning (Eisler, 1986). Numerous wildlife mortality incidents in the United States involving diazinon include 54 documented incidents in 17 states and involve 23 species of birds, mostly waterfowl, and two species of mammals, including rabbits and pocket gophers (Stone and Gradoni, 1985a). Mass mortality incidents have been documented for brown-headed cowbirds (Molothrus ater; Anderson and Glowa, 1985), Canada geese (Branta canadensis; Frank et al., 1991; Zinkl et al., 1978), American brant (Branta bernicla; Stone and Gradoni, 1985b; Stone and Knoch, 1982), and American wigeon (Anas americana; Kendall et al., 1992). The mass mortality of American brant documented by Stone and Gradoni (1985b) is the largest known incident involving diazinon, where 700 individuals were found dead from an intentional poisoning incident on a golf course in New York. Although diazinon applications to agricultural fields constitutes a relatively small percentage of the reported mortality incidents, it is likely that this category is grossly underreported since such incidents generally are less conspicuous than those that occur on turf farms and golf courses (Stone and Gradoni, 1985a). Mammals generally are less sensitive to diazinon and are also often less conspicuous than birds, thereby making it more difficult to document mammalian mortality due to diazinon exposure. The U.S. Fish and Wildlife Service has determined that certain uses of diazinon, including uses on corn and sorghum, may jeopardize the continued existence of endangered species

(USEPA, 1988).

Chronic toxicity

Little is known about the subchronic or chronic toxicity of diazinon (Eisler, 1986). The few chronic toxicity tests conducted with mammals suggest that daily intake exceeding 5 - 10 mg/kg BW diazinon is probably fatal over time to pigs, Sus scrofa, and dogs, Canis familiaris (Earl et al., 1971). Diazinon (9 mg/kg BW) fed to pregnant lab mice during gestation was associated with significant mortality of pups prior to weaning (Spyker and Avery, 1977). A chronic no-effect level of 0.1 mg/kg BW in the diet was calculated for lab rats (Kenaga, 1979). Chronic effects of diazinon on aquatic or terrestrial organisms in their natural environment or on communities and ecosystems are not presently known (Eisler, 1986).

Toxicokinetics

Animal studies have found rapid absorption of diazinon following oral administration (Iverson et al., 1975; Janes et al., 1973; Machin et al., 1971, 1974; Mucke et al., 1970). Diazinon, like parathion, is largely metabolized to diethylthiophosphoric acid (Iverson et al., 1975; Matsumura, 1985). It can be further oxidized at the side chain, and glutathione (GSH) attaches to the pyrimidyl ring, thereby facilitating GSH S-aryltransferase action (Matsumura, 1985). Diazinon also induces the mixed-function oxidases (MFO) detoxifying enzyme system, which, through desulfuration, forms the immediate metabolite of diazinon, the oxygen analog diazoxon (Eisler, 1986; Matsumura, 1985).

Diazinon can also be oxidized to form diazoxon. No human or animal studies have reported the presence of unchanged diazinon in the urine following exposure, although unchanged diazinon has been detected in animal feces following exposure (Mucke et al., 1970). A variety of polar metabolites have been detected in animal urine and feces, including 2-isopropyl-4-methyl-6-hydroxypyrimidine, dimethyl- and diethylphosphorothioic acids and diethylphosphoric acid (Iverson et al., 1975; Machin et al., 1975; Mucke et al., 1970; Yang et al., 1971). Excretion of diazinon is rapid in the laboratory rat, requiring about 12 hrs. for 50% completion. For either ^{14}C-ring-labeled or ^{14}C-ethyl-labeled compounds, 69 - 80% were excreted in the urine and 18 - 25% in the feces (Mucke et al., 1970).

Effect of formulation on toxicity

Pesticide formulation is another variable involved in the potential toxicity of diazinon. Some formulations of diazinon, particularly emulsifiable formulations, can be converted to much more toxic compounds on contact with air (Gaines, 1969; Gallo and Lawryk, 1991) and UV irradiation (Machin et al., 1971). Some formulations of diazinon contain 0.2 - 0.7% (2000 - 7000 mg/kg) of the compound TEPP (tetraethyl pyrophosphate) as a manufacturing impurity. TEPP is one of the most toxic OP compounds known, having an oral LD_{50} in lab rats of 1 mg/kg BW (Eisler, 1986). Formulations of diazinon contain inert ingredients that affect toxicity. For example, diazinon 4E contains approximately 48% active ingredient, with the remainder consisting of organic solvents such as xylene and ethylbenzene. Xylene and ethylbenzene by themselves are potentially toxic, and both

are on a list of inert ingredients that the EPA strongly encourages pesticide registrants to remove or substitute from their products.

Pesticide effects on communities/ecosystems

Almost no information exists on the short- and long-term effects of diazinon or other OP or carbamate pesticides on communities and ecosystems. The findings of Woodwell (1970) for effects of OC pesticides on ecosystem structure and function probably hold true for anti-ChE insecticides as well, including affecting every trophic level, reducing reproductive capacity, altering behavioral patterns, and disrupting competitive relationships between species thus favoring the generalist (or broad-niched) species. The loss of ecosystem structure involves a shift away from complex arrangements of specialized species toward generalist species, which results in decreased species diversity of plants and animals, decreased nutrient cycle efficiency leading to system nutrient depletion, and a decrease in stability, especially in regard to sizes of populations of small, rapidly reproducing organisms such as insects and rodents. The effects of diazinon on an old-field ecosystem were examined by Malone (1969) and Shure (1971). They found that species diversity, net primary productivity (NPP), total density of vegetation, and species diversity and density of soil microarthropods were reduced in the diazinon-exposed fields. These negative impacts affect rates of succession, decomposition, and nutrient cycling, ultimately affecting the whole old-field ecosystem.

Transfer of diazinon and other OP pesticides in communities and ecosystems may occur through bioaccumulation and biomagnification through food webs, which could

serve to further disrupt community and ecosystem structure and function. Although bioaccumulation and biomagnification of OC pesticides is well-documented, little information exists on these phenomena involving OP and carbamate pesticides. Bioaccumulation of anti-ChE insecticides in prey can result in secondary poisoning (Fleming et al., 1982; Mendelssohn and Paz, 1977; White et al., 1979). Hall and Kolbe (1980) found that parathion and fenthion, and to a lesser extent malathion, acephate, and dicrotophos, were bioaccumulated in bullfrogs (Rana catesbiana) in levels lethal to their avian predator. In mammals, McEwen et al. (1972) found that white-footed mice (Peromyscus leucopus) captured 6-8 days after a diazinon application (5.0-8.0 oz/acre - very low application rate) to shortgrass prairie contained 0.10-0.17 ppm diazinon. Mendelssohn and Paz (1977) showed that an OP insecticide (monocrotophos) applied at two times the recommended label rate can bioaccumulate in rodents at levels high enough to cause significant secondary posioning of avian predators.

Currently, there is little evidence for diazinon or other OP pesticides causing alterations to community and ecosystem structure and function. Barrett and Darnell (1967) found that a field application of the OP insecticide dimethoate had no overall effect on small mammal density, but there was a shift in species composition from omnivores to herbivores which was attributed to a decrease in insect availability. Baker (1986) and Clark and Bunck (1991) found that, through the analyses of barn owl diets over several decades, small mammal communities in the United States have been altered (from mostly insectivorous to mostly herbivorous species), and that the widespread application of anti-ChE pesticides (including diazinon) may play a major role in the

alteration.

Sublethal effects

A major concern in ecotoxicology is the frequency and extent to which organisms may survive the impact of environmental contamination but function less effectively in some way, or suffer sublethal effects (Moriarty, 1988). These sublethal effects can be significant because they increase the likelihood of mortality of the exposed organism. Numerous sublethal effects of diazinon exposure have been demonstrated in the laboratory with various species of birds and mammals (Eisler, 1986). Sublethal effects in mammals have been seen at exposures as low as 0.18 mg/kg BW daily through gestation in pregnant lab mice, 0.5 mg/kg BW for 5 weeks in lab rats, and at single doses of 1.8 mg/kg BW for lab rats and 2.3 mg/kg BW for Peromyscus leucopus (Eisler, 1986; Spyker and Avery, 1977). Exposure to diazinon has been reported to results in reduced daily food consumption/anorexia in lab mice (Spyker and Avery, 1977), ring-necked pheasants (Phasianus colchicus; Stromborg, 1977) and bobwhite quail (Colinus virgianinus; Stromborg, 1981); food avoidance in ring-necked pheasants (Bennett and Prince, 1981); depression of plasma/RBC/brain acetylcholinesterase activity in lab rats (Davies and Holub, 1980a, 1980b; Tomokuni and Hasagawa, 1985), dogs (Iverson et al., 1975), and white-footed mice (Montz, 1983; Montz and Kirkpatrick, 1985a); reduced body temperature (hypothermia) and lowered resistance to cold stress in white-footed mice (Montz and Kirkpatrick, 1985b); altered immune function (Barnett et al., 1980) and decreased number of peripheral blood lymphocytes in lab mice (Lopez et al., 1986);

altered blood chemistry including decreased clotting ability in lab rats (Lox, 1983; Lox, 1987; Lox and Davis, 1983), increased serum B-glucuronidase activity in lab rats (Kikuchi et al., 1981), and altered blood and brain monoamines and amino acids (Rajendra et al., 1986); altered endocrine (adrenal) function in lab mice (Spyker-Cranmer et al., 1978); altered reproductive function (testicular atrophy) in dogs (Earl et al., 1971); decreased productivity (litter/clutch size) in lab mice (Spyker and Avery, 1977), ring-necked pheasants (Stromborg, 1977), bobwhite quail (Stromborg, 1981), and robins (Turdus migratorius; Decarie et al., 1993); decreased reproductive success (% eggs hatching/% nests successful) in mourning doves (Zenaida macroura; Brehmer and Anderson, 1992); delayed sexual maturity (in progeny where the pregnant female was dosed) in lab mice (Spyker and Avery, 1977); impaired endurance and motor coordination in lab mice (Spyker and Avery, 1977); and altered visual acuity in lab rats (Plestina and Piukovic-Plestina, 1978).

Other sublethal effects with possible significance have been documented for a number of other anti-cholinesterase OP and carbamate pesticides, including loss of motor coordination (Clark, 1986), reduced predator escape response (Galindo et al., 1985; Hunt et al., 1992), reduced nest attentiveness during incubation (Grue et al., 1982; King et al., 1984; White et al., 1983), altered immune function (Fan et al., 1978; Street and Sharma, 1975), reduced plasma LH levels (Rattner and Michael, 1985) and altered steroidogenesis (Civen et al., 1980), altered hearing ability (Reischl et al., 1975), decreased daily food and water consumption (anorexia; Costa and Murphy, 1982; Glow et al., 1966), altered neurochemistry and motor and learning abilities (Boyd et al., 1990), decreased ability to

learn (Bignami et al., 1975; Reiter et al., 1973; Russell, 1969), frontal brain lobe impairment (Korsak and Sato, 1977), reduced aggressive behavior (Durda et al., 1989), decreased discrimination behavior (Richardson and Glow, 1967), altered behavior (Kurtz, 1977), decreased serial problem-solving behavior (Banks and Russell, 1967), and spatial memory impairment and central muscarinic receptor loss (McDonald et al., 1988).

Wildlife species recovering from diazinon poisoning may face increased predation, aberrant behavior, learning disabilities, vision and hearing impairment, decreased endurance, motor coordination, and immunocompetence, anorexia, hypothermia, and reproductive impairments (Montz, 1983, Sheffield, ch. 2, ch. 3). There is no data at all on reptilian or amphibian species recovering from diazinon exposure.

Physiological effects

A decrease in body temperature has been found in diazinon-and other OP insecticide-exposed rodents in the laboratory (Ahdaya et al., 1976; Meeter and Wolthuis, 1968; Montz and Kirkpatrick, 1985b). However, Sheffield (ch. 3) found this negative physiological effect under field conditions in three species of wild small mammals exposed to diazinon. A possible correlation may be made between ChE inhibition and decreased body temperature in small mammals, a correlation also seen by Ahdaya et al. (1976). Although overall body temperatures in small mammals from low application rate (1X) and high application rate (8X) enclosures were significantly decreased in relation to control small mammals, the decreases in body temperature generally were small (0.5 to 2.0°C) and not all 1X and 8X animals experienced decreased body temperatures. Body

temperatures can drop dramatically following OP insecticide exposure. Meeter and Wolthuis (1968) and Montz and Kirkpatrick (1985b) found that core temperatures of lab rats exposed to OP insecticides decreased up to 6°C and 4.5°C, respectively, within just a few hours. It is not exactly clear what the effects of decreased body temperature are both at the individual and population levels. Any hypothermic condition in small mammals could potentially cause significant physiological problems, although severity of effects might vary with ambient temperature. There is evidence for enhanced toxicity of OP pesticides during heat and cold exposure in mammals (Chattopadhyay et al., 1982) and birds (Maguire and Williams, 1987; Rattner et al., 1987).

Cholinesterase inhibition

Brain and plasma ChE inhibition has been found in small mammals following exposure to diazinon and its metabolites (particularly diazoxon) in the lab and the field. In the field, the degradation products of diazinon have also been found to inhibit ChE activity (Gallo and Lawryk, 1991). Under controlled experimental conditions in the field (enclosures), plasma ChE activity was found to be significantly depressed at days 2, 16, and 30 post-spray in three wild small mammal species following application of diazinon 4E (Sheffield, ch. 3). In addition, brain ChE activities were found to be significantly depressed in day 30 diazinon-exposed animals. These results indicate that small mammals were exposed to diazinon or its ChE-inhibiting metabolites and degradation products throughout the 30-day field trials. Few studies assessing diazinon exposure using ChE activity in avian species have been completed. In an urban environment,

robins whose nests had been sprayed with diazinon had little change in brain ChE activity, but had significant depression (up to 72%) of plasma ChE activity (Decarie et al., 1993).

In laboratory studies, female lab rats fed just 2 ppm diazinon in their food for 7 days showed significant (29%) plasma ChE depression (Davies and Holub, 1980a). Feeding 25 ppm diazinon for 30 days produced more significant depression of plasma ChE activity (by 22-30%) and brain (by 5-9%) among treated females compared to corrsponding males. Edson and Noakes (1960) found that feeding 25 or 125 ppm diazinon to lab rats caused severe depression in red blood cell (RBC), plasma, and brain ChE activities, and that recovery of ChE activity was appreciably faster in plasma than in RBCs. The presence of 1 ppm diazinon in the rat diet for 16 weeks proved to be apparently harmless, but did result in non-significant (<20%) depression of RBC ChE activity. White-footed mice orally dosed with 18.8 mg diazinon/kg BW had maximal brain ChE activity depression (50.9%) 12 h after dosing, were still significantly depressed at 40 h after dosing, and did not recover to control brain ChE levels until 15 days after dosing (Montz and Kirkpatrick, 1985a). Brain ChE activities of diazinon-exposed female mice were significantly lower than those of corresponding males. In lab studies, female small mammals generally have been found to be more susceptible to ChE inhibition than males (Agarwal et al., 1982; Davies and Holub, 1980a; Montz and Kirkpatrick, 1985a).

Observations using wild rodents (Rattner and Hoffman, 1984; Sheffield, ch.3; Westlake et al., 1982) indicate that plasma ChE activity is a more sensitive index of anticholinesterase insecticide exposure than brain AChE activity. However, the

predictive value of plasma ChE in wild animals is limited because activity is not readily correlated with lethality, recovery is rapid, and activity can also be affected by age, sex, reproductive state, stress, and pathophysiological conditions (Fleming and Bradbury, 1981; Ludke et al., 1975; Rattner and Hoffman, 1984; Rattner, 1982). Sequential, non-lethal measurement of plasma ChE activity has been used successfully for monitoring exposure to diazinon (Sheffield, ch. 3) and other OP insecticides (Fairbrother et al., 1989; Hill and Fleming, 1982), but is not a good indicator of mortality from anti-ChE exposure and may be of less use in the field if many of the experimental animals are not recaptured. Previous studies have demonstrated a marked decrease in small mammal brain ChE activity following field exposure to other OP insecticides (Jett, 1986; Montz et al., 1983; Westlake et al., 1982; Zinkl et al., 1980). Roberts et al. (1988) found that feral S. hispidus and M. musculus were much more sensitive to brain ChE inhibition from methyl parathion exposure than were laboratory rats and mice. Brain ChE activities had recovered in lab rats by day 7 (males) and day 14 (females), whereas activities in male and female S. hispidus had not recovered until day 28.

Pathological effects

Organisms exposed to diazinon may suffer a number of pathological effects which may alter organ function and lead to debilitating health problems and possibly death. Sheffield (ch. 3) found a slight decrease in liver weights in wild S. hispidus exposed in the field to diazinon. Decreased liver weight could potentially impact liver function, including its mechanisms for detoxifying contaminants such as pesticides. Cecil et al.

(1974) found that the OP insecticide malathion had an effect on liver weight and liver lipid and vitamin A content of lab rats. Liver weight was significantly increased in female rats, but decreased slightly in male rats, and lipid and vitamin A content both decreased in female rats and increased slightly in male rats. Montz et al. (1984) found slightly increased liver weights, decreased adrenal weights, and significantly decreased kidney fat indices and perirenal fat pads in female cottontail rabbits (Sylvilagus floridanus) exposed to the OP insecticide parathion.

Significant histopathological changes have been documented in various internal organs of animals exposed chronically to sublethal doses of diazinon. Earl et al. (1971) found histopathology of liver and intestinal tract, including duodenal ulcers in pigs and hemorrhaging in the small intestine, occasional rupture of intestinal wall, and testicular atrophy in dogs exposed to diazinon. At a dosage level of 10 mg diazinon/kg BW/day, the liver was yellow and fatty in appearance and microscopic examination revealed cirrhosis. Other organs showed some degree of atrophy confirmed by histopathology. Sublethal doses of diazinon have also been documented to cause acute pancreatitis in dogs and guinea pigs (Frick et al., 1987) and cellular damage and necrosis of hepatocytes in livers of laboratory rats (Anthony et al., 1986; Dikshith et al., 1975). The acute pancreatitis is thought to result from the inhibition of pancreatic butyrylcholinesterase (BChE), leading to cholinergic hyperstimulation of the acinar cells. In the liver, a significant increase in lipid peroxidation causes cellular lipid accumulation, leading to necrosis of affected parencymal cells. The pups of lab mice that received diazinon orally at a rate of 9.0 mg diazinon/kg BW/day during pregnancy had significantly small adrenal

glands (Spyker-Cranmer et al., 1978). When lab rats received a single intraperitoneal injection of 21.6 mg diazinon/kg BW, degenerative changes were found in the liver and necrosis, edema, and reduction of tubular size were found in the testes (Dikshith et al., 1975). Immunological effects over the lifespan of lab mice exposed in utero to diazinon have been demonstrated (Avery et al., 1981; Barnett et al., 1980).

Reproductive effects

One of the most important parameters for assessing the sublethal effects of pesticides appears to be to what degree they affect the reproductive processes (Matsumura, 1985). Negative effects on reproduction can impact recruitment, population density, long-term population stability, and ultimately affect other co-existing populations in the community.

In the lab, it has been demonstrated that female rats and dogs are more sensitive to diazinon than males (Davies and Holub, 1980a, 1980b; Earl et al., 1971). Following field exposure to diazinon, Sheffield (ch. 2, 3) found that females of three species of wild small mammals (Sigmodon hispidus, Microtus ochrogaster, and Reithrodontomys fulvescens) were generally more sensitive to diazinon exposure as measured by reproductive activity. This finding has been seen using other OP insecticides as well. Agarwal et al. (1982) found that female lab rats were more sensitive to the ChE inhibiting effects of parathion than were male rats. Administration of testosterone to castrated males and ovariectomized females led to recovery from increased sensitivity to parathion and indicated that testosterone played an important role in determining parathion toxicity (as reflected by ChE activity). It may be that the presence of high testosterone levels in

males conveys a detoxification advantage to males over females that have only low levels of testosterone.

Several recent studies have clearly demonstrated that OP insecticides can potentially negatively impact reproduction in mammals. However, few studies have examined the potential reproductive effects of diazinon exposure in wildlife species. Sheffield (ch. 2, 3) found significantly depressed reproductive activity in males of three wild small mammal species following field exposure to diazinon. In addition, weights of testes, epididymides, and seminal vesicles were found to be slightly decreased in diazinon-exposed males (Sheffield, ch. 2, 3). In male rodents, other OP insecticides have been shown to cause numerous alterations in the testes which would subsequently impact reproduction. Mathew et al. (1992) found that the OP insecticide parathion, administered in the diet of lab mice, induced sperm shape abnormalities. Chou and Cook (1994) found that paraoxon inhibited in vitro fertilization of gametes in lab mice. Sperm motility was not affected, but capacitation of sperm was altered. Chou and Cook (1995) subsequently found that acetylcholine prevents the inhibition of fertilization. Alterations of the seminiferous epithelium and Leydig cells of testes has been found in lab rats exposed to the OP insecticide dichlorvos (Krause and Homola, 1974). A decrease in testicular sperm density, steroidogenesis, and enzyme activity, along with damage to the spermatogenic cells was found in testes of lab mice subchronically orally exposed to the OP insecticide phosphamidon (Bhatnagar and Soni, 1990).

It has been shown that diazinon can cause severe negative effects on the female reproductive system, particularly during pregnancy. Spyker and Avery (1977) found

significant mortality of pups prior to weaning in pregnant lab mice fed 9 mg/kg BW diazinon during gestation. Sheffield (ch. 2, 3) found that reproductive activity (as measured by pregnancy, lactation, and vaginal condition) was significantly depressed in diazinon-exposed females of three wild small mammal species. Further, reproductive productivity was significantly reduced in these females as well. In all three species, diazinon exposure resulted in a significant reduction in the number of females giving birth as seen by numbers of recent placental scars in the uterus. In S. hispidus and M. ochrogaster, the number of females with embryos also decreased significantly in diazinon-exposed animals, and in female M. ochrogaster, the number of embryos was found to decrease significantly in diazinon-exposed animals. Other OP insecticides have been found to cause severe negative effects on the female reproductive system. Fish (1966) found that lab rats exposed to single doses of either DFP, parathion, or methyl parathion resulted in maternal weight loss and toxicity, embryonic ChE inhibition, an increase in stillbirths and neonatal deaths accompanied by a reduction in juvenile weight gain. However, incidence of fetal abnormalities and fetal deaths, fetal weight, and average birth weight were unaffected. Slightly decreased uterine and ovarian weights, mean number of embryos per female, and significant decrease in stage of pregnancy were found in cottontail rabbits (Sylvilagus floridanus) exposed to parathion (Montz et al., 1984). In addition, placental transfer of OP's (malathion) and bioconcentration in fetal tissues has been shown in lab rats (Ackermann and Engst, 1970; Gustave et al., 1994). The OP insecticide ethion was found to concentrate in the milk of goats, >10 times higher than that in plasma after i.v. exposure and > 20 times higher than that in plasma after

dermal exposure (Mosha et al., 1991).

Little information is available regarding the effects of diazinon on reproductive hormones. However, several other OP insecticides have been found to alter reproductive hormones. Civen et al. (1977) found that lab rats exposed to the oxon metabolites of dichlorvos and chlorpyrifos had reduced cholesteryl esterification, an important step in the production of steroid hormones, and hydrolysis of cholesteryl esters. Rattner and Michael (1985) found that Peromyscus leucopus exposed to acephate exhibited reduced plasma LH titres after being dosed by oral gavage, although not after dietary exposure producing similar AChE inhibition. Ray et al. (1991, 1992) found that male lab rats exposed to quinalphos over 13 days had reduced levels of plasma FSH and those exposed to quinalphos over 26 days had reduced levels of plasma LH and testosterone. These hormonal changes were accompanied by decreased testicular testosterone and weight, increased spermatid degeneration, and reduced total sperm counts (up to 63%). 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.

Diazinon is not known to be a teratogen in mammals, but is a potent teratogen in birds (Eisler, 1986). Diazinon was found to produce visible Type I and II deformities when injected into ckicken embryos (Misawa et al., 1981, 1982; Wyttenbach and Hwang, 1984). Diazinon adversely affected survival of developing mallard embryos when the eggshell surface was subjected for 30 sec to concentrations 25 - 34 times higher than

recommended field application rates (Hoffman and Eastin, 1981). However, no Type I deformities, significant differences in egg viability or hatchability, and only slightly decreased chick immunocompetence were seen in northern bobwhites exposed as embryos in the field to two different recommended field application rates of diazinon (Dabbert et al., 1996). Diazinon, administered orally between the 5th and 15th day of gestation, was not found to be teratogenic in either the rabbit (at 7 or 30 mg/kg) or the hamster (at 0.125 or 0.25 mg/kg) even though severe cholinergic signs, death, and decreased average fetal weights were seen in rabbits at 30 mg/kg (Robens, 1969). OP insecticides have generally been found to be embryotoxic and fetotoxic in lab rodents, including diazinon (Robens, 1969), azinphosmethyl (Short et al., 1980), chlorpyrifos (Deacon et al., 1980), methyl parathion (Tanimura et al., 1967), and dimethoate and fenthion (Budreau and Singh, 1973). Prenatal exposure to diazinon may produce subtle dysfunctions that appear later in life (Spyker and Avery, 1977). Diazinon fed to pregnant mice resulted in offspring with slower growth rates, forebrain neuropathology, delayed sexual maturity, impaired endurance and motor coordination, slower running speeds, and adverse behavioral modifications that did not become apparent until later in life (Spyker and Avery, 1977).

Wildlife Species as biomonitors

Many wildlife species have served as biomonitors, or sentinels, of exposure and subsequent effects from field applications of OP insecticides. However, differences in behavior, foraging habits, and habitat could affect routes and degree of exposure, and thus

render some species more vulnerable to OP insecticide exposure in the field (Rattner and Hoffman, 1984). It has been shown that lab mice and wild mice were equally sensitive to the OP insecticide acephate when maintained under uniform laboratory conditions (Rattner and Hoffman, 1984). On the other hand, Meyers and Wolff (1994) found that lab mice were not representative of deer mice or gray-tailed voles with respect to sensitivity to the OP insecticide azinphosmethyl, but provided a conservative estimate for risk assessment. Roberts et al. (1988) found that wild rodents (Sigmodon, Mus) were more susceptible to toxicity and ChE inhibition by methyl parathion than lab rodents (Mus, Rattus). Cholakis et al. (1981) found that lab rodents appeared to be more susceptible than wild rodents to 6 of the 10 pesticides tested, but also found differences in sensitivity to pesticides between vole species (Microtus sp.). With little data available, it is not at all clear as to whether wild rodent populations are more susceptible or resistant to pesticide exposure than lab rodents. However, there are considerable limitations in the use of lab rodents in toxicological studies which attempt to predict toxicant-induced effects on ecological systems (Schaeffer and Beasley, 1989). In birds, little comparative data are available. Hill et al. (1984) found that the acute toxicity of diazinon was similar for northern bobwhite from eight different game farms in the United States.

Conclusions

Exposure to the OP insecticide diazinon has been shown to negatively affect small and medium-sized mammals and avian species in a laboratory setting, and has been shown to negatively affect small mammal and avian populations in the field. In addition, there is

some evidence that small mammal communities in the field may be impacted by diazinon exposure. Little work has been done examining exposure and effects of diazinon on reptilian or amphibian species. The responses of small mammal, avian, or other wildlife communities to multiple applications of diazinon or a combination of diazinon and other pesticides remains to be studied in a replicated field experiment. I hypothesize that under conditions of multiple applications of diazinon or a combination of diazinon and other pesticides, negative impacts would be more severe and responses would likely be more pronounced and prolonged. Since diazinon and other OP insecticides are widely used throughout the world, continued characterization of exposure and toxicity of these compounds is necessary in order to better evaluate the hazards they pose to wildlife and humans.

Although many studies have examined diazinon effects on lab animals in the laboratory, relatively few studies have done this with wild species in the field. Lab studies have the advantage of control of many variables, but cannot come close to mimicking realistic exposures and physical conditions (climatic factors), and cannot examine effects at higher levels of ecological organization (population, community, ecosystem). Further controlled field and mesocosm studies (e.g., Sheffield, ch. 2, ch. 3) are necessary to more precisely assess the impact of diazinon exposure on animal populations and communities (Hoffman et al., 1990; Sheffield, ch. 2, ch.3).

Sublethal effects can serve as biomonitors of environmental contamination, elucidate mechanisms of action of a contaminant, and provide signs of contaminant exposure, all assisting us in predicting negative impacts of diazinon and other anti-ChE insecticides

and other contaminants on wildlife populations. More studies that document sublethal effects of diazinon in the field and provide proof that these effects can lead to death and reproductive impairment in wild animal populations are badly needed (Heinz, 1989).

Avian species appear to be the most sensitive terrestrial vertebrate species to diazinon exposure. Little work has been done in the field with wild avian species or small mammals in the ecological risk assessment of diazinon. It appears that upland gamebirds and waterfowl appear to be the most sensitive groups of birds and that rodents are the most sensitive group of mammals yet tested with diazinon, and these groups have proven to be sensitive to other OP and carbamate insecticides as well. The validity of using domesticated species in a laboratory environment to represent wild species in a field situation is highly questionable (Schaeffer and Beasley, 1989).

It seems essential to human, wildlife, and ecosystem health to re-examine the continued widespread use of the OP insecticide diazinon, and to more closely scrutinize recommended uses and label application rates and pesticide registration and re-registration procedures of many of diazinon due to its high acute toxicity and potential for debilitating sublethal effects and subsequent effects on populations, and perhaps communities, of non-target organisms. In that regard, more work needs to be done to fine-tune the EPA's hazard assessment for diazinon, including the Quotient Method (QM) to more accurately characterize realistic exposure to diazinon and other anti-ChE insecticides in the field (Urban and Cook, 1986). We urgently need to examine further the effects of multiple and chronic exposure to low levels of diazinon and other widely used anti-ChE insecticides in all taxa, but particularly in mammals, so that we can obtain

a better understanding of how these compounds are affecting humans.

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CHAPTER 2

EFFECTS OF FIELD EXPOSURE TO DIAZINON ON SMALL MAMMALS INHABITING A SEMI-ENCLOSED PRAIRIE GRASSLAND ECOSYSTEM

I. ECOLOGICAL AND REPRODUCTIVE EFFECTS

EFFECTS OF FIELD EXPOSURE TO DIAZINON IN SMALL MAMMALS INHABITING A SEMI-ENCLOSED PRAIRIE GRASSLAND ECOSYSTEM

I. ECOLOGICAL AND REPRODUCTIVE EFFECTS

Abstract-The widespread use of anti-cholinesterase (anti-ChE) pesticides in the environment presents increasing concerns about their effects on human, wildlife, and ecosystem health. As a group, they are generally highly toxic and have great potential for negatively affecting non-target organisms. Using 12 0.1-ha terrestrial enclosures, we examined the effects of low-level diazinon exposure on small mammal communities consisting of Sigmodon hispidus, Microtus ochrogaster, Reithrodontomys fulvescens, and Mus musculus inhabiting semi-enclosed grassland ecosystems. Our primary objective was to examine potential ecological and reproductive effects resulting from exposure to relatively low levels of diazinon in small mammals inhabiting a controlled field mesocosm. Diazinon 4E was applied at two different maximum recommended label application rates, 0.5 lbs a.i./acre (1X) and 4.0 lbs a.i./acre (8X), and controls remained unsprayed, with four enclosures (replicates) per treatment. Two 30-day experimental trials were conducted during peak rodent breeding seasons and enclosures were trapped on days 2, 16, and 30 of each trial. Survival of small mammals was not significantly different among treatments, although fewer animals were recovered from the diazinon-exposed enclosures in both trials. Trapping data suggested that the normally strong competitive relationship between Sigmodon and Microtus may have been altered by

diazinon, favoring Microtus in the diazinon-exposed enclosures. Reproductive activity of males and females was found to be reduced 20 - 80 % and 33 - 100%, respectively, following diazinon exposure. The percentage of females becoming pregnant during the 30-day trials was significantly reduced in diazinon-exposed animals (13.6 - 43.5%) compared to control animals (40 - 80%). Generally, the effects seen suggested that diazinon was relatively persistent in the sprayed enclosures and that diet, possibly through the consumption of dead and dying arthropods, may have been an important route of exposure. 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. Negative impacts on community structure and function may persist longer than the pesticide persists in the environment.

Keywords- Diazinon, Small Mammals, Ecological Effects, Reproductive Effects, Mesocosm, Terrestrial Ecotoxicology

INTRODUCTION

Anti-cholinesterase (anti-ChE) pesticides (including organophosphate (OP) and carbamate pesticides) have been the most widely used classes of pesticides in North America, which has resulted in concern over their possible effects on humans, wildlife species and ecosystems (Brown, 1978; Grue et al., 1983; Smith, 1987). Currently, there are more than 100 different anti-ChE chemicals registered as the active ingredient in thousands of different pesticide products in the United States. Total pesticide usage in the U.S. was estimated at about 2.2 billion pounds of active ingredient in 1993 (Aspelin, 1994). A relatively small percentage of this amount may actually hit target species, thereby resulting in exposure of non-target organisms and movement to surrounding ecosystems (Pimentel and Edwards, 1982). Although OP and carbamate pesticides are relatively less persistent in the environment than organochlorine (OC) pesticides and rarely bioaccumulate in food chains, they generally are more acutely toxic and lack target specificity, tending to exert a potentially more widespread effect on non-target organisms (Brown, 1978).

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 (Grue et al., 1983). 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 (Eisler, 1986; Grue et al., 1983; Shore and Douben, 1994).

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 (Mineau, 1991), including studies with carbaryl (Barrett, 1968; Pomeroy and Barrett, 1975), dimethoate (Barrett and Darnell, 1967), malathion (Giles, 1970), and azinphosmethyl (Edge et al. in press, Schauber et al. in press). These studies have documented mild to severe reductions in population size, inhibited reproduction, and increased population turnover rates, leading to alterations in the structure of small mammal communities.

Diazinon (O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate) is a broad spectrum organophosphate (OP) insecticide that is widely used in agriculture, range, commercial, and home and garden settings for the control of a wide variety of insect, acarine, and nematode pests (Eisler, 1986). Diazinon has been registered for use since 1952, and since 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 (Aspelin, 1994; Gianessi and Anderson, 1995). Diazinon was chosen for the study due to its widespread usage, which has resulted in an alarming increase in diazinon concentrations in all environmental media (Agency for Toxic Substances and Disease Registry, 1996), and its high toxicity to a wide variety of organisms, potential for persistence in the environment, and our relative lack of data regarding its effects 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 (Eisler, 1986).

There have been relatively few field experimental studies that have examined short- or long-term effects of pesticides or other contaminants on natural ecosystems (Pimentel and Edwards, 1982). Controlled field and mesocosm studies are necessary to more precisely assess the impact of pesticide exposures on mammalian populations and communities (Hoffman et al., 1990). Mesocosms have been shown to be useful in examining potential effects of pesticides and other environmental contaminants on experimental organisms (Crossland, 1994; Crossland and LaPoint, 1992; Gillett, 1989; Kimball and Levin, 1985; Odum, 1984) because they provide realism that is not possible in the lab and allow for control of certain experimental parameters (e.g., movements, predation, food resources, community structure, density). In addition, replication and simultaneous investigation of populations, communities and ecosystems are possible. Further, mesocosms allow the accurate analysis of reproductive effects, something that cannot be done in open study areas. Therefore, the experimental design approach of this study was to conduct replicated field exposures (subchronic) under the controlled conditions of a mesocosm to test the effects of diazinon on experimental small mammal communities.

The objective of this study was to measure potential ecological and reproductive effects of subchronic field exposure to the OP insecticide diazinon in wild small mammals living in a controlled prairie grassland ecosystem. Specifically, this study

evaluates the responses of experimental small mammal communities to diazinon exposure, including survival, behavioral interactions (recapture rates, trappability), and reproductive activity and 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. Vegetation in the study area consisted mainly of grasses (little bluestem Schizachyrium scoparium, Indiangrass Sorghastrum nutans), forbs (coralberry Symphoricarpos orbiculatus) and woody shrubs (smooth sumac Rhus glabra, sandplum Prunus angustifolia). Small mammals that occur locally in this habitat include least shrews (Cryptotis parva), short-tailed shrews (Blarina hylophaga), hispid cotton rats (Sigmodon hispidus), prairie voles (Microtus ochrogaster), pine voles (Microtus pinetorum), fulvous harvest mice (Reithrodontomys fulvescens), plains harvest mice (Reithrodontomys montanus), deer mice (Peromyscus maniculatus), white-footed mice (P. leucopus), eastern woodrats (Neotoma floridana) and house mice (Mus musculus). The area had no prior history of pesticide use but was subjected to a controlled burn in Spring 1990.

Enclosure design and construction

A series of 12 0.1-ha enclosures (32 m x 32 m) were constructed of galvanized sheet metal (1.27 m above ground, 0.15 m below ground). Mowed strips inside (0.75 m) and

outside (1.5 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, climbing) along the walls.

Experimental animals

Experimental small mammal communities consisted of Sigmodon hispidus, Microtus ochrogaster, Reithrodontomys fulvescens, and Mus musculus. Animals chosen for the study consisted of wild-caught individuals of the desired species, sex, and age from nearby grasslands habitats. All small mammals were held in captivity for varying periods of time (1 - 90 d) prior to each trial.

Sigmodon hispidus generally is the most numerous rodent in the grasslands of the southern Great Plains, and is predominantly herbivorous, but is known to eat arthropods, seeds, and soil on occasion (Cameron and Spencer, 1981; Garten, 1980). R. fulvescens is omnivorous, eating plant material, especially seeds, as well as insects and other invertebrates. M. musculus, although an exotic, coexists with cotton rats and fulvous harvest mice in areas where they have become feral. M. musculus is omnivorous, although they tend to eat more insects and other animal matter than most omnivores. M. ochrogaster is mainly herbivorous, but will eat arthropods in warmer months when available. S. hispidus, M. ochrogaster, and R. fulvescens naturally coexist together and comprise the major component of the small mammal community in prairie grassland habitats in northern Oklahoma (Caire et al., 1990; Grant and Birney, 1979). The strong competitive relationships between S. hispidus and R. fulvescens (Cameron and Spencer,

1981; Spencer and Cameron, 1982) and S. hispidus and M. ochrogaster (Glass and Slade, 1980a, 1980b; Stalling, 1990; Terman, 1974; Terman and Johnson, 1971) are well-documented. Gestation periods for these species range from 21-27 days (Cameron and Spencer, 1981; Stalling, 1990) and placental scars normally remain in the uterus 6-7 weeks (Corthum, 1967).

Field methodology

A completely randomized split plot design was employed for the group of 12 enclosures, which allowed for four replicates of each of three experimental treatments (0X = control, 1X = 0.5 lbs. active ingredient (a.i.)/acre, and 8X = 4.0 lbs. a.i./acre). 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 5 x 4 matrix with two Sherman live traps at each station (40 traps/enclosure). Traps were baited with rolled oats, set near dusk, and checked early the following morning. All trap doors were kept shut during non-trapping days. In addition, six pieces of sheet metal, approximately 1.0 m x 1.3 m in size, were placed in each enclosure to provide cover for nesting sites. Prior to each trial, we conducted removal trapping in each enclosure for 14 days to ensure that enclosures were free of non-experimental animals. Small mammals were released into enclosures 3 - 5 h prior to spraying at densities similar to densities of natural populations. During trial 1 (31 July - 1 September 1993), each enclosure contained 12 S. hispidus (120/ha), 5 M. musculus (50/ha), and 3 R. fulvescens (30/ha) at the start (day 0). During

trial 2 (9 June - 10 July 1994), each enclosure contained 12 S. hispidus (120/ha), 12 M. ochrogaster (120/ha), and 5 R. fulvescens (50/ha) at the start. At least one pregnant female S. hispidus was included in each enclosure in both trials. Prior to their release into enclosures, animals were toe-clipped for identification, and we recorded weight, reproductive condition, and general condition (e.g., ectoparasites, overall health). Both experimental trials were conducted during peak small mammal breeding seasons.

Diazinon 4E (4 lbs/gal), an emulsifiable liquid formulation consisting of 47.5% active ingredient (Estes Chemical Co., Oklahoma City, OK), was mixed with water and applied to the experimental mesocosms at a low (0.5 lbs. a.i./acre = 1X) or high (4.0 lbs. a.i./acre = 8X) maximum recommended field application rate and was compared to controls (no spraying = 0X). The two 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 CO_2 powered backpack unit with 1.83-m boom at a constant rate under 40 lbs pressure using 20 gal H_2O/acre, and applied as close to the ground (<0.5 m) as possible. The exposure scheme using diazinon was that of a "pulse", or one-time, exposure (Bender et al., 1984), applied at the start of each trial and terminating on day 30 following diazinon application, the approximate half-life of diazinon in the environment (Bartsch, 1974; Wauchope et al., 1992).

During each trial, we trapped animals on days 2, 16 and 30 post-spray 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 activity, and body weight (nearest 0.5 g except for S. hispidus (1 g). For

each species, capture days were recorded, and survival rate (percent of marked animals recaptured at day 30/total number of marked animals at day 0), recapture rate (percent of marked animals captured/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 was defined as the percentage of scrotal males or the percentage of females with a perforate vagina, pregnant, or lactating (McCravy and Rose, 1992). Pregnancy was determined through a combination of visual inspection and palpation. Following the day 30 sampling, we trapped for an additional 14 days to insure that all individuals that survived were recorded.

All animals caught on day 30 were returned to the laboratory, and euthanized through a heavy inhalation dose of Metofane (Pitman-Moore, Inc., Mundelein, IL) followed by cervical dislocation, and necropsied. The percentage of breeding females (pregnant or had given birth during the trial) was determined by examination of uteri for embryos and recent placental scars. 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

Differences in survival, recapture rate, trappability, reproductive activity and productivity were tested using a repeated measures analysis of variance using PROC GLM (SAS Institute, Inc., 1990) among replicated treatments (control, 1X, 8X) for each

species at each sampling day. Trials were not temporal replicates of each other and were analyzed independently. 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 (SE) for treatment effects are presented; a significance level of $P < 0.05$ was used for all comparisons.

RESULTS

Small mammal survival

There was no evidence of acute mortality in any species following 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 post-spray, 45, 39, and 30 animals were recovered from control, 1X, and 8X enclosures, respectively, for a total recovery of 114 animals out of 224, or 50.9% (Fig. 1). Over the three trapping days for all species, 108, 104, and 94 animals were trapped from control, 1X, and 8X enclosures, respectively. Although fewer S. hispidus and R. fulvescens and more M. musculus were trapped from diazinon-exposed enclosures, none of these differences were statistically significant for any species. Overall survival totals for trial 1, and percentage of those that survived, 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 post-spray, 91, 79, and 77 animals were recovered from control, 1X, and 8X enclosures, respectively, for a total recovery of 247 animals out of 346, or 71.4% (Fig. 1). Over the three trapping days for all species, 177, 170, and 161 animals were captured from control, 1X, and 8X enclosures, respectively. Survival rate was lower for S. hispidus from diazinon-exposed enclosures overall ($P<0.087$), with significant differences between control and 1X enclosures ($P<0.049$) and nearly significant differences between 1X and 8X enclosures ($P<0.063$). Survival rate was higher for M. ochrogaster from diazinon-exposed enclosures overall ($P<0.008$) and between control and 8X enclosures ($P<0.003$) but was not significant between 1X and 8X enclosures ($P<0.323$). No differences in survival rate for R. fulvescens from diazinon-exposed enclosures was seen overall ($P<0.600$) or between control and 1X enclosures ($P<0.432$) and control and 8X enclosures ($P<0.362$). 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 on days 2, 16, and 30 post-spray (Fig. 1). Recapture rates for S. hispidus were highest in 8X animals and decreased slightly in 1X animals and again in 0X animals at both days 16 and 30. Trappability generally decreased in diazinon-exposed S. hispidus, with differences between control and diazinon-exposed (8X) animals ranging from 12 - 23%. Due to escapes, there was not enough trapping success for M. musculus or R.

fulvescens to calculate recapture rates or trappability with confidence. No R. fulvescens were trapped from 8X enclosures after day 2. No significant differences in trapping sex ratios between control, 1X, and 8X enclosures were seen. For S. hispidus, females outnumbered males in 1X and 8X enclosures and males outnumbered females in control enclosures. For both R. fulvescens and M. musculus, the opposite trend was seen.

In trial 2, behavioral interactions and effects between small mammal species, as reflected by trap occupancies, were more apparent. Significantly more S. hispidus were trapped than were M. ochrogaster or R. fulvescens on days 2 and 16, but approximately equal proportions of each species were trapped at day 30, but only after the first night of trapping removed many of the S. hispidus (Fig. 1). The ratio of M. ochrogaster to S. hispidus taken was increased significantly in the diazinon-exposed 1X and 8X enclosures on days 2, 16, and 30 post-spray (Fig. 2). In control enclosures, ratios of trapped S. hispidus to M. ochrogaster ranged from 3.5:1 - 4.4:1, whereas in 1X and 8X diazinon-treated enclosures ratios ranged from 1.7:1 - 2.9:1 and 1.3:1 - 1.6:1, respectively. Recapture rates for S. hispidus were lowest in 8X enclosure animals at both days 16 and 30, ranging from 11 - 39% less than in control animals. Recapture rates for M. ochrogaster and R. fulvescens were higher in diazinon-exposed animals than in control animals at both days 16 and 30, with differences ranging from 89 - 430% for M. ochrogaster and 291 - 332% for R. fulvescens. Trappability of S. hispidus generally decreased sharply in diazinon-exposed animals, with differences between diazinon-exposed animals and control animals ranging from 11 - 35%. In contrast, 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 - 81% and 103 - 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

A. Reproductive activity

Over both trials, reproductive activities of diazinon-exposed males and females of all species generally decreased sharply when compared to control animals. At the start of each trial, there were no significant differences between treatments in the percentage of reproductively active males or females of any species. Reproductive activity of diazinon-exposed male S. hispidus showed a significant decrease in both trials 1 ($P<0.004$) and 2 ($P<0.0008$) when compared to controls (Fig. 3). Significant differences in male activities were apparent at day 16 and 30 post-spray throughout both trials. Reproductive activity of diazinon-exposed female S. hispidus decreased significantly in both trials 1 ($P<0.002$) and 2 ($P<0.006$) when compared to controls (Fig. 4). Differences in female reproductive activity were apparent as early as day 2 post-spray, and these differences persisted throughout both trials 1 and 2 (Fig. 4).

In M. ochrogaster, significant declines were observed in reproductive activities of diazinon-exposed males ($P<0.01$) and females ($P<0.0001$) when compared to controls (Figs. 3, 4). Declines in reproductive activity in diazinon-exposed males and females were apparent at day 16 (40.0 - 50.0% decline in 1X animals, 80.0 - 100% decline in 8X animals when compared to controls), and activity continued to be severely reduced (39.1

- 50.8% decline in 1X animals, 70.9 - 81.6% decline in 8X animals when compared to controls) on day 30 post-spray. In R. fulvescens, the declines in reproductive activity in diazinon-exposed males were apparent and nearly significant ($P<0.061$; Fig. 3). Significant declines in reproductive activity were observed in diazinon-exposed females ($P<0.001$) when compared to control animals (Fig. 4). Little reproductive activity was observed in either male or female R. fulvescens captured in the high rate (8X) enclosures throughout both trials.

B. Reproductive productivity (females)

There was a significant reduction observed in percent of breeding females and percent of females giving birth in diazinon-exposed female S. hispidus during both trials 1 and 2 (Table 1). Mean number of embryos per female were increased in diazinon-exposed female S. hispidus in trial 1, but showed no pattern in diazinon-exposed female S. hispidus in trial 2 (Table 1). The percent of breeding females declined in diazinon-exposed animals (20.0% in 1X and 25.0% in 8X animals in trial 1; 26.3% in 1X and 13.6% in 8X animals in trial 2) when compared to control animals (40.0% in trial 1, 43.4% in trial 2; Table 1). The percent of diazinon-exposed female S. hispidus giving birth during trials 1 and 2 declined significantly (10.0% in 1X and 0.0% in 8X animals in trial 1; 10.5% in 1X and 9.1% in 8X animals in trial 2) when compared to control animals (40.0% in trial 1, 21.7% in trial 2; Table 1). These findings represented a significantly lower ($P<0.001$ in trial 1, $P<0.003$ in trial 2) female reproductive productivity in diazinon-exposed S. hispidus.

In M. ochrogaster and R. fulvsecens in trial 2, a significant reduction in the percent of breeding females and females giving birth was observed in diazinon-exposed females (Table 1). The percent of breeding female M. ochrogaster significantly declined in diazinon-exposed animals (43.5% in 1X and 30.4% in 8X animals) when compared to control animals (80.0%; Table 1). The percent of female M. ochrogaster giving birth during the trial was significantly lower in diazinon-exposed animals (17.4% in 1X and 13.0% in 8X enclosures) when compared to control animals (50.0%; Table 1). A non-significant decline in mean number of embryos/female was seen in diazinon-exposed females. The percent of breeding female R. fulvescens significantly declined in diazinon-exposed animals (33.3% in 1X and 14.3% in 8X animals) when compared to control animals (57.2%; Table 1). The percent of females giving birth during the trial also was significantly lower in diazinon-exposed animals (0.0% in 1X and 8X enclosures) when compared to control animals (28.6%; Table 1). These findings represented a significantly lower female reproductive productivity in diazinon-exposed M. ochrogaster ($P<0.001$) and R. fulvescens ($P<0.01$).

DISCUSSION

Ecological effects on small mammal populations/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. Escapes from assigned enclosures by R. fulvescens and M. musculus occurred, with movements mainly from sprayed enclosures to control enclosures. The relatively poor recovery of R.

fulvescens and M. musculus prevented us from examining the impacts of diazinon on their individual populations or on community interactions in trial 1. Overall, generally fewer S. hispidus and R. fulvescens and more M. musculus were taken in the diazinon-exposed 1X and 8X enclosures. 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. 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 consistently less trappable in the diazinon-exposed enclosures, but showed an increase from day 2 to day 30.

Recoveries of S. hispidus, M. ochrogaster, and R. fulvescens was substantially greater in trial 2 and allowed an examination of interactions among species in the small mammal community. Generally, fewer animals were recovered from diazinon-exposed 1X and 8X enclosures than from control enclosures. Significantly more M. ochrogaster and R. fulvescens and significantly fewer S. hispidus were captured in the diazinon-exposed 1X and 8X enclosures. It appeared that the normally strong competitive relationship between the herbivorous S. hispidus and M. ochrogaster may have been altered in the diazinon-exposed enclosures. Recapture rates and trappability were decreased for S. hispidus and increased for M. ochrogaster and R. fulvescens in diazinon-exposed 1X and 8X enclosures, suggesting that competitive interactions were not as prevalent. Generally, dominant species and individuals are more readily trapped in Sherman traps, as they mark

traps in their territory and tend to keep other species away (Wilson et al., 1996). In control enclosures, more S. hispidus and fewer M. ochrogaster were trapped on days 2, 16, and 30 post-spray, indicating normal dominance behavior.

Currently, few studies have examined the impact of any anti-ChE pesticide on structure or function at the community or ecosystem levels. Diazinon has been found to impact rates of succession, decomposition, and nutrient cycling, and to decrease species diversity and density of soil microarthropods in an old-field ecosystem (Malone, 1969; Shure, 1971). Barrett and Darnell (1967) found that a field application of the OP insecticide dimethoate had no overall effect on small mammal density, but it caused a shift in species composition from omnivores to herbivores which was attributed to a decrease in insect availability. Pomeroy and Barrett (1975) and Barrett (1988) found that small mammal population composition remained altered for several months following an application of the carbamate insecticide carbaryl, 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. 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 (Barrett, 1988). Densities of M. musculus in were 50-75% higher than controls, whereas densities of M. pennsylvanicus were 50-140% smaller than controls following carbaryl application. Trapping efficiencies did not differ significantly between treatments for either community type examined, although Mus was recaptured nealy twice as much as Microtus within both community types (Barrett, 1988). 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 (Pomeroy and Barrett, 1975). Baker (1986) and Clark and Bunck (1991) found that, through analyses of barn owl diets, small mammal communities have changed favoring herbivorous over insectivorous small mammals in the United States over the later half of the twentieth century, possibly through the widespread application of pesticides.

Survival rate was not profoundly affected by low-level diazinon exposure in this study, although some evidence for reduced survival was evident for S. hispidus in trial 2. Morris (1972) found that endrin caused more than 50% mortality in experimental Microtus pennsylvanicus populations immediately after treatment. Recruits entering the experimental population during post-spray periods survived significantly better than young entering the more crowded control population. This increased survival, combined with active post-spray breeding, yielded a final experimental population which significantly exceeded the control. Edge et al. (in press) found that enclosed populations of gray-tailed voles (Microtus canicaudus) responded in a dose-dependent manner to a single application of the OP insecticide azinphosmethyl. 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 azinphosmethyl-exposed animals. Schauber et al. (in press) found that azinphosmethyl applied at 3.61 kg/ha caused reduced recruitment, survival and body growth in M. canicaudus, resulting in vole densities <40% of control densities, that population recruitment and growth rates of P. maniculatus in mowed enclosures was also significantly reduced, and that these conditions persisted over

6 weeks post-spray.

Reproductive effects

The reproductive endpoints examined in this study (reproductive activity, productivity) proved to be the most sensitive endpoints of diazinon exposure in our experimental trials. Both trials were conducted during peak breeding season as indicated by the high levels of reproductive activity of males and females from control enclosures. Reproductive activity in all small mammal species of both sexes generally decreased significantly or increased slower from initial or control activity levels in diazinon-exposed animals during the two trials. The negative impact of diazinon on reproductive activity was most striking for M. ochrogaster. Activities for males and females reached 100% by day 16 in control populations, but remained low in diazinon-exposed populations throughout trial 2. Differences in reproductive activity were also reflected in the percentage of breeding females as revealed by necropsy for all small mammal species.

Reproductive productivity in females of all small mammal species was significantly impacted by diazinon exposure. There was a significant reduction in the number of breeding females and in the number of females giving birth from diazinon-exposed populations during both trials. 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.

Significant reproductive effects have been demonstrated in enclosed S. hispidus (Barrett, 1968; Pomeroy and Barrett, 1975) and M. pennsylvanicus (Barrett, 1988)

populations exposed to the carbamate insecticide carbaryl. These effects resulted in significant delays in reproduction in these populations, which was speculated to have been due to the relatively high embryotoxicity of carbaryl in mammals (Barrett, 1988). Schauber et al. (in press) found that reproductive activity or the proportion of adults that were pregnant or lactating was not significantly affected for either M. canicaudus or P. maniculatus exposed to azinphosmethyl, even at the high application rate. However, they did not analyze male reproductive condition and used a different, less reliable method for assessing female reproductive condition in the field.

Our results suggested that the reproductive activity of females was more sensitive than males to diazinon exposure. In the lab, it has been demonstrated that female rats and dogs are more sensitive to diazinon than males (Davies and Holub, 1980a, 1980b; Earl et al., 1971). It may be that the presence of high testosterone levels in males conveys a detoxification advantage to males over females that have only low levels of testosterone. In male rodents, OP insecticides have been shown to cause numerous alterations in the testes, including sperm shape abnormalities (Mathew et al., 1992), altered sperm capacitiation and inhibition of fertilization (Chou and Cook, 1994; 1995), alterations of the seminiferous epithelium and Leydig cells (Krause and Homola, 1974), decreased testicular weight, increased spermatid degeneration and reduced total sperm counts (Ray et al., 1991, 1992) and decreased testicular sperm density, steroidogenesis, and enzyme activity, along with damage to spermatogenic cells (Bhatnagar and Soni, 1990). OP insecticides have also been shown to cause severe negative effects on the female reproductive system and developing young, including increased maternal toxicity and

weight loss, decreased birth and weanling weights (Montz, 1983), embryonic ChE inhibition, an increase in stillbirths and neonatal deaths, and a reduction in juvenile weight gain (Fish, 1966), decreased uterine and ovary weights, mean number of embryos per female, and significant decrease in stage of pregnancy (Montz et al., 1984), and increased weanling mortality (Spyker and Avery, 1977). In addition, placental transfer of OP's, bioconcentration in fetal tissues (Ackermann and Engst, 1970; Gustave et al., 1994), and lactational transfer of OP's have been documented (Mosha et al., 1991). Reproductive hormones have been found to be altered by exposure to OP insecticides, including reduced plasma FSH, LH and testosterone titres, reduced testicular testosterone, and reduced cholesteryl esterification, an important step in the production of steroid hormones (Civen et al., 1977; Rattner and Michael, 1985; Ray et al., 1991, 1992). Interference with the production or metabolism of reproductive hormones may considerably impact mating success, fertility, and neonatal survival in small mammals. This may, in turn, negatively impact exposed small mammal populations. Negative effects on reproduction can impact recruitment, population density, long-term population stability, and ultimately affecting other co-existing populations in the community.

Exposure

It was apparent that significant exposure to diazinon occurred in many of the animals in sprayed enclosures. Little rainfall fell during either 30-day trial (7.6 and 1.3 cm, respectively), allowing continued persistence of diazinon in enclosures. Preliminary soil residue analysis for diazinon indicated that it persisted in sprayed enclosures throughout

the 30-day trials. Soil samples were found to contain up to 5.3 (1X) and 7.7 ppm (8X) diazinon at day 2, 2.8 (1X) and 4.2 ppm diazinon at day 16, and 0.6 (1X) and 2.3 ppm (8X) diazinon at day 30 (TIWET, Clemson University). The relatively high water solubility (40-60 mg/l at 20^{o}C) and high estimated K_{oc} (1000) of diazinon result in a relatively longer environmental persistence than many anti-ChE insecticides (Eisler, 1986; Wauchope et al., 1992). Diazinon does not bind tightly to soil particles and seldom penetrates below the top 5 cm of soil (Kuhr and Tashiro, 1978; Malone et al., 1967); therefore, it 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 (Freed et al., 1979). The 50% persistence rate of diazinon in soil is estimated to be from 2-4 weeks (Bartsch, 1974) to 40 days (Wauchope et al., 1992). Diazinon may remain biologically active in soils for up to 1 year or more under certain environmental conditions (Eisler, 1986). The bioconcentration factor (BCF) for diazinon has been estimated at 35 - 77, a relatively high figure for an OP insecticide (Kenaga, 1980). McEwen et al. (1972) found that white-footed mice (Peromyscus leucopus) captured 6-8 days after a diazinon application (5.0-8.0 oz/acre - very low application rate) to shortgrass prairie contained 0.10-0.17 ppm diazinon. Mendelssohn and Paz (1977) 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.

Arthropod communities in the sprayed enclosures were severely impacted by diazinon; dead and dying arthropods were routinely seen in diazinon-exposed enclosures

(Sheffield, 1996). Potential routes of exposure for small mammals included dietary, inhalation, and dermal. However, dietary exposure appears to be the most significant route of exposure, and may have occurred through the opportunistic consumption of dead and/or dying arthropods and vegetation. The opportunistic consumption of arthropods is not surprising considering the fact that all species used in this study consume relatively large numbers of arthropods (Cameron and Spencer, 1981; Spencer and Cameron, 1982; Stalling, 1990). Both trials were conducted during the peak rodent breeding season when additional protein is necessary for reproduction. Stehn et al. (1976) found a 70%, 300%, and 400% increase in weakened or dead arthropods in the diets of the short-tailed shrew (Blarina brevicauda), white-footed mouse (Peromyscus leucopus), and red-backed vole (Clethrionomys gapperi), respectively, over control animals following 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, 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. Both the dermal LD50 and inhalation LC50 in rabbits for diazinon 4E are the lowest values (highest toxicity) found for any diazinon formulation (Eisler, 1986). Weeks et al. (1977) suggest that a greater hazard exists from inhalation than from ingestion of equivalent amounts of malathion in rabbits and quail.

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 - 900 mg/kg BW in lab rats, but only 65 - 96 mg/kg in lab mice, ranking it as one of the more acutely toxic OP insecticides (Gallo and Lawryk, 1991; Kenaga, 1979). 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 (Gallo and Lawryk, 1991) and UV irradiation (Machin et al., 1971). Other components of the diazinon 4E formulation (xylene, ethylbenzene, etc.) 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 EPA strongly encourages pesticide registrants to remove or substitute from their products.

Little is known about the subchronic or chronic toxicity of diazinon (Eisler, 1986). The few chronic toxicity tests conducted with mammals suggest that daily intake exceeding 5-10 mg/kg BW diazinon is probably fatal over time to pigs, Sus scrofa, and dogs, Canis familiaris (Earl et al., 1971). A chronic no-effect level of 0.1 mg/kg BW in the diet has been calculated for lab rats (Kenaga, 1979).

Diazinon exposure has been demonstrated in the laboratory (Eisler, 1986) and in the field (Sheffield, 1996) to result in various sublethal effects using several species of mammals. Sublethal effects in mammals have been seen at exposures as low as 0.18 mg/kg BW daily through gestation in pregnant lab mice, 0.5 mg/kg BW for 5 weeks in lab rats, and at single doses of 1.8 mg/kg BW for lab rats and 2.3 mg/kg BW for Peromyscus leucopus (Eisler, 1986; Spyker and Avery, 1977). Sublethal effects can be significant because they increase the likelihood of mortality of the exposed organism

(Moriarty, 1988). Small mammal species recovering from diazinon poisoning may face increased predation, aberrant behavior, learning disabilities, decreased endurance, motor coordination, and immunocompetence, anorexia, and vision and hearing impairment in addition to hypothermia, cholinesterase inhibition, pathological changes and reproductive impairments (Sheffield, 1996).

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 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. These 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, it appears that rodents 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 (Rattner and Hoffman, 1984). With little clear data available, it is not at all clear as to whether wild rodent populations are more susceptible or resistant to pesticide exposure than lab rodents (Cholakis et al., 1981; Meyers and Wolff, 1994; Rattner and Hoffman, 1984; Roberts et al., 1988). However, there are considerable limitations in the use of lab rodents in toxicological studies which attempt to predict toxicant-induced effects on ecological systems (Schaeffer and Beasley, 1989; Shore and Douben, 1994). Further, at least one study (Johnson and Barrett, 1975) found that a toxic chemical had a negative effect on a rodent population under field conditions at doses considered safe in laboratory populations.

Based on the findings of this study and those of Sheffield (1996), it may be necessary to re-examine recommended label application rates for diazinon due to its high acute toxicity and potential for debilitating sublethal effects and subsequent effects on populations, and perhaps communities, of non-target organisms. The EPA's Quotient Method (QM), widely used during pesticide registration evaluations for assessing risk of contaminant exposure to wildlife (Urban and Cook, 1986), has proven unreliable in many cases (Grue et al., 1983; Schauber et al, in press) and a re-evaluation of expected environmental concentration (EEC) and overall hazard quotient for diazinon from lab to field should be considered. We urgently need to examine further the effects of chronic exposure to low levels of widely used pesticides, particularly when a single application at recommended label application rates is found to negatively impact small mammal species. 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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Table 1. Effects of subchronic diazinon exposure on female reproductive productivity in small mammals. Mean numbers are given for each treatment (control, 1X = 0.5 lbs a.i./acre, 8X = 4.0 lbs a.i./acre). Significant differences ($P < 0.05$) are indicated by a difference in superscript lettering using Bonferroni tests for pair-wise differences between treatment means.

Species	Diazinon application rate	n	x no. embryos/ female	% pregnant females	% females giving birth
S. hispidus[1]	control	15	0.00^{A}	40.0^{A}	40.0^{A}
	1X	20	3.50^{B}	20.0^{B}	10.0^{B}
	8X	16	4.00^{B}	25.0^{B}	0.0^{B}
S. hispidus[2]	control	23	6.00^{A}	43.4^{A}	21.7^{A}
	1X	19	5.67^{A}	26.3^{B}	10.5^{B}
	8X	22	7.00^{A}	13.6^{B}	9.1^{B}
M. ochrogaster[2]	control	20	3.17^{A}	80.0^{A}	50.0^{A}
	1X	23	2.17^{A}	43.5^{B}	17.4^{B}
	8X	23	2.50^{A}	30.4^{B}	13.0^{B}
R. fulvescens[2]	control	7	3.00^{A}	57.2^{A}	28.6^{A}
	1X	6	4.00^{A}	33.3^{B}	0.0^{B}
	8X	7	3.00^{A}	14.3^{C}	0.0^{B}

[1] Trial 1
[2] Trial 2

Figure 1. Percentage of animals surviving through 30-day trials following application of diazinon (controls = not sprayed, 1X = 0.5 lbs a.i./acre, 8X = 4.0 lbs a.i./acre).

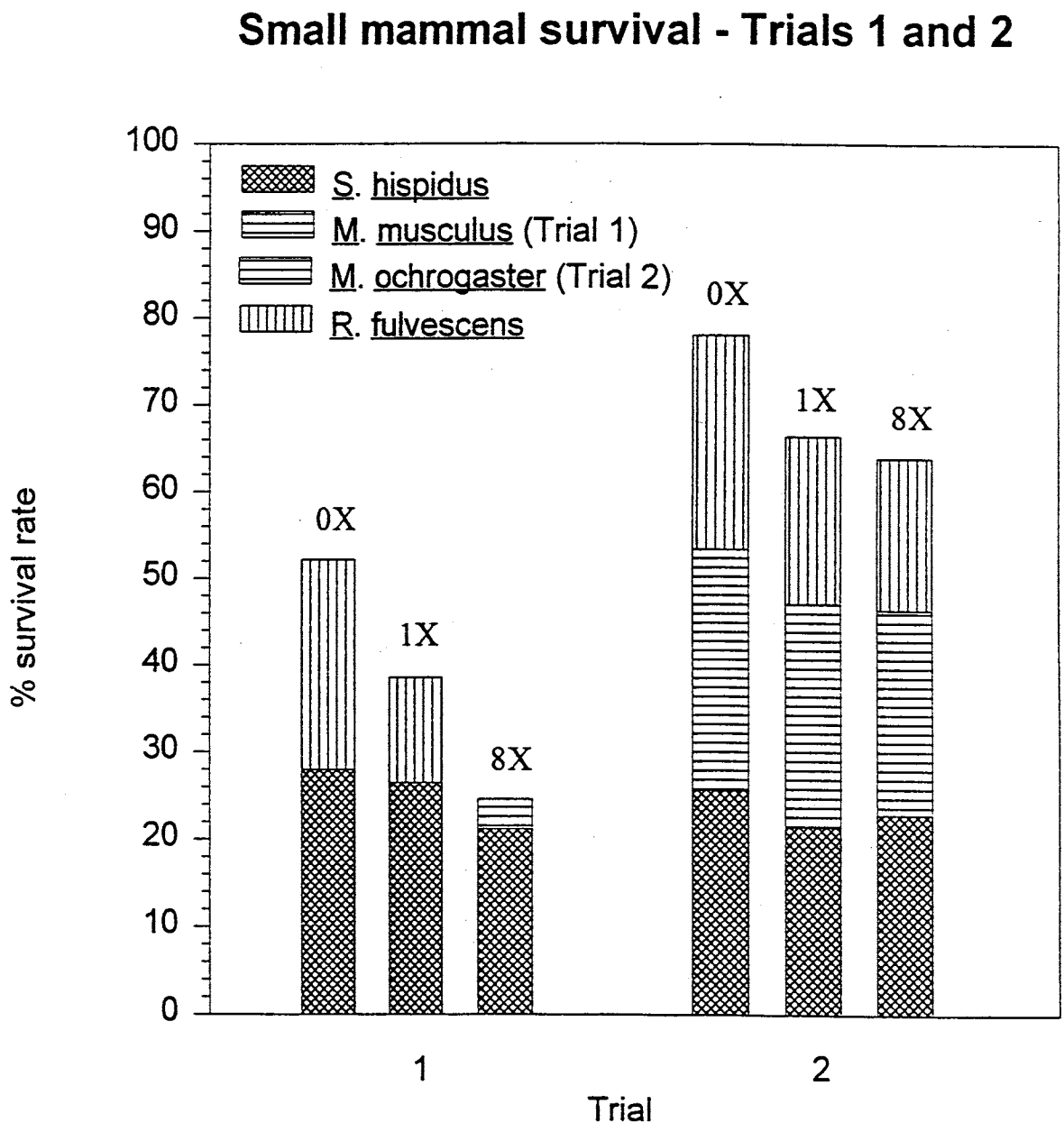

Small mammal survival - Trials 1 and 2
% survival rate
100
90
80
70
60
50
40
30
20
10
0
S. hispidus
M. musculus (Trial 1)
M. ochrogaster (Trial 2)
R. fulvescens
0X
1X
8X
0X
1X
8X
1
2
Trial

Figure 2. Trapping results for S. hispidus and M. ochrogaster (includes day 2, 16 and initial day of day 30 trapping) following application of diazinon (controls = not sprayed, 1X = 0.5 lbs a.i./acre, 8X = 4.0 lbs a.i./acre) - Trial 2

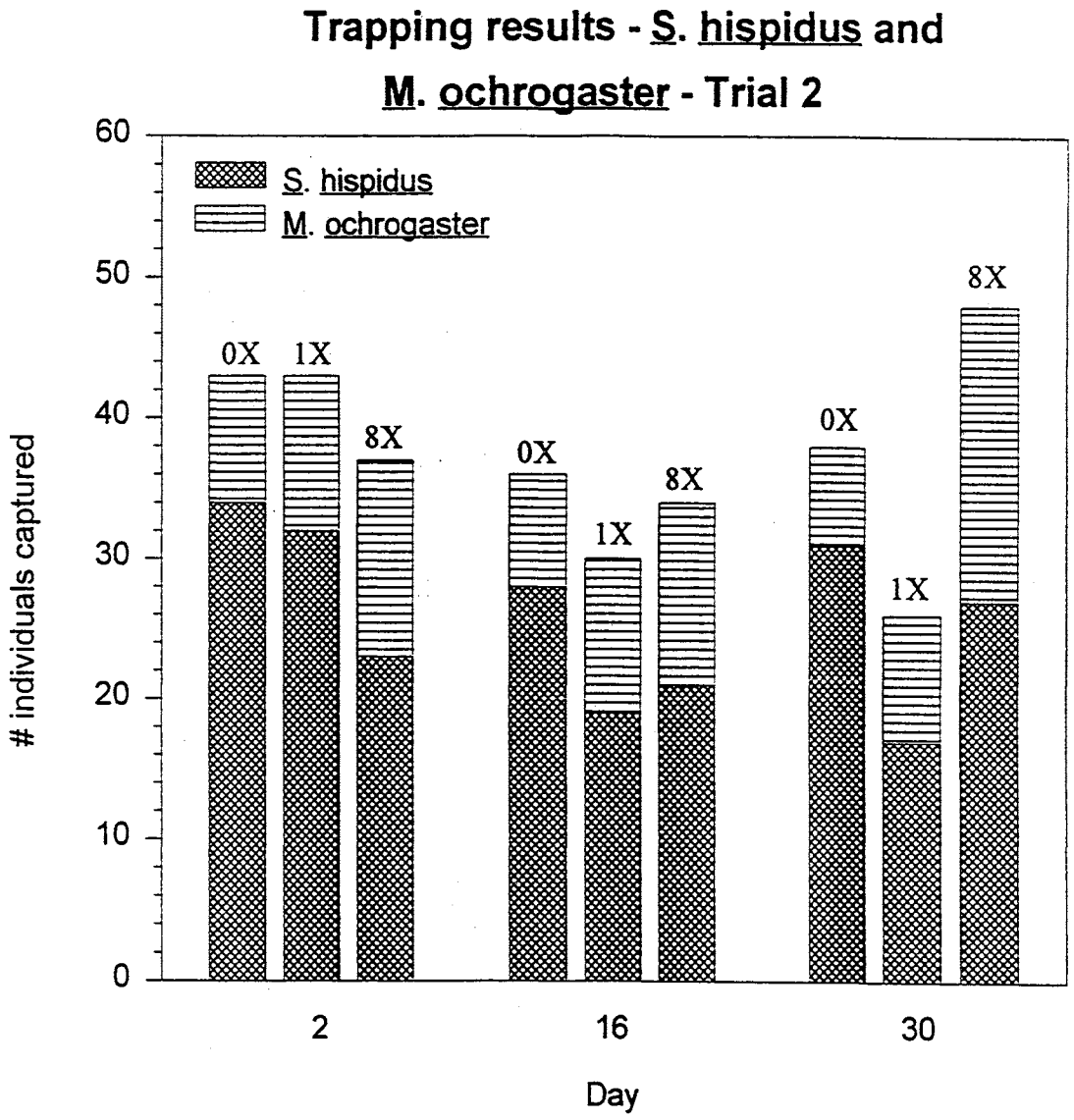
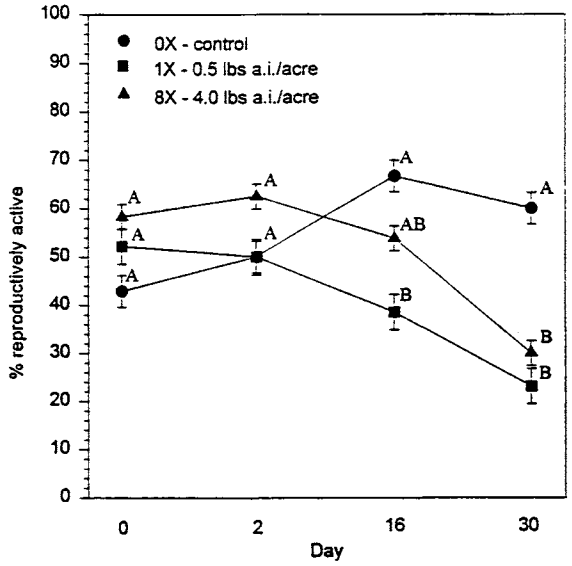
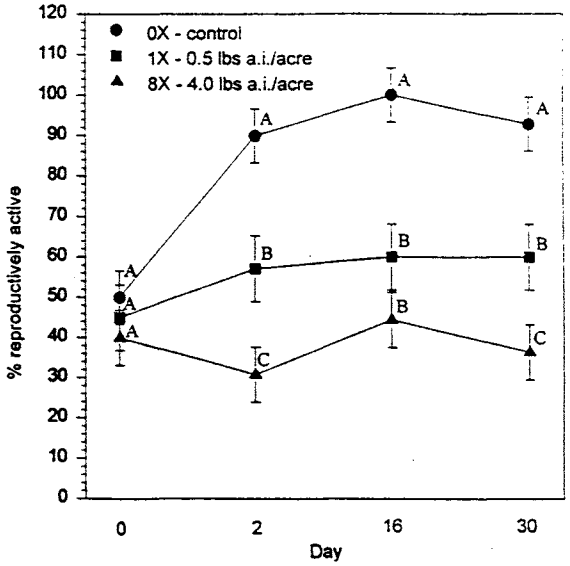

Trapping results - S. hispidus and M. ochrogaster - Trial 2
S. hispidus
M. ochrogaster
individuals captured
60
50
40
30
20
10
0
0X
1X
8X
0X
1X
8X
0X
1X
8X
2
16
30
Day

Figure 3. Percentage of reproductively active male small mammals found throughout 30-day trials following exposure to diazinon (controls = not sprayed, 1X = 0.5 lbs a.i./acre, 8X = 4.0 lbs a.i./acre)

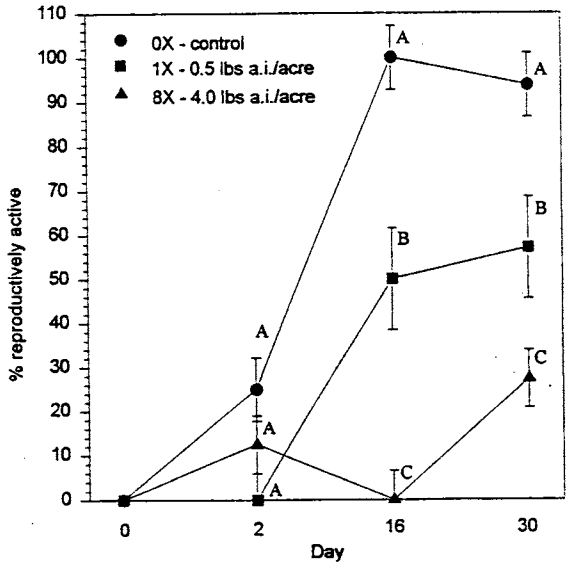
male S. hispidus - Trial 1



male S. hispidus - Trial 2



male M. ochrogaster - Trial 2



male R. fulvescens - Trial 2

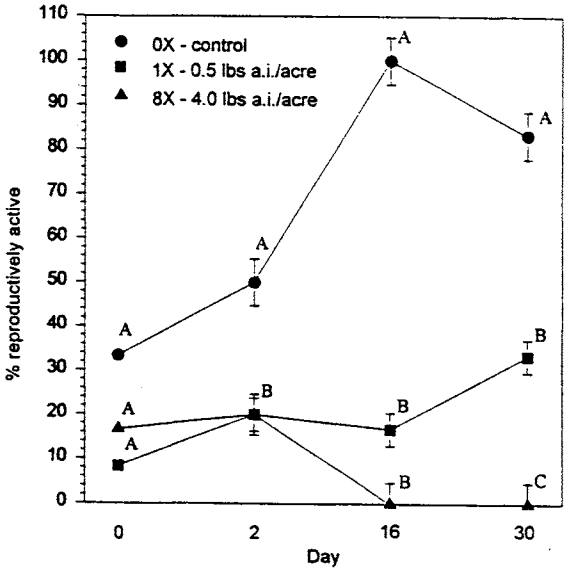
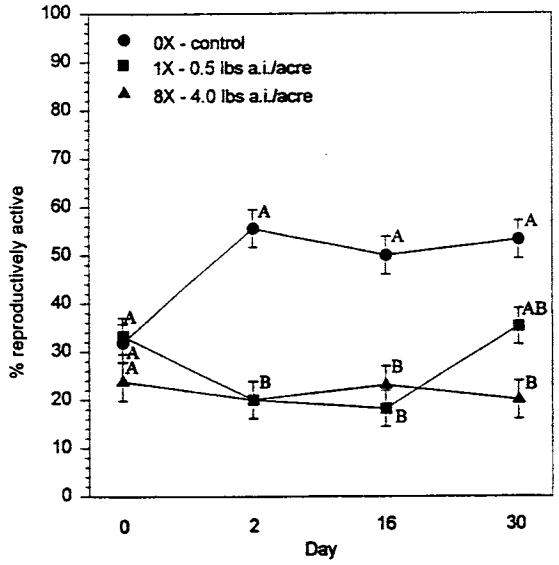
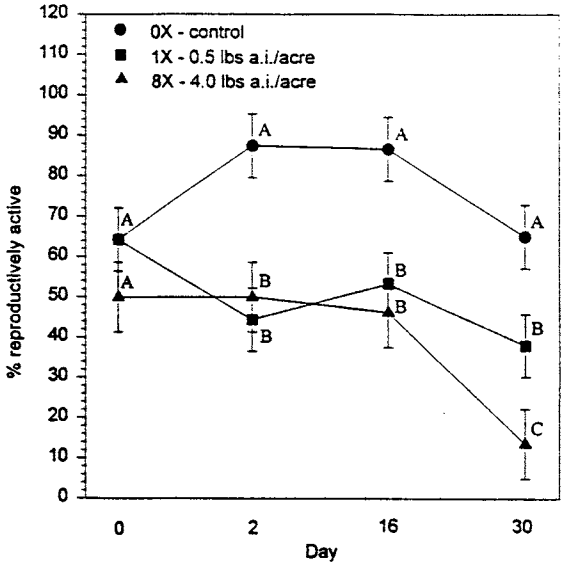


Figure 4. Percentage of reproductively active female small mammals found throughout 30-day trials following exposure to diazinon (controls = not sprayed, 1X = 0.5 lbs a.i./acre, 8X = 4.0 lbs a.i./acre)

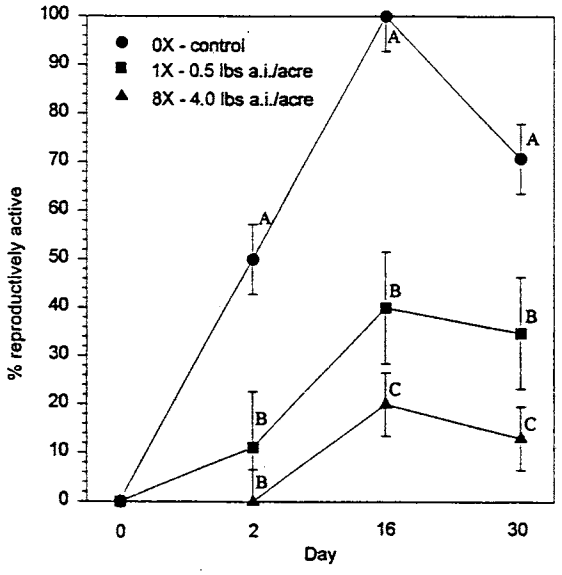
female S. hispidus - Trial 1



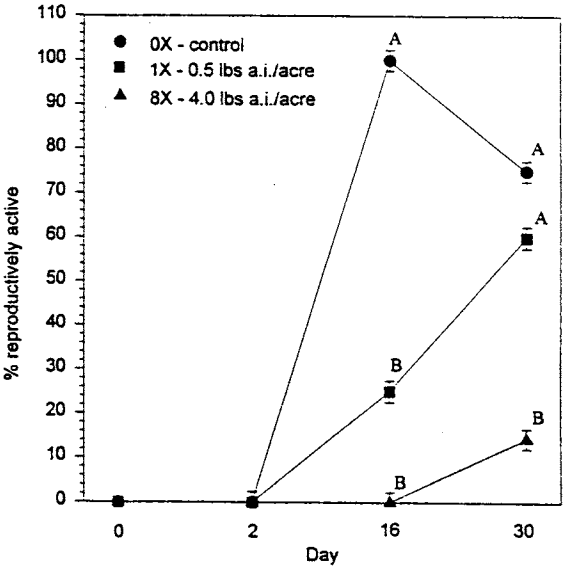
female S. hispidus - Trial 2



female M. ochrogaster - Trial 2



female R. fulvescens - Trial 2



CHAPTER 3

EFFECTS OF FIELD EXPOSURE TO DIAZINON ON SMALL MAMMALS INHABITING A SEMI-ENCLOSED PRAIRIE GRASSLAND ECOSYSTEM

II. SUBLETHAL EFFECTS

EFFECTS OF FIELD EXPOSURE TO DIAZINON IN SMALL MAMMALS INHABITING A SEMI-ENCLOSED PRAIRIE GRASSLAND ECOSYSTEM

II. SUBLETHAL EFFECTS

Abstract-Anti-cholinesterase (anti-ChE) pesticides are widely used throughout the world, and their presence in the environment presents concerns about their effects on human, wildlife, and ecosystem health. Potential exposure and effects of a widely used anti-ChE insecticide, diazinon, was studied using wild small mammals in a natural field setting using an enclosure (mesocosm) system. Our primary objective was to examine the potential sublethal physiological, biochemical, and pathological effects resulting from exposure to diazinon in small mammals inhabiting a controlled field mesocosm. Experimental small mammal communities consisting of Sigmodon hispidus, Microtus ochrogaster, Reithrodontomys fulvescens, and Mus musculus were established inside 12, 0.1-ha enclosures. Diazinon 4E was applied at a low rate (0.5 lbs a.i./acre) and high rate (4.0 lbs a.i./acre), or remained unsprayed (controls), with four enclosures (replicates) per treatment. Two 30-day experimental trials were conducted during the peak breeding season and small mammal communities were monitored on days 2, 16, and 30 post-spray. Body temperature and plasma cholinesterase activity were found to decrease in a dose-dependent manner in diazinon-exposed individuals for all species in both trials. Body temperatures were found to be 0.6 - 2.8°C (1.2 - 7.9%) lower in diazinon-exposed animals as compared to controls. Similarly, plasma ChE activities in diazinon-exposed animals

were found to be 17.1 - 37.0% of control values on day 2 and 47.5 - 60.5% of control values by day 30 post-spray. Liver weights in S. hispidus were found to decrease significantly in both trials; however, testes, epididymides, and seminal vesicle weights were found to decrease only slightly in diazinon-exposed individuals. Results suggested that diazinon was relatively persistent in the sprayed enclosures and suggests that diet. possibly through the consumption of dead and dying arthropods, may be a major route of exposure. Both herbivorous and omnivorous mammals were negatively impacted by diazinon exposure. Overall, sublethal effects seen in this study could act to disrupt populations and communities of small mammals in the enclosed grassland ecosystem. In particular, reproductive effects, possibly resulting from reduced cholinesterase levels, decreased reproductive organ weights, and other effects (e.g., behavioral changes) may negatively impact small mammal populations and communities.

Keywords- Sublethal Effects, Small Mammals, Diazinon, Mesocosm, Terrestrial Toxicology

INTRODUCTION

Field applications of cholinesterase-inhibiting (anti-ChE) pesticides (including organophosphate (OP) and carbamate insecticides) occasionally result in substantial non-target organism mortalities despite prior testing which supposedly establishes their safety to wildlife (Grue et al., 1983; Smith, 1987). Anti-ChE are relatively less persistent than organochlorine (OC) pesticides in the environment and rarely bioaccumulate in food chains; however, they generally are much more acutely toxic than OC pesticides and lack target specificity, tending to exert a potentially more widespread effect on non-target organisms (Brown, 1978). Although the potential for lethal effects following application of anti-ChE pesticides has been well-documented, we know relatively little about the more subtle sublethal effects that can occur in wild animal species. Various physiological, biochemical, immunological, behavioral, and reproductive alterations that are critical for survival and reproduction have been reported in a number of wildlife species (Grue et al., 1983). These types of sublethal effects of OP pesticide applications have the potential to increase the likelihood of mortality and therefore alter the distribution and abundance of wildlife species, but the extent to which these effects may alter recruitment, population size and community structure and function is not known (Eisler, 1986; Grue et al., 1983; Moriarty, 1988; Rattner and Hoffman, 1984).

There have been few intensive studies examining exposure and effects of anti-ChE insecticides on wild small mammal communities under controlled field conditions.

Mesocosms have been shown to be useful in examining potential effects of pesticides and other environmental contaminants on experimental animal populations (Crossland, 1994; Crossland and LaPoint, 1992; Gillett, 1989; Kimball and Levin, 1985; Odum, 1984). The primary advantage of an experimental mesocosm system is that it provides realism that is not possible in the laboratory, and allows for control of certain crucial parameters (e.g., movements, predation, food resources, density).

Among the most widely used anti-ChE insecticides is diazinon. Diazinon has been registered for use since 1952, and since 1985, an average of 10 million pounds or more of active ingredient has been applied annually in the United States, making it among the most widely used insecticides (Aspelin, 1994; Gianessi and Anderson, 1995). In 1993, approximately 11 - 16 million pounds of the active ingredient diazinon was applied in the United States in both agricultural and non-agricultural uses (Aspelin, 1994). Diazinon (O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate) is a broad spectrum OP insecticide that is widely used in agriculture, range, commercial, and home and garden settings for the control of a wide variety of insect, acarine, and nematode pests (Eisler, 1986). Currently, diazinon poses a major environmental concern due to its widespread usage, which has resulted in its presence in all environmental media and its high toxicity, potential for persistence in the environment, and our relative lack of understanding regarding its health effects on wildlife and humans (Agency for Toxic Substances and Disease Registry, 1996).

The primary objective of this study was to evaluate potential sublethal effects of subchronic exposure to the widely used OP insecticide diazinon in small mammal

assemblages established in mesocosms located in a prairie grassland ecosystem. Specifically, we evaluated interspecific responses at the individual level, including physiological (body temperature), biochemical (plasma cholinesterase), and pathological (organ weights) effects.

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. Vegetation on the study area consisted of a mixture of grasses and forbs with scattered woody shrubs, and site topography is mainly flat. Small mammals that occur locally in this habitat include least shrews (Cryptotis parva), short-tailed shrews (Blarina hylophaga), hispid cotton rats (Sigmodon hispidus), prairie voles (Microtus ochrogaster), pine voles (Microtus pinetorum), fulvous harvest mice (Reithrodontomys fulvescens), plains harvest mice (Reithrodontomys montanus), deer mice (Peromyscus maniculatus), white-footed mice (P. leucopus), and house mice (Mus musculus). The area had no prior history of pesticide application but was subjected to a controlled burn in Spring 1990.

Enclosure design and construction

A series of 12, 0.1-ha enclosures (32 m x 32 m) were constructed of galvanized sheet metal (1.27 m above ground, 0.15 m below ground). Mowed strips inside (0.75 m) and outside (1.5 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 along the walls (i.e., digging, trying to climb).

Experimental Design

A completely randomized split plot design was employed for the group of 12 enclosures, which allowed for four replicates of three experimental treatments (control, low (=1X) diazinon application rate, and high (=8X) diazinon application rate. The low (0.5 lbs. active ingredient (a.i.)/acre) and high (4.0 lbs. a.i./acre) application rates were compared to a control (no spraying). The two application rates used represent maximum recommended label application rates for diazinon to treat different pests. This design was repeated twice during the peak rodent breeding seasons in August/September 1993 and June/July 1994. Treatments were applied randomly to enclosures and enclosures received the same treatments during each of two trials in the study.

Each 0.1 ha enclosure contained a trapping grid consisting of 20 trapping stations in a 5 x 4 matrix. Two Sherman live traps were used at each station (40 traps/enclosure). Traps were baited with rolled oats, set near dusk, and checked early the following morning. All trap doors were kept shut during non-trapping days. Enclosures were removal trapped for 14 days to ensure that they were free of non-experimental animals prior to the start of each trial. Experimental animals consisted of wild-caught individuals of the desired species, sex, and age from surrounding grasslands. Mammals were released into the enclosures at densities similar to those of natural populations. During trial 1, each enclosure contained 12 S. hispidus (120/ha), 5 M. musculus (50/ha), and 3 R.

fulvescens (30/ha) at the start (day 0). During trial 2, each enclosure contained 12 S. hispidus (120/ha), 12 M. ochrogaster (120/ha), and 5 R. fulvescens (50/ha) at the start. Prior to release into enclosures, all mammals were toe-clipped for identification, and weight, reproductive condition, and general body condition (parasites, injuries, etc.) were recorded.

The hispid cotton rat (S. hispidus) generally is the most numerous rodent in the grasslands of the southern Great Plains, and is predominantly herbivorous, but known to consume arthropods, seeds, and soil (Cameron and Spencer, 1981; Garten, 1980). The fulvous harvest mouse (R. fulvescens) is omnivorous, eating plant material, especially seeds, as well as insects and other invertebrates. The house mouse (M. musculus), although an exotic, also coexists with cotton rats and fulvous harvest mice in areas where they have become feral. House mice are also omnivorous, although they tend to eat more insects and other animal matter than most omnivores. The prairie vole (M. ochrogaster) is mainly herbivorous, but will eat arthropods when available. S. hispidus, M. ochrogaster, and R. fulvescens naturally coexist together and comprise the major component of the small mammal community in prairie grassland habitats in northern Oklahoma (Caire et al., 1990; Grant and Birney, 1979).

Diazinon 4E (4 lbs/gal), an off-the-shelf formulation consisting of 47.5% active ingredient, was obtained (Estes Chemical Co., Oklahoma City, OK) and was mixed with water and applied to the experimental enclosures. Diazinon 4E was applied at day 0 of each trial using a CO_2 powered backpack unit with 1.83-m boom at a constant rate under 40 lbs pressure using 20 gal H_2O/acre and applied as close to the ground (<0.5-m) as

possible. The exposure scheme using diazinon was that of a one-time "pulse" exposure (Bender et al., 1984) applied at the start of each trial. Experimental trials were then run for 30 days, the approximate half-life of diazinon in the environment (Bartsch, 1974; Wauchope et al., 1992).

During each trial, small mammals were trapped and sampled on days 2, 16 and 30 post-spray using Sherman traps that were set and baited the previous night. Data recorded for each animal captured included identification number, capture location, reproductive condition, general condition (parasites, injuries), and body weight (to the nearest 0.5 g except for S. hispidus (1 g). Body temperature was measured (generally between 0700 - 1000 h) for each animal captured to the nearest 0.1°C using a 10-50°C quick-reading rectal thermometer (Miller and Weber, Inc., Queens, NY). A blood sample was obtained (generally between 0700 - 1000 h) from the retro-orbital sinus using heparinized microcapillary tubes, placed in labelled 250 µl microtubes containing 10 µl heparin, immediately placed on ice, and returned to the lab for analysis. All mammals were anesthetized using methoxyflurane (Metofane; Pitman-Moore, Inc., Mundelein, IL) inhalation prior to handling. Following the day 30 sampling, all small mammals collected were returned to the laboratory for necropsy.

Lab methodology

Blood samples were centrifuged for 1 min at 10,000 rpm to separate blood plasma from the packed cells. Plasma was removed and placed in labelled microtubes for storage at -87°C until analysis. Plasma ChE activities were determined using the procedure of

Ellman et al. (1961) as modified in our laboratory for use on a Flow Titertek Multiskan Plus MKII 96-well plate spectrophotometer (Flow Laboratories, Inc., McLean, VI) set in kinetic mode at a wavelength of 405 nm with a run time of 3 min, read at 10 sec intervals, with a 0 sec lag time, and with a final volume of 270.5 µl/well. All samples were run in duplicate with blanks and controls at room temperature (25°C). Acetylthiocholine iodide (ACTI) (Sigma Chem. Co., St. Louis, MO) was used as the substrate in this method. The compound 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) (Sigma Chem. Co., St. Louis, MO) was added to a mixture of buffer, plasma, and ACTI which completed the Ellman reaction. The resulting yellow-colored end-product was quantified using a spectrophotometer for calculation of ChE activity.

Animals returned to the laboratory on day 30 were euthanized through a heavy inhalation dose of Metofane followed by cervical dislocation. Necropsies included measuring weights (to the nearest 0.1 mg) of liver, spleen, kidney, adrenal glands, testes, epididymides, and seminal vesicles using an analytical balance (Ohaus GA 110; Ohaus Corp., Florham Park, NJ). Relative organ weights (mg/g body mass) were calculated for each organ examined.

Data analysis

Differences in body temperature, growth rate, ChE activity, and relative organ weights were tested using a repeated measures analysis of variance using PROC GLM in the statistical package SAS (SAS Institute, Inc., 1990) among replicated treatments (control, 1X, 8X) for each species at each sampling day in both trials. Replicates were treated as

statistically independent and not pooled before analyses in order to derive an estimate of variation among enclosures (replications). Bonferroni tests were used to test for pair-wise differences between treatment means. Means and standard errors (SE) for treatment effects are presented for most parameters. A significance level of $P<0.05$ was used for all comparisons.

RESULTS

Physiological effects

Body temperature was significantly reduced in all diazinon-exposed small mammals on all sampling days of both trials (Fig. 1). Compared to control animals, body temperatures of diazinon-exposed (low and high rates) animals were lowest on day 2 and gradually increased on days 16 and 30 in all species in both trials. In trial 1, body temperature of S. hispidus clearly decreased in a dose-dependent manner on days 2, 16, and 30, with differences between body temperatures of diazinon-exposed animals versus control animals ranging from about 0.6 to 1.3°C, representing a decrease of about 1.2 to 3.6 %; Fig. 1). Low recpature numbers of R. fulvescens and M. musculus made interpretation of results difficult, but a similar trend in body temperature was observed for both species (data not shown). In trial 2, body temperatures of S. hispidus, M. ochrogaster, and R. fulvescens also were significantly decreased in a dose-dependent manner on days 2, 16, and 30. Differences between body temperatures of diazinon-exposed animals versus control animals in trial 2 ranged from 1.4 to 2.4°C (4.1 - 6.7 %) lower in S. hispidus, 2.0 to 2.8°C (6.0 - 7.9 %) lower in M. ochrogaster, and 1.7 to 2.1°C

(5.0 - 6.2%) lower in R. fulvescens (Fig. 1).

Biochemical effects

Plasma ChE activity (measured in nmol ACTI hydrolyzed/min) was significantly inhibited in a dose-dependent manner in diazinon-exposed animals when compared to control animals for all species in both trials (Fig. 2). In trial 1, plasma ChE activity in S. hispidus decreased sharply at day 2, where ChE activities of diazinon-exposed animals were 39.2% (low rate) and 17.1% (high rate) of control values. ChE activity in S. hispidus showed a slight recovery by day 16 (45.3% for low rate, 38.3% for high rate) and continued to increase at day 30 (57.7% for low rate, 49.1% for high rate; Fig. 2). Plasma ChE activity remained well below control levels at day 30 (Fig. 2). We observed a similar trend during trial 1 for R. fulvescens and M. musculus, but low recapture numbers precluded statistical analysis of ChE activity results (data not shown).

In trial 2, plasma ChE activities in S. hispidus, M. ochrogaster, and R. fulvescens were greatly inhibited on day 2 (Fig. 2). Plasma ChE activities in diazinon-exposed S. hispidus were 29.7% (low rate) and 20.0% (high rate) of control values. On day 2, ChE activities in diazinon-exposed M. ochrogaster were 37.0% (low rate) and 22.6% (high rate) of control values. ChE activities in diazinon-exposed R. fulvescens were 33.5% (low rate) and 28.2% (high rate) of control values. Plasma ChE activities for all species showed gradual recovery by day 16 (45.1 to 51.9% for low rate, 36.1 to 39.7% for high rate) and continued to increase to day 30 (58.9 to 60.5% for low rate, 47.5 to 49.4% for high rate; Fig. 2). Plasma ChE activities remained well below control levels in all species

by day 30 (Fig. 2).

Pathological effects

Although there appeared to be a general trend towards decreased relative weights of all organs measured in diazinon-exposed animals, only the relative liver weight (mg/g body weight) in S. hispidus from high rate enclosures in both trials were reduced in size compared to controls (Table 1). We observed no treatment effects on the relative weights of testes, adrenal glands, kidneys, spleen, or other reproductive organs for any species during either trial (Table 1). Relative testes weights of diazinon-exposed S. hispidus were nearly two times smaller than those of the control animals, but this difference was not statistically different in trial 1. Relative testes, epididymides, and seminal vesicle weights generally tended to decrease in diazinon-exposed animals compared to control animals in all three small mammal species in trial 2, but differences were not statistically different from controls.

DISCUSSION

Exposure

It is apparent that significant exposure to diazinon occurred in many of the animals in sprayed enclosures and that diazinon persisted in sprayed enclosures throughout the 30-day trials. Arthropod communities in the sprayed enclosures were severely impacted by diazinon. There was little rainfall (7.6 and 1.3 cm, respectively) during each of the 30-day trials, allowing for a greater persistence of diazinon in enclosures. Potential routes of

exposure included dietary, inhalation, and dermal, but we suspect that dietary exposure, through the opportunistic consumption of arthropods or vegetation, was the primary route of exposure. The fact that S. hispidus and M. ochrogaster may have consumed large amounts of arthropods is not surprising; these two species are not strict herbivores and are known to take relatively large numbers of arthropods when available (Cameron and Spencer, 1981; Stalling, 1990). Significant increases in arthropod consumption by small mammals following an OP insecticide application has been reported (Stehn et al., 1976). Other possible means of oral intake of diazinon by small mammals included ingestion of soil and grooming of fur containing diazinon residues.

Its relatively high water solubility, high estimated K_{OC}, tendency to remain on the top 5 cm of the soil, and an environmental half-life of about 40 days made diazinon bioavailable to small mammals throughout the 30-day trials (Kuhr and Tashiro, 1978; Malone et al., 1967; Wauchope et al., 1992). Diazinon may remain biologically active in soils for up to 1 year or more under certain environmental conditions (Eisler, 1986). McEwen et al. (1972) found that white-footed mice (Peromyscus leucopus) captured 6-8 days after a diazinon application (5.0-8.0 oz/acre) to shortgrass prairie contained 0.10-0.17 ppm diazinon. Mendelssohn and Paz (1977) showed that an 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.

Physiological effects

The decrease in body temperatures we observed in diazinon-exposed animals has been

demonstrated in laboratory rodents exposed to OP insecticides (Ahdaya et al., 1976; Meeter and Wolthuis, 1968; Montz and Kirkpatrick, 1985b). However, to our knowledge, this is the first demonstration of this negative physiological effect under field conditions. Although body temperatures in small mammals from diazinon-exposed 1X and 8X enclosures were decreased relative to control small mammals, the magnitude of these decreases was generally small (0.5 to 2.0°C) and not every animal from diazinon-treated enclosures experienced reductions in body temperature. In comparison, Meeter and Wolthuis (1968) and Montz and Kirkpatrick (1985b) found that core body temperatures of laboratory rats exposed to OP insecticides decreased 4.5 to 6°C within a few hours post-exposure. As natural variation in diurnal body temperature can vary from <1 to several °C in small rodents, we read body temperatures at approximately the same time (early morning) during each trapping day in order to avoid incorporating this variation. The correlation between ChE inhibition and decreased body temperature we observed in small mammals was reported for laboratory mice by Ahdaya et al. (1976). Acetylcholine is known to influence body temperature regulation (Meeter and Wolthuis, 1968) and chemicals which inhibit cholinesterases might be expected to influence regulation of body temperature. Any hypothermic condition in small mammals could potentially cause significant thermoregulatory problems, although the severity of effects may vary with ambient temperature. It is not exactly clear what the effects of decreased body temperature are both at the individual and population levels, but metabolic costs, disease resistance problems, and subsequent population declines could likely occur. There is evidence for enhanced toxicity of OP pesticides during heat and cold exposure in

mammals (Chattopadhyay et al., 1982).

Cholinesterase inhibition

The finding that plasma ChE was significantly inhibited by both application rates of diazinon in all species of small mammals in both trials is consistent with findings from laboratory studies. It also indicates that all small mammals (and other vertebrates) are at risk of ChE inhibition-related sublethal effects when exposed to diazinon in the field. The relatively slow recovery of ChE activity in all species in both trials was somewhat surprising. Plasma ChE activity levels were still depressed well below those of controls by day 30, suggesting that small mammals were receiving a continual exposure to diazinon or its cholinesterase-inhibiting metabolites and degradation products throughout the trials.

Observations from this study and others with wild rodents (Rattner and Hoffman, 1984; Westlake et al., 1982) indicate that plasma ChE activity is a more sensitive index of anti-ChE insecticide exposure than brain AChE activity. In addition, assessing plasma ChE inhibition required very small amounts of blood (<100 μl) and is a non-lethal method of exposure determination. However, the value of plasma ChE in wild animals is limited for predicting lethality because recovery is rapid and activity can also be affected by age, sex, reproductive state, stress, and a variety of pathophysiological conditions (Fleming and Bradbury, 1981; Rattner, 1982; Rattner and Hoffman, 1984). The use of sequential measurements of plasma ChE activity has been used successfully for monitoring exposure to OP insecticides (Fairbrother et al., 1989; Hill and Fleming, 1982),

but is not a good indicator of mortality from anti-ChE exposure and may be of less use in the field due to the fact that many experimental animals are not recaptured as was found in this study. Previous studies have demonstrated a marked decrease in small mammal brain ChE activity following field exposure to OP insecticides (Jett, 1986; Montz et al., 1983; Westlake et al., 1982; Zinkl et al., 1980). Roberts et al. (1988) found that feral S. hispidus and M. musculus were much more sensitive to brain ChE inhibition from methyl parathion exposure than were laboratory rats and mice. Brain ChE activities had recovered in lab rats by day 7 (males) and day 14 (females), whereas activities in male and female S. hispidus had not recovered until day 28.

Pathological effects

The trend towards slightly decreased organ weights in diazinon-exposed animals was consistent in both trials of the study. Most of these differences were not significant, except for the decrease in liver weights in S. hispidus in both trials. Decreased liver weight could potentially impact liver function, including its mechanisms for detoxifying contaminants such as pesticides. Cecil et al. (1974) found that the OP insecticide malathion significantly increased liver weight of exposed female rats, but caused a slight decrease in male rats; lipid and vitamin A content both decreased in female rats and increased slightly in male rats. Montz et al. (1984) found slightly increased liver weights and decreased adrenal weights in female cottontail rabbits (Sylvilagus floridanus) exposed to the OP insecticide parathion. Spyker-Cranmer et al. (1978) found no significant change in liver weights in males or females exposed to 0.18 or 9.0 mg

diazinon/kg BW/day, but diazinon exposure resulted in persistent liver malfunction (decreased hepatic catabolism). Significant histopathological changes have been documented in the liver of animals exposed chronically to sublethal doses of diazinon, including cellular damage and necrosis of liver hepatocytes (Anthony et al., 1986; Dikshith et al., 1975; Earl et al., 1971). Relative adrenal gland weights were found to decrease slightly in diazinon-exposed animals in both trials but were not significantly different from those of controls. Both male and female pups of lab mice exposed to diazinon orally at a rate of 9.0 mg diazinon/kg BW/day during pregnancy were found to have significantly smaller adrenal glands, and adrenal function (steroidogenesis) was negatively affected in males exposed to 0.18 mg diazinon/kg BW/day (Spyker-Cranmer et al., 1978).

Sublethal effects

Numerous sublethal effects resulting from diazinon exposure have been demonstrated in the laboratory with various species of birds and mammals (Eisler, 1986). Sublethal effects in mammals have been seen at exposures as low as 0.18 mg/kg BW daily through gestation in pregnant lab mice, 0.5 mg/kg BW for 5 weeks in lab rats, and at single doses of 1.8 mg/kg BW for lab rats and 2.3 mg/kg BW for Peromyscus leucopus (Eisler, 1986; Spyker and Avery, 1977). Exposure to diazinon has been reported to result in reduced daily food consumption/anorexia (Spyker and Avery, 1977); depression of plasma/RBC/brain acetylcholinesterase activity (Davies and Holub, 1980a, 1980b; Iverson et al., 1975; Montz and Kirkpatrick, 1985a; Tomokuni and Hasagawa, 1985);

reduced body temperature (hypothermia) and lowered resistance to cold stress (Montz and Kirkpatrick, 1985b); altered immune function (Avery et al., 1981; Barnett et al., 1980), altered blood chemistry including decreased clotting ability (Lox, 1983) and increased serum B-glucuronidase activity (Kikuchi et al., 1981); altered hepatic and endocrine (adrenal) function (Spyker-Cranmer et al., 1978); altered reproductive function (testicular atrophy; Earl et al., 1971); decreased productivity (litter/clutch size; Spyker and Avery, 1977); delayed sexual maturity (in progeny where the pregnant female was dosed; Spyker and Avery, 1977); impaired endurance and motor coordination (Spyker and Avery, 1977); and altered visual acuity in (Plestina and Piukovic-Plestina, 1978). Other sublethal effects with possible significance to the present study have been documented for a number of other related anti-ChE pesticides, including loss of motor coordination (Clark, 1986), reduced predator escape response (Galindo et al., 1985; Hunt et al., 1992), altered immune function (Fan et al., 1978; Street and Sharma, 1975), reduced plasma LH levels (Rattner and Michael, 1985) and altered steroidogenesis (Civen et al., 1977, 1980), altered hearing ability (Reischl et al., 1975), decreased daily food and water consumption (anorexia; Glow et al., 1966), altered neurochemistry and motor and learning abilities (Boyd et al., 1990), decreased ability to learn (Reiter et al., 1973; Russell, 1969), reduced aggressive (Durda et al., 1989) and discrimination (Richardson and Glow, 1967) behavior, altered behaviors (Kurtz, 1977), decreased serial problem-solving behavior (Banks and Russell, 1967), and spatial memory impairment and central muscarinic receptor loss (McDonald et al., 1988). Overall, little is known about what sublethal effects are induced in wild mammals following exposure to OP insecticides and

what their ecotoxicological significance may be due to the fact that exposure and uptake are difficult to quantify in feral mammals and effects cannot be related to residue levels (Shore and Douben, 1994).

Toxicity

Diazinon is classified as highly toxic or very highly toxic to warm water fishes, invertebrates, birds, and mammals (USEPA, 1989), and has been documented to cause mass mortalities in birds and mammals (Eisler, 1986; Grue et al., 1983). Diazinon is one of the few OP insecticides that has metabolites with greater toxicity than the parent compound (Eisler, 1986). Some formulations of diazinon, particularly emulsifiable formulations, can be converted to much more toxic compounds on contact with air (Gallo and Lawryk, 1991) and UV irradiation (Machin et al., 1971).

Little is known about the subchronic or chronic toxicity of diazinon (Eisler, 1986). The few chronic toxicity tests conducted with mammals suggest that daily intake exceeding 5-10 mg/kg BW diazinon is probably fatal over time to pigs, Sus scrofa, and dogs, Canis familiaris (Earl et al., 1971). Diazinon (9 mg/kg BW) fed to pregnant lab mice during gestation was associated with significant mortality of pups prior to weaning (Spyker and Avery, 1977). A chronic no-effect level of 0.1 mg/kg BW in the diet was calculated for lab rats (Kenaga, 1979), but chronic effects of diazinon on any terrestrial organism including rodents in natural environments are presently unknown (Eisler, 1986).

Conclusions

Application of the OP insecticide diazinon resulted in the manifestation of detectable sublethal effects in small mammals in this field experiment. All species of small mammals used in this study that were exposed to diazinon were found to have decreased body temperature, significant inhibition of plasma ChE, and slightly reduced liver weights. Although it is not clear exactly what the ramifications of the observed sublethal effects are at the population or community level, they have the potential to negatively impact their structure and function. To our knowledge, this is the first study that has demonstrated a correlation between a sublethal effect and inhibition of ChE activity in wild small mammals in the field. It is significant to note that a single application of diazinon used in each trial of this study; 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, sublethal effects in small mammals would be more severe and lethality would be a possibility in some individuals.

In this study, small mammals have served as biomonitors, or sentinels, of exposure and subsequent effects from field applications of the widely used OP insecticide diazinon. From the results of this study and those of other studies, it appears that rodents are the most sensitive group of mammals yet tested with diazinon. Interspecific differences were apparent, probably because OP insecticide exposure in the field is influenced by behavior, foraging habits, and habitat use affecting routes and degree of exposure (Rattner and

Hoffman, 1984). With little data available, it is not clear whether wild rodent species are more susceptible or resistant to diazinon or other OP insecticide exposure than laboratory rodents (Meyers and Wolff, 1994; Rattner and Hoffman, 1984). However, there are considerable limitations in the use of laboratory rodents in toxicological studies which attempt to predict toxicant-induced effects on populations and communities in the field (Schaeffer and Beasley, 1989).

Sublethal effects are useful in serving as biomonitors of environmental contamination, elucidating mechanisms of action of a contaminant, and providing signs of contaminant exposure, all assisting us in predicting negative impacts of contaminants on wildlife populations. More studies that document sublethal effects in the field and provide proof that these effects can lead to mortality and reproductive impairment in wild populations are badly needed (Heinz, 1989).

Based on the findings of this study and those of Sheffield (1996), it seems that a re-examination of recommended label application rates and pesticide registration and re-registration procedures of diazinon and other widely used OP pesticides are in order due to their high acute toxicity and potential for debilitating sublethal effects and subsequent effects on populations, and perhaps communities, of non-target organisms. We urgently need to examine further the effects of chronic exposure to low levels of widely used pesticides, particularly when a single application at recommended label application rates is found to negatively impact small mammal species. 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 these higher levels of ecological organization.

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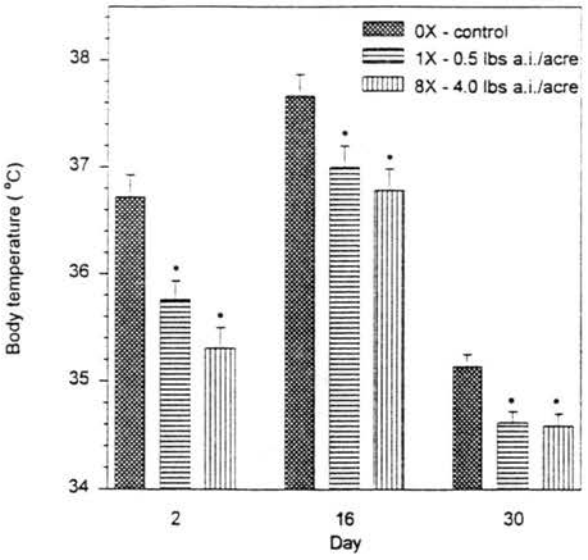
Table 1. Effects of subchronic diazinon exposure on selected organ relative weights (mg/g body weight) in small mammals. Means (± s.e.) are given for each treatment (control, 1X = 0.5 lbs a.i./acre, 8X = 4.0 lbs a.i./acre). Significant differences ($P < 0.05$) are indicated by a difference in superscript lettering using Bonferroni tests for pair-wise differences between treatment means.

Species	Diazinon application rate	n	Liver	Kidney	Spleen	Adrenal glands
S. hispidus[1]	control	25	$41.8(0.70)^{A}$	$6.3(0.18)^{A}$	$1.4(0.28)^{A}$	$0.27(0.06)^{A}$
	1X	30	$40.6(0.64)^{AB}$	$6.3(0.16)^{A}$	$1.3(0.26)^{A}$	$0.24(0.05)^{A}$
	8X	24	$39.5(0.73)^{B}$	$6.2(0.19)^{A}$	$1.4(0.29)^{A}$	$0.24(0.06)^{A}$
S. hispidus[2]	control	34	$33.2(0.57)^{A}$	$7.0(0.22)^{A}$	$1.4(0.08)^{A}$	$0.40(0.04)^{A}$
	1X	28	$31.6(0.71)^{AB}$	$6.9(0.27)^{A}$	$1.3(0.10)^{A}$	$0.38(0.05)^{A}$
	8X	33	$30.7(0.58)^{B}$	$6.7(0.22)^{A}$	$1.3(0.08)^{A}$	$0.35(0.04)^{A}$
M. ochrogaster[2]	control	34	$46.3(2.76)^{A}$	$11.8(0.30)^{A}$	$2.2(0.16)^{A}$	$0.53(0.05)^{A}$
	1X	36	$45.5(2.86)^{A}$	$11.4(0.31)^{A}$	$1.9(0.17)^{A}$	$0.49(0.06)^{A}$
	8X	34	$44.9(2.90)^{A}$	$11.2(0.31)^{A}$	$1.8(0.17)^{A}$	$0.45(0.06)^{A}$
R. fulvescens[2]	control	10	$57.4(2.93)^{A}$	$15.3(0.88)^{A}$	$1.3(0.29)^{A}$	$0.24(0.05)^{A}$
	1X	7	$56.0(2.38)^{A}$	$14.5(1.02)^{A}$	$1.3(0.37)^{A}$	$0.18(0.06)^{A}$
	8X	8	$54.7(2.76)^{A}$	$14.1(0.96)^{A}$	$1.0(0.33)^{A}$	$0.17(0.06)^{A}$

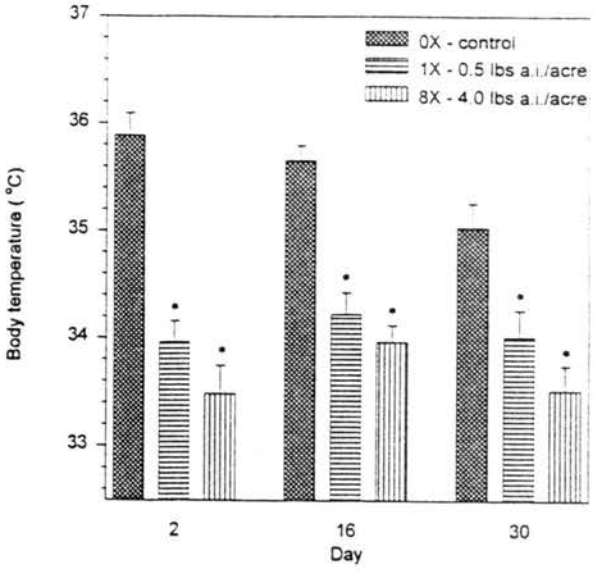
[1] Trial 1
[2] Trial 2

Figure 1. Effects of subchronic diazinon exposure on rectal body temperature in small mammal species over 30-day trials. * = a significant difference from control, ** = a significant difference from 1X (low rate).

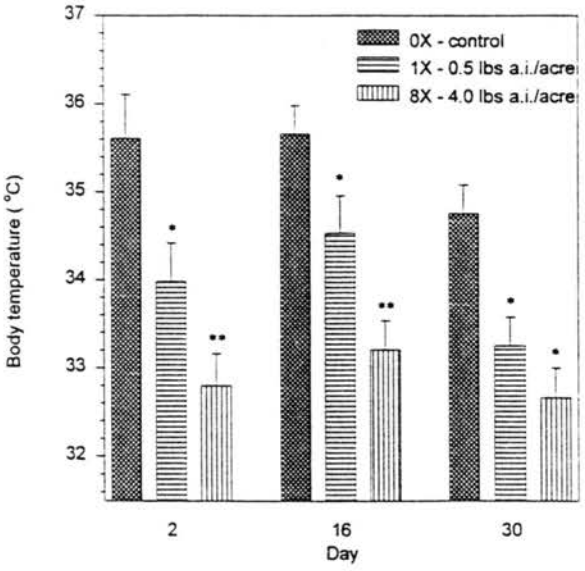
S. hispidus - Trial 1



S. hispidus - Trial 2



M. ochrogaster - Trial 2



R. fulvescens - Trial 2

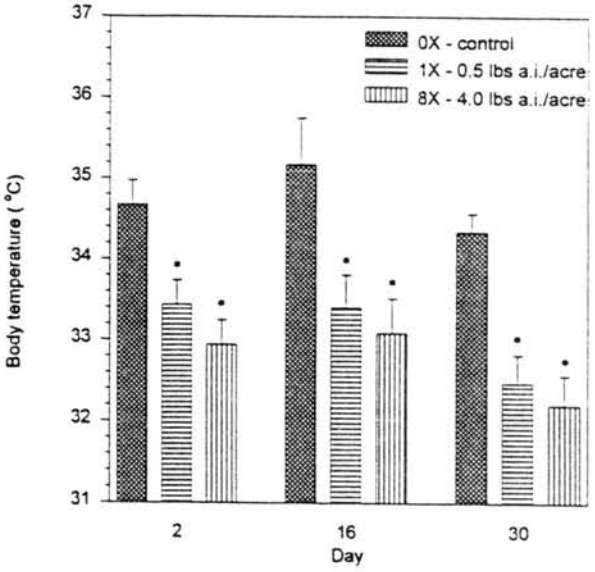


Figure 2. Effects of subchronic diazinon exposure on plasma cholinesterase (ChE) activity in Sigmodon hispidus (SH) over 30-day trials (SH1 = trial 1, SH2 = trial 2). * = a significant difference from control, ** = a significant difference from 1X (low rate).

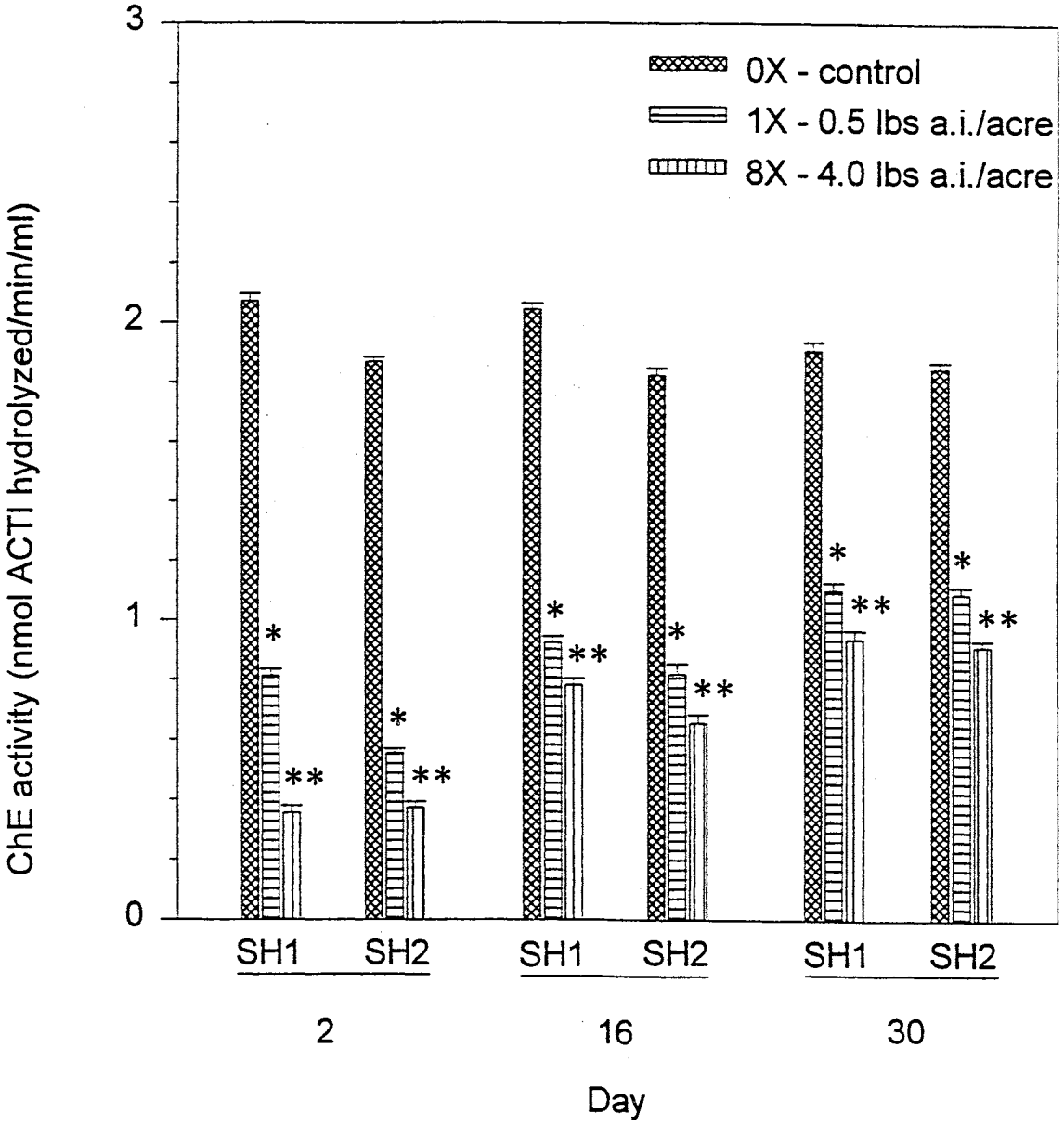
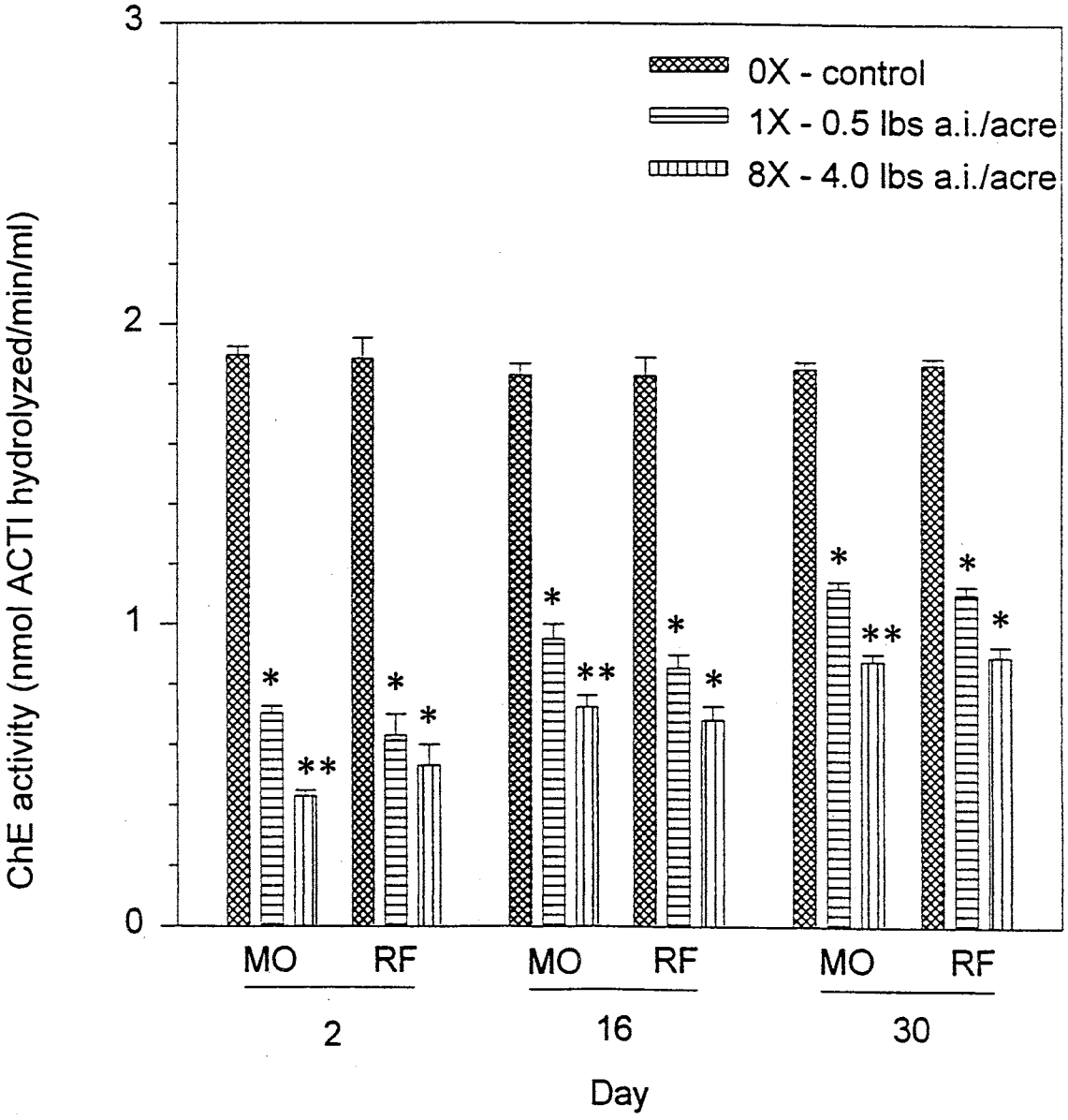

3
2
1
0
ChE activity (nmol ACTI hydrolyzed/min/ml)
0X - control
1X - 0.5 lbs a.i./acre
8X - 4.0 lbs a.i./acre
*
**
SH1
SH2
2
16
30
Day

Figure 3. Effects of subchronic diazinon exposure on plasma cholinesterase (ChE) activity in Microtus ochrogaster (MO) and Reithrodontomys fulvescens (RF) over a 30-day trial (trial 2). * = a significant difference from control, ** = a significant difference from 1X (low rate).


3
2
1
0
ChE activity (nmol ACTI hydrolyzed/min/ml)
0X - control
1X - 0.5 lbs a.i./acre
8X - 4.0 lbs a.i./acre
*
**
MO
RF
2
16
30
Day