POTENTIAL AGRICULTURAL INSECTICIDE EXPOSURE OF INDIANA BATS
(*MYOTIS SODALIS*) IN MISSOURI

A Thesis
presented to
the Faculty of the Graduate School
University of Missouri-Columbia

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
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DECEMBER 1998

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(*MYOTIS SODALIS*) IN MISSOURI

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ACKNOWLEDGEMENTS

I would like to express my sincere thanks to all of the members of my committee, Dr. Ronald Drobney, thesis advisor, and Drs. Susan B. Jones, Chada Reddy, and Mark Ellersieck for their continued assistance and invaluable support throughout the duration of this project. I also want to thank Richard Clawson, Missouri Department of Conservation, for his expert advice and input during the past three years.

I would like to extend a special thanks to my advisor, Ron Drobney, in particular for the opportunity to be included in this phase of the Indiana bat study. He valued and considered all my ideas and provided guidance and pertinent suggestions throughout my tenure at the University of Missouri.

Appreciation is offered to all those students and technicians who helped in the field and with experiments, sometimes continuing late into the early morning. Tom Thompson, David Rubenstein, Todd Farrand, Adrianne Nold, Nancy Miller, and Jennifer Paris helped to make this project run smoothly.

I would also like to thank Dr. Donald R. Clark, Jr. for providing scientific counsel and suggestions for this research.

I would be remiss in failing to acknowledge the following scientists and researchers for their assistance in making this project a reality: Anastasia Becker, Kelly Davis, Jo Ann Grant, Dr. Jim Jarman, Dr. Armon Keaster, Mike Klote, Dr. Charles Knowles, Jim Lovelass, Dr. Carl Orazio, Dr. George Smith, and Tim Spurgeon.

And finally, I want to thank my family for their support and unyielding confidence in my academic pursuits.

This study was funded through the Pittman Robertson Wildlife Act administered by the United States Fish and Wildlife Service, the Missouri Department of Conservation, Region III of the United States Fish and Wildlife Service, and the Missouri Cooperative Fish and Wildlife Research Unit.

ABSTRACT

The type and distribution of agrochemical insecticides routinely applied to the major field crops in northern Missouri were described and shown to be coincident with the summer/maternity range of the endangered Indiana bat (*Myotis sodalis*). The use of insecticides was found to be greatest in the production of corn. In addition, agriculturally significant insect pests causing field crop damage along with those that provide a resource base for the Indiana bat were identified.

Two congeneric surrogate bat species (*M. lucifugus*, the little brown bat, and *M. septentrionalis keenii*, the northern long-eared bat) were collected from northern Missouri agricultural sites and from winter hibernacula in non-agricultural southern parts of the state to assess the potential impact insecticides have on the Indiana bat. Residues of 8 historically applied organochlorine insecticides were identified in all surrogate specimens, with pp-DDE found in greatest amounts. Two synthetic pyrethroids, permethrin and esfenvalerate, were recovered from 16 individuals and both surrogates. Two biomarker indices suggestive of organophosphate and/or carbamate insecticide exposure, brain acetylcholinesterase (AChE) and muscarinic receptor number (B_{max}), were depressed in 10 little brown and 4 northern long-eared bats ($\alpha = 0.05$) collected from 6 northern Missouri agricultural sites. The current findings suggest that some individual bats of both surrogate species living in northern Missouri during the summer months are being exposed to sublethal levels of organophosphate and /or carbamate insecticides.

The sensitivity, as measured by 24h oral LD₅₀ tests in the little brown bat, was

determined to an agricultural field grade and an analytical reference grade of the insecticide permethrin. The field grade product had an LD₅₀ of 38 mg/kg, while the analytical grade had an LD₅₀ of 514 mg/kg. Bats surviving analytical grade LD₅₀ tests were examined for their flight ability at 24 hours. An effective dose (ED₅₀) of 79 mg/kg impaired flight in 50% of tested bats, a dose approximately 15% of that found to produce lethality.

Flight performance of little brown bats was evaluated in obstacle avoidance tests after exposure to analytical grade permethrin. No relationship was found between performance as measured by the total number of obstacle avoidance passes or the percentage of hits and oral permethrin dosage or brain tissue permethrin concentrations. A brain tissue permethrin concentration of 1-3 ppm appears to be a toxic threshold, below which high performance variability is noted. Above a brain concentration of 1-3 ppm, death prior to completion of the experiment at 96h was common.

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CHAPTER I

AGRICULTURAL INSECTICIDE USE IN THE SUMMER/MATERNITY RANGE OF THE INDIANA BAT (*MYOTIS SODALIS*) IN MISSOURI

INTRODUCTION

The Indiana bat (*Myotis sodalis*) is a medium-sized migratory insectivore with a forearm length of 35-41mm, head and body length of 41-49mm, and a weight of 6-9g (Humphrey 1978, Hall 1981, U.S. Fish and Wildlife Service 1983). The scientific name, *Myotis*, is a combination of 2 Greek words and means "mouse ear". The species name, *sodalis*, is Latin for "companion" and refers to the bat's habit of hibernating in great masses (Schwartz and Schwartz 1981). The distribution (Figure 1) of this monotypic species (there are no subspecies) is associated with the major cavernous limestone areas and areas north of cave regions in the Midwestern and eastern United States (Hall 1962, Brady et al. 1983). Winter hibernacula are located in caves and abandoned mines throughout most of its range, but the largest wintering populations are in the Ozark and southern Appalachian mountain regions (Klaas 1986).

The Indiana bat was officially listed as an endangered species on 11 March 1967 by the U.S. Fish and Wildlife Service under the Endangered Species Preservation Act of 15 October 1966 (80 Stat. 926; 16 U.S.C. 668aa[c]). Endangered status was originally applied in 1966 in recognition that 97% of the estimated population of 412,000 (Hall 1962) hibernated in only 4 caves during winter (Humphrey 1978). It is also listed as endangered in several states, including Missouri (MDC 1991).

Figure 1. Known and suspected range of the Indiana bat (*Myotis sodalis*).
(Adapted from Brady et al. 1983)

More than 85% of the range-wide population of the Indiana bat occupies 9 Priority 1 hibernacula (hibernation sites with a recorded population > 30,000 bats since 1960) (Brady et al. 1983). There are 3 Priority 1 hibernacula in each of the 3 states; Indiana, Kentucky, and Missouri. Pilot Knob Mine (Iron county), Bat Cave (Shannon county), and Great Scott Cave (Washington county) are the 3 Priority 1 hibernacula in Missouri. There are also 13 Priority 2 hibernacula in Missouri (hibernation sites with a recorded population >1000 but < 30,000 bats since 1960) (Brady et al. 1983).

Since 1983, the overall Missouri population has shown a cumulative decline of over 250,000 individuals, a reduction of more than 80% of the population (Clawson, personal observation). All 3 Priority 1 and 12 of the 13 Priority 2 hibernacula in the state have experienced declines in colony size. Colonies in other major hibernation caves in Kentucky and Indiana have been able to maintain some measure of stability or demonstrated population increases during the same period. This notable decrease in the Missouri Indiana bat population has occurred despite efforts such as the construction of "bat friendly" gates at cave entrances. Although gates or fences at 8 of the 13 Missouri caves have been constructed to prevent human disturbance (MDC 1992), no link between population trend and protection from disturbance is discernible. Earlier hopes that protection of hibernating populations would provide recovery of the Indiana bat now appear too optimistic and Clawson (1985) suggests that factors other than those pertaining to hibernacula may be causing the decline of the species (Klaas 1986). The Indiana Bat Recovery Plan (Brady et al. 1983) called for more attention to summer foraging and maternity/nursery habitats.

Current knowledge of the Indiana bat summer ecology indicates that following hibernation in caves or abandoned mines in the Missouri Ozarks, females migrate north

in spring to predominantly agricultural regions in Missouri (Esterla and Watkins 1969, LaVal and LaVal 1980, Clawson 1986), Iowa (Bowles 1981), and possibly Illinois (Gardner and Gardner 1980, Brack 1983) to give birth and raise their young. Most of the land in northern Missouri was a tall grass prairie and forest mosaic. Current land use is devoted almost entirely to row-crop agriculture and pasture, with forest being limited to narrow strips along streams and river corridors and small upland tracts. Many of these forested riparian areas are often bordered by cropland.

In summer, females and their young typically roost under loose bark (Mumford and Cope 1958, Cope et al. 1974, Gardner et al. 1991a). They have also been found within tree crevices (Gardner et al. 1991a) and cavities (Humphrey et al. 1977). Parturition occurs in late June and early July and the young are capable of flight at about 4 weeks of age.

Earlier studies of maternity colonies in Indiana (Humphrey et al. 1977), Illinois (Gardner et al. 1991b), Michigan (Kurta et al. 1993), and Missouri (Callahan 1993, Callahan et al. 1997) have provided valuable information concerning roost tree characteristics and roosting behavior. Callahan (1993) defined a maternity colony as a group of bats that shared the same set of maternal roost trees. These 4 studies showed that Indiana bats usually select large diameter trees (living or dead) with loose or exfoliating bark. It was also shown that a roost tree's spatial orientation and resultant exposure to sunlight is a critical factor influencing roost tree suitability. Callahan's (1993) work suggests that an optimal maternity roost would be one that exhibits those thermodynamic properties that would minimize energy expended by female bats for thermoregulation during pregnancy and lactation. Also important is a tree's location in relation to other trees and its relationship to water sources and foraging areas.

The Illinois, Michigan, and Missouri studies had the benefit of radio telemetry and found that multiple roosts were used by maternity colony members, and that the numbers of bats varied among roost trees and temporally within a given roost. Miller's (1996) landscape level study found that sites in north Missouri at which reproductively active Indiana bats had been captured had significantly more large ($> 30\text{cm dbh}$) trees than sites at which Indiana bats were not captured.

Callahan et al. (1997) located 52 roost trees (any tree a bat roosts in during the day) at 4 Indiana bat maternity colony sites in northern Missouri in 1990. The forest components at each of the colony sites was primarily comprised of oaks (*Quercus* spp.) and hickories (*Carya* spp.). Streams traversed in close proximity to each of the 4 colony sites. The number of roosts located at a colony ranged from 8 to 20 and, within a colony, were not widely dispersed. The maximum distance between 2 roost trees used by bats from the colony was less than 3 km. Roost tree ranges (the size of a circle that would encompass all roost trees in a colony) for the 4 colonies had a minimum radius of 0.81 to 1.48 km.

Although Indiana bats are most often captured in the riparian zone, they are not considered an obligate riparian species (Bowles 1981, Clark et al. 1987, Callahan 1993). Indiana bats forage in and around the tree canopy of flood plain, riparian, and upland forest. Cope et al. (1978) characterized woody vegetation with a width of at least 30 m on both sides of a stream as excellent foraging habitat. Indiana bats also forage over clearings with early successional vegetation (e.g., old fields), along the borders of croplands, along wooded fence rows, and over farm ponds in pastures (Clark et al. 1987, Gardner et al. 1991b).

Indiana bats tend to forage within 1 km of their roosts (Belwood 1979), though a maximum foraging range of 3 km was found by Gardner et al. (1991b). Indiana bats are obligate insectivores, feeding strictly upon volant insects; selection of prey items reflects the environment in which they forage. Both aquatic (Belwood 1979, Lee 1993) and terrestrial (Brack and LaVal 1985, Lee 1993) insects are utilized. Reproductively active females and juveniles exhibit greater dietary diversity than males and non-reproductively-active adult females, possibly due to higher energy demands of lactation and growth. Presumably, higher energy demands would result in more opportunistic foraging and in a more general diet. Reproductively-active females eat more aquatic insects than do adult males and juveniles (Lee 1993).

The diet of Indiana bats has been studied by examination of feces (Belwood 1979, Brack and LaVal 1985, Whitaker 1988). According to a comparison of insects eaten with insects collected from the foraging habitat, Lepidopterans (moths) were eaten throughout the summer, often in excess of proportional availability. Trichoptera (caddisflies) and Diptera (flies, midges, mosquitoes) (Belwood 1979), were also identified as major prey items (Kurta and Whitaker, in review). Other prey includes Hymenoptera (bees, wasps, flying ants), Coleoptera (beetles, weevils), Plecoptera (stoneflies), Homoptera (leafhoppers, aphids, greenbugs), Neuroptera (lacewings), and Hemiptera (chinch bugs) (Whitaker 1972, Belwood 1979, Gardner and Brack, unpubl. data).

Land use practices in the Indiana bats' maternity range has changed dramatically from pre-settlement conditions: forest has been highly fragmented, fire has been suppressed, and prairie has been supplanted with agricultural systems composed primarily of row crops and pasture/hayland. Simplification and increased uniformity of

the habitat that is typical of modern agriculture could have profound effects on availability, abundance, and diversity of insects on which bats depend (Lawton and Schroder 1977, George Smith, personal communication).

The high proportion of land in northern Missouri devoted to agriculture raises concerns as to the influence it may have on the quality of Indiana bat habitat and life history patterns (Ricklefs 1990) such as fecundity, juvenile recruitment, and survivorship. It also seems reasonable that some portion of the Indiana bat's insect prey base could be at risk because of its proximity to row-crop agriculture and the routine application of insecticides. Whether any link exists between northern Missouri agricultural systems and the significant population changes seen in the Indiana bat, it is likely that subtle effects on physiology and/or behavior (e.g., thermoregulation, food consumption, reproduction) has occurred to impact this endangered species (Grue et al. 1997).

Insecticides have been implicated in the declines of a number of insectivorous bats in North America (Mohr 1972, Reidinger 1972, 1976, Clark and Prouty 1976, Geluso et al. 1976, Clark et al. 1978, Clark 1981). Little is known about the extent of this problem in Indiana bats. Indiana bat population losses in Missouri, despite extensive efforts to protect bats during hibernation, point to a mortality factor or factors as yet unidentified. Agricultural insecticides in the environment are potential causes.

Despite the ban on their use in the late 1970s, organochlorines (OCs), and/or their metabolites, can persist in the environment for many years (Korschger 1971). The bats' insect diet (especially insects that have developmental stages in contaminated soils or sediments) exposes them to these long-lived compounds. Clark (1981) suggested that even if direct mortality does not occur, sublethal effects on fitness could

be equally devastating. Thus, dieldrin, heptachlor epoxide, DDE, and other breakdown products may still be present and affecting bat populations even though their parent compounds have been banned for agricultural field-application for over 20 years.

The OCs have been replaced by organophosphate (OP), carbamate (CB), and synthetic pyrethroid insecticides. These newer classes of purportedly safer products are used extensively in contemporary agriculture. Although not as likely to bioaccumulate, they have the potential to adversely impact nontarget organisms. The transfer of pesticides from agricultural uses to wildlife populations can be significant (Grue et al. 1983). The usage patterns of currently applied agricultural insecticides in modern agricultural ecosystems could provide insight and explanations into the continuously declining Indiana bat populations.

AGRICULTURE AND ECOLOGY

Agricultural ecosystems are land areas in which physical, chemical, and biological processes are manipulated by humans for the production of food and/or fiber products. The structure and function of natural ecosystems over much of the world have been dramatically changed by agriculture (Stinner and Stinner 1989). The differences between natural- and agroecosystems are intentionally and purposefully introduced human alteration (disturbance) of the ecosystem to enhance specific agricultural production (Gliessman 1990).

Farmers have custody of more environment than does any other group (Paarlberg 1980). Because of periodic and chronic disturbances inherent in agricultural management, agroecosystems are some of the fastest changing landscapes of any ecosystem type. As a result, agroecosystems have contributed significantly to the

degradation of our environment while at the same time providing us with a high standard of living because of relatively low food and fiber costs (Elliott and Cole 1989).

The agricultural transformation of natural areas, involving over 190 million ha of land in the United States (Clawson 1972, Chapin 1983), is historical and ongoing and affects ecological processes at the population, community, and ecosystem levels as well as being one of the powerful anthropogenic forces shaping landscapes (Cox and Atkins 1979, Chapin 1983, Forman and Godron 1986). When a forest or prairie is cleared and plowed, the most obvious change is the dramatic alteration in vegetation structure. This change, in turn, profoundly impacts both above- and below-ground plant-animal distribution and interactions (Stinner and Stinner 1989).

Although there is little direct evidence for how agriculture affects the different kinds of species interactions (e.g., antagonism, mutualism, commensalism), it has been suggested that there is an increase in antagonistic (predator, competition) interactions (e.g., crop-weeds, plant-pathogens, plant-herbivores) in agricultural, versus natural, ecosystems (Crossley et al. 1984, Stinner and House 1989). It is these antagonistic interactions in particular, where a great deal of mechanical and chemical energy (e.g., pesticides, fertilizers) is expended in trying to control influences such as insects, weeds, and disease (Stinner and Stinner 1989).

The spread of agriculture throughout the world has so profoundly influenced the distribution of many plants and animals, that it can be compared to major geological forces, such as continental drift, in determining the distribution of species. This is true for both the species intentionally selected for agricultural production and the pest species (Cox and Atkins 1979). In this context, the term pest includes any harmful, destructive, or troublesome animals, plants, or microorganisms (Ecobichon 1996).

When exotic crop species are introduced to an area, associated pest species can also be inadvertently introduced. Such is the case with the European corn borer (*Ostrinia nubilalis*), a noctuid moth that gained worldwide distribution as a result of the spread of maize (corn) agriculture. This insect originated in eastern Europe. The first record of its presence in North America was in the Boston area in 1917 (Caffrey and Worthley 1927). It has subsequently spread over North America to become one of the most serious, and costly, pests of corn. Also, there is evidence that in the last 50 - 60 years *O. nubilalis* has divergently evolved into several ecotypes, each locally adapted to different geographic and farming conditions (Showers et al. 1975).

In the 1992 Missouri pesticide survey, the Integrated Pest Management Unit (IPMU) of the University of Missouri-Columbia (UMC) reported that corn producers ranked *O. nubilalis* as the insect having the second greatest economic impact (Becker et al. 1992). Missouri agriculture, with its emphasis on monoculture planting systems has, by design, exposed fields of a single crop to pest infestations by providing concentrated resources and uniform physical conditions that encourage specialized insect invasions (Root 1973).

Lawton and Schroder (1977) showed that insect numbers and species richness became increasingly depauperate in the sequence: forest > shrubs > perennial herbs > weeds and other annuals > monocotyledons (corn, wheat, sorghum, rice). In natural populations of arthropods, high species diversity provides a measure of the complexity of the ecological system in which they are found and can also yield as estimate of stability of the local ecosystem web (Price 1975). Some suggest that ecosystem health can be measured as a function of the species diversity to be found there (Steiner et al. 1995).

In natural ecosystems, insect herbivores have major impacts on the characteristics of plants and plant communities, including physiology, morphology, population dynamics, species diversity, and production (Stinner and Stinner 1989). In agricultural ecosystems, protecting against insect pests and diseases are major problems in maintaining production continuity (Altieri 1987). The omnipresence of unwanted insects has naturally lead to the development and encouragement of measures that can limit their damage (Morse and Buhler 1997).

INSECTICIDES AND RESISTANCE

Human control of insect herbivory in cropping systems is substantial. At one time it was thought that insect pests could be eradicated with chemical toxicants (Hunter and Hinds 1904). At first, inorganic poisons such as arsenic, copper, and cyanide compounds and natural plant toxins such as pyrethrum, nicotine, and derris were used to kill insects (Smith 1978, Morse and Buhler 1997). The 1930's ushered in the era of modern synthetic chemicals (Ecobichon 1996). By the beginning of World War II, DDT (dichlorodiphenyltrichloroethane), an environmentally persistent OC insecticide, emerged as a major insect-control product. DDT was highly effective in killing insects and was extensively used (Luckman and Metcalf 1982). However, within 10 years of it's introduction, resistance was being noticed to what had promised to be a panacea for insect control. The loss in efficacy of DDT and many other OCs, coupled with their environmentally persistent effects, caused toxicologists to search for newer and safer chemical compounds to control insect pests (Stinner and Stinner 1989). Within a few years of their introduction and use, the synthetic pyrethroids were also becoming less

effective in controlling those insect populations that had previously developed OC resistance (Mlot 1985).

Insect resistance to insecticides is an example of evolution, an experiment in natural selection (Feyereisen 1995), on a time scale that is sufficiently brief to allow easy detection and quantification. It is a population phenomenon that results from repeated exposure over a number of generations to a compound that kills the most susceptible members of that population. In time, a population is produced that can withstand dosages of a given insecticide that were previously lethal (Cochran 1995). Such individuals become the nucleus for subsequent populations largely comprising resistant individuals (Way and Cammell 1985).

There are 3 major mechanisms of resistance: 1) decreased penetration of the insecticide into the insect's body (Plapp and Hoyer 1968), 2) metabolic resistance in which the resistant insects have enhanced enzymatic processes to degrade the toxin before it becomes lethal (Georghiou 1987), and 3) target-site (molecular chemical receptors) insensitivity in which the nervous system has been modified in such a way that the insecticide no longer has a significant adverse effect on it (Soderlund and Bloomquist (1990). The mechanism of target-site alteration is common in arthropods and is seen in all of the major classes of insecticides (Metcalf 1989, Lee et al. 1993).

Resistance mechanisms could readily allow for a variety of sublethal effects to be expressed at higher insecticide concentrations than would be seen in non-resistant individuals and with many levels of impairment (Haynes 1988). Insect behavioral patterns affecting reproduction, host-finding and feeding, and dispersal and locomotion can be altered (Moriarty 1969). Certain insecticides stimulate or depress locomotor behavior such as walking or flight (Haynes 1988). It has been suggested that if an

insect's mobility (e.g., flight) were impaired by toxins it would be subject to a higher level of predation (Forbes and Forbes 1994). These resistant (R), compromised insects would carry greater insecticidal body burdens than susceptible (S) individuals (Narahashi 1964, 1971) and potentially transfer these chemicals to predators upon their ingestion, obeying classic food-chain dynamics (Ricklefs 1990, Francis 1994).

At the present, 3 classes of insecticides are used in modern agriculture: organophosphate (OP), carbamate (CB), and pyrethroid compounds. The effects of these chemicals are based on an understanding of biological and biochemical mechanisms of pest control and are often effective at very low application rates (IARC 1991). As with all poisons, it must be remembered that reduced quantities does not necessarily mean reduced toxicity (Morse and Buhler 1997). All of the chemical insecticides in use today are neurotoxicants and act by poisoning the nervous system of the target organism (Smith and Stratton 1986). The central nervous system of insects is highly developed biochemically and not unlike that of many vertebrates (O'Brien 1960). Given the fact that insecticides are not selective and can affect nontarget species as readily as target organisms, it is not surprising that a chemical that acts on the insect nervous system will elicit similar effects in higher life forms (Ecobichon 1996).

Finally, a distinction needs to be made between the direct (resulting from the immediate toxicity of a particular compound on select target species) and indirect effects (those resulting from changes in habitat or food resources) brought about by pesticides (Hardy and Stanley 1984). Many environmentally applied chemicals, because of their lack of target organism specificity, can readily impact wildlife species through resource (e.g., insects) or habitat loss or change. For example, if nontarget prey insect species in these environments are reduced or eliminated for variable periods of time, dependent

predators (vertebrate and invertebrate) are left with little to feed upon and migrate elsewhere, or decline in numbers because of starvation (Smith and Stratton 1986, Mian and Mulla 1992). Environmental disturbance with sublethal effects on wildlife can be just as deadly as an acute poisoning event. (Sublethal as defined here is any environmental alteration, chemical or otherwise, which does not directly or acutely cause death.)

The agricultural settlement of Missouri during the last 200 years has, by design and intent, altered and disturbed the structure and function of the natural environment in which it occurred (Stinner and Stinner 1989, Gliessman 1990). Other than environmental contamination from pesticides (Clement 1993, Vyas et al. 1995, Grue et al. 1997), habitat loss and fragmentation (Mader 1984, Fahrig and Merriman 1985, Henderson et al. 1985, Lovejoy et al. 1986) are probably the most common, and accepted, examples of environmental alteration leading to sublethal effects on wildlife. A few background remarks about Missouri agriculture are important in understanding its hypothesized influence on the Indiana bat.

MISSOURI AGRICULTURE

Agriculture plays a key role in Missouri's economy. According to the 1992 Census of Agriculture (U.S. Department of Commerce 1992), over 28.5 million acres, or 64.7% of the 44,094,720 total acres of land in Missouri, were in 98,082 farms. In 1990, 17.5% (> 519,000) of all employment was in farm and farm-related jobs (Pauley 1994). Cash farm receipts for 1995 exceeded 4.3 billion dollars (Sallee 1996). Of that total, 51.5% was from livestock and 48.5% from crops, principally soybeans and corn.

Land use patterns in Missouri were originally highly constrained and dictated by natural factors such as physiography, climate, geologic features, soils, and topography

(Thom and Wilson 1980). With the advent of modern technology and science, agriculture developed and expanded into areas that were previously impossible or impractical to farm. Technological advancements made it unnecessary to completely accept the environment as it was because it could be changed. Modern equipment and new farming techniques such as the steel plow, the internal combustion engine, practical drainage methods of low areas, chemical fertilizers and pesticides, and many other advancements that characterize the “systems approach” of modern farming, have lead to what has become known as the American “agricultural miracle” (Ebeling 1979).

The agricultural settlement of Missouri occurred principally throughout the 19th century. In a relatively short period of time, the state had undergone the transition from a land predominantly covered by prairie grasses and forests to that of an area used mainly for agriculture (Rutledge 1941). Due to the topography and geologic features of Missouri, the land use/land cover varies greatly (Figure 2). Since the karst topography of the Ozarks does not hold highly productive soil, it has more forested land and cattle production than elsewhere in the state. In the northern parts of the state, arable agriculture is the primary land use (Gordon and Bullock 1995).

In 1978 Missouri was organized into a regional scheme that divides the state into 6 major divisions (Glaciated Plains, Big Rivers, Ozark Border, Osage Plains, Ozark, and Mississippi Lowlands) by integrating geologic history, soils, bedrock geology, topography, plant and animal distribution, presettlement vegetation, and other natural factors. These 6 relatively distinct natural divisions (Figure 3) have proved useful in providing a general understanding of the natural history of Missouri as well as for specific applications in natural, and agricultural, resource management and conservation (Thom and Wilson 1980).

Figure 2. Major patterns of land use and cover in Missouri. Note the predominance of agricultural land in the northern part of the state. (From Gordon and Bullock 1995)

- Urban or built-up land
- Agricultural land
- Rangeland
- Forest land
- Water

Note: This map was produced from USGS 1:250,000 Land Use and Data Analysis (LUDA) database. Land use/land cover maps in LUDA were compiled from 1972-1976 aerial photography.

Land use polygons less than 500 acres were dropped.

Figure 3. The natural divisions of Missouri and their sections (From Thom and Wilson 1980)

The Natural Divisions of Missouri and Their Sections

- Glaciated Plains
 - a. Western
 - b. Grand River
 - c. Eastern
 - d. Lincoln Hills
- Big Rivers
 - a. Upper Missouri
 - b. Lower Missouri
 - c. Upper Mississippi
 - d. Lower Mississippi
- Ozark Border
 - a. Missouri River
 - b. Mississippi River
- Osage Plains
- Ozark
 - a. Springfield Plateau
 - b. Upper Ozark
 - c. St. Francois Mountains
 - d. Elk River
 - e. White River
 - f. Lower Ozark
- Mississippi Lowlands
 - a. Crowley's Ridge
 - b. Lowlands

The Glaciated Plains division extends southward from Iowa to the Osage Plains and Ozark Border division. In presettlement times about 45 % of this division consisted of upland forest and prairie (Thom and Wilson 1980). The Glaciated Plains are so named because 2 glacial events (Nebraskan and Kansan Stages) covered approximately the northern third of the state (Figure 4) during the Pleistocene epoch with an ice sheet that nearly extended to the present Missouri River (Mehl 1962). Upon receding, what was left was a mantle of drift from 10 to 100 or more feet in thickness, consisting of the ground-up material of the underlying shales, limestones, and sandstones, together with soil material and boulders brought down from the north (Miller and Krusekopf 1929). In time, these materials became the loam and clay loam till that provides northern Missouri the highly productive soils to support agriculture as the primary land use (Figure 3).

Missouri is divided into 8 crop reporting districts (Figure 5); only the 3 northernmost districts (Northwest, North Central, and Northeast) are considered to contain significant Indiana bat summer/maternity habitat (Myers 1964, LaVal and LaVal 1980, Bowles 1981). The 3 districts comprise most of the northern third of the state north of the Missouri River and are composed of 37 counties. The soils and topography of this region are characteristic of the Glaciated Plains.

Acreage in northern Missouri allotted to field crops was planted, in decreasing order of occurrence (1992) with soybeans (Figure 6), corn (Figure 7), wheat (Figure 8), sorghum (Figure 9), and alfalfa. Despite the fact that northern Missouri contains only 28% of the states' total land area, approximately 41% of all harvested acres were within the 3 crop reporting districts (U.S. Dept. of Commerce 1994). Except for the southeast

Figure 4. Approximate maximum extent of Nebraskan and Kansan ice sheets in Missouri. Some major features of preglacial drainage in northwestern Missouri are indicated. (From Mehl 1962)

Figure 5. Missouri crop reporting districts. (From Becker et al. 1992)

Figure 6. Location of soybean production (harvested bushels), 1987 and 1992.
(From U. S. Department of Commerce, 1992 Census of Agriculture)

1987

Missouri = 148,272,506 Bushels

1992

Missouri = 150,385,224 Bushels

Figure 7. Location of corn production (harvested bushels), 1987 and 1992.
(From U. S. Department of Commerce, 1992 Census of Agriculture)

1987

Missouri = 218,093,408 Bushels

1992

Missouri = 308,784,225 Bushels

Figure 8. Location of wheat production (harvested bushels), 1987 and 1992.
(From U. S. Department of Commerce, 1992 Census of Agriculture)

1987

Missouri = 31,939,663 Bushels

1992

Missouri = 58,143,633 Bushels

Figure 9. Location of sorghum production (harvested bushels), 1987 and 1992.
(From U. S. Department of Commerce, 1992 Census of Agriculture)

1987

Under 5,000

5,000 to 24,999

25,000 to 99,999

100,000 or More

Missouri = 50,759,064 Bushels

1992

Under 5,000

5,000 to 24,999

25,000 to 99,999

100,000 or More

Missouri = 53,048,665 Bushels

“Bootheel” region, a disproportionate amount of the states’ total cropland acreage was north of the Missouri River in 1987 in addition to 1992 (Figure 10).

Efficient row-crop agriculture has become absolutely dependent upon insecticides and some minimal pesticide application requirement will continue if current production levels are to be maintained (Pimentel et al. 1990). On a seasonal or yearly basis, insecticide usage will vary to address specific local or regional insect control patterns (Becker et al. 1992). Despite these fluctuations in insecticide usage, crop acreage and production levels can give additional usage information because they are often closely correlated with insecticide mass and distribution patterns. In addition, insecticide patterns provide the basis for determining the degree of exposure in studies of risk assessment.

My goal was to identify the insecticides applied to the major field crops in northern Missouri during the period when Indiana bats are foraging and raising their young in this highly agricultural region of the state. My specific objectives were to: 1) determine types, amounts, and distributions of agricultural insecticides used in the summer/maternity range of the Indiana bat in Missouri, 2) identify agriculturally significant insect pests causing field crop damage along with those that provide a resource base for the Indiana bat, and 3) identify specific, commonly used field crop insecticides and product formulations.

METHODS

In 1978 (English 1978), 1989 (Smith and Fairchild 1990), 1991 (Smith et al. 1991), and 1992 (Becker et al. 1992), random surveys of agricultural producers

Figure 10. Missouri total cropland acreage 1987. (From Missouri Farm Facts 1987) Wilson 1980)

throughout Missouri were conducted to determine pesticide usage patterns during the routine production of 5 major field crops (Marlowe Schlegel, personal communication). Data for alfalfa is from a 1991 survey and has been adjusted for and included with 1992 information. The surveys were a cooperative venture of the IPMU of the University of Missouri-Columbia (UMC), the Missouri Agricultural Statistics Service (MASS), the Missouri Department of Natural Resources (MDNR), and the Missouri Department of Agriculture (MDA). Survey results provided the necessary information to identify the types and amounts of insecticides applied in areas of the state considered critical Indiana bat summer/maternity habitat.

Estimated rates of insecticide use for each crop and each survey year were made using: 1) total planted acres for each reporting district, 2) percent acres treated for each reporting district, and 3) total state acres treated with each insecticide listed by brand name and active ingredient (common name). All named insecticides were grouped into 3 major classes by similar mechanism of action, chemical structure, and metabolism. Insecticide groups and examples are, 1) organochlorines (heptachlor, toxaphene, and methoxychlor), 2) organophosphates/carbamates (chlorpyrifos, terbufos, and carbofuran), and 3) synthetic pyrethroids (permethrin, tefluthrin, and esfenvalerate). A proportion of the total state treated acres was determined for each class; this allowed the calculation of treated acres for each of the 3 crop reporting districts by insecticide class. In 1992, the proportion of treated acres for each insecticide class was based on use patterns of the northern 3 crop districts as provided by the IPMU of UMC.

Additional information was obtained from the U.S. Department of Commerce (1994), U. S. Department of Agriculture (1995), and the U.S. Geological Survey (1994).

These and other sources provided information on the occurrence and distribution of pesticides in ground water and rural wells from primarily agricultural settings throughout the state of Missouri. Geology, soils, and topography in a particular region determine groundwater vulnerability to contamination from pesticides applied to cropland (Ritter 1990). Contaminated groundwater samples recovered in agricultural regions help to support the notion of aggressive and irresponsible use of pesticides.

Commercially available insecticide products can differ greatly in toxicity and exposure potential making clear comparisons of insecticidal risk difficult. Because the number of treated acres is a reliable indicator of the scale of insecticide use and is more closely associated with the likelihood of bat exposures, it is an important criteria used to access the relative risk to these chemical compounds. The identification of selected insecticides from Missouri groundwater samples also suggests a level of risk Indiana bats may experience when in their summer/maternity range.

Agriculturally significant insect pests causing field crop damage were identified along with those that provide a food resource base for the Indiana bat. Insect life cycles within or near the agricultural environment (terrestrial and aquatic) help to provide the link in the movement of insecticides from the field and into the food chain. The duration of insecticide concentrations in contaminated soil is an integral part in the overall determination of risk (Kumar 1984, McEwen et al. 1986, Beyer and Linder 1995). The IPMU of UMC assisted in providing methods and timing of insecticide applications and their formulations. These steps show the biological connection between the field application of an insecticide and potential risks to the Indiana bat.

RESULTS

AGRICULTURAL INSECT PESTS

Indiana bats are obligate insectivores. The dietary variation of *M. sodalis* is associated with the habitat in which they forage (Belwood 1979, Lee 1993); both aquatic and terrestrial insects are utilized. There are 9 important orders of insects that make up the majority of the diet of Indiana bats: (a) Lepidoptera, (b) Coleoptera, (c) Diptera, (d) Homoptera, (e) Hemiptera, (f) Hymenoptera, (g) Trichoptera, (h) Plecoptera, and (i) Neuroptera. Of these 9 orders (Table 1), the first 6 are considered significant agricultural pests (Johansen 1985). The common insect pests in field crops in Missouri typically belong to 5 major orders (Table 2).

One method of classifying insects is by their type of growth or development. The change in form during the development of an insect is called metamorphosis and is either simple or complex (complete) in most insect orders. In simple metamorphosis there is a relative gradual change in the external appearance in the molting steps from egg to adult. Immatures are called nymphs and have feeding habits similar to those of the adult (Pfadt 1985). Chinch bugs, corn leaf aphids, stoneflies, and green bugs are common examples of insects with simple metamorphosis (Harwood 1985). Of the insect orders that comprise most of the Indiana bat's diet, only Homopterans, Hemipterans, and Plecopterans have a simple form of development.

The greatest external and internal structural changes take place among insects with complex metamorphosis. The postembryonic form is a larva, which feeds actively and may have locomotory appendages developed to varying degrees. After several molts the inactive pupa is formed. At the termination of the pupal stage a highly active

Table 1. Characteristics of Insect Orders that comprise the majority of the diet of Indiana bats.

Order	Examples	Metamorphosis	Mouthparts	Habitat ^a	Agricultural Importance ^b
Lepidoptera	Moths, Butterflies	Complex	Chewing in Larvae Siphoning in Adults	T	5
Coleoptera	Beetles, Weevils	Complex	Chewing	T & A	5
Diptera	Flies, Midges, Mosquitoes	Complex	Piercing, Sucking, or Sponging . Chewing in Larvae	T & A	5
Homoptera	Aphids, Leafhoppers, Scales, Greenbugs	Simple	Piercing-Sucking	T	5
Hemiptera	Chinch Bugs	Simple	Piercing-Sucking	T & A	4
Hymenoptera	Flying ants, Bees, Wasps	Complex	Chewing or Lapping	T	2
Trichoptera	Caddisflies	Complex	Chewing	A	0
Plecoptera	Stoneflies	Simple	Chewing	A as nymphs	1
Neuroptera	Lacewings	Complex	Chewing	T & A	0

Source: Adapted from Fundamentals of Applied Entomology, Robert E. Pfadt, ed.1985.

^a Habitat Type: A = Aquatic; T = Terrestrial

^b Value indicates extent of agricultural importance (5 = most injurious).

Table 2. Classification of common insect pests in field crops in Missouri by Order.

Lepidoptera	Coleoptera	Homoptera	Hemiptera	Diptera
Black cutworm	Wireworms	Green bug	Chinch bug	Sorghum midge
European corn borer	Corn Rootworm Seed corn beetle	Corn leaf aphid		Seed corn maggot
Armyworm	Cereal leaf beetle			
Corn earworm	Bean leaf beetle			
Southwestern corn borer	Alfalfa weevil			

Source: Grain Crop Pesticide Use Survey Missouri 1992.

adult results. These variations in structure are often accompanied by different habits; larvae and adults may have different food needs and occupy radically distinct environments. Corn rootworms, cutworms, sorghum midge, and mosquitoes are common representatives (Peters 1988). A majority of the insects upon which Indiana bats forage have complex developmental patterns.

CORN

Corn has the highest value of any crop produced in the United States (Figure 11). Over 25 major destructive insect species and many more minor ones are responsible for significant damage every year. Some of these pests are readily visible as they feed on the leaves, stems, or silks, but the majority hide and feed inside the stalk and roots, in the ears, in the whorl (a ring-like arrangement of like parts, usually leaves, around the stem of a plant) or underground (Burkhardt 1985). There are more treated acres and a greater mass of insecticide active ingredients applied to corn in Missouri, and in the United States, than any other crop (Becker et al. 1992, Gianessi and Puffer 1992).

In a 1992 Missouri pesticide use survey (Figure 12), corn producers indicated that 3 insects caused the greatest economic losses: the black cutworm (15%), European corn borer (14%), and wireworms (10%). Corn rootworms, chinch bugs, and armyworms were also significantly damaging. Together these 6 insects accounted for over 45% of all responses from corn producers; 52% of the producers indicated no insect problems in their corn crop (Becker et al. 1992).

Except for chinch bugs (Hemipteran), the major corn pests are either Lepidopteran or Coleopteran species. Many of these pests make up the soil insect

Figure 11. Ten major corn producing states, 1994. (From U. S. Department of Agriculture, 1994 Field Crop Summary)

Figure 12. Producer survey of insects causing the greatest economic loss to 1992 corn crop.

Source: Grain Crop Pesticide Use Survey Missouri 1992

* 52% of the responses indicated no insect problems.

complex, a highly destructive and diverse group of species that spend a portion of their life cycle within the soil causing damage to root systems. Black cutworms, wireworms, and corn rootworms are examples of species belonging to the soil insect complex. Corn borers and armyworms enter the above-ground plant and feed on the folded leaves often destroying the central bud (Burkhardt 1985).

When these larval feeding stages have reached adult reproductive status, egg laying begins. Adults, particularly Lepidopterans, are strong fliers and lay many eggs during their lifetime on corn plants. Chinch bug adults are also volant and disperse widely during egg laying. Chinch bug larvae have piercing-sucking mouth parts and do considerable damage by withdrawing enormous quantities of plant fluids (Bland and Jaques 1978).

Granular formulations (G) were used with most of the insecticides applied to corn in 1992 (Becker et al. 1992). They are often more effective than other formulations against soil insect pests. Some of the OPs (terbufos and phorate) and CBs (carbofuran) act systemically affecting insects that feed upon plant tissues and fluids. Granular forms are applied by broadcast methods or in bands along the rows, either before or at planting time, usually between March and early May. Granules can be applied over the developing leaf whorl to control European corn borer and corn rootworm larvae. Applied in this manner, insecticide granules roll down into the whorl of growing leaves where larvae congregate (Burkhardt 1985).

Growers of corn can also apply insecticide solutions made from emulsifiable concentrates (EC) such as permethrin or wettable powders (WP) like chlorpyrifos (Fronk 1985) with the use of high-clearance sprayers or airplanes. Treatment of entire fields is sometimes necessary with repeat spring and summer applications dependent on the

degree of infestation. Foliar insecticide applications volatilize quickly after spraying, leaving a deposit of toxicant after the water or other carrier solvent has evaporated. In this way, flying adult insects contacting treated plant surfaces are exposed to and absorb (Welling 1977, Lankford 1994) chemicals during egg laying periods of their reproductive cycle.

The OP and CB, and pyrethroid classes of insecticides were applied to 52% and 48% of treated corn acres, respectively, in 1992. Approximately 33% of total corn acreage was treated for insect pests that year (Becker et al. 1992).

SORGHUM

In 1992, Missouri sorghum producers indicated that 4 insect pests caused the greatest economic losses (Figure 13): the green bug (9%), armyworms (7%), sorghum midge (6%), and chinch bug (4%). These insects were responsible for 26% of the total responses from sorghum producers; however, 66% of the responses indicated no insect problems in their sorghum crop (Becker et al. 1992).

Common sorghum pests in Missouri can belong to 4 different insect orders: (a) Homoptera (green bug), (b) Lepidoptera (armyworms), (c) Diptera (sorghum midge), and (d) Hemiptera (Chinch bug). Depending on the season and conditions, wireworms can also be a problem. The green bug and chinch bug impoverish plants by sucking juices from leaves or stems (Pfadt 1985). The larvae of the sorghum midge inflict injury by feeding on the early developing seeds (Davidson and Lyon 1987).

Granular systemic insecticides such as carbofuran, phorate, and terbufos were used in most sorghum insect pest infestations in 1992. The soil and surface-active insecticide chlorpyrifos was used to control soil (e.g., wireworms) and foliar feeding

Figure 13. Producer survey of insects causing the greatest economic loss to 1992 sorghum crop.

Source: Grain Crop Pesticide Use Survey Missouri 1992

* 66% Of The Responses Indicated No Insect Problems.

** Includes Grasshoppers, Ants, and Leafhoppers

insects (e.g., armyworms, greenbugs, and sorghum midge) when applied as either a granular formulation or as an EC (Becker et al. 1992).

The OP and CB classes combined, were applied to over 95% of treated acres. Only 5% of sorghum acres was insecticide treated in 1992 (Becker et al. 1992).

WHEAT

In 1992, the only insect pest causing any significant wheat damage was the armyworm (Figure 14). An unusually heavy outbreak of armyworms was responsible for 36% of reported losses. This outbreak was centered primarily in the southwest and west central crop reporting districts (Becker et al. 1992).

Armyworm outbreaks are notable for their suddenness and severity. Aggregations of the adult moths, flying or wind carried, alight in fields of grain at night to deposit millions of eggs (Pfadt 1985). Insecticide sprays applied by ground equipment or airplane are effective. The OP and CB classes combined were applied to over 95% of the treated acres in Missouri. Only approximately 1.6% of the total wheat acreage was insecticide treated in 1992 (Becker et al. 1992).

SOYBEANS

Soybeans in 1992 (Figure 15) encountered insect problems with only the bean leaf beetle (7%). Eighty six percent of soybean producers indicated that insects pests were not a problem (Becker et al. 1992). Historically, soybeans have been the field crop least plagued by insects in Missouri and consequently are least insecticide treated. Less than 1% of soybean acreage was treated for insect control in 1992 (Becker et al. 1992). The bean leaf beetle causes injury to the host plant by adults devouring the leaves,

Figure 14. Producer survey of insects causing the greatest economic loss to 1992 wheat crop.

Source: Grain Crop Pesticide Use Survey Missouri 1992

* 62% Of The Responses Indicated No Insect Problems.

**Includes Cutworms and Grasshoppers

Figure 15. Producer survey of insects causing the greatest economic loss to 1992 soybean crop.

Source: Grain Crop Pesticide Use Survey Missouri 1992

* 86% Of The Responses Indicated No Insect Problems.

pods, and stems and the larvae feeding on the roots (Davidson and Lyons 1987). As with the alfalfa weevil, surface-active chemicals are applied with either ground equipment or airplanes (Haws and Davis 1985). The OP and CB, and pyrethroid insecticides were applied to 66% and 34% of treated acres, respectively, in 1992 (Becker et al. 1992).

ALFALFA

Alfalfa, a forage legume, is attractive to many insects (Haws and Davis 1985). In 1991 (Figure 16), the alfalfa weevil was cited as causing the most damage (71%; Smith et al. 1991).

The alfalfa weevil adult emerges from hibernation in the spring to feed on the young growth and females begin egg laying within a few days of emergence. Chemical controls for alfalfa weevils are usually aimed at the larvae and can be applied by either ground or air (Haws and Davis 1985). Chlorpyrifos and carbofuran, applied on 50% and 32% of treated acres, respectively, were the most commonly used insecticides and accounted for 84% of active ingredients by mass applied to alfalfa acreage (Smith et al. 1991). The OP and CB insecticide classes combined were applied to 99% of the treated acres in the northern 3 crop reporting districts in 1992. Approximately 30% of alfalfa acreage was insecticide treated in 1992.

INSECTICIDE USE IN THE CORN BELT

Nationwide, and in Missouri, the OP insecticide chlorpyrifos ranked number 1 as the most applied (mass) active ingredient (a.i.) for the year 1992 (Gianessi and Puffer 1992, Becker et al. 1992). Of the top 20 insecticide active ingredients used in the United

Figure 16. Producer survey of insects causing the greatest economic loss to 1992 alfalfa crop.

Source: Pesticide Use Survey Missouri 1991

* 9% Of The Responses Indicated No Insect Problems.

** Includes Blister Beetles, Cutworms, and Armyworms

States for all crop production (pounds a.i. per year), 15 were either OP or CB class insecticides (Gianessi and Puffer 1992).

There are 10 major corn producing states (identified by two-letter postal abbreviation) in the Corn Belt (Figure 11). The greatest use of pesticides occurs in this region of the United States (Ritter 1990). Gianessi and Puffer (1992) rank corn as receiving more insecticide mass a.i. per year than any other crop, with amounts exceeding those applied to citrus crops and cotton. Of the 10 major corn producing states in 1994 (Table 3), Missouri ranked 10th in area planted and total mass of all insecticides applied (USDA 1995). Two neighboring corn states, Iowa and Illinois, ranked number 1 and number 2, respectively, in total acres planted in corn. Both of these states are considered important summer maternity habitat for migrating Indiana bats (Bowles 1981, Brack 1983, Gardner and Gardner 1980).

Iowa ranks number 3 (2.9 million pounds) and Illinois ranks number 2 (>3.0 million pounds) in insecticide mass used (Table 3). Except for Bt (*Bacillus thuringiensis*), all of the insecticides used in the Corn Belt states belong to either the OP or CB class (Table 4). Bt is a bacterial organism which causes disease in certain insects, thereby controlling them (Thomson 1994). Chlorpyrifos and terbufos were the most commonly used individual insecticides, with application to 8% and 6% of the report acreage, respectively (USDA 1995). Of the 10 major corn producing states, 27% of the total area (62.5 million acres) planted in corn in 1994 received insecticide treatment, higher than the 16% used in Missouri in 1994 (Table 3).

In a comparison made of the accepted range of the Indiana bat (Figure 1) and the 10 major corn producing states (Figure 11), all or part of 7 corn states are contained within the accepted Indiana bat range (Figure 17). In addition, 6 of the 9 Priority 1

Table 3. Corn acreage and percent of area receiving pesticide applications and amount applied, major states, 1994.

State ^c	Area Planted (1000 acres)	Area Receiving and Total Applied ^a			
		Insecticide ^b		Herbicide	
		Percent	(1000 lbs.)	Percent	(1000 lbs.)
Iowa (3)	13,000	27	2,947	99	38,199
Illinois (2)	11,600	31	3,009	99	37,870
Nebraska (1)	8,600	53	3,468	97	18,735
Minnesota (8)	7,000	8	491	98	15,218
Indiana (4)	6,100	24	1191	97	19,601
South Dakota (9)	3,800	7	402	93	7,022
Wisconsin (5)	3,750	25	906	97	8,456
Ohio (7)	3,700	19	537	98	11,382
Michigan (6)	2,550	24	575	97	6,987
Missouri (10)	2,400	16	170	96	6,749
Total	62,500	27	13,695	98	170,221

Source: Crop Production - 1994 Summary and Agricultural Chemical Usage Survey.

National Agricultural Statistics Service, USDA.

^a Refers to percent area planted receiving 1 or more applications of a specific pesticide class.

^b Total applied excludes Bt (*Bacillus thuringiensis*), quantities are not available because amounts of active ingredient are not comparable between products.

^c Number in parentheses () = state ranking by mass of applied insecticides.

Table 4. Agricultural insecticide applications, major states, 1994.^a

Insecticides: ^b	Percent Area Applied ^c	Number Applications	Rate per Application ^d	Rate per Crop Year	Total Applied ^e
Bt (<i>B. thuringiensis</i>) ^f	1	1.0			
Carbofuran	1	1.0	0.88	0.88	326
Chlorpyrifos	8	1.0	1.07	1.08	5,206
Fonofos	2	1.0	1.03	1.06	1,549
Methyl Parathion	2	1.4	0.39	0.53	738
Permethrin	3	1.0	0.11	0.11	222
Phorate	1	1.0	0.97	0.97	895
Tefluthrin	4	1.0	0.11	0.11	242
Terbufos	6	1.0	1.06	1.07	4,270

Source: Crop Production - 1994 Summary and Agricultural Chemical Usage Survey.
National Agricultural Statistics Service, USDA.

^a Area planted in 1994 for the 10 major corn states was 62.5 million acres.

^b Insufficient reports to publish data for the following insecticides: carbaryl, chlorethoxyfos, dimethoate, disulfoton, esfenvalerate, petroleum distillates, and trimethacarb.

^c Refers to percent area planted receiving 1 or more applications of a specific insecticide.

^d Pounds per acre.

^e Million of pounds.

^f Rates and total applied are not available, amounts of active ingredient are not comparable between products.

Figure 17. Comparison of accepted range of the Indiana bat (shaded area) and 10 major corn producing states. (From Brady et al. 1983 and USDA-Field Crops summary 1994)

hibernacula are in 2 states (Missouri and Indiana) in the southern portion of the Corn Belt.

GROUND WATER AND RURAL WELL CONTAMINATION

In the United States in 1985 pesticides were being used at a rate approaching 1 billion pounds per year. It was then predicted that these chemicals would continue to play a major role in plant protection for the foreseeable future (Hall 1985). When a pesticide is applied to soil, it may undergo microbial decomposition, photodecomposition, chemical degradation, volatilization, plant uptake, or adsorption. It may also be transported in surface runoff or sediment or be leached through the soil profile (Ritter 1990).

Northern Missouri contains extensive areas of land used for agricultural crop and livestock production. The percent of cropland in corn and soybeans during 1990 ranged from 95% in Nodaway county to 63% in Audrain county (Wilkison and Maley 1994). Agriculture of such a scale has lead to a growing concern in Missouri regarding the quality of water resources. There is documentation to suggest that in states surrounding Missouri the widespread use of insecticides, and herbicides, on agricultural land is beginning to pollute ground water resources, particularly in shallow aquifers, either through pesticide application or point-source contamination at the well (Steichen et al. 1988, Kross and Hallberg 1990). Because agricultural practices in nearby Midwest states are similar to those in Missouri, it is reasonable to assume that ground water in rural Missouri could be similarly affected (Sievers and Fulhage 1992).

In 1992 Sievers and Fulhage sampled 8 areas of the state (Figure 18) where agricultural practices dominated land use and where geology indicated a potentially high

Figure 18. Sampling areas (A, B, C, D, E, F, G, H) for well survey. Description of areas are in Table 5. (From Sievers and Fulhage 1992)

vulnerability to ground water pollution from pesticides (Table 5). A total of 201 wells (<300-foot deep) were chosen and were within or immediately adjacent to cropping or livestock areas. Water wells in each area were sampled by collecting directly from spigots. Samples were analyzed for 13 insecticides, 12 herbicides, and nitrate.

For the entire survey, samples from 87 wells (43%) had at least 1 pesticide detection. This represents 19 different chemicals: 8 insecticides and 11 herbicides. Of the 36 confirmed detections (≥ 1 ppb), all but 1 came from areas along or north of the Missouri River (Figure 18 - Areas A, B, C, D, G). With the exception of the southeast Bootheel region, these 5 areas contain the majority of corn, soybeans, and sorghum acres and account for a large proportion of Missouri's pesticide usage (Smith and Fairchild 1990, Becker et al. 1992).

Table 6 shows 3 major field crops and the number of pesticide detections. Both carbaryl (3) and diazinon (1) were recovered at ≥ 1 ppb. Table 7 lists detections by area and month of the year. Well D-7 is unique in that during a dry summer it was filled with water siphoned from a nearby pond. The pond was below a field previously cropped in corn. In May an analysis of this well revealed the presence of carbofuran (0.5 ppb) and heptachlor (0.8 ppb).

In this study average well depth was significantly correlated ($R^2 = 0.96$) with the number of positive pesticide detections; shallow wells were much more likely to be contaminated. It would seem logical that shallow wells and surface waters (ponds, streams) would be subject to similar levels of contamination from spills, runoff, and drift (Nations and Hallberg 1992, Smith 1990). These surface waters could be a contaminant source that might expose bats and the aquatic insect prey they depend upon. Sievers and Fulhage (1992) concluded that the number of pesticide detections appeared to

Table 5. Vulnerable areas chosen for sampling, general ground water region, principal agriculture, and general soil type.

Area (Figure 1)	Counties Included	Ground Water Region ^a	Principal Agriculture of Counties ^b	General Soil of Area ^c
A	Nodaway Atchison	Glaciated Plains	Corn(1), Soybeans(5), Hogs(4) Corn(3), Soybeans(9)	Deep Loess Hills
B	Carrol Chariton	Glaciated Plains	Corn(8), Soybeans(4), Wheat(2) Corn(7), Soybeans(7), Wheat(3)	Heavy Till Plain
C	Saline Lafayette	Osage Salt Plains	Corn(2), Soybeans(10), Hogs(10) Corn(4), Hogs(1)	Loess Hills
D	Monroe Shelby Audrain	Osage Salt Plains	Wheat(9), Sorghum(7), Hogs(7) Hogs(8) Soybeans(2), Wheat(7), Sorghum(3)	Claypan
E	Webster Greene	Ozarks	Dairy(2), Beef(3), Dairy(6)	Ozark Highland
F	Wright Texas	Ozarks	Dairy(1) Beef(9), Dairy(8)	Ozark Highland
G	Lafayette Saline Carrol Howard Boone	Missouri Alluvium	Corn(4), Soybeans(15) Corn, Soybeans	Alluvium
H	Barton Jasper Lawrence	Ozarks	Wheat(1), Sorghum(12) Sorghum(17), Wheat(11), Beef(18), Dairy(16) Beef(1), Dairy(5)	Cherokee Prairie

^a Source: Missouri Department of Natural Resources (1986)^b Number in parentheses () = state ranking by county. Source: Bay and Bellinghausen (1986)^c Source: USDA (1975)

Table 6. Percent of corn, soybean, and sorghum acres in areas A, B, C, D, G, and H treated with insecticides and total amount used.

Insecticides:	Percent of Total Treated Acres in the State			Total Pounds A.I. ^a Used in Areas	Number Detections ^b
	Soybeans	Corn	Sorghum		
Carbaryl		0.1		1141	3
Carbofuran		4.3	7.2	24,419	0
Chlorpyrifos	0.1	19.2	9.9	107,867	0
Fonofos		1.7		9,733	0
Terbufos		6.6		36,820	0
Diazinon		2.0		354	1
Parathion			2.6		0
Ethoprop		0.5		2,866	0
Lindane		2.0		326	0

Source: Pesticide Use Survey in Missouri Field Crops 1989.

^a Active Ingredient

^b Greater than or equal to 1 ppb (part per billion).

Table 7. Individual wells with insecticide detections equal to or greater than 1 ppb.

Well	Insecticides:	Level of Insecticide Detected (ppb)			
		December	March	May	September
B-16	Carbaryl			1.1	
B-21	Diazinon	1.0			
C-10	Carbaryl	1.3			
C-24	Carbaryl	1.0			
	Lindane (0.5 ppb) ^a				
D-7	Carbofuran			0.5	
	Heptachlor			0.8	
G-4	Carbaryl			< 1 ppb	
	Diazinon			< 1 ppb	

Source: Adapted from Sievers and Fulhage, 1992.

^a Month of detection not given.

correspond with pesticide-use patterns associated with the major crops of corn, soybeans, and sorghum.

In another study, Cohen, Creeger, Carsel, and Enfield (1984) found that the carbamate aldicarb was recovered in Missouri ground water in the range of 1 to 50 ppb. An Ozark Plateau study in 1993 detected p,p'-DDE in 4 samples from domestic wells and springs in a concentration range of 0.002 to 0.003 ppb. Carbaryl, chlorpyrifos, lindane, and dieldrin were also detected with concentrations ranging from 0.003 to 0.032 ppb. Except for lindane, p,p'-DDE (a metabolite of DDT), and dieldrin, carbaryl aldicarb, and chlorpyrifos are routinely used agricultural insecticides (Adamski and Pugh 1996).

In Iowa, Nations and Hallberg (1992) sampled rainfall for pesticides to assess their occurrence in precipitation. Both rural and urban settings were sampled. Four OP insecticides were detected; dimethoate (0.23 ppb), fonofos (0.12 ppb), malathion (0.17 ppb), and methyl parathion (2.77 ppb). Pesticide detections were seen from April to August during typical applications periods and when greater volatilization rates occurred during warmer weather. Insecticides concentrations were greater at sampling sites near agricultural fields where applications occurred. Volatilization of agricultural chemicals was seasonal and greatest a few days after application.

AGROCHEMICAL INSECTICIDE USE IN NORTH MISSOURI

Over 2000 producers of more than 2.0 million acres of corn, sorghum, wheat, soybeans, and alfalfa responded to the 1991 and 1992 Missouri Pesticide Use Surveys (Smith et al. 1991, Becker et al. 1992). Prior to 1992, a comparable survey was conducted in 1989 with funding by the Environmental Protection Agency through the Missouri Department of Natural Resources. Before 1989, the last survey year was 1978.

Insecticide use patterns vary and reflect specific insect control problems unique to a particular crop, year, season, or region within the state. Despite this variability, insecticide use from 1978 to 1992 indicated notable trends and patterns relative to the class and amounts of insecticides used and the particular crops upon which they were applied. Land use in northern Missouri is predominantly field crop agriculture and results in more uniform insecticide application patterns than in the southern Ozark Plateau portion of the state (Figure 2), which has a more variable and dissected topography and is primarily forest land. The southeastern corner of Missouri (Bootheel) is a highly agricultural alluvial plain known for its cotton, rice, soybean, and corn production.

Total insecticide treated acres in northern Missouri has shown a consistent decline during the 3 survey periods (Figure 19). Thirty-two and 21% reductions in treated acres were seen from 1978 to 1989 and 1989 to 1992, respectively. This same general pattern is seen in each of the 3 crop districts, except for an 11% increase in the northeast in 1992. This increase was due to a 67% addition in acreage treated with pyrethroids in that district between 1989 and 1992 (Table 8, Figure 22).

Statewide, of all harvested cropland in 1992 (12.16 million acres), nearly 5 million acres, or approximately 41%, were in the northern 3 crop districts, composed of 37 counties (U.S. Dept. of Commerce 1994). When total state insecticide treated acres is considered for that same year (1.79 million acres), the northern part of the state accounts for 36% or nearly 637,000 acres of those treated acres (U.S. Dept. of Commerce 1994). The 637,000 figure is approximately 22% larger than that reported by the 1992 Pesticide Use Survey (Becker et al. 1992). The Becker survey was limited to 5 field crops only. The data presented by the U.S. Dept. of Commerce in 1994 includes "sprays, dusts, granules, fumigants, etc., to control insects on hay and other crops."

Figure 19. Total insecticide treated acres by region and survey year for northern Missouri.

Source: Grain Crop Pesticide Use Surveys Missouri 1978, 1989, and 1992.

Table 8. Insecticide treated acres^a by class and survey year in the 3 northern crop reporting districts.

Insecticide Class	Northwest			North Central			Northeast		
	1978	1989	1992	1978	1989	1992	1978	1989	1992
Organochlorines	53.7	12.4	0.3	22.7	3.3	0.041	29.0	4.2	0.1
OP/CB ^b	441.3	302.1	147.0	185.3	97.0	64.6	238.0	123.1	100.8
Pyrethroids	0	116.9	87.2	0	31.6	51.5	0	41.4	69.2
Total	495.0	431.4	234.5	208.0	131.9	116.1	267.0	168.7	170.1

Source: Grain Crop Pesticide Use Surveys Missouri 1978, 1989, and 1992.

^a Thousands of acres

^b Organophosphate/Carbamate

75

Table 9. Insecticide treated acres^a and percent by class and survey year for northern Missouri.

Insecticide Class	Survey Year/Percent					
	1978	%	1989	%	1992	%
Organochlorines	105.4	10.9	19.9	2.7	0.4	< 0.01
OP/CB ^b	864.6	89.1	522.2	71.3	312.3	60.0
Pyrethroids	0	0	189.9	26.0	208.0	40.0
Total	970.0	100	732.0	100	520.7	100

Source: Grain Crop Pesticide Use Surveys Missouri 1978, 1989, and 1992.

^a Thousands of acres

^b Organophosphate/Carbamate

Figures 20-22 (top to bottom). Insecticide treated acres by class and survey year in the Northwest, North Central, and Northeast crop reporting districts.

Source: Grain Crop Pesticide Use Surveys Missouri 1978, 1989, and 1992.

Additional crops included in this accounting would be cotton, rice, oats, hay other than alfalfa, tobacco, and greenhouse crops. Some of these crops are not typically planted in northern Missouri making insecticidal exposures to Indiana bats less likely.

In northern Missouri both OC and OP/CB usage declined during the survey periods (Figure 23). In 1978, toxaphene and heptachlor were the primary OCs in use and accounted for nearly 11% of all insecticides applied that year (Table 9). By 1989, only 2.7% of treated acres had any OCs applied (lindane was used as a seed treatment on corn). And by 1992, less than 500 alfalfa acres in northern Missouri were treated with the OC methoxychlor. OP/CB usage declined approximately 40% from both 1978 to 1989 and 1989 to 1992. OP/CBs were used on 60% of all crop treated acres in 1992, down from 71.3% in 1989 and 89.1% in 1978 (Table 9, Figure 23).

The synthetic photostable pyrethroids became commercially available in the late 1970s. Their use was first evident in the 1989 survey and that year were applied to 26% of treated acres in northern Missouri. By 1992, the application of pyrethroids had increased to 40% (Table 9).

When the specific insecticide classes are examined within the 3 crop reporting districts, both the OCs and OP/CBs decline in their application rates over the 3 survey periods (Figures 20, 21, 22). Pyrethroid use, on the other hand, increased in both the northeast and north central regions, while decreasing in the northwest between 1989 and 1992. The explanation for this decline in the northwest district was that this region had an exceptionally high proportion of corn acreage treated in 1989 (52.5%) versus 1992 (27.8%). That contributed to a relative decline in pyrethroid use in 1992. Even with this decline in 1992, pyrethroid treated acreage in the northwest (87,200 acres) exceeded that in the north central (51,500 acres) and northeast (69,200 acres) districts

Figure 23. Insecticide treated acres by class and survey year for northern Missouri.

Source: Grain Crop Pesticide Use Surveys Missouri 1978, 1989, and 1992.

(Table 8). For all 3 surveys, more total insecticide treated acres were in the northwest than in the north central or northeast districts (Table 8, Figure 19).

When a comparison is made of the total insecticide treated acres for all 5 field crops, corn always ranks the highest (Figure 24). Between 76% and 81% of the total treated acres for each of the survey years was planted in corn (Table 10). Table 11 shows the decrease in mass (a.i.) for the major insecticides and treated acres for corn for each survey period. Except for soybeans in 1978, alfalfa is typically 2nd in the number of acres requiring insecticides. The other field crops have considerably fewer treated acres as compared to corn and alfalfa.

Corn and alfalfa also have a much greater proportion of planted acres requiring insecticides (Figure 25). From 33% to 54% of corn and 29% to 36% of alfalfa was treated during the survey periods. Approximately 5% to 16% of the sorghum was treated. Typically less than 2% of the soybean and wheat acreage received insecticides.

Table 12 lists the 37 counties by crop reporting region for northern Missouri. Within each region, county size and total and percent insecticide treated acres is noted for 1992.

DISCUSSION

Indiana bat hibernacula populations initially were surveyed in the late 1950s (Hall 1962). Many of these populations, especially in Kentucky and Missouri, have decreased in number since monitoring began. Human disturbance, vandalism and improper cave gates are major documented causes of decline. Despite enhanced protective measures undertaken to reduce and minimize disturbance of wintering, hibernating Indiana bats, population numbers continue to decrease. Other possible significant factors contributing

Figure 24. Total insecticide treated acres by crop and survey year for northern Missouri.

Source: Grain Crop Pesticide Use Surveys Missouri 1978, 1989, and 1992.

Table 10. Total Insecticide treated acres^a and percent by crop/year for north Missouri.

Field Crop	Survey Year					
	1978	%	1989	%	1992	%
Corn	739.0	76.2	537.2	81.6	419.1	80.5
Sorghum	32.0	3.3	23.7	3.6	9.9	1.9
Wheat	0	0	3.6	0.6	7.7	1.5
Soybeans	133.0	13.7	19.0	2.9	19.4	3.7
Alfalfa	66.0	6.8	74.8	11.3	64.6	12.4
Total	970.0	100	658.3	100	520.7	100

Source: Grain Crop Pesticide Use Surveys Missouri 1978, 1989, and 1992.

^a Thousands of acres

Table 11. Pounds of active ingredients and treated acres for the major insecticides applied to corn^a in Missouri from 1978 to 1992.

Insecticides:	Pounds Active Ingredient (x1000)			Treated Acres (x 1,000)		
	1978	1989	1992	1978	1989	1992
Chlorpyrifos	192	337	217	130	337	202
Carbofuran	476	75	74	453	75	81
Terbufos	172	116	72	176	116	69
Phorate	29	100	30	36	100	31
Permethrin	NL ^b	38	27	NL ^b	190	219
Fonofos	74	31	7	140	31	10
Ethoprop	75	9	0	93	9	0
Other Insecticides	252	61	99	219	117	155
Totals:	1,270	767	526	1,247	975	767

Source: Grain Crop Pesticide Use Survey Missouri 1992

^a 2.4 million acres of corn were planted in each of the 3 survey years.

^b Not labeled

Figure 25. Percent of planted crop acres insecticide treated by survey year for northern Missouri.

Source: Grain Crop Pesticide Use Surveys Missouri 1978, 1989, and 1992.

Table 12. County size and total and percent insecticide treated acres by region in northern Missouri 1992.

<u>Northwest</u>				<u>North Central</u>				<u>Northeast</u>			
County	Area ^a	Treated ^b	Percent	County	Area	Treated	Percent	County	Area	Treated	Percent
Andrew	278,534	20,542	7.38	Adair	363,342	7,167	1.97	Audrain	443,748	31,755	7.16
Atchison	348,636	51,568	14.79	Carroll	444,535	17,024	3.83	Clark	324,699	19,005	5.85
Buchanan	262,247	27,197	10.37	Chariton	483,784	36,094	7.46	Knox	323,668	16,289	5.03
Caldwell	274,801	7,934	2.89	Grundy	278,945	14,580	5.23	Lewis	323,233	20,055	6.20
Clay	253,733	5,661	2.23	Linn	397,049	10,447	2.63	Marion	280,381	16,515	5.89
Clinton	268,026	10,138	3.78	Livingston	342,169	11,645	3.40	Monroe	413,454	22,215	5.37
Daviess	362,882	10,514	2.90	Macon	514,440	20,290	3.94	Pike	430,673	30,709	7.13
De Kalb	271,493	8,454	3.11	Mercer	290,854	7,481	2.57	Ralls	301,456	10,658	3.54
Gentry	314,594	17,963	5.71	Putnam	331,491	14,106	4.26	Scotland	280,640	19,059	6.79
Harrison	464,121	17,860	3.85	Randolph	308,749	11,388	3.69	Shelby	320,582	13,025	4.06
Holt	295,617	28,651	9.69	Schuyler	197,054	5,661	2.87				
Nodaway	561,075	36,004	6.42	Sullivan	416,642	8,215	1.97				
Platte	269,037	15,418	5.73								
Ray	364,486	10,021	2.75								
Worth	170,584	5,671	3.32								
MEAN	317324	18240	5.75		364088	13675	3.76		344253	19929	5.79
Total	4,759,866	273,596			4,369,054	164,098			3,422,534	199,285	

Source: U.S. Department of Commerce 1994, Census of Agriculture.

^a Approximate land area in acres

^b Total insecticide treated acres by county

Bold: County mist-net sites 1996 field season

to the decline of the species include stream channelization and bank modification, conversion of forest land to other uses, agricultural development, and surface mining. In 1983 the Indiana Bat Recovery Plan (Brady et al. 1983) called for more attention to summer foraging and maternity/nursery habitats (Klass 1986). As pointed out by Humphrey (1978), Tuttle (1979a), and the recovery plans, protecting caves will be to no avail if summer foraging habitat is so degraded that it will not sustain an adequate food supply sufficient to allow bats to increase or even maintain their populations (LaVal and LaVal 1980).

Earlier studies in the 1970s and 1980s in Missouri and elsewhere implicated the routine use of agricultural insecticides in the declines of a number of North American insectivorous bats (Mohr 1972, Reidinger 1972, 1976, Clark and Prouty 1976, Geluso et al. 1976, Clark et al. 1978, 1980, 1983, Clawson 1984a). When Indiana bats migrate each spring to agricultural areas of northern Missouri, it is likely that they, too, are exposed to insecticides.

The northern 3 crop reporting districts (37 counties) comprise ca 28.5% of the state's total land area. Within this region is 36% of all insecticide treated acres and 41% of all the harvested cropland in the state. Concentration of agriculture and related activities could lead to insecticidal exposure to Indiana bats during that part of the year when they are in their summer/maternity range (Figure 10).

The most probable source of insecticides is associated with the production of corn, the heaviest insecticide treated field crop in Missouri, and in the United States. In 1992, over 33% of all corn acres in northern Missouri received insecticides (Figure 25). Approximately 80% of all treated acres in the Indiana bat's maternity range in Missouri are planted in corn (Table 10). The yearly agrochemical usage patterns of corn, more

than any other field crop, would be the best predictor and provide the closest approximation of insecticide exposure of Indiana bats. Also, corn production (bushels) in an area can give a reasonable estimate of insecticide use (Figure 7).

Both insecticide and herbicide treatments in corn are generally made early in the growing season when the potential for erosion and runoff losses are great due to lack of vegetative cover and high precipitation probability. In addition, a larger percentage of corn is grown on land classified as highly erosive by the 1977 National Resources Inventory than any other crop except tobacco (Heimlich and Bills 1984). In northern Missouri, insecticidal applications precede and occur coincident with the summer foraging season and maternity range of the Indiana bat. It is my belief that pesticide exposure probabilities of the Indiana bat are closely associated with habitat use patterns.

The OP and CB insecticides were the most frequently used and accounted for 60% of the total treated acreage in northern Missouri in 1992 (Table 9). The synthetic pyrethroids were used on approximately 40% of the total acres. Environmental fate and degradation of these compounds depends on many factors including physical and chemical properties, method and timing of application, weather and climatic conditions, and the land (i.e., soils, slopes, crops).

Estimates of soil half-lives for different OP compounds vary, but most are in the range of 1 to 8 weeks (Mass et al. 1984). Despite differences in solubility, carbamates are lost from agricultural fields almost entirely in the dissolved phase of runoff. These chemicals are moderately persistent with half-lives from 2 to 50 weeks. The pyrethroids are readily degraded in soils maintained under aerobic conditions (Leahey 1985).

Permethrin, one of the most common pyrethroids, has a half-life of 1 to 3 weeks in silt and clay soils, and up to 15 weeks in organic soils (Smith and Stratton 1986).

Depending on the concentration, the longer a particular chemical is maintained in the environment (soil, water, air) the greater it's opportunity to impact or reduce all insect species. Previous studies have demonstrated an exposure-response relationship with higher chemical concentrations correlating with increased toxicity and reduced numbers of invertebrates.

Mian and Mulla (1992) reviewed the literature to determine the impact pyrethroids have on nontarget aquatic species. They found that in almost all field studies, nontarget insects such as Ephemeroptera, Odonata, Plecoptera, Hemiptera, Coleoptera, Trichoptera, and a variety of crustaceans groups, were more severely affected by pyrethroids than other invertebrates (Table 1). Insect mortality was found to range from 50% to greater than 95% and populations of affected organisms generally recovered to pretreatment levels within weeks to months after application. In some situations species composition was different before and after a series of pyrethroid applications. A change in the composition and abundance of insects of such a magnitude, even if only localized and for a couple of weeks, would have the potential to severely compromise the ability of any colony of bats to acquire sufficient nutrients to support growing young, gain weight for fall migration, or meet basic maintenance needs without the added stress and adjustment of colony relocation.

Because bats have a small body size, they need a high caloric intake to balance radiation heat losses to the surrounding air. As the mass of an animal decreases, proportionally more of its body heat is lost due to a relatively greater surface to volume ratio (Altringham 1996). Given the relatively unreliable nature of insectivorous bats' food

supply in an often harsh climate, it does not seem unreasonable that even a 10 to 20% reduction in calories resulting from fewer available insects could decrease foraging efficiency such that it would lead to a concomitant reduction in other activities required for survival (e.g., grooming, care of young, territorial defense, mate attraction, continued foraging). On average, each night a bat must catch enough insects to provide the energy to get it through to the next night. The high energetic demands of lactation in females and growth in newly volant young particularly put these individuals at risk. Hence, an important requirement for a bat is collecting sufficient food to satisfy their maintenance, energy, and nutrient requirements. Any factor, contaminant or otherwise, that upsets food intake has the potential to jeopardize survivability.

Agrochemical influences on the Indiana bat are mediated through the resource food base of this insectivore. By definition, insecticides are poisons designed to kill and remove insect biota from the area being treated. Because of the development of insecticide resistance and sublethal or subinhibitory exposure of insects in natural agricultural settings, a variable proportion of the Indiana bat's insect prey species survive chemical exposures and contaminate bats with toxins when ingested. Bats intoxicated in this manner typify the direct pathways Lacher (1990) spoke of when he said the chemical acts specifically on the vertebrate species either through direct physical contact or ingestion.

Though this paper is principally concerned with direct pathways of pesticide use and exposure, agrochemicals can result in death by starvation of those bats dependent upon the eliminated prey species (an indirect lethal effect). In cases of indirect lethality, attempts to relate the mortality of bats to chemical exposure by looking for tissue residues may reveal nothing if the chemical was degraded by the prey species or the

dead insects were unavailable for ingestion by bats. The long-term ramifications of these more indirect effects can be greater than direct mortality. Many agricultural chemicals now have low acute mammalian toxicity (e.g., pyrethroids) and much mortality may be indirect. Palmer (1995) found that in North Carolina modern farming systems appeared to reduce quail recruitment indirectly by reducing food resources for young chicks, primarily through control of weeds in field crops.

Of the 9 insect orders commonly utilized by Indiana bats as a food source (Belwood 1979, Brack and LaVal 1985, Lee 1993), 6 are known to cause significant economic damage to Missouri field crops. These pests are terrestrial species with life cycles closely associated with the agricultural field environment. In addition to terrestrial insects, Indiana bats also forage aquatic invertebrate prey (Belwood 1979, Lee 1993).

Because of their proximity to agricultural fields, agricultural insecticides can contaminate both aquatic and terrestrial habitats through drift, wind and soil erosion and runoff. Nontarget aquatic insects become exposed to insecticides (Tang and Siegfried 1996) when runoff events contaminate nearby streams and ponds (Khan 1983) or from drift during aerial insecticide applications (Crossland et al. 1982). The single most relevant feature of agricultural systems in this regard is that they are open systems. Chemical pesticides are not confined by property lines or fences, and typically, as production intensifies, the chemical leakage also increases (Carroll 1990). Hall (1985) found that it was not unusual for 1 to 3% of an agrochemical to reach the intended site of action and contribute to the desired biological effect. Therefore, significant amounts of field applied insecticides are potentially available to contaminate soil, air and water. This is especially true in the Midwest where agriculture is the largest nonpoint source of water pollution (Ritter 1990).

Numerous published studies and reviews describe ambient water monitoring programs for pesticides. These studies addressed field runoff, ground water concentration, and pesticides in surface waters (Mass et al. 1984). Under such conditions, numerous opportunities exist for exposure of Indiana bats to agricultural chemicals.

Conceptually, there are 3 basic management options to reduce environmental exposure of nontarget organisms to agricultural insecticides (Mass et al. 1984).

- 1.) Reduce the amount of product applied by improving application efficiency (restricting aerial spraying, using larger droplet sizes, restricting application when runoff events are predicted).
- 2.) Substitute less toxic, less persistent, less mobile, and more selective insecticides.
- 3.) Reduce or retard the transport of applied insecticides from fields to aquatic systems.

A reduction of insecticides or the substitution of less toxic compounds to be effective would need to occur not only on a statewide basis in Missouri but also on a much larger landscape scale. The routine use of these chemicals in the neighboring corn producing states (Figure 11) would preclude a significant decline in overall exposure if changes were limited to Missouri only (Figure 17). Indiana bats foraging during the spring and summer in this multistate region known for its heavy and systematic use of insecticides, would be expected to continue to have a high probability of exposure to environmental agrochemicals.

As intuitive as a reduction in insecticides or lessened toxicity sounds, results could be disappointing. Without long-term toxicology-population studies to suggest a

safe threshold/exposure level (bat tissues, insects, soil, water), changes in insecticides could lead to the same outcome that improved protection (i.e., gates) of Indiana bat hibernacula did during the 1980s, population numbers continued to drop. The level of a toxin that could impair function(s) may be extremely low, in fractions of parts per billion, such that an exposure at a critical time would be sufficient to disrupt a metabolic or behavioral process and produce lethality.

Overall insecticide usage has declined in Missouri, and nationwide, starting in the mid 1970s. Most of this decrease is attributable to the increased use of more effective synthetic pyrethroids, improved application efficiencies, and integrated pest management programs. Despite these reductions, total Indiana bat numbers have not improved. The Indiana bat population in the state of Indiana has increased approximately 47% from 1980 to the most recent surveys in 1995-1997 (Richard L. Clawson, personal communication). If agricultural insecticides are responsible in negatively impacting bat populations, safe threshold levels are still being exceeded in that part of the Indiana bat range where population numbers continue to decrease.

As previously suggested, the acreage and insecticides used in the production of corn are probably more closely associated with the degree of insecticidal exposure of Indiana bats than any other factor. But what influences a farmer's choice in deciding where and what to produce? The decisions made determine the degree of ecosystem disturbance, changes in community structure, and resulting loss in species diversity or abundance (Gerard 1995).

Agricultural markets are a central force influencing land-use practices. In the 1970s, a 28-million ha expansion of crop land occurred when demand increased in Europe and the former Soviet Union.

Changes in consumer food habits are a significant factor, as in the declining consumption of red meat, eggs, and other foods once considered the mainstay of the American diet (USDA 1991c). About a third of United States corn production is fed to livestock and poultry (Mercier 1989).

The availability and cost of agricultural mechanization, chemical inputs, and genetic advances exert a powerful influence on land-use. Synthetic fertilizers and pesticides allow farmers to decrease their dependence on livestock wastes and crop rotation for the maintenance of soil fertility and crop yields.

Our modern capitalist economy grew out of agriculture. Production beyond subsistence formed the basis for economic development. The more that people leave the land to work in urban centers, the more demand there is in urban markets for agricultural goods.

Although market forces are important in determining the what, where, and how of farm production patterns, public or government policy is clearly influential. Federal agricultural policies have encouraged export markets, subsidized inputs (i.e., energy, chemicals), and stimulated production over a larger area than would otherwise have occurred (Doering 1992).

National Commodity Support and Adjustment programs were developed to adjust supplies, support prices, encourage exports, and maintain income for farmers. These programs have also helped promote the conversion of heterogeneous, habitat-rich agricultural landscapes into homogenous, habitat-poor landscapes (Berner 1988), while discouraging sustainable farming practices that may benefit biological diversity, such as crop rotations, cover crops, decrease in agricultural chemical use, and farm diversification (Robinson 1991). These programs are a significant force in United States

agriculture. From 1984 to 1993, 18 to 42% of net farm income came from direct government payments (Gerard 1995).

The past 25 years has seen considerable growth of export markets for commodities such as corn, wheat, and soybeans. These markets promoted cash crop specialization, with a consequent expansion of land devoted to cultivated crops. An average of 25% of corn, 58% of wheat, and 40% of soybeans production was exported between 1976 and 1989. The USDA encouraged farmers to increase production for these markets (MacFadyen 1984).

Long-term declines in wildlife populations are associated with agricultural land-use practices and related habitat loss (Paoletti et al. 1992). Many soil-dwelling and terrestrial arthropod species are declining due to modern farming activities (Pimental et al. 1992, Tucker 1992). The ring-necked pheasant (*Phasianus colchicus*), northern bobwhite (*Colinus virginianus*), and cottontails (*Sylvilagus* spp.) showed a decrease in population size of 33 to 96% in the midwestern states (varies with state and species) from the late 1950s to the late 1970s primarily due to changes in farming practices (Farris and Cole 1981, Mankin 1993).

The distribution and timing of the use of agricultural insecticides within the summer/maternity range of the Indiana bat suggests a potential for exposure of this species. The recovery and identification of pesticides in surface and groundwater samples would argue that bats foraging in riparian habitats are probably, temporarily at least, coming into direct contact with a variety of agrochemicals. Though many of these compounds have short half-lives under field conditions, some are repeatedly applied during a growing season to maintain some minimal concentration and insecticidal efficacy. It is suspected that when Indiana bats return to their hibernacula in the fall of

the year that the potential for agrochemical exposure diminishes until the following spring when migration, again, repeats the cycle. In ecological risk assessments, environmental exposure information, along with toxicity data (Kendall 1992), provides a logical and scientific structure to evaluate the likelihood that adverse ecological effects will occur or are occurring in the region and species of interest.

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CHAPTER II

POTENTIAL AGROCHEMICAL INSECTICIDE EXPOSURE IN INDIANA BATS (*MYOTIS SODALIS*) IN NORTH MISSOURI

INTRODUCTION

The endangered Indiana bat (*Myotis sodalis*) is distributed over most of the eastern United States, but its summer/maternity range is a highly fragmented woodland region of riparian-bottomland forests in an otherwise agriculturally-dominated (nonforested) landscape. In the western portion of their range, Indiana bats that hibernate in caves in the Missouri Ozarks migrate north each spring to agricultural areas in northern Missouri (Easterla and Watkins 1969, LaVal and LaVal 1980, Clawson 1986), southern Iowa (Myers 1964, Bowles 1981), and possibly Illinois (Gardner and Gardner 1980, Brack 1983). Indiana bats, and other wildlife species, living adjacent to farmlands may benefit from the crops grown but may also be inadvertently exposed to pesticides used to reduce weeds, diseases, and insect pests of those crops.

It is well documented that certain agricultural environments are contaminated enough to pose hazards to wildlife (Pimentel et al. 1980, Fleming and Clark 1983, Kendall 1988). Since the 1950s, agricultural pesticides have had a variety of deleterious influences on many wildlife populations (Pimentel et al. 1980, Ware 1980, Sotherton et al. 1985, Hooper et al. 1989, Chessser et al. 1990). In birds, mass mortalities occurred when DDT was used for a variety of pest eradication efforts (Rudd and Genelly 1955).

Over 1100 birds of 10 species at Tule Lake National Wildlife Refuge died during 1960 to 1962 from agricultural runoff (Keith 1966), while dramatic local population reductions occurred in species such as bald eagle, brown pelican, Bermuda petrel, California condor, western grebe, peregrine falcon, osprey, and Cooper's hawk. Studies of residues accumulated in wildlife give additional evidence of the intense exposure to insecticides that some animals receive (Keith 1970, Cooke 1972, Reidinger 1976, Geluso et al. 1981, Wilson et al. 1991).

The first bat deaths directly attributable to chemical contaminants in the environment occurred in 1949 and 1950 (Benton 1951, Dalquest 1953) when DDT was applied to bats or to their roosts. About this time, it was first suggested that insecticides might be a cause of observed population declines in bats (Mohr 1953). Although the effects of insecticides on Indiana bats have not been studied, insecticides have been implicated in the decline of other North American bat species (Mohr 1972, Clark and Prouty 1976, Geluso et al. 1976). In Missouri, insecticides have been known or suspected as a cause of a number of bat die-offs, including the endangered gray bat (*Myotis grisescens*) (Clark et al. 1978, Clark et al. 1983).

The organochlorine (OC) group of chemicals has been of environmental concern since 1962 when the concept of biomagnification was first widely publicized (Carson 1962). The persistence of these compounds in soils is measured in decades, and major losses from soil appear to be due to volatilization, leaching, or erosion rather than to degradation (Francis 1994). The characteristics of low water solubility, chemical stability, and the propensity to accumulate in lipids of organisms led to severe restrictions in the use of many OC pesticides in the early 1970s.

Bats possess certain characteristics that increase their likelihood of being harmed. Like other insectivorous mammals and birds, insectivorous bats encounter more OC in their diets than do herbivores or frugivores because of food chain buildup. Their insect diet exposes them to these persistent chemicals carried by their prey, especially insects whose larval stages develop in contaminated soils or sediments. Bats are long-lived and have years to accumulate OCs, even when exposed only to low levels or trace amounts. Also, the higher metabolic rates of bats associated with small size and the activity of flight demand increased rates of food consumption than found in larger, less active birds and mammals. Greater food intake causes greater amounts of OCs to be available for concentration in the fat of bats (Clark 1981).

Most bat contaminant studies in the 1970s and early 1980s were surveys that quantitatively measured residues of OCs in tissues of free-living bats (Jefferies 1972, Best 1973, Dunsmore et al. 1974, Clark et al. 1983). These surveys provided the evidence that bat populations could experience major mortality due to OC insecticides applied agriculturally and subsequently carried through the food chain (Geluso et al. 1976, Clark et al. 1978).

Polychlorinated biphenyls (PCBs) are synthetic compounds used extensively for various industrial purposes. Like OCs, they are extremely persistent and widespread contaminants of the ecosystem and are a potential health problem due to their presence in the food chain.

Since OCs were banned during the 1970s and 1980s, other purportedly safer products have been developed and are routinely being used. Although not as likely to bioaccumulate, they are toxins and so have the potential to adversely impact nontarget organisms. The organophosphate (OP) and carbamate (CB) classes of insecticides

were initially developed during the 1950s as replacements for the OCs which had problems of environmental persistence and bioaccumulation.

The principal toxic effect of OP/CB compounds is the inhibition of cholinesterase (ChE) enzyme activity, resulting in the accumulation of the neurotransmitter acetylcholine (ACh) at nerve synapses and excess stimulation and consequent disruption of nerve function (O'Brien 1967). With exposure to these insecticides the potential for altering physiological and behavioral responses essential for survival and reproduction is great (Grue et al. 1997). In Missouri in 1992 approximately 60% of all insecticide treated acres had OPs/CBs applied.

The muscarinic cholinergic receptor (MChR) is a cellular membrane protein which interacts and binds with ACh. An increase in ACh as a result of an inhibition of ChE can result in an adaptation by the cell manifested by a decrease in receptor number (Costa et al. 1981, Sivam et al. 1983). MChRs can be readily evaluated with radioligand binding techniques (Byland et al. 1982, Jones and King 1995). B_{max} (tissue number or density of receptors) and K_D (affinity of receptors for the radioligand) are 2 methods frequently used to evaluate MChRs. A decrease in B_{max} (i.e., downregulation) coupled with a measurable decrease in ChE activity would provide presumptive evidence of sublethal exposure to OP/CB insecticides (McDonald et al. 1988).

The newest major class of insecticides is the synthetic pyrethroids, a group of chemicals entering the marketplace in 1980 but, by 1982, accounting for approximately 30% of the worldwide insecticide usage (Ecobichon 1996). These compounds have a large differential toxicity to mammals and insects (Hill 1985). This means that pyrethroids present a significantly greater intoxication potential for insects than

heretofore studied mammalian species. Chronic feeding studies yield high “no-effect” levels, suggesting that there is little storage or accumulation of a body burden of these agents and, perhaps, an efficient detoxification of the chemicals (Ecobichon 1996). In 1992 Missouri farmers applied pyrethroid insecticides to approximately 40% of all insecticide treated acres.

The purpose of this study was to determine in 2 surrogate bat species obtained from winter hibernacula and the summer/maternity range of the Indiana bat in Missouri if there is evidence of a potential for exposure of Indiana bats to agricultural insecticides. The study will provide tissue contaminant levels and other biomarkers of exposure and effect for comparison with future investigations. The particular objectives were to: 1) determine ChE activity and MChR number and affinity in brain tissue, 2) evaluate OC, PCB, and pyrethroid residues in carcass/body tissues, 3) analyze gastrointestinal tissues and contents for OP residues, and 4) measure blood plasma ChE activity

METHODS

SPECIMEN COLLECTION AND PREPARATION

Little brown (*Myotis lucifugus*) and northern long-eared (*Myotis septentrionalis keenii*) bats were collected in northern Missouri, north of the Missouri River, from June 14 to August 25, 1996. These two surrogate species were used in an attempt to increase overall and individual site sample size and to better represent potential Indiana bat insecticide exposures and the extent of insecticide use in northern Missouri. Site selection was based on the results of previous investigations (La Val and LaVal 1980, Clawson 1986, Callahan 1993, Miller 1996) where collection efforts had recovered either

or both congeneric surrogate species. In addition, we attempted to survey only those sites where the 2 most recent studies (Callahan 1993, Miller 1996) had failed to indicate the presence of Indiana bats. It was not always possible to satisfy both conditions when trying to sample the maximum number of sites and when Indiana bats and the 2 surrogate species are often locally sympatric.

Five of the 15 total locations in northern Missouri where a collection effort was made were non-Indiana sites in 1993 (Callahan 1993) or 1996 (Miller 1996) but did formerly capture Indiana bats in 1980 (LaVal and LaVal 1980) or 1986 (Clawson 1986). Surrogate bats were successfully collected from 4 of these 5 locations. Overall, a sampling effort was made at 15 riparian sites on 17 occasions collecting 27 little brown and 15 northern long-eared bats. One or both surrogate species were collected at 8 of these 15 sites. Sites in Knox and Scotland counties were sampled twice during the summer, in July and August, and are designated Knox I and Knox II, and Scotland I and Scotland II, respectively.

Bats were netted over streams at night commencing at approximately 2030 CDT and terminating about 0100 CDT. A portable mist net-set previously described (Gardner et al. 1989) was placed across riparian flyways, perpendicular to the bank, 12.8 m in length and to a height of 6 m. When possible, the net was placed beneath overhanging trees so that all the available flight space between the surface of the water and the tree canopy was covered by netting. Mist nets were set at dusk and checked every 10 to 15 minutes.

Reference specimens of the same species are routinely recommended for the support of diagnoses in cases of toxicologic exposures (Clark 1988). Reference specimens were cave-collected individuals thought to represent minimal to no recent (4

to 8 months) exposure to agricultural pesticides and consequently were used to provide reference ("control") values for comparison to those obtained from field-collected bats in northern Missouri during the summer 1996. *M. lucifugus* were collected from 3 cave hibernacula in the Ozark Plateau region of southern Missouri on April 18, 1996. *M. s. keenii* were collected from 2 cave hibernacula in the same region of southern Missouri from April 17 to 20, 1997. Eighteen specimens of each species were collected. Bats were captured with a double-frame Tuttle trap (Tuttle 1974) placed near cave entrances or manually removed from cave ceilings or walls.

Collecting reference bats late in the hibernation period maximizes the time to metabolize and eliminate insecticides that may have been ingested during the previous spring or summer. Therefore, I assumed that insecticide, ChE, and MChR levels in reference bats would be as similar as possible to values found in a population unexposed to insecticides. Only laboratory raised bats would be expected to have no prior insecticide exposure.

Each animal was assigned a permanent identification code that indicated whether the individual bat was captured from northern Missouri or was a cave-reference specimen from southern Missouri. The code also provided the species, age, and gender of each bat. Age class was determined by examination of the fifth metacarpal phalangeal joint. Juvenile bats were recognized by incomplete ossification or closure of the cartilaginous epiphyseal growth plates (Barbour and Davis 1969) and their small overall body size; all other bats were classed as adults. Bats that were not 1 of the 2 designated surrogate species were immediately released at the point of capture.

Specimens were euthanized according to accepted Animal Care and Use Committee (ACUC; University of Missouri, Columbia, Mo.) protocol. The physical

method of cervical dislocation was determined to be the most appropriate for the specific circumstances of this study and is in accordance with the American Veterinary Medical Association's Panel on Euthanasia (1993).

Initially, all specimens for diagnostic study had blood samples drawn into heparinized microhematocrit tubes. The tubes were centrifuged and the separated plasma transferred to microcentrifuge tubes for later ChE assay. The red blood cells were discarded. The brain was removed and placed in appropriately identified cryovials. Also, the gastrointestinal tract, including the contents, from the distal esophagus to the rectum was removed and placed into labeled cryovials.

For preparation of the carcass, wings were removed at the shoulder and feet were removed at the ankle. Specimens were skinned to prevent the inclusion of contaminants that may have been on the skin or coat at the time of capture. Any fat adherent to the skin was removed and placed with the body (Thies and McBee 1994). The term carcass refers to the entire body minus skin, wings, feet, gastrointestinal tract, and brain (Geluso et al. 1981).

All tissue samples were placed on dry ice in an insulated container and placed in a refrigerator freezer (Hill and Fleming 1982, Fairbrother et al. 1991). The dry ice allowed for storage of tissues at -20°C until transfer 24 to 48 hours later to a -80°C biological freezer at the Columbia Environmental Research Center (USGS), Columbia, Missouri. We estimate a maximum of 6 hours elapsed between live specimen capture and the freezing of tissues.

ANALYTICAL PROCEDURES

Cholinesterase Assay

Frozen brain tissue (~125 mg) was thawed and diluted with ice-cold 0.05 M pH 7.4 Trizma buffer (Tris 7.4) and manually homogenized in teflon-on-glass homogenization tubes. The final dilution of bat brain tissue was 100:1. Blood plasma was also diluted 100:1 with Tris 7.4.

Brain tissue samples and blood plasma were analyzed for total cholinesterase (ChE) activity according to the methods of Ellman et al. (1961) as modified by Gard and Hooper (1993) and Beauvais (1997). The procedure used a V_{max} 96-well microplate reader (Labsystems Multiskan MS) set in kinetic mode at a wavelength of 405 nm with a run time of 2 min, read at 10 sec intervals, with a 5 sec lag time, and with a final volume of 250 μ l/well. The microplate reader was interfaced with an IBM compatible PC loaded with Labsystems genesis V2.16 software package. All samples were run in triplicate at 22 to 25°C (Gard and Hooper 1993).

The optimum concentration of the substrate acetylthiocholine iodide (AThCh) and the colorimetric agent 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) were determined on non-study samples prior to analysis (Hunt and Hooper 1993). Final concentrations were 8.33×10^{-3} M for AThCh, and 1×10^{-3} M for DTNB.

The mean value of triplicate analyses were used in all calculations. Triplicates with a coefficient of variation (CV) greater than 10% were rerun. All activities were converted from optical density units/min to μ moles AThCh hydrolyzed/min/mL plasma or g brain tissue, wet weight.

Muscarinic Receptor Assay and Radioligand Binding

Brain tissue homogenates (100:1) were diluted by the addition of sufficient ice-cold Tris 7.4 buffer to bring the samples to 10 mL. The tissues were further homogenized for 20 sec with a Tekman Tissumizer on setting 90. Ice-cold Tris 7.4 was added to bring the final volume to 25 mL, and the samples were centrifuged at 20,000 X g at 4°C for 15 min. The supernatant was discarded and the process repeated. The resulting pellet was resuspended in 20 volumes ice-cold ultra-pure H₂O, and samples were removed for protein determinations. Ice-cold 0.05 M pH 8.0 Trizma buffer (Tris 8.0) was added to bring the final volume of the crude membrane particulate preparation to 2400V. Protein content was determined by the method of Lowry et al. (1951), with bovine serum albumin as a standard.

Radioligand binding assay for MChR followed the method as described by Bylund et al. (1982) and Jones and King (1995). The tissue suspension (970 µL) was incubated in tubes with increasing concentrations of 20 µL of the radioligand [³H] N - methyl scopolamine ([³H] NMS) for 45 min at 22°C to determine total binding. Nonspecific binding was characterized as [³H] NMS binding in the presence of 10 µL of 1 µM atropine in a set of parallel tubes. Specific muscarinic receptor binding is the difference between total and nonspecific binding. Incubations were terminated with filtration through Whatman GF/B glass fiber filters. The filters were rinsed with 12 mL of ice-cold Tris 8.0 buffer and transferred to vials containing 10 mL scintillation cocktail (Ecolume® from ICN). Radioactivity was determined by scintillation spectroscopy at a 46% efficiency.

Individual determinations within an experiment were done in duplicate with binding data represented as mean values for use in analysis. Data were analyzed with non-linear regression using Graphpad Inplot® software to obtain all values of dissociation constant (K_D) and maximum number of binding sites (B_{max}). K_D values are expressed in pM and B_{max} values in fmol/mg protein.

[^3H] NMS was purchased from Dupont New England Nuclear (Boston, MA). All other chemicals and buffers were purchased from Sigma Chemical Co. (St. Louis, MO).

OC, PCB, Pyrethroid, and OP Assays

Frozen carcass/body and gastrointestinal tissues were submitted to a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) approved laboratory, Analytical Biochemical Laboratories, Inc. (ABC Labs), in Columbia, Missouri. Analytical methodology and cleanup procedures generally followed those outlined in the Food and Drug Administration's (FDA) Pesticides Analytical Manual (PAM). All tissues for analysis were delivered frozen and identified by a code unique for each individual sample.

Gastrointestinal samples were chosen and submitted based on the results of previous analysis of brain tissue ChE activity. Four composite and 3 individual gastrointestinal samples were submitted from surrogate bats collected from the summer/maternity range of the Indiana bat having brain ChE activity levels 20% less than the mean of the same species cave-collected reference specimens. Also, two composite gastrointestinal samples were submitted from surrogates having brain ChE levels not less than and similar to the mean of the same species cave-collected reference specimens. Thus, a total of 9 gastrointestinal samples were submitted, 7 from

bats with depressed brain ChE levels and 2 from bats with nondepressed brain ChE levels. Except for the difference in mass between the 2 types of tissues, extraction and cleanup procedures for carcass/body (~ 5g) and gastrointestinal (~ 100-500 mg) samples were handled similarly.

Tissue was weighed into 500 mL french square glass jars. Fortifications were made at this time. The sample was ground using a polytron homogenizer (fitted with a blade) for 5-10 seconds. Methylene chloride/acetone (100 mL of 75%/25%) and 50 g sodium sulfate were added and the sample blended for 3 minutes. The sample was vacuum filtered into a 250 mL flat-bottom flask through GF/A filter paper. The container and filter cake were rinsed with 2 X 25 mL portions of 75%/25% methylene chloride/acetone and these rinses were added to the 250 mL flat-bottom flask. The sample was rotary evaporated to dryness with approximately 1 mL of oily residue remaining.

A small plug of glass wool was added to a glass column, followed by 4 inches (8.5 mL by volume) of 100% activated florisil (measured from the top of the glass wool) and 1 inch of muffled sodium sulfate (measured from the top of the florisil). A 25 mL portion of petroleum ether was added to pre-rinse and prepare the florisil column. The residue was quantitatively transferred to the prepared florisil column using 4 X 2 mL of 50%/50% ethyl ether/petroleum ether (v/v). The eluant from the load fraction was collected in a 125 mL flat-bottom flask. The analytes were further eluted with 50 mL of 50%/50% ethyl ether/petroleum ether (v/v) into the same 125 mL flat-bottom flask. The sample was rotary evaporated to dryness with 0.5-1 mL of oily residue remaining. The extract was qualitatively transferred to a 5 mL calibrated test tube using 1 mL ethyl acetate followed by 3 X 1 mL iso-octane with a final volume adjustment using iso-octane.

A Hewlett Packard Model 5890 Series II Gas Chromatograph (GC) with an electrolytic conductivity detector was used to analyze body tissues for OCs. The column used during the analysis was a J&W® DB-1701 (30 m X 0.53 mm internal diameter). The injector temperature was 200°C and the detector temperature was 275° C. The carrier gas (He) flow rate was 9.2 mL/min, make up gas (He) was 20 mL/min, and the reaction gas (H₂) flow rate was 110 mL/min. The volume of sample injected was 2 µL.

The initial temperature of the GC oven was 90°C with a hold time of 1 minute. The initial ramp program increased temperature from 90°C to 140°C at 30°C/min. The secondary ramp program increases the temperature from 140°C to 280°C at 10°C/min with a hold of 5 minutes.

The same instrument and detector were used to analyze body tissues for pyrethroids. A J&W® DB-17 column (30 m X 0.53 mm internal diameter) was used and the initial ramp program increased temperature from 90°C to 140°C at 25°C/min. All other parameters were the same.

A Hewlett Packard Model 5890 Gas Chromatograph (GC) with a flame photometric detector was used to analyze gastrointestinal contents and tissues for OPs. The column used during the analysis was a J&W® DB-17 (30 m X 0.53 mm internal diameter). The injector temperature was 250°C and the detector temperature was 300°C. The carrier gas (He) flow rate was 11 mL/min, make up gas (He) was 33 mL/min, the H₂ flow rate was 75 mL/min, and the air flow rate was 109 mL/min. The volume of sample injected was 1 µL.

The initial temperature of the GC oven was 90°C with a hold time of 1 minute. The initial ramp program increased temperature from 90°C to 140°C at 25°C/min. The

secondary ramp program increases the temperature from 140°C to 280°C at 10°C/min with a hold of 5 minutes.

Body samples were analyzed for the OC insecticides *o,p'*- and *p,p'*-DDE, *o,p'*- and *p,p'*-DDT, *o,p'*- and *p,p'*-DDD, dieldrin, heptachlor epoxide, and oxychlordan, total PCBs (Aroclors® 1021, 1248, 1254, 1260, and 1268 congeners), and the synthetic pyrethroids tefluthrin, cyhalothrin, permethrin, and esfenvalerate. Individual and composite gastrointestinal samples were analyzed for the OPs phorate, terbufos, fonofos, methyl parathion, and chlorpyrifos.

The limits of quantitation (LOQ) were 10 parts per billion (ppb) for all OCs, the PCBs, and the pyrethroids tefluthrin and cyhalothrin. The LOQ for permethrin and esfenvalerate was 50 ppb. For all OPs the LOQ was 10 ppb. Body tissue recovery efficiency for the OCs ranged from 83% for dieldrin to 329% for *o,p'*-DDT and for the pyrethroids from 71% for permethrin to 308% for esfenvalerate. Recovery efficiency for the OP gastrointestinal samples ranged from 61 to 88%. Residue data were not adjusted on the basis of these derived recoveries. All recovered analytes were quantified by peak area and are reported in ng/g (ppb) by weight of fresh (wet) tissue.

STATISTICAL PROCEDURES

One-factor analysis of variance (ANOVA:PROC GLM, SAS Inst. Inc. 1990) was initially used to test for differences in brain B_{max} , and ChE activity by site location. Individual sample values for brain B_{max} , K_D , and ChE activity from northern Missouri collected bats were analyzed in reference to a 95% confidence interval derived from cave data and a rejection region for a two-tailed test in the upper and lower tail of the

Student's t distribution. The statistical, and especially biological, significance of each northern Missouri bat specimen was determined by a comparison to the 95% confidence interval which set the diagnostic biological threshold for low brain $B_{\max}$, K_D , and ChE values. Because data can be highly variable combined with small sample sizes in many wildlife studies, a slightly less stringent $\alpha=0.10$ was also reported for comparison purposes with the more conventional $\alpha=0.05$ in tests of the null hypothesis. In addition, contrast statements were used to focus more closely on any potential differences between individual sites.

Statistical analyses of OC insecticides were limited to 4 compounds known to be dangerous to bats and detected more frequently and in higher concentrations. Oxychlorane (OXYCH), heptachlor epoxide (HE), p,p'-DDE (PPDDE), dieldrin (DLDRN), and total OCs (TOC) were included in the analysis. TOCs are the sum of all 9 possible OC residues recovered in any particular bat sample. OC residues in the Tables are expressed as geometric means rather than arithmetic means because of the wide variation in the level of residues among samples. Most data sets were positively skewed with many values occurring near the low end of the range. ANOVA/PROC UNIVARIATE tests for normality indicated that 6 of the 18 total sites had data that were not normally distributed. Therefore, the nonparametric method of rank transformation of residue concentrations was used to normalize the data prior to statistical analysis (Conover and Iman 1981). Subsequently, the usual parametric Student's t-tests and ANOVA F tests of rank-transformed data were used to test for differences in mean levels of OC insecticides between and among sites. Residues reported as below the LOQ (U. S. Environmental Protection Agency 1992) or not detected (ND) were entered into

computations as one-half of the observed LOQ for that compound to eliminate zeros in the data set. The $P < 0.05$ significance level was used for rejection of the null hypothesis.

An ANOVA/PROC REG procedure and a simple linear regression model were run using ordinary least squares to investigate potential relationships between both brain B_{max} and ChE activity and the rank transformed OC concentrations. Levels of significance were $P < 0.05$.

Residue levels of the 4 synthetic pyrethroid compounds, tefluthrin, cyhalothrin, permethrin, and esfenvalerate were rank transformed to normalize the data prior to analysis. Data were then analyzed to test for differences in mean pyrethroid concentrations between and among sites. Geometric means and ranges were also determined and reported in tabular form.

Statistical analyses and results were performed and reported separately for each surrogate species and with no consideration for possible differences in gender and age. No statistical interspecific comparisons were made as a part of this thesis. Though residue comparisons according to season were not directly made, such an analysis is implied when residue comparisons are categorized to cave and farm site locations. This is because cave specimens were collected in the spring in April and farm specimens collected during the summer from June to August.

RESULTS

PHYSIOLOGICAL PARAMETERS

When mean brain $B_{\max}$, K_D , and ChE levels for both little brown and northern long-eared bats were compared among cave sites no significant differences were noted. A $P < 0.05$ and $0.05 \leq P < 0.10$ level for rejection of the null hypothesis was calculated from all species-specific cave data to determine if individual bats collected from northern Missouri agricultural areas had biochemical values significantly lower than the mean value for bats collected from the reference cave sites.

Ten little brown bats (37%), out of a total of 27 collected from 6 north Missouri farm sites, were collected in Knox and Scotland counties and found to have significantly ($P < 0.05$) lower brain $B_{\max}$ or ChE values as compared to mean values from cave collected little browns (Table 1, Appendix Table D2). An additional 7 bats (26%) were significant at $P < 0.10$. Twelve of the 27 little brown bats (44%) had a minimum of 20% depression in brain ChE levels while another 5 individuals were depressed at least 30% when compared to cave collected bats.

Four northern long-eared bats (27%), out of a total of 15 collected from 7 north Missouri agricultural sites, were collected in Monroe, Macon, and Schuyler counties and found to have significantly ($P < 0.05$) lower brain ChE (Table 2, Appendix Table E2). An additional bat (7%) was significantly lower at $P < 0.10$. One of 15 (7%) had a significantly ($P < 0.10$) depressed brain $B_{\max}$. A single specimen of each of the surrogate species collected from north Missouri sites had a significantly ($P < 0.05$) low K_D value.

Table 1. Comparison of significant values for brain muscarinic cholinergic receptor density (B_{max}) and cholinesterase activity (ChE) in little brown bats collected from cave and farm sites.^a

Cave Sites		B_{max}	ChE
Scotia Hollow	A	0/6	0/6
	B	0/6	1/6
Great Scott	A	0/6	0/6
	B	0/6	1/6
Onyx	A	0/6	0/6
	B	0/6	0/6
Total ^b		0/18	2/18

Farm Sites		B_{max}	ChE
Monroe	A	0/2	0/2
	B	0/2	0/2
Knox I	A	1/8	3/8
	B	1/8	2/8
Scotland I	A	2/6	1/6
	B	3/6	2/6
Knox II	A	2/6	1/6
	B	0/6	0/6
Scotland II	A	0/4	0/4
	B	0/4	0/4
Sullivan	A	0/1	0/1
	B	0/1	0/1
Total ^b		9/27	9/27

A = Significant @ $P < 0.05$.

B = Significant @ $0.05 \leq P < 0.10$.

^a Number of specimens with significant values/total sample size from each site.

^b Total number of specimens from cave and farm sites significant @ $P < 0.10$.

Table 2. Comparison of significant values for brain muscarinic cholinergic receptor density (B_{max}) and cholinesterase activity (ChE) in northern long-eared bats collected from cave and farm sites.^a

Cave Sites		B_{max}	ChE
Pilot Knob	A	0/9	0/9
	B	0/9	1/9
Great Scott	A	1/9	1/9
	B	0/9	0/9
Total ^b		1/18	2/18
Farm sites			
Monroe	A	0/3	1/3
	B	0/3	0/3
Macon	A	0/1	0/1
	B	0/1	0/1
Pike	A	0/3	1/3
	B	0/3	0/3
Scotland I	A	0/2	0/2
	B	0/2	0/2
Schuyler	A	0/2	2/2
	B	1/2	0/2
Putnam	A	0/2	0/2
	B	0/2	0/2
Sullivan	A	0/2	0/2
	B	0/2	1/2
Total ^b		1/15	5/15

A = Significant @ $P < 0.05$.

B = Significant @ $0.05 \leq P < 0.10$.

^a Number of specimens with significant values/total sample size from each site.

^b Total number of specimens from cave and farm sites significant @ $P < 0.10$.

In addition to the analysis of individual bats for $B_{\max}$, K_D , and ChE levels, these same parameters were also examined and analyzed for significance on a site-specific basis. An ANOVA/PROC GLM approach determined that bats collected from cave and north Missouri farm sites did not differ significantly with respect to mean brain $B_{\max}$, K_D , or ChE activity for either of the 2 surrogate species. The only exceptions were little brown bat farm sites in Sullivan county where a single K_D value was low, and another in Knox county where elevated K_D values were noted. The differences in K_D at these 2 sites were not considered biologically significant, and therefore, were not included in any individual or site-specific statistical analysis.

When PROC GLM contrast statements were used, mean brain ChE and $B_{\max}$ values were observed to be different at 3 individual collection sites in north Missouri. At the Knox I site significantly lower brain ChE values were noted in little brown bats when compared to the 3 cave sites ($P = 0.0115$) and when compared to the group of 5 other farm sites ($P = 0.0351$) (Table 3). $B_{\max}$ values were also significantly depressed at the Scotland I farm site when compared to all cave sites ($P = 0.0009$) and to the group of 5 other farm sites ($P = 0.0053$).

Northern long-eared bats showed results similar to little brown bats when contrast statements were used to compare for differences between sites (Table 4). Brain ChE levels at the Schuyler site were depressed when compared to the 2 cave sites ($P = 0.0173$) and when compared to the group of 6 other farm sites ($P = 0.0270$).

Table 3. Variation in brain muscarinic cholinergic receptor density^a ($B_{\max}$) and affinity^b (K_d) and cholinesterase activity^c (ChE) in little brown bats collected from cave and farm sites.^d

Cave Sites	N	$B_{\max}$	K_d	ChE
Scotia Hollow	6	355.5 ± 28.1	54.3 ± 2.1	11.62 ± 0.91
Great Scott	6	294.0 ± 10.1	44.8 ± 3.8	10.94 ± 0.78
Onyx	6	345.2 ± 15.2	46.8 ± 3.3	11.21 ± 0.79
Mean $\pm$ SEM		331.5 ± 12.36	48.7 ± 1.98	11.25 ± 0.46
Total N	18			
Farm Sites				
Monroe	2	370.0 ± 6.0	45.0 ± 6.0	10.30 ± 1.31
Knox I	8	303.3 ± 17.6	58.5 ± 3.2	8.97 ± 0.67^e
Scotland I	6	244.8 ± 12.0^f	50.7 ± 2.4	9.65 ± 1.01
Knox II	6	322.3 ± 32.5	47.0 ± 1.5	10.72 ± 0.61
Scotland II	4	330.0 ± 23.9	55.8 ± 6.4	10.28 ± 0.76
Sullivan	1	276.0	32.0	14.54
Total N	27			

^a Expressed as [³H] NMS binding (fmol /mg protein).

^b Units in pM.

^c Micromoles AThCh hydrolyzed/min/g brain or mL plasma, wet weight.

^d All values expressed as mean $\pm$ SEM.

^e Significant compared to cave ($p = 0.0115$) and farm sites ($p = 0.0351$).

^f Significant compared to cave ($p = 0.0009$) and farm sites ($p = 0.0053$).

Table 4. Variation in brain muscarinic cholinergic receptor density^a ($B_{\max}$) and affinity^b (K_d) and cholinesterase activity^c (ChE) in northern long-eared bats collected from cave and farm sites.^d

Cave Sites	N	$B_{\max}$	K_d	ChE
Pilot Knob	9	330.1 ± 9.0	52.3 ± 1.8	11.54 ± 0.43
Great Scott	9	340.6 ± 20.9	58.8 ± 6.5	11.5 ± 0.62
Mean $\pm$ SEM		335.3 ± 11.08	55.6 ± 3.37	11.52 ± 0.37
Total N	18			
Farm Sites				
Monroe	3	308.7 ± 22.6	53.0 ± 9.2	10.49 ± 0.93
Macon	1	322.0	37.0	12.43
Pike	3	326.7 ± 29.0	49.7 ± 6.1	10.78 ± 1.60
Scotland I	2	358.5 ± 45.5	55.0 ± 10.0	11.61 ± 1.64
Schuyler	2	300.5 ± 35.5	60.5 ± 14.5	7.83 ± 0.26^e
Putnam	2	301.5 ± 6.5	45.5 ± 7.5	12.87 ± 2.49
Sullivan	2	293.5 ± 14.5	34.5 ± 6.5	10.13 ± 0.82
Total N	15			

^a Expressed as [³H] NMS binding (fmol /mg protein).

^b Units in pM.

^c Micromoles AThCh hydrolyzed/min/g brain or mL plasma, wet weight.

^d All values expressed as mean $\pm$ SEM.

^e Significant compared to cave ($p = 0.0173$) and farm sites ($p = 0.0270$).

ORGANOCHLORINE ANALYSIS

In examining Table 5 several observations can be made concerning OC insecticide residues in little brown bats. PPDDE had the highest concentrations of all residues and was recovered in 43 (96%) of 45 bat samples. The levels detected at Onyx Cave were significantly higher than those at all of the 6 farm sites and the 2 other cave sites. DLDRN residues were recovered in 44 (98%) of 45 samples and had the second highest concentrations. Four farm sites and Great Scott Cave had significantly elevated levels as compared to Scotia Hollow and Onyx Caves.

OXYCH and HE were compounds with next 2 highest concentrations and accounted for 43 (96%) and 37 (82%) recoveries, respectively, in a sample of 45 bats. OXYCH ranked number 3, and HE number 4, with respect to mean total body burden levels. There were no significant differences in mean OXYCH levels among all little brown bat locations, however, HE at the Knox II site was elevated compared to both Scotia Hollow and Onyx Caves. Though not statistically significant ($P > 0.05$), TOC concentrations at all 3 cave locations were higher than at any of the 6 farm sites.

Concentrations of OC recoveries in northern long-eared bats followed the same tissue concentration rank order as in the little brown bat with PPDDE > DLDRN > OXYCH > HE (Table 6). PPDDE was identified in 30 (91%) of 33 total samples. The Monroe farm site had values significantly higher than at the Sullivan farm site and at both Pilot Knob and Great Scott Caves. DLDRN residues were recovered in 26 (79%) of 33 samples. Bats collected from 6 of the 7 summer/maternity sites had DLDRN concentrations significantly elevated above those levels in bats collected at both of the cave sites.

Table 5. Geometric means and ranges of organochlorine residues^a in carcasses of little brown bats.

Cave Sites	N	Oxychlorthane	Heptachlor Epoxide	o,p'-DDE	p,p'-DDE	Dieldrin	o,p'-DDD	o,p'-DDT	p,p'-DDD	p,p'-DDT	Total Organochlorines
Scotia Hollow	6	78.4 (13.5-376.2)	14.9 (5.0-123.1)	47.5 (5.0-1844.3)	527.6 (286.2-994.3)	69.1 (28.8-346.0)	5.0 (5.0-5.0)	125.2 (5.0-10373.6)	65.8 (5.0-3708.2)	7.4 (5.0-54.3)	1577.8
Great Scott	6	132.0 (20.7-672.2)	33.7 (5.0-179.4)	11.9 (5.0-168.3)	1185.6 (494.2-5116.0)	347.8 (60.8-2658.9)	5.0 (5.0-5.0)	37.1 (5.0-1157.9)	32.4 (5.0-600.7)	16.5 (5.0-1302.2)	2129.4
Onyx	6	78.1 (14.0-460.8)	25.7 (5.0-282.7)	69.5 (5.0-449.6)	1214.4 (624.6-3301.0)	61.6 (5.0-647.3)	5.0 (5.0-5.0)	61.3 (5.0-439.7)	24.3 (5.0-290.0)	11.2 (5.0-135.1)	2050.8
Farm Sites											
Monroe	2	112 (57.6-217.6)	59.3 (39.5-88.8)	12.1 (5.0-29.4)	456.8 (273.0-764.4)	307.4 (150.4-628.3)	5.0 (5.0-5.0)	16.2 (5.0-52.7)	17.1 (5.0-58.5)	31.6 (23.8-42.1)	1089.9
Knox I	8	65.8 (5.0-308.1)	47.5 (5.0-155.6)	5.0 (5.0-5.0)	411.2 (92.0-874.2)	247.6 (41.9-653.3)	5.0 (5.0-5.0)	32.9 (5.0-123.0)	50.6 (5.0-153.3)	19.1 (5.0-57.3)	960.4
Scotland I	6	104.2 (16.9-641.2)	54.1 (13.7-311.8)	6.1 (5.0-17.3)	412.5 (155.5-981.3)	454.1 (83.9-1635.3)	5.0 (5.0-5.0)	42.6 (5.0-165.0)	58.2 (14.1-210.4)	22.4 (5.0-128.6)	1223.9
Knox II	6	154.4 (54.0-516.1)	102.8 (46.3-222.6)	7.6 (5.0-63.5)	127.3 (5.0-982.0)	511.1 (265.5-1036.2)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	14.8 (5.0-167.7)	16.7 (5.0-73.0)	1190.3
Scotland II	4	99.6 (54.0-156.0)	55.0 (35.1-120.0)	6.0 (5.0-10.4)	486.6 (252.8-1161.3)	355.8 (247.2-677.9)	5.0 (5.0-5.0)	14.0 (5.0-43.4)	13.1 (5.0-35.0)	14.9 (11.1-25.8)	1101.2
Sullivan	1	93.9	13.0	5.0	415.0	78.3	5.0	30.3	17.1	60.6	718.0

^a Results are reported in ng/g (ppb) by weight of fresh (wet) tissue.

The Limit of Quantitation (LOQ) for all analytes was 10ppb.

Residues less than the LOQ or not detected (ND) are reported and entered into computations as one-half of the observed LOQ for that compound.

Table 6. Geometric means and ranges of organochlorine residues^a in carcasses of northern long-eared bats.

Cave Sites	N	Oxychlorane	Heptachlor Epoxide	o,p'-DDE	p,p'-DDE	Dieldrin	o,p'-DDD	o,p'-DDT	p,p'-DDD	p,p'-DDT	Total Organochlorines
Pilot Knob	9	13.3 (5.0-45.8)	5.0 (5.0-5.0)	5.6 (5.0-15.0)	95.8 (5.0-1033.1)	20.0 (5.0-46.3)	5.0 (5.0-5.0)	6.5 (5.0-26.5)	5.0 (5.0-5.0)	5.9 (5.0-21.7)	205.6
Great Scott	9	11.4 (5.0-20.00)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	69.3 (27.3-92.6)	8.2 (5.0-19.5)	5.0 (5.0-5.0)	7.0 (5.0-37.1)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	128.6
Farm Sites											
Monroe	3	80.5 (41.5-175.5)	38.4 (32.4-51.7)	5.0 (5.0-5.0)	305.6 (217.1-389.4)	172.5 (114.7-265.3)	5.0 (5.0-5.0)	7.9 (5.0-19.9)	9.8 (5.0-37.3)	14.2 (5.0-26.9)	681.7
Macon	1	18.8	27.0	5.0	183.8	73.0	5.0	5.0	11.2	5.0	333.8
Pike	3	106.4 (51.4-270.1)	29.8 (21.2-55.1)	13.7 (5.0-103.6)	118.1 (5.0-1877.1)	210.1 (134.4-450.7)	5.0 (5.0-5.0)	8.3 (5.0-22.5)	7.7 (5.0-18.1)	14.6 (5.0-123.9)	705.9
Scotland I	2	11.1 (5.0-24.5)	14.9 (10.5-21.2)	5.0 (5.0-5.0)	160.6 (156.8-164.5)	114.6 (93.5-140.6)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	333.0
Schuyler	2	26.3 (24.6-28.2)	20.8 (17.0-25.6)	5.0 (5.0-5.0)	186.0 (182.1-190.0)	84.5 (60.7-117.5)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	7.1 (5.0-10.0)	349.2
Putnam	2	8.4 (5.0-14.1)	10.8 (5.0-23.5)	5.0 (5.0-5.0)	142.8 (64.9-313.9)	63.0 (49.8-79.6)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	287.4
Sullivan	2	12.5 (5.0-31.3)	8.6 (5.0-14.6)	13.2 (5.0-34.8)	28.9 (5.0-167.3)	42.9 (26.3-70.0)	5.0 (5.0-5.0)	13.6 (5.0-36.9)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	156.6

^a Results are reported in ng/g (ppb) by weight of fresh (wet) tissue.

The Limit of Quantitation (LOQ) for all analytes was 10ppb.

Residues less than the LOQ or not detected (ND) are reported and entered into computations as one-half of the observed LOQ for that compound.

OXYCH and HE residues were identified in 26 (79%) and 13 (39%) northern long-eared bat carcasses, respectively, in a sample of 33 bats. HE was either ND or below the LOQ in bats from all cave-site samples but elevated in bats collected at 4 of the 7 summer/maternity sites. OXYCH was higher in bats at 3 of the 7 summer/maternity sites and both cave locations. TOC concentrations in northern long-eared bats were significantly higher at 5 of the 7 summer/maternity sites when compared to the levels at cave sites.

CORRELATION OF BRAIN ChE AND OCs

In little brown bats collected from northern Missouri agricultural sites, but not cave sites, brain ChE activity was negatively correlated to increasing OC concentrations for each of the 5 compounds that were analyzed. There was no significant correlation, however, between any of the OC concentrations and B_{max} . Northern long-eared bats differed from little brown bats in that no significant correlations were seen with OC concentrations and brain ChE activity or B_{max} .

As the rank-transformed OC concentrations increased in individual specimens, brain ChE activity decreased (Figures 1-5). P values ranged from 0.0023 to 0.0117 and negative correlation coefficients (r) ranged from -0.562 to -0.455. Evaluation of correlation coefficients suggests that of the 5 OCs analyzed, the TOC group influences the variability of brain ChE activity the greatest ($r = -0.562$). The suggested influence of OXYCH on brain ChE activity is the least of all OCs ($r = -0.455$).

Figure 1. Relationship of Brain ChE activity (μ moles AThCh hydrolyzed/min/g brain, wet weight) to ranked Oxychlordan residues (ppb) in carcasses of little brown bats (*Myotis lucifugus*) collected from northern Missouri farm sites.

Figure 2. Relationship of Brain ChE activity (μ moles AThCh hydrolyzed/min/g brain, wet weight) to ranked Heptachlor Epoxide residues (ppb) in carcasses of little brown bats (*Myotis lucifugus*) collected from northern Missouri farm sites.

Figure 3. Relationship of Brain ChE activity (μ moles AThCh hydrolyzed/min/g brain, wet weight) to ranked p,p'-DDE residues (ppb) in carcasses of little brown bats (*Myotis lucifugus*) collected from northern Missouri farm sites.

Figure 4. Relationship of Brain ChE activity (μ moles AThCh hydrolyzed/min/g brain, wet weight) to ranked Dieldrin residues (ppb) in carcasses of little brown bats (*Myotis lucifugus*) collected from northern Missouri farm sites.

Figure 5. Relationship of Brain ChE activity (μ moles AThCh hydrolyzed/min/g brain, wet weight) to ranked Total Organochlorine residues (ppb) in carcasses of little brown bats (*Myotis lucifugus*) collected from northern Missouri farm sites.

PYRETHROIDS

Of 312 possible individual pyrethroid-specimen recoveries in both surrogate species, only 17 were greater than the LOQ or ND. Eleven of the 17 recoveries were in little brown bats and 6 were in northern long-eared bats. Ten of the 17 pyrethroid identifications were for the compound permethrin and 9 of the 10 were detected in little brown bats.

All 6 reference little brown bats collected from Onyx Cave had permethrin levels greater than the LOQ or ND (Appendix, Table B1). Permethrin concentrations detected at this site were found to be normally distributed and significantly higher than those concentrations at all other cave and farm sites ($P < 0.0001$). The geometric mean of permethrin at Onyx Cave was 248ppb and with a range of 82 to 892ppb (Table 7).

Permethrin was also significantly higher at Scotia Hollow Cave when compared to other cave and farm sites (Appendix, Table B1) with P values from 0.0358 to 0.0001. The geometric mean of permethrin at Scotia Hollow was 78ppb and with a range of 25 to 2547ppb (Table 7).

Esfenvalerate was recovered in only 2 little brown bats, 1 from Scotia Hollow Cave and the other from Great Scott Cave (Appendix Table B1). Tefluthrin and cyhalothrin residues were either below the LOQ or ND at all farm and cave sites for both surrogate species.

In northern long-eared bats the only specimen to contain permethrin residues was collected from Great Scott Cave (Appendix, Table C1). Esfenvalerate residues were recovered in 5 specimens with 3 from Pilot Knob Cave, 1 from Great Scott Cave, and 1 from the Putnam farm site (Appendix, Tables C1 and C2). The geometric mean of esfenvalerate at Pilot Knob Cave was 40ppb and ranged from 25 to 140ppb (Table 8).

Table 7. Geometric means and ranges of synthetic pyrethroid residues^a in carcasses of little brown bats.

Cave Sites	N	Tefluthrin	Cyhalothrin	Permethrin	Esfenvalerate
Scotia Hollow	6	5.0 (5.0-5.0)	5.0 (5.0-5.0)	77.8 (25.0-2546.9)	33.4 (25.0-141.1)
Great Scott	6	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	36.7 (25.0-251.8)
Onyx	6	5.0 (5.0-5.0)	5.0 (5.0-5.0)	247.7 (82.1-891.9)	25.0 (25.0-25.0)
Farm Sites					
Monroe	2	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)
Knox I	8	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)
Scotland I	6	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)
Knox II	6	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)
Scotland II	4	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)
Sullivan	1	5.0	5.0	25.0	25.0

^a Results are reported in ng/g (ppb) by weight of fresh (wet) tissue.

The Limit of Quantitation (LOQ) for tefluthrin and cyhalothrin was 10ppb and 50ppb for permethrin and esfenvalerate.

Residues less than the LOQ or not detected (ND) are reported and entered into computations as one-half of the observed LOQ for that compound.

Table 8. Geometric means and ranges of synthetic pyrethroid residues^a in carcasses of northern long-eared bats.

Cave Sites	N	Tefluthrin	Cyhalothrin	Permethrin	Esfenvalerate
Pilot Knob	9	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	40.0 (25.0-140.4)
Great Scott	9	5.0 (5.0-5.0)	5.0 (5.0-5.0)	27.2 (25-52.6)	34.1 (25.0-411.1)
Farm Sites					
Monroe	3	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)
Macon	1	5.0	5.0	25.0	25.0
Pike	3	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)
Scotland I	2	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)
Schuyler	2	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)
Putnam	2	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	52.1 (25.0-108.5)
Sullivan	2	5.0 (5.0-5.0)	5.0 (5.0-5.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)

^a Results are reported in ng/g (ppb) by weight of fresh (wet) tissue.

The Limit of Quantitation (LOQ) for tefluthrin and cyhalothrin was 10ppb and 50ppb for permethrin and esfenvalerate.

Residues less than the LOQ or not detected (ND) are reported and entered into computations as one-half of the observed LOQ for that compound.

Though 3 out of the 9 bats collected at the Pilot Knob site contained detectable esfenvalerate residues, this site was not statistically different ($P = 0.6941$) when compared to all other sites.

CARCASS PCBs, GASTROINTESTINAL OPs, AND PLASMA ChE

In an effort to better assess the potential overall contaminant status of Indiana bats in north Missouri, tissue samples from surrogate little brown and northern long-eared bats were analyzed for PCB congeners, gastrointestinal OPs, and plasma ChE activity. Though the environmental persistence of PCBs and the OC insecticides is similar, no bat specimens were found that contained residues of PCB congeners. Gastrointestinal sample analysis also failed to recover residues of any of the 5 selected OPs.

Finally, RBC hemolysis and inadequate plasma volumes introduced a degree of variability such that valid statistical comparisons could not be made. Therefore, plasma ChE results will only be reported in tabular form and without statistical analysis (Appendix, Tables D1, D2, E1, E2).

DISCUSSION

PHYSIOLOGICAL PARAMETERS

Bats of both surrogate species collected from cave sites did not exhibit significant differences in brain $B_{\max}$ or ChE activity. Uniformity of reference site parameters provides the basis with which to compare and identify potentially insecticide exposed bats collected from northern Missouri agricultural sites. The anticholinesterase

OP/CB insecticides, as opposed to the OC class, are of a nonpersistent nature and so it is reasonable to expect that bats entering hibernation in the fall would have no significantly altered biochemical effects indicative of recent anticholinesterase exposure when leaving the hibernacula during the spring of the following year (Grue et al. 1983). The near total cessation of foraging activity during the winter season and lack of available OP/CB insecticide contaminated prey provide a relatively high degree of assurance that hibernating bats are not being exposed to the OP/CB class of agricultural insecticides during the winter.

One method of providing evidence of exposure to anticholinesterase insecticides is to identify individual bats with brain ChE values below a predetermined diagnostic threshold. Information on individual organisms, particularly biochemical or cellular such as ChE activity, has always had great utility because physiological and toxicological responses occur at the level of the individual. When whole organism responses can be monitored, events occurring at the population level are made more understandable.

Two different biochemical criteria are available to make a determination of exposure to anticholinesterase compounds and both are based on levels of ChE activity in sampled organisms. Statistically, we identified 9 of 27 little brown bats (33%) collected in northern Missouri having depressed brain ChE levels ($P < 0.10$) when compared to reference specimens collected from cave sites in southern Missouri. Brain ChE levels in 5 of the 27 bats (19%) were found to be significant at $P < 0.05$ when compared to reference specimens (Table 1). In northern long-eared bats, 5 of 15 specimens (33%) collected in northern Missouri had significantly depressed brain ChE levels ($P < 0.10$) when compared to reference specimens, while 4 of these 15 bats (27%) were significant at $P < 0.05$ (Table 2). Although the amount or specific anticholinesterase insecticides

that these affected bats were exposed to is unknown, comparison of brain ChE levels between northern Missouri and reference surrogate bats indicates that, statistically at least, significantly lower levels of a major enzyme (ChE) have been identified and documented in bats collected from agricultural regions of northern Missouri as compared with bats collected from nonagricultural cave sites in southern Missouri.

A second criterion to diagnose patterns of depressed ChE activity was developed by Ludke et al. (1975). This method of exposure assessment was originally developed to diagnose and monitor the effects of selected ChE inhibition in birds, but it has since been generally accepted as being applicable to other wildlife. Ludke proposed that inhibition (depression) of brain ChE by 20% below mean normal values is indicative of exposure to OPs/CBs, while death accompanied by ChE inhibition of 50% or greater can be attributed to OP/CB intoxication. Inhibition of brain ChE activity is thus a strong indicator of both exposure and to and impact of OP and CB insecticides. Twelve of 27 little brown bat samples exhibited a minimum of 20% depression in brain ChE activity. It is apparent that this approach is more liberal than the statistical comparisons in that 3 additional bats were considered as exposed when the 20% criterion was used. Use of the 20% criterion for northern long-eared bats however, did not increase the number of exposed bats above that determined by statistical comparisons.

Another biochemical indication of exposure to anticholinesterase insecticides is a change in B_{max} , or muscarinic receptor number. B_{max} responds to elevated acetylcholine neurotransmitter levels by reducing the number of receptor membrane proteins. Changes or modulations in these membrane proteins are considered mechanisms of adaptation or the apparent development of tolerance to overstimulation by OP/CB

insecticides. It has been demonstrated that repeated sublethal doses of an OP/CB compound can lead to the development of tolerance to its toxicity (Costa et al. 1981). Of the 27 little brown bats captured at northern Missouri farm sites, 9 had significantly ($P < 0.10$) lower $B_{\max}$ values as compared to the reference cave bats (Table 1). Five of these 9 bats have $B_{\max}$ significant at $P < 0.05$. Only 1 of 15 northern long-eared bats had a depressed $B_{\max}$ at $P < 0.10$ (Table 2).

Although identification of potentially contaminant exposed individual bats is important, site or location analysis can broaden and strengthen suggestions that contaminant exposure may be occurring on a larger scale. For example, site analysis may identify particular geographic areas where routine agricultural practices allow for a surfeit of agricultural chemicals to be released into local environments. Patterns of wider agrochemical usage usually lead to questions of contamination at the local, and landscape scale, and ultimately consider the negative ecological impacts on populations at these higher levels of organization (e.g., energy transfer between trophic levels, life history effects).

Scotland and Knox, 2 northern Missouri little brown bat agricultural sampling sites, had significantly lower $B_{\max}$ and ChE values, respectively, when sampled in early July as compared to mean values obtained from reference cave sites and the 5 other agricultural sites (Table 3). When sampled approximately 5 weeks later in August, $B_{\max}$ and ChE values for bats from the Scotland and Knox sites were not significantly different from the reference cave sites and the 5 other agricultural sites. It would be tempting to argue that the bats at these 2 agricultural sites “recovered” from an exposure to anticholinesterase insecticides experienced in July. Without the opportunity to follow and

retest individual bats, “recovery” as an explanation of the differences on these 2 occasions is not justified. Though physiological and toxicological parameters are dynamic and continuously adjust and “recover” from a variety of physical and chemical perturbations to maintain homeostasis, differences in $B_{\max}$ and ChE between these 2 sampling periods are possibly observations from a normal distribution and represent a range of possible values obtained from individual bats along a continuum of exposures to OP/CB insecticides.

Both northern long-eared bats collected at the Schuyler site in July, 1996 had significantly lower brain ChE activity as compared to mean values obtained from cave sites and to the remaining 6 farm sites (Table 4). However, no repeat sampling data were obtained from the Schuyler site later in the summer to compare with that from the initial sampling period as was done for the Scotland and Knox locations.

An important consideration in the toxicology of ChE and muscarinic receptor modulation is the degree of exposure required for such changes to occur. Costa et al. (1981) found in a time-course study that between 7 and 9 days were required to significantly decrease muscarinic receptors in the brains of mice. Though differences were noted among tissues with regard to the rate of recovery of receptor number, generally 7 to 14 days were needed. These response times probably mean that bats must experience exposures to OP/CB insecticides over some minimal time period to significantly lower ChE and $B_{\max}$ values. It is my belief that insectivorous bats are ingesting some unknown amount of OP/CB insecticides while foraging on insect prey for a sufficient duration that brain ChE activity is inhibited and muscarinic cholinergic receptor numbers decrease in number (downregulate) to compensate.

It would be difficult to overstate the impact that OP/CB compounds have on a normally functioning cholinergic nervous system. It has been stated, "Most impressive is the singular fact that ChE is the only substance that can influence every physiological or behavioral response thus far examined." (Myers 1974). What are some of the biological consequences of biochemical, and suspected sublethal, alterations in brain neurochemicals? Depending on the degree of insecticide exposure and ACh buildup, increased levels of the neurotransmitter ACh can lead to overstimulation of the central and peripheral nervous systems with their classical, attendant symptoms (e.g., increased secretions, gastrointestinal cramping, alterations in cardiac rate, muscle weakness, convulsions). Increases in ACh levels are followed by a downregulation in muscarinic receptor number characterized by critical physiological and behavioral changes (Costa et al. 1981, McDonald 1988, Bushnell 1991, Costa 1996, Grue et al. 1997). Rats dosed with OPs developed deficits in cognitive and motor functions (Bushnell et al. 1991). McDonald (1988) found that inhibition of ChE caused by repeated OP exposure alters spatial memory functions in rats, as well as causing a loss of muscarinic receptors. If bats incur changes in spatial memory function this aberration could impair the fall migration of bats back to southern Missouri hibernacula.

Grue et al. (1997) reviewed the effects on thermoregulation (hypothermia), food consumption (anorexia and altered foraging behavior), and reproduction (altered hormone levels, reduction in litter size, alterations in reproductive behavior). He found that studies, primarily with captive small mammals, have documented significant effects of ChE inhibitors on thermoregulation; cholinergic synapses are believed to be directly involved in heat regulation and arranged in discrete pathways governing heat production and heat loss. Reduced tolerance to cold appears to be related to changes in endocrine

activity, particularly suppressed adrenocortical function, while direct impairment of thermoregulation is associated with alterations in the function of the hypothalamus.

Because bats have a small body mass, a higher proportion of heat is lost due to a relatively greater surface area to volume ratio (Altringham 1996). This is 1 of the reasons bats have such a high caloric requirement. Any factor, contaminant or otherwise, that affects thermoregulatory capacity in bats has the potential to jeopardize survivability.

Given the number of physical symptoms indicative of gastrointestinal distress in birds and mammals following acute and chronic exposure to ChE inhibitors (diarrhea, nausea, vomiting), it is not surprising that reductions in food and water intake have been observed in intoxicated animals. Some authors believe that reductions in food consumption are largely the result of physiological distress caused by chemical ingestion ("pesticide-induced anorexia"), and not repellency of contaminated food. As previously suggested, alterations of caloric intake have the potential to upset a bats' capacity to maintain thermoregulatory homeostasis.

Studies with small mammals indicate that stress, such as that associated with food restriction, can alter reproductive success and result in skewed sex ratios and reduced litter size in mammals. Though most contaminant studies use rodent species, it is believed that the responses seen in mammalian studies are qualitatively similar and vary only in the intensity of the interspecific reactions. It is highly probable that insecticide exposed bats would undergo the same suite of physiological responses exhibited in laboratory studies that utilize rodents as test animals.

Heinz (1989) proposed one of the better definitions of sublethal effects. He described sublethal effects as "those that would not normally lead to an animal's death

or reproductive impairment (which is often the death of its eggs, embryo or young) in the protected laboratory pen or pond in which it is being studied.” The sublethal effects associated with low-dose exposure to OP/CB insecticides are interesting no doubt, but sublethal responses in affected organisms require more attention than “interesting” alone would engender. Of the influences that anticholinesterase compounds have on wildlife, decreased food intake and hypothermia are probably the changes most likely to impair survivability in free-living bats. Reduction in food intake for 12-24 hours following acute exposure could result in starvation of bats and other small animals with high metabolic rates (Grue et al. 1991). Though hypothermia and starvation are easily understood sequela to OP/CB exposure, in reality the biological (ecological) significance of sublethal levels of enzyme inhibition in bats is unknown. What is needed is the ability to associate probabilities of effects, particularly on reproduction and survival, at different levels of enzyme inhibition. Clearly, laboratory and field studies are needed in which both ChE inhibition and population effects are measured (Grue et al. 1991).

ORGANOCHLORINE ANALYSIS

The recovery of OCs from carcass tissues indicates the continued presence of this class of chemicals in the environment and that food chain, bioaccumulation processes are still ongoing in the 2 sampled surrogate bat species. In a review of bats and environmental contaminants, Clark (1981) observed that the most common OC found in bats is DDE, with the less frequently recovered insecticides including DLDRN, DDT metabolites, HE, OXYCH, and various fractions (isomers) of chlordane. Except for the chlordane isomers which were not analyzed for in this study, the OCs recovered from the carcasses of the 2 designated surrogate bat species supports Clark’s findings

that this class of insecticides continues to be environmentally persistent and ubiquitous, at least within the sampling area of northern Missouri. When the soil half-lives (the time required for contaminant concentrations in the soil to have decreased by 50%) of DDT and DLDRN are estimated at 57 and 4-7 years, respectively, it is understandable that soil dwelling organisms could remain a source of residues for decades to come (Newton 1984).

It has been acknowledged that animals dependent upon aquatic habitats generally contain higher OC residues than terrestrial species. Higher OC concentrations are also reflected in samples analyzed from the habitat (e.g., vegetation, soil, invertebrates). Exceedingly low insecticide levels in water have often been the source of much higher concentrations found in fish and wildlife and even minute residues in water must be regarded as potentially hazardous to animals dependent on aquatic or wetland environments (Keith and Hunt 1966). In an aquatic medium, insecticides adsorb onto particulate, organic matter with concentrations ranging up to 78ppm. Organic matter OC concentrations have been found to be 10,000 to 100,000 times higher than that in the filtrate. Organic portions of the suspended material and sediments serve as a basic source of energy for the lower trophic levels in aquatic food chains. Adherence of contaminants to organic particulate matter may be of major importance in transfer of residues to higher forms of wildlife (Keith and Hunt 1966). Bats foraging in riparian habitats on aquatic insect prey are, therefore, potentially at risk to accumulate elevated OCs.

Of all farm and cave sites sampled, ranked levels of the 5 analyzed OCs were more uniformly distributed and with less variability at all little brown bat sites when compared to all northern long-eared bat sites. Variability in residue concentrations by

site can be explained as credibly by physiological and ecological differences between species as by the variability in contaminant levels within the physical environment (i.e., geographic distribution). DLDRN was the only insecticide in little brown bats found to vary significantly among sites. OC concentrations were also higher across all sites in little brown bats than northern long-eared bats. The relatively low residue accumulation by northern long-eared bats could be the result of lower dietary intake, different insect prey species, or more efficient excretion of OC compounds (Clark and Prouty 1976). The higher levels noted in little browns, if also characteristic of Indiana bats, could have important management implications for this endangered species (Clark and Prouty 1976). Without direct analysis of Indiana bats it would be impossible to say with certainty whether this species is accumulating OC body burdens as great, or greater, than those observed in little brown bats.

Seasonal (cave sites vs. farm sites) differences in body weight and OC levels were noted in the 2 surrogate species (Tables 5 and 6). During hibernation it is usual for endogenous fat and water reserves to be utilized to varying degrees with the result that weight loss can be significant. Little brown cave bats weighed an average of 0.76g (17%) less than their farm site congeners while northern long-eared cave bats weighed an average of 1.13g (27%) less than their farm site congeners. As a consequence of weight loss during hibernation, body contaminant residues become more concentrated in the spring. Lee (1993) found that little brown, northern long-eared, and Indiana bats showed temporal and spatial resource partitioning within co-occurring areas. Morphological differences in jaw lengths also contributed to the interspecific dietary variation among the 3 species. Differences in ecology and jaw morphology in little brown

and northern long-eared bats might explain the observed seasonal differences between these 2 surrogates.

Examination of body burdens for the 5 OCs revealed that both bat species had the same rank order carcass concentration. Comparable ranking of tissue insecticides would suggest that contaminants in the environment, particularly the food base, are similarly ranked and are being quantified by foraging free-living bats. The relative magnitude of these compounds does not necessarily equate to greater, or lesser, original use of any of the insecticides, but more likely to environmental fate processes (transport, transformation, degradation) and differences in persistence and toxicity within the animals themselves (Newton 1984).

CORRELATION OF BRAIN ChE AND OCs

The neurotoxic effects of the OC insecticides in mammals are well known and include such signs as hyperexcitability, incoordination, tremors, hyperthermia, and convulsions (Eriksson et al. 1984). Until recently, little was known about the biochemical correlates of various neurotoxic effects produced by DDT and related pesticides. Hrdina et al. (1975) and Woolley (1982) reviewed some of the possible neurochemical mechanisms that include alterations in the levels of ACh and norepinephrine and a change in serotonin metabolism in the brain. DDT has also been shown to decrease the number of muscarinic cholinergic receptors (B_{max}) in the adult mouse brain (Eriksson et al. 1984).

Data collected from brain ChE analyses and ranked carcass OC residue concentrations were examined for a possible relationship. It was found that in carcasses

of little brown bats collected from northern Missouri farm sites brain ChE activity was negatively correlated to the ranked concentrations of all 5 OC insecticides (Figures 1-5). Muscarinic receptor number, which is known to decrease with elevated ChE activity, however, was not correlated with changes in carcass OC concentrations. Though our data suggests that B_{max} levels were not linked to changes in OC concentrations, it is known that the cholinergic nervous system, as monitored by B_{max} , ACh, and ChE levels, is a system of feedback mechanisms in which changes in one part of the system lead to reciprocal effects (modulation) elsewhere (Eriksson et al. 1990). Changes observed in brain ChE activity suggests an OC influence at some organizational level of the cholinergic nervous system. Lack of apparent effects on B_{max} could be due to variation in species sensitivity, specific chemical types and exposure levels, and the duration of exposure. The relatively low environmental OC levels that little brown bats are probably being exposed to may be inadequate in dosage or duration to elicit a response in B_{max} .

Another explanation needs to be offered for the observed correlation between brain ChE activity and OC levels. The OC chemicals recovered in body tissues strongly suggest low level, “background” exposures are occurring in little brown bats within the study sampling range in northern Missouri. OCs are not known to have a direct effect on the cholinergic nervous system, but OP and CB insecticides do. An alternative explanation is that bats are being coexposed during foraging in farm site areas to insect prey contaminated by both OC and OP/CB insecticides. It is much more likely that exposure to the OP/CB insecticides, and not the OCs simultaneously ingested, are having a direct effect in causing the observed depression in brain ChE levels. It should also be noted that little brown cave bats had mean OC levels greater than bats collected

from farm sites and did not have depressed $B_{\max}$ or ChE activity as observed in the bats collected from farm sites.

OCs are highly persistent environmental chemicals and were readily recovered and identified with carcass tissue analysis. Analysis of brain tissue suggested exposure to anticholinesterase insecticides when $B_{\max}$ and brain ChE activity were found to be significantly depressed in bats collected from northern Missouri agricultural sites. Recovery of gastrointestinal OP compounds, coupled with an inhibition of brain $B_{\max}$ and ChE, would strengthen our diagnosis of OP exposure in little brown and northern long-eared bats. Unfortunately, OP compounds were not recovered with G.I. tissue and contents' analysis. Small sample mass and rapid metabolism oftentimes makes recovery of OP compounds difficult.

Srikanth and Seth (1990) found that in rats, brain ChE activity was significantly inhibited (42%) in animals coexposed to endosulfan (a cyclodiene OC) and malathion. These authors suggested that endosulfan may potentiate the toxicity of malathion by interfering with glutathione and carboxylesterase routes of malathion detoxification. Owing to the higher rate of persistence of OCs in the environment compared to OP/CBs, there are chances of simultaneous exposure to these 2 insecticide classes. Studies correlating recovery of OCs, or any other pesticide, with changes in neurotransmitter or neurochemical levels must be interpreted with caution because of the potential for exposure of wildlife to multiple chemical compounds with interactive effects.

PYRETHROIDS

Of 78 total individual surrogate bats, randomly submitted and analyzed for pyrethroid insecticides, 16 had detectable residues above the LOQ. (Samples were randomly submitted for laboratory analysis to ensure initial equivalence between the samples and to minimize the influence and potential bias/error laboratory techniques could have on pre-grouped samples). Permethrin was recovered in 10 of the 16 specimens. All 6 little brown bats collected from Onyx Cave on 4/18/96 were found to contain permethrin ranging from 82.1 to 891.9ppb. Three of the 6 little browns from Scotia Hollow Cave also contained permethrin and with levels ranging from 55 to 2547ppb.

The identification of any field site where a significant proportion of the sampled specimens are found to be contaminated would invariably generate interest and concern. Interest would be in the origin or source of the contaminant and concern would be raised because such a finding suggests a localized and/or concentrated exposure event. Because only a single surrogate bat out of 45 collected from northern Missouri had residues of one pyrethroid insecticide (esfenvalerate) in its body tissues suggests that contaminated reference bats possibly acquired permethrin not while foraging in north Missouri, but locally, near the hibernaculum itself. If 2 surrogate species could be collected from a cave site location and found to contain pyrethroid residues a case could be made that contamination of bats by such compounds is more likely to be occurring near the cave site itself, and not being transported back from other, more distant areas in northern Missouri.

Uptake (Tang and Sigefried 1995) and bioconcentration (Muir et al. 1985) of pyrethroids by aquatic insects has been previously shown to occur. The

bioconcentration factor (BCF) of a chemical is the ratio of the average concentration of test chemical accumulated in the tissues of the test organisms under steady-state conditions to the average measured concentration in the water to which the organisms are exposed. Depending on whether insect larvae are held in contaminated sediments, or the water column itself, BCFs can range from 135 for *trans*-permethrin to 316 for deltamethrin (Muir et al. 1985). In a laboratory setting with measured concentrations, long-term (up to 28 days) BCFs for permethrin in stoneflies (*Pteronarcys dorsata*) ranged from 43 to 570 (Anderson 1982). In soils, most synthetic pyrethroids are immobile and cannot be readily leached (Kaufman et al. 1981) and have half-lives of approximately 3 weeks (Williams and Brown 1979). Regardless of the specific source or location of pyrethroids, availability of these compounds to biota is a possibility in areas where they are applied.

A counterargument to contamination of the local cave area is that the Missouri Ozark region is not known for row crop agriculture and the use of insecticides that accompanies it. Additionally, it is not considered likely that in mid April when these bats were collected that springtime foraging had begun (Richard L. Clawson, personal communication), precluding exposure and acquisition of insecticides. It is possible that the source of these chemicals will never be known.

Metabolism of pyrethroid insecticides has been studied extensively in mammals (Elliot et al. 1976, Marei et al. 1982). These compounds are relatively quickly excreted and do not persist significantly in tissues with half-lives in rats of 5 to 6 days for *trans*-permethrin. Although the major portion of an oral pyrethroid dose undergoes rapid metabolism, a small amount partitions into fatty tissues and persists in amounts detectable for at least 3 weeks. I propose that little brown bats could have been exposed

to pyrethroid insecticides during the late summer or early fall in northern Missouri agricultural areas and retained some contaminant load throughout the hibernation period due to a lower metabolic rate and an extended period of elimination from the body. Subsequently, when the spring collected reference bats were chemically analyzed, residues of pyrethroid insecticides were identified. Repeat sampling and analysis of 2 surrogate species collected from the same cave site could help to answer some of these questions.

CONCLUDING REMARKS

The objective of this chapter was to collect tissues from 2 surrogate bat species, both considered to be ecologically similar to the endangered Indiana bat, and to analyze for contaminants of agricultural origin and other biomarkers of exposure and effect. The identification of these biomarkers, or physiological responses, would then provide some evidence, albeit indirect, that the Indiana bat is potentially at risk because of exposure to the routine use of agriculturally applied insecticides in Missouri.

Data have shown that all 36 little brown and northern long-eared reference bats and all 42 surrogate bats collected from northern Missouri demonstrate prior exposure to OCs. Of the 78 individual surrogates, 20 little brown and 8 northern long-eared bats show biochemical effects of exposure to OP/CB anticholinesterase insecticides with depressed B_{max} or brain ChE activity. In addition, 17 bats of both surrogate species tested positive for the newer synthetic pyrethroid insecticides, 10 of which were for the compound permethrin.

The biomarkers we have described are of 2 general types. Biomarkers signify that an organism has been exposed to an exogenous contaminant, may predict future harm, or may themselves indicate a harmful effect. The OCs and pyrethroids are indicators of exposure and tell us that an organism has retained some of these chemicals in its tissues but gives no information on the degree of adverse effect that it may cause. Changes in $B_{\max}$ or ChE activity are biomarkers of toxic effect and demonstrate that a biochemical response has been elicited and that some unknown measure of adverse effect may have occurred.

One assumption of most toxicological studies using wild animals is that even a subtle, sublethal toxic effect can ultimately alter population size, recruitment, and species composition in a natural community (McBee and Bickham 1990). It is also assumed that exposure to chemicals that may have adverse metabolic, immunosuppressive, or pharmacologic properties will so dramatically alter the normal physiology of resident wildlife that species richness, diversity, and abundance characteristics will be negatively impacted.

Though the assumptions of the harmful effects of contaminants on populations and communities are likely valid, demonstrating a clear connection between physiology and ecology is often quite tenuous. The biological (i.e., ecological) significance of sublethal inhibition of brain ChE activity in our sampled bats can only be guessed at (McEwen et al. 1986). The recovery and identification of OCs and pyrethroid tissue residues is also without clear consequences. For example, we do not know whether a 25% inhibition of brain ChE in a little brown bat will affect its ability to thermoregulate or

forage efficiently or if 300ppb of o,p'-DDT in body tissues of a bat will impair reproductive behavior or hormone cycles.

The utility of any biochemical or physiological biomarker as an ecological indicator of potential impact on a population will ultimately depend on being able to correlate observed changes in an individual with population-level effects. However, predicting population-level effects of OCs or OPs from LD₅₀ toxicity tests, carcass residue levels, or biomarker responses is not a straightforward process (Gard and Hooper 1995). Our ability to predict from the cellular to the individual is far from perfect and, realistically, predicting from the cellular or physiological to the population will be especially challenging (Mineau 1998).

In order for physiological end points to be useful data, quantitative models need to be developed that permit extrapolation to population responses (Emlen 1989). Given our current state of knowledge, biomarkers alone are unlikely to shed light on population-level properties (Mineau 1998). Up to this point, very few studies have been conducted dealing with the delayed effects of chemical toxins on the demographics of natural populations. More field investigations should be undertaken that evaluate sublethal effects and biochemical changes and relate such findings to critical population characteristics such as fecundity and survival. Studies are needed to provide the necessary linkages between, and make more meaningful, the oftentimes separate disciplines of ecology and toxicology.

And finally, a few remarks concerning possible bias in the collection or gathering of field specimens. An integral early component of any chemical risk assessment process is the characterization of the extent of exposure associated with the suspected contaminant. With wild populations in natural settings, exposure is typically determined

by analysis of the sampled or recovered specimens. The potential mistake occurs in the evaluation of this exposure and is a function of the recovered specimens or, more specifically, the specimens that are not recovered.

The suggestion that the level, or extent, of pesticide exposure presented by our data may possibly misrepresent what had actually occurred under natural field conditions came from the discussion section in an article from a reputable wildlife management journal. The statement read, in part, "it is important to remember that we only examined birds that had died" (Spalding et al. 1994). The possible bias that our collection procedures were possibly generating was in the opposite direction, i.e., we were sampling the survivors. Bats analyzed in our study were definitely not dead, but alive, and apparently functioning well enough to engage in nocturnal foraging activities. It could also be argued that collected bats were not totally unimpaired, but in some unknown and subtle physiological way compromised, which then increased their likelihood of capture.

The collection bias I refer to is probably unavoidable and due to constraints inherent in the sampling process itself. For a bat to be collected in a mist net it needs to have the ability to fly. So what is the "health" status of bats not collected? I suspect that some bats were uncollected due to a variety of incapacitating physiological and/or neurological symptoms. It is clear that dead, dying, or moribund bats would never be discovered.

The idea of collection bias also ties in to the concern over the "sublethal" effects of pesticides. Agricultural pesticides often affect the physiology, behavior, or nutrition of an organism without causing overt or immediate death to an individual, yet decrease the reproductive fitness or chances of survival (Tome et al. 1995). For every bat collected

and analyzed and found to have evidence of exposure to agricultural insecticides, there may be many more undetected individuals with more serious impairments.

We suggest that data collected in this study could show a collection bias and, accordingly, should be interpreted carefully. Keeping these considerations in mind, we suggest that more emphasis be placed on the few collected individuals that show evidence of greater levels of chemical exposure. Given the lower probability of collecting the more seriously affected bats, there is reason to believe that they could be the "tip of the iceberg" (Mineau and Peakall 1987).

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CHAPTER III

TOXICITY OF PERMETHRIN TO LITTLE BROWN BATS (*MYOTIS LUCIFUGUS*): LOSS OF FLIGHT ABILITY AND MORTALITY

INTRODUCTION

The pyrethroids are among the most potent insecticides known. They constitute a major class of highly active synthetic organic chemicals derived from pyrethrin I, one of the 6 active components of pyrethrum, which is an extract from the dried flowerheads of the pyrethrum daisy, *Chrysanthemum cinerariaefolium* and *C. coccineum* (Smith and Stratton 1986). In the late 1960s and 1970s research efforts produced the first synthetic analogues with greater insecticidal activity and environmental stability than the parent compounds (Soderlund 1992).

Pyrethroids are much more effective against a wide spectrum of economically important pests than the organochlorine (OC), organophosphate (OP), and carbamate (CB) insecticides, yet they have a low oral toxicity to mammals and, in general, their insect (topical) to mammal (oral) toxicity ratio is much higher than that of the other classes of insecticides (Vijverberg and Bercken 1990). Favorable properties have promoted widespread agricultural and environmental health applications (Eriksson and Nordberg 1990). In the past 10 years, the major chemical companies have been marketing pyrethroids that have since gained an important position on the world market of insecticides, replacing many of the earlier compounds (Vijverberg and Bercken 1990).

Pyrethroids, like other major classes of insecticides (e.g., OCs, OPs, CBs) interfere with the function of the nervous system. Initial symptoms of poisoning include incoordination and locomotor instability (knockdown), followed by hyperexcitation, tremors, and convulsions. The precise mode of action of these insecticides involves an interaction with the sodium channel in nerve membranes (Vijverberg et al. 1982 and 1983). Their principle effect is to induce a continuous series of nerve impulses, referred to as repetitive activity, which completely upsets the proper functioning of the entire nervous system and eventually results in death (Smith and Stratton 1986).

In mammals 2 distinct toxic syndromes have been described. The T-syndrome is induced by pyrethrins and noncyano pyrethroids, e.g., permethrin, and this syndrome is named after the prominent symptom of whole-body tremors. The CS-syndrome, induced by deltamethrin and most other cyano pyrethroids, is characterized by choreoathetosis (ceaseless and involuntary body movements, where rapid/jerky and slow/sinuous movements predominate) and salivation. Some pyrethroids produce tremors and salivation, classified as the intermediate TS-syndrome (Vijverberg and Bercken 1990).

The announcement of the discovery of permethrin in 1973 provided the first evidence that sufficient photostability could be achieved in pyrethroids to permit their widespread use in agriculture. The development of these compounds was to produce non-persistent insecticides which possessed low mammalian toxicity and were safe to use on food and edible crops (Kumar 1984). According to the 1989 pesticide use survey, Missouri producers applied pyrethroid insecticides to approximately 190,000 acres or 26% of the treated acres in northern Missouri. By 1992, the application of pyrethroids had increased to 40%, equating to more than 208,000 treated acres (Becker et al. 1992). Permethrin, a synthetic pyrethroid marketed under the trade names

Ambush® and Pounce®, was the most commonly used corn insecticide in Missouri in 1992. The purported safety of these compounds, along with their desirable persistence in the field, indicates that this class of insecticides will become increasingly more important in agriculture and therefore increasingly present in the environment.

Concern about the loss of bat populations to insecticides, first stated by Mohr (1953), has stimulated toxicological research to quantify the sensitivity of bats to these chemicals (Clark 1988). Evaluation of the hazards of pesticides to any species begins with the development of the dose-effect relationship. In toxicology this relationship is expressed in the median lethal dose (LD_{50}) and is usually the first experiment performed on a new chemical. The LD_{50} is the statistically derived single dose of a substance that can be expected to cause death in 50% of the animals tested (Eaton and Klaassen 1996).

The classical LD_{50} test first introduced by Trevan (1927), though having wide acceptance as a general measure of acute toxicity, is not without criticism for ethical and scientific reasons (Zbinden 1986). US regulatory agencies, such as the FDA (Food and Drug Administration) and the EPA (Environmental Protection Agency), discourage the use of the classical LD_{50} test, and accept submission of data from alternative methods that use fewer animals (EPA 1988, FDA 1988). The 1985 European Chemical Industry Ecology and Toxicology Centre (ECETOC) report on acute oral toxicity took a similar position (ECETOC 1985). While it is generally accepted that the determination of a precise LD_{50} value is not necessary, it is widely agreed that evaluation of acute oral toxicity is still necessary to determine the toxic properties of a chemical (ECETOC 1985, EPA 1988, FDA 1988, Zbinden 1986).

Very limited information is available on the impact of pyrethroids on wild terrestrial vertebrates, even less on mammalian species. Most acute pyrethroid toxicity data for mammals are limited to studies with laboratory mice and rats (Smith and Stratton 1986). Though necessary to understand the behavior of these chemicals, much of the work with pyrethroids is basic research and deals with toxicokinetics (absorption, distribution, biotransformation, excretion) and residue recovery studies, oftentimes using radiolabeled compounds (Elliott et al. 1976, Gaughan et al. 1977, Marei et al. 1982).

Despite the ca 25 year history and wide use of pyrethroids in agriculture, the environmental risk and toxicity of this class of insecticides to bats have not been investigated. For these reasons and because the increased use of pyrethroid insecticides occurs coincident with the summer/maternity range of the Indiana bat (*Myotis sodalis*) (Easterla and Watkins 1969, LaVal and LaVal 1980, Clawson 1986, Smith and Fairchild 1990, Becker et al. 1992), research into the potentially harmful impacts of pyrethroids on the ecology of this declining species should be determined.

My objective was to determine the sensitivity, as measured by the 24-h oral LD₅₀, of the little brown bat to 2 different formulations of the synthetic pyrethroid insecticide permethrin, an agricultural field grade and an analytical reference standard grade. I also gathered data on how that the analytical grade of permethrin affected the flight capabilities of the little brown bat. Additionally, brain and carcass tissues were analyzed for permethrin residues to enable the future evaluation of these compounds recovered from wild bat populations. It is intended that these results will increase knowledge of the effects of pyrethroid insecticides on nontarget bat species, particularly the endangered congener of the little brown bat, the Indiana bat.

METHODS

This study was conducted at the Charles Green Wildlife Area near Columbia, Missouri. Adult little brown bats were captured with a double-frame Tuttle trap (Tuttle 1974) as they left their hibernaculum in Great Scott Cave located in Washington County, Missouri on July, 22, 1997. Bats collected at this time were dosed with the agricultural field-grade permethrin Pounce®. Little brown bats were again collected from Great Scott Cave on August 1, 1997 and were subsequently dosed with an analytical grade of permethrin.

The dosage levels initially selected for the analytical grade of permethrin to be administered to the bats collected on August 1 were later proved too low to produce a lethality distribution that would enable the estimation of an LD₅₀. Therefore, the LD₅₀ test was conducted a second time with bats collected August 8, 1997 and were dosed with higher levels of analytical-grade permethrin.

All little brown bats collected from Great Scott Cave in April, 1996 were found to have carcass permethrin concentrations below the limit of quantitation (LOQ) or not detected (ND). However, in April, 1997, a single northern long-eared bat collected from Great Scott Cave had a permethrin concentration of 52.5 parts per billion (ppb). Though the recovery of permethrin insecticides in bat tissues could indicate the use of these compounds within the feeding range of bats using Great Scott Cave as a hibernaculum, I believe that the influence of any prior exposure to permethrin would be minimal and have a negligible impact on the results of bats undergoing LD₅₀ tests.

Bats were captured the evening prior to testing and randomly placed into 1 of 5 galvanized wire mesh cages (30 x 25 x 50 cm), each cage corresponding to a common dosage level. Lighting was both natural and artificial and approximated 18-h light:6-h dark periods. Water only, and not food, was provided the first day of capture. Bats were weighed and hand fed Coleopteran mealworm larvae (*Tenebrio molitor*) on the second day.

Because of the difficulty of maintaining bats for an extended period in captivity (Clark 1986, Clark and Rattner 1987, Philip Jen, personal communication), LD₅₀ exposure tests were begun 48 hours after capture. Preliminary range-finding tests were conducted on July 16, 1997 to reduce the number of dosage levels and the number of animals required.

The field-grade formulation of permethrin (Pounce® 3.2 EC, 38.4%, cis 51: trans 49), obtained from FMC Corp. (Philadelphia, PA), was dissolved in distilled water. The analytical grade (99.4% w/w, cis 52:trans 48), obtained from Zeneca Agricultural Products (Richmond, CA), was dissolved in corn oil. A dosing procedure was conducted using 1 of 4 predetermined dose levels (i.e., 15, 45, 135, and 405 mg/kg body weight) selected on the basis of results of the range-finding study. The second LD₅₀ trial using analytical grade permethrin utilized higher levels of exposure (i.e., 135, 405, 1215, and 3645 mg/kg body weight).

Four animals were randomly assigned to 1 of 5 dose levels, including a control group. Field-grade control animals received only the Pounce® 3.2 EC solvent/emulsifier carrier, while analytical-grade dosed animals received only corn oil. The dose was administered by gastric gavage with a 1mL tuberculin syringe attached to a 7.5 cm

Sovereign® polypropylene catheter. Based on an estimated maximum gastric capacity, concentrations of both permethrin formulations were adjusted to deliver no more than 0.1 mL.

All bats were dosed at approximately 2200 CDT and observed for signs of intoxication or death for 4h and again at 18h and 24h. Upon death, all bats were immediately frozen for later chemical analysis. At 24h all surviving bats were tested for their ability to fly by releasing each individual from a height of 2 m and observing for active flight behavior (i.e., not passive gliding) for a distance of 5-10 m. Each bat was given 3 opportunities to demonstrate some capacity for active flight.

Surviving bats were euthanized 24h after dosing in accordance with accepted Animal Care and Use Committee (ACUC; University of Missouri, Columbia, Mo.) protocol. The physical method of cervical dislocation was determined to be the most appropriate for the specific circumstances of this study and is in agreement with the American Veterinary Medical Association's Panel on Euthanasia (1993).

The carcass was prepared by removing the wings at the shoulder and the feet at the ankle. Specimens were skinned to prevent the inclusion of insecticides that may have been on the skin or coat at the time of capture. Any fat adherent to the skin was removed and placed with the body (Thies and McBee 1994). The brain was removed and placed in labeled cryovials. Gastrointestinal tract tissue, and contents, from the distal esophagus to the rectum was removed and discarded. The term carcass refers to the entire body minus skin, wings, feet, gastrointestinal tract, and brain (Geluso et al. 1981).

All tissue samples (carcass and brain) were frozen and submitted to a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) approved laboratory, Analytical Biochemical Laboratories, Inc. (ABC Labs), in Columbia, Missouri. Analytical methodology generally followed those outlined in the Food and Drug Administration's (FDA) Pesticides Analytical Manual (PAM). Carcass and brain tissue for permethrin analysis were delivered frozen and identified by a code unique for each individual sample. Tissue samples from little brown bats collected on August 1, 1997 were not submitted for permethrin residue analysis.

The entire tissue sample was weighed into a 250 mL Nalgene bottle. Fortifications were made at this time. The sample was processed using a homogenizer (fitted with a blade) for 5-10 seconds. Methylene chloride (100 mL) and sodium sulfate (5x the sample weight) were added and the sample was blended for 3 minutes. The sample was vacuum filtered into a 250 mL flat-bottom flask through GF/A filter paper. The container and filter cake were rinsed with 2 x 25 mL portions of methylene chloride and these rinses were added to the 250 mL flat-bottom flask. The sample was rotary evaporated to dryness. The extract was quantitatively transferred to a 7 mL test tube with methylene chloride and blown to dryness with nitrogen. The residue was dissolved in 1 mL of 80% iso-octane/20% ethyl acetate if the sample matrix was brain, or 5 mL of 80% iso-octane/20% ethyl acetate if the sample matrix was carcass.

A Hewlett Packard Model 5890a Series II gas chromatograph (GC) with a mass selective detector was used to analyze the tissue samples for the insecticide permethrin. The column used during the analysis was a HP® -5ms (30 meters x 0.25 mm internal diameter, 0.25 μ m film thickness). The injector temperature was 250°C and the detector

temperature was 280°C. The carrier gas (He) flow rate was 0.8mL/min (constant flow). The volume of sample injected was 1µL.

The initial temperature of the GC oven was 150°C with a hold time of 1 minute. The temperature was ramped from 150 to 300°C at 25°C/minute, with a hold time of 3 minutes. A longer hold time (10 minutes) was used for sample sets. Select Ion Monitoring was chosen for compound quantitation. The ions selected were 163, 165, and 183. Quantitation was based on the sum of the selected ions.

The LOQ in carcass and brain tissue for both field-grade and analytical permethrin were 50 ppb. Recovery efficiency for the field-grade permethrin ranged from 70% to 82%, while recovery for the analytical-grade permethrin ranged from 103% to 127%. Residue data were not adjusted on the basis of these derived recoveries. All recovered permethrin was quantified by peak area and reported in µg/g (ppm) by weight of fresh (wet) tissue.

Median lethal dosages (LD_{50}) and median effective dosages (ED_{50} - an inability to fly in 50% of the treated bats) were estimated using the Spearman-Kärber method (Finney 1978). LD_{50} and ED_{50} dosages were also estimated using the probit method for purposes of comparison and statistical validation. The combined results from both LD_{50} experiments using the analytical grade of permethrin generated data that was used to estimate the ED_{50} dosage and provide a more valid assessment of time to death after permethrin administration.

RESULTS

Many of the little brown bats dosed with both the field- and analytical-grade permethrin formulations exhibited a similar pattern of clinical signs and progressive intoxication including hyperventilation, mild to severe muscular tremors, vocalization, and seizures (Table 1). The ability of a bat to right itself after being placed in a supine position (i.e., lying on its back) ranged from slightly impaired to, in some cases, total loss. Twenty-four hour survivors that had received the higher doses of permethrin were typically extremely weak and fatigued. Lower dosed individuals, particularly those bats exhibiting minimal neurological signs, displayed relatively normal flight and righting behaviors at 24h. Signs of toxicity observed in the field-grade exposed bats progressed more rapidly and preceded signs observed in analytical-grade bats by between 2 and 12 hours. Nearly all bats, particularly field-grade treated individuals, progressed to death once whole-body, generalized tremors were noted.

Table 2 summarizes mortality, flight ability, and brain and carcass concentrations of field- and analytical-grade permethrin dosed bats and relates these findings to dosage levels of both permethrin formulations. Spearman-Kärber LD_{50} for the field-grade product (Pounce® 3.2 EC) in little brown bats was estimated at 45 mg/kg with a 95% C.I. of 19 to 107 mg/kg. The probit method gave a similar LD_{50} of 38 mg/kg. FMC, the manufacturer of Pounce® 3.2 EC, permethrin field-grade insecticide, reported in its Material Safety Data Sheet (MSDS) an LD_{50} in rats of 1030 mg/kg, or approximately 25 times greater (less toxic) than my findings with the little brown bat. The Spearman-Kärber LD_{50} for the analytical-grade permethrin was considerably higher (less toxic) than

Table 1. Chronology of clinical signs in little brown bats dosed with field- and analytical grade permethrin.

Clinical Signs	Dose (mg/kg)	Time of Onset(h) ^a	
		Field-Grade	Analytical-Grade
Tremors	15	4.0	----
	45	0.40	----
	135	0.33	4.50
	405	1.25	4.0
	1215	----	NO
	3645	----	0.90
Seizures	15	NO	----
	45	3.50	----
	135	3.0	NO
	405	1.25	NO
	1215	----	16.0
	3645	----	0.90
Righting	15	3.0	----
Reflex ^b	45	1.5	----
	135	1.0	4.50
	405	1.25	24
	1215	----	23
	3645	----	NO
Vocalization	15	4.0	----
	45	1.5	----
	135	2.0	4.5
	405	2.0	18
	1215	----	NO
	3645	----	NO
Hyperventilation	15	NO	----
	45	NO	----
	135	NO	2.5
	405	NO	2.0
	1215	----	NO
	3645	----	NO
Weakness	15	10.0	----
	45	NO	----
	135	0.33	19
	405	9.0	24
	1215	----	18
	3645	----	16

^a Time when first noted after dosage in at least 1 bat.

^b Depressed or abolished.

NO = Not observed.

Table 2. Effects of orally administered field- and analytical-grade permethrin on mortality and flight ability in little brown bats.

Formulation	Dose (mg/kg)	Deaths/ Sample Size	Unable to fly/number survivors ^b	Mean Permethrin Concentration ^a			
				Survivors (24h)		Died (<24h)	
				Brain	Carcass	Brain	Carcass
Field Grade	Control	0/5	0/5	0	0.169	----	----
	15	1/4	1/3	0.148	2.40	0.713	2.06
	45	2/4	1/2	0.79	4.82	4.66	22.95
	135	4/4	----	----	----	5.95	73.73
	405	3/4	1/1	3.67	179.0	9.34	283.0
Analytical Grade	Control	0/4	0/4	1.765	0.572	----	----
	135	1/4	2/3	1.11	55.32	0.389	1.37
	405	1/4	2/3	12.99	228.13	5.02	34.6
	1215	3/4	1/1	1.77	15.2	144.19	464.0
	3645	4/4	----	----	----	9.42	392

^a Mean values expressed in parts per million (ppm).

^b 24-h survivors only.

the field-grade product and was estimated at 533 mg/kg with a 95% C.I. of 234 to 1215 mg/kg. The probit method gave a comparable estimated LD₅₀ of 502 mg/kg with a 95% C.I. of 66 to 1978 mg/kg. Rat LD₅₀s reported in the literature for analytical permethrin range from 500 to 1000 mg/kg (Litchfield 1985).

The dosage estimated by the Spearman-Kärber method that impaired flight in 50% of the analytical-permethrin dosed bats (ED₅₀) was 79 mg/kg with a 95% C.I. of 31 to 201 mg/kg. The probit method gave an estimated ED₅₀ value of 81 mg/kg. Both estimated ED₅₀ dosages are ca 16%, or 1/6th, of the estimated LD₅₀ dosage.

It is apparent from Tables 2 and 3 that the concentration of both permethrin formulations was considerably higher in carcass tissue (approximately 10x greater) than in brain tissue. When mean permethrin concentrations were considered across multiple dosage levels, and bats were subdivided between survival to 24h or death prior to 24h, the analytical grade was detected in higher concentrations than the field grade in both tissue types (Table 3). However, when tissue permethrin concentrations at 2 comparable dosage levels (i.e., 135 and 405 mg/kg) with both formulations were compared in bats that died prior to 24h, the field-grade product was detected in greater concentrations (Table 2).

It is also noteworthy that at the 135 and 405 mg/kg dosage levels, 2 of 8 (25%) bats died when dosed with the analytical-grade product while 7 of 8 (87.5%) died when dosed with the field-grade product (Table 2). A comparison of time to death between the 2 permethrin formulations shows that the field-grade product killed bats more quickly than the analytical grade (Figures 1 and 2). This observation is compatible with earlier

Table 3. Mean permethrin concentration in tissues of little brown bats.^a

	Survivors (24h)		Died (<24h)	
	Brain	Carcass	Brain	Carcass
Field Grade	0.95 $\pm$ 1.38 (6)	32.64 $\pm$ 71.78 (6)	6.19 $\pm$ 3.98 (10)	119.2 $\pm$ 116.8 (10)
Analytical Grade	6.29 $\pm$ 11.2 (7)	123.65 $\pm$ 142.7 (7)	52.85 $\pm$ 134.82 (9)	332.89 $\pm$ 308.13 (9)

^a Mean values expressed in parts per million (ppm) $\pm$ SD.

Number in parentheses () = number of bats per group and includes all appropriate dosage levels.

Figure 1. Temporal patterns in deaths of little brown bats during the 24h period dosing with analytical-grade permethrin.

Figure 2. Temporal patterns in deaths of little brown bats during the 24h period dosing with field-grade permethrin.

and more rapidly progressive clinical signs in field- as compared to analytical-grade dosed bats (Table1).

Orally administered permethrin dosages were related to brain and carcass concentrations for both insecticide formulations. Tissue concentrations with all 4 regression analysis combinations exhibited a positive dose-dependent relationship (Figures 3, 4, 5, and 6). The correlation of the field-grade formulation to tissue concentrations was found to be stronger when compared to the analytical grade for comparable tissues. Carcass tissue permethrin concentrations and field-grade dosage rates had a correlation coefficient of 0.970 (Figure 6).

And finally, brain and carcass tissue concentrations of both field- and analytical-grade permethrin were found to be positively correlated. As with the tissue-dosage relationship, the brain and carcass levels of field-grade permethrin were more closely correlated (Figure 8, $r = 0.711$) than with the analytical grade (Figure 7, $r = 0.420$).

DISCUSSION

The insecticide permethrin is a potent neurotoxin and upon uptake by mammals results in a pattern of toxicological features typified by increased sensitivity to external stimuli, restlessness, incoordination, enhanced startle response, whole-body tremor, and finally, prostration and death (Miyamoto 1976, Lawrence and Casida 1982, Crofton and Reiter 1984, Litchfield 1985). I observed the same general pattern of signs in little brown bats after permethrin administration, with the field-grade product eliciting toxic responses much earlier than the analytical grade. Solvent and carrier chemicals are present in most agricultural insecticides (e.g., Pounce® 3.2 EC) intended for field

Figure 3. Relationship of dosage (mg/kg) of analytical-grade permethrin to brain concentration (ppm) in LD₅₀ tests with little brown bats.

Figure 4. Relationship of dosage (mg/kg) of analytical-grade permethrin to carcass concentration (ppm) in LD₅₀ tests with little brown bats.

Figure 5. Relationship of dosage (mg/kg) of field-grade permethrin to brain concentration (ppm) in LD₅₀ tests with little brown bats.

Figure 6. Relationship of dosage (mg/kg) of field-grade permethrin to carcass concentration (ppm) in LD₅₀ tests with little brown bats.

Figure 7. Relationship of brain and carcass permethrin concentrations (ppm) in LD₅₀ tests with little brown bats when administering an analytical-grade formulation.

Figure 8. Relationship of brain and carcass permethrin concentrations (ppm) in LD₅₀ tests with little brown bats when administering a field-grade formulation.

application and, by themselves, are not particularly toxic in amounts bats would normally acquire during the routine agricultural use of these products.

The differences in toxic responses noted in this study between the field- and analytical-grades of permethrin are most likely due to the effect of the organic solvents in aiding gastrointestinal absorption and potentiating the toxicity of the primary active ingredient permethrin. The solvents/carriers (vehicle) present in Pounce® 3.2 EC, and most other field-applied liquid insecticides, are carefully researched chemicals employed by agrochemical manufacturers to improve the insecticides' efficacy against insect pest species by enhancing the penetration (i.e., toxicity) of the insecticide through the insect cuticle exoskeleton. In this study, the aromatic hydrocarbon solvents such as benzene and xylene present in Pounce® 3.2 EC apparently aided in the absorption of the insecticide permethrin in the little brown bat and resulted in an earlier and more rapid progression of neurological signs than with the insecticide administered without the solvents. Permethrin has a very low water solubility (i.e., lipophilic), and to have practical application must be dissolved in an appropriate solvent prior to agricultural use. The same vehicle can have a similar influence in both target pest insects and nontarget bat species.

At 135 mg/kg, a dosage we used for both insecticide formulations, brain permethrin levels were 15 times greater with the field-grade product than with analytical permethrin, and 54 times greater when comparing carcass concentrations (Table 2). These differences in permethrin levels support our observations of enhanced toxicity of permethrin in combination with organic solvents when compared to our results with the analytical grade of the same chemical in a laboratory setting.

The mammalian toxicity of the pyrethroids has become better understood since their initial introduction in the early 1970s. The predecessors of the modern synthetic pyrethroids are the active ingredients of pyrethrum which has been used since the last century for insect control. Because these were naturally derived products it was always assumed that they were inherently safe to use. Their very early introduction precluded the comprehensive toxicological assessment which has been required of new pesticides in the last 20 years. Their toxicological evaluation has been established slowly and is still not equivalent to present-day requirements for a new insecticide (Litchfield 1985).

As previously stated, tests of lethality with newly introduced chemicals provide some of the most basic information about the potential toxicological hazards such compounds may have to wild populations. Acute oral tests have attained general acceptance among environmental toxicologists as a general measure of relative toxicity in vertebrates because these tests are rapid, uncomplicated, and inexpensive, yet statistically reliable within the lethality curve and slope of dosage to death or recovery. Caution should be used, however, when extrapolating results from a relatively controlled set of circumstances to situations where more inherent variability may exist under natural conditions in the field.

Twenty-four hours after dosing 24 little brown bats with 4 dosage levels of the analytical grade of permethrin (i.e., 15, 45, 135, and 405 mg/kg body weight), 21 survivors were tested for their ability to actively fly for a distance of 5-10m. Although 18 of the 21 surviving bats were able to right themselves at 24h, only 10 were capable of active flight. Data combined from 2 LD₅₀ tests had sufficient sublethal dosages and allowed for the testing and discrimination of individuals at a lower toxic threshold.

This study showed that a dosage of 79 mg/kg (i.e., ED₅₀) incapacitated approximately 50% of the treated bats preventing them from active flight. Seventy-nine mg/kg was calculated to be about 16% of the lethal dosage in the same proportion (50%) of dosed individual bats. Clark (1986) found that ED₅₀ (righting ability) dosages with the big brown bat (*Eptesicus fuscus*) were one-third or less of the LD₅₀ for that species. Ten of the little brown bats in this study that were able to fly when tested at 24h, 18 were able to right themselves in tests of incoordination, suggesting that loss of flight ability is a more sensitive test, with the insecticide permethrin at least, and potentially more environmentally and ecologically relevant. Because nonvolant bats are unable to feed and maintain a regular intake of calories, thermoregulatory capacity and survivability quickly become compromised.

Behavioral aberrations in wildlife have been shown to be manifested at 1 or 2 orders of magnitude below lethal levels of environmental contaminants, and therefore can be regarded as sensitive toxic response indicators (Hoffman 1995). We believe that behavioral, sublethal tests can provide definable, interpretive endpoints that can be used to assess a level of impairment and integrate the effects of toxicants on wild populations within an ecological context.

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CHAPTER IV

TOXICITY OF PERMETHRIN TO LITTLE BROWN BATS (*MYOTIS LUCIFUGUS*): AVOIDANCE OF OBSTACLES

INTRODUCTION

Behavior consists of a series of observable activities that operate through the central nervous system and enable an animal and its progeny to exist in an environment beneficial to its survival (Little et al. 1993). Behavioral responses are critical to survival because they are necessary to perform essential life functions such as habitat selection, competition, predator avoidance, prey selection, and reproduction (Little et al. 1993). For example, changes in the ability of a bat to detect, pursue, capture, and consume insect prey will have a considerable influence on its growth and survival.

The rationale for behavioral toxicity studies consists of 3 main points. First, the behavior of an organism represents the final integrated result of a diversity of biochemical and physiological processes. Thus, a single behavioral parameter is generally more comprehensive than a physiological or biochemical parameter. Second, behavioral patterns are known to be highly sensitive to changes in the steady state of an organism. This sensitivity is one of the key values for its use in investigating sublethal intoxication. Last, behavioral measurements can usually be made without causing direct physical harm to the organism. Behavioral toxicity tests can, if correctly designed, be

used with other standard tests to add ecological relevancy to toxicant assessments (Peakall 1996).

In 1953, Donald R. Griffin (Griffin 1953) suggested that insectivorous bats use their high frequency sounds not only for avoiding obstacles, but also for locating and capturing flying insects. Since the early pioneering work of Griffin in the 1940s and 1950s, echolocation in bats is now appreciated as one of the most demanding adaptations of hearing to be found in any animal. Transforming the information carried by sounds into perceptual images depicting the location and identity of objects rapidly enough to control the decisions and reactions of a swiftly flying bat is a prodigious task for the auditory system to accomplish (Simmons et al. 1995). The ability of auditory function to achieve spatial imaging reflects the vital role of hearing in the lives of bats - for finding prey and perceiving obstacles to flight (Neuweiler 1990).

The toxicity of pyrethroids on the nervous system of mammals has been amply discussed elsewhere (Kumar 1984, Vijverberg et al. 1982, Smith and Stratton 1986, Vijverberg and Bercken 1990). What is less certain are the effects of the pyrethroid insecticides on the processes of echolocation in the bat. Carlton (1977) found that in rabbits cismethrin (a type I pyrethroid like permethrin) caused a lowering of the threshold of some sensory receptors and the appearance of spontaneous activity in some afferent fibers that were previously silent. Forshaw and Ray (1986) reported that cismethrin impaired the normal auditory response in rats at doses which facilitated the startle response (a motor response to an unexpected sudden auditory stimulus). Crofton and Reiter (1988) reported that exposure to Type I pyrethroids, permethrin included, resulted in increased acoustic startle response, suggesting that this process may reflect the operation of a neural pathway that modulates the startle response. Hijzen and

Slangen (1988) also concluded that Type I pyrethroids increase the amplitude of the startle reflex and is independent of the presence of overt signs of toxicity. How these findings relate to neurological echolocation mechanisms and physiology in the bat is unknown, but the suggestion is made that sensory receptors and/or auditory function is involved, thus making a potential connection with echolocation processes.

The widespread use of pyrethroids in northern Missouri encourages the potential exposure of Indiana bats (*Myotis sodalis*) to sublethal levels of these insecticides. Sublethal pyrethroid exposure may impair a bats' survival or reproductive success, while slight effects on coordination during flight could increase the risk of collisions. Less efficient prey capture (i.e., foraging success) could also endanger the survival of adults and any young they are nursing.

Numerous approaches to evaluate the toxicity of insecticides to mammals have been described (Clark 1978, Heinz et al. 1979, Tucker and Leitzke 1979, Grue et al. 1997). Appropriate examinations should always attempt to assess those parameters and characteristics which are relevant to that species' unique survival requirements. Insectivorous bats monitor their environment by emitting ultrasonic signals and listening to their echoes (Griffin 1958). The simplest and most direct way to evaluate this ability is by the obstacle-avoidance test in which a bat flies through an array of stationary lines and the number of hits and misses is recorded (Jen et al. 1980, Schnitzler and Henson 1980). In behavioral experiments involving wildlife, it is essential to present the animal with tasks which are relevant to situations encountered in its natural environment (Jen and McCarty 1978).

This study tested to what degree flying performance of little brown bats, as surrogates for the Indiana bat, was affected by low levels of the pyrethroid insecticide

permethrin. I measured little brown bats' ability to avoid collision with obstacles (obstacle avoidance) by flying through a tier of stationary lines placed in the flight path (Jen and McCarty 1978, Jen et al. 1980, Schnitzler and Henson 1980). Also, brain and carcass tissues were analyzed for permethrin residues to compare these compounds to levels recovered from wild bat populations. This study was designed to evaluate the influence of sublethal permethrin exposure on the flight capabilities and performance of the little brown bat, an experimental test surrogate for the Indiana bat.

METHODS

The study was conducted at the Charles Green Wildlife Area near Columbia, Missouri. A group of 20 to 25 little brown bats was captured at a time with a double-frame trap (Tuttle 1974) as they left their hibernaculum in Great Scott Cave (Washington County, Missouri) during the months of July and August, 1997. Prior to testing, bats were allowed to adjust to captivity for a period of 7-10 days. A necessary part of the adjustment process was an initial 2-3 days of hand feeding Coleopteran mealworm larvae (*Tenebrio molitor*) that trained bats to eat voluntarily while in captivity. All bats collected for obstacle-avoidance testing were dosed with an analytical grade of permethrin.

Bats that were chosen for testing demonstrated consistently good performance. Only those individuals that readily adapted to life in captivity, in that they ate mealworms well, flew freely, and avoided obstacles, were considered. Griffin et al. (1963) found that bats selected for obstacle-avoidance tests usually improved during the first few days of testing, and then remained at approximately peak performance for 2-3 weeks. Bats that

exhibited consistent behavior remained in the study. Uniformity of behavioral patterns minimized effects of unspecified and potentially influential variables (learning, age, gender) that could confound performance interpretation.

Two, or three, groups of bats consisting of 6 individuals per group (i.e., 6 dosage levels in a group) were tested during each testing period. A total of 42 bats were tested during the months of August and September, 1997 (i.e., 12 bats were started August 30 and September 6, and 18 were started September 13). Six bats, each uniquely identified and corresponding to a particular dose, were housed together in galvanized wire mesh cages (30 x 25 x 50 cm). Lighting was both natural and artificial and approximated 18-h light:6-h dark periods. Both water and mealworm larvae were offered free choice.

The dosage levels selected were based on results of lethality testing (LD_{50}) previously obtained during an earlier part of this study. I wanted a range of permethrin doses at which all exposure levels were less than the lowest determined lethal dose (i.e., 135 mg/kg). A range of sublethal exposures, correlating to expected changes in flight performance, was selected.

All little brown bats collected from Great Scott Cave in April, 1996 were found to have carcass permethrin concentrations below the limit of quantitation (LOQ) or not detected (ND). However, in April, 1997, a single northern long-eared bat collected from Great Scott Cave had a permethrin concentration of 52.5 parts per billion (ppb). Though the recovery of permethrin insecticides in bat tissues could indicate the use of these compounds within the feeding range of bats using Great Scott Cave as a hibernaculum. I believe that the influence of any prior exposure to permethrin would be minimal and have a negligible impact on the results of bats undergoing obstacle-avoidance testing.

The analytical grade (99.4 % w/w, cis 52:trans 48), obtained from Zeneca Agricultural Products (Richmond, CA), was dissolved in corn oil. A dosing procedure consisting of 1 of 5 predetermined dose levels (i.e., 10, 18, 32.4, 58.3, and 105 mg/kg body weight) was selected on the basis of results of LD₅₀ tests previously conducted in this study.

Seven animals were randomly assigned to 1 of 6 dose levels, including a control group. Control-group dosed animals received only corn oil. The dose was administered by gastric gavage with a 1mL tuberculin syringe attached to a 7.5 cm Sovereign® polypropylene catheter. Based on an estimated maximum gastric capacity, concentrations of the permethrin we adjusted to deliver no more than 0.1 mL.

Testing was conducted in a constructed enclosure approximately 11.6 m long 2.3 m wide, and 2.1 m high. A wooden frame containing 6 vertically strung nylon monofilament lines (1mm in diameter) was placed in the center of the enclosure. The distance between the lines was 30 cm. The total opening of this frame through which a bat flew was 1.68 m wide and 1.73 m high. All interior wooded surfaces of the room were covered with cheese cloth to minimize echoes and to provide a surface for attachment for tested bats. The exterior of the room was covered with sheet polypropylene to minimize air currents.

Attached to the end of each of the 6 monofilament lines was a piezoelectric crystal “preloaded” with 2 ounce (56.7gm) lead weights. When a line was touched by a bat flying between the vertical lines, a voltage was produced by the crystal as a result of the force of the impact on the line. The voltage was fed to an electric amplifier and threshold detection circuit. The output of the individual amplifier/detection circuits was

electronically combined so that when a threshold was exceeded (due to contact by a flying bat) on any one of the channels, an output lamp was activated. Thus, a hit of the line by a bat was determined by the activation of a small lamp observed by the experimenters standing on each side of the flight room. During testing a bat was made to fly through the nylon lines and the total number of passes and hits was recorded.

Prior to dosing with permethrin, obstacle-avoidance tests were conducted on all bats to establish pre-exposure (baseline) performance. Except for day one, all testing was conducted at approximately 2130 CDT. Immediately after baseline testing, bats were dosed with analytical-grade permethrin. Approximately 6 and 9 hours after pre-exposure testing, bats were again tested and their performance recorded. Bats were later tested at 24, 48, 72, and 96h for a total of 7 obstacle-avoidance performance tests.

At 96h all surviving bats were euthanized in accordance with accepted Animal Care and Use Committee (ACUC; University of Missouri, Columbia, Mo.) protocol. The physical method of cervical dislocation was determined to be the most appropriate for the specific circumstances of this study and is in agreement with the American Veterinary Medical Association's Panel on Euthanasia (1993). At the completion of the study, or if death occurred sooner, all bats were immediately frozen for later chemical analysis.

The carcass was prepared by removing the wings at the shoulder and the feet at the ankle. Specimens were skinned to prevent the inclusion of insecticides that may have been on the skin or coat at the time of capture. Any fat adherent to the skin was removed and placed with the body (Thies and McBee 1994). The brain was removed and placed in labeled cryovials. Gastrointestinal tract tissue, and contents, from the distal esophagus to the rectum was removed and discarded. The term carcass refers to

the entire body minus skin, wings, feet, gastrointestinal tract, and brain (Geluso et al. 1981).

All tissue samples (carcass and brain) were frozen and submitted to a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) approved laboratory, Analytical Biochemical Laboratories, Inc (ABC Labs), in Columbia, Missouri. Analytical methodology generally followed those outlined in the Food and Drug Administration's (FDA) Pesticides Analytical Manual (PAM). Carcass and brain tissue for permethrin analysis were delivered frozen and identified by a code unique for each individual sample.

The entire tissue sample was weighed into a 250 mL Nalgene bottle. Fortifications were made at this time. The sample was processed using a homogenizer (fitted with a blade) for 5-10 seconds. Methylene chloride (100 mL) and sodium sulfate (5x the sample weight) were added and the sample was blended for 3 minutes. The sample was vacuum filtered into a 250 mL flat-bottom flask through GF/A filter paper. The container and filter cake were rinsed with 2 x 25mL portions of methylene chloride and these rinses were added to the 250 mL flat-bottom flask. The sample was rotary evaporated to dryness. The extract was quantitatively transferred to a 7 mL test tube with methylene chloride and blown to dryness with nitrogen. The residue was dissolved in 1 mL of 80% iso-octane/20% ethyl acetate if the sample was brain, or 5 mL of 80% iso-octane/20% ethyl acetate if the sample was carcass.

A Hewlett Packard Model 5890a Series II gas chromatograph (GC) with a mass selective detector was used to analyze the tissue samples for the insecticide permethrin. The column used during the analysis was a HP® -5ms (30 meters x 0.25 mm internal

diameter, 0.25 μm film thickness). The carrier gas (He) flow rate was 0.8 mL/min (constant flow). The volume of the sample injected was 1 μL .

The initial temperature of the GC oven was 150°C with a hold time of 1 minute. The minutes. A longer hold time (10 minutes) was used for sample sets. Select Ion Monitoring was chosen for compound quantitation. The ions selected were 163, 165, and 183. Quantitation was based on the sum of the selected ions.

The LOQ in carcass and brain tissue for analytical-grade permethrin was 50 ppb. Recovery efficiency ranged from 10% to 78%. Residue data were not adjusted on the basis of these derived recoveries. All recovered permethrin was quantified by peak area and reported in $\mu\text{g/g}$ (ppm) by weight of fresh (wet) tissue.

This experiment has aspects of a repeated measures design in which an individual bat is used as both a control and as a treated subject. With an ANOVA approach significant relationships were examined between permethrin dosage and brain concentrations and flight performance. Flight performance was evaluated both as total number of passes and the percentage of hits during a test period.

RESULTS

Thirteen of a total of 42 (31%) treated little brown bats died prior to the completion of obstacle-avoidance testing. There was at least one dead specimen representing each dosage level, including the controls. With the doses used in this study no gross signs indicative of permethrin neurotoxicity (tremors, restlessness, enhanced startle response) were demonstrated in any of the 42 bats. Seven of the 13 bats died at 48h, 4 at 24h, and the remaining 2 at 72h. An inability to fly in at least one testing

session prior to death (minimum of 24h) was noted in 11 of 13 bats (85%). Two bats that died prior to 96h died suddenly at 24h and without previous signs. It was observed in only two specimens (control and 105 mg/kg) that a tested individual bat unable to fly did not die before the completion of the study at 96h.

Griffin et al. (1960) described 3 flight phases to describe flight behavior of tested bats. I observed similar patterns of flight in tested little brown bats. The *approach phase* represents when a bat was released at one end of the flight room and flew directly toward the monofilament line barrier. When the bat was very close to and finally negotiated the obstacles, it was in the *final phase*. The final phase was especially unique in some bats in that as they approached the obstacles, flight speed slowed and the course of flight became elevated. Bats appeared to “pause” or “float” in front of the barrier, pull in their wings, and then carefully pass between the lines. The *pass phase* is that period after a bat has passed beyond the obstacles (Jen and Kamada 1982).

Though the majority of bats flew consistently during each test period, a few individuals would circle in one-half of the flight room and required “encouragement” to pass through the obstacles. Encouragement took the form of hand and arm waving and finger snapping. In a few rare instances, an “uncooperative” bat would not pass between the obstacles, precluding any data collection on that particular test day. A few individuals were exceptionally fast, agile, and flew continuously until stopped after 60-70 passes. Data were not collected after 70 obstacle passes.

Figures 1 and 2 illustrate 2 different levels of correlation between orally administered analytical-grade permethrin and brain and carcass tissue permethrin concentrations. There was essentially no relationship between permethrin dosage and brain tissue levels ($P = 0.2375$; $r = 0.0055$). However, a degree of correlation was seen

Figure 1. Relationship of dosage (mg/kg) of analytical-grade permethrin to brain concentrations (ppm) in obstacle avoidance tests with little brown bats.

Figure 2. Relationship of dosage (mg/kg) of analytical-grade permethrin to carcass concentration (ppm) in obstacle avoidance tests with little brown bats.

between permethrin dosage and carcass concentration ($P = 0.0555$; $r = 0.3315$). As previously noted in this study (Chapter III, "Toxicity of Permethrin to Little Brown Bats (*Myotis sodalis*): Loss of Flight Ability and Mortality," Figures 3 and 4), carcass permethrin levels were much more responsive (correlated) to orally administered analytical-grade permethrin than were brain tissue permethrin levels. Carcass and brain tissue concentrations of permethrin when compared (Figure 3) were positively correlated and significant ($P = 0.0017$; $r = 0.5191$).

As originally proposed, correlations between analytical permethrin dosage and flight performance were examined. No statistically significant relationships between permethrin dosage and flight performance were found, as measured by either the total number of passes through the obstacles or the percentage of hits ($P > 0.05$).

Brain permethrin concentrations when compared to performance during obstacle-avoidance testing failed to show a significant relationship (Figure 4a). As brain permethrin concentrations increased, the total number of obstacle avoidance passes decreased for the treatment period ending at 96h, though not significantly ($P = 0.2766$; $r = 0.2263$).

I was also unable to find a significant relationship between flight performance as measured by the percentage of hits and brain permethrin concentration (Figure 4b: $P = 0.9067$; $r = 0.0775$). Figures 4a and 4b illustrate that a high proportion of brain permethrin levels were restricted to a narrow region between approximately 0.1 and 0.3ppm and with a high variability in total number of passes and percent hits, respectively. A similar relationship was seen when mean performance over all testing periods was compared to brain permethrin concentrations in which most permethrin brain concentrations were closely grouped and typically less than 1ppm (Figure 5).

Figure 3. Relationship of brain and carcass concentrations (ppm) in obstacle avoidance tests with little brown bats when administering an analytical-grade formulation.

Figures 4a and 4b. Relationship of brain permethrin concentrations (ppm) and flight performance as measured by the number of passes and percent hits at 96h in obstacle avoidance tests with little brown bats.

Figure 5. Relationship of brain permethrin concentrations (ppm) and flight performance as measured by mean percent hits measured over all testing periods in obstacle avoidance tests with little brown bats.

A visual comparison between Figures 6a and 6b suggests a similarity in the frequency distribution of percent hits in bats prior to permethrin treatment and at 96h. Over 50% of the bats in both groups contacted obstacle avoidance lines in 5 to 15% of the passes during a testing period.

DISCUSSION

Of the 700 living species of echolocating bats in the mammalian order Chiroptera, about 600 actively pursue prey, probably with partial or complete guidance by sonar (Simmons et al. 1979). The great majority of these bats are insectivorous and chase airborne insects when they forage for food. In laboratory studies on their pursuit of airborne targets, bats use echolocation for detecting, locating, identifying, and tracking prey to a successful capture (Griffin et al. 1965).

Insectivorous bats are found in arable regions of Missouri and consequently may be unintentionally exposed to pyrethroid insecticides applied to crops. As far as I am aware, there are no published data on the effects that such exposure may have on these animals. This study is the first to investigate the behavioral and performance effects of permethrin insecticide exposure in bats. The results suggest that exposure to permethrin at the dosages used in this study display a threshold capacity in causing impairment of flight performance as measured in obstacle-avoidance tests.

Data central to behavioral obstacle-avoidance testing is the determination of a bat's ability to avoid collisions with an object in space. During each testing session, the number of times a bat touches a vertical monofilament line is recorded and these are expressed as a percentage of the total number of passes.

Figures 6a and 6b. Relative frequency distribution of percent hits prior to and 96h after the administration of analytical-grade permethrin in obstacle avoidance tests with little brown bats.

An initial dose-response (i.e., dose-behavioral relationship) was sought with the oral administration of analytical-grade permethrin dissolved in corn oil. Although 8 bats with brain permethrin concentrations greater than 1ppm died or could not fly at 24h, no prior overt or gross neurotoxic responses were demonstrated in any of the treated little brown bats. Behavioral changes as measured by obstacle-avoidance testing (total number passes and % hits) were not found to be correlated to permethrin dosage levels. I believe that the high variability in individual performance and obstacle-avoidance skills observed in many of the testing sessions throughout the study contributed to a lack of correlation with dosage.

Orally administered chemicals are dependent upon consistency in gastrointestinal absorption for uniform effect. Factors influencing absorption (gastrointestinal blood flow, presence of foods, status of hepatic function, weight/obesity, age, general condition of subject) also likely contributed to a lack of predictability between oral permethrin dosage and results of obstacle-avoidance testing. Figures 1 and 2 support the same lack of a consistent relationship between orally administered permethrin and brain and carcass tissue concentrations.

In addition to the recovery of tissue toxicant burdens, a functional approach was made to relate permethrin to behavior by comparing permethrin levels recovered in brain tissue to obstacle-avoidance performance. Part of the data collection in obstacle-avoidance tests was recording the percent hits made by an individual during each testing period. It was thought that this variable may be responsive to orally administered permethrin. Starting at 24h, and continuing to completion of all tests at 96h, the mean percent hits for all tested bats increased. Admittedly, an increase in percent collisions with vertically strung monofilament nylon lines over time would be difficult to differentiate

from fatigue and/or other stresses related to captivity. The percent hits, if related to permethrin exposure, would probably be a better measure of physical endurance and stamina than a test of echolocation.

If exposure to permethrin, or brain tissue concentrations, are related to inactivity (i.e., fatigue, loss of endurance), such a state could have serious consequences for the survival of individuals such as bats, which have a high metabolic rate and need to feed regularly. For their size, insectivorous bats use expensive modes of transportation during hunting, a situation which, coupled with factors relating to body surface area and volume, may seriously impose on a balanced energy budget (Fenton 1982).

Analytical permethrin was administered orally and therefore gastrointestinal processes of absorption become critical in the distribution of these toxicants. Lipophilic compounds (e.g., permethrin) pass from the gastrointestinal tract to the liver through the portal blood flow for "first-pass" uptake of ingested toxic chemicals. Lipophilic compounds readily diffuse into liver cells. Fat and renal tissue are also known to accumulate pyrethroid compounds in high concentrations relative to other body tissues (Gaughan et al. 1977). Storage, temporary or long-term, of toxicants by tissues is a primary method to decrease the availability of poisons for their target sites. The protective defenses available after oral dosing such as absorption and redistribution are combined with the rapid rate of detoxifying metabolism to dissipate the inherent toxicity of the pyrethroid molecule (Litchfield 1985). Comparison of Figures 1 and 2 show how permethrin residues are higher in carcass, as compared to brain tissue, at equivalent doses. Based on results in other studies residence time is short-lived with permethrin residues being rapidly metabolized and essentially eliminated from the body by 4 days (Elliott et al. 1976, Gaughan et al. 1977).

At 96h all surviving bats were euthanized and brain tissue analyzed for permethrin residues. The level of performance of 25 bats as measured by the total number of passes or percentage of hits immediately prior to 96h was not significantly correlated to brain permethrin concentrations ($P = 0.2766$ and $P = 0.9067$, respectively).

Figures 4a/b and 5 show that 24 bats had brain permethrin levels less than 1ppm. I am unaware of any published literature reporting permethrin in bats and suspect that 1ppm in brain tissue is partially, at least, influenced by dosage level and/or duration of exposure. Administering higher permethrin levels and a wider variation in doses by 50-100% would possibly allow this insecticide greater access to brain tissue and produce chemical residues covering a broader range.

Nine bats had a brain permethrin concentration greater than 1ppm, indicating that the dosages selected in this study allowed permethrin entry into the brain at higher levels. What is noteworthy is that only 2 of these 9 bats were alive at 96h, and only 1 had the ability to fly. I believe that brain concentrations greater than 1-3ppm suggests a threshold of significant neurotoxicity in little brown bats. Tissue concentrations greater than this threshold kills or incapacitates bats, whereas lower tissue levels appear to be relatively innocuous. At concentrations less than 1ppm high variability in flight performance becomes evident (Figures 4a/b and 5) and is suspected to have no detectable relationship to brain permethrin concentrations. With a large sample size a normal distribution of flight behaviors would probably be seen as suggested by the relative frequency distribution of percentage of hits in Figure 6a.

CONCLUSIONS:

- Protective absorption, storage, and detoxification mechanisms minimize permethrin residue accumulation in the brain.
- A relatively discrete neurotoxic threshold exists for the residues of permethrin in brain tissue of little brown bats.
- Low brain permethrin concentrations (less than threshold) are not overtly toxic and obstacle-avoidance testing bats under these conditions, with an adequate sample size, will likely demonstrate a normal distribution of flight capabilities.
- Recommend the design and development of more subtle and discriminating tests of echolocation with the pyrethroid insecticides to identify potentially sublethal exposure effects on changes in auditory function.

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APPENDIX

Table A1. County size and total and percent insecticide treated acres by region in northern Missouri 1992.

Northwest Region				North Central Region				Northeast Region			
County	Area ^a	Treated ^b	Percent	County	Area	Treated	Percent	County	Area	Treated	Percent
Andrew	278,534	20,542	7.38	Adair	363,342	7,167	1.97	Audrain	443,748	31,755	7.16
Atchison	348,636	51,568	14.79	Carroll	444,535	17,024	3.83	Clark	324,699	19,005	5.85
Buchanan	262,247	27,197	10.37	Chariton	483,784	36,094	7.46	Knox	323,668	16,289	5.03
Caldwell	274,801	7,934	2.89	Grundy	278,945	14,580	5.23	Lewis	323,233	20,055	6.20
Clay	253,733	5,661	2.23	Linn	397,049	10,447	2.63	Marion	280,381	16,515	5.89
Clinton	268,026	10,138	3.78	Livingston	342,169	11,645	3.40	Monroe	413,454	22,215	5.37
Daviess	362,882	10,514	2.90	Macon	514,440	20,290	3.94	Pike	430,673	30,709	7.13
De Kalb	271,493	8,454	3.11	Mercer	290,854	7,481	2.57	Ralls	301,456	10,658	3.54
Gentry	314,594	17,963	5.71	Putnam	331,491	14,106	4.26	Scotland	280,640	19,059	6.79
Harrison	464,121	17,860	3.85	Randolph	308,749	11,388	3.69	Shelby	320,582	13,025	4.06
Holt	295,617	28,651	9.69	Schuyler	197,054	5,661	2.87				
Nodaway	561,075	36,004	6.42	Sullivan	416,642	8,215	1.97				
Platte	269,037	15,418	5.73								
Ray	364,486	10,021	2.75								
Worth	170,584	5,671	3.32								
MEAN	317324	18240	5.75		364088	13675	3.76		344253	19929	5.79
Total	4,759,866	273,596			4,369,054	164,098			3,422,534	199,285	

Source: U.S. Department of Commerce 1994, Census of Agriculture.

^a Approximate land area in acres^b Total insecticide treated acres by county**Bold:** County mist-net sites 1996 field season

Table B1. OC and pyrethroid residues^a in carcasses of little brown bats collected from cave sites in southern Missouri.

ORGANOCHLORINES											PYRETHROIDS			
CAVE SITES	Carcass Weight (g)	Oxychlordan	Heptachlor Epoxide	o,p'-DDE	p,p'-DDE	Dieldrin	o,p'-DDD	o,p'-DDT	p,p'-DDD	p,p'-DDT	Tefluthrin	Cyhalothrin	Permethrin	Esfenvalerate
SCOTIA HOLLOW														
C.101 LBAM	4.52	145.5	123.1	1844.3	994.3	55.1	ND	10373.6	3708.2	ND	ND	ND	2547	ND
C.102 LBAF	3.98	136.6	ND	72.7	535.9	64.6	ND	793.4	260.3	ND	ND	ND	ND	ND
C.103 LBAF	3.64	376.2	49.6	66.2	393.0	346.0	ND	ND	ND	54.3	ND	ND	ND	141.1
C.104 LBAM	3.52	37.4	ND	ND	426.9	49.9	ND	ND	ND	ND	ND	ND	ND	ND
C.105 LBAM	3.32	61.6	14.5	51.5	842.7	61.4	ND	127.2	106.2	ND	ND	ND	54.6	ND
C.106 LBAM	3.92	13.5	ND	ND	286.2	28.8	ND	147.3	31.8	ND	ND	ND	101.7	ND
GREAT SCOTT														
C.201 LBAM	3.24	20.7	<10.0	ND	508.9	60.8	ND	93.3	ND	ND	ND	ND	<50.0	ND
C.202 LBAM	3.74	611.7	85.5	168.3	5116.0	968.8	ND	1157.9	600.7	1302.2	ND	ND	ND	ND
C.203 LBAF	3.85	29.4	19.6	ND	610.6	81.2	ND	ND	ND	ND	ND	ND	ND	ND
C.204 LBAF	3.42	68.5	25.3	ND	494.2	147.5	ND	ND	ND	ND	ND	ND	<50.0	ND
C.205 LBAM	3.58	672.2	38.6	ND	2796.7	943.8	ND	ND	317.4	ND	ND	ND	ND	ND
C.206 LBAM	3.34	308.6	179.4	26.6	1263.7	2658.9	ND	192.1	48.8	25.1	ND	ND	ND	251.8
ONYX														
C.301 LBAM	4.18	74.8	31.9	68.5	833.1	68.4	ND	279.9	128.7	ND	ND	ND	130.3	ND
C.302 LBAM	3.32	14.0	ND	ND	3301.0	ND	ND	109.3	ND	ND	ND	ND	255.8	ND
C.303 LBAM	3.70	460.8	58.8	156.3	2376.8	647.3	ND	ND	ND	ND	ND	ND	891.9	ND
C.304 LBAM	3.67	161.2	282.7	449.6	708.0	36.0	ND	ND	ND	ND	ND	ND	388.7	ND
C.305 LBAM	3.14	154.1	21.6	102.6	1109.5	147.7	ND	439.7	290.0	135.1	<10.0	ND	243.7	ND
C.306 LBAM	3.82	18.9	ND	45.5	624.6	46.5	ND	157.9	44.7	22.9	ND	ND	82.1	ND

^a Results are reported in ng/g (ppb) by weight of fresh (wet) tissue. NS = Not Detected

The Limit of Quantitation (LOQ) for all analytes was 10ppb except for permethrin and esfenvalerate which was 50ppb.

Residues less than the LOQ or ND are reported and entered into computations as one-half of the observed LOQ for that compound.

Table B2. OC and pyrethroid residues^a in carcasses of little brown bats collected from northern Missouri farm sites.

		ORGANOCHLORINES									PYRETHROIDS			
NORTHERN MISSOURI FARM SITES	Carcass Weight (g)	Oxychlordane	Heptachlor Epoxide	o,p'-DDE	p,p'-DDE	Dieldrin	o,p'-DDD	o,p'-DDT	p,p'-DDD	p,p'-DDT	Tefluthrin	Cyhalothrin	Permethrin	Esfenvalerate
MONROE														
T.201 LBAFPG	6.61	57.6	39.5	ND	273.0	150.4	ND	52.7	58.2	23.8	ND	ND	ND	ND
T.203 LBAM	4.08	217.6	88.8	29.4	764.4	628.3	ND	ND	ND	42.1	<10.0	ND	ND	ND
KNOX I														
T.601 LBJF	3.39	82.0	76.7	ND	442.0	457.0	ND	ND	49.0	32.2	<10.0	ND	ND	ND
T.602 LBJM	3.32	207.5	100.0	ND	641.7	535.2	ND	73.7	85.1	47.9	ND	ND	ND	ND
T.603 LBJM	3.59	308.1	155.6	ND	614.8	653.3	ND	119.6	153.3	57.3	ND	ND	ND	ND
T.604 LBJM	3.58	126.3	73.1	ND	555.0	310.0	ND	74.1	65.6	28.0	ND	ND	<50.0	ND
T.605 LBJF	3.15	200.9	136.5	ND	645.4	489.6	ND	ND	111.6	56.5	ND	ND	ND	ND
T.606 LBJM	3.33	102.6	86.4	ND	874.2	312.5	ND	123.0	113.8	ND	ND	ND	ND	ND
T.607 LBAF	4.34	<10.0	<10.0	ND	162.9	41.9	ND	31.1	ND	ND	<10.0	ND	ND	ND
T.608 LBAF	4.40	<10.0	<10.0	ND	92.0	44.5	ND	22.2	16.2	ND	ND	ND	ND	ND
SCOTLAND I														
T.701 LBJM	3.30	641.2	311.8	ND	981.3	1039.4	ND	137.5	210.4	80.5	ND	ND	ND	ND
T.702 LBJF	4.59	16.9	13.7	ND	155.5	83.9	ND	15.8	14.1	<10.0	ND	ND	ND	<50.0
T.703 LBLF	4.66	45.2	23.7	ND	190.0	288.5	ND	ND	26.4	ND	ND	ND	ND	ND
T.704 LBJF	3.71	162.9	109.5	17.3	754.3	1264.7	ND	73.1	98.0	36.3	<10.0	ND	<50.0	ND
T.707 LBAM	4.16	242.4	84.3	ND	779.6	1635.3	ND	165.0	139.0	128.6	ND	ND	ND	ND
T.708 LBAF	4.73	66.1	26.9	ND	288.7	168.5	ND	46.0	36.6	13.5	ND	ND	ND	ND
KNOX II														
T.1401 LBJF	5.21	266.8	222.6	ND	533.1	1036.2	ND	ND	ND	33.6	ND	ND	ND	<50.0
T.1402 LBJF	4.55	516.1	145.9	63.5	982.0	998.5	ND	ND	167.7	70.6	ND	ND	ND	ND
T.1403 LBJF	4.05	106.4	80.8	ND	577.8	345.4	ND	ND	ND	ND	ND	ND	ND	ND
T.1404 LBJF	4.32	109.9	115.8	ND	ND	477.0	ND	ND	ND	ND	ND	ND	ND	ND
T.1405 LBAF	4.90	54.0	46.3	ND	ND	265.5	ND	ND	ND	<10.0	ND	ND	ND	ND
T.1406 LBJF	4.98	156.0	84.0	ND	562.6	393.8	ND	ND	100.4	73.0	ND	ND	<50.0	ND

Table B2. (Continued)

SCOTLAND II														
T.1501 LBJF	4.13	106.4	54.0	10.4	451.8	247.2	ND	43.4	33.5	11.1	ND	ND	ND	ND
T.1502 LBJF	4.23	109.9	120.0	ND	1161.3	677.9	ND	ND	ND	25.8	ND	ND	ND	ND
T.1503 LBJF	3.10	54.0	40.2	ND	422.6	379.8	ND	35.5	35.0	12.3	ND	ND	ND	ND
T.1504 LBAF	4.70	156.0	35.1	ND	252.8	251.9	ND	ND	ND	14.0	ND	ND	ND	ND
SULLIVAN														
T.1603 LBAM	4.63	93.9	13.0	<10.0	415.0	78.3	ND	30.3	17.1	60.6	ND	ND	ND	ND

^a Results are reported in ng/g (ppb) by weight of fresh (wet) tissue. NS = Not Detected

The Limit of Quantitation (LOQ) for all analytes was 10ppb except for permethrin and esfenvalerate which was 50ppb.

Residues less than the LOQ or ND are reported and entered into computations as one-half of the observed LOQ for that compound.

Table C1. OC and pyrethroid residues^a in carcasses of northern long-eared bats collected from cave sites in southern Missouri.

		ORGANOCHLORINES									PYRETHROIDS			
CAVE SITES	Carcass Weight (g)	Oxychlordan	Heptachlor Epoxide	o,p'-DDE	p,p'-DDE	Dieldrin	o,p'-DDD	o,p'-DDT	p,p'-DDD	p,p'-DDT	Tefluthrin	Cyhalothrin	Permethrin	Esfenvalerate
PILOT KNOB														
C.401KBAM	3.12	20.3	<10.0	<10.0	120.2	46.3	ND	ND	<10.0	ND	ND	ND	<50.0	ND
C.402KBAF	3.23	<10.0	<10.0	ND	41.2	<10.0	ND	<10.0	ND	<10.0	ND	ND	ND	140.4
C.403KBAM	3.20	16.2	<10.0	ND	1033.1	25.9	ND	ND	<10.0	<10.0	ND	ND	ND	89.8
C.404KBAM	3.34	22.3	<10.0	15.0	116.8	28.2	ND	26.5	11.0	<10.0	ND	ND	ND	ND
C.405KBAM	3.65	16.1	<10.0	ND	131.1	46.2	ND	ND	ND	ND	ND	ND	ND	86.1
C.406KBAF	3.11	45.8	<10.0	ND	ND	31.8	ND	ND	ND	ND	ND	<10.0	ND	ND
C.407KBAF	3.34	<10.0	<10.0	ND	89.2	<10.0	ND	10.6	<10.0	<10.0	ND	ND	ND	<50.0
C.408KBAM	2.83	<10.0	<10.0	ND	112.2	11.5	ND	ND	ND	ND	ND	ND	ND	ND
C.409KBAF	3.48	18.9	<10.0	<10.0	173.1	35.9	ND	ND	ND	21.7	<10.0	ND	<50.0	ND
GREAT SCOTT														
C.501KBAM	2.50	16.5	<10.0	ND	89.3	13.2	ND	ND	ND	ND	ND	ND	ND	ND
C.502KBAM	3.10	10.0	ND	ND	71.4	<10.0	ND	ND	ND	ND	ND	ND	ND	ND
C.503KBAM	2.73	<10.0	ND	ND	77.7	ND	ND	ND	ND	ND	ND	ND	ND	ND
C.504KBAM	2.76	12.6	<10.0	ND	27.3	<10.0	ND	37.1	ND	ND	ND	ND	ND	ND
C.505KBAM	2.96	10.5	<10.0	ND	59.2	12.9	ND	14.5	ND	ND	ND	ND	ND	ND
C.506KBAF	2.84	20.0	<10.0	ND	71.2	19.5	ND	ND	ND	ND	ND	ND	ND	ND
C.507KBAF	2.83	10.6	<10.0	ND	77.8	<10.0	ND	ND	ND	ND	ND	ND	ND	ND
C.510KBAM	2.73	11.6	<10.0	ND	92.6	<10.0	ND	ND	ND	ND	ND	ND	ND	ND
C.511KBAM	2.95	12.5	<10.0	ND	89.9	16.8	ND	ND	ND	ND	ND	ND	52.6	411.1

^a Results are reported in ng/g (ppb) by weight of fresh (wet) tissue. NS = Not Detected

The Limit of Quantitation (LOQ) for all analytes was 10ppb except for permethrin and esfenvalerate which was 50ppb.

Residues less than the LOQ or ND are reported and entered into computations as one-half of the observed LOQ for that compound.

Table C2. OC and pyrethroid residues^a in carcasses of northern long-eared bats collected from northern Missouri farm sites.

NORTHERN MISSOURI FARM SITES														
ORGANOCHLORINES														
PYRETHROIDS														
MONROE														
MACON														
PIKE														
SCOTLAND														
SCHUYLER														
PUTNAM														
T.202 KBAM														
T.204 KBAFPG														
T.205 KBAFPG														
T.301 KBAF														
T.401 KBAM														
T.402 KBJM														
T.403 KBJM														
T.705 KBAF														
T.706 KBAF														
T.1001 KBJF														
T.1002 KBJM														
T.1101 KBAM														
T.1102 KBAF														

Table C2. (Continued)

SULLIVAN															
T.1601 KBAM	3.45	31.3	14.6	34.8	167.3	70.0	ND	36.9	<10.0	<10.0		ND	ND	ND	ND
T.1602 KBAF	3.45	<10.0	<10.0	ND	ND	26.3	ND	ND	ND	<10.0		ND	ND	ND	ND

^a Results are reported in ng/g (ppb) by weight of fresh (wet) tissue. NS = Not Detected

The Limit of Quantitation (LOQ) for all analytes was 10ppb except for permethrin and esfenvalerate which was 50ppb.

Residues less than the LOQ or ND are reported and entered into computations as one-half of the observed LOQ for that compound.

Table D1. Brain muscarinic cholinergic receptor density^a (B_{max}) and affinity^b (K_d), and brain and plasma cholinesterase activity^c (ChE) in little brown bats collected from cave sites in the Ozark Plateau region of southern Missouri.^d

CAVE SITES	Bmax(fM)	Kd(pM)	BRAIN ChE	PLASMA ChE
SCOTIA HOLLOW				
C.101 LBAM	326	51	11.60	6.21
C.102 LBAF	426	53	12.75	3.64
C.103 LBAF	268	53	10.38	6.66
C.104 LBAM	316	48	14.54	12.34
C.105 LBAM	349	60	8.01**	7.35
C.106 LBAM	448	61	12.42	7.03
GREAT SCOTT				
C.201 LBAM	269	36	12.91	18.71
C.202 LBAM	302	38	12.42	4.21
C.203 LBAF	262	47	7.92**	8.58
C.204 LBAF	294	37	12.01	6.66
C.205 LBAM	327	59	10.84	12.66
C.206 LBAM	310	52	9.53	5.07
ONYX				
C.301 LBAM	301	43	12.01	5.68
C.302 LBAM	348	60	14.38	8.91
C.303 LBAM	337	46	11.36	8.58
C.304 LBAM	310	36	11.03	8.37
C.305 LBAM	381	45	8.99***	6.00
C.306 LBAM	394	51	9.48	8.58

^a Expressed as [³H] NMS binding (fmol /mg protein).

^b Units in pM.

^c Micromoles AThCh hydrolyzed/min/g brain or mL plasma, wet weight.

^d Identification code unique for each specimen listed below cave sites.

LB = Little brown bat; AM = Adult Male; AF = Adult Female.

* Sign. @ P < 0.05 ** Sign. @ P < 0.10 *** > 20% inhibition.

Table D2. Brain muscarinic cholinergic receptor density^a (B_{max}) and affinity^b (K_d), and brain and plasma cholinesterase activity^c (ChE) in little brown bats collected from northern Missouri farm sites.^d

NORTHERN MISSOURI FARM SITES	Bmax(fM)	Kd(pM)	BRAIN ChE	PLASMA ChE
MONROE				
T.201 LBAFPG	376	51	11.60	Missing
T.203 LBAM	364	39	8.99***	Missing
KNOX I				
T.601 LBJF	281	63	9.40	Unidentifiable
T.602 LBJM	240*	54	9.07	Missing
T.603 LBJM	260**	60	7.59*	Missing
T.604 LBJM	273	52	7.6*	7.68
T.605 LBJF	348	52	8.66**	Missing
T.606 LBJM	305	76	7.76*	6.86
T.607 LBAF	330	48	8.33**	Missing
T.608 LBAF	389	63	13.32	Missing
SCOTLAND I				
T.701 LBJM	249**	52	8.50**	Missing
T.702 LBJF	209*	54	9.23	20.59
T.703 LBLF	261**	57	12.26	19.61
T.704 LBJF	242**	53	6.64*	7.92
T.707 LBAM	218*	47	8.25**	11.19
T.708 LBAF	290	41	12.99	19.12
KNOX II				
T.1401 LBJF	331	43	9.07	16.67
T.1402 LBJF	376	47	9.29	14.30
T.1403 LBJF	393	46	13.24	15.69
T.1404 LBJF	240*	46	11.17	10.46
T.1405 LBAF	212*	54	10.78	14.22
T.1406 LBJF	392	46	7.57*	Missing
SCOTLAND II				
T.1501 LBJF	333	53	8.99***	12.01
T.1502 LBJF	281	73	11.11	14.71
T.1503 LBJF	394	55	8.99***	12.66
T.1504 LBAF	312	42	12.01	10.62
SULLIVAN				
T.1603 LBAM	276	32*	14.54	19.12

^a Expressed as [³H] NMS binding (fmol /mg protein).

^b Units in pM.

^c Micromoles AThCh hydrolyzed/min/g brain or mL plasma, wet weight.

^d Identification code unique for each specimen listed below farm sites.

LB = Little brown bat; AM = Adult Male; AF = Adult Female; JM = Juvenile Male; JM = Juvenile Female; PG = Pregnant.

* Sign. @ P < 0.05 **Sign. @ P < 0.10 *** > 20% inhibition.

Table E1. Brain muscarinic cholinergic receptor density^a (B_{max}) and affinity^b (K_d), and brain and plasma cholinesterase activity^c (ChE) in northern long-eared bats collected from cave sites in the Ozark Plateau region of southern Missouri.^d

CAVE SITES	Bmax(fM)	Kd(pM)	BRAIN ChE	PLASMA ChE
PILOT KNOB				
C.401NLBAM	344	40	10.21	2.36
C.402NLBAM	336	57	12.75	1.35
C.403NLBAM	371	47	11.19	2.37
C.404NLBAM	289	54	11.36	0.8
C.405NLBAM	325	54	12.42	1.23
C.406NLBAF	324	57	12.58	Missing
C.407NLBAF	309	56	10.95	2.61
C.408KNLBM	365	52	13.15	0.84
C.409NLBAF	308	54	9.23**	3.35
GREAT SCOTT				
C.501NLBAM	319	71	13.15	Not Sampled
C.502NLBAM	452	64	10.95	0.53
C.503NLBAM	324	62	10.29	2.21
C.504NLBAM	301	47	12.99	Not Sampled
C.505NLBAM	379	103	9.97	0.82
C.506NLBAF	242*	42	12.09	Inadequate Amt.
C.507NLBAF	384	41	14.62	Inadequate Amt.
C.510NLBAM	291	47	8.58*	Inadequate Amt.
C.511NLBAM	373	52	10.87	Inadequate Amt.

^a Expressed as [³H] NMS binding (fmol /mg protein).

^b Units in pM.

^c Micromoles AThCh hydrolyzed/min/g brain or mL plasma, wet weight.

^d Identification code unique for each specimen listed below cave sites.

NLB = Northern long-eared bat; AM = Adult Male; AF = Adult Female.

*Sign. @ P < 0.05 **Sign. @ P < 0.10 *** > 20% inhibition.

Table E2. Brain muscarinic cholinergic receptor density^a (B_{max}) and affinity^b (K_d), and brain and plasma cholinesterase activity^c (ChE) in northern long-eared bats collected from the summer maternity range of the Indiana bat.^d

NORTHERN MISSOURI FARM SITES	Bmax(fM)	Kd(pM)	BRAIN ChE	PLASMA ChE
MONROE				
T.202 KBNLB	47	301	8.66*	Inadequate Amt.
T.204 KBNLBPG	71	351	11.71	13.64
T.205 NLBAFPG	41	274	11.11	Mising
MACON				
T.301 NLBAF	37	322	12.34	13.24
PIKE				
T.401 NLBAM	60	305	7.03*	4.09
T.402 NLBJM	39	291	13.64	10.78
T.403 NLBJM	50	384	11.66	8.58
SCOTLAND I				
T.705 NLBAF	45	404	9.97	9.15
T.706 NLBAF	65	313	13.24	4.09
SCHUYLER				
T.1001 NLBJF	46	336	8.09*	1.47
T.1002 NLBJM	75	265**	7.57*	4.00
PUTNAM				
T.1101 NLBAM	53	308	10.38	2.61
T.1102 NLBAF	38	295	15.36	1.72
SULLIVAN				
T.1601 NLBAM	28*	279	9.31**	2.57
T.1602 NLBAF	41	308	10.95	3.35

^a Expressed as [³H] NMS binding (fmol /mg protein).

^b Units in pM.

^c Micromoles AThCh hydrolyzed/min/g brain or mL plasma, wet weight.

^d Identification code unique for each specimen listed below field sites.

NLB = Northern long-eared bat; AM = Adult Male; AF = Adult Female;

JM = Juvenile Male; JF = Juvenile Female; PG = Pregnant.

*Sign. @ P < 0.05 **Sign. @ P < 0.10 *** > 20% inhibition.

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