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AVAILABILITY OF ORGANOCHLORIDES AND ACCUMULATION IN POPULATIONS OF Tadarida brasiliensis (Free-Tailed Bat) AND Eptesicus fuscus (BIG BROWN BAT)

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The College of Science

The Graduate Program in Environmental Science

Availability of Organochlorides and Accumulation in Populations of *Tadarida brasiliensis* (Free-Tailed Bat) and *Eptesicus fuscus* (Big Brown Bat)

A Thesis in
Environmental Science
by
Michelle Lee Smith

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

August 2002

I have submitted this thesis in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Organochlorides were historically effective in controlling a wide range of pests. However, organochlorides have proved to present many problems to the health and welfare of other organisms. Due to these problems, especially declines in wildlife populations, organochlorides were restricted in the 1970's and a majority of them were banned in the mid to late 1980's. The effects of organochlorides on bats such as *Tadarida brasiliensis* and *Eptesicus fuscus* have been intensively studied. The effects can range from problems with reproduction to mortality. My analysis of populations of *T. brasiliensis* and *E. fuscus* showed that organochlorides are still present in the environment, although in trace amounts. A wide range of organochlorides was found in the livers. When *T. brasiliensis* and *E. fuscus* were compared using a Wilcoxon signed-rank test, no significant difference in the types of organochlorides was found between the species. Also, a significant difference was found between juvenile and adult *T. brasiliensis* using the Wilcoxon signed-rank test.

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Acknowledgements

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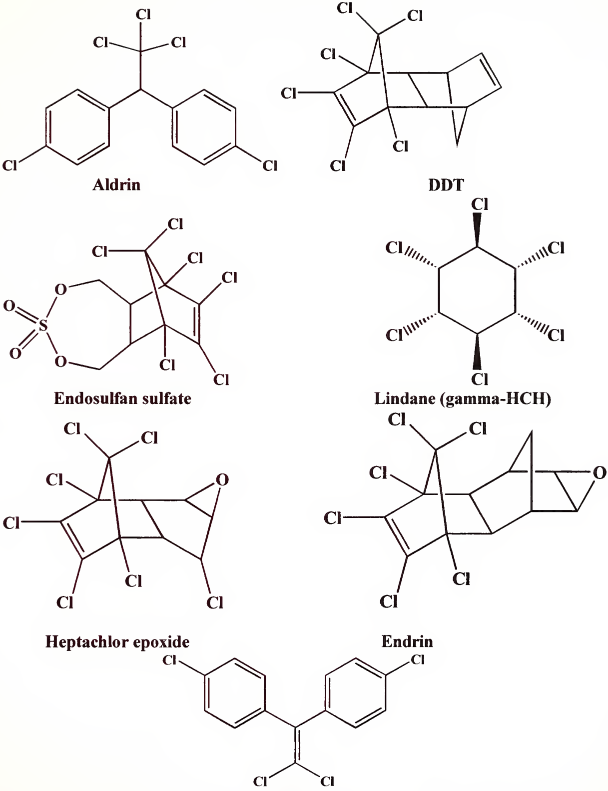
Introduction

In 1962, Rachel Carson made the public aware of the dangers of organochlorides such as 1,1,1-trichloro-2, 2-bis (p-chlorophenyl) ethane (DDT) in the environment. With her book, *Silent Spring*, she helped to change the public view of pesticides and their detrimental effects on wildlife (Carson 1962).

Organochlorides (Figure 1) are hydrocarbon compounds with varying numbers of chlorine atoms (Manahan 2000). Historically, organochlorides such as DDT, dieldrin, and others have been thought to cause enormous environmental problems. Since these compounds are fat-soluble, when consumed, they accumulate in high fat regions such as the brain, liver and reproductive organs. When organisms are exposed to these pesticides that occur at low levels for prolonged periods of time, pesticides accumulate in their organs and the toxicity of the pesticides increases and may cause chronic damage. The exposure to organochlorides results in a bioaccumulation in the food chain (Chalupka 2001).

The length of a pesticide's half-life can depend upon a combination of soil, water, and air conditions. The half-life of organochlorides such as DDT in the atmosphere is about two days, but in soil can range from two to 15 years (Agency for Toxic Substances and Disease Registry (ATSDR) 1995). Other organochlorides such as chlordane may persist four to 20 years in the soil (Pakdeesusuk and Huddleston 1998). Table 1 shows the half-life of several organochlorides.



Aldrin

DDT

Endosulfan sulfate

Lindane (gamma-HCH)

Heptachlor epoxide

Endrin

DDE

Figure 1. Organochloride structures

Table 1. Organochlorides half-lives

Type of organochloride pesticide	**Half-life in soil**	**Half-life in water**	**Half-life in air**
Aldrin	113 days	24 days	113 hours
Dieldrin	868 days	~60 days	153 hours
Endrin	~14 years	~4 years	1.45hours-1.8days
Heptachlor	25-41 days	3.5 days	1.5-6 hours
Heptachlor epoxide	4 years	~60 days	121 hours
HCH	Up to 15 months	30 days	17 weeks
Endosulfan	50 days	19 days	N/a
DDT	2-15 years	28-56 days	2days

Aldrin and dieldrin are similar in chemical structure. When exposed to sunlight and bacteria in the environment, aldrin breaks down into dieldrin. Both aldrin and dieldrin were broad-spectrum insecticides that were widely applied until 1974. After 1974, they were restricted to termite control by direct soil injection and non-food seed and plant treatment. In 1989, the Environmental Protection Agency (EPA) banned all uses of these chemicals (ASTDR 2001a).

Heptachlor epoxide is a chemical and biological derivative of heptachlor, produced as a result of exposure to sunlight and microorganisms. Heptachlor epoxide is absorbed readily into soil and is very resistant to biodegradation. Heptachlor can persist in the soil for 25 to 41 days. While in water, heptachlor half-life is about four days (ASTDR 2001b). Heptachlor epoxide has an even longer residence time than heptachlor lasting up to four years in soil. In water it can persist up to 60 days (ASTDR 2001b). In 1978, the EPA canceled the registration of heptachlor and chlordane, which contained 10% heptachlor. Heptachlor and chlordane could only be used for underground termite control after July 1, 1983 (Spectrum Laboratories 2002). By 1988, the use of heptachlor for termite control ended. Heptachlor is currently approved only for commercial use for fire ant control in power transformers (ASTDR 2001b).

Endrin is an insecticide and rodenticide primarily used to treat agricultural and ornamental plants and may remain in the environment up to 14 years (Table 1). When exposed to high temperatures or light, endrin breaks down into endrin ketone and endrin aldehyde. Although the amount of endrin ketone and endrin aldehyde produced is small, it is unknown what happens to these products after they are released (ASTDR 2001c). In 1986, the production and sale of endrin halted in the United States.

Lindane, ς-hexachlorocyclohexane (ς-bhc), α-hexachlorocyclohexane (α-bhc), and β-hexachlorocyclohexane (β-bhc) are hexachlorocyclohexanes (HCH), which are not naturally produced in the environment. They have historically and inappropriately been called benzene hexachloride or BHC. There are eight forms of HCH but the four most common are lindane (γ-bhc), ς-bhc, α-bhc, and β-bhc. HCHs were used as insecticides for agriculture crops and forest crops. HCH continues to be used in ointments to treat head and body lice and scabies. However, HCH ceased to be produced in 1977, it continues to be imported and formulated in the United States. The technical grade has not been produced since 1983. The isomers cannot be legally produced or used commercially in the United States. HCH breaks down quickly in water and only persists about 30 days. In air, HCH can last up to 17 weeks and can travel long distances depending upon prevailing winds. In soil, HCH can remain up to fifteen months. Health problems that can occur after exposure to HCH are decreases in reproductive capabilities, blood disorders and death (ASTDR 2001d; Extoxnet 1996c).

Endosulfan was a pesticide used to control insects on food and non-food crops. Also it was used as a wood preservative. Endosulfan sulfate is a reactive product of endosulfan and is found in the environment after endosulfan photoylsis. Endosulfan sulfate can be found in organisms as a result of oxidation by biotransformation after ingestion of endosulfan. Endosulfan is currently on the EPA's Restricted Use Pesticide

(RUP) list. Endosulfan breaks down chemically on crops in a few weeks. It does not dissolve readily in water. In an average agricultural field, the half-life of endosulfan in soil is about 50 days. In mammals, damage to the kidneys, testes, liver, and immune system occurs. Also, damage to the central nervous system might occur from exposure to endosulfan and endosulfan sulfate (ASTDR 2001e; Extoxnet 1996b).

DDT and 1,1-dichloro-2,2-bis (chlorophenyl) ethylene (DDE) were used on agricultural crops to control insects. Also DDT and DDE were used to control disease-carrying mosquitoes. DDE was also used medically to treat adrenal gland cancer. These compounds were manufacted and do not occur naturally. 1,1-dichloro-2, 2-bis (p-chlorophenyl) ethane (DDD) results from a biological breakdown of DDT and DDE. It has no commercial use. DDT was banned from use in 1972 except in public health emergencies. DDE was completely banned at the same time. The half-life in air for DDT is two days, in soil it can persist for up to 14 years and in water, from 28 to 56 days. DDT, DDE, and DDD damage mammalian nervous and reproduction system (ATSDR 1995; Extoxnet 1996a).

The biodegradation pathways, under natural conditions, have been described for certain organochlorides such as DDT (Appendix A) and HCH (Appendix B).

Studies have shown that pesticides such as organochlorides have a significant detrimental affect on bat populations, in both rural and urban areas (Clark *et al.* 1975; Clark *et al.* 1978; Clark 1981; White and Krynitsky 1986; Schmidt *et al.* 2001). Gray bats in Missouri were found with organochlorides such as dieldrin in their brains, which were presumably derived from their food base of insects. Consequently, the pesticides were found to have passed from mother to young through lactation (Clark *et al.* 1978). As juvenile bats accumulate a lethal amount of pesticides, the risk of mortality from falls from the roost increases. They become too weak from the pesticides in their system to cling to the wall and fall to the floor of the bat roost (Clark *et al.* 1975).

Studies have reported the effect pesticides have on the health of wildlife such as increased mortality and reproductive problems (Clark 1981; White and Krynitsky 1986; Schmidt *et al.* 2001). White and Krynitsky (1986) demonstrated that organochloride residues in certain wildlife populations in New Mexico and Texas might be restricted to locations where the bats forage. Bats often have similar migration patterns but different foraging patterns. One cave of bats was found to contain high levels of (DDE), which suggests that they were getting DDE or DDT from their foraging area, since insects consumed by the bats contained organochlorides (Schmidt *et al.* 2001). Ryckman *et al.* (1997) examined how organochloride pesticides such as DDE, DDT, and 2,3,7,8-TCDD affected Herring Gull eggs. A 25- year study revealed several of the pesticides levels decreased while some, like DDE, remained constant.

The application of pesticides (herbicides, insectides, or rodenticides) on lawns in urban areas is a possible, non-point source of contamination of surface water. Pesticides have been found, but not in high levels, in the streams and detention ponds of Hamilton and Guelph, Canada. Urban application (lawns and gardens) rates were generally higher than agricultural (farms, orchards and forests) rates for the same number of compounds (Struger 1994).

When Brazilian Free-tailed bats (*Tadarida brasiliensis)* were forced into starvation, which possibly mimicked migration conditions, the bats appeared to be poisoned from pesticides being released from depleted fat stores (Geluso *et al.* 1976). Organochlorides such as dieldrin can be passed to young *T. brasiliensis* through milk and placental transfer (Clark *et al.* 1975; Thies and McBee 1994). Al-Hachim and Fink (1968) suggested that DDT might interfere with the development of conditioned avoidance response (CAR) in mouse offspring exposed to DDT. If the DDT was given during the first trimester, there was no affect on CAR. Yet if DDT was given during the second or third trimester, CAR was delayed until after the offspring's first month after birth. Additional evidence of DDT transfer through lactation and placental transport was given by Al-Hachim and Fink (1968).

When the DNA variation in *T. brasiliensis* was analyzed by flow cytometry, it was found that the compounds might alter the genome of the bats. In males, who had measurable levels of DDE in their livers and testes, there was a positive correlation with percent coefficients of variation in their DNA indicating a possible change in DNA content potentially causing an alteration in the genome (Armstrong 2000). Bennett (2000) examined the DNA content variation and found that a majority of males collected with high levels of organochlorides exhibited a lack of spermatogenesis. A positive correlation between the levels of pesticides and the amount of chromosomal breakage has also been observed in T. *brasiliensis* (Thies 1993).

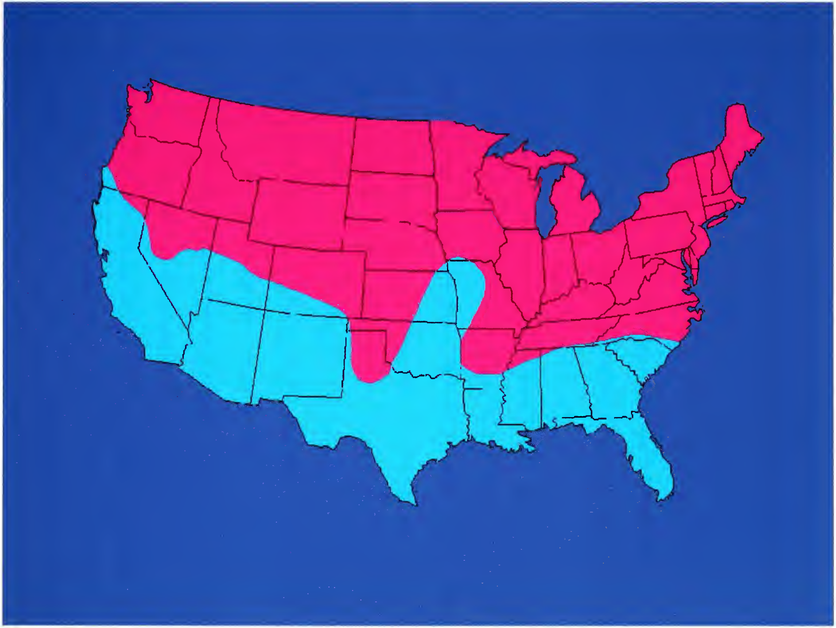
T. brasiliensis is one of the most widely distributed mammals in the western hemisphere. There are three species of *Tadarida* in the United States, in which *T. brasiliensis* is the smallest in size. The subspecies in the southeastern United States is *T. b. cynocephala*. The key characteristics of *T. brasiliensis* are deep grooves or wrinkles on the upper lip, a z-shaped upper third molar, the tail extending beyond the uropatagium (membrane surrounding the tail) and the ears laying forward but not extending beyond the muzzle (Hall 1981). The pelage is a uniform brown and the hair is short on the head and body. The range of *T. brasiliensis* in the United States of America is depicted in Figure 2. The Brazilian free-tailed bat is found throughout Central America and much of South America. The range for South American populations is not well known. *T. brasiliensis* is found on the Caribbean islands, both the Greater and Lesser Antilles (Hall 1981; Lee and Marsh 1978; Glass 1982; Villa-R. 1966; Koopman 1982; Baker and Genoways 1978). *T. brasiliensis* may inhabit many different types of roosts such as caves, culverts, bridges, trees and buildings (Cockrum 1969). They tend to aggregate in large numbers, especially maternity colonies, which are almost exclusively female (Davis *et al.* 1962).

Breeding in *T. brasiliensis* occurs in February and March in most of the range. Gestation lasts for about 11 weeks. Males reach sexual maturity by their second year. Females reach sexual maturity at nine months (Sherman 1937). The longest lifespan recorded for *T. brasiliensis* is eight years (LaVal 1973). Body fat content of *T. brasiliensis* varies significantly by season. Fat loss occurs during the dry season when insects are fewer in number (McNab 1976; Pagels and Jones 1974). Females have greater fat reserves than males. *T. brasiliensis* in the western United States migrate and tend to have high body fat, while *T. brasiliensis* populations in the eastern United States do not migrate and individuals have less body fat (Herreid 1963; Pagels and Jones 1974).

The feeding range for *T. brasiliensis* has been documented having a 10 to 15 kilometers radius (Williams *et al.* 1976). *T. brasiliensis* feed primarily on winged ants, chalcids, dytiscid beetles, chironomid midges, and small lepidopterans (Hamilton and Whitaker 1979). Predators of *T. brasiliensis* are skunks, raccoons, opossums, snakes, and predatory birds such as kestrels, kites, hawks, owls, and roadrunners (Constantine 1948; Twente 1956; Davie et al. 1962; Taylor 1964; Black 1976).

Eptesicus fuscus (Big brown bat) is a medium size, heavy bodied bat. The pelage coloration can range from pinkish tan to rich chocolate brown. The unfurred areas are blackish. *E. fuscus* can be found through out North and Central America. The big brown bat's southern distributional limits are northern South America (Colombia, Venezuela, and Brazil). They are also found in the islands of the Caribbean (Bahamas, Greater Antilles, and Lesser Antilles) (Manville and Young 1965; Davis 1966; Villa-R. 1966; Piccinini 1974; Hall 1981; Koopman 1982; Van Zyll de Jong 1985; Buden 1985).

Figure 2. Geographic range (in blue) for T. brasiliensis (Hall 1981; Lee and Marsh 1978; Glass 1982)



Maternity colonies of *E. fuscus* are formed by the females in summer months. The males either live in singly, roost with the females, or roost in an all-male colony. The maternity colonies in eastern North America are usually located in man-made structures but are occasionally found in hollow trees (Barbour and Davis 1969). In Canada, the roosts are frequently in rock cervices and ponderosa pines (Brigham 1988). Colonies can be as small as five individuals, or as large as 700 individuals. Most colonies in eastern North America average 20 to 75 individuals (Barbour and Davis 1969; Kurta 1980; Mills *et al.* 1975).

Reproduction in *E. fuscus* occurs from September through March (Mumford 1958; Phillips 1966). The gestation is about sixty days. Normally one young is born, but twins occasionally occur (Barbour and Davis 1969; Silva Taboada 1979). Females deposit fat early in their pregnancy. In late pregnancy, some of the fat is utilized to accelerate fetal growth (Stack 1985). Lactation lasts 32 to 40 days. Juveniles begin to fly at 18 to 35 days. The males reach sexual maturity by their first autumn, while females may not reach sexual maturity until the end of their first year (Christian 1956; Schowalter and Gunson 1979;Paradiso and Greenhall 1967). Colonies disperse in early August.

Hibernation does not occur until November (Barbour and Davis 1969). Females deposit fat a month earlier than males (Pistole 1989). However, males go into hibernation a month earlier than females (Phillips 1966). *E. fuscus* can leave hibernation any time through winter (Mumford 1958). They generally hibernate singly or in small clusters

(Mumford 1958; Nagorsen 1980). In warmer climates, *E. fuscus* does not hibernate but goes through torpor in the cooler months. Winter and summer roosts tend to be about eighty kilometers apart (Mills *et al.* 1975).

E. fuscus is a generalist in foraging areas. *E. fuscus* have no preference over types of foraging habitats (Furlonger *et al.* 1987; Geggie and Fenton 1985). *E. fuscus* forage throughout the night, but most of their activity is within the second hour after sunset (Kunz 1973). The flight foraging radius for feeding for a big brown bat is between one and two kilometers (Brigham 1988). *E. fuscus* feed primarily on small Coleoptera, Hymenoptera and Diptera (Hamilton and Whitaker 1979). Vegetation and non-flying prey make up four percent of their stomach content (Whitaker 1972). Predators of *E. fuscus* are birds such as owls and grackles, long tailed weasels, housecats, rats, snakes and bullfrogs (Rysgaard 1942; Beer 1953; Long 1971; Black 1976; Kirkpatrick 1982).

Initial pesticide studies (Clark and Lamont 1976a; Clark and Lamont 1976b; Clark and Prouty 1977) on *Eptesicus fuscus* reported the effects of organochlorides on reproduction and nursing of young. The females were found to have DDT, dieldrin, heptachlor epoxide, HCB, mirex, trans-nonachlor and cis-nonachlor. However, DDE was shown to transfer most readily through lactation (Clark and Lamont 1976b). Clark and Prouty (1977) found big brown bats that were forced into a starvation mode had higher residue levels of DDE in their brains. When the fat tissue was analyzed, the pesticides are found in a more concentrated form.

The objective of this study was to examine the presence of organochlorides in *T.*

brasiliensis and *E. fuscus* and the relationship between accumulation and availability of pesticides in bat populations in southwestern Georgia. This study is different from previous studies since a comparison was made between two species of bats, *T. brasiliensis* and *E. fuscus* in the presence of organochlorides, which has not been done before. This study examined whether or not pesticides such as organochlorides are still accumulating in organisms, such as bats, even after organochlorides were resisted or banned.

Experimental material and methods

T. brasiliensis individuals were captured by mist nets in the roost and using forceps to pluck bats from the wall and ceiling of an inhabited commercial building in Chattahoochee County, Georgia. The approximate feeding range for this colony can be seen in Figure 4. The feeding range was estimated from a study reported by Williams *et al.* (1976) working on bats from caves near San Antonio, Texas.

E. fuscus individuals were captured by placing a mist net over a house attic louver the bats were occupying and agitating the bats to fly into the net. These specimens were located in an inhabited residential building in Muscogee County, Georgia. The feeding range for this colony can be seen in Figure 5. *E. fuscus* were also taken from an inhabited residential home in Harris County, Georgia. The estimated feeding range for this colony can be seen in Figure 6. The feeding range was approximated from a study reported by Brigham (1988) in Ontario, Canada.

Appendix C shows an overview of the feeding ranges and approximate locations for the three selected bat populations. In Europe, many bat roosts have been destroyed, which has caused many bats to be endangered or extirpated. The protection of the roosts insures that the bats have a place to live in the future (Racey 1992). Consequently, the exact locations of the bat roosts have not been disclosed because of possible destruction of the roost sites.

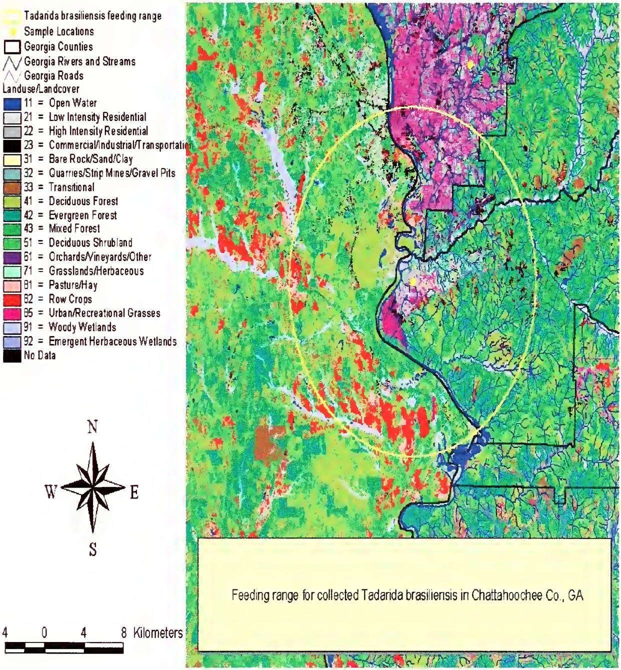


Figure 4. Feeding range for collected *Tadarida brasiliensis* in Chattahoochee Co, GA

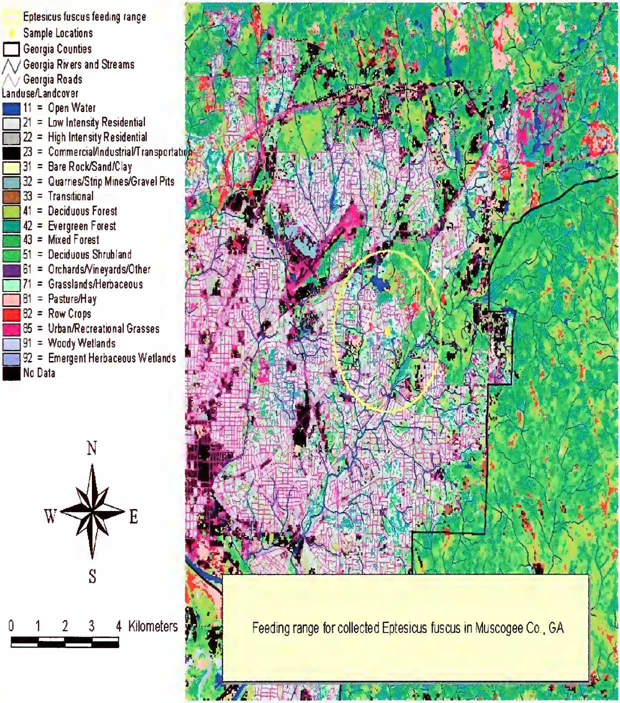


Figure 5. Feeding range for collected *Eptesicus fuscus* in Muscogee Co, GA

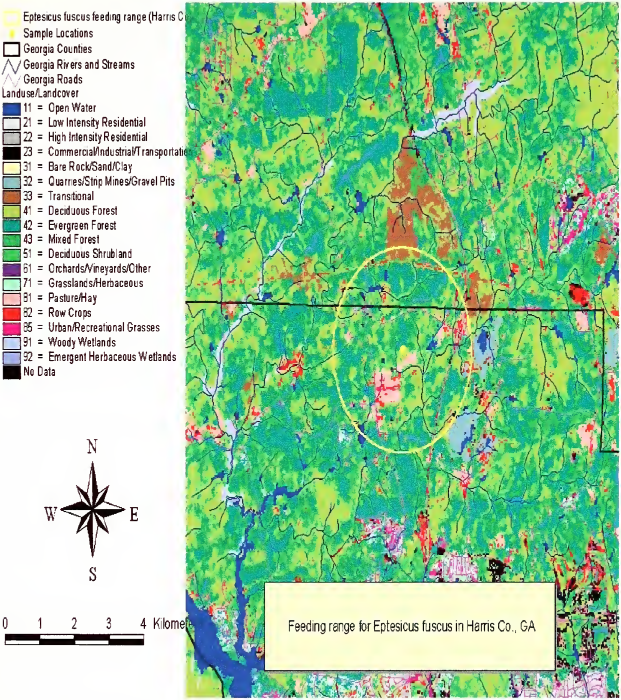


Figure 6. Feeding range for collected *Eptesicus fuscus* in Harris Co, GA

Carbon dioxide was used to euthanize the bats in compliance with the standards established by the American Society of Mammalogists (1987). The euthanized bats were frozen at -78° C within two hours of collection and maintained at this temperature until needed for analysis.

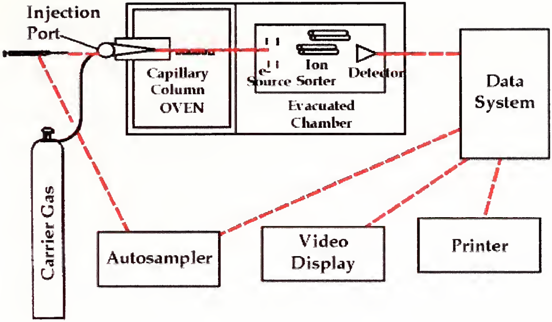
The livers were utilized because this organ processes or filters toxins and because of their high fat content. The method for isolation of the pesticides from the liver was described by Peterson (1976). Extraction of the liver was carried out immediately after the bats were thawed. The liver of each specimen was placed in a tared test tube, weighed, and mixed with five times its weight of anhydrous sodium sulfate. The sample was chilled in an ice-bath until brittle and ground with a mortar and pestle. The ground material was transferred to sterile test tubes. An extraction solvent containing 20% acetone in isooctane was placed in the test tubes. The solvent was administered in the ratio of 10 mL solvent per one-gram solid sample. The test tubes were placed in an ultrasonic cleaner for 15 minutes to mix the sample. The samples were cleaned for analysis with Florisil and placed in a solution of five percent methanol in isooctane. All mortars, pestles and evaporating dishes were acid-cleaned with a solution of sulfuric acid and distilled water between each use.

As a control for comparison with the bat livers, spiked livers from euthanized laboratory-reared rats with known quantities of each pesticide were used. The laboratory-reared rats were used since the rats most likely were never exposed to organochlorides.

The pesticide array was obtained from ChemService, Inc. and certified as pure.

Samples were analyzed using a Finnigan MAT GCQ gas chromatograph (Figure 7). Instrument settings and operating condition were as follows: injector temperature 190ºC, detector temperature 300º C, carrier gas helium, flow rate 60º mL/min. The oven temperature was held at 190º C for 30 seconds, and then ramped from 190º C to 240º C at 2º C/min. Oven temperature was held at 240º C for 30 seconds then ramped from 240ºC to 275ºC at 10º C/min, and held at 275º C for 9 seconds. The GC/MS detection limit was 1000 µL/g. Data acquisition was conducted using the Finnigan Mat GC/MS computer program and library.

The Wilcoxon signed-rank test was used in this study because it allowed to samples from populations that are not normally distributed to be compared. The Wilcoxon is a nonparametric test that does not examine the two populations individually, but it focuses on the differences in values for each pair of observation as a median difference equal to zero. Also, the Wilcoxon test takes into account the magnitude of the differences (Pagano and Gauvreau 1993). This is the appropriate test since the organochlorides could not be quantified and a normal distribution could not be assumed.



http://www.state.ia.us/government/dps/dci/lab/drugchem/gas_chromatograph.htm

Figure 7. Gas chromatograph and Mass spectrometer

Results

Analysis of 191 individuals collected from the sites confirmed the presence of pesticide residues. One juvenile and three adult T. *brasiliensis* and six *E. fuscus* did not have detectable organochlorides. Levels were low and appeared to be trace amounts in the specimens. Several organochlorides were found in the bat livers, but in trace amounts. The trace amounts were too small to obtain an accurate quantitative analysis, so a non-parametric test was performed.

The organochlorides were not detected until background "noise" was removed. Background may be described as the presence of other compounds such as metabolic products and their isomers that mask pesticide peaks on the spectrograph (Appendix D) If the background noise was not removed, the compounds in low or trace amounts would not have been observed. Between *T. brasiliensis* and *E. fuscus*, similar organochlorides appeared. Four organochlorides were in the majority of the bats, both *T. brasiliensis* and *E. fuscus*: endrin, heptachlor epoxide, aldrin and dieldrin. Other organochlorides found were ς-bhc, β-bhc, α-bhc, lindane, heptachlor, DDE, DDT, DDD, endosulfan, and endosulfan sulfate (Figures 8-10). A complete list of organochlorides detected can be seen in Table 2.

The presence of the same types of organochlorides in juvenile *T. brasiliensis* (Table 3) was seen as well as in adult *T. brasiliensis* (Table 4). *E. fuscus* did not vary significantly in types of organochlorides present compared to *T. brasiliensis* (Table 5). Adult *T. brasiliensis* population had one individual with heptachlor and DDD, which did not appear in the juvenile *T. brasiliensis* and *E. fuscus* populations.

Using the Wilcoxon signed-rank test, a comparison between *T.* brasiliensis and *E. fuscus* was performed to determine any difference in types of organochlorides occurring between the groups (Appendix E). The Wilcoxon signed-rank test indicated no significant difference between the types of organochlorides occurring between the two species (Table 6); however, there was a significant difference in the amounts and types of organochlorides in adult and juvenile *T. brasiliensis* (Table 7).

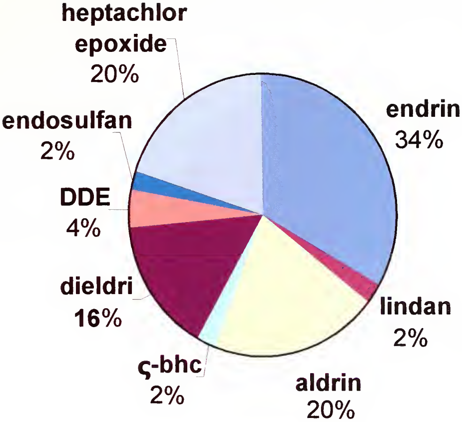


Fig. 8. Organochlorides (percentage) occurring in *T. brasiliensis* juveniles

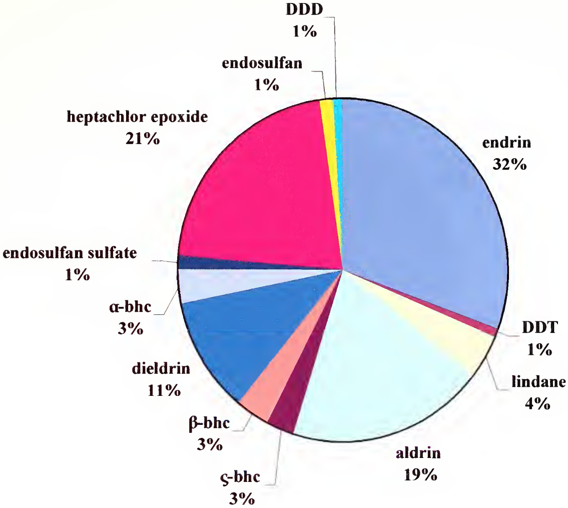


Figure 9. Organochlorides (percentage) occurring in *T. brasiliensis* adults

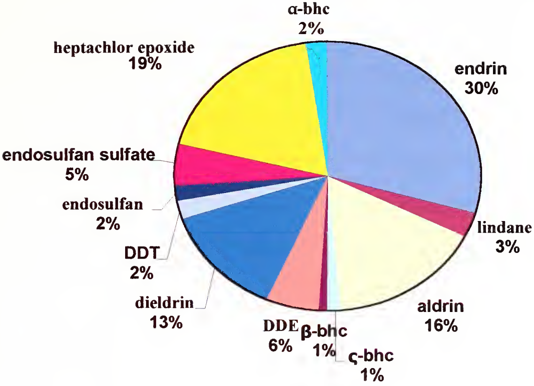


Figure 10. Organochlorides (percentage) occurring in *E. fuscus*

Table 2. List of organochlorides in both *T. brasiliensis* and *E. fuscus*

T. Brasiliensis		*E.fuscus*
Adults	**Juveniles**	**Adults**
Endrin	**Endrin**	**Endrin**
Lindane	**Lindane**	**Lindane**
Aldrin	**Aldrin**	**Aldrin**
ς-bhc	**ς-bhc**	**ς-bhc**
β-bhc	**Dieldrin**	**β-bhc**
DDE	**DDE**	**DDE**
Dieldrin	**Endosulfan**	**Dieldrin**
DDT	**Heptachlor epoxide**	**DDT**
Endosulfan		**Endosulfan**
Endosulfan sulfate		**Endosulfan sulfate**
Heptachlor epoxide		**Heptachlor epoxide**
α-bhc		**α-bhc**
Heptachlor		
DDD		

Table 3. Pesticides present in 22 juvenile *T. brasiliensis*

Tadarida brasiliensis **juveniles**	
Pesticides	**Number of bats**
Endrin	15
Lindane	1
Aldrin	9
ς-bhc	1
Dieldrin	7
DDE	2
Endosulfan	1
Heptachlor epoxide	9
Endosulfan sulfate	0
DDT	0
α-bhc	0
β-bhc	0

Table 4. Pesticides present in 111 adult *T. brasiliensis*

***Tadarida brasiliensis* adults**	
Pesticides	**Number of bats**
Endrin	83
Lindane	11
Aldrin	52
ς-bhc	8
β-bhc	9
DDE	1
Dieldrin	29
DDT	2
Endosulfan	4
Endosulfan sulfate	4
Heptachlor epoxide	58
α-bhc	9
Heptachlor	1
DDD	2

Table 5. Pesticides present in 58 *E. fuscus*

Eptesicus fuscus	
Pesticides	**Number of bats**
Endrin	42
Lindane	4
Aldrin	23
ς-bhc	2
β-bhc	1
DDE	8
Dieldrin	19
DDT	3
Endosulfan	3
Endosulfan sulfate	7
Heptachlor epoxide	27
α-bhc	3

Table 6. Wilcoxon signed rank table for comparison for *T. brasiliensis* and *E. fuscus*

Difference between pairs	n	Rank sum	Mean rank
Positive	9	70.0	7.78
Negative	3	8.0	2.67
Zero	2		

Difference between medians	7.500	
95.1% CI	1.000	to 23.5

Wilcoxon's W statistic	70
2-tailed p	0.0122

Table 7. Wilcoxon signed rank table comparison between adult and juvenile *T. brasiliensis*

Difference between pairs	**n**	**Rank sum**	**Mean rank**
Positive	1	1.5	1.50
Negative	13	103.5	7.96
Zero	0		

Difference between medians	-9.500	
95.1% CI	-29.000	to -4.000

Wilcoxon's W statistic	1.5
2-tailed p	0.0004

Discussion

The pesticide concentrations from bats in this study were lower, in trace amounts, than reported in previous studies (Clark *et al.* 1975; Clark and Lamont 1976a; Clark and Lamont 1976b; Geluso *et al.* 1976; Clark and Prouty 1977; Clark *et al.* 1978; Thies 1993; Thies and McBee 1994; Armstrong 2000; Bennett 2000). The acquisition of pesticides such as organochlorides from the environment was obviously from food sources. The food source could have been contaminated by pesticides used for agricultural and urban uses. The residues of pesticides, even though no longer in common use, could still remain in soil for years and may cause problems in organisms through the food chain (Clark 1981; White and Krynitsky 1986; Schmidt *et al.* 2001).

Four organochlorides endrin, heptachlor epoxide, aldrin and dieldrin, appeared in a majority of the bats, both *T. brasiliensis* (juveniles and adults) and *E. fuscus*. The wide variety of different organochlorides detected suggests, in the past that heavy use occurred in the areas currently occupied by the bats. Also, the organochlorides could occur from used organic biomass or transported from another site. Since the background noise on the gas chromatograph had to be removed to detect the organochlorides, this implies that organochlorides are occurring in trace amounts.

Juvenile *T. brasiliensis* taken from in early July had organochlorides in their livers. Since the juveniles were not able to forage on their own, the organochloride present may have come through lactation or by cross-placental transfer as suggested by past studies (Clark and Lamont 1976a; Clark and Lamont1976b; Clark *et al.* 1978;Thies and McBee 1994).

Conclusion

Previous pesticide studies have demonstrated effects that organochlorides have had upon organisms' health. Since organochlorides are fat-soluble, organisms that use their stored fats have a greater chance of being affected when hibernating or migrating long distances (Geluso *et al.* 1976).

Since bats are roosting in buildings used or occupied by humans, the chances for exposure to pesticides increases since bats are in proximity to pesticides used around the buildings. From this study, it can be seen that use of organochlorides by humans, has caused a residual pesticide effect upon the environment and bats, since organochlorides were detected long after many were banned from use. This may suggest that the life span of organochlorides is longer than previously thought or some banned pesticides are still in use. This study provides evidence that organochlorides continue to persist in the environment long after the substances have been banned.

Further monitoring of pesticide levels should be undertaken with more sophisticated instrumentation in various habitats to determine possible long-term impact upon bat populations. The sustained monitoring of organochlorides, even in trace amounts, should be performed to see if organochlorides continue to decrease in the environment.

Researchers involved in similar studies should take care to remove the background "noise" from their GC/MS's before assuming pesticides are not present. Although this is an obvious procedure in standard analyses, it needs to be emphasized when low levels of pesticides are anticipated. Many different organochlorides were found when the background noise was removed from the analysis.

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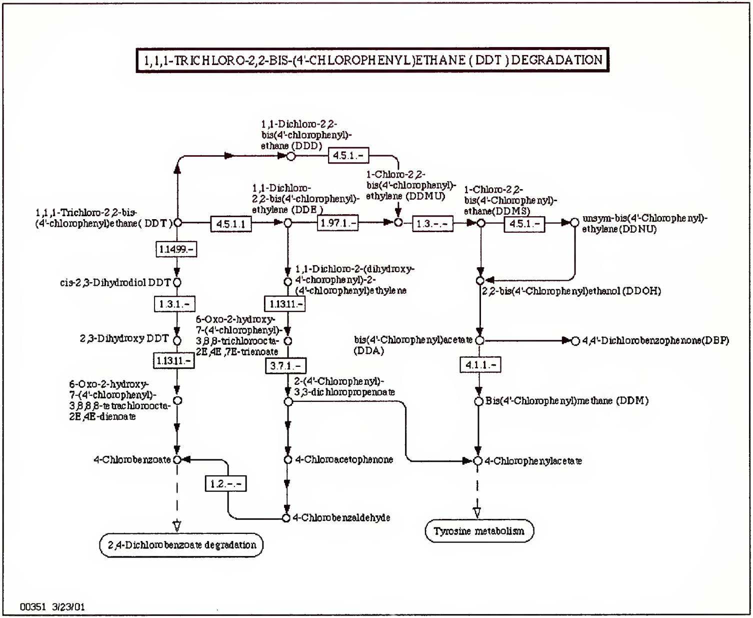
Appendix A

DDT biodegradation pathway

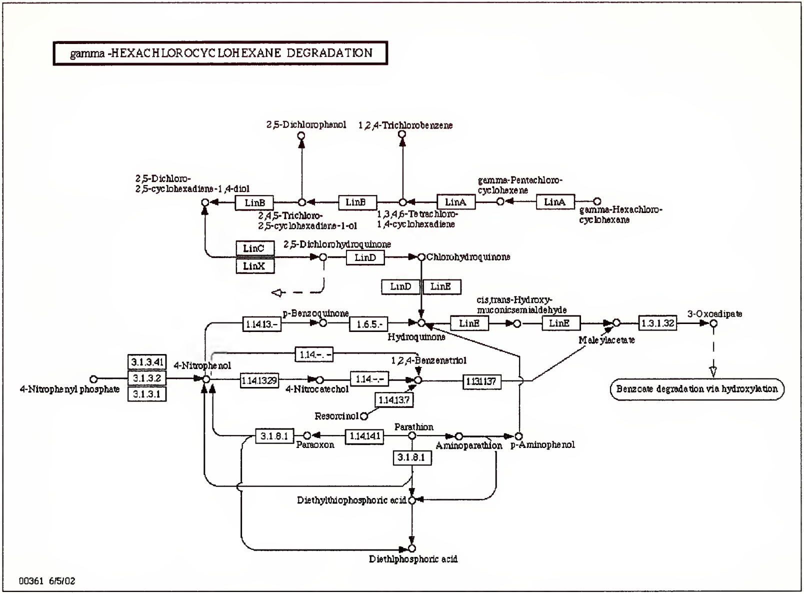
(KEGG Metabolic Pathways 2002)


1,1,1-TRICHLORO-2,2-BIS-(4'-CHLOROPHENYL)ETHANE (DDT) DEGRADATION
1,1-Dichloro-2,2-bis(4'-chlorophenyl)-ethane (DDD)
4.5.1.-
1-Chloro-2,2-bis(4'-chlorophenyl)-ethylene (DDMU)
1,1-Dichloro-2,2-bis(4'-chlorophenyl)-ethylene (DDE)
1-Chloro-2,2-bis(4'-Chlorophenyl)-ethane(DDMS)
1,1,1-Trichloro-2,2-bis-(4'-chlorophenyl)ethane(DDT)
4.5.1.1
1.97.1.-
1.3.-.-
4.5.1.-
unsym-bis(4'-Chlorophenyl)-ethylene (DDNU)
1.14.99.-
cis-2,3-Dihydrodiol DDT
1,1-Dichloro-2-(dihydroxy-4'-chorophenyl)-2-(4'-chlorophenyl)ethylene
2,2-bis(4'-Chlorophenyl)ethanol (DDOH)
1.3.1.-
1.13.11.-
6-Oxo-2-hydroxy-7-(4'-chlorophenyl)-3,8,8-trichloroocta-2E,4E,7E-trienoate
2,3-Dihydroxy DDT
bis(4'-Chlorophenyl)acetate (DDA)
4,4'-Dichlorobenzophenone(DBP)
1.13.11.-
3.7.1.-
4.1.1.-
6-Oxo-2-hydroxy-7-(4'-chlorophenyl)-3,8,8,8-tetrachloroocta-2E,4E-dienoate
2-(4'-Chlorophenyl)-3,3-dichloropropenoate
Bis(4'-Chlorophenyl)methane (DDM)
4-Chlorobenzoate
4-Chloroacetophenone
4-Chlorophenylacetate
1.2.-.-
4-Chlorobenzaldehyde
Tyrosine metabolism
2,4-Dichlorobenzoate degradation
00351 3/23/01

Appendix B

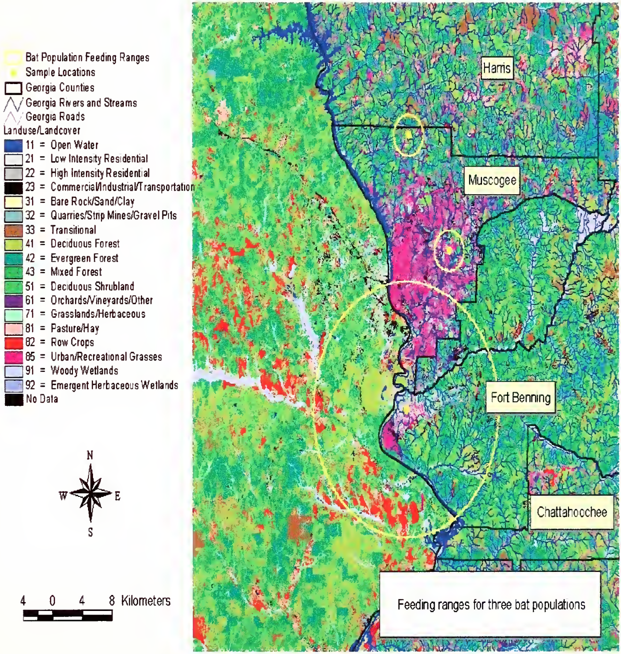
HCH biodegradation pathway

(KEGG Metabolic Pathways 2002)


gamma -HEXACHLOROCYCLOHEXANE DEGRADATION
2,5-Dichlorophenol
1,2,4-Trichlorobenzene
2,5-Dichloro-
2,5-cyclohexadiene-1,4-diol
gamma-Pentachloro-
cyclohexene
LinB
LinB
LinA
LinA
gamma-Hexachloro-
cyclohexane
2,4,5-Trichloro-
2,5-cyclohexadiene-1-ol
1,3,4,6-Tetrachloro-
1,4-cyclohexadiene
LinC
LinX
2,5-Dichlorohydroquinone
LinD
Chlorohydroquinone
LinD
LinE
cis,trans-Hydroxy-
muconicsemialdehyde
p-Benzoquinone
1.14.13.-
1.6.5.-
LinE
LinE
1.3.1.32
3-Oxoadipate
Hydroquinone
Maleylacetate
1.14.-.-
1,2,4-Benzenetriol
3.1.3.41
3.1.3.2
3.1.3.1
4-Nitrophenol
4-Nitrophenyl phosphate
1.14.13.29
1.14.-.-
4-Nitrocatechol
1.13.11.37
Benzoate degradation via hydroxylation
1.14.13.7
Resorcinol
Parathion
3.1.8.1
Paraoxon
1.14.14.1
Aminoparathion
p-Aminophenol
3.1.8.1
Diethylthiophosphoric acid
Diethlphosphoric acid
00361 6/5/02

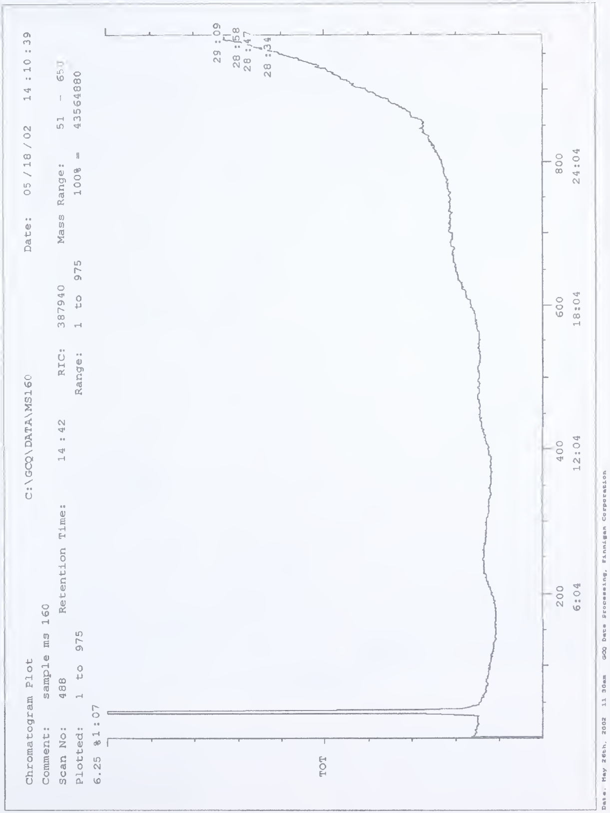
Appendix C

Feeding ranges for three bat populations



Appendix D

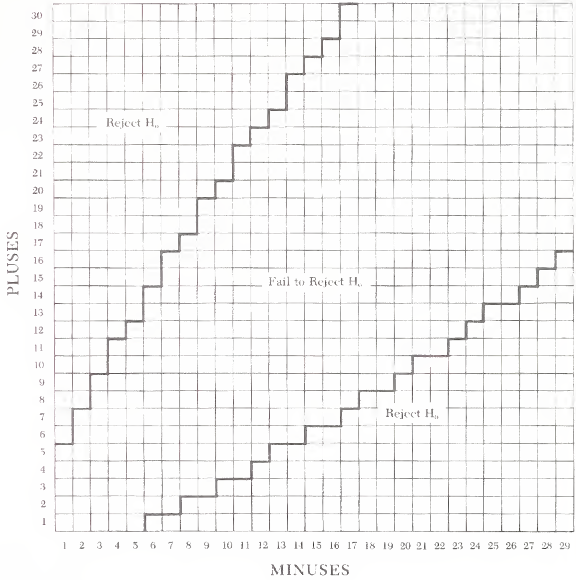
Representive chromatograph


Chromatogram Plot
C:\GCQ\DATA\MS160
Date: 05/18/02 14:10:39
Comment: sample ms 160
Scan No: 488
Retention Time: 14:42
RIC: 387940
Mass Range: 51 - 650
Plotted: 1 to 975
Range: 1 to 975
100% = 43564880
6.25 %
1:07
TOT
29:09
28:58
28:47
28:34
200
400
600
800
6:04
12:04
18:04
24:04
Date: May 26th, 2002 11 30am GCQ Data Processing, Finnigan Corporation

Appendix E

Sign test table

Table for the Sign Test at the alpha level of .05.[1]



Ambrose and Ambrose 1995