CYTOGENETIC ABERRANCY AND ORGANOCHLORINE PESTICIDE ACCUMULATION IN THE MEXICAN FREE-TAILED BAT: A COMPARISON BETWEEN OKLAHOMA AND NEW MEXICO POPULATIONS

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CHAPTER I

INTRODUCTION

Accumulation of pesticide residues in tissues of wild vertebrates is related to food habits and presumably, is a primary reflection of contamination of the food supply (Dustman and Stickel 1969). Because of similarities in structural, metabolic, and genetic components of all life forms, absolute species specificity in the development of agricultural pesticides is frequently difficult to attain. Improper use of such toxic chemicals may engender biological effects beyond those for which they were originally manufactured and results in expanding concerns about potential environmental health problems resulting from increasing risks of exposure to toxic chemicals (Hutton 1982).

Although a number of studies have demonstrated the accumulation and movement of organochlorine pesticide residues in the food chain, effects other than acute toxicity (i.e., death) have not been adequately addressed. Similarly, effects of chronic exposure or potential genetic and populational effects have not been properly examined. This study was designed to address whether observed cytogenetic aberrancy frequencies and flow cytometric analysis of nuclear

DNA content variation could be correlated with measured organochlorine pesticide residues in body tissues of the Mexican free-tailed bat, Tadarida brasiliensis (Chiroptera: Molossidae).

Organochlorine Pesticides

Organochlorine (OC) pesticides are a group of aromatic and non-aromatic ring synthetic insecticides that contain chlorine. The first member of this group, formulated in 1874 but not introduced onto the pesticide market until 1939, was DDT (1,1,1-trichloro-2,2-bis[p-chlorophenyl]ethane). Because of its low mammalian acute toxicity, it was used widely and in large quantities as a nonsystemic stomach and contact insecticide for human health applications such as mosquito control, agriculture, horticulture, and forestry (Hay 1981). Use of OC chemicals peaked 1958-59, during which 78 million pounds of DDT were used in the United States alone (Dustman and Stickel 1969). Worldwide, more than 1.8 million metric tons have been used to control insects and insect-transmitted diseases since its discovery (Miller 1992).

Due to the aromatic nature of some of these compounds and the lack of hydrophilic groups in all members, OC pesticides are highly hydrophobic, very resistant to chemical degradation, and act as universal poisons. Mechanisms by which these compounds exhibit toxic effects are not well understood, but all appear to dissolve in fatty membranes surrounding nerve fibers and produce central nervous system

excitation. DDT, in particular, acts as a nervous stimulant by delaying closing of the Na^+ gate and preventing full opening of the K^+ gate, which can result in paresthesia, tremors, confusion, and convulsions (Baselt 1982).

Recently, it was discovered that, due to their nonreactivity and hydrophobic properties, DDT and its residues are extremely persistent in the environment and tend to bioconcentrate in the environment, accumulating in the lipid stores of long-lived mammals and birds (Dustman and Stickel 1969; Fleming et al. 1983; McBee and Bickham 1990). Rates of accumulation and storage in fatty tissues vary among species and are dependent upon exposure concentrations, duration and route of exposure, and individual fat content (Hayes 1965).

Use of other OC compounds that were developed after DDT, including dieldrin (HEOD: 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene), aldrin (1,2,3,4,10,10-hexachloro-1,4,4α,5,6,7,8,8α-hexahydro-endo-1,4-exo-5,8-dimethanonaphthalene), heptachlor (1,4,5,6,7,8,8-heptachloro-3α,4,7,7α-tetrahydro-4,7-methanoindene), and endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-exo-1,4-exo-5,8-dimethanonaphthalene), has resulted in more serious persistence and accumulation problems (Dustman and Stickel 1969; Fleming et al. 1983; McBee and Bickham 1990). Dieldrin was used as a seed treatment and sheep dip and caused widespread mortality of predatory birds

and birds eating newly sown grain. Use of DDT was canceled except for limited applications relating to human health in the United States in 1972, followed by restrictions or cancellations of other OC products in the United States (EPA 1979). Use of the more toxic products is also severely restricted in most countries. However, lindane (γ-BHC: 1,2,3,4,5,6-hexachloro-cyclohexane) is still used for many applications due to its lower mammalian toxicity and more rapid breakdown in soils and tissues.

Residues of OC pesticides including DDT and its metabolites are still being detected in both aquatic and terrestrial species from identified and/or unknown sources (Fleming et al. 1983; McBee and Bickham 1990). Technical grade DDT consisted of a mixture of o,p'-DDT (67-85%), p,p'-DDT (8-21%), and related compounds. The p,p'- isomer dehydrochlorinates to form p,p'-DDE (1,1-dichloro-2,2-bis[p-chlorophenyl]ethylene) and does not degrade further. This breakdown product is less active as an insecticide and is more toxic to mammals and birds than the parent compound. Pfaender and Alexander (1972), although able to demonstrate extensive degradation of DDT in vitro under anaerobic conditions, attributed the environmental persistence of this compound to one of two hypotheses. Complete destruction through bacterial degradation requires both anaerobiosis and subsequent aerobiosis. Binding to clay minerals may prevent movement of the insecticide from anaerobic to aerobic microhabitats, although free O_2 may still move to the site

where the chemical is bound. A more plausible hypothesis is that the pesticide is degraded by only a very small number of microorganisms, none of which use the compound as a carbon or energy source.

Metabolism of DDT to DDA (bis[p-chlorophenyl]acetic acid), which can be readily excreted by mammals, has been characterized in the laboratory (Peterson and Robison 1964). Replacement of a chlorine with hydrogen yields saturated DDD (1,1-dichloro-2,2-bis[p-chlorophenyl]ethane), which degrades through a series of intermediates to the excretable DDA. However, this mechanism does not include DDE, which results from dehydrochlorination of DDT. If exposed directly to DDT, Rhesus monkeys (Macaca mulatta), laboratory rats (Rattus spp.), and pigeons (Columba livia) can form DDE and DDA, but appear to be incapable of producing DDA from DDE. Dietary DDE was not converted into DDA (Matsumura 1985). Both Rhesus monkeys (Durham et al. 1963) and humans (Morgan and Roan 1971) will readily excrete the highly soluble DDA detoxification product while retaining DDE in adipose tissue indefinitely.

Genotoxicity Assay Background

Genotoxic effects are considered among the most serious of the possible side effects of agricultural chemicals and include heritable genetic diseases, carcinogenesis, reproductive dysfunction, and birth defects. There is strong evidence that somatic mutation plays a role in cancer

induction and may evoke multiple deleterious effects (Ames 1979, Hook 1982, Hartman 1983). Carcinogenesis and mutagenesis are of special concern because of their irreversible nature and the long latency associated with their manifestations. Special attention has been focused on inherently toxic substances applied to crops or soil, where there is a possibility of direct entry into the food cycle (Waters et al. 1982).

Three basic types of genetic damage may result from exposure to different agricultural chemicals: 1) point/gene mutation, 2) primary DNA damage and repair, and 3) chromosomal alterations. Point/gene mutations are changes in the DNA sequence of a gene and are usually brought about through insertions, deletions, or substitutions of single DNA base pairs. DNA damage and repair systems may be affected to the point that DNA itself is altered directly or the processes that synthesize or repair DNA fails to function properly. Chromosomal alterations involve changes in number or structure of entire chromosomes and may include breakage, nondisjunction, translocations, and other forms of structural change. Such changes may be observed with microscopy or indirectly with flow cytometry and provide direct evidence that a chemical can alter organized genetic material (Waters et al. 1982).

A number of in vitro assays have been developed and applied to test the genotoxicity of pesticides. Point/gene mutation assays include the Salmonella typhimurium plate

incorporation and Escherichia coli reverse mutation assays in prokaryotes and the S. cerevisiae D7 reverse mutation, mouse lymphoma forward mutation, and Drosophila melanogaster sex-linked recessive lethal assays in eukaryotes. Primary DNA damage assays include the E. coli polA, S. typhimurium SL4525(rec^+)/SL4700(rec^-) differential toxicity assays in prokaryotes, and the human lung fibroblast unscheduled DNA synthesis assay in eukaryotes. Chromosomal effects also may be examined using sister chromatid exchange (SCE), mouse micronucleus, mouse dominant lethal assays (Waters et al. 1982), spermatogonia and spermatocyte, and oocyte and early embryo assays (Preston et al. 1981). The bone marrow cytogenetics assay is well defined for both in vitro and in vivo evaluation of the genotoxicity of chemicals in mammalian systems (Preston et al. 1981). Flow cytometry also is being used as a mechanism for detecting and quantitatively measuring chromosomal damage induced by physical and chemical agents (Otto and Oldiges 1980; Otto et al. 1981a, 1981b; Fantes et al. 1983; Lovett et al. 1984; Hacker-Klom et al. 1989).

Limited genotoxicity data are available for most pesticides. Thirty-five of 65 pesticides tested by Waters et al. (1982) were positive for one or more of 14 genotoxicity test systems and could be divided into two major categories representing the damage observed. Nineteen agents caused point/gene mutations, and 16 caused primary DNA damage but not measurable gene mutation. Methoxychlor (2,2-bis[p-

methoxyphenyl]-1,1,1-trichloroethane) and endrin tested negative in two prokaryotic and three eukaryotic point/gene mutation assays and three prokaryotic and three eukaryotic DNA damage assays. Using lymphocyte cultures, Yoder et al. (1973) showed a marked increase in the frequency of chromatid lesions in pesticide applicators during heavy spraying periods. Galloway et al. (1987) demonstrated positive genotoxic responses from dieldrin and p,p'-DDE for SCEs; evidence also indicated an increase in chromosome aberrations for o,p'-DDD but SCE results were negative. Georgian (1975) observed chromosome lesions in human lymphocyte cultures exposed to aldrin within a narrow range of doses that were near the limits for cell survival. She postulated that chromosome lesions resulting from aldrin exposure probably were not perpetuated in other abnormal cells.

Cytogenetic studies of in vitro exposure of Chinese hamster (V76) cell lines to DDT and its metabolites indicated that DDE-treated cells had a significant increase in chromosomal aberrations (Kelly-Garvert and Legator 1973). Exchange figures and chromatid breaks also were evident. However, DDT produced no significant increase in chromosome abnormalities. Cytotoxicity also was observed at doses between 35 and 40 µg/ml DDE or DDT. A two-fold increase in chromosome abnormalities in rat kangaroo (Potorous tridactylis apicalis) cell cultures exposed to p,p'- and o,p'- isomers was also observed by Palmer et al. (1972). Only p,p'- isomers produced exchange figures. Cytotoxicity

was observed at 20 µg/ml DDT, with complete inhibition of mitosis at concentrations of 40 µg/ml (Palmer et al. 1972).

Cell death associated with genotoxicity depends upon the type of tissue involved. If cell death occurs in an embryo, it probably will result in some type of malformation or death of the embryo (Kalter 1971). If it occurs in somatic or germinal cells, it will result in nothing more than the loss of the cell. If the cell survived but was damaged, a tumor in a somatic cell or mutation in a germinal cell could result. Chromosome damage, specifically single chromatid breaks or exchange figures, is probably of great mutagenic significance because it is incorporated in cells that are most likely to survive and carry an alteration of the genetic material (Palmer et al. 1972).

Cells exposed to a clastogenic compound would be expected to have (1) an increased incidence of genetic damage, (2) failure of repair mechanisms to properly repair resulting damage, and (3) a greater variation in mean nuclear DNA content in daughter cells following mitosis (Otto and Oldiges 1980). Continued exposure to a clastogen would be expected to produce a population of cells with a significantly higher coefficient of variation (CV) for mean nuclear DNA content than a normal population of cells. If environmental conditions place greater selective pressure on cells with an increased or decreased amounts of genetic material than normal cell lines, resulting cell populations may actually demonstrate a lower CV than the original cell

lines (Otto et al. 1981b).

Species and Site Background

Accumulation of OC pesticide residues in body tissues of insectivorous bats is well documented for a number of species (Clark 1981, 1988; Clark and Krynitsky 1983; Clark et al. 1983; Clawson and Clark 1989). Extensive studies of T. brasiliensis in maternity roosts in Texas, New Mexico, California, and Arizona have addressed concerns expressed by the U. S. National Park Service about population declines at Carlsbad Caverns National Park (Geluso et al. 1976, 1981). The size of the summer population declined from an estimated 8.7 million in 1936 to approximately 200,000 in 1973 (Altenbach et al. 1979). The 1991 population was estimated at 700,000 individuals (David Ek, Carlsbad Caverns National Park, pers. comm.). Body burdens of DDE during the years of decline are not known, but levels of DDE present at Carlsbad Caverns have been high enough to be potentially hazardous to young bats, primarily during their first migration (Geluso et al. 1981). Although other maternity roosts have been examined for pesticide accumulation in young bats and have demonstrated elevated levels of OC pesticide residues (Geluso et al. 1981), no Oklahoma populations have ever been examined for the presence of pesticides. No studies have specifically examined chronic accumulation in adults or potential seasonal variation in accumulation rates due to changes in reproductive and activity patterns.

After their return to the south-central U. S. from southern Mexico in late April and early May, T. brasiliensis forage extensively in cotton-producing and truck-farming areas. These highly agricultural areas accounted for more than 80% of the domestic use of DDT prior to its U. S. ban in 1972. These chemical residues have remained in the soils and continue to be biomagnified through the food chain even without subsequent applications at any given site (Dustman and Stickel 1969; Clark and Krynitsky 1983). In late September and early October, adults and their young migrate southward into agricultural regions of Central America and Mexico, where DDT and other similar pesticides are still used (Miller 1992).

Nursing young accumulate maximum lifetime residues in their tissues through the milk of their mothers and remain protected from the toxic effects because they contain sufficient body fat for pesticide storage as long as they are nonvolant. Subsequent release of pesticides when fat is mobilized during migration, however, is potentially hazardous. Geluso et al. (1976) have demonstrated that pesticide body burdens in young T. brasiliensis from Carlsbad Caverns were high enough to cause symptoms of poisoning when OCs stored in body fats were mobilized and reached the brain. Mobilization of accumulated pesticides during an individual's first fall migration may be the primary cause for the observed declines at Carlsbad Caverns. Prolonged effects of sublethal doses of pesticides on those individuals that

survive the first migration are unknown. Genotoxic responses to OC pesticide exposure also are unknown.

Vickery Cave is one of approximately seven maternity colonies in northwestern Oklahoma and supports a summer colony of approximately one million T. brasiliensis. Migratory patterns of this population have been studied (Glass 1958, 1959, 1982; Short et al. 1960; Constantine 1967): data collected by Short et al. (1960) and Glass (1958, 1959, 1982) have indicated that Oklahoma populations move through western and central Texas in the spring northerly migration but shift to a more western route through the Carlsbad area on their return to Mexico and Central America in the fall. Perry (1965) and Perry and Herreid (1969) examined population structure and age distribution; however, accurate population estimates and significant changes in population numbers have not been reported.

Because of differences in agricultural practices associated with wheat farming and cattle ranching, it was hypothesized that the northwestern Oklahoma area would not have been exposed to heavy pesticide applications in the United States. These bats should have a significantly lower potential for OC pesticide residue accumulation. Personal communications with personnel from the Oklahoma Department of Agriculture and the Woodward County Agricultural Extension Service have indicated that DDT compounds were used primarily for home pest control in this area. Total quantities applied for commercial agricultural uses are not available. Because

wheat and alfalfa production are threatened to a greater extent by plant than animal pests, applications of herbicides such as 2,4-d (2,4-dichlorophenoxyacetic acid) and atrazine (6-chloro-N-ethyl-N'-[1-methylethyl]-1,3,5-triazine-2,4-diamine) have greatly outweighed the use of insecticidal compounds. Recent insecticide use also has included carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) and parathion (O,O-diethyl O-[4-nitrophenyl] phosphorothioate), synthetic chemicals that are much less persistent in the environment than were OC pesticides (Hay 1981).

Current pesticide use in agricultural areas of southern New Mexico and the Rio Grande River valley of Texas include acephate (N-[methoxy{methylthio}phosphinoyl] acetamide), carbofuran, malathion (diethyl [{dimethoxyphosphinothioyl} thio]butanedioate), and other synthetic organophosphate and pyrethroid compounds which have low environmental persistence (Hay 1981). Problems associated with OC use also have led to the development of stricter application procedures. Herbicides including pendimethalin (N-[1-ethylpropyl]-3,4-dimethyl-2,6-dinitro-benzamine), dimethoate (O,O-dimethyl-S-[2-{methylamino}-2-oxoethyl]phosphorodithioate), and glysophate (N-[phosphoromethyl] glycine) are also being used for botanical controls (Lonnie Mathew, New Mex. Dept. Agr., pers. comm.).

Study Objectives

This study examined changes in OC tissue residues through two migratory/reproductive seasons to evaluate differences in contamination between sites and seasons. Objectives were to: 1) examine and compare identifiable OC pesticides in random samples of adult bats from populations of migratory T. brasiliensis in summer maternity roosts in northwestern Oklahoma and Carlsbad Caverns, New Mexico, using gas chromatography; 2) evaluate fluctuations of pesticide contamination through one summer at Vickery Cave; 3) examine potential genotoxic effects of OC pesticide contamination using the standard bone marrow chromosomal aberration assay (SBMCA) and flow cytometry (FCM) to measure nuclear DNA content variation in spleen and testicular cells; and 4) compare OC pesticide residue results with genotoxicity assays.

Null hypotheses were:

1) the Oklahoma and Carlsbad Caverns populations demonstrate similar types and levels of OC pesticide accumulation;

2) both populations demonstrate similar types and frequencies of chromosomal aberrations in bone marrow and similar coefficients of variation in nuclear DNA content variation for spleen and testicular tissues;

3) observed frequencies of chromosomal aberrancy and nuclear DNA content variation are not correlated with OC

contamination;

4) frequencies of chromosomal aberrancy, nuclear DNA content variation, and concentrations of OC pesticides in body tissues do not vary within or among seasons, geographic locations, and sexes; and

5) OC pesticides in the developing embryo are significantly lower than in the mother's brain and carcass tissues and do not affect fetal development.

This study compares data collected from a declining population documented to be severely contaminated by organochlorine pesticides with a stable population that theoretically should have low contamination levels. It may be possible to directly assess the effects of OC pesticide accumulation on genetic and populational stability in T. brasiliensis and further clarify the role that pesticides have played in observed populational declines of this and other species.

CHAPTER II

ORGANOCHLORINE PESTICIDE ACCUMULATION AND GENOTOXICITY IN MEXICAN FREE-TAILED BATS

Abstract. The summer population of Mexican free-tailed bats (Tadarida brasiliensis) at Carlsbad Caverns, New Mexico, declined from 8.7 million in 1936 to 200,000 in 1974. This decline has been attributed to organochlorine (OC) pesticide contamination and habitat disturbance and has been observed in other populations of this species. This study examined the potential genotoxic effects of OC pesticide contamination on two populations of T. brasiliensis. Pesticide accumulation, frequencies of chromosomal aberrancy, and nuclear DNA content variation in spleen and testicular tissues were monitored in specimens collected from Carlsbad Caverns in southern New Mexico and Vickery Cave in northwestern Oklahoma in 1990 and 1991. Pesticide residues in brain and carcass tissues were identified and quantified using electron capture gas chromatography. Genotoxicity was examined with the standard bone marrow chromosomal aberration assay and flow cytometry. Relationships among pesticide content, observed chromosomal aberrancy, and nuclear DNA content variation were examined for statistical differences and possible correlations. Both populations demonstrated

significant levels of DDE contamination; however, the Carlsbad Caverns population showed consistently higher pesticide loads. Males also demonstrated higher levels than females. No statistical differences in chromosomal aberrancy or nuclear DNA content variation were observed among sexes, sites, or collection periods. Positive correlations were detected between brain and carcass DDE concentrations for all bats examined. A significant negative relationship was found between brain DDE concentration and coefficients of variation in spleen DNA content only for males.

Accumulation of pesticide residues in tissues of wild vertebrates is related to food habits and presumably, is a primary reflection of contamination of the food supply (Dustman and Stickel 1969). Because of similarities in structural, metabolic, and genetic components of all life forms, absolute species specificity in the development of agricultural pesticides is frequently difficult to attain. Improper use of such toxic chemicals may engender biological effects beyond those for which they were originally manufactured and results in expanding concerns about potential environmental health problems resulting from increasing risks of exposure to toxic chemicals (Hutton 1982).

Although a number of studies have demonstrated the accumulation and movement of organochlorine (OC) pesticide residues in the food chain, effects other than acute toxicity (ie., death) have not been adequately addressed. Nor have

the questions of chronic exposure or potential genetic and populational effects been properly examined. This study was designed to address whether observed cytogenetic aberrancy frequencies and flow cytometric analysis of nuclear DNA content variation could be correlated with measured organochlorine pesticide residues in body tissues of the Mexican free-tailed bat, Tadarida brasiliensis.

Organochlorine Pesticides

Due to the aromatic nature of some of these compounds and the lack of hydrophilic groups in all members, OC pesticides are highly hydrophobic, very resistant to chemical degradation, and act as universal poisons. DDT (1,1,1-trichloro-2,2-bis[p-chlorophenyl]ethane) and its residues have been extremely persistent in the environment and tend to bioconcentrate, accumulating in the lipid stores of long-lived mammals and birds (Dustman and Stickel 1969; Fleming et al. 1983; McBee and Bickham 1990).

Use of other OC compounds, including dieldrin (HEOD: 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-exo-1,4 endo-5,8-dimethanonaphthalene), aldrin (1,2,3,4,10,10-hexachloro-1,4,4α,5,6,7,8,8α-hexahydro-endo-1,4-exo-5,8-dimethanonaphthalene), heptachlor (1,4,5,6,7,8,8-heptachloro-3α,4,7,7α-tetrahydro-4,7-methanoindene), and endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-exo-1,4-exo-5,8-dimethanonaphthalene), has also resulted in serious accumulation and persistence problems

(Dustman and Stickel 1969; Fleming et al. 1983; McBee and Bickham 1990). Dieldrin was used as a seed treatment and sheep dip and caused widespread mortality of predatory birds and birds eating newly sown grain (Hayes 1965). Use of DDT was suspended except for limited applications relating to human health in the United States in 1972, followed by federally mandated restrictions or cancellations of other OC products in the United States through the 1980's (EPA 1979). Use of the more toxic products is now severely restricted in most countries; however, OC pesticides are still being used in Mexico and Central America (Miller 1992). However, lindane (γ-BHC: 1,2,3,4,5,6-hexachloro-cyclohexane) is still in limited use due to its lower mammalian toxicity and more rapid breakdown in soils and tissues.

Genotoxicity Assay Background

Genotoxic effects are considered among the most serious of the possible side effects of agricultural chemicals. These effects include heritable genetic diseases, carcinogenesis, reproductive dysfunction, and birth defects (Ames 1979; Hook 1982; Hartman 1983). Carcinogenesis and mutagenesis are of special concern because of the irreversible nature of the processes and the long latency associated with their manifestations. This concern focuses special attention on inherently toxic substances applied to crops or soil, where there is a possibility of direct entry into the food cycle (Waters et al. 1982).

Genetic damage in the form of chromosomal alterations may result from exposure to different agricultural chemicals, for which limited genotoxicity data are available (Palmer et al. 1972; Kelly-Garvert and Legator 1973; Yoder et al. 1973; Georgian 1975; Waters et al. 1982; Galloway et al. 1987). These alterations involve changes in number or structure of entire chromosomes and may include breakage, nondisjunction, translocations, and other forms of structural change. Such change may be observed microscopically with the bone marrow cytogenetic assay or indirectly with flow cytometry. These assays may provide direct evidence that a chemical can alter organized genetic material (Preston et al. 1981; Waters et al. 1982). The bone marrow cytogenetics assay is well defined for both in vitro and in vivo evaluation of chemical genotoxicity in mammalian systems (Preston et al. 1981). Flow cytometry has also been used as a mechanism for detecting and quantitatively measuring chromosomal damage induced by physical and chemical agents (Otto and Oldiges 1980; Otto et al. 1981a, 1981b; Fantes et al. 1983; Lovett et al. 1984; Hacker-Klom et al. 1989).

The result of cell death associated with genotoxicity depends upon the type of tissue involved. If cell death occurs in an embryo, it will probably result in some type of malformation or death of the embryo (Kalter 1971). If it occurs in somatic or germinal cells, it will result in nothing more than the loss of the cell. If the cell survives but is damaged, the damage could result in a tumor for a

somatic cell or mutation if a germinal cell is involved. Chromosome damage, specifically single chromatid breaks or exchange figures, is probably of great mutagenic significance because they are incorporated in the cells that are most likely to survive and carry an alteration of the genetic material (Palmer et al. 1972).

In a population of cells exposed to a clastogenic compound, increased incidence of genetic damage would be expected to result in a greater coefficient of variation (CV) in mean nuclear DNA content in daughter cells following mitosis (Otto and Oldiges 1980). Continued exposure to a clastogen would, therefore, be expected to produce cells with a significantly higher CV as compared to a normal population of cells. If environmental conditions place greater selective pressure on cells with an increased or decreased amounts of genetic material as compared to the normal cell line, then the resulting cell population may actually demonstrate a lower CV than the original cell line (Otto et al. 1981b).

Species and Site Background

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the U. S. National Park Service about population declines at Carlsbad Caverns National Park (Geluso et al. 1976, 1981). The size of the summer population has declined from an estimated 8.7 million in 1936 to approximately 200,000 in 1973 (Altenbach et al. 1979). The 1991 population was estimated at 700,000 individuals (David Ek, Carlsbad Caverns National Park, pers. comm.). Body burdens of OC pesticides during the years of decline are not known, but levels of p,p'-DDE (1,1-dichloro-2,2-bis[p-chlorophenyl]ethylene) present at Carlsbad Caverns have been high enough to be potentially hazardous to young bats, primarily during their first migration (Geluso et al. 1981). Although other maternity roosts have been examined for pesticide accumulation in young bats and have demonstrated elevated levels of OC pesticide residues (Geluso et al. 1981), no Oklahoma populations have ever been examined for the presence of pesticides. No studies have specifically examined chronic accumulation in adults or potential seasonal variation in accumulation rates due to changes in reproductive and activity patterns.

After their return to the south-central U. S. from southern Mexico and Central America in late April and early May, T. brasiliensis forage extensively in cotton-producing and truck-farming areas. Application of pesticides to these highly agricultural areas accounted for more than 80% of the domestic use of DDT prior to its U. S. ban in 1972. These chemical residues have remained in the soils and continue to

be biomagnified through the food chain even without subsequent applications at any given site (Dustman and Stickel 1969; Clark and Krynitsky 1983). In late September and early October, adults and their young migrate southward into agricultural regions of Central America and Mexico, where DDT and other similar pesticides are still used (Miller 1992).

Pesticide use in agricultural areas of southern New Mexico and the Rio Grande River valley of Texas include acephate (N-[methoxy{methylthio}phosphinoyl] acetamide), carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate), malathion (diethyl [{dimethoxyphosphinothioyl} thio]butanedioate), and other synthetic organophosphate and pyrethroid compounds that have low environmental persistence (Hay 1981). Problems associated with OC use also have led to the development of much stricter application procedures. Herbicides including pendimethalin (N-[1-ethylpropyl]-3,4-dimethyl-2,6-dinitro-benzamine), dimethoate (O,O-dimethyl-S-[2-{methylamino}-2-oxoethyl]phosphorodithioate), and glysophate (N-[phosphoromethyl] glycine) are also being used for botanical controls (Lonnie Mathew, New Mex. Dept. Agr., pers. comm.).

Nursing young accumulate maximum lifetime residues in their tissues through the milk of their mothers and remain protected from the toxic effects because they contain sufficient body fat for pesticide storage as long as they are nonvolant. Subsequent release of pesticides when fat is

mobilized during migration, however, is potentially hazardous. Geluso et al. (1976) have demonstrated that pesticide body burdens in young T. brasiliensis from Carlsbad Caverns were high enough to cause symptoms of poisoning such as nervous dysfunction and/or death when OCs stored in body fats were mobilized and reached the brain. Mobilization of DDT metabolites during an individual's first fall migration may be the primary cause for the observed declines at Carlsbad Caverns. Prolonged effects of sublethal doses of pesticides on those individuals that survive the first migration are unknown. Genotoxic responses to OC pesticide exposure also are unknown.

Vickery Cave, one of approximately seven maternity colonies in northwestern Oklahoma, supports a summer colony of approximately one million bats. This population has been studied for migratory patterns (Glass 1958, 1959, 1982; Short et al. 1960; Constantine 1967). Data collected by Short et al. (1960) have indicated that Oklahoma populations move through western and central Texas in the spring northerly migration but shift to a more western route through the Carlsbad area on their return to Mexico and Central America in the fall. Perry (1965) and Perry and Herreid (1969) examined population structure and age distribution; however, accurate population estimates and significant changes in population numbers have not been reported.

Because of differences in agricultural practices associated with wheat farming and cattle ranching, it was

postulated that northwestern Oklahoma would not have been exposed to heavy pesticide applications. These bats should have a significantly lower potential for OC pesticide residue accumulation during their summer residence at Vickery Cave. Personal communications with personnel from the Oklahoma Department of Agriculture and the Woodward County Agricultural Extension Service have indicated that DDT compounds were used primarily for home pest control. Total quantities applied for commercial agricultural uses are not available. Because wheat and alfalfa production are threatened to a greater extent by plant than animal pests, applications of herbicides such as 2,4-d (2,4-dichlorophenoxyacetic acid) and atrazine (6-chloro-N-ethyl-N'-[1-methylethyl]-1,3,5-triazine-2,4-diamine) have greatly outweighed the use of insecticidal compounds. Insecticide use has focused on carbofuran and parathion (O,O-diethyl O-[4-nitrophenyl] phosphorothioate), synthetic chemicals which are much less persistent in the environment than OC pesticides (Hay 1981).

Study Objectives

This study examined changes in OC tissue residues through two migratory/reproductive seasons to evaluate differences in contamination between sites and seasons. Objectives were to: 1) examine and compare identifiable OC pesticides in random samples of adult bats from populations of migratory T. brasiliensis in summer maternity roosts in

northwestern Oklahoma and Carlsbad Caverns, New Mexico, using gas chromatography; 2) evaluate fluctuations of pesticide contamination through one summer at Vickery Cave; 3) examine potential genotoxic effects of OC pesticide contamination using the standard bone marrow chromosomal aberration assay (SBMCA) and flow cytometry (FCM) to measure nuclear DNA content variation in spleen and testicular cells; and 4) compare OC pesticide residue results with genotoxicity assays.

Null hypotheses were:

1) the Oklahoma and Carlsbad Caverns populations demonstrate similar types and levels of OC pesticide accumulation;

2) both populations demonstrate similar types and frequencies of chromosomal aberrations in bone marrow and similar coefficients of variation in nuclear DNA content variation for spleen and testicular tissues;

3) observed frequencies of chromosomal aberrancy and nuclear DNA content variation are not correlated with OC contamination; and

4) frequencies of chromosomal aberrancy, nuclear DNA content variation, and concentrations of OC pesticides in body tissues do not vary within or among seasons, geographic locations, and sexes.

This study compares data collected from a declining population documented to be severely contaminated by organochlorine pesticides with a stable population that

theoretically should have low contamination levels. It may be possible to directly assess effects of OC pesticide accumulation on genetic and populational stability in T. brasiliensis and further clarify the role that pesticides have played in observed populational declines of this and other species.

MATERIALS AND METHODS

Specimen Collection

Specimens were collected using hoop nets or a single-tier mist net following protocols established by Geluso et al. (1976). Animals were caught in the entrances of Carlsbad Caverns and Vickery Cave as they exited for nightly feeding excursions. To assess levels of chronic exposure to OC pesticides and potential genetic effects observable with SBMCA and FCM, bats were classified into subjective age classes by examining canine tooth wear, with only those individuals judged as belonging to the oldest age class retained. Aging protocols were adapted from guidelines presented by Anthony (1988). Females with canines worn approximately half way to the gums were retained: maximum wear in males was a flattening of tips of the canines. Males did not exhibit wear equivalent to that of females in either population, which supports the hypothesis that older males do not migrate north but remain on their winter range throughout the year (Caire et al. 1989). Specific ages could not be

assigned because no known-age series of specimens exist for this species. Tooth wear, complete epiphyseal-diaphyseal fusion of phalangeal joints, and pelage wear and coloration were used to verify that males were at least 9 - 12 months old and had survived at least one migration.

During summer 1990, 12 adult females were collected at 4-week intervals from Vickery Cave and returned to the laboratory. In August 1990 and May and August 1991, 12 adult males and females were collected from both Carlsbad Caverns and Vickery Cave (Appendix).

Each animal was randomly assigned a permanent identification number to eliminate possible bias resulting from knowledge of collection history during analyses. Animals were sacrificed by cervical dislocation within 24 hr of capture, and tissues were collected and processed for individual analyses.

Pesticide Content

Extraction: Analytical procedures for determining OC pesticide content followed Geluso et al. (1981) as modified from Peterson et al. (1976). Pesticide extraction procedures were applied to brain and carcass tissues for each specimen collected. Livers, spleens, and kidneys from 18 randomly selected bats also were analyzed for pesticide residues as a method of determining quantities of pesticide residues lost to removal of these organs for other analyses.

For sample preparation, wings were removed at the

shoulder, and feet were removed at the ankle. Specimens were skinned, with any fat adhering to the skin removed and placed with the body. The stomach, small intestine, and large intestine were removed to avoid analyzing insect remains or other contaminants in the gastrointestinal tract. The liver, spleen, one kidney, and both testes from males were removed for other analyses. Embryos and placenta, when present, were removed from the uterus, which remained with the mother. The head was removed at the first cervical vertebra and the brain carefully removed for analyses. Major cranial musculature was removed from the skull and placed with the carcass for analysis. Thus, carcass refers to the entire body minus skin, wings, feet, gastrointestinal tract, liver, spleen, embryo, testes, one kidney, skull, and brain.

Following Geluso et al. (1976, 1981), immediately after removal, brains were placed in tared test tube, weighed, and mixed with five times their weight of anhydrous sodium sulfate. Carcass also were weighed, placed in jars with five times their weight of anhydrous sodium sulfate, frozen, homogenized individually in a blender, and transfered to flasks. An extraction solvent (40 ml for carcass; 3ml for brain) consisting of 20% acetone in isooctane (v/v) was placed in each test tube and flask. Flasks were shaken for 10 min and test tubes sonicated in an ultrasonic cleaner for 15 min. Both were agitated periodically for 24 hr and allowed to stand an additional 24 hr. One ml of brain extract and 3 ml of carcass extract were evaporated in a

tared vial to determine fat content. Remaining extracts were retained for pesticide identification and quantification. The latter were cleaned prior to analysis by the addition of activated Florisil and concentrated into a final volume of isooctane for gas chromatography. Liquid-liquid partitioning was omitted because it has been found to be unnecessary (Geluso et al. 1981).

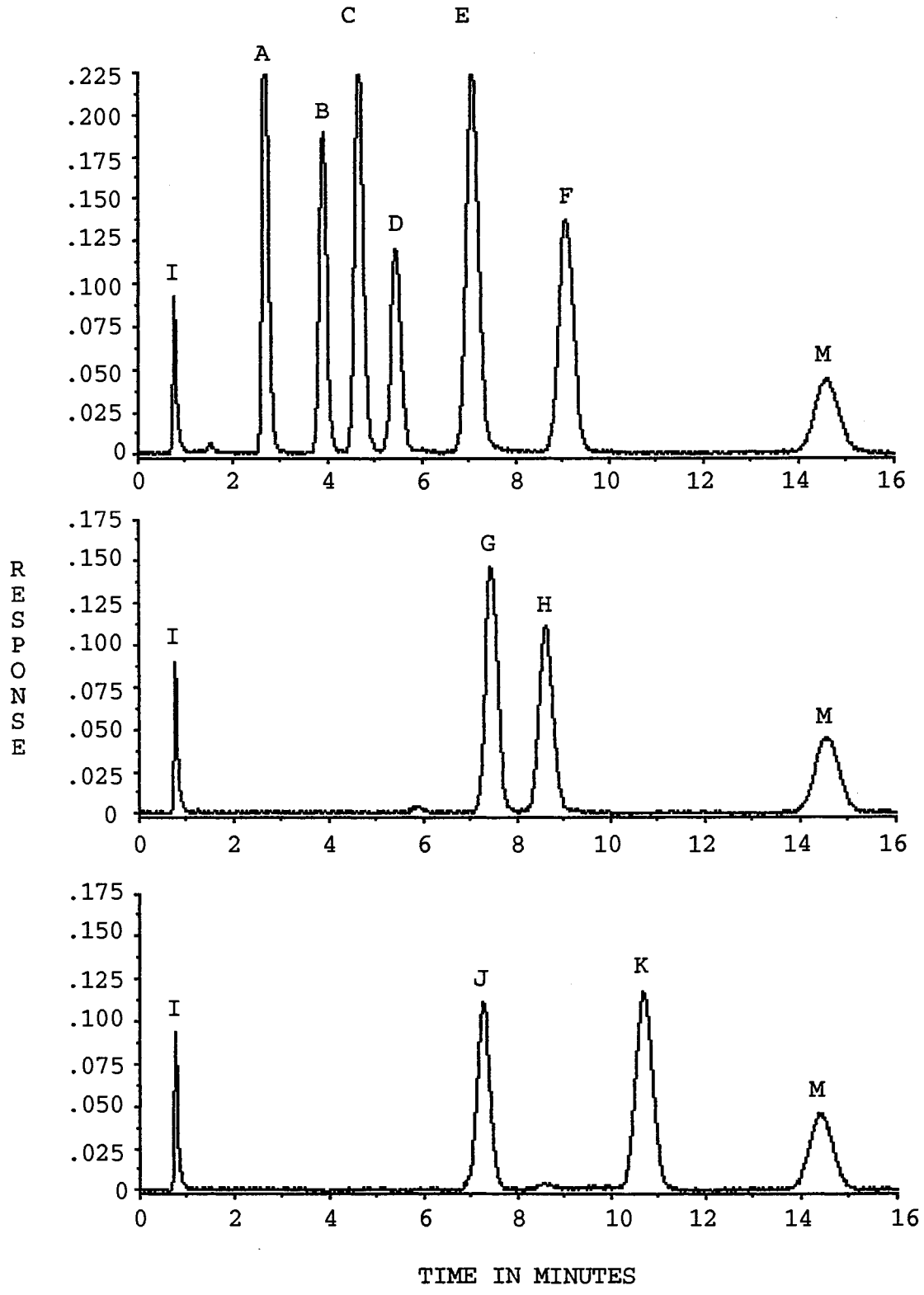
Livers, spleens, kidneys, and testes from 18 random bats also were prepared following similar procedures. This was done to estimate pesticide loss resulting from the removal of selected organs, as well as to provide information on the distribution of contaminants within different body tissues. Quality control/quality assurance protocols included extractions of pure anhydrous sodium sulfate as procedural blanks and spiking carcass and brain samples of laboratory-reared Peromyscus maniculatus with known quantities of each pesticide to determine extraction efficiencies. Results for all analyses were reported in µg/g wet weight.

Gas Chromatography: Samples were analyzed for identifiable OC pesticides including p,p'-DDE, o,p'- and p,p'-DDT, o,p'- and p,p'-DDD (1,1-dichloro-2,2-bis[p-chlorophenyl]ethane), aldrin, dieldrin, lindane, heptachlor, heptachlor epoxide (1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene), and PCBs using Tracor 560 and Perkin Elmer Sigma 1B gas chromatographs. Instrument settings and operating conditions for the Tracor 560 were: column packing 5% OV-17 and 1.95% QF-1, oven T

300°C, injection port T 250°C, detector T 300°C, carrier gas flow rate 40 ml/min. Perkin Elmer conditions and settings were: column packing 3% OV-1; oven T 230°C; injection port T 275°C; detector T 350°C; carrier gas flow rate 50 ml/min; detecter purge rate 40 ml/min. Each instrument was configured with a single electron capture detector and dissimilar glass columns (1.8 m X 0.6 mm id). All samples were analyzed simultaneously on both instruments to confirm identified and quantified residues. Peaks in chromatograms were identified by comparing retention times of unknown peaks with standard compounds. Peaks with retention times that failed to match standard chromatograms within a 5% window on both instruments were not identified. Instrument detection limits were: 0.010 µg/g for lindane, heptachlor, and heptachlor epoxide; 0.015 µg/g for aldrin; 0.020 µg/g for p,p'-DDE, o,p'- and p,p'- DDD, p,p'-DDT, and dieldrin; and 0.025 µg/g for o,p'-DDT.

Data acquisition was conducted using the Maxima (Dionyx, Millipore Corporation) computer interface system. A 2-µl spike of methoxychlor (2,2-bis[p-methoxyphenyl]-1,1,1-trichloroethane) was added to all brain and organ samples and 0.4 µl were added to all carcasses prior to injection for use as internal standards. Resulting methoxychlor peaks were used to quantify identified OC residues based on observed peak areas for the methoxychlor spike and a series of standards obtained immediately prior to analysis of tissue samples (Fig. 1).

Fig. 1. Standard gas chromatographs of organochlorine pesticides used to identify and quantify detected peaks in tissue residue analyses. I: isooctane solvent; A: Lindane; B: Heptachlor; C: Aldrin; D: Heptachlor Epoxide; E: p,p'-DDE; F: o,p'-DDT; M: Methoxychlor internal standard; G: Dieldrin; H: p,p'-DDD; J: o,p'-DDD; K: p,p'-DDT.


C
E
A
.225
.200
B
.175
.150
F
D
.125
I
.100
.075
M
.050
.025
0
0
2
4
6
8
10
12
14
16
.175
G
.150
H
.125
I
.100
M
.075
.050
.025
0
0
2
4
6
8
10
12
14
16
RESPONSE
.175
.150
J
K
.125
I
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M
.050
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0
0
2
4
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12
14
16
TIME IN MINUTES

Analyses of P. maniculatus carcass and brain tissues spiked with standard compounds demonstrated a greater efficiency in residue recovery for carcasses than for brains. Reduced efficiencies in brain tissue recovery were attributed to smaller volumes of tissue being extracted. Efficiencies for carcasses ranged from 88 percent for dieldrin and p,p'-DDE to 116 percent for o,p'-DDT. Brain recoveries were consistently lower than carcasses and ranged from 50 percent for heptachlor epoxide to 100 percent for aldrin. Recovery of p,p'-DDE from brain tissue was 74 percent.

Differences in pesticide levels in bats within and between Vickery Cave and Carlsbad Caverns were analyzed using three-factor ANOVA. Because pesticide data were positively skewed, data were log(x + 1) transformed (Clark and Lamont 1976, Clark et al. 1978, Geluso et al. 1976, 1981) prior to parametric analyses. The Duncan new multiple range test was applied to significant ANOVA results to examine differences among means. Probability values ≤ 0.05 were considered statistically significant for all comparisons. Samples from individual collection periods were also analyzed with three-factor ANOVA and Duncan multiple range tests to determine significant differences between sexes for carcass and brain weight and percent fat differences.

Standard Bone Marrow Chromosomal Aberrancy

Protocol for chromosomal analyses followed McBee et al. (1987). Both humeri were excised and cleaned of all muscle

tissue. Epiphyses were clipped from both ends and the marrow flushed into centrifuge tubes with warm (37°C) 0.075 M potassium chloride (KCl) solution. Marrow clumps were dispersed by gentle agitation and the cell suspensions were incubated at 37°C for 27 min. Swollen metaphase cells were centrifuged out of suspension (3000 rpm, 1.5 min, IEC clinical centrifuge), the supernatant discarded, and the pellet resuspended in modified Carnoy's fixative (methanol:glacial acetic acid, 3:1). Following three successive washes of fixative, the cell suspension was resuspended in approximately 1.0 ml of fixative. Three or four drops of suspension were then placed on a clean, dry microscope slide and immediately ignited with a flame. Extinguished slides were air dried and stained for 10 minutes in a 2% Giemsa-phosphate buffer solution (pH = 7.0). A sufficient number of slides was prepared for each individual to perform the analyses; the remaining cell suspension was stored at -20°C.

Fifty complete (2N = 48) metaphase spreads from each animal were scored for observable chromosome and chromatid aberrations (McBee et al. 1987) using an Olympus BH-2 light microscope. Chromosomal aberrations with definite breaks were recorded and documented with an Olympus PM-10AD photomicrographic system. Chromatid and chromosome breaks (breaks in a single chromatid and breaks in identical places in sister chromatids, respectively) and acentric fragments (chromosomes with no apparent centromere) were scored as

single, unrepaired lesions. Chromosome rearrangements such as translocation figures, ring chromosomes, and dicentric chromosomes were scored as resulting from at least two lesions. Data were converted to number of lesions per metaphase cell and number of aberrant cells per individual.

Results of SBMCA were compared among sampling periods and collection sites. Statistical analyses of cytogenetic data included three-factor ANOVA and the Duncan new multiple range test to examine differences among means. Levels of significance were 0.05.

Flow Cytometry

Methods for flow cytometric analyses followed those of Otto (1990) and Otto et al. (1981b). The spleen from each individual was thawed and thoroughly minced using a tissue-tearor® (Biospec, Inc.) in a pretreatment solution of 0.2M citric acid with 0.5% Tween 20. Testes were minced in a 0.1M citric acid/0.5% Tween 20 pretreatment solution with a tissue-tearor® to produce a cell suspension. Following incubation at room temperature for 20 minutes, suspensions were centrifuged at 200g for 10 minutes, the supernatent removed, and pellets resuspended in cold 70% ethanol. Cell suspensions were then filtered through 37µm filter cloth (Small Parts, Inc.) and stored in 70% ethanol at -20°C for subsequent analyses.

At the time of staining, samples were thawed and an aliquant centrifuged for 10 min at 200g. After removal of

the supernatant, pellets were resuspended in 0.1 ml of pretreatment solution (spleens, 0.2M; testes, 0.1M) and allowed to incubate at room temperature for 10 min. A minimum of 2 ml of disodium hydrogen phosphate containing 5μM of DAPI (4,6-diamidino-2-phenylindole), a stable, DNA proportional binding dye, was added to the ethanol-fixed cell suspensions. Cells were allowed to stain overnight in the dark at room temperature before being analyzed. Nuclear fluorescence was measured using a Partec PAS II microscope-based flow cytometer. The system was aligned and optimized using calf thymocyte nuclei at the beginning of each session and whenever system drift occurred. Epi-illumination was accomplished with a mercury vapor bulb and filter cube with a wavelength range of 340-380 nm.

Samples were number coded and analyzed randomly. A minimum of 20,000 cells was counted for each of five replicates per individual for both spleen and testicular tissues. Coefficients of variation in mean nuclear DNA content within and among samples were examined for spleen and testicular cells. The 1C peak, representing elongated spermatids, also was counted for testicular samples.

Flow histograms were analyzed using the Multicycle computer package (Phoenix Flow Systems) to calculate CVs for each sample run. Data were compared among sexes, sampling periods and collection sites using parametric three-factor ANOVA. Levels of significance were 0.05.

Correlations Among Assays

Relationships among OC pesticide accumulation, SBMCA, and FCM data were examined using simple linear correlation. Data were compared among collection periods, sexes, and sites to assess pesticide exposure on the genetic stability of this species. If significant correlations were detected, correlation coefficients and slopes were determined ($P \leq 0.05$).

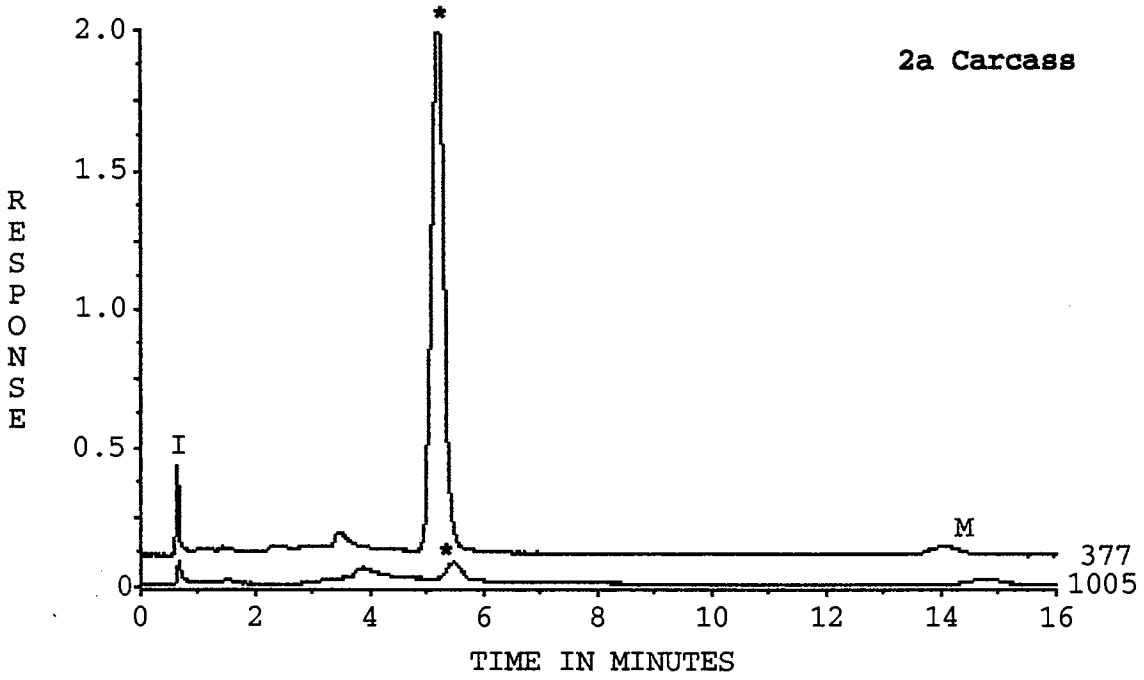
RESULTS

Two hundred *T. brasiliensis* were collected from Carlsbad Caverns and Vickery Cave (Appendix). All individuals were examined for OC pesticide content and nuclear DNA content variation. A total of 177 was examined for chromosomal aberrations and 18 for pesticide content in organ systems.

Pesticide Accumulation

Gas chromatographs of carcass and brain residues for *T. brasiliensis* (Fig. 2) were analyzed for OC pesticide content. Means and standard deviations in carcass weight and percent fat (Table 1), brain weight and percent fat (Table 2), and geometric means and ranges in carcass and brain concentrations of *p,p'*-DDE (Table 3) were determined for all specimens examined for pesticide content. Distribution patterns of brain and carcass weights and percent fat were also plotted for comparison (Figures 3 and 4, respectively).

Fig. 2. Comparative gas chromatographs of organochlorine pesticide residues in carcass (2a) and brain (2b) tissues from female Tadarida brasiliensis OK00377 (Vickery Cave, Oklahoma) and OK01005 (Carlsbad Caverns, New Mexico). I: isooctane solvent; *: p,p'-DDE; M: Methoxychlor internal standard.


2a Carcass
RESPONSE
2.0
1.5
1.0
0.5
0
I
M
*
377
1005
0
2
4
6
8
10
12
14
16
TIME IN MINUTES

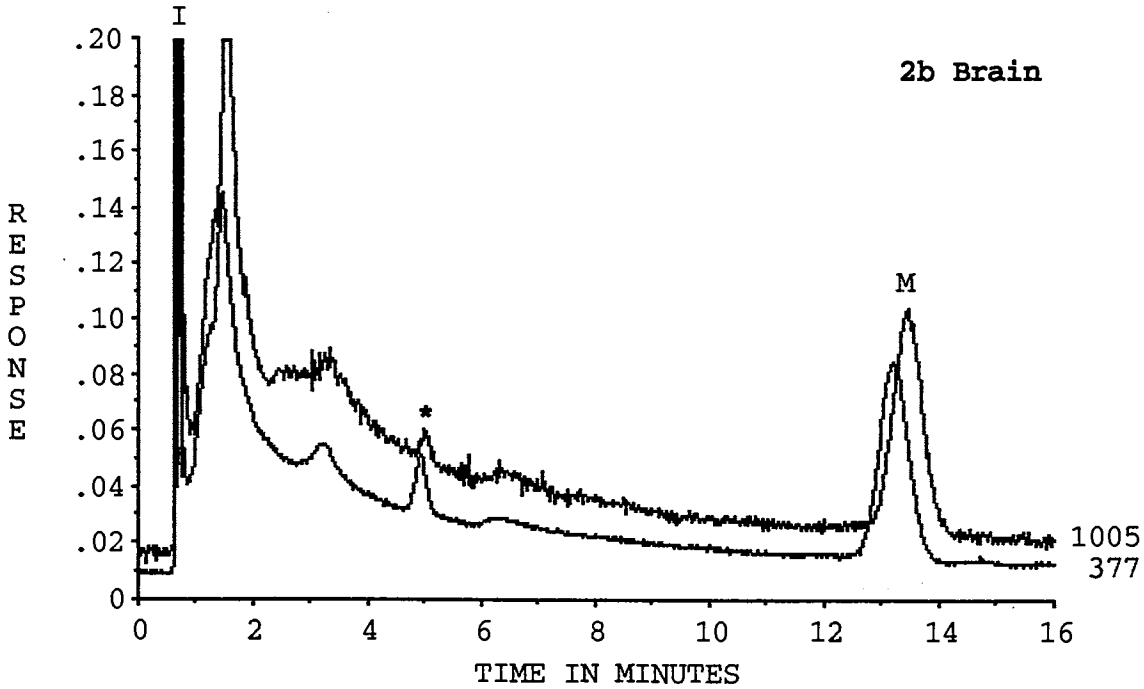

2b Brain
RESPONSE
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
0
I
M
*
1005
377
0
2
4
6
8
10
12
14
16
TIME IN MINUTES

Table 1. Mean carcass weights (in grams), percent fat, and standard deviations for Tadarida brasiliensis collected from Vickery Cave, Oklahoma, and Carlsbad Caverns, New Mexico. Data are for 12 individuals unless otherwise noted.

	Sex	Weight		Percent Fat	
		Vickery Cave	Carlsbad Caverns	Vickery Cave	Carlsbad Caverns
1990					
May[a]	F	4.932 ± 0.609		6.12 ± 2.43	
June[b]	F	6.090 ± 0.544		8.72 ± 2.33	
July	F	5.377 ± 0.619		14.90 ± 3.75	
August	F	5.001 ± 0.448	5.031 ± 0.335	6.58 ± 1.59[c]	6.85 ± 2.58
	M	4.696 ± 0.327	4.833 ± 0.468	7.54 ± 1.43[c]	6.51 ± 2.47
1991					
May	F	5.951 ± 0.465[d]	5.833 ± 0.358[e]	10.71 ± 3.09[f]	12.78 ± 3.12[g]
	M	4.876 ± 0.356[d]	4.982 ± 0.437[e]	5.37 ± 1.82[f]	7.85 ± 3.06[g]
August	F	5.398 ± 0.451[h]	5.202 ± 0.296	9.57 ± 1.17[i]	6.99 ± 1.49[j]
	M	4.789 ± 0.414[h]	5.238 ± 0.383	8.38 ± 1.78[i]	10.40 ± 3.84[j]

[a] N = 10; [b] N = 11

[c-j] significant differences between sexes within site and collection period.

Table 2. Mean brain weights (in grams), percent fat, and standard deviations for Tadarida brasiliensis collected from Vickery Cave, Oklahoma, and Carlsbad Caverns, New Mexico. Data are for 12 individuals unless otherwise noted.

		Weight		Percent Fat	
	Sex	Vickery Cave	Carlsbad Caverns	Vickery Cave	Carlsbad Caverns
1990					
May[a]	F	0.169 ± 0.010		8.02 ± 0.78	
June[b]	F	0.181 ± 0.014		8.32 ± 1.86	
July	F	0.171 ± 0.017		7.67 ± 0.95	
August	F	0.185 ± 0.074[c]	0.154 ± 0.021[d]	7.72 ± 0.81	7.88 ± 0.94
	M	0.185 ± 0.019[c]	0.186 ± 0.014[d]	7.17 ± 1.27	8.36 ± 0.88
1991					
May	F	0.180 ± 0.018	0.168 ± 0.017[e]	8.20 ± 0.79[f]	9.45 ± 2.58
	M	0.192 ± 0.017	0.189 ± 0.009[e]	8.68 ± 0.89[f]	7.80 ± 1.04
August	F	0.178 ± 0.017	0.173 ± 0.013	8.04 ± 1.06	9.43 ± 1.24[g]
	M	0.187 ± 0.013	0.183 ± 0.017	7.80 ± 1.33	7.85 ± 0.89[g]

[a] N = 10; [b] N = 11

[c-g] significant differences between sexes within site and collection period.

Table 3. Geometric means and ranges in p,p'-DDE concentrations (µg/g wet weight) in carcass and brain tissues for Tadarida brasiliensis collected from Vickery Cave, Oklahoma, and Carlsbad Caverns, New Mexico. Data are for 12 individuals unless otherwise noted.

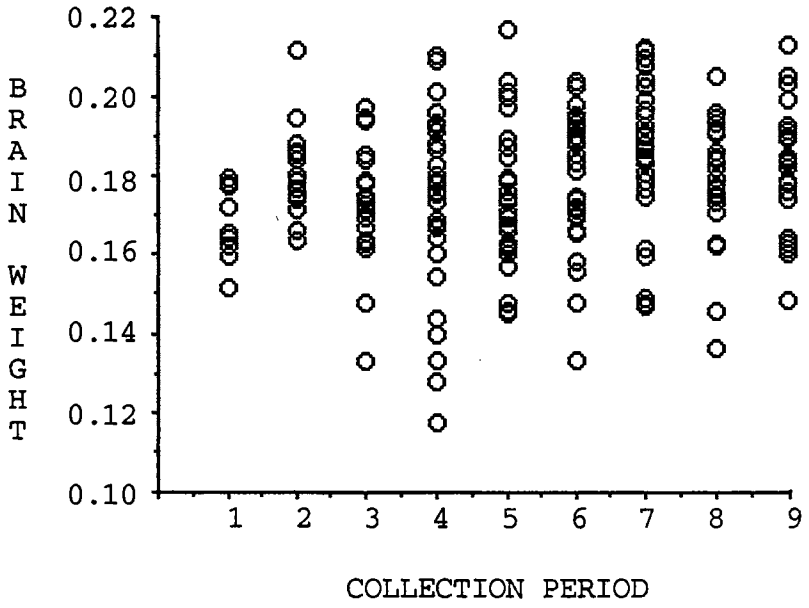
		Carcass		Brain	
	Sex	Vickery Cave	Carlsbad Caverns	Vickery Cave	Carlsbad Caverns
1990					
May[a]	F	1.706 (0.398 - 99.315)		0.042 (0.010 - 7.295)	
June[b]	F	0.252 (0.043 - 0.758)		0.014 (0.010 - 0.026)	
July	F	0.303 (0.010 - 3.022)		0.019 (0.010 - 0.099)	
August	F	0.045 (0.010 - 0.228)[c]	2.455 (0.423 - 9.342)[c]	0.013 (0.010 - 0.066)	0.018 (0.010 - 0.236)[h]
	M	0.899 (0.437 - 13.802)	4.682 (1.329 - 34.693)	0.023 (0.010 - 0.156)	0.055 (0.010 - 0.226)[h]
1991					
May	F	0.578 (0.010 - 2.550)[d]	1.845 (0.010 - 7.559)[d]	0.021 (0.010 - 0.110)[i]	0.043 (0.010 - 0.271)
	M	0.891 (0.010 - 2.709)[e]	4.617 (2.098 - 10.473)[e]	0.074 (0.010 - 1.001)[i]	0.036 (0.010 - 0.475)
August	F	0.067 (0.010 - 0.208)[f]	4.687 (0.010 - 14.031)[f,j]	0.016 (0.010 - 0.102)	0.049 (0.010 - 0.484)
	M	1.301 (0.550 - 21.883)[g]	14.261 (2.911 - 37.474)[g,j]	0.057 (0.010 - 1.000)	0.079 (0.010 - 0.629)

[a] N = 10; [b] N = 11

[c-g] significant difference between sites within sex and collection period.

[h-j] significant difference between sexes within site and collection period.

Fig. 3. Observed distribution of brain and carcass weights (in grams) for pooled male and female samples of Tadarida brasiliensis collected from Vickery Cave, Oklahoma (VC), and Carlsbad Caverns, New Mexico (CC). Collection periods: 1, May 1990 VC; 2, June 1990 VC; 3, July 1990 VC; 4, August 1990 CC; 5, August 1990 VC; 6, May 1991 CC; 7, May 1991 VC; 8, August 1991 CC; 9, August 1991 VC.


BRAIN WEIGHT
0.22
0.20
0.18
0.16
0.14
0.12
0.10
1
2
3
4
5
6
7
8
9
COLLECTION PERIOD

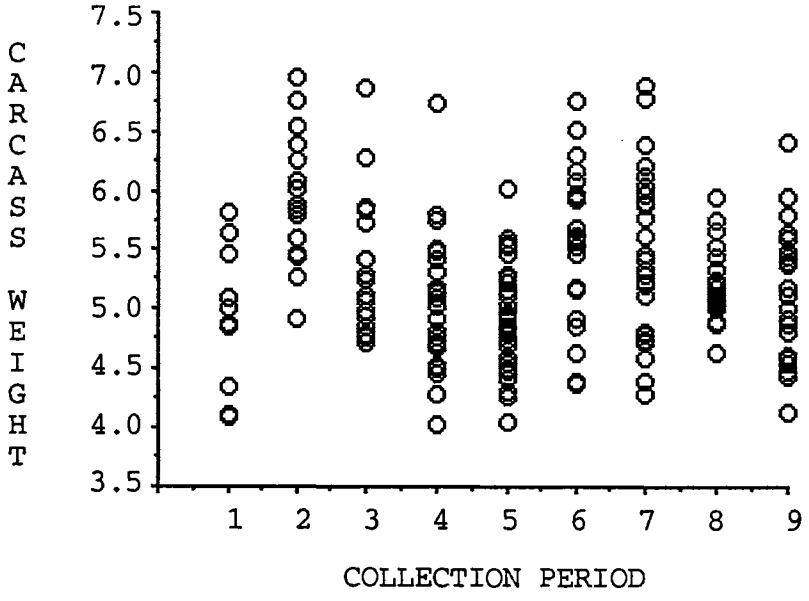
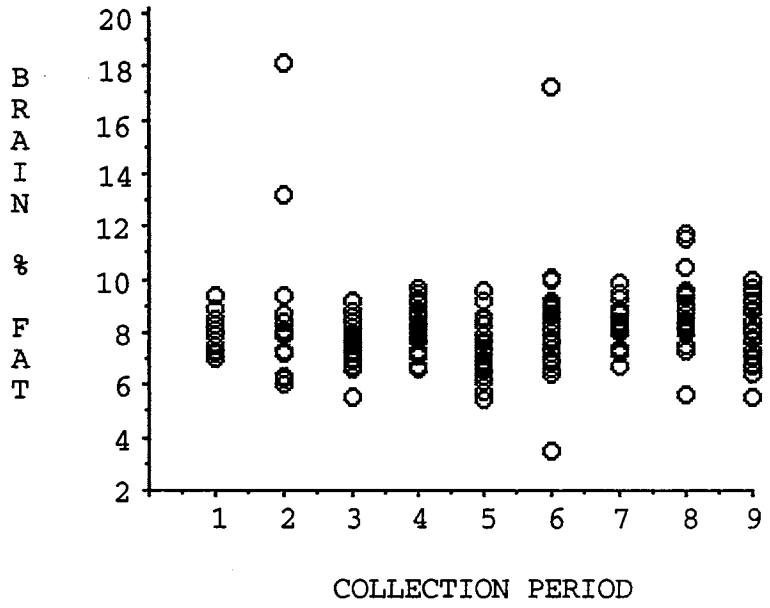
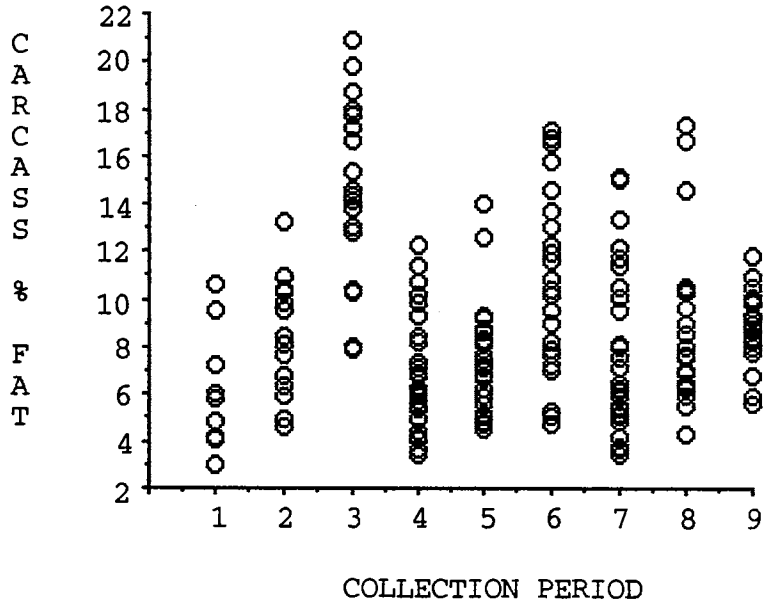

CARCASS WEIGHT
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
1
2
3
4
5
6
7
8
9
COLLECTION PERIOD

Fig. 4. Observed distribution of percent fat in brains and carcasses for pooled male and female samples of Tadarida brasiliensis collected from Vickery Cave, Oklahoma (VC), and Carlsbad Caverns, New Mexico (CC). Collection periods: 1, May 1990 VC; 2, June 1990 VC; 3, July 1990 VC; 4, August 1990 CC; 5, August 1990 VC; 6, May 1991 CC; 7, May 1991 VC; 8, August 1991 CC; 9, August 1991 VC.


BRAIN % FAT
20
18
16
14
12
10
8
6
4
2
1 2 3 4 5 6 7 8 9
COLLECTION PERIOD


CARCASS % FAT
22
20
18
16
14
12
10
8
6
4
2
1 2 3 4 5 6 7 8 9
COLLECTION PERIOD

Other compounds, including *p,p'*-DDT, lindane, and heptachlor, were detected in several specimens from both sites; however, the low frequencies of occurrence made statistical comparisons impossible.

In a three-way ANOVA of carcass weights, females were significantly heavier than males ($P = 0.0001$), with average carcass weights of 5.470 g for females and 4.973 g for males (Table 1). Significant sex-by-collection period and sex-by-site interactions were also found. May samples were heavier than both August samples, which also differed significantly. Carlsbad individuals also were heavier than their corresponding Vickery samples. A significant three-way interaction among site, sex and collection period was determined for percent carcass fat ($P = 0.0108$; Table 1). Fat content was greater in females from both populations as compared to males ($P = 0.0116$). Samples from 1991 also were higher in fat content as compared to 1990 ($P = 0.0001$).

Males, contrary to carcass weight analyses, had significantly heavier brains ($P = 0.0001$), with an average weight of 0.187 g vs. 0.171 g for females (Table 2). Vickery Cave individuals also had heavier brains than Carlsbad Caverns. August 1991 brains did not differ from either August 1990 or May 1991 samples: heaviest brains were found in the May 1991 sample. Fat content in brain tissue demonstrated a significant sex-by-site-by-collection period interaction ($P = 0.0053$; Table 2). Fat content was higher in females than males ($P = 0.0185$), Carlsbad vs. Vickery ($P =$

0.0140), and 1991 vs. 1990 collections (P = 0.0115).

Only p,p'-DDE was detected in sufficient numbers of individuals for adequate statistical analyses (Table 3). Higher concentrations of p,p'-DDE were detected in males as compared to females (P = 0.0001) and in Carlsbad Caverns bats as compared to Vickery Cave (P = 0.0001). Pesticide levels were also higher in August 1991 as compared to August 1990 and May 1991 (P = 0.0001). ANOVA results also showed significant sex-by-site, sex-by-collection period, and site-by-collection period interactions among the data.

The four sets of females collected from Vickery Cave in 1990 demonstrated significant difference (P = 0.0042; Table 3) in carcass p,p'-DDE content. Concentrations of p,p'-DDE were highest in May followed by July, June, and August. Brain p,p'-DDE concentrations did not differ (P = 0.2034).

Carcass, brain, and organ weights (Table 4), percent fat (Table 5) and geometric means and ranges in p,p'-DDE concentrations (Table 6) were determined for eighteen specimens to examine distribution of pesticides among body tissues. No differences in weights, percent fat, or p,p'-DDE were determined among samples. Significant correlation coefficients (Table 7) were determined for p,p'-DDE concentrations among all tissues examined, indicating a significant relationship in the distribution of p,p'-DDE among organ systems. On a weight basis, livers contained between 2 and 5 percent of the total body burden of p,p'-DDE; the remainder was in the carcass.

Table 4. Means and ranges in weight (in grams) of different body tissues of Tadarida brasiliensis collected from Vickery Cave, Oklahoma (VC), and Carlsbad Caverns, New Mexico (CC). N = 3 for each collection.

	Carcass	Brain	Liver	Kidney	Spleen	Testes
June 1990	5.965	0.171	0.460	0.056	0.049	
(VC)	5.466 - 6.389	0.166 - 0.174	0.394 - 0.584	0.048 - 0.063	0.030 - 0.095	
July 1990	4.807	0.166	0.371	0.047	0.080	
(VC)	4.773 - 4.860	0.163 - 0.171	0.364 - 0.377	0.043 - 0.052	0.054 - 0.119	
August 1990	5.993	0.190	0.507	0.066	0.045	
(CC)	5.520 - 6.726	0.176 - 0.201	0.487 - 0.528	0.063 - 0.068	0.025 - 0.061	
August 1990	5.588	0.187	0.349	0.060	0.049	0.013
(VC)	5.231 - 6.003	0.173 - 0.217	0.340 - 0.356	0.055 - 0.064	0.041 - 0.066	0.011 - 0.014
May 1991	6.211	0.172	0.361	0.058	0.036	
(CC)	5.63 - 6.756	0.158 - 0.188	0.318 - 0.411	0.054 - 0.059	0.022 - 0.054	
May 1991	6.163	0.174	0.376	0.060	0.036	
(VC)	5.893 - 6.387	0.148 - 0.193	0.347 - 0.411	0.055 - 0.070	0.033 - 0.040	

Table 5. Means and ranges in percent fat in different body tissues of Tadarida brasiliensis collected from Vickery Cave, Oklahoma (VC), and Carlsbad Caverns, New Mexico (CC). N = 3 for each collection.

	Carcass	Brain	Liver	Kidney	Spleen	Testes
June 1990	5.17	6.59	2.54	3.95	0.87	
(VC)	4.68 - 5.94	6.25 - 7.23	2.01 - 3.02	3.351 - 4.70	0.55 - 1.40	
July 1990	10.42	6.78	5.27	4.09	1.94	
(VC)	7.89 - 13.86	6.60 - 7.00	4.58 - 5.96	3.751 - 4.80	1.30 - 2.45	
August 1990	8.26	7.23	3.11	4.11	2.20	
(CC)	6.95 - 9.87	6.75 - 7.70	2.83 - 3.36	3.700 - 4.70	1.90 - 2.80	
August 1990	11.36	6.71	4.21	4.33	2.36	4.11
(VC)	8.27 - 14.06	6.63 - 6.80	2.99 - 6.77	3.35 - 5.90	2.00 - 2.80	3.20 - 5.30
May 1991	10.49	5.48	4.05	5.04	0.74	
(CC)	7.735 - 13.690	3.48 - 7.18	3.39 - 4.80	4.70 - 5.60	0.10 - 2.95	
May 1991	7.80	7.18	4.17	3.68	1.24	
(VC)	5.94 - 10.54	6.73 - 7.43	3.48 - 4.99	2.95 - 4.50	0.75 - 2.05	

Table 6. Geometric means and ranges in concentrations of p,p'-DDE (µg/g wet weight) in different body tissues of Tadarida brasiliensis collected from Vickery Cave, Oklahoma (VC), and Carlsbad Caverns, New Mexico (CC). N = 3 for each collection.

	Carcass	Brain	Liver	Kidney	Spleen	Testes
June 1990	0.447	0.012	0.325	0.138	0.086	
(VC)	0.169 - 1.457	0.010 - 0.018	0.122 - 0.868	0.056 - 0.359	0.041 - 0.148	
July 1990	2.331	0.026	1.294	0.400	0.172	
(VC)	0.951 - 11.783	0.010 - 0.095	0.207 - 11.020	0.152 - 2.581	0.070 - 0.862	
August 1990	3.337	0.010	1.685	0.615	0.194	
(CC)	1.968 - 4.913	0.010 - 0.010	0.562 - 3.261	0.439 - 0.818	0.094 - 0.314	
August 1990	1.284	0.010	0.274	0.150	0.019	0.134
(VC)	0.707 - 3.962	0.010 - 0.010	0.176 - 0.428	0.084 - 0.251	0.010 - 0.068	0.116 - 0.149
May 1991	10.485	0.028	1.896	0.657	0.189	
(CC)	7.735 - 13.690	0.010 - 0.050	1.500 - 2.574	0.322 - 1.612	0.110 - 0.260	
May 1991	0.969	0.010	0.430	0.230	0.047	
(VC)	0.793 - 1.283	0.010 - 0.010	0.221 - 0.699	0.168 - 0.313	0.010 - 0.140	

Table 7. Correlation matrix of p,p'-DDE concentrations in different body tissues of Tadarida brasiliensis collected from Vickery Cave, Oklahoma, and Carlsbad Caverns, New Mexico. All coefficients are significant at the 0.05 level.

	Carcass	Brain	Liver	Kidney
Brain	0.996			
Liver	0.954	0.938		
Kidney	0.891	0.905	0.908	
Spleen	0.979	0.963	0.988	0.895

Standard Bone Marrow Chromosomal Aberrancy

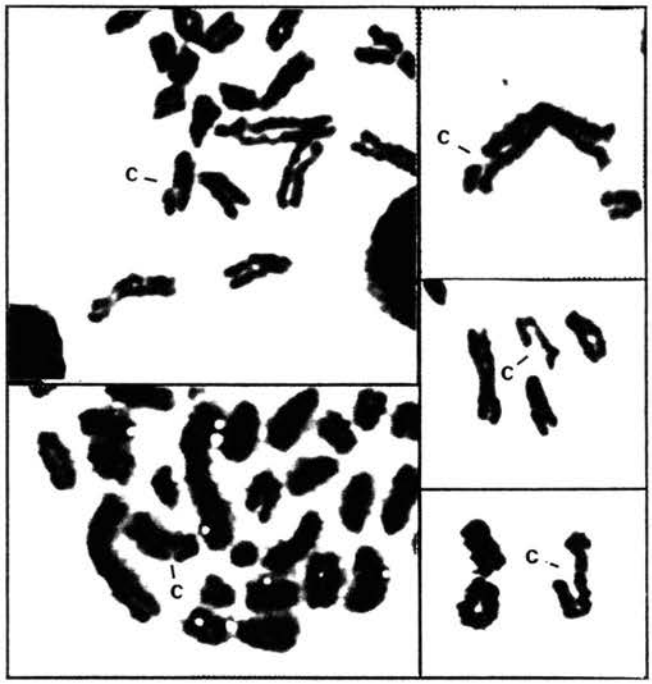
As described by Hsu and Benirschke (1970), the standard karyotype of T. brasiliensis consists of 48 chromosomes (Figure 5): six metacentric and submetacentric and 40 acrocentric and subtelocentric chromosomes. A number of the acrocentric chromosomes have distinct short arms and at least one pair have a secondary constriction near the centromere. The X chromosome is similar to the second pair of largest metacentric autosomes, but its arms are slightly shorter. The Y is the smallest element of the complement.

Chromatid breaks were observed at a much greater frequency than all other forms of visible structural damage (Fig. 6; Table 8). Chromatid breaks were observed in 60 of 177 T. brasiliensis examined in this study. Frequencies of occurrence per 50 cells ranged from 49 with single chromatid breaks, nine with two, and one each having three and five lesions (Table 8). Single acentric fragments were seen in six specimens: four females from Vickery Cave in May 1990 (N = 1), June 1990 (N = 2), and August 1990 (N = 1), and single Vickery Cave males from July 1990 and August 1990. A single Carlsbad female collected in May 1991 had acentric fragments in two of 50 cells examined. Acentric fragments in four (two males and two females) of the seven individuals were the only aberrations seen; the other three also had chromatid breaks. Deletions of a chromatid arm were observed in five individuals, single females from Vickery Cave in June 1990,

Fig. 5. Standard karyotype of Tadarida brasiliensis. Inset contains representative sex chromosomes.

X X
X Y

Fig. 6. Examples of chromosomal damage observed in standard bone marrow chromosomal preparations of Tadarida brasiliensis (c = chromatid break; d = deletion; ac = acentric fragment).


c
c
c
c


d
ac

Table 8. Mean number (and range) of observed chromatid breaks per individual for 50 bone marrow cells from Tadarida brasiliensis collected from Vickery Cave, Oklahoma, and Carlsbad Caverns, New Mexico. Data are for 12 individuals unless otherwise noted.

	Sex	Vickery Cave	Carlsbad Caverns
1990			
May[a]	F	0.33 (0 - 2)	
June[b]	F	0.23 (0 - 1)	
July	F	0.55 (0 - 2)	
August	F	0.33 (0 - 1)	0.75 (0 - 2)
	M	0.40 (0 - 2)	0.33 (0 - 1)
1991			
May	F	0.50 (0 - 5)	0.33 (0 - 1)
	M	0.50 (0 - 1)	0.42 (0 - 2)
August	F	0.42 (0 - 2)	0.17 (0 - 1)
	M	0.33 (0 - 1)	0.33 (0 - 1)

[a] N = 10

[b] N = 11

August 1990, and May 1991, and Carlsbad Caverns in May 1991. A single Vickery Cave male from May 1991 also showed a single deletion. Only one female from the August 1990 Carlsbad sample showed a chromosome break.

Three-factor ANOVA of the chromatid break data indicated no significant differences between sexes and collection sites, and among collection periods. Chromosome breaks, acentric fragments, and deletions occurred at frequencies that were too low to be tested.

Flow Cytometry

Flow histograms were generated for 200 spleen and 72 testicular samples (Fig. 7). Coefficients of variation were calculated and compared between sexes and sites, and among collection periods.

Spleens: No statistical differences in nuclear DNA content variation were found in spleen cells among the four samples of females collected from Vickery Cave during 1990 ($P = 0.3008$, Table 9). Mean CVs ranged from 3.052 to 3.240.

A significant difference ($P = 0.0053$) in CVs was found between sexes but not between sites. Females had a higher mean CV than males (3.086 vs. 2.664; $P = 0.0001$). May and August 1991 spleen samples demonstrated lower CVs than the August 1990 samples ($P = 0.0001$). August 1990 Carlsbad females had a higher mean CV (2.762) than Carlsbad males (2.471; $P = 0.0022$) and Vickery Cave females (2.562; $P = 0.0001$). CVs for Vickery females did not differ from Vickery

Fig. 7. Representative Multicycle data analysis of nuclear DNA content variation in spleen cells from female Tadarida brasiliensis OK00377 collected from Vickery Cave, Oklahoma.

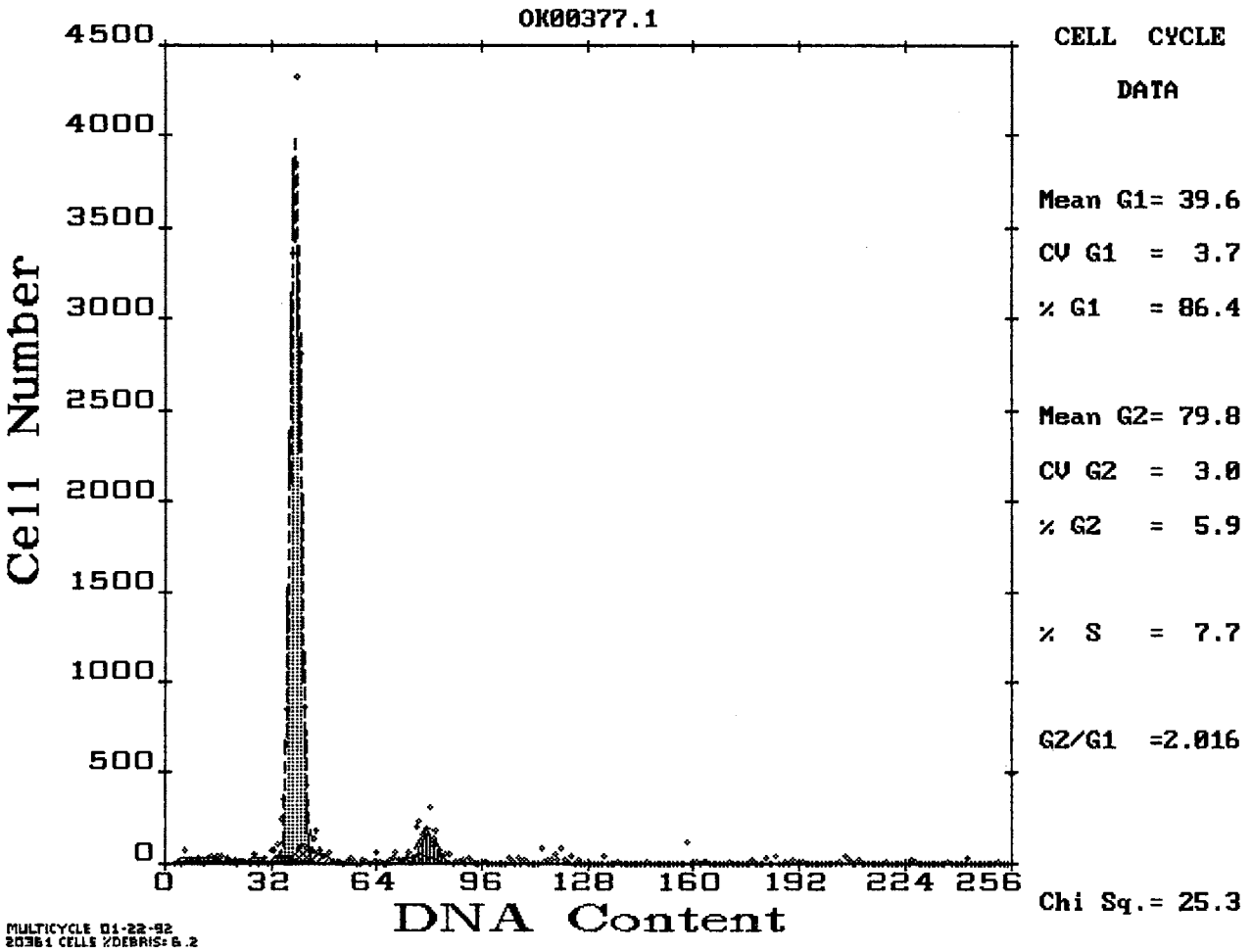

OK00377.1
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Cell Number
0
32
64
96
128
160
192
224
256
DNA Content
CELL CYCLE
DATA
Mean G1= 39.6
CV G1 = 3.7
% G1 = 86.4
Mean G2= 79.8
CV G2 = 3.0
% G2 = 5.9
% S = 7.7
G2/G1 =2.016
Chi Sq.= 25.3
MULTICYCLE 01-22-92
20361 CELLS %DEBRIS= 6.2

Table 9. Mean coefficients of variation and ranges in nuclear DNA content in spleen and testicular cells of Tadarida brasiliensis as measured by flow cytometry. Results are for five replicates from 12 individuals unless otherwise noted.

		Spleen		Testes	
	Sex	Vickery Cave	Carlsbad Caverns	Vickery Cave	Carlsbad Caverns
1990					
May[a]	F	3.18 (2.32 - 4.32)			
June[b]	F	3.24 (2.24 - 4.10)			
July	F	3.05 (2.64 - 3.74)			
August	F	3.11 (2.20 - 4.32)	3.09 (2.32 - 4.20)		
	M	2.58 (2.04 - 3.12)	2.76 (2.34 - 3.82)	2.21 (1.62 - 2.64)	1.96 (1.70 - 2.36)
1991					
May	F	2.37 (2.02 - 3.02)	2.33 (1.96 - 2.72)		
	M	2.24 (1.90 - 3.34)	2.33 (1.80 - 3.00)	2.43 (1.92 - 2.80)	2.30 (1.62 - 3.06)
August	F	2.21 (1.58 - 3.82)[c]	2.87 (1.92 - 5.38)[c,d]		
	M	2.25 (1.58 - 3.26)	2.33 (1.82 - 3.18)[d]	2.48 (2.25 - 2.76)	2.39 (1.82 - 2.96)

[a] N = 10; [b] N = 11

[c] significant difference between sites within sex and collection period.

[d] significant difference between sexes within site and collection period.

males (P = 0.4285).

Testes: Means CVs and standard deviations were calculated for each collection period (Table 9). Analysis of flow histograms showed a lack of spermatogenesis in most of the males collected, as would be expected because T. brasiliensis breed in the early spring. Peaks representing haploid (1C) spermatozoa were observed in several individuals but were not present in sufficient numbers for adequate statistical analysis.

CVs of nuclear DNA content in mitotic testicular tissues were, however, compared among the males to assess differences among the samples collected. Significant differences in testicular DNA content variation were found between Carlsbad and Vickery males (P = 0.0001). The 1991 samples also demonstrated significantly higher CVs as compared to August 1990 (P = 0.0007). A significant interaction between collection period and site was also found (P = 0.0064). A paired t-test of male data comparing CVs from spleen and testes indicated no significant differences between tissues (P = 0.1046).

Correlation analyses

Correlation coefficients were determined by comparing p,p'-DDE concentrations in carcass and brain tissues with frequencies of chromatid breaks and FCM analyses of spleen and testis cells (Table 10). Significant positive correlations were detected between carcass and brain tissues

Table 10. Correlation matrices of p,p'-DDE concentrations in carcass (C DDE) and brain (B DDE), mean coefficients of variation in DNA content for spleen (S CV) and testicular cells (T CV), and chromatid breaks (cB) for Tadarida brasiliensis collected from Vickery Cave, Oklahoma, and Carlsbad Caverns, New Mexico.

Vickery Cave				
Females				
	C DDE	B DDE	S CV	
B DDE	.328			
S CV	-.185	-.047		
cB	-.165	-.106	-.171	
Males				
	C DDE	B DDE	S CV	T CV
B DDE	.522*			
S CV	-.197	-.410*		
T CV	-.051	-.016	.087	
cB	-.143	.086	.250	.190

Carlsbad Caverns				
Females				
	C DDE	B DDE	S CV	
B DDE	.369*			
S CV	-.310	.060		
cB	.028	.009	.044	
Males				
	C DDE	B DDE	S CV	T CV
B DDE	.364*			
S CV	-.222	-.355*		
T CV	.355*	.304	-.426*	
cB	-.037	-.091	-.198	-.226

* $P \leq 0.05$

for both sexes (males r = 0.278, P = 0.0181; females r = 0.475, P = 0.0001).

In analyses by site, significant positive correlations between brain and carcass DDE were found for Vickery and Carlsbad males and Carlsbad females (Table 10). Significant negative correlations between spleen FCM results and brain DDE concentrations were also found for both Vickery and Carlsbad males (Table 10). Carlsbad males also showed significant correlations between testes FCM and carcass p,p'-DDE (P = 0.0362, Table 10) and spleen FCM (P = 0.0106, Table 10). All other correlations were nonsignificant.

DISCUSSION

Pesticides

As hypothesized, bats collected from Carlsbad Caverns had OC pesticide levels (specifically p,p'-DDE) greater than those from Vickery Cave. The Vickery Cave population did, however, have detectable levels of p,p'-DDE in both carcass and brain samples, which indicated a potential for pesticide-induced populational effects.

Significant differences of carcass residue levels of p,p'-DDE for females collected from Vickery Cave during 1990 may represent changes in body composition associated with parturition and nursing. High levels obtained in May represent pesticides accumulated since the preceding fall. Lower levels observed in June, July, and August may be attributed to giving birth and transferring OCs to the

juvenile through lactation and cross-placental transfer. If further contamination was obtained in summer foraging areas, it is reasonable to expect a decline in carcass concentrations of p,p'-DDE in females through the summer. This was demonstrated in this data set because August's sample was lower than that of June and July. However, July's results were not significantly lower than May's. Several individuals in July may have had higher tissue levels at the beginning of spring and retained proportionally higher levels at the beginning of lactation. Eleven of the 15 specimens examined showed evidence of lactation in the form of enlarged mammary glands and hair worn from around the nipples. The higher tissue levels may indicate that a secondary source of contamination existed within the summer foraging area of the Oklahoma population.

Higher pesticide loads also were expected in males from both populations than females due to the loss of pesticides by females through lactation. Although carcass residue levels did not differ significantly between sexes, brain concentrations in Vickery males were approximately ten times greater than for females, indicating a potentially serious exposure to toxic effects. This indicates that males were potentially at a greater risk to direct toxic neurological effects of p,p'-DDE. Increasing levels of brain residues may have resulted from pesticides mobilized from body fats during migration being sequestered in brain tissues at sublethal levels. Once transferred into nervous tissues, DDE may not

be readily transported back across the blood-brain barrier into body tissues but may instead remain within brain tissues.

These pesticide data for chronically exposed T. brasiliensis reinforce the findings of Geluso et al. (1976, 1981) about p,p'-DDE contamination at Carlsbad Caverns. Unlike previous research that examined exposure in juveniles, this study examined adults that presumably were undergoing chronic pesticide exposure. As would be expected if juveniles are truely those members of a population to aquire maximum lifetime loads before their first migrations, residues observed in the adults examined here were consistently lower than those of Geluso et al. (1976, 1981). Although they did not specifically look at the oldest individuals within the Bracken Cave, Texas, population, my findings were consistent with those of Clark et al. (1975).

This study also expands the information available on pesticide contamination to another population of T. brasiliensis which had not previously been examined. Bats from Vickery Cave demonstrated exposure to similar pesticides; however, tissue levels were lower than those from Carlsbad Caverns. This may be explained, in part, by differences in agricultural practices occurring in areas surrounding each of the summer caves (pers. comm., personnel from Okla. and New Mex. Dept. Agr.).

August 1991 data would suggest an additional source for p,p'-DDE contamination within the summer foraging range of

the Carlsbad Caverns population. Tissue levels were approximately double that of the spring sample. Although some differences in results may be attributed to sampling, it is unlikely that sampling would affect both sexes equally. Both sexes showed an approximate three-fold increase in p,p'-DDE carcass concentrations. Without an additional source of contamination, it also would appear unlikely that lactating females would demonstrate higher pesticide loads after nursing than before parturition. Loss of p,p'-DDE was observed in females from Oklahoma; it was stationary in males. This further supported the theory that lactation provides a successful mechanism for shedding pesticides in the adult female that is not available to males. However, because of observed increases in the Carlsbad females, additional residue accumulation must be occurring at a rate greater than can be shed through the milk. Reports have noted significant DDE contamination in a number of resident vertebrate species of the Rio Grande and Pecos River drainages of New Mexico and Texas where Carlsbad's T. brasiliensis are known to forage (White and Krynitsky 1986, Clark and Krynitsky 1983). This contamination cannot be attributed to either residual or recent pesticide use and warrents further investigation.

In addition to human disturbance, continued summer contamination may be a significant contributing factor to the observed declines at Carlsbad Caverns. Further accumulation monitoring and analyses of effects of chronic exposure of OC

pesticides on this population of T. brasiliensis may provide the information necessary to determine how this valuable member of the ecosystem may be preserved.

Standard Bone Marrow Chromosomal Aberrancy

McBee et al. (1987) demonstrated that metaphase chromosomal analysis could document chromosomal aberrations in rodent species contaminated with mutagenic petrochemical compounds. Yoder et al. (1973) also reported an observed increase in lymphocyte aberrancy rates in agricultural workers exposed to organochlorine pesticides over background rates of damage in unexposed individuals. The types of aberrations seen in this study are in agreement with those of Yoder et al. (1973) in which they demonstrated cultures from exposed individuals during periods of heavy spraying showed a marked increase in the frequency of chromatid lesions. This study also demonstrated consistently low frequencies of observed chromosomal damage within samples of T. brasiliensis collected from populations with both high and low levels of organochlorine pesticide exposure, which does not correspond with their findings. Frequencies of chromatid aberrancy in T. brasiliensis in this study were lower (0.32 - 1.5% cells with chromosomal lesions) than observed in populations of wild rodents (2.83 - 3.57% cells with aberrations; McBee et al. 1987).

Three hypotheses may explain low frequencies of aberrancy and lack of aberrations other than chromatid breaks

in Mexican free-tailed bats. First, this species may be able to prevent or control cellular damage during normal activities by sequestering pesticides in body fat where they remain ineffective. Rapid mobilization of pesticides from lipid storage during migration may result in acute toxicity and death of the individual, and cytogenetic effects may not be observed with SBMCA. Second, natural selection may have eliminated populations of cells with a DNA damage repair mechanism susceptible to chronic pesticide exposure, and thereby bats that are intolerate of pesticides. Continued exposure to small doses of pesticides during the period of heavy OC pesticide use beginning in the 1930's may have effectively acted as a selective mechanism. Only those individuals able to withstand genotoxic effects of pesticides under conditions of chronic exposure survived. A third hypothesis is that pesticide levels may be high enough to cause cell lethality (Kelley-Garvert and Legator 1972, Palmer et al. 1972), resulting in lower frequencies of observed chromosomal aberrancy than would be expected in an unexposed population. Data are not currently available to determine whether these bats are any more or less susceptible to pesticides than the mammal cell lines used by Kelley-Garvert and Legator (1972) and Palmer et al. (1972).

Result of cell death associated with genotoxicity depends upon the type of tissue involved. If cell death occurs in an embryo, it will probably result in some type of malformation or death of the embryo (Kalter 1971). If it

occurs in somatic or germinal cells, it will result in nothing more than the loss of the cell. Should the cell survive but is damaged, damage could result in a tumor for a somatic cell or mutation if a germinal cell is involved. Chromosomal damage, specifically single chromatid breaks or exchange figures, are probably of great mutagenic significance because they are incorporated in the cells that are most likely to survive and carry an alteration of the genetic material (Palmer et al. 1972).

Further genetic analyses, particularly of specimens collected during migration, are needed to answer questions about chromosomal effects of organochlorine pesticides in this species of bat.

Flow Cytometry

This study was intended to examine potential effects of chronic exposure of p,p'-DDE on variability in DNA content of spleen and testicular cells in T. brasiliensis using flow cytometry. Unlike the approximate two-fold elevation in CVs obtained by McBee and Bickham (1988) for wild rodents exposed to hazardous materials, T. brasiliensis showed a much smaller degree of difference; males demonstrated a slightly lower (but insignificant) mean CV than females. Because males are unable to shed accumulated pesticides and must suffer the effects of continuous accumulations, it is possible that they are under more stringent genotoxic controls. Entire cell lines within populations of T. brasiliensis exposed to OC

pesticides may be more susceptible to environmental constraints and cannot survive if genetic damage results in a significant decrease or increase in DNA content.

Relatively low CVs observed in these bats may be indicative of prolonged exposure to organochlorine pesticides eliminating much of the inherent variability in DNA content within this species. Through generations of exposure, those individuals with either too little or too much genetic variability may have been affected or selected against more harshly than those with what might be considered the proper complement of genetic material. These selective pressures could potentially be translated into a selective event against the population as a whole, potentially contributing to the events which led to the populational declines observed at Carlsbad Caverns. With additional research, it may be possible to fully understand exactly how organochlorine pesticides have affected T. brasiliensis at Carlsbad Caverns and other populations throughout the southwestern United States.

Correlations

Because highly lipophilic compounds are actively transported throughout body tissues and able to freely cross cell embranes (Goldstein and Betz 1986), the positive correlation detected between carcass and brain tissues was expected. As carcass concentrations increase, it would be reasonable to assume that all body tissues would show a

proportional increase in pesticide concentration. This was demonstrated by the positive relationship between brain and carcass DDE concentrations, as well as the significant correlations found among liver, spleen, and kidney.

A positive correlation between mitotic spleen and testicular DNA content variation in all specimens examined was expected but was not significant. The negative correlation for Carlsbad males was, however, not expected. If both tissues were actively undergoing mitosis without any extraneous influences affecting DNA replication, all cells within an individual should have a relatively consistent cellular DNA content. However, if exposure to mutagenic agents affects mitotic activity in different body tissues differently, then one tissue may demonstrate a departure in CVs as compared to anothers. Because both tissues have different body functions and testicular tissue also undergoes meiotic cell division, FCM analyses may innately show a greater variability in DNA content in testes. Although not significantly different, this hypothesis may be supported by a slightly lower mean CV for testes (2.30) vs. spleens (2.41). Similar differences in tissue response to mutagenic compounds have been demonstrated by Bickham et al. (1992a, 1992b).

Significant negative correlations also were found among spleen CVs and brain p,p'-DDE concentrations. These differences may indicate that as DDE concentrations increase in the brain, DNA and/or cell replication in the spleen is

either under more stringent replication control or those cells with excessive DNA content variation are unable to survive. Because a negative correlation was observed only in males, which tended to have higher pesticide loads than females, it is likely that as brain concentrations increased, a simultaneous selection against cells with excessive DNA content vatiation also occurred.

Conclusion

Three explanations can be proposed for the genotoxicity results in this study. First, bats may have evolved defensive mechanisms to counter adverse effects of chronic exposure to OC pesticides. Second, chronic exposures may have eliminated individuals that were most susceptible to exposure and genotoxic effects of contamination, leaving only those individuals who are relatively unaffected.

The third, and perhaps most likely, reason for failure to observe a positive relationship among all assays performed involves sampling and resolution of the analytical data. Inability to accurately determine ages of individuals may have contributed heavily to variability both within and between collection periods and sites. The best time for specimen collection to address genetic effects of contamination would appear to be during migration, when individuals are actively burning fat and accumulated pesticides are mobilized in body tissues. Analyses conducted on specimens collected during migration would be expected to

show maximum levels of tissue damage, which should be detectable using both flow cytometry and chromosomal aberrancy assays. Neither flow cytometry or chromosomal aberration assay may be sensitive enough to detect subtle genetic differences in specimens collected before or after migration that are due to chronic pesticide exposure.

CHAPTER III

CROSS-PLACENTAL TRANSFER OF ORGANOCHLORINE PESTICIDES IN MEXICAN FREE-TAILED BATS

Abstract. Forty pregnant Mexican free-tailed bats (Tadarida brasiliensis) were collected from Carlsbad Caverns, New Mexico, and Vickery Cave, Oklahoma, in May 1989 and 1991. Residues of p,p'-DDE were detected by gas chromatography in all embryos at levels highly correlated with contaminant levels in mothers' carcasses and brains. Residue levels were not correlated with fat concentrations, which suggested that placental membranes offered minimal protection for developing embryos against exposure to organochlorine pesticides.

Because of similarities in structural, metabolic, and genetic components of all life forms, absolute species specificity in the development of agricultural pesticides is frequently difficult to attain. As a result, improper use of toxic chemicals may cause biological effects beyond those originally intended and result in expanding concerns about potential environmental health problems from exposure to toxic chemicals (Hutton 1982).

Accumulation of organochlorine (OC) pesticide residues in body tissues has been documented for a number of

insectivorous bat species (Clark 1981, 1988; Clark et al. 1983; Clark and Krynitsky 1983; Clawson and Clark 1989). Extensive studies of the Mexican free-tailed bat, Tadarida brasiliensis (Chiroptera: Molossidae), occupying a summer maternity roost at Carlsbad Caverns National Park have been conducted by Geluso et al. (1976, 1981) in response to concerns of the U. S. National Park Service. The size of the summer population of free-tailed bats has declined from an estimated 8.7 million in 1936 to approximately 200,000 in 1973 (Altenbach et al. 1979). Body burdens during the years of decline are not known, but levels of DDE (1,1-dichloro-2,2-bis[p-chlorophenyl]ethylene) at Carlsbad Caverns National Park were high enough to be potentially hazardous to young bats, primarily during their first migration (Geluso et al. 1981). Young bats from Bracken Cave, Texas, Eagle Creek Cave, Arizona, and Newman Bridge, California, had OC pesticide residues at levels lower than those at Carlsbad Caverns (Geluso et al. 1981). No other populations, including those from Oklahoma, have been examined.

Although transmission of pesticide residues from mother to offspring have been attributed primarily to lactation (Ottoboni and Ferguson 1969; Clark et al. 1975; Clark and Lamont 1976; Geluso et al. 1976, 1981), studies also have documented transfer of OC compounds across the placenta. In wild-caught big brown bats (Eptesicus fuscus), Clark and Lamont (1976) suggested that Arochlor 1260, a polychlorinated biphenyl (PCB), caused young to be stillborn. Clark and

Krynitsky (1978) also reported possible stillbirths in little brown bats (Myotis lucifugus) due to high concentrations of PCBs, DDE, and/or oxychlordane (isomers of 1,2,4,5,6,7,8,8a-octachloro-2,3,3a,4,7,7a-hexahydro-4,7,methaon-1H-indene). In 18 pregnant T. brasiliensis collected during June 1973 from Bracken Cave, Texas, Clark et al. (1975) found a positive relationship between embryonic and adult pesticide content.

This study examines and compares transfer of OC pesticides across placental membranes in T. brasiliensis from Vickery Cave, Oklahoma, and Carlsbad Caverns, New Mexico. The Vickery Cave population has been studied for migratory patterns (Glass 1958, 1959, 1982; Constantine 1967) and population structure (Perry 1965; Perry and Herreid 1969), but never for the presence of pesticides. The population at Carlsbad Caverns has demonstrated consistently higher tissue levels of pesticides in juveniles than other populations of the species (Geluso et al. 1976, 1981), but embryonic transfer data are lacking. Specific null hypotheses were: 1) the Oklahoma and Carlsbad Caverns populations demonstrate similar types and rates of OC pesticide contamination in the adult females examined and 2) OC contamination is lower in embryonic tissue than adult brain and carcass due to differences in lipid content and/or restricted movement of contaminants across the placental membrane.

MATERIALS AND METHODS

Specimen Collection

Specimens were collected using a hoop net or single-tier mist net erected in the entrance of both caves to catch animals as they exited for nightly feeding excursions following protocols of Geluso et al. (1976). To assess potential effects from chronic pesticide exposure, individuals were classified into relative age groups by examining canine tooth wear (Anthony 1988). Females with canines worn approximately half way to the gums were retained for analysis. No attempts were made at the time of collection to distinguish between pregnant and nonpregnant individuals.

Each animal was randomly assigned a permanent identification number to eliminate possible bias resulting from knowledge of collection history during analyses. Animals were sacrificed by cervical dislocation within 24 hr of capture and processed for pesticide analyses. Pregnant females were then identified by dissection so that embryos could be removed from the uterus and analyzed separately.

Pesticide Extraction

Analytical procedures for determining OC pesticide content followed Geluso et al. (1981) as modified from Peterson et al. (1976). Pesticide extraction procedures were applied to brain, carcass, and embryo for each pregnant

specimen collected.

For sample preparation, wings were removed at the shoulder and feet were removed at the ankle. Specimens were skinned, and fat adhering to the skin was removed and placed with the body. Stomach, small intestine, and large intestine were removed to avoid analyzing insect remains or other contaminants in the gastrointestinal tract. The liver, spleen, and one kidney also were removed for other analyses. The head was removed at the first cervical vertebra and the brain carefully removed for analysis. Major cranial musculature was removed from the skull and placed with the carcass for analysis. Embryos and placenta were removed from the uterus and visually examined for observable abnormalities. Thus, carcass refers to the entire body minus skin, wings, feet, gastrointestinal tract, liver, spleen, one kidney, embryo, skull, and brain.

Immediately after removal, brains, carcasses, and embryos were placed in tared flasks or test tubes, weighed, and mixed with five times their weight of anhydrous sodium sulfate. Samples were individually homogenized in a blender or mortar and pestle and placed in centrifuge tubes. Extraction solvent consisting of 20% acetone in isooctane (v/v) was added to each centrifuge tube (carcass 40 ml; brain 3 ml; embryo 5 ml). Tubes were then shaken for 10 min, agitated periodically for 24 hr, and allowed to stand an additional 24 hr. One ml of extracts were evaporated in tared vials to determine percent fat. Remaining extracts

were reserved for pesticide identification and quantification. The latter were cleaned prior to analysis by the addition of activated Florisil and concentrated into a final volume of isooctane. Liquid-liquid partitioning was omitted because it has been found to be unnecessary (Geluso et al. 1981).

Quality control/quality assurance protocols included extractions of pure anhydrous sodium sulfate as procedural blanks and spiking carcass and brain samples of laboratory-reared Peromyscus maniculatus with known quantities of pesticide standards to determine extraction efficiencies. Results for all analyses were reported in μg/g wet weight.

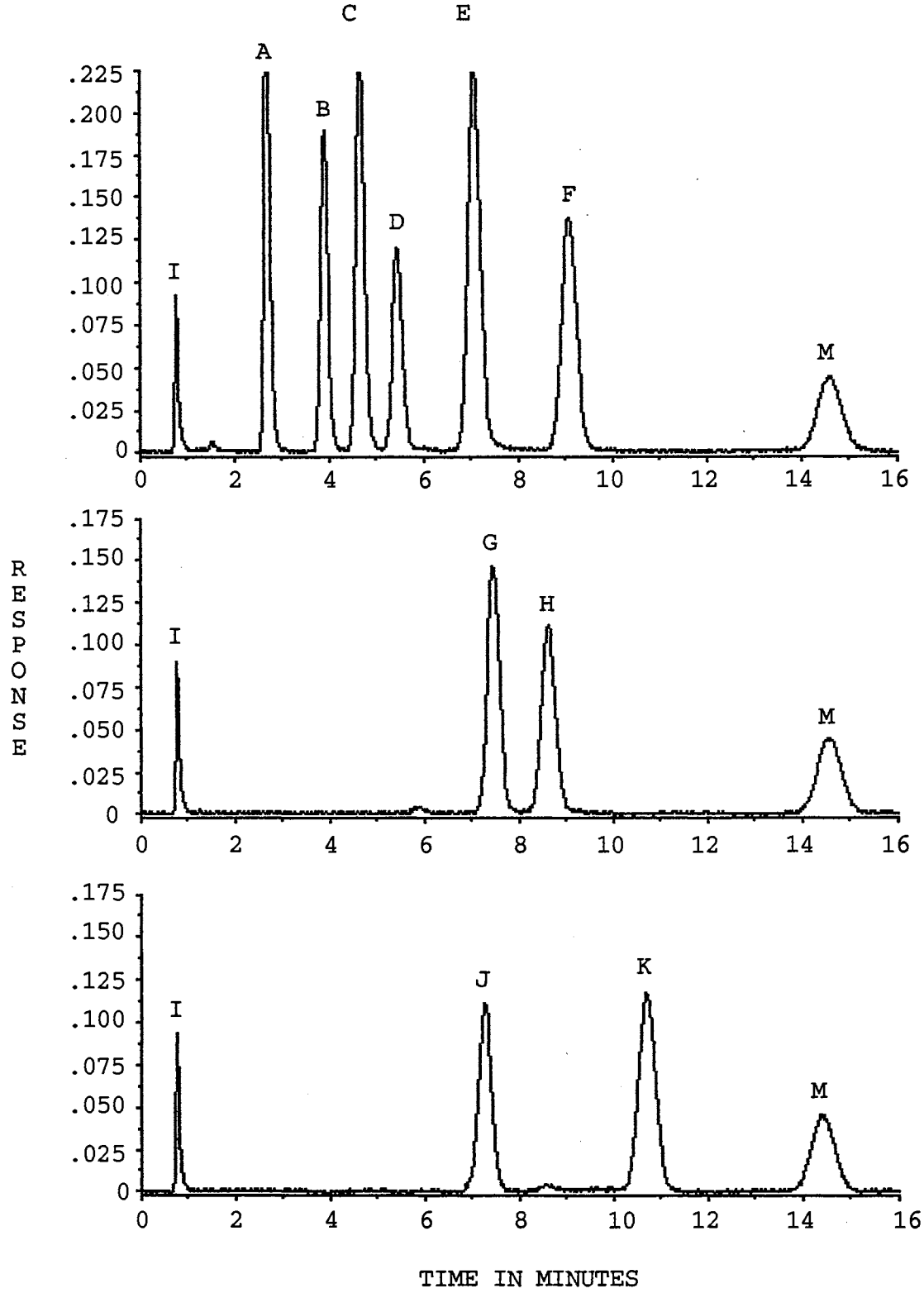
Gas Chromatography

Samples were analyzed for identifiable OC pesticides including p,p'-DDE, o,p'- and p,p'-DDT (2,2-bis[p-chlorophenyl]1,1,1-trichloroethane), o,p'- and p,p'-DDD (2,2-bis[p-chlorophenyl]1,1-dichloroethane), aldrin (1,2,3,4,10,10-hexachloro-1,4,4α,5,6,7,8,8α-hexahydro-endo-1,4-exo-5,8-dimethanonaphthalene), dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-exo-1,4 endo-5,8-dimethanonaphthalene), endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-exo-1,4-exo-5,8-dimethanonaphthalene), lindane (1,2,3,4,5,6-hexachloro-cyclohexane), heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene), heptachlor epoxide (1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-

4,7-methanoindene), and PCBs using Tracor 560 and Perkin Elmer Sigma 1B gas chromatographs. Each instrument was configured with a single electron capture detector and dissimilar glass columns (1.8 m X 0.6 mm id). Instrument settings and operating conditions for the Tracor 560 were: column packing 5% OV-17 and 1.95% QF-1, oven T 300°C, injection port T 250°C, detector T 300°C, carrier gas flow rate 40 ml/min. Perkin Elmer conditions and settings were: column packing 3% OV-1; oven T 230°C; injection port T 275°C; detector T 350°C; carrier gas flow rate 50 ml/min; detector purge rate 40 ml/min. All samples were analyzed simultaneously on both instruments to confirm identified and quantified residues. Instrument detection limits were: 0.010 µg/g for lindane, heptachlor, and heptachlor epoxide; 0.015 µg/g for aldrin; 0.020 µg/g for p,p'-DDE, o,p'- and p,p'-DDD, p,p'-DDT, and dieldrin; and 0.025 µg/g for o,p'-DDT.

Data acquisition was conducted using the Maxima (Dionyx, Millipore Corporation) computer interface system. A 2-µl spike of methoxychlor (2,2-bis[p-methoxyphenyl]1,1,1-trichloroethane) was added to all brains and embryos and 0.4 µl were added to all carcasses prior to injection for use as internal standards. Resulting methoxychlor peaks were used to quantify identified OC residues based on observed peak areas for the methoxychlor spike and a series of standards obtained immediately prior to analysis of tissue samples (Fig. 8). Analyses of P. maniculatus carcass and brain tissues spiked with standard compounds demonstrated a greater

Fig. 8. Standard gas chromatographs of organochlorine pesticides used to identify and quantify detected peaks in tissue residue analyses. I: isooctane solvent; A: Lindane; B: Heptachlor; C: Aldrin; D: Heptachlor Epoxide; E: p,p'-DDE; F: o,p'-DDT; M: Methoxychlor internal standard; G: Dieldrin; H: p,p'-DDD; J: o,p'-DDD; K: p,p'-DDT.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TIME IN MINUTES

efficiency in residue recovery for carcasses than for brains. Reduced efficiencies in brain tissue recovery were attributed to smaller volumes of tissue being extracted. Efficiencies for carcasses ranged from 88 percent for dieldrin and p,p'-DDE to 116 percent for o,p'-DDT. Brain recoveries were consistently low and ranged from 50 percent for heptachlor epoxide to 100 percent for aldrin. Recovery of p,p'-DDE from brain tissue was 74 percent.

Differences in pesticide levels within and between the Vickery Cave and Carlsbad Caverns populations were statistically examined using three-factor ANOVA. Because the pesticide data were positively skewed, they were log(x + 1) transformed prior to analyses. Specific analyses included comparisons in pesticide content among females by site and collection period, as well as content and transfer of pesticides in embryos. Probability values ≤ 0.05 were considered statistically significant for all analyses.

RESULTS

In May 1990, all 10 females collected from Vickery Cave were pregnant. From 13 females collected at Vickery Cave in June for other studies, an additional two pregnant females were obtained. The remaining 11 were lactating or had parous nipples, indicating that they had recently suckled young. In May 1991, all 15 females collected from Carlsbad Caverns and 14 of 15 collected from Vickery Cave were pregnant.

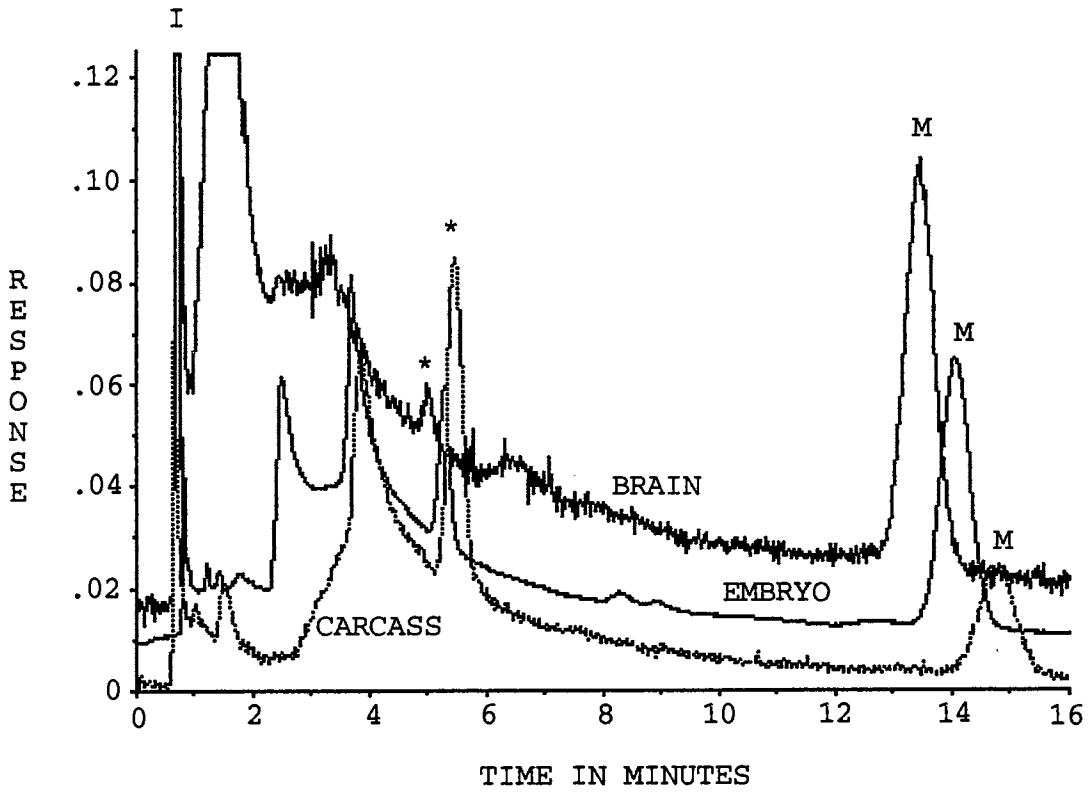
Comparative gas chromatograms of carcass, brain, and

embryo residues for OK00377, collected from Vickery Cave in May 1990, are shown in Fig. 9. Means and standard deviations for carcass, brain, and embryo weights, percent fat, and observed concentrations of p,p'-DDE were determined for comparison (Table 11).

Identified pesticide residues detected in the 41 embryos examined were restricted primarily to p,p'-DDE (N = 40). Heptachlor was found in 14 embryos: nine from the 1991 Vickery sample, three from 1990 Vickery, and two from Carlsbad Caverns. Although frequencies of occurrence were strongly biased toward Vickery Cave, concentrations ranged from 0.017 µg/g to 0.117 µg/g and did not differ significantly among samples. Nine embryos also contained detectable quantities of p,p'-DDT ranging from 0.035 to 0.067 µg/g. Four were from the 1990 Vickery and five were from the 1991 Vickery samples; no Carlsbad embryos showed the presence of DDT. A single individuals also showed lindane at 0.324 µg/g. Only p,p'-DDE was detected in sufficient quantities and frequencies in carcass and brain tissues of the female parents to compare with embryonic data.

Among 40 specimens with measurable embryonic p,p'-DDE content, strong positive correlations were found between embryo concentration and concentration in the mother's carcass ($r = 0.993$; $P = 0.0001$) and brain ($r = 0.999$; $P = 0.0001$). A strong positive correlation was also found between the mother's carcass and brain concentrations ($r = 0.993$; $P = 0.0001$). Significant correlations also were found

Fig. 9. Comparative gas chromatographs of organochlorine pesticide residues in carcass, brain, and embryo from female Tadarida brasiliensis OK00377 collected from Vickery Cave, Oklahoma. I: isooctane solvent; *: p,p'-DDE; M: Methoxychlor internal standard.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TIME IN MINUTES

Table 11. Means and standard deviations for carcass, brain, and embryo weights (in grams), percent fat, and concentrations of p,p'-DDE (µg/g wet weight) for all pregnant Tadarida brasiliensis examined.

		Vickery 1990 N=12	Carlsbad 1991 N=15	Vickery 1991 N=14
Mother				
Carcass	Weight	5.030 ± 0.598	5.912 ± 0.416	6.035 ± 0.417
	Percent Fat	6.50 ± 2.52	12.38 ± 3.10	10.39 ± 3.08
	p,p'-DDE	9.333 ± 28.348	3.639 ± 1.994	0.923 ± 0.573
Brain	Weight	0.172 ± 0.011	0.169 ± 0.016	0.181 ± 0.018
	Percent Fat	7.88 ± 0.92	8.71 ± 2.85	7.97 ± 0.86
	p,p'-DDE	0.641 ± 2.096	0.063 ± 0.072	0.029 ± 0.035
Embryo				
	Weight	1.520 ± 0.790	1.130 ± 0.331	1.699 ± 0.366
	Percent Fat	1.58 ± 0.39	1.57 ± 1.45	2.50 ± 2.60
	p,p'-DDE	4.352 ± 14.052	0.110 ± 0.081	0.087 ± 0.121

between weight and percent fat for carcasses ($r = 0.672$; $P = 0.0001$) and brains ($r = -0.324$; $P = 0.0385$) but not for embryos ($r = 0.018$; $P = 0.9109$).

Analyses of the female carcasses and percent fat among sites showed the 1990 Vickery females to weigh significantly less ($P = 0.0001$) and contain lower percent fat ($P = 0.0001$) than both 1991 samples, which did not differ (Table 11). DDE concentrations, however, were not significantly different among groups ($P = 0.3762$). Brain weights, percent fat, and DDE concentrations did not differ significantly among any of the three groups. Embryo percent fat and DDE concentrations did not differ among groups. The 1991 Vickery embryos were significantly heavier than those from Carlsbad Caverns ($P = 0.0162$), but neither 1991 embryo samples differed in weight from the 1990 Vickery embryos.

DISCUSSION

Although a number of studies have demonstrated the accumulation and movement of OC pesticide residues in the food chain, effects other than acute toxicity (i.e. death) have not been adequately addressed. Results obtained in this study indicate that both populations are exposed to OC pesticides. Data obtained from the mothers examined indicate that both populations are accumulating p,p'-DDE in their body tissues and passing those accumulations to the developing embryos. Unlike Clark et al. (1975), females examined here did not contain DDT or dieldrin; however, levels of DDE were

similar. Embryo concentrations also were lower than those reported by Clark et al. (1975). These lower values and lack of evidence for DDT contamination may be the direct result of significant changes in pesticide use and reduced exposure of the parent to OC pesticides that were occurring 15 to 20 years ago.

Although fat content was consistently higher in the mother's carcass than in the embryo, p,p'-DDE is apparently freely transmitted across the placental membranes to the developing embryo. This transfer may be occurring as nutrients and wastes are exchanged. It cannot be determined from these data if the developing embryonic tissue has a natural affinity for absorbing pesticides from the parental bloodstream. It appears, however, that placental membranes are less effective at preventing p,p'-DDE from crossing to the embryo than is the blood-brain barrier because brain concentrations were consistently lower than for embryos even though they may have twice the fat content.

Because all females collected at Carlsbad Caverns were pregnant and appeared relatively healthy, these data failed to prove that pesticide exposure was having detrimental reproductive effects. Because all specimens were frozen immediately after collection, embryonic viability was not examined. Questions regarding embryonic viability cannot fully be resolved without additional studies into effects of pesticide exposure on fetal development and survivability.

CHAPTER IV

SUMMARY

Historically, the population of T. brasiliensis at Carlsbad Caverns National Park has consistently demonstrated levels of OC pesticides (specifically DDE) higher than other populations examined throughout the southwestern United States. Past studies have provided information on the types and quantities of OC pesticides in body tissues. They have not, however, contributed toward determination of long-term effects resulting from subacute or chronic exposure.

This study was designed to combine newly developed cytogenetic monitoring techniques with classical residue analyses. Observed levels of cytogenetic aberrancy may be correlated with OC pesticide tissue residues to directly assess potential genetic hazards and long-term populational effects of pesticide exposures on non-target organisms in the environment, and to establish base-line genotoxicity effects for a species.

Data collected in this study provided answers to a number of questions about the relationships among pesticide contamination and cytogenetic aberrancy in the Oklahoma and Carlsbad Caverns populations of T. brasiliensis. Because both populations winter in Central America where OC pesticide

use still occurs, I expected to find OC pesticide contamination in both populations. However, because of the differences in agricultural practices between the Oklahoma and Carlsbad Caverns study areas and published data for other free-tail colonies, I expected pesticide contamination to be significantly lower in Oklahoma. I also expected to find that females would have lower levels of contamination than males because females can shed pesticides through lactation. I also expected to observe a decline in OC pesticides in fall female samples as compared to spring samples if no additional contamination was occurring in the summer foraging areas and they were efficiently shedding pesticides through milk.

As expected, both populations showed OC pesticide contamination, which was restricted primarily to p,p'-DDE. Carlsbad Caverns specimens demonstrated a trend toward higher levels of OC pesticide accumulation, although sex and seasonal differences were not significantly different. Data also failed to show a significant decline in female pesticide content from spring to fall. Fall Carlsbad females, in fact, showed an increase in DDE concentrations, indicating that additional contamination may, in fact, be occurring while bats are in the U. S..

Both populations demonstrated similar types and frequencies of chromosome lesions. Chromatid breaks were the only structural lesion that was observed consistently in both populations. Marginal differences in nuclear DNA content variation were detected for both spleen and testicular

tissues but patterns among samples were not detected. Neither frequencies of chromosomal aberrancy nor DNA content variation in testicular cells could be correlated with OC residue levels. A negative correlation between brain DDE concentration and spleen FCM results was found for males from both Carlsbad Caverns and Vickery Cave. This indicate that the higher tissue levels of DDE in males may cause a genotoxic effect on proliferating spleen cells. This relationship was not detected in testicular cells from the same males, or for spleen cells in females that were collected concurrently. An examination of embryonic transfer of pesticides across placental membranes also demonstrated the potential for teratogenic or other hazardous developmental effects in developing embryos.

Although data collected in this study show correlations among pesticide exposure and observable genetic effects, the long-term exposure of bats to pesticides may be too subtle for assays used in this study to detect. In specimens collected either before or after migration, neither flow cytometry nor chromosomal aberration assay may be sensitive enough to detect subtle differences in genetic structure resulting from chronic pesticide exposure. Analyses conducted on specimens collected during migration would be expected to show maximum levels of tissue damage, which should be detectable using both flow cytometry and chromosomal aberrancy assays. Further examinations of both wild and captive populations of this and other species of

bats may provide critical information about prolonged effects of pesticides in the environment.

LITERATURE CITED

Altenbach JS, Geluso KN, Wilson DE (1979) Population size of Tadarida brasiliensis at Carlsbad Caverns in 1973. In: Genoways HH Baker RJ (eds) Biological investigations in the Guadelupe Mountains National Park, Texas. Natl Park Service Proc Trans Ser No 4, pp 341-348

Ames BN (1979) Identifying environmental chemicals causing mutation and cancer. Science 204:587-593

Anthony ELP (1988) Age determination in bats. In: Kunz TH (ed) Ecological and behavioral methods for the study of bats. Smithsonian Inst, pp 47-58

Baselt RC (1982) Disposition of toxic drugs and chemicals in man, 2nd ed. Biomedical Publ, Davis, California

Bickham JW, Sawin VL, Burton DW, McBee K (1992a) Flow-cytometric analysis of the effects of triethylenemelamine on somatic and testicular tissues of the rat. Cytometry 13:368-373

Bickham JW, Sawin VL, McBee K, Smolen MJ, Derr JN (1992b, accepted pending revisions) Further flowcytometric studies of the effects of triethylenemelamine on somatic and testicular tissues of the rat. Cytometry

Caire W, Tyler JD, Glass BP, Mares MA (1989) Mammals of Oklahoma. Univ Oklahoma Press, Norman

Clark DR Jr (1981) Bats and environmental contaminants: a review. US Fish and Wildl Serv Spec Sci Rep - Wildl 235

Clark DR Jr (1988) How sensitive are bats to insecticides? Wildl Soc Bull 16:399-403

Clark DR Jr, Bunck CM, Cromartie E (1983) Year and age effects on residues of dieldrin and heptachlor in dead gray bats, Franklin County, Missouri - 1976, 1977, and 1978. Environ Toxicol Chem 2:387-393

Clark DR Jr, Krynitsky AJ (1983) DDT: Recent contamination in New Mexico and Arizona. Environment 25:27-31

Clark DR Jr, Lamont TG (1976) Organochlorine residues in females and nursing young of the big brown bats. Bull Environ Contam Toxicol 15:1-8

Clark DR Jr, Martin CO, Swineford DM (1975) Organochlorine insecticide residues in the free-tailed bat (Tadarida brasiliensis) at Bracken Cave, Texas. J Mammal 56:429-443

Clawson RL, Clark DR Jr (1989) Pesticide contamination of endangered gray bats and their food base in Boone County, Missouri, 1982. Bull Environ Contam Toxicol 42:431-437

Constantine DG (1967) Activity patterns on the Mexican free-tailed bat. Univ New Mexico Publ Biol No 7

Durham WF, Ortega P, Hayes WJ Jr (1963) The effect of various dietary levels of DDT on liver function, cell morphology, and DDT storage in the Rhesus monkey. Arch Int Pharmacodyn 141:111-129

Dustman EH, Stickel LF (1969) The occurrence and significance of pesticide residues in wild animals. Ann NY Acad Sci 160:162-172

EPA (1979) Suspended and cancelled pesticides. Office of Public Awareness, Washington DC

Fantes JA, Green DK, Elder JK, Malloy P, Evans HJ (1983) Detecting radiation damage to human chromosomes by flow cytometry. Mutat Res 119:161-168

Fleming WJ, Clark DR Jr, Henny CJ (1983) Organochlorine pesticides and PCB's: A continuing problem for the 1980s. In: Transactions of the 48th North American Wildlife and Natural Resources Conference. Wildlife Management Institute, Washington DC, pp 186-199

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin BH, Resnick MA, Anderson B, Zeigler E (1987) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. Environ Molec Mutagen Vol 10 Supl 10:1-175

Geluso KN, Altenbach JS, Wilson DE (1976) Bat mortality: Pesticide poisoning and migratory stress. Science 194:184-186

Geluso KN, Altenbach JS, Wilson DE (1981) Organochlorine residues in young Mexican free-tailed bats from several roosts. Amer Midl Nat 105:249-257

Georgian L (1975) The comparative cytogenetic effects of aldrin and phosphamidon. Mutat Res 31:103-108

Glass BP (1958) Returns of Mexican freetail bats banded in Oklahoma. J Mammal 39:435-437

Glass BP (1959) Additional returns from free-tailed bats banded in Oklahoma. J Mammal 40:542-545

Glass BP (1982) Seasonal movement of the Mexican freetail bats Tadarida brasiliensis banded in the Great Plains. Southwestern Nat 27:127-133

Goldstein GW, Betz AL (1986) The blood-brain barrier. Sci Amer 255:74-83

Hacker-Klom U, Heiden T, Otto FJ, Mauro F, Göhde W (1989) Radiation-induced diploid spermatids in mice. Int J Radiat Biol 55:797-806

Hay RKM (1981) Chemistry for agriculture and Ecology. Blackwell Sci Publ, Boston

Hayes WJ Jr (1965) Review of the metabolism of chlorinated hydrocarbon insecticides especially in mammals. Annual Review Pharmacol 5:27-52

Hook EB (1982) Contribution of chromosome abnormalities to human morbidity and mortality. Cytogenet Cell Genet 33:101-106

Hsu TC, Benirschke K (1970) An atlas of mammalian chromosomes Vol 4 Folio 159. Springer Verlag, New York

Hutton M (1982) The role of wildlife species in the assessment of biological impact from chronic exposure to persistent chemicals. Ecotox Environ Safe 6:471-478

Kalter H (1971) Correlations between teratogenic and mutagenic effects of chemicals in mammals. In:

Hollaender A (ed) Chemical mutagens: Principles and methods for their detection Vol 1. Plenum Press, New York, pp 57-82

Kelley-Garvert F, Legator MS (1972) Cytogenetic and mutagenic effects of DDT and DDE in a Chinese hamster cell line. Mutat Res 17:223-229

Lovett EJ III, Schnitzer B, Keren DF, Flint A, Hudson JL, McClatchey KD (1984) Application of flow cytometry to diagnostic pathology. Lab Invest 50:115-140

Matsumura F (1985) Toxicology of pesticides, 2nd ed. Plenum Press, New York

McBee K, Bickham JW (1988) Petrochemical-related DNA damage in wild rodents detected by flow cytometry. Bull Environ Contam Toxicol 40:343-349

McBee K, Bickham JW (1990) Mammals as bioindicators of environmental toxicity. In: Genoways HH (ed) Current Mammalogy Vol 2. Plenum Publ Corp, New York, pp 37-88

McBee K, Bickham JW, Brown KW, Donnelly KC (1987) Chromosomal aberrations in native small mammals (Peromyscus leucopus and Sigmodon hispidus) at a petrochemical waste disposal site: I. Standard karyology. Arch Environ Contam Toxicol 16:681-688

Miller GT Jr (1992) Living in the environment: An introduction to environmental science. Wadsworth Publ Co, Belmont, California

Morgan DP, Roan CC (1971) Absorption, storage, and metabolic conversion of ingested DDT and DDT metabolites

in man. Arch Environ Health 22:301-308

Otto F (1990) DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. In: Darzynkiewicz Z Crissman HA (eds) Methods in cell biology Vol 33: Flow cytometry. Academic Press, San Diego, pp 105-110

Otto F, Oldiges H, Göhde W, Dertinger H (1981a) Flow cytometric analysis of mutagen induced chromosomal damage. Acta Pathol Microbiol Scand Sect A Supl 274:284-286

Otto FJ, Oldiges H, Göhde W, Jain VK (1981b) Flow cytometric measurement of nuclear DNA content variations as a potential in vivo mutagenicity test. Cytometry 2:189-191

Ottoboni A, Ferguson JI (1969) Excretion of DDT compounds in rat milk. Tocicol Appl Pharmacol 15:56-61

Palmer KA, Green S, Legator MS (1972) Cytogenetic effects of DDT and derivatives of DDT in a cultured mammalian cell line. Toxicol Appl Pharmacol 22:355-364

Perry AE (1965) Population analysis of the guano bat Tadarida brasiliensis mexicana (Saussure) using the lens-weight method of age determination. Unpubl PhD dissertation, Oklahoma State University, Stillwater

Perry AE, Herreid CF II (1969) Comparison of the tooth-wear and lens-weight methods of age determination in the guano bat, Tadarida brasiliensis mexicana. J Mammal 50:357-360

Peterson JE, Robison WH (1964) Metabolic products of p,p'-DDT in the rat. Toxicol Appl Pharmacol 6:321-327

Peterson JE, Stahl KM, Meeker DL (1976) Simplified extraction and cleanup for determining organochlorine pesticides in small biological samples. Bull Environ Contam Toxicol 15:135-139

Phaender FK, Alexander M (1972) Extensive microbial degredation of DDT in vitro and DDT metabolism by natural communities. J Agr Food Chem 20:842-846

Preston RJ, Au W, Bender MA, Brewen JG, Carrano AV, Heddle JA, McFee AF, Wolff S, Wassom JS (1981) Mammalian in vivo and in vitro cytogenetic assays: A report of the US EPA's Gene-Tox Program. Mutat Res 87:143-188

Short HL, Davis RB, Herried CF II (1960) Movements of the Mexical free-tailed bat in Texas. Southwestern Nat 5:208-216

Wares MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R, Garrett NE (1982) Study of pesticide genotoxicity. In: Fleck RA Hollaender A (eds) Genetic toxicology: An agricultural perspective. Plenum Press, New York, pp 275-326

White DH, Krynitsky AJ (1986) Wildlife in some areas of New Mexico and Texas accumulate elevated DDE residues, 1983. Arch Environ Contam Toxicol 15:149-157

Yoder J, Watson M, Benson WW (1973) Lymphocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides. Mutat Res 21:335-340

APPENDIX

COLLECTION INFORMATION AND ASSAYS PERFORMED FOR SPECIMENS EXAMINED

APPENDIX. Collection localities and assays performed for specimens examined.

Number	Sex	Site	Date	SBMCA	FCM	Gas Chromatography			
						Carcass	Brain	Embryo	Organs
OK00371	F	Vickery	30 May 1990	x	x	x	x	x	
OK00372	F	Vickery	30 May 1990	x	x	x	x	x	
OK00373	F	Vickery	30 May 1990	x	x	x	x	x	
OK00374	F	Vickery	30 May 1990	x	x	x	x	x	
OK00375	F	Vickery	30 May 1990	x	x	x	x	x	
OK00376	F	Vickery	30 May 1990	x	x	x	x	x	
OK00377	F	Vickery	30 May 1990	x	x	x	x	x	
OK00378	F	Vickery	30 May 1990	x	x	x	x	x	
OK00379	F	Vickery	30 May 1990	x	x	x	x	x	
OK00380	F	Vickery	30 May 1990	x	x	x	x	x	
OK00381	F	Vickery	27 June 1990	x	x	x	x	x	
OK00382	F	Vickery	27 June 1990	x	x	x	x		
OK00383	F	Vickery	27 June 1990	x	x	x	x		
OK00384	F	Vickery	27 June 1990	x	x	x	x		
OK00385	F	Vickery	27 June 1990	x	x	x	x		
OK00386	F	Vickery	27 June 1990	x	x	x	x		
OK00387	F	Vickery	27 June 1990	x	x	x	x		
OK00388	F	Vickery	27 June 1990	x	x	x	x		
OK00389	F	Vickery	27 June 1990	x	x	x	x	x	
OK00390	M	Vickery	27 June 1990	x	x	x	x		
OK00391	F	Vickery	27 June 1990	x	x	x	x		
OK00392	F	Vickery	27 June 1990	x	x	x	x		
OK00393	F	Vickery	27 June 1990		x	x	x		x
OK00394	F	Vickery	27 June 1990		x	x	x		x
OK00395	F	Vickery	27 June 1990		x	x	x		x
OK00396	F	Vickery	23 July 1990	x	x	x	x		

APPENDIX (CONT.)

Number	Sex	Site	Date	SBMCA	FCM	Gas Chromatography			
						Carcass	Brain	Embryo	Organs
OK00397	F	Vickery	23 July 1990	x	x	x	x		
OK00398	M	Vickery	23 July 1990	x	x	x	x		
OK00409	M	Vickery	23 July 1990	x	x	x	x		
OK00410	F	Vickery	23 July 1990	x	x	x	x		
OK00411	F	Vickery	23 July 1990	x	x	x	x		
OK00412	F	Vickery	23 July 1990	x	x	x	x		
OK00413	F	Vickery	23 July 1990	x	x	x	x		
OK00414	M	Vickery	23 July 1990	x	x	x	x		
OK00415	M	Vickery	23 July 1990	x	x	x	x		
OK00416	F	Vickery	23 July 1990	x	x	x	x		
OK00417	F	Vickery	23 July 1990	x	x	x	x		
OK00418	F	Vickery	23 July 1990	x	x	x	x		
OK00419	F	Vickery	23 July 1990	x	x	x	x		
OK00420	F	Vickery	23 July 1990	x	x	x	x		
OK00421	F	Vickery	23 July 1990		x	x	x		x
OK00422	F	Vickery	23 July 1990		x	x	x		x
OK00423	F	Vickery	23 July 1990		x	x	x		x
OK00424	F	Carlsbad	14 August 1990	x	x	x	x		
OK00425	F	Carlsbad	14 August 1990	x	x	x	x		
OK00426	F	Carlsbad	14 August 1990	x	x	x	x		
OK00427	F	Carlsbad	14 August 1990	x	x	x	x		
OK00428	F	Carlsbad	14 August 1990	x	x	x	x		
OK00429	F	Carlsbad	14 August 1990	x	x	x	x		
OK00430	F	Carlsbad	14 August 1990	x	x	x	x		
OK00431	F	Carlsbad	14 August 1990	x	x	x	x		
OK00432	F	Carlsbad	14 August 1990	x	x	x	x		
OK00433	F	Carlsbad	14 August 1990	x	x	x	x		

APPENDIX (CONT.)

Number	Sex	Site	Date	SBMCA	FCM	Gas Chromatography			
						Carcass	Brain	Embryo	Organs
OK00434	F	Carlsbad	14 August 1990	x	x	x	x		
OK00435	F	Carlsbad	14 August 1990	x	x	x	x		
OK00436	M	Carlsbad	14 August 1990	x	x	x	x		
OK00437	M	Carlsbad	14 August 1990	x	x	x	x		
OK00438	M	Carlsbad	14 August 1990	x	x	x	x		
OK00439	M	Carlsbad	14 August 1990	x	x	x	x		
OK00440	M	Carlsbad	14 August 1990	x	x	x	x		
OK00441	M	Carlsbad	14 August 1990	x	x	x	x		
OK00442	M	Carlsbad	14 August 1990	x	x	x	x		
OK00443	M	Carlsbad	14 August 1990	x	x	x	x		
OK00444	M	Carlsbad	14 August 1990	x	x	x	x		
OK00445	M	Carlsbad	14 August 1990	x	x	x	x		
OK00446	M	Carlsbad	14 August 1990	x	x	x	x		
OK00447	M	Carlsbad	14 August 1990	x	x	x	x		
OK00448	F	Carlsbad	14 August 1990		x	x	x		x
OK00449	F	Carlsbad	14 August 1990		x	x	x		x
OK00450	M	Carlsbad	14 August 1990		x	x	x		x
OK00483	M	Vickery	22 August 1990		x	x	x		x
OK00484	M	Vickery	22 August 1990		x	x	x		x
OK00485	M	Vickery	22 August 1990		x	x	x		x
OK00486	F	Vickery	22 August 1990	x	x	x	x		
OK00487	F	Vickery	22 August 1990	x	x	x	x		
OK00488	F	Vickery	22 August 1990	x	x	x	x		
OK00489	F	Vickery	22 August 1990	x	x	x	x		
OK00490	F	Vickery	22 August 1990	x	x	x	x		
OK00491	F	Vickery	22 August 1990	x	x	x	x		
OK00492	F	Vickery	22 August 1990	x	x	x	x		

APPENDIX (CONT.)

Number	Sex	Site	Date	SBMCA	FCM	Gas Chromatography			
						Carcass	Brain	Embryo	Organs
OK00493	F	Vickery	22 August 1990	x	x	x	x		
OK00494	F	Vickery	22 August 1990		x	x	x		
OK00495	F	Vickery	22 August 1990	x	x	x	x		
OK00496	F	Vickery	22 August 1990	x	x	x	x		
OK00497	F	Vickery	22 August 1990	x	x	x	x		
OK00498	M	Vickery	22 August 1990	x	x	x	x		
OK00499	M	Vickery	22 August 1990	x	x	x	x		
OK00500	M	Vickery	22 August 1990	x	x	x	x		
OK00514	M	Vickery	22 August 1990	x	x	x	x		
OK00515	M	Vickery	22 August 1990	x	x	x	x		
OK00516	M	Vickery	22 August 1990		x	x	x		
OK00517	M	Vickery	22 August 1990	x	x	x	x		
OK00518	M	Vickery	22 August 1990	x	x	x	x		
OK00519	M	Vickery	22 August 1990	x	x	x	x		
OK00520	M	Vickery	22 August 1990	x	x	x	x		
OK00521	M	Vickery	22 August 1990	x	x	x	x		
OK00522	M	Vickery	22 August 1990	x	x	x	x		
OK00625	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00626	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00627	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00628	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00629	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00630	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00631	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00632	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00633	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00634	F	Carlsbad	21 May 1991	x	x	x	x	x	

APPENDIX (CONT.)

Number	Sex	Site	Date	SBMCA	FCM	Gas Chromatography			
						Carcass	Brain	Embryo	Organs
OK00635	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00636	F	Carlsbad	21 May 1991	x	x	x	x	x	
OK00637	M	Carlsbad	21 May 1991	x	x	x	x		
OK00638	M	Carlsbad	21 May 1991	x	x	x	x		
OK00639	M	Carlsbad	21 May 1991	x	x	x	x		
OK00640	M	Carlsbad	21 May 1991	x	x	x	x		
OK00641	M	Carlsbad	21 May 1991	x	x	x	x		
OK00642	M	Carlsbad	21 May 1991	x	x	x	x		
OK00643	M	Carlsbad	21 May 1991	x	x	x	x		
OK00644	M	Carlsbad	21 May 1991	x	x	x	x		
OK00645	M	Carlsbad	21 May 1991	x	x	x	x		
OK00646	M	Carlsbad	21 May 1991	x	x	x	x		
OK00647	M	Carlsbad	21 May 1991	x	x	x	x		
OK00648	M	Carlsbad	21 May 1991	x	x	x	x		
OK00649	F	Carlsbad	21 May 1991		x	x	x	x	x
OK00650	F	Carlsbad	21 May 1991		x	x	x	x	x
OK00651	F	Carlsbad	21 May 1991		x	x	x	x	x
OK00652	F	Vickery	28 May 1991	x	x	x	x	x	
OK00653	F	Vickery	28 May 1991	x	x	x	x	x	
OK00654	F	Vickery	28 May 1991	x	x	x	x	x	
OK00655	F	Vickery	28 May 1991	x	x	x	x	x	
OK00656	F	Vickery	28 May 1991	x	x	x	x	x	
OK00657	F	Vickery	28 May 1991	x	x	x	x	x	
OK00658	F	Vickery	28 May 1991	x	x	x	x	x	
OK00659	F	Vickery	28 May 1991	x	x	x	x	x	
OK00660	F	Vickery	28 May 1991	x	x	x	x	x	
OK00661	F	Vickery	28 May 1991	x	x	x	x	x	

APPENDIX (CONT.)

Number	Sex	Site	Date	SBMCA	FCM	Gas Chromatography			
						Carcass	Brain	Embryo	Organs
OK00662	F	Vickery	28 May 1991	x	x	x	x	x	
OK00663	F	Vickery	28 May 1991	x	x	x	x	x	
OK00664	M	Vickery	28 May 1991	x	x	x	x		
OK00665	M	Vickery	28 May 1991	x	x	x	x		
OK00666	M	Vickery	28 May 1991	x	x	x	x		
OK00667	M	Vickery	28 May 1991	x	x	x	x		
OK00668	M	Vickery	28 May 1991	x	x	x	x		
OK00669	M	Vickery	28 May 1991	x	x	x	x		
OK00670	M	Vickery	28 May 1991	x	x	x	x		
OK00671	M	Vickery	28 May 1991	x	x	x	x		
OK00672	M	Vickery	28 May 1991	x	x	x	x		
OK00673	M	Vickery	28 May 1991	x	x	x	x		
OK00674	M	Vickery	28 May 1991	x	x	x	x		
OK00675	M	Vickery	28 May 1991	x	x	x	x		
OK00676	F	Vickery	28 May 1991		x	x	x	x	x
OK00677	F	Vickery	28 May 1991		x	x	x	x	x
OK00678	F	Vickery	28 May 1991		x	x	x	x	x
OK00993	F	Carlsbad	9 August 1991	x	x	x	x		
OK00994	F	Carlsbad	9 August 1991	x	x	x	x		
OK00995	F	Carlsbad	9 August 1991	x	x	x	x		
OK00996	F	Carlsbad	9 August 1991	x	x	x	x		
OK00997	F	Carlsbad	9 August 1991	x	x	x	x		
OK00998	F	Carlsbad	9 August 1991	x	x	x	x		
OK00999	F	Carlsbad	9 August 1991	x	x	x	x		
OK01000	F	Carlsbad	9 August 1991	x	x	x	x		
OK01001	F	Carlsbad	9 August 1991	x	x	x	x		
OK01002	F	Carlsbad	9 August 1991	x	x	x	x		

APPENDIX (CONT.)

Number	Sex	Site	Date	SBMCA	FCM	Gas Chromatography			
						Carcass	Brain	Embryo	Organs
OK01003	F	Carlsbad	9 August 1991	x	x	x	x		
OK01004	F	Carlsbad	9 August 1991	x	x	x	x		
OK01005	M	Carlsbad	9 August 1991	x	x	x	x		
OK01006	M	Carlsbad	9 August 1991	x	x	x	x		
OK01007	M	Carlsbad	9 August 1991	x	x	x	x		
OK01008	M	Carlsbad	9 August 1991	x	x	x	x		
OK01009	M	Carlsbad	9 August 1991	x	x	x	x		
OK01010	M	Carlsbad	9 August 1991	x	x	x	x		
OK01011	M	Carlsbad	9 August 1991	x	x	x	x		
OK01012	M	Carlsbad	9 August 1991	x	x	x	x		
OK01013	M	Carlsbad	9 August 1991	x	x	x	x		
OK01014	M	Carlsbad	9 August 1991	x	x	x	x		
OK01015	M	Carlsbad	9 August 1991	x	x	x	x		
OK01016	M	Carlsbad	9 August 1991	x	x	x	x		
OK01017	F	Vickery	20 August 1991	x	x	x	x		
OK01018	F	Vickery	20 August 1991	x	x	x	x		
OK01019	F	Vickery	20 August 1991	x	x	x	x		
OK01020	F	Vickery	20 August 1991	x	x	x	x		
OK01021	F	Vickery	20 August 1991	x	x	x	x		
OK01022	F	Vickery	20 August 1991	x	x	x	x		
OK01023	F	Vickery	20 August 1991	x	x	x	x		
OK01024	F	Vickery	20 August 1991		x	x	x		
OK01025	F	Vickery	20 August 1991	x	x	x	x		
OK01026	F	Vickery	20 August 1991	x	x	x	x		
OK01027	F	Vickery	20 August 1991	x	x	x	x		
OK01028	F	Vickery	20 August 1991	x	x	x	x		
OK01029	M	Vickery	20 August 1991	x	x	x	x		

APPENDIX (CONT.)

Number	Sex	Site	Date	SBMCA	FCM	Gas Chromatography			
						Carcass	Brain	Embryo	Organs
OK01030	M	Vickery	20 August 1991	x	x	x	x		
OK01031	M	Vickery	20 August 1991	x	x	x	x		
OK01032	M	Vickery	20 August 1991	x	x	x	x		
OK01033	M	Vickery	20 August 1991	x	x	x	x		
OK01034	M	Vickery	20 August 1991	x	x	x	x		
OK01035	M	Vickery	20 August 1991	x	x	x	x		
OK01036	M	Vickery	20 August 1991	x	x	x	x		
OK01037	M	Vickery	20 August 1991	x	x	x	x		
OK01038	M	Vickery	20 August 1991	x	x	x	x		
OK01039	M	Vickery	20 August 1991	x	x	x	x		
OK01040	M	Vickery	20 August 1991	x	x	x	x		
OK01041	F	Vickery	20 August 1991	x	x	x	x		

VITA

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Candidate for the Degree of

Doctor of Philosophy

Thesis: CYTOGENETIC ABERRANCY AND ORGANOCHLORINE PESTICIDE ACCUMULATION IN THE MEXICAN FREE-TAILED BAT: A COMPARISON BETWEEN OKLAHOMA AND NEW MEXICO POPULATIONS

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