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Residues of Lead, Cadmium, and Arsenic in Livers of Mexican Free-Tailed Bats

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Since 1936, the size of the summer population of Mexican free-tailed bats, Tadarida brasiliensis (Chiroptera: Molossidae), at Carlsbad Caverns, New Mexico, declined from an estimated 8.7 million to slightly more than 200,000 in 1973 (Altenbach et al. 1979). In 1991, the population was estimated at 700,000 (David Ek, Carlsbad Caverns Natl. Park, pers comm). This decline has been attributed primarily to human disturbance and the heavy agricultural use of organochlorine pesticides (principally DDT) in Central America, Mexico, and the southwestern United States. Members of this species forage extensively over heavily agricultural areas, feeding on insects potentially contaminated with high levels of insecticides and trace metals. Accumulation studies (Geluso et al. 1976, 1981) reported levels of DDE present in body tissues of juvenile bats at Carlsbad Caverns during the 1970's as high enough to be potentially hazardous, primarily during their first migration.

However, contamination from elements such as lead, cadmium, and arsenic have not been examined. The accumulation of these elements in the tissues of wild vertebrates is often related to their food habits and, presumably, a primary reflection of contamination of the food supply (Dustman and Stickel 1969). Insects constitute an important dietary ingredient for many birds and mammals, allowing for passage of environmental contaminants to higher trophic levels where they are accumulated and exert their toxic effects (McBee and Bickham 1992). The presence of elemental contaminants in body tissues of bats is poorly documented (Clark et al. 1986, Clark 1979, 1981), with studies of T. brasiliensis restricted to a survey of mercury residues

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in breast muscle and guano from specimens collected in Arizona (Reidinger 1972).

The objectives of this study were to examine and compare lead, cadmium, and arsenic contamination in livers of adult T. brasiliensis from Carlsbad Caverns and Vickery Cave, a maternity colony in northwestern Oklahoma. No contamination data or populational declines have been reported for the Oklahoma population. Nor has the Carlsbad Caverns population been examined for the possibility that environmental contaminants such as lead,cadmium, and arsenic may have contributed to observed historic declines.

Lead, cadmium, and arsenic were specifically selected because of their documented toxic and/or reproductive effects and their potential availability to this species. Large quantities of tetraethyl lead have been released into the environment and other lead compounds continue to be released by industrial manufacturing and petroleum refinement processes. At high doses, lead induces a reduction in the number of pregnancies in mice and provokes an early mortality in mouse embryos before implantation. After implantation, mouse fetuses demonstrate a delay in growth, post-implantation mortality, and an increase in skeletal malformations (Tachon et al. 1983). Other effects include impaired function of the nervous, gastrointestinal, muscular, and hematopoietic systems (Eisler 1988b). Cadmium is used in a number of industrial processes such as metal plating and fabrication of alloys and is released from phosphate fertilizers and combusted coals. Levels exceeding 10.0 µg/g in liver may be considered indicative of cadmium contamination (Eisler 1985, Puls 1990). Teratogenicity appears to be greater for cadmium than for other elements, including lead and arsenic. Laboratory studies with rats and mice have demonstrated that cadmium metals or salts also cause malignancies (Eisler 1985). Arsenical compounds have been commonly used as herbicides and defoliants, containing highly soluble trivalent or arsenite forms. These compounds have been demonstrated to cause abnormal embryonic development, degenerative tissue changes, cancer, chromosomal damage, and death in domestic animals. However, documented episodes of wildlife contamination are rare (Eisler 1988a).

MATERIALS AND METHODS

Bats were collected using a hoop net as they emerged for nightly feeding excursions from both caves in May and August 1991. Bats were classified into subjective age groups by examining canine tooth wear following Anthony

(1988), with only those individuals judged as belonging to the oldest age class for each sex retained. Because of the lack of adequate known-age series of specimens of this species with which to calibrate tooth wear patterns, specific ages could not be assigned. Females in which the canine tips were worn approximately half way to the gums were retained whereas maximum wear observed in males was a flattening of the tips of the canines. Complete epiphyseal-diaphyseal fusion of phalangeal joints, obvious tooth wear, and pelage wear and coloration were used to verify that males were a minimum of nine to 12 months old and had survived at least one migration. Six individuals of each sex were collected from each site for measurement of lead, cadmium, and arsenic concentrations in livers. Animals were sacrificed by cervical dislocation within 12 hr of capture and livers removed and stored in liquid nitrogen until analyzed. Measurements of canine tip widths were used in statistical analyses for possible age-related accumulation of contaminants.

Tissue preparation followed protocols used by the Oklahoma Animal Disease Diagnostic Laboratory as adapted from Perkin-Elmer (1980). For lead and cadmium, half (0.2 - 0.25 g) of each liver was digested in 10 ml concentrated HNO_3 for 24 hr on a 60° C hot plate. For arsenic, the remaining liver tissue was digested in 10 ml concentrated HNO_3 and 2 ml $Ni(NO_3)_2 \cdot 6H_2O$ for 24 hr on a 60° C hot plate. Sufficient distilled, deionized water was then added to bring the final volume to 100 ml for lead and cadmium, and 10 ml for arsenic. Residues were determined by stabilized temperature graphite furnace atomic absorption spectrophotometry using a Perkin-Elmer Zeeman 5000 equipped with an HGA graphite furnace, Model 3600 data station, and AS40 Autosampler. Due to the small size of extraction samples, instrument detection limits were 0.2 µg/g (= parts per million) wet weight for all metals.

Atomic absorption analyses cannot provide a true zero concentration. Therefore, values representing half the detection limit (0.1 µg/g wet weight) were entered into the data sets for those samples found below accepted instrument detection limits. Because all data sets were positively skewed and log-transformed data were also nonnormally distributed, nonparametric Kruskal-Wallis analysis of variance by ranks and Mann-Whitney two-sample t-test were applied to nontransformed data for significant differences at the 0.05 level. Simple correlation coefficients between residue concentrations and canine tip widths were calculated and tested for significance using an F test. Because differences in canine morphology and wear patterns between males and females represents an indeterminate difference in age between sexes, comparisons between sexes were invalid and therefore not made. Results were also examined for correlations between carcass and brain concentrations of DDE (1,1-dichloro-2,2-bis[p-chlorophenyl]ethylene) in these same individuals (Thies 1993).

RESULTS AND DISCUSSION

Measurable levels of lead were found in all 48 samples (Table 1). Cadmium above 0.20 µg/g wet weight was detected in all but four males, a single male from the May Vickery sample and three from the August Vickery Cave sample (Table 2). Only one bat, a male from the August Vickery Cave sample, had an arsenic concentration (0.353 µg/g) above instrument detection limits.

Although ranges in lead concentration were observably greater in the Vickery Cave samples, the only significant difference was between August Vickery females and May Carlsbad females ($P = 0.0104$). Lead concentrations did not correlate with canine tip width in either sex, indicating that liver accumulation may not be age related in this species. DDE concentrations in carcasses and brain tissues were not correlated. With the exception of a single May Vickery male, six of the seven individuals with levels of lead > 10.0 µg/g were collected in August, which may indicate local exposure.

Sixteen of the 48 individuals had lead levels greater than 4.0 µg/g; this concentration has been found associated with chronic toxic effects in cattle (Puls, 1990). All specimens in this study had lead levels higher than those reported by Clark et al. (1986) in southeastern myotis (Myotis austroriparius) from central Florida. None of their bats, which were potentially receiving contamination from a battery salvage plant, contained liver residues greater than 0.58 µg/g. Because bats were collected at the onset of nightly feeding excursions, it was assumed that all individuals were capable of normal feeding behavior and, therefore, apparently healthy. All females in both May collections were pregnant and gross examination of the embryos showed no observable abnormalities. Although the sample sizes were small, these data would seem to indicate no adverse reproductive effects from lead contamination.

Major sources of environmental contamination of lead have historically included exhaust products of leaded gasoline, coal and oil combustion, metal smelting plants, lead arsenate pesticides, and phosphate fertilizers. Carlsbad Caverns was expected to

Table 1. Geometric mean and ranges (N=6 for each sample) of lead in livers (µg/g wet weight) of _Tadarida brasiliensis_ collected at Carlsbad Cavern, New Mexico, and Vickery Cave, Oklahoma.

	May 1991	August 1991
Females		
Carlsbad Cavern	1.91(1.41-2.96)[a]	4.35(1.11-13.70)
Vickery Cave	2.22(0.74-7.07)	5.07(2.47-34.90)[a]
Males		
Carlsbad Cavern	3.51(1.25-7.72)	3.67(1.45-16.00)
Vickery Cave	5.37(1.84-49.44)	5.34(1.40-27.64)

[a] significant difference between these two samples ($P = 0.0104$).

demonstrate higher lead levels due to its proximity to petrochemical refinery industries and heavy tourist automobile traffic associated with the national park. Vickery Cave, situated in an area of northwestern Oklahoma noted for agriculture and cattle grazing, was expected to have lesser opportunities for lead exposure. However, our results indicate that the Vickery Cave environment may actually be more conducive for lead accumulation in this species. Increased levels of contamination in the Vickery population may be attributable at least in part to oil and natural gas exploration and production which releases significant quantities of lead compounds into the environment (Edwards 1985). Insects exposed to these residues may also provide a mechanism for passage of lead to higher trophic levels, although Price et al. (1974) found that the insect component of the ecosystem does not act as a reservoir for large quantities of lead.

Liver cadmium levels demonstrated a number of significant differences among samples when examined by sex and collection period (Table 2). Analyses of the data, however, failed to indicate a clear pattern in tissue contamination rates. Males, with the exception of the Fall Carlsbad sample, and the Spring Carlsbad females do not differ in cadmium content from samples of _M. austroriparius_ from Gainesville, Florida (Clark et al. 1985). Six of our eight samples showed levels similar to their Judges Cave, Florida, site, with the

Table 2. Geometric mean and range (N=6 for each sample) of cadmium in livers (μg/g wet weight) of *Tadarida brasiliensis* collected at Carlsbad Cavern, New Mexico, and Vickery Cave, Oklahoma.

	May 1991	August 1991
Females		
Carlsbad Caverns	0.53(0.22-1.31)	0.80(0.52-1.55)
Vickery Cave	0.88(0.47-1.52)	1.16(0.60-1.98)
Males		
Carlsbad Caverns	0.44(0.28-0.67)[a]	0.88(0.51-1.46)[a,c]
Vickery Cave	0.44(BDL*-1.02)[b]	0.20(BDL-0.22)[b,c]

[a,b,c] pairs of means sharing the same letter differ significantly ($P < 0.05$) from each other.

* Below Detection Limits

exception of the Fall Vickery males (significantly lower) and Fall Vickery females (significantly higher). As with lead, cadmium concentrations did not correlate with canine tip width. Puls (1990) reported toxic liver levels at 80 μg/g for horses and 13 μg/g in pigs. If this species shows similar toxic responses to cadmium, data obtained in this study do not demonstrate a potentially harmful situation for either population.

Liver arsenic levels, with the exception of a single August Vickery Cave male, were below established instrument detection limits. Toxicity occurs in dogs, horses, and pigs at liver concentrations greater than 10 μg/g, 7 - 15 μg/g, and 6.3 - 28.0 μg/g, respectively. Concentrations less than 0.5 μg/g in these animals may result from environmental exposure to natural sources (Puls 1990).

Pesticide data for these individuals show DDE present in both populations (Thies 1993), but at levels much lower than reported by Geluso et al. (1976, 1981). No relationships or interactions among DDE concentrations in carcass and brain tissues, canine tip width (indicator of age), and lead, cadmium and arsenic were found. Among the three elements examined in this study, only lead may constitute a potential problem to the free-tailed bat. However, our results only document its presence and provide no further toxicity information beyond an apparent lack of effect on reproductive success. A similar increase in selenium above levels found to be toxic in domestic animals have been reported by Clark et al. (19890 in raccoons at Kesterson Reservoir, Merced Co., California, with no apparent adverse effects. It is possible that wild mammals undergo selective pressures which eliminate those individuals susceptible to increased contaminant exposures. Further studies will be required to determine if a relationship occurs between lead and pesticide exposure in juveniles during their first fall migration. Additional data are also required to verify whether contamination increases from May to August indicating that the collection sites are the major sites of uptake.

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