The Consumption of Metallic Lead and Its Effects on Tissue Lead Levels of Urban Eastern Gray Squirrels (*Sciurus carolinensis*)

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ABSTRACT: Eastern gray squirrels (*Sciurus carolinensis*) are known to routinely consume or be exposed to lead from many anthropogenic sources, including ingesting bullet fragments, and gnawing on flashing. However, there is little research on consumption of metallic lead in squirrels. To determine if squirrels purposefully consume and metabolize lead, we supplied lead in the form of ingots to determine if squirrels are primarily gnawing lead, but not ingesting any, or incidentally ingesting relatively small amounts and compared that to lead levels from untreated squirrels from the same area. We found that squirrels readily consumed the provided lead ingots. The pooled mean liver and muscle lead levels of treated squirrels was 2.790 ppm (n = 6; CI $\pm$ 3.478) and 0.524 ppm (n = 5; CI $\pm$ .159), respectively, compared with the pooled mean liver 0.374 ppm (n = 6; CI $\pm$ 0.079) and muscle 0.252 ppm (n = 6; CI $\pm$ 0.094) lead levels from untreated squirrels. Even though this was a relatively large effect size between the liver of the squirrels fed lead (Cohen's d = 1.00) and a smaller effect size between muscle tissue (Cohen's d = 0.28), the 2 groups were not statistically different, likely due to the small sample size. Because squirrels will readily consume anthropogenic lead, raptors and other predators may bioaccumulate this lead through their foraging behaviors.

Key Words: lead consumption, eastern gray squirrel, *Sciurus carolinensis*, lead toxicosis, environmental contamination

Proceedings of the 16th Wildlife Damage Management Conference.
(L.M. Conner, M.D. Smith, Eds). 2016. Pp. 83-87.

INTRODUCTION

Eastern gray squirrels (*Sciurus carolinensis*) have been known to routinely gnaw lead and damage items constructed from lead (McKinnon et al. 1976, Lewis et al. 2001, Pokras and Kneeland 2008). Though this phenomena is well documented, the cause for this behavior is not known (Pokras and Kneeland 2008) and there is little research on deliberate consumption of metallic lead in squirrels. Medvedev (1999) examined lead levels in several species of wildlife in Russia, including a native species of squirrel (*Sciurus vulgaris*), and found elevated liver lead levels. The authors speculated this liver lead elevation to be due to the squirrel's preference for mushrooms. In addition, liver lead levels were higher than muscle lead levels. We know that eastern gray squirrels will metabolize large quantities of lead (McKinnon et al. 1976, Lewis et al. 2001); however, the source of lead exposure in eastern gray squirrels seems to be variable. McKinnon et al. (1976) speculated the source of lead in their study to be both inhaled from leaded gasoline and ingested while foraging in the urban environment. While aerosolized lead is no longer a significant problem (EPA 2012), metallic lead is still commonly found in association with human activity. Eastern gray squirrels were found to have elevated tissue lead levels in a study conducted at the Federal Law Enforcement Training Facility in Glynn County, Georgia. This study determined that lead was being ingested in the form of lead bullet fragments as the animals foraged at a firing range, but it was unclear if this was deliberate or a consequence of foraging in areas with large amount of lead fragments. The authors also speculate that some species may have been attracted to the lead bullets because of the taste of the oxidized lead salts that formed on the fragments over time (Lewis et al. 2001), but no evidence was provided that squirrels purposefully seek out and consume lead. We have also observed squirrels gnawing lead from buildings in Murray, Kentucky and Hollywood, Maryland, but it is unclear if they are gnawing or consuming lead.

While we know that squirrels commonly gnaw metallic lead, we do not know if they are gnawing it for behavioral objectives and inadvertently ingesting it, if they metabolize it when they ingest lead, it, or if they are ingesting lead but it is passing it through the digestive system without being metabolized. In order to address these two questions we supplied anthropogenic lead in the form of ingots to determine if squirrels are primarily gnawing lead, but not ingesting any or incidentally ingesting relatively small amounts; and, if squirrels are ingesting lead, are they metabolizing it or is the lead being passed rapidly through the digestive tract without significant absorption.

STUDY AREA AND METHODS

Our study area was Murray, Kentucky, a small city of approximately 18,000 residents, and is also located in the Jackson Purchase region of western Kentucky. We collected by trapping and shooting with a pellet rifle using Gamo© PBA Raptor non-lead pellets. In addition, road-killed squirrels were collected when available. Live captured squirrels were euthanized with inhaled carbon dioxide (American Veterinary Medical Association 2013). Treated squirrels were collected from locations where they were actively removing metallic lead from soft lead ingots that we placed at sites where squirrels were known to have damaged lead components on homes in the past.

We collected liver and muscle samples from each squirrel. We combined muscle samples into ~6g pools consisting of ~1g of tissue from 6 squirrels and combined liver samples into ~5g pools consisting of ~1g of tissue from 5 squirrels for analysis. Tissue samples were analyzed by the Breathitt Veterinary Center Toxicology Laboratory (BVCTL) with atomic absorption spectroscopy. A Cohen's *d* effect size test was performed on tissue lead levels in addition to a one-tailed Welch's T-test to test for statistical significance between the squirrels in Murray and the squirrels collected from LBL. We also calculated a 95% confidence intervals (CI) to examine differences between the treated populations. Institutional Animal Care and Use Committee (IACUC) approval was obtained prior to the research (IACUC Number: 2012-016).

RESULTS

We collected squirrels from 30 untreated squirrels and 30 treated squirrels from the city of Murray. Squirrels in untreated and treated areas were pooled in to groups of 5-6 squirrels for testing. In a 3-month period (May-July 2013) approximately 184 g of metallic lead were removed from one site. Over a 2.25-year period, approximately 1,360 g of placed metallic lead was consumed. We recovered only a few small fragments of lead from the ground underneath locations where we placed the ingots.

Mean liver lead levels of treated squirrels was 2.790 ppm (n = 6; SE $\pm$ 1.353; CI $\pm$ 3.478) and 0.524 ppm (n = 5; SE $\pm$ 0.057; CI $\pm$ 0.159) from untreated squirrels. Pooled liver samples ranged from 0.25-9.24 ppm in treated squirrels and 0.42-0.73 ppm in untreated squirrels. Mean muscle lead levels from treated squirrels was 0.288 ppm (n = 6; SE $\pm$ 0.045; CI $\pm$ .1282) and 0.252 ppm (n = 6; SE $\pm$ 0.037; CI $\pm$ 0.094) in untreated squirrels. Pooled muscle samples ranged from 0.15-0.41 ppm in treated squirrels and 0.14-0.35 ppm in untreated squirrels. There was no statistically significant difference in the liver ($t_{1,5}$ = -1.67, P = 0.155) or the muscle ($t_{1,5}$ = -0.59; P = 0.284) between the treated and untreated squirrels; however, we did see a large effect size between liver (Cohen's *d* = 1.00) and a small effect size between muscle tissue (Cohen's *d* = 0.28).

DISCUSSION

Our research indicates that squirrels will actively seek out and consume lead, and that it is ingested and readily stored in the liver. While we could not positively demonstrate a difference in liver lead levels between the treated and untreated squirrels in Murray, the large difference between the means and the large effect size provide some evidence that this is the case. This supports previous evidence from Lewis et al. (2001) that squirrels will consume lead if it is readily available in the environment.

While we did see a large difference between the means, small sample size and large variance tempers these results. The large range in the pooled liver samples fed lead in Murray (0.25-9.24 ppm) indicate that it is likely that some squirrels were shot without having consumed lead. Squirrels were shot if they were in close proximity to our lead ingots; however, we had no way to determine if they had previously consumed lead. Ideally, individual squirrels would have been tested to account for this; however, it was necessary to pool the samples to provide enough tissue for sampling using the standard protocols at BVCTL. But even with the lack of statistical significance, the effect size, lack of lead fragments and shavings under limbs where lead was provided, and large range in liver levels indicate that squirrels purposefully consumed lead.

There was little difference in lead concentrations within muscle tissues between the two groups, suggesting that although squirrels readily metabolize and store lead in the liver, less lead is stored in muscle tissue. This is consistent with studies in swine (*Sus domesticus*) and cattle that found metabolized lead was stored more readily in liver tissue than in muscle tissue (Neimi et al. 1991). Medvedev (1997) also found higher concentrations of lead in squirrels (*Sciurus vulgaris*) than other tissues examined and relatively low lead levels in muscle tissue; however, concentrations of lead levels among other species and tissues were variable.

MANAGEMENT IMPLICATIONS

Squirrels have been known to metabolize lead in the vicinity of firearms ranges (Lewis et al. 2001). The precedent to issue consumption advisories due to possibly high lead concentrations in squirrel meat (EPA 2007, Division of Epidemiology 2009) could have consequence for sport hunting and consumption of squirrels near the thousands firing ranges and other areas where large quantities of available anthropogenic lead exist. While squirrel liver is not typically consumed by hunters , raptors and other carnivores may commonly consume squirrel liver and other organs when eating these prey item. Lead fragments from bullets are often cited as the primary source of lead for lead toxicosis in raptors (Kendall et al. 1995); however, if increased lead levels in squirrels and other rodents is wide spread, it may be that consumption of lead from anthropogenic sources are also a significant contributor. Sources of anthropogenic lead, including flashing, are still sold in home improvement stores, though acceptable non-lead alternatives area available. If increased lead concentrations in squirrels and other rodents is a concern for biomagnification, legislation may need to be enacted reducing the availability of commonly consumed anthropogenic lead sources.

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