CONTAMINANTS AT A SHOOTING RANGE: TOXICOLOGICAL AND NUTRITIONAL SIGNIFICANCE TO BIRDS AND MAMMALS

by

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(ABSTRACT)

Target shooting in the United States has become an increasingly popular sport in the last century. In addition to the large quantity of lead pellets littering range grounds and surrounding land, considerable amounts of clay target fragments cover shooting range areas as well as adjoining habitats. Polycyclic aromatic hydrocarbons (PAHs) within the target, as well as lead pellets from shotguns pose multiple threats to a variety of wildlife. To determine the effects of clay target and lead pellet ingestion on wildlife, I conducted controlled experiments on Coturnix quail exposed to clay targets in the lab, and collected wild birds and mammals exposed to lead pellets at a shooting range.

The first Coturnix study determined whether quail voluntarily consumed target fragments or limestone fragments. In both fall ($F=29.2$, $P<0.01$) and spring ($F=6.45$, $P=0.02$) experiments, I found that quail consistently selected limestone fragments, but almost completely rejected clay target fragments. In the second study, quail were force-fed varying amounts of target dust on a weekly basis to simulate sporadic exposure to clay target dust. In both summer ($F=1.63$, $P=0.23$) and winter ($F=0.34$, $P=0.8$) trials, male quail did not have significant weight loss. Female quail

had insignificant weight losses in summer trials ($F=1.63$, $P=0.23$) but experienced weight gains in winter trials ($F=3.53$, $P=0.04$). In the third and final Coturnix study, varying amounts of target dust were incorporated into daily feed rations to simulate frequent exposure to clay target dust. Male quail experienced weight loss in both summer ($F=16.13$, $P<0.01$) and winter ($F=8.47$, $P<0.01$) trials. Female quail also suffered weight loss in both summer ($F=15.62$, $P<0.01$) and winter ($F=17.50$, $P<0.01$) trials. Weight loss likely resulted from inadequate nutrition as opposed to target poisoning. However, because there were no biochemical analyses performed to test for PAH presence, no definite conclusions can be made.

The second study focused on lead contamination in Passeriformes, perching birds, and small mammals. Seventeen of 20 birds (85%) (Passerine spp) captured at the shooting range had elevated lead levels ($F=5.21$, $P<0.028$), when compared to birds (n=20) at the control site. Nine of 26 (35%) white-footed mice (*Peromyscous leucopus*), trapped at the shooting range had elevated liver ($F=9.78$, $P=0.0029$) and kidney ($F=22.49$, $P<0.01$) lead levels. These results indicate that Passerine species as well as *Peromyscous* species around shooting ranges inadvertently consume lead, either as lead pellets, mistaking them for grit or dietary items, or through environmental sources such as water, soil, and vegetation.

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INTRODUCTION

Baer et al. (1995) determined that clay target use in the USA averages about 560 million targets/year. Studies on clay targets indicate that each target contains approximately 67% limestone, 32% petroleum pitch, and 1% aqueous paint, if the target is painted (Baer et al., 1995). The toxic components are polycyclic aromatic hydrocarbons (PAHs) found within the petroleum matrix. These are environmental contaminants that have been identified in a variety of media, including the air, soil, and water (Eisler, 1987). PAHs have both mutagenic and carcinogenic properties, making them potentially harmful to wild animals that may inadvertently ingest target fragments as food or through secondary consumption. Thus it is important to know the effects of ingested PAHs on wildlife species.

Few studies have been conducted on the toxic effects of clay targets in wildlife or human subjects. Baer et al. (1995) concluded that PAHs likely would not leach from the clay target's petroleum substrate and harm aquatic species. Despite this conclusion, Baer et al. (1995) suggested that paucity of data and lack of tests warranted further wildlife studies. However, no studies have been published since their paper. LaFontaine et al. (1999), who studied human subjects working at a clay target manufacturing plant, concluded that petroleum was not as harmful as coal-tar pitch, the predecessor to petroleum pitch. However, they recommended further studies to determine overall long-term effects and exposure routes.

Lead pellets, from shotgun shells used for shooting clay targets, have been studied thoroughly because of their adverse effects resulting from accidental ingestion. Birds and mammals inadvertently consume lead pellets, mistaking them for grit and seed (Vyas et al., 2001), and may become sick or die. Although lead shot has been banned in the use of

waterfowl hunting (Anderson, 1992), shooting ranges across the country still allow the use of lead for target practice, likely resulting in the deaths of many wildlife species. Numerous studies have addressed the issue of lead shot ingestion, yet few have examined the effects of lead on small mammals and perching birds.

This study focused on the toxicity of PAHs in clay targets to captive Coturnix quail, (*Coturnix coturnix japonica)*, and investigated the presence of lead in small mammals and Passerine species at the shooting range on the Blacksburg Ranger District of the Jefferson National Forest. My specific objectives were to:

1. Measure the effects of ingested clay targets on captive coturnix quail as a model for avian species in general.
2. Investigate the presence of lead in wild avian and mammalian species residing near the Blacksburg shooting range on the Jefferson National Forest.

The goal of this research was to provide information that would aid in future management and clean-up at shooting ranges.

LITERATURE REVIEW

Coal-Tar Pitch

In 1933, several herds of pigs grazing near an abandoned shooting range ingested clay target fragments, developed symptoms such as appetite loss, weakness, depression, and subsequently died (Quin et al, 1933). Necropsies of these animals revealed anemia, jaundice, liver necrosis, and further indications of severe liver damage. Graham et al. (1940) conducted several tests on experimental pigs to determine the toxic component of the clay targets. Their initial concern was lead in the paint, but when they completed their analyses, they found that coal-tar pitch in the target matrix was fatal to the majority of experimental subjects. Necropsies of the study animals revealed extensive liver damage, as had been observed in the grazing pigs near the shooting range.

Libke and Davis (1967) completed additional studies on coal-tar pitch poisoning. The first study involved 21 pigs that were fed finely ground target material mixed with milk replacer for 14 days. Eleven pigs died and the remaining 10 were euthanized for necropsy. Four of the initial 11 pigs died with no symptoms, while the remaining 7 developed various symptoms prior to their death such as weakness, lack of coordination, anemia, anorexia, and jaundice. Necropsies of all pigs indicated that their livers were similar in appearance: congested, enlarged, stippled with brown and dark red areas, and friable.

In a second study, 9 pigs were force-fed powdered clay targets for 4 days (Davis and Libke, 1968). The target dust was mixed with water and administered through a stomach tube. As the experiment progressed, 8 pigs died and the ninth was euthanized for necropsy. Similar to

the first experiment, 5 pigs developed symptoms such as weakness, lack of coordination, anemia, and anorexia. The remaining 3 pigs were found dead with no previous symptoms. Necropsies on these 8 pigs showed congested and enlarged livers stippled with brown and dark red areas, analogous to the first study. Davis and Libke (1968) surmised that the cause of these illnesses was the coal-tar pitch binder within the clay target matrix. This relatively unknown substance acted quickly and resulted in death in most cases. Coal-tar pitch is a substance rich in PAHs, compounds that have proven toxic and deadly in many circumstances involving both animal and human subjects (Samanta et al., 2002). Modern clay targets substitute coal-tar pitch with petroleum pitch, a substance less rich in PAHs (Lafontaine et al., 1999). However, the full extent of petroleum pitch toxicity to animals is not yet known (Baer et al., 1995).

Petroleum Pitch

Despite the scarcity of data concerning petroleum pitch in clay targets, a recent study confirmed that petroleum substances yield fewer PAHs than coal-tar pitch. LaFontaine et al. (1999) examined the occurrence of PAH indicators in atmospheric zones, as well as urine samples of several individuals working in a clay target factory. The study monitored 5 workers who spent each day handling petroleum pitch targets on manufacturing lines. The research team took urine and air samples at different times throughout the study, in addition to samples taken after the workers had not been exposed to clay targets for 3 days. They determined that benzo(a)pyrene (BaP), a highly toxic PAH indicator, was 10x less abundant within the body, when compared to workers who had handled coal-tar pitch targets in the past. Additionally, this study proposed that a minimum of 70% of indicator excreted in the urine was related to dermal absorption, indicating

the likelihood of dermal contact in the environment was much higher than the risk from inhalation. However, further studies were suggested to determine main exposure routes.

A recent study investigated the possible effects of PAHs in the petroleum matrix using crushed clay targets and aquatic species. Baer et al. (1995), performed the tests on a host of marine and freshwater species, including fathead minnows (*Pimephales promelas)*, daphnia (*Daphnia magna)*, opossum shrimp/mysid (*Mysidopsis bahia)*, silversides (*Menidia menidia)*, eastern oysters (*Crassostrea virginica)*, and marine diatoms (*Skeletonema costatum)*. Targets were crushed to 2 to 5 mm with a specified amount placed directly into the water with the species of interest. In a second experiment, water was combined with crushed target in a 1:4 ratio by volume to create a leachate that was placed in the same container as the study species. The final results for both experiments indicated low acute toxicosis to all aquatic organisms, relative to EPA standards. Baer et al. (1995) concluded that the PAHs in the targets were unlikely to leach because of manufacturing methods: PAHs in the petroleum pitch are bound under pressure and heat, thus minimizing the release of any substance. However, Baer et al. (1995) recommended that target shooting near aquatic areas be minimized due to scarcity of tests dealing with toxicity effects on sensitive life stages, and the paucity of tests associated with freshwater species.

PAHs in the Environment

Few studies have examined the toxicosis of PAHs in clay targets, but a plethora of studies have examined the effects of PAHs on various organisms in diverse environments. PAHs are made up of hydrogen and carbon molecules in 2 or more fused benzene rings (Eisler, 1987). Those of a

lower molecular weight (2 to 3 rings) exhibit significant toxicity, but are noncarcinogenic; conversely, those of a higher molecular weight (4 to 7 rings) are carcinogenic as well as mutagenic (Eisler, 1987). Environmental concerns center around PAHs that weigh between 128.16-300.36 grams (Eisler, 1987). Unfortunately, few generalities can be made about most PAHs, as they are extremely variable in both physiochemical and toxicological properties, as well as the effect they have on individual species (Lee and Grant, 1981).

Evidence of PAH contamination has been found in a range of settings, including plant and animal tissues, soils, air, sediments, drinking water, surface water, well water, and ground water (EPA, 1980). Examples of PAH production include coke production in the steel and iron industry, manufacture of asphalt and coal tar pitch, open burning, and emissions from internal combustion engines most commonly used for transportation (Eisler, 1987). As a result of anthropogenic activities across the globe, approximately 230,000 tons of PAHs enter aquatic habitats each year and 43,000 tons are discharged into the atmosphere (Eisler, 1987).

PAHs occur naturally in the wild, through volcanic activity as well as microorganism synthesis; however, artificial production vastly outweighs the synthesis and degradation of natural PAHs (Suess 1976; Sims and Overcash, 1983). It is possible for PAHs to degrade in a natural setting, either through microbial transformation or photodegradation; it is also possible for animals to metabolize PAHs to end products, which may undergo degradation (Edwards 1983). Despite these assertions, most PAH degradation is still not understood. This is especially disconcerting in light of the great excess of PAHs dispensed into the environment on a daily basis. Some PAHs are transformed by animals, metabolized by liver mixed-function oxidases, to intermediates that are highly mutagenic, carcinogenic, and toxic (Sims and Overcash 1983), thus affecting the survival of various species.

Benzo(a)pyrene

According to the World Health Organization and the U.S. Environmental Protection Agency, 16 PAHs have been designated as priority pollutants (Beyer et al, 1996). These 16 PAHs were found in the clay target dust used in this study (Table 1.12). One of the more carcinogenic and thus more damaging of the 16 compounds is benzo(a)pyrene, BaP. Industrial processes account for the entry of approximately 1,045 tons of BaP into the atmosphere each year (Lo and Sandi, 1978; Neff, 1979; Edwards, 1983; Sims and Overcash, 1983). In addition, approximately 700 tons of BaP is released annually worldwide through petroleum spillage, surface land runoff, and wastewaters (ibid). BaP has produced tumors in rats, rabbits, mice, ducks, and monkeys through dermal, oral, and intraperitoneal exposure (Pucknat, 1981). In most cases of PAH ingestion, higher concentrations tend to occur in the liver, gonads, and stomach tissue while smaller concentrations appear in muscle tissue (Beyer et al., 1996) and fatty tissue (Halbrook, 1990). However, attempts to relate PAH levels in mammals to those standardized in sediments has been cited as premature because of the minute amount of information available on such a complex subject (Beyer et al., 1996). Researchers continue to investigate the effects of BaP and other toxic PAHs, with hope of determining malignant and benign levels of exposure.

PAHs in Birds

Patton and Dieter (1980) fed mallards (*Anas platyrhynchos*) 4,000 mg PAHs/kg (phenanthrene and naphthalene – both found in clay targets) in their diet for 7 months, and found no physical signs of illness throughout exposure, and no mortality. However, when compared to control

ducks, blood flow to the liver of experimental ducks had increased by 30%, and liver weight had increased 25%.

Hoffman and Gay (1981) attempted to measure the embryotoxicity of PAHs applied directly to the surface of mallard eggs. A 0.002 mg application resulted in a 26% mortality rate within 18 days. Among the survivors, there was a significant increase in the percent of anomalies, including brain, liver, feather, and eye defects, and a notable reduction in embryonic growth. In a second experiment, 0.05 mg of BaP was applied in the same fashion; within 3 days, there was 75% mortality.

Although some birds are tolerant of large doses of PAHs for extended periods of time (Patton and Dieter, 1980), stresses such as spring and winter migration could exacerbate the effects of these contaminants on general health and survival (Custer et al., 2000). Increased mortality was observed in ducks fed petroleum oil (a substance rich in PAHs) when they were placed in a cold climate and shifted from freshwater to seawater (Holmes et al., 1978). In addition, birds fed petroleum products produced fewer eggs (Grau et al., 1977, Coon and Dieter, 1981) or ceased to lay eggs completely (Hartung, 1965). When the Exxon Valdez oil spill occurred, decreased reproduction by Harlequin ducks (*Histrionicus histrionicus*) purportedly resulted from continued consumption of blue mussels contaminated with PAHs (Patten, 1993).

PAHs in Mammals

Dipple (1985) discovered that tumors could be induced on mouse skin by sustained applications of minute doses of PAHs or by one large dose of the same PAH. Further manipulations indicated one application of a sub-carcinogenic dose followed by the repeated application of a specific

sub-carcinogenic dose induced the formation of a tumor. Lab studies have shown that many PAHs, including mostly carcinogenic PAHs, negatively affect the immune system; the greater the carcinogenic properties of a PAH, the greater the tendency to suppress the immune system (Ward et al., 1985).

Dipple (1985) performed a second study using BaP. Incorporated into the diets of experimental mice, BaP led to lung adenoma, leukemia, and stomach tumors. Repeated exposure to BaP in mammal subjects can destroy lymphoid tissue, intestinal and respiratory epithelium, and have negative effects on reproductive functions (EPA 1980, Lee and Grant 1981).

PAH Effect on Wild Animals

From the laboratory experiments cited above, we know how PAHs affect animals in a controlled setting. However, there are circumstances that wild animals encounter which cannot be simulated in a laboratory. Custer et al. (2000) completed a study looking at the effect of PAHs on lesser scaup wintering on the Indiana Harbor Canal, an area encompassing the Little National Wildlife Refuge. The canal does not freeze in the winter, making the area appealing to many migrating birds (Root, 1988). However, the refuge is situated within a concentrated steel and petrochemical complex (Crawford and Wangness, 1987). In 1989 and 1990, high levels of PAHs were identified in sediments taken from the canal area. Twenty-two lesser scaup were collected and analyzed for contamination; in comparison with reference scaup, the level of PAHs was 4 to 5x higher in each carcass, suggesting significant and repeated exposure to PAHs. Because of scarcity of data regarding the consequences of PAH contamination, Custer et al. (2000)

suggested that further studies be conducted to determine the effects of PAHs on reproduction and survival.

Biotransformation of PAHs

Most PAH research indicates that PAHs have solely negative effects on avian and mammalian species. However, there are studies indicating that PAHs are quickly metabolized, leaving little or no lasting effect on the organism. Naf et al. (1992) completed a study supporting the notion that birds have superior biotransformation capabilities that expel PAHs before any long-term damage can occur. In this experiment, several different PAHs (including many of the 16 priority PAHs) were injected into 14 common eider (*Somateria mollissima)* eggs collected from the wild and 26 chicken (*Gallus domesticus*) eggs; 0.2ml of solution was injected into the eider eggs, and 0.1ml of solution was injected into the chicken eggs. On the 18^{th} day of incubation, approximately 90% of total PAH material had been metabolized. In the treated embryos, the highest concentrations were found in the gall bladder. Lower concentrations were discovered in the kidney, liver, and adipose tissue. This experiment was valuable in demonstrating that PAHs move quickly through a system. However, it would be prudent to test the hypothesis of speedy biotransformation on juvenile and adult birds, in addition to embryos.

Conclusion

From the wealth of literature investigating the detrimental effects of PAHs, it is apparent that main exposure routes are dermal, oral, and/or respiratory. PAHs pose numerous threats to a

variety of animal and plant species that may come into contact with these compounds. However, it is uncertain if PAHs within the petroleum matrix, which comprises 32% of clay targets, leach from the petroleum component of the targets. If PAHs escaped the substrate and were picked up by living animals, they could enter the bloodstream, concentrate in the kidneys and liver, become metabolized and quickly excreted through the urine; however, it is likely that small amounts would be retained in fatty tissues for longer periods of time (Halbrook, 1990). Unfortunately, there are no standards of comparison to determine what constitutes high levels of exposure in wildlife. If there were no bioaccumulation, target fragments would possibly progress through the body without any lasting damage. Further studies are needed to determine if these circumstances are fact or if clay target PAHs pose real danger to the lives and well-being of wild avian and mammalian species.

Lead

Lead (Pb) is a soft, metallic element occurring naturally in a variety of environmental matrices (Scheuhammer and Norris, 1996). Because of its malleability, low melting point, low cost, and general ease of processing, use of lead has resulted in a wide array of products, including ammunition and fishing weights (Scheuhammer and Norris, 1996). Prior to the detection of its lethal effects, lead was often used in paints, plumbing pipes, pottery glazes, and gasoline; however, due to adverse effects, its use has been reduced and/or banned for commercial purposes (Scheuhammer and Norris 1996). Despite the known toxicity, shotgun pellets are still a large source of lead in the environment. They are made up of >90% lead, with minute amounts of antimony (between 1 and 9%), and zinc or copper as a jacketing (VanCantfort, 1999).

Lead poisoning from shotgun pellets is a well-documented cause of mortality among domestic animals and wildlife (Trainer, 1982). In 1991, the United States banned the use of lead shot for waterfowl hunting (Anderson, 1992). Before this ban, the U.S. Fish & Wildlife Service (1986) reported, that on average, 1- 3 million North American geese and ducks died annually from lead poisoning as a result of shot ingestion. Such large numbers seem unrealistic, yet when examining the amount of lead shot deposited on shooting ranges, it is quite comprehensible. Roscoe et al.(1989) studied sediments within a shotfall zone of a trap and skeet range. They found that the top 7.5 cm of sediments contained approximately 29 million pellets/ha. Lewis and Legler (1968) studied lead shot accumulation at a Tennessee dove management field. Prior to the season's commencement, they found 26,800 spent lead shot/ha in the top 1 cm of soil; 2 days following the start of the season, the number had risen to 107,600 lead shot/ha.

Jorgensen and Willems (1987) completed a study in Denmark examining the process of lead pellet transformation in soils on and around shooting ranges. They demonstrated that metallic lead from shotgun pellets deposited in soil was transformed into a compound consisting of $Pb_3(CO_3)_2(OH)_2$ and $PbCO_3$; these compounds are easily transformed into lead ions, having quick and damaging effects. They calculated that, on uncultivated grasslands with a pH of 5.5, a pellet's metallic lead content would be transformed into lead ions within 54-63 years. However, if any agricultural or mechanical treatment occurred that turned the soil, the time periods would be shortened to 15-17 years. In acidic soil, the compound is quickly dissolved by H^+ ions, thus producing pure Pb^+ ions. This form of lead is rapidly dissolved in the soil, enters aquatic and plant systems throughout the area, and affects humans, birds, mammals, and fish.

Lewis et al. (2001) conducted a study on the Federal Law Enforcement Training Center in Glynn County, Georgia. Previous to the study, a gray squirrel and yellow-rumped warbler had

been found at the center incapacitated; it was presumed that they were victims of neurological disorders. However, after preliminary tests, they were diagnosed with lead poisoning. Traps were set nightly to collect mammals, and mist nets were opened daily to collect birds. The animals were necropsied, and kidney and liver samples were obtained for histopathological testing. In total, 37 mammals and 35 birds were collected; 33% of the animals examined had elevated lead levels, >1.00 ppm. Approximately 17% of the collected specimens had levels >2.00 ppm, indicative of clinical lead exposure. Although the sample size was not large enough to indicate mass mortality from lead, it was an adequate number to deduce that a portion of annual mortality was attributable to lead poisoning. Lewis et al (2001) concluded the squirrel and warbler deaths were not isolated incidents. Unfortunately, it has become an increasing trend across the country for such events to occur on shooting ranges.

Lead Poisoning in Birds

Lead poisoning in birds has been studied in various orders, including Anseriformes, Falconiformes, Galliformes, Charadriiformes, and Columbiformes (Bellrose, 1959; Locke and Friend, 1992). Lead pellets are particularly dangerous to avian species because of the nature of the gizzard, an anatomical feature unique to birds. Lead pellets, often mistaken for grit, become lodged in the gizzard. Because of the grinding action of the gizzard and its acidic environment, ionic lead is released into the bloodstream, tissues, and organs, resulting in acute or chronic lead poisoning (Scheuhammer and Norris 1996). Lead poisoning leads to drooping wings, anemia, and emaciation (USFWS 1986). Affected birds often die within 2-3 weeks of ingestion,

assuming that they are not preyed upon in their weakened and decrepit condition (USFWS 1986).

Despite the wealth of information regarding avian lead poisoning, few studies have been conducted on Passeriforme species: perching birds. Vyas et al. (2000) conducted a study on Passerine toxicology on the Patuxent Research Refuge in Laurel, Maryland. Specimens were collected from a local trap and skeet range in operation since 1970. Soil samples were taken, mist nets were opened daily, and an outdoor aviary was set up on site. Blood was drawn from both captive and netted birds at the refuge and analyzed for protoporphyrin levels. Soil analyses indicated that 91% of the lead shot was present in the top 3 cm of soil. In both the captive and netted bird groups, protoporphyrin levels were substantially higher than reference levels. Vyas et al. (2000) concluded that birds were likely mistaking lead pellets for seed or grit. Spent shot can erode to a brown or pale gray color, bearing a strong resemblance to seeds or grit. Nestlings and young birds with weak immune systems may also be in danger of lead poisoning, since grit ingestion occurs 3 days after hatching (Potter, 1983; Mayoh and Zach, 1986). The overall results suggested that ground foraging birds were most susceptible to lead ingestion, making them vulnerable to predation due to degrading effects of lead on the body.

A second study by Vyas et al. (2001) examined the effects of lead on dosed cowbirds. The first group of dosed birds was fed a commercial diet and given one new single size 7.5 lead shot. The second experimental group was given both new and weathered shot doses and fed a combination of wild bird seed mix, cracked corn, and the Zeigler® commercial diet. Green, watery feces were noted for all dosed birds, yet no mortality was observed for the first group of birds. Three birds from the second group died, leading to conclusions that the more natural diet of seed and corn increased lead toxicity resulting in mortality. Diets low in calcium and protein

may increase lead toxicity, as low protein diets tend to increase lead absorption into tissues (Kendall et al., 1982). Additionally, the weathered shot given to the second group may have eroded more quickly in the gizzard, releasing greater amounts of lead ions, thus altering further gizzard function and ultimately leading to death. Despite the small sample size, Vyas et al. (2001) concluded that only one 7.5 shot was needed to kill a medium-sized songbird. Results from other studies have suggested that Passerines are more sensitive to lead shot than other birds, such as waterfowl (Beyer et al., 1988).

Lead Poisoning in Mammals

Although mammals do not have a gizzard, they are subject to many adverse effects from lead. Shrews in particular are susceptible to lead poisoning because of their diet of earthworms and beetles – earthworms are known to ingest lead because they swallow a great deal of soil (Ma, 1989), typically 20-30% of their body weight (Beyer et al., 1994). A strong indication of lead toxicity in mammals is weight loss (Ma, 1989), as well as increased kidney and liver-to-body weight ratios (Stansley and Roscoe, 1996). However, in mammals a large portion of lead tends to collect in bone.

In a study by Ma (1989), mammal specimens were collected on a shooting range that had been used exclusively as a target shooting range. The study was conducted 20 years after lead pellets use had been prohibited on the target range. However, the majority of pellets were still present in the metallic form in the soil. Ma (1989) found that animals on the range were chronically exposed to lead. Mammals that were trapped had high levels of lead in their kidneys and bone tissue. Shrews on average had higher lead levels than other mammals that consumed

plants and fruits. ANOVA analyses showed that lead concentration in liver and kidneys differed significantly ($P<0.001$) between populations inside and outside the downfall area of lead pellets.

Stansley and Roscoe (1996) conducted a small mammal study on a trap and skeet range with approximately 266,000 kg lead shot/ha present in the top 7.5 cm of soil. There was a control site and a study site located within the shot fall zone. They necropsied white-footed mice and short-tail shrews, and obtained kidney, liver, and femur samples. Soil samples were collected along the traplines in the shot fall zone. Soil samples from the study site were 1000-fold higher in lead concentration than samples from the control site, and soil pH was more acidic on the study site. Compared to control samples, femur, kidney, and liver concentrations were elevated 5-to 64-fold in study site samples suggesting soil ingestion, including the consumption of earthworms, was a potentially significant route of exposure. In white-footed mice, the majority of lead was concentrated in the bone, in accordance with other lead and mammal studies (Talmage and Walton, 1991). Although mammals are mostly at risk through incidental soil consumption, lead concentrations around shooting ranges are adequately high to contribute significantly to overall lead body burden if ingestion occurs.

Shooting Range Design

Vyas et al. (2001) noted that several factors increased the magnitude of shot effect on local habitats surrounding shooting ranges. Among these were land slope, soil type, range design, type of edge habitat, and prevalent shooting direction. Land slope may result in pellet run-off, thus carrying lead onto adjoining woodlands following heavy rains and/or snowmelts. Soil type influences the availability of pellets on the top layers of the soil, in addition to the amount of lead

ions present; Schrank and Dollahon (1975) noted a greater number of shot in firm versus muddy soil. Additionally, Ma (1989) established that a substantial amount of pellets were still present in the topmost layers of an acidic, sandy soil located at a range that had been abandoned for 20 years.

Concerning range design, Vyas et al. (2001) speculated that accumulation of shot was a result of the positioning and number of trap houses. Edge habitat influences the nature of the woodland surrounding the range. Large, deciduous trees tend to reduce shot penetration into forested habitat, relative to evergreens such as pines and cypress species. Shrub habitat allows for a greater amount of pellets to infiltrate more pristine areas. Because the demand for shooting ranges is not likely to decline, it is important to consider all these factors when altering or constructing ranges.

Conclusion

Lead is an incredibly toxic substance, which acts quickly on the body and has caused the demise of many animals. The amount of lead can be correlated with the amount of target material found on shooting ranges because lead pellets are used in target shooting; therefore, the more target fragments found across range habitat, the more lead pellets are likely to be embedded in the trees and soil, and the greater number of animals that are likely to suffer as a result of consumption. Many steps can be taken to reduce the amount of lead that is introduced into the environment everyday; landscape changes as well as lead substitutions such as steel, can be made to improve the survival of a diversity of plants and animals that reside on and around shooting ranges.

CHAPTER 1 - EFFECTS OF INGESTED CLAY TARGETS ON CAPTIVE COTURNIX QUAIL

INTRODUCTION

Clay Targets

Clay targets are used nationally on shooting ranges for target practice and shooting competition. Since 1970, clay target use has averaged approximately 560 million targets/year (Baer et al., 1995), and shooting clay targets has become an increasingly popular sport with an average 12,800,000 participants each year (Peddicord et al., 1993). A study on a shotgun range on the shore of Lake Michigan reported that several tons of clay targets were discharged over a period of 78 years; when the lake bottom was surveyed, an accumulation of targets one meter in depth was noted for the surrounding area (Pott et al., 1993). Contrary to its name, the composition of a clay target is 67% limestone, 32% petroleum pitch, and 1% aqueous fluorescent paint (Baer et al., 1995). Ecological and toxicological concerns focus on the petroleum pitch, an organic binder containing polycyclic aromatic hydrocarbons (Pott et al., 1993), also known as PAHs.

Polycyclic Aromatic Hydrocarbons

PAHs occur in a range of compounds, many of which have been thoroughly researched and examined. They are considered environmental contaminants known to have carcinogenic,

mutagenic, and toxicological effects (Naf et al., 1992). PAHs have been found in plant and animal tissues, soils, sediments, air, surface water, drinking water, and groundwater (EPA 1980, Eisler 1987). The primary concern is that PAHs will leach from the target substrate, dissolve in water, and run into surface waters; transformation processes could release PAHs into the soils and sediments, where they could be absorbed by vegetation (Peddicord et al., 1993). PAHs may also be released directly onto vegetation (Eisler, 1987). Once the toxin is in the vegetation, it is a component of the food chain and available to a range of animals. A chief concern with clay targets is that birds and mammals will inadvertently ingest fragments strewn across the range as they forage for food. Despite these concerns, there have been few studies investigating the toxicity of clay targets. While it is possible that PAHs present in clay targets are noncarcinogenic, there is little information available on toxicological properties of PAHs that are noncarcinogenic to wildlife (EPA 1980; Lee and Grant, 1981). There is an urgent need for further investigation of clay target toxicity and existing threats to wildlife. The objective of this study was to determine if clay target fragments and dust had any effect on captive Coturnix quail.

METHODS

I obtained Japanese quail (*Coturnix coturnix japonica)* for all experiments from the Department of Animal and Poultry Science at Virginia Polytechnic Institute and State University (VPI&SU) and the Department of Poultry Science at the University of Georgia at Athens. I conducted 3 experiments at the captive animal facility in Center Woods, adjacent to the VPI&SU campus. The facility has a roof and wood-slat/chain-link fence walls, which protected study animals from

rain, high winds, and predators. Quail were housed in 50 x 30 x 23 cm metal cages, either alone or in pairs. During June and July 2002, temperatures ranged from 21-32^{o}C during the day (average temperature: 28^{o}C) and 8-19^{o}C in the evening (average temperature: 16^{o}C). In the winter, the weather was consistently cold, with multiple snowstorms. In January and February, 2003, temperatures during the day ranged from -9-13^{o}C (average temperature: 3^{o}C) and temperatures at night ranged from -16-9^{o}C (average temperature: -5^{o}C). However, we placed multiple heat lamps above the cages and tarps around the cages to protect against wind currents. Daily and average temperatures were obtained from the National Weather Service Forecast Office in Blacksburg, VA.

The Consumption Experiment

The first experiment, known as the consumption experiment, was designed to determine if quail would eat clay target fragments as grit. Thirty-six Coturnix quail were confined individually in cages during October 1, 2001 – November 1, 2001, and offered crushed samples of clay targets or ground limestone after a 1-week acclimation period. Fragments were obtained using 2 sieves with size dimensions of 3.36 and 1.19mm. The quail were fed a commercial wild birdseed diet (Southern States® Wild Birdseed), *ad libitum*. Ten grams of crushed targets (size range: 1.19 to 3.36mm, Remington Blue Rock®) or 10g of limestone (1.19 to 3.36mm) were offered to each quail in containers separate from feed containers. As quantities decreased, the cups were replenished to ensure experimental birds had constant access to target or limestone. Sixteen quail (8M, 8F) received target fragments and 12 (6M, 6F) received limestone fragments. All quail were weighed at the beginning and end of the experiment. Additionally, target and limestone

samples were weighed at the beginning and end of the trial to determine how much of each substance was consumed throughout the experiment.

We replicated the experiment in spring of 2003 (March 10-April 7). This experiment used just 20 quail (10M, 10F). Ten quail (5M, 5F) received target fragments and 10 received limestone fragments. Instead of wild birdseed, the quail were fed a commercial poultry mash (Southern States® Sporting Bird Mash), *ad libitum*, with adequate protein and fat components. In both trials, birds had the opportunity to consume target fragments or limestone, 10.0g amounts. If quantities decreased, amounts were replenished. Upon completion of the experiment, bird weights as well as target and limestone weights were recorded and compared to those taken at the start of the experiment. The weather in the fall and spring seasons was somewhat similar: in the fall, daily temperatures ranged from 9-26^{o}C (average temperature: 17^{o}C) and evening temperatures ranged from 2-14^{o}C (average temperature: 8^{o}C). In the spring, daily temperatures ranged from 3-23 ^{o}C (average temperature: 15 ^{o}C) and evening temperatures ranged from –7-10 ^{o}C (average temperature: 1 ^{o}C). Temperatures were obtained from the National Weather Service Forecast Office in Blacksburg, VA.

The Dose Experiment

The second experiment, the dose experiment, began June 1, 2002 and ended June 30, 2002. Forty quail, caged in pairs (1M, 1F) were separated into 4 treatments of 10 birds (5 pairs) each. After a 1-week acclimation period, each bird was dosed with target dust once a week for 3 weeks using plastic tubing attached to a miniature funnel. Target dust for all experiments was obtained by crushing whole targets with equipment used for grinding dog food to a fine powder. Dosage rates

(treatments) were 0g (control), 0.5g, 1.0g, and 1.5g – in the control treatment, the birds were intubated, yet no target material was forced. Weekly body weight measurements were taken at the time of dosing: M0=weight at the first dose, M7=weight at the second dose, M14=weight at the third dose, and M21=weight at the fourth and final dose. A commercial poultry diet (Southern States® Sporting Bird Mash), was given in 120.0g amounts and measured daily to determine how much was consumed/pair. At the end of the dose experiment, the birds were sacrificed and necropsied, and livers were collected and weighed.

This experiment was replicated in the winter of 2003, January 13 – February 10, with 36 quail. Treatment groups were the same as the summer trial, but only 8 birds were included in the 1.0g and 0.5g treatment group. As in the summer treatment, weekly body weight measurements were taken at each dosing, and feed was measured daily to determine amount consumed. At the end of the trial, the birds were sacrificed and necropsied, and livers were collected and weighed.

The Incorporation Experiment

The third experiment, the incorporation experiment, began July 5, 2002 and lasted 8 days. Sixty quail, caged in pairs (1M, 1F) were separated into 3 treatment groups of 20 birds (10 pairs) each. Following a 1-week acclimation period, each bird was fed a commercial poultry diet (Southern States® Sporting Bird Mash), with clay target dust incorporated into their daily ration at a rate of 0% (control), 10%, or 20%. The total amount of feed given was 120.0g; therefore if the treatment was 20%, the quail received 96.0g mash and 24.0g target dust. Quail in the 10% treatment received 108.0g mash and 12.0g target dust. Each quail was weighed at the start of the experiment (M0), after 4 days (M4), and at the end of the trial, on the 8th day (M8). Feed was

measured daily to determine how much was consumed/pair. At the end of the incorporation experiment, the birds were sacrificed and necropsied, and livers were collected and weighed.

This experiment was partially replicated in winter 2003, February 3-11, with 46 quail; the treatment groups were not synonymous to the summer group. The majority of birds in the 20% summer incorporation trial became emaciated after several days, indicating that this target incorporation quantity was too large, and the birds were not receiving adequate nutrition. Therefore, treatments were 0% (control), 5%, and 10%. We used 14 birds (7 pairs) in the control group and 16 birds (8 pairs) in both the 5% and 10% groups. Feed was measured daily to determine food consumption. At the end of the trial, the birds were sacrificed and necropsied, and livers were collected and weighed.

Organs for all experiments were stored in a freezer at -20^{o}C. However, liver-to-body weight ratios were determined as the fresh weight of the liver divided by the fresh weight of the body, multiplied by 100, to obtain grams of liver / 100 grams of body weight.

Chemical Analysis

A sample of the target dust used in all the experimental trials was sent to the Department of Environmental and Occupational Health at the Texas A&M University System Health Science Center in College Station, TX for PAH analysis.

Benzo(a)pyrene

Total benzo(a)pyrene (BaP) was presented in ng/dry g in PAH analyses. BaP amounts were multiplied by total target dust used in each treatment to obtain BaP present for each treatment. In

the dose experiment, BaP values were multiplied by 0.5g, 1.0g, and 1.5g. In the incorporation experiment, the amount of consumed target dust per pair was determined, multiplied by the BaP amount, and then averaged for each treatment.

Statistical Analysis

I used the Statistical Analysis System (SAS Institute Inc.; Cary, NC) for all analyses. The PROC MIXED option of analysis of variance (ANOVA) was used to determine if weight change was related to treatments, seasons, and sex. PROC GLM was used to test if there was an increase or decrease in food consumption for each treatment. PROC GLM also was used to compare liver weights and proportions across treatments, season, and sex. F-values and P-values were obtained for all analyses. Means were obtained for weight changes, feed consumed across treatments in all experiments, as well as target and limestone consumed in the choice experiment. I used $\alpha=0.05$ as my significance level.

RESULTS

The Consumption Experiment

In both fall ($F=29.2$, $P<0.01$) and spring ($F=6.45$, $P=0.02$) experiments, quail consumed more limestone than target material (Table 1.1). Consumption of clay target fragments did not differ seasonally ($F=3.0$, $P=0.09$), but limestone intake was higher in the fall ($F=17.4$, $P=0.0002$). Overall consumption of target ($F=1.2$, $P=0.29$) and limestone ($F=0$, $P=0.99$) was similar for male

Table 1.1 – Ingestion of clay target fragments and limestone fragments in the consumption experiment conducted at the Virginia Tech Center Woods Research Station, October-November, 2001 (fall) and March-April, 2003 (spring). Fall birds were fed Southern States® Wild Birdseed; spring birds were fed Southern States® Sporting Bird Mash.

	Amount Consumed (g)					
	N	Target ($\bar{x}$)[a]	SE	N	Limestone ($\bar{x}$)[a]	SE
Fall[b]						
Male[c]	8	0.15	0.13	6	3.94	1.41
Female[c]	8	0.47	0.20	6	4.09	1.02
Total	16	0.62		12	8.03	
Spring[b]						
Male	5	0	0	5	0.36	0.18
Female	5	0.042	0.042	5	0.25	0.15
Total	10	0.042		10	0.61	

a. Total limestone vs. target consumption: fall – $P<0.01$,
spring – $P=0.02$

b. Overall seasonal comparison of limestone and target consumption: target: $P=0.09$,
limestone: $P=0.002$

c. Sex comparison of limestone and target consumption: fall - target: $P=0.29$, limestone: $P=0.99$
spring – target: $P=0.33$, limestone:$P=0.68$
fall x spring – target: $P=0.29$, limestone:$P=0.98$

and female quail. Regardless of specific amounts consumed, birds receiving limestone grit consumed significantly more grit than those receiving target grit. No experimental birds died throughout the experiment.

The Dose Experiment

No quail died in either summer or winter dose experiments. In both summer ($F=1.77$, $P=0.2$) and winter ($F=0.34$, $P=0.8$) trials, weight changes for male quail did not differ among treatments (Tables 1.2, 1.3). Female quail (Tables 1.2, 1.3) experienced no weight change in the summer ($F=1.63$, $P=0.23$) but had large weight gains ($F=3.53$, $P=0.04$) in the 0.5g and 1.0g winter trials, in addition to a weight loss in the winter 1.5g trial. Both sexes gained more weight ($P<0.01$) in winter than summer; in summer, males gained more weight, and in the winter, females gained more weight, suggesting a sex x season interaction ($P<0.01$).

A pattern formed during the 23-day feeding trial for the summer dose experiment. Average food intake levels dropped the first day following dosing (Days 0, 7, 14, 21), steadily increased for 3 to 4 days, and slowly decreased until the following dose day (Fig. 1.1). In the winter experiment, I noted similar trends, with average food intake sharply decreasing on the day following a dose treatment, steadily increasing, and eventually decreasing until the following dose day (Fig 1.2).

During summer, daily consumption rates varied between treatments ($F=11.09$, $P<0.01$). Birds in the 1.5g treatment consumed the most feed (41.61g), followed by the control group (38.34g), the 0.5g group (35.21g), and the 1.0g (29.39g) group (Table 1.4). In the winter trials, daily consumption rates also varied between treatments ($P<0.01$). The control group consumed

Table 1.2 – Average weight gain/loss (g) for Coturnix quail in a dose experiment conducted at the Virginia Tech Center Woods Research Station, June, 2002.

Treatment	N	$\bar{x}$	SE
Male[a]			
Control, 0g	5	1.94	0.81
0.5g	5	1.06	1.57
1.0g	5	1.46	2.18
1.5g	5	-2.46	1.56
TOTAL	20	2.00	
Female[b,c]			
Control, 0g	5	3.08	0.83
0.5g	5	0.79	1.27
1.0g	5	-0.93	1.90
1.5g	5	-0.1	1.40
TOTAL	20	2.84	

a. Male weight change across treatments: P=0.20
b. Female weight change across treatments: P=0.23
c. Sex comparison across treatments: P=0.88

Table 1.3 - Average weight gain/loss (g) for Coturnix quail in a dose experiment conducted at the Virginia Tech Center Woods Research Station, January-February, 2003.

Treatment	N	$\bar{x}$	SE
Male[a]			
Control, 0g	5	5.65	1.58
0.5g	4	4.57	1.99
1.0g	4	3.46	1.52
1.5g	5	4.94	1.21
TOTAL	18	18.62	
Female[b,c]			
Control, 0g	5	4.77	4.57
0.5g	4	10.54	3.71
1.0g	4	9.4	1.45
1.5g	5	-3.63	2.72
TOTAL	18	21.08	

a. Male weight change across treatments: P=0.8
b. Female weight change across treatments: P=0.04
c. Sex comparison across treatments: P=0.99

Figure 1.1 – Average daily feed intake for Coturnix quail in a dose experiment conducted at the Virginia Tech Center Woods Research Station, June, 2002. Vertical arrows indicate dosing days.

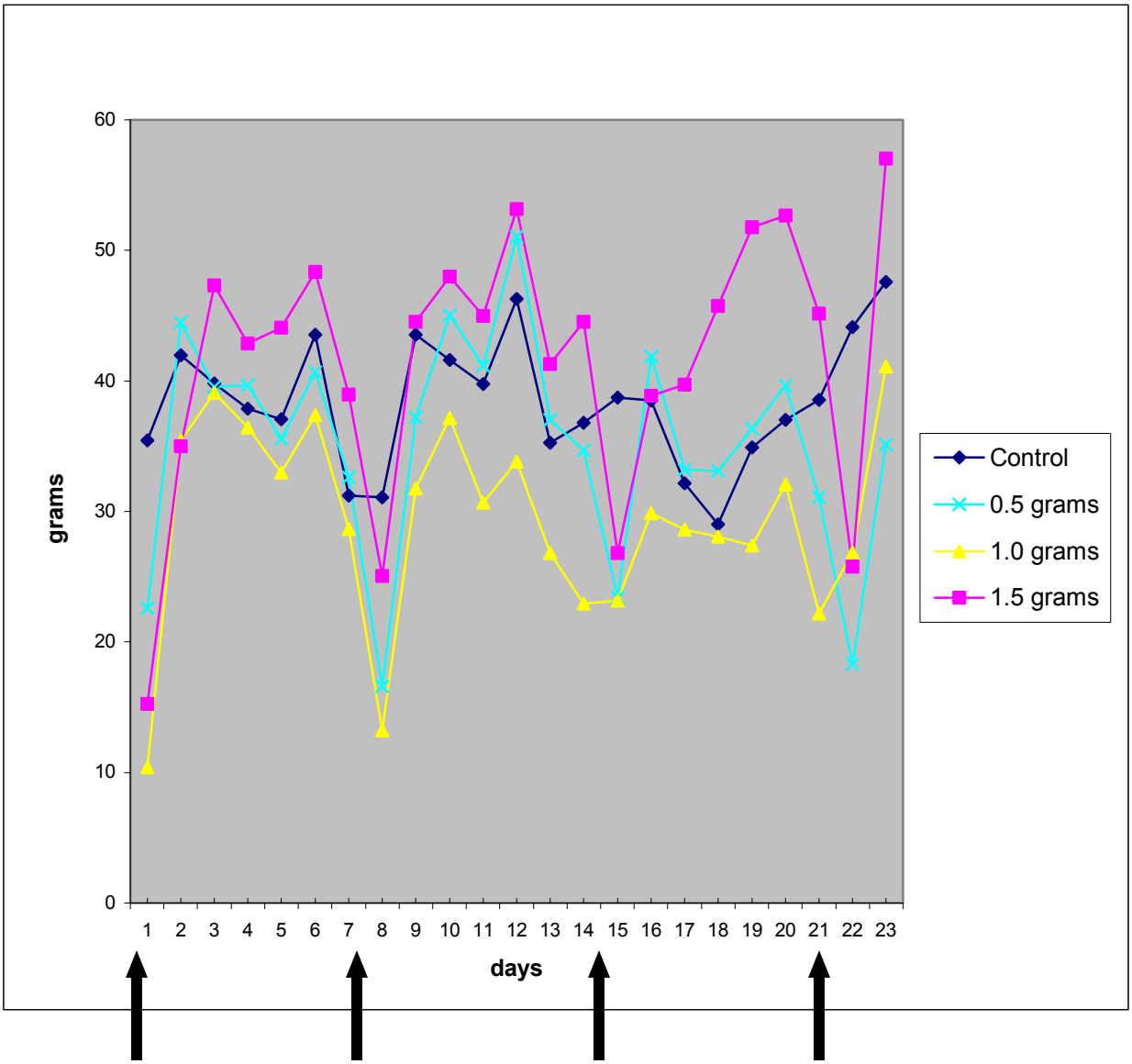


Figure 1.2 – Average daily feed intake for Coturnix quail in a dose experiment conducted at the Virginia Tech Center Woods Research Station, January-February, 2003. Vertical arrows indicate dosing days.

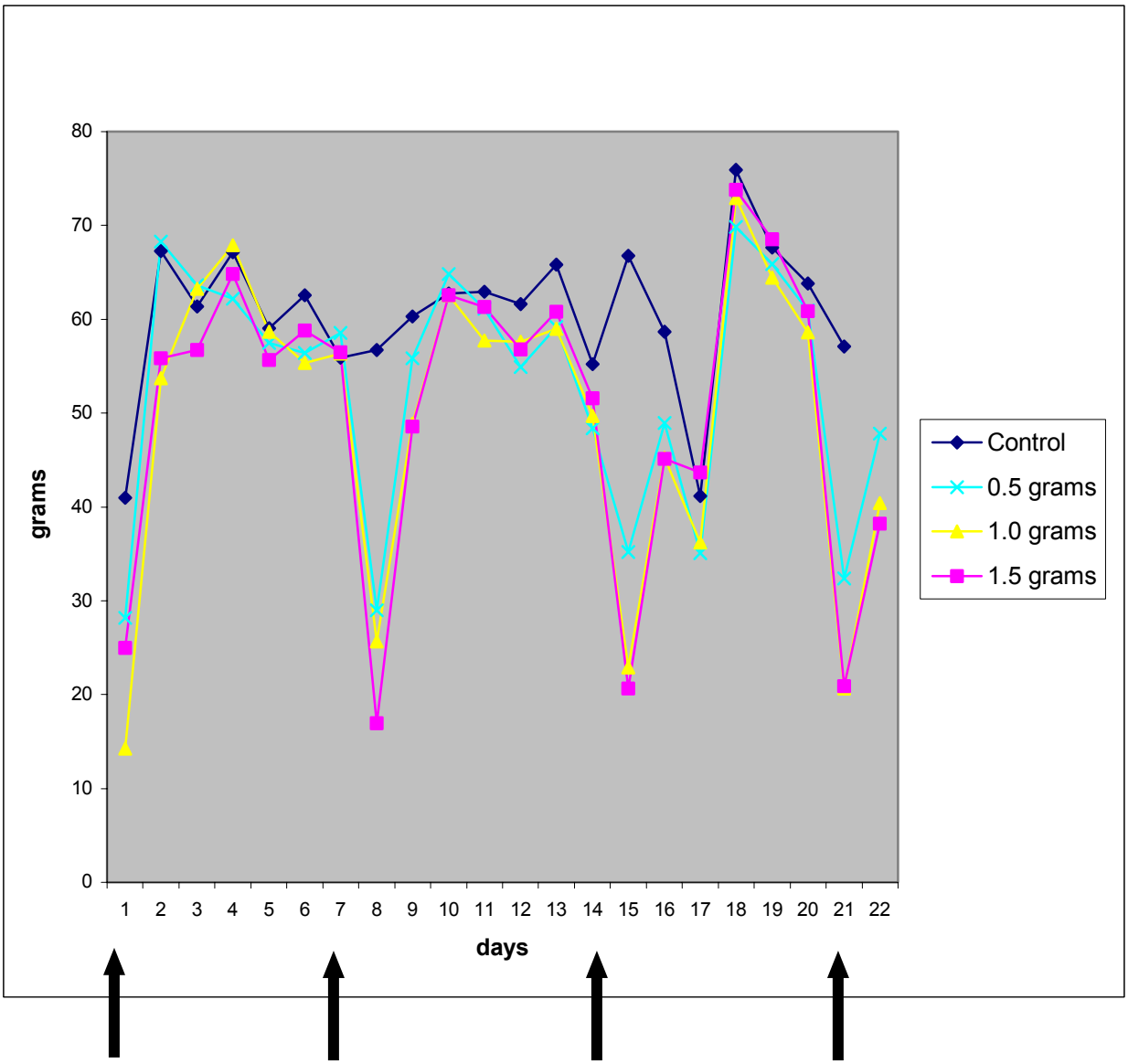


Table 1.4 – Average daily feed consumption / treatment (g) for Coturnix quail in a dose experiment conducted at the Virginia Tech Center Woods Research Station, June, 2002 (summer) and January-February, 2003 (winter).

	Summer[a]			Winter[b,c]		
Treatment	N	$\bar{x}$	SE	N	$\bar{x}$	SE
Control (0 grams)	5	38.34	2.64	5	60.51	1.40
0.5 grams	5	35.21	1.21	4	52.90	2.41
1.0 grams	5	29.39	1.22	4	49.63	1.49
1.5 grams	5	41.61	1.20	5	50.16	2.13

a. Change in daily feed consumption across treatments: $P<0.01$
b. Change in daily feed consumption across treatments: $P<0.01$
c. Seasonal comparison across treatments: $P<0.01$

the greatest amount of feed (60.51g), followed by the 0.5g group (52.9g), the 1.5g group (50.16g), and the 1.0g group (49.63g; Table 1.4). Quail in the winter experiment on average ate greater amounts ($P<0.01$) of feed for all treatments.

Liver weights and liver-to-body weight ratios for males and females in both summer (liver: $F=5.49$, $P=0.0038$; ratio: $F=4.33$, $P=0.012$) and winter (liver: $F=7.94$, $P=0.0005$; ratio: $F=7.53$, P0.0008) trials increased as treatment amounts increased (Tables 1.5, 1.6). Liver weights in summer ($F=12.51$, $P=0.0013$) and winter ($F=7.94$, $P=0.0005$) as well as liver-to-body weight ratios in summer ($F=12.56$, $P=0.0013$) and winter ($F=95.73$, $P<0.01$) indicate that female organs were consistently larger than males for all treatments.

The Incorporation Experiment

No study birds died during the incorporation experiment, but some experienced severe weight loss. In both the summer ($F=16.13$, $P<0.01$) and winter ($F=8.47$, $P=0.002$) trials, male quail lost large amounts of weight in the 10%, 20% and 5%, 10% groups, respectively (Tables 1.7, 1.8). Because the 20% trial suffered a severe weight loss in the summer, we eliminated the 20% trial and added a 5% trial for the winter experiments. Females in the summer ($F=15.62$, $P<0.01$) and winter ($F=17.50$, $P<0.01$) trials also suffered large weight losses in the 10%, 20% and 5%, 10% groups, respectively (Tables 1.7, 1.8). Control groups in summer and winter gained weight.

Weights differed within sex (MF: $F=21.93$, $P<0.01$) and treatment (MF: $F=24.11$, $P<0.01$) between the summer and winter trials – only control and 10% groups were compared. Summer males in the control group gained more than winter males and 10% summer males lost more than winter males. Winter females in the control group gained more weight than the

Table 1.5 – Average liver weight (g), final body weight (MF, g), and liver-to-body weight ratio (liver ratio, g/100g body weight) for Coturnix quail in a dose experiment conducted at the Virginia Tech Center Woods Research Station, June, 2002.

Treatment	N	liver	SE	MF	SE	liver ratio	SE
Male[a]							
Control, 0g	5	3.0	0.18	119.30	3.53	2.52	0.19
0.5g	5	3.5	0.17	122.58	6.24	2.89	0.21
1.0g	5	3.48	0.24	121.82	2.52	2.86	0.18
1.5g	5	3.65	0.24	122.76	2.27	2.98	0.22
Female[b,c]							
Control, 0g	5	3.28	0.22	120.61	4.46	2.72	0.16
0.5g	5	4.09	0.17	120.43	2.70	3.41	0.17
1.0g	5	4.28	0.31	117.35	0.76	3.64	0.24
1.5g	5	4.24	0.28	117.39	3.89	3.63	0.30

a. Male: liver weight change across treatments: P=0.18,
liver ratio change across treatments: P=0.44
b. Female: liver weight change across treatments: P=0.03,
liver ratio change across treatments: P=0.03
c. Sex comparison: overall liver weight change across treatments: P=0.005,
overall liver ratio change across treatments: P=0.004

Table 1.6 – Average liver weight (g), final body weight (MF, g), and liver-to-body weight ratio (liver ratio, g/100g body weight) for Coturnix quail in a dose experiment conducted at the Virginia Tech Center Woods Research Station, January-February, 2003.

Treatment	N	liver	SE	MF	SE	liver ratio	SE
Male[a]							
Control, 0g	5	2.98	0.37	124.41	2.72	2.39	0.29
0.5g	4	3.82	0.28	122.90	2.90	3.10	0.18
1.0g	4	3.71	0.28	122.79	3.29	3.02	0.23
1.5g	5	3.83	0.25	124.03	1.70	3.09	0.21
Female[b,c]							
Control, 0g	5	5.69	0.37	149.03	3.52	3.81	0.20
0.5g	4	7.59	0.37	152.21	5.85	5.03	0.40
1.0g	4	8.13	0.54	146.53	6.57	5.58	0.40
1.5g	5	6.85	0.34	143.14	6.55	4.81	0.25

a. Male: liver weight change across treatments: P=0.17,
liver ratio change across treatments: P=0.13
b. Female: liver weight change across treatments: P=0.004,
liver ratio change across treatments: P=0.008
c. Sex comparison: overall liver weight change across treatments: P<0.01,
overall liver ratio change across treatments: P<0.01

Table 1.7 – Average weight gain/loss (g) for Coturnix quail in an incorporation experiment conducted at the Virginia Tech Center Woods Research Station, July, 2002.

Treatment	N	$\bar{x}$	SE
Male[a]			
Control, 0%	10	6.99	2.39
10%	10	-9.29	6.21
20%	10	-32.96	5.55
TOTAL	30	-35.26	
Female[b,c]			
Control, 0%	10	5.93	1.93
10%	10	-9.21	3.98
20%	10	-22.15	4.28
TOTAL	30	-25.43	

a. Male weight change across treatments: $P<0.01$
b. Female weight change across treatments: $P<0.01$
c. Sex comparison across treatments: $P<0.01$

Table 1.8 – Average weight gain/loss (g) for Coturnix quail in an incorporation experiment conducted at the Virginia Tech Center Woods Research Station, February 2003.

Treatment	N	$\bar{x}$	SE
Male[a]			
Control, 0%	7	5.15	2.46
5%	8	-9.22	2.41
10%	8	-8.19	3.07
TOTAL	23	-12.26	
Female[b,c]			
Control, 0%	7	11.14	5.13
5%	8	-17.69	3.44
10%	8	-21.58	4.01
TOTAL	23	-28.13	

a. Male weight change across treatments: P=0.002
b. Female weight change across treatments: P<0.01
c. Sex comparison across treatments: P<0.01

summer birds, but 10% winter females lost more weight than summer females.

During summer feed trials, the control group consumed consistently more feed than the treatment groups (Fig. 1.3). Birds in the 10% and 20% trials began the treatment period by consuming small amounts of feed, but steadily increased consumption until the third day and stabilized until the final day. The control group consumed an average of 40.92g/pair, followed by the 10% group (35.83g/pair) and 20% group (25.75g/pair; Table 1.9). In the winter feed trial, the same trend occurred, i.e., the control group consistently ate more feed than the 5% and 10% treatment groups (Fig. 1.4). The control group consumed 64.05g/pair, followed by the 5% group (51.56g/pair) and 10% group (43.9g/pair; Table 1.9). Unlike the summer experiment, food consumption in winter increased only slightly from the first to second day and remained relatively stable throughout the 8-day period. All quail in the winter trials had higher ($P<0.01$) daily consumption rates than quail in summer trials.

Liver weights between sexes in the summer treatments were not significantly different ($F=0.26$, $P=0.77$; Table 1.10). In the winter trials, there was an increase in liver weight ($F=3.69$, $P=0.034$). However, liver-to-body weight ratios in the summer trial increased ($F=0.52$, $P<0.01$) with increasing amounts of clay target dust in the diet, as was the case in the winter trials ($F=11.98$, $P<0.001$; Tables 1.10, 1.11). Liver weights in the summer ($F=10.76$, $P=0.0018$) and winter ($F=36.75$, $P<0.0001$) were larger for females compared to males. However, female liver-to-body weight ratios were only higher ($F=15.16$, $P=0.0004$) than male liver-to-body weight ratios in the winter.

Figure 1.3 – Average daily feed intake for Coturnix quail in an incorporation experiment conducted at the Virginia Tech Center Woods Research Station, July, 2002.

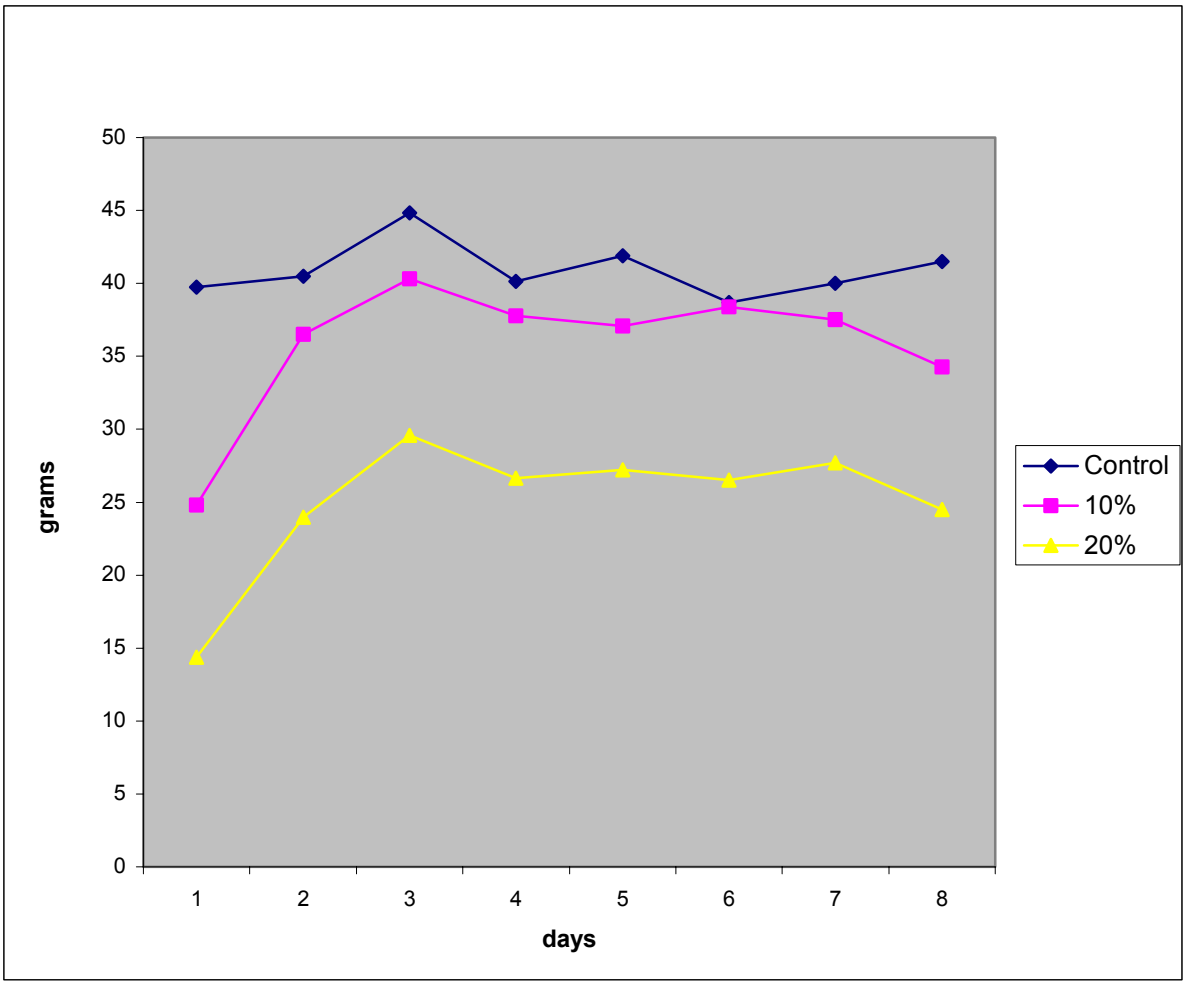


Figure 1.4 – Average daily feed intake for Coturnix quail in an incorporation experiment conducted at the Virginia Tech Center Woods Research Station, February, 2003.

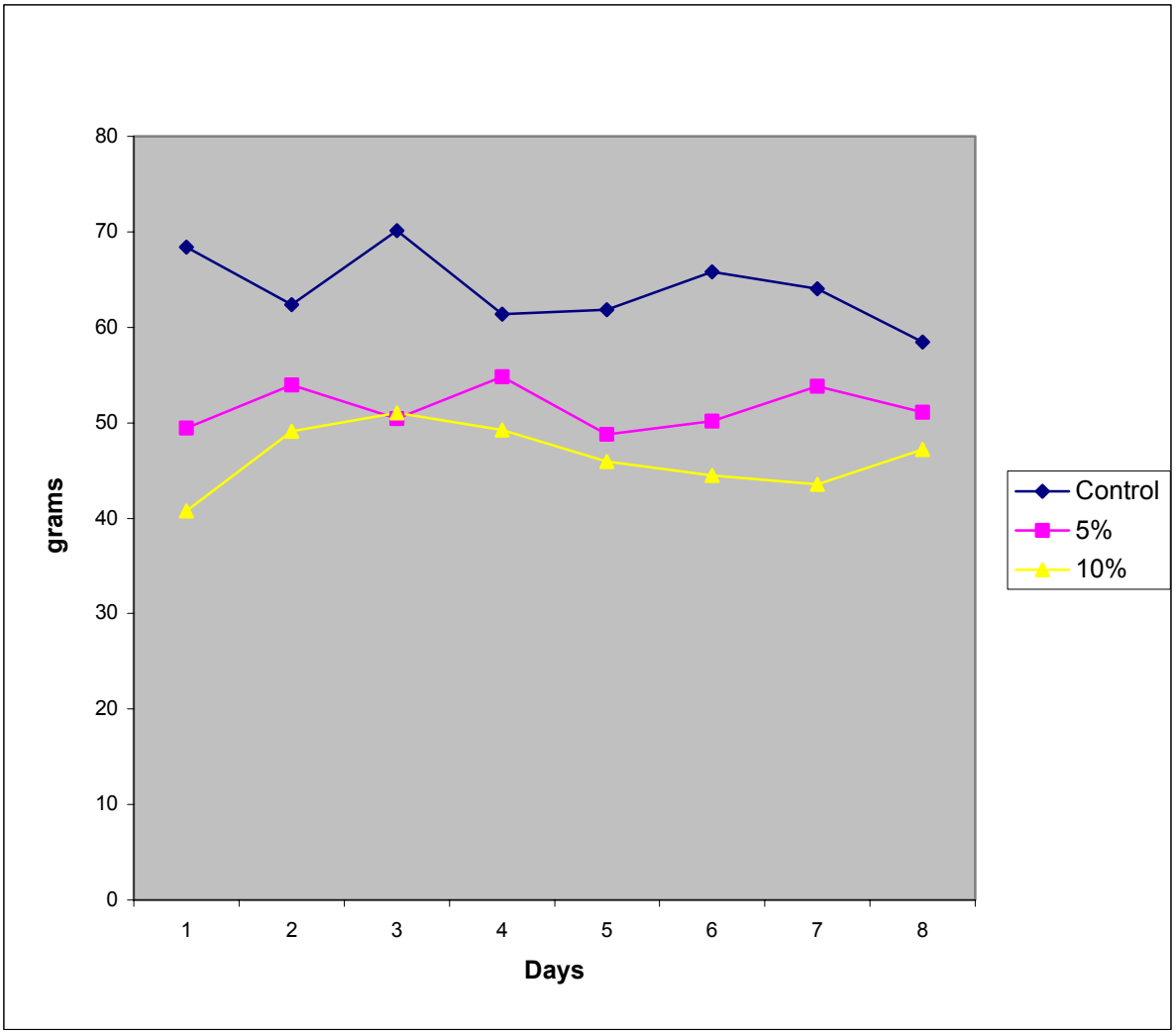


Table 1.9 – Average daily feed consumption / treatment (g) for Coturnix quail in an incorporation experiment conducted at the Virginia Tech Center Woods Research Station, July, 2002 (summer) and February, 2003 (winter).

	Summer[a]			Winter[b,c]		
Treatment	N	$\bar{x}$	SE	N	$\bar{x}$	SE
Control (0%)	10	40.92	2.24	7	64.05	3.47
5%				8	51.56	3.44
10%	10	35.83	2.47	8	43.9	2.74
20%	10	25.75	2.82			

a. Change in daily feed consumption across treatments: P=0.03
b. Change in daily feed consumption across treatments: P=0.94
c. Seasonal comparison across treatments: P<0.01

Table 1.10 – Average liver weight (g), final body weight (MF, g), and liver-to-body weight ratio (liver ratio, g/100g body weight) for Coturnix quail in an incorporation experiment conducted at the Virginia Tech Center Woods Research Station, July, 2002.

Treatment	N	liver	SE	MF	SE	liver ratio	SE
Male[a]							
Control, 0%	10	3.77	0.33	117.67	3.17	3.20	0.24
10%	10	3.66	0.22	98.95	5.47	3.73	0.19
20%	10	3.48	0.27	77.85	4.68	4.46	0.17
Female[b,c]							
Control, 0%	10	4.13	0.24	126.44	2.40	3.28	0.23
10%	10	4.62	0.38	108.64	4.27	4.23	0.26
20%	10	4.47	0.25	98.44	4.23	4.57	0.24

a. Male: liver weight change across treatments: P=0.77,
liver ratio change across treatments: P=0.0008
b. Female: liver weight change across treatments: P=0.49,
liver ratio change across treatments: P=0.002
c. Sex comparison: overall liver weight change across treatments: P=0.002,
overall liver ratio change across treatments: P=0.31

Table 1.11 – Average liver weight (g), final body weight (MF, g), and liver-to-body weight ratio (liver ratio, g/100g body weight) for Coturnix quail in an incorporation experiment conducted at the Virginia Tech Center Woods Research Station, February, 2003.

Treatment	N	liver	SE	MF	SE	liver ratio	SE
Male[a]							
Control, 0%	7	3.11	0.26	128.45	4.28	2.45	0.24
5%	8	3.55	0.21	117.38	3.12	3.06	0.25
10%	8	4.05	0.23	110.37	4.47	3.73	0.29
Female[b,c]							
Control, 0%	7	4.53	0.48	147.68	4.45	3.07	0.31
5%	8	5.51	0.30	127.14	4.81	4.36	0.24
10%	8	4.95	0.22	115.84	5.86	4.33	0.25

a. Male: liver weight change across treatments: P=0.032,
liver ratio change across treatments: P=0.009
b. Female: liver weight change across treatments: P=0.15,
liver ratio change across treatments: P=0.004
c. Sex comparison: overall liver weight change across treatments: P<0.01,
overall liver ratio change across treatments: P=0.002

Results from the clay target analysis are presented in Table 1.12. The same PAHs from Baer et al.(1995) were found in the sample of clay target dust used in this study (Table 1.12), however, select PAHs, specifically BaP, were found in higher quantity in our study's sample of clay target.

Benzo(a)pyrene

In the dose experiment, total amount of BaP (based on results from clay target PAH toxicity test) in target dust increased as dose amounts increased: 0.167mg in the 0.5 dose, 0.335mg in the 1.0 dose, and 0.503mg in the 1.5 dose (Fig. 1.5). In the incorporation experiment, average amount of BaP in target dust per trial increased as percent incorporation increased: 1.20mg and 1.73mg in the 10% and 20% summer trials, respectively, and 0.864mg and 1.47mg in the 5% and 10% winter trials, respectively (Fig. 1.6).

DISCUSSION

This study examined the potential toxicity of Remington Arms Company Blue Rock® target fragments to captive Coturnix quail. The concern centers on the PAHs, toxic substances bound within the petroleum pitch, which comprises approximately 32% of each target. Baer et al. (1995) concluded that PAHs would not leach from the petroleum substrate because of the heat and pressure from manufacture under which they are bound to the clay substrate; however, based on the scarcity of empirical evidence, it is impossible to know if this is fact or speculation.

Table 1.12 – Comparison of PAH values in clay target analysis from Texas A&M Univeristy to Baer et al. study (1995) for studies completed at theVirginia Tech Center Woods Research Station in October, 2001, June- July, 2002, and January-March, 2003.

Compound[a]	Mean	SD	Mean: Baer et al. (1995)
Naphthalene	179	6.9	185
2-Methylnaphthalene	836	34	1,495
1-Methylnaphthalene	351	32.5	459
Biphenyl	128	22.1	N/A
2,6-Dimethylnaphthalene	1,340	26.5	2,119
Acenaphthylene	87.2	18.1	N/A
Acenaphthene	161	6	209
2,3,5-Trimethylnaphthalene	105	5.2	690
Fluorene	410	18.8	699
Phenanthrene	9,350	566	27,085
Anthracene	2,140	130	5,074
1-Methylphenanthrene	6,513	239	12,042
Fluoranthene	5,167	280	10,587
Pyrene	63,067	3,107	126,532
Benz(a)anthracene	231,133	20,997	182,366
Chrysene	467,400	42,778	296,556
Benzo(b)fluoranthene	118,933	4,271	66,384
Benzo(k)fluoranthene	34,733	1,950	N/A
Benzo(e)pyrene	333,800	2,536	127,921
Benzo(a)pyrene	335,233	7,605	113,554
Perylene	87,767	1,380	30,518
Indeno(1,2,3-c,d)pyrene	40,033	3,252	9,256
Dibenzo(a,h)anthracene	64,800	2,691	23,646
Benzo(g,h,I)perylene	159,100	3,236	38,657
Total PAHs	1,962,766	95,188.1	1,076,032

a. Concentration (ng / dry g)

Figure 1.5 – Average benzo(a)pyrene / treatment in a dose experiment conducted at the Virginia Tech Center Woods Research Station, June, 2002 and January-February, 2003.

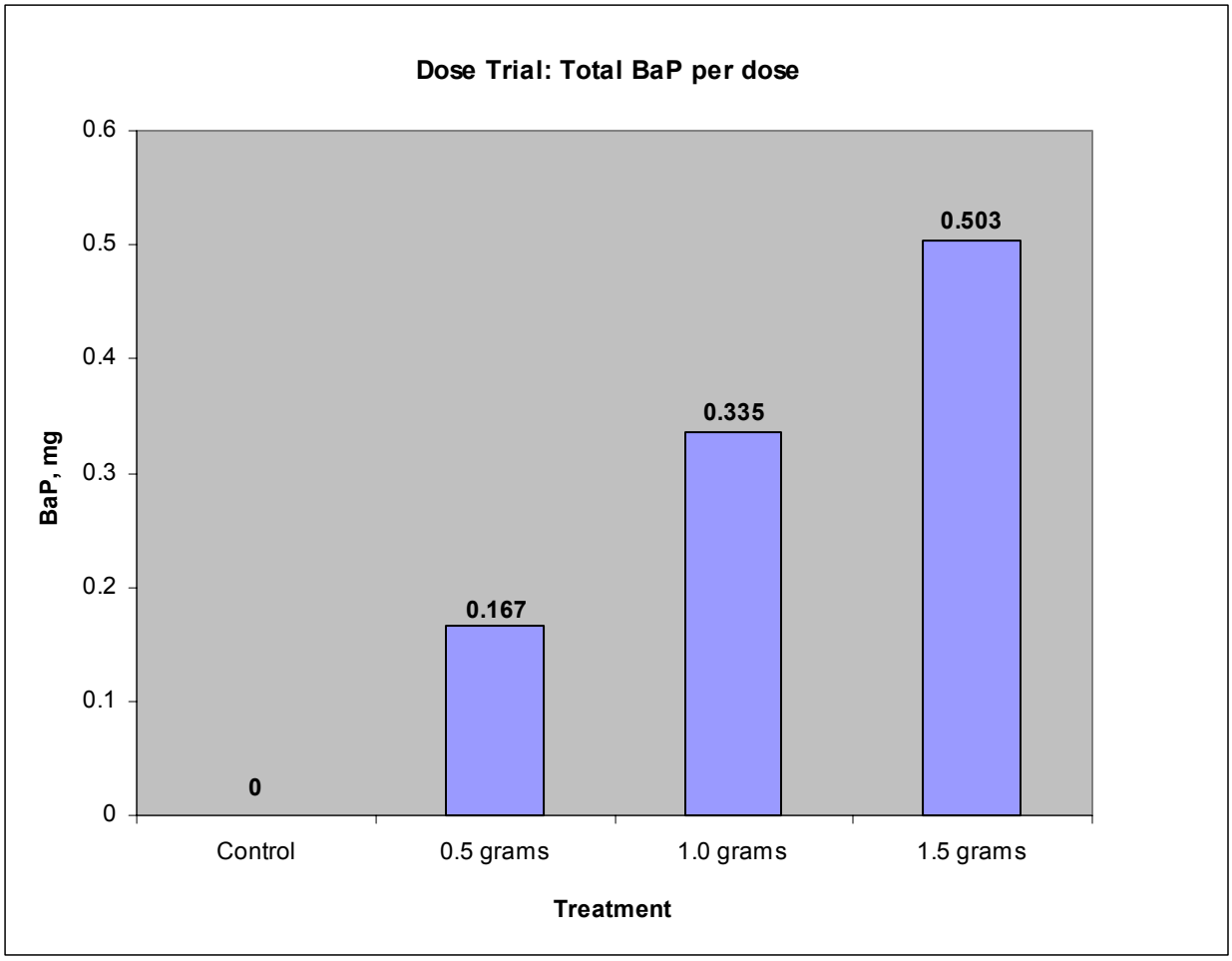
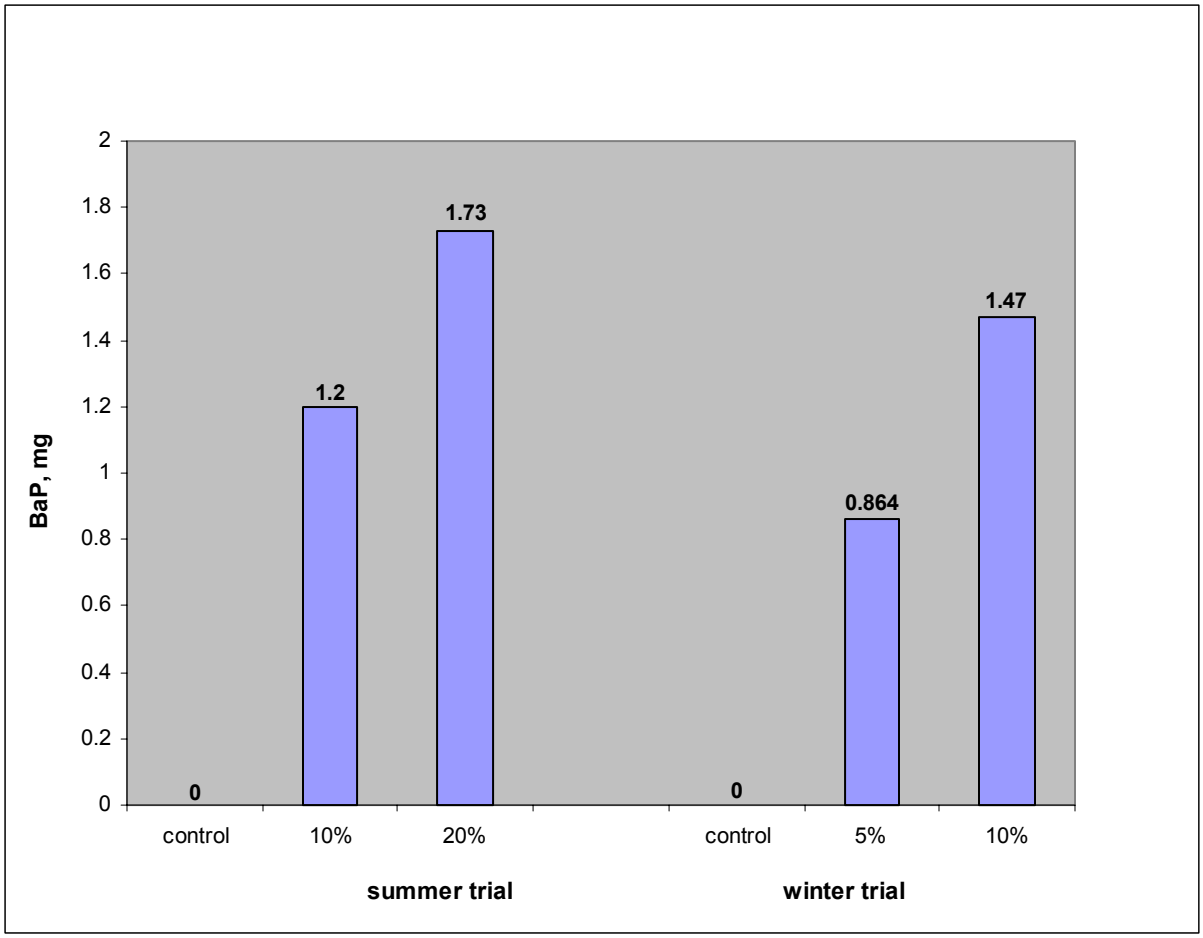


Figure 1.6– Average benzo(a)pyrene / treatment in an incorporation experiment conducted at the Virginia Tech Center Woods Research Station in July, 2002 (summer) and February, 2003 (winter).



The Consumption Experiment

The consumption experiment focused on the likelihood of birds consuming target fragments if given the opportunity. The fall and spring experiments differed in the type of feed offered. In the fall, the experimental birds were fed a wild birdseed diet, consisting largely of millet and sunflower seeds; however, this diet did not contain the nutritional requirements necessary to sustain the quail. Therefore, birds in the spring trial were fed a commercial poultry diet with a higher protein and fat content. These birds did not lose weight, as was seen in the fall experiment. Despite the dietary discrepancy, one major tendency appeared throughout all experimentation. The birds consistently consumed more limestone, a substance likely to be ingested in the wild, than clay target fragments as a grit component.

Experimental birds ate more limestone and target in fall than in spring, likely in response to the diet they received from the wild birdseed in fall. Gionfriddo and Best (1996) observed that diet strongly influences the amount of grit used by birds, particularly the tendency to eat more grit with a seed diet. Despite the change from seed to a commercial poultry diet, more limestone was consumed than target in both trials. Confounding factors such as dietary disparities affect the accuracy of this particular experiment; however, our results imply that birds in controlled settings are not likely to choose target fragments on a frequent basis. Instead, they may choose something synonymous to limestone or nothing at all. Wild birds would likely have more access to seeds and insects, objects encouraging the consumption of grit; however, based on our study, birds would not necessarily consume target fragments if they were available. Nevertheless, because this study was completed in a controlled setting, only speculative statements are possible.

Another aspect of the consumption experiment gave insight into how ingested target fragments affected the experimental birds. Despite evidence that some birds consumed target fragments, there was no mortality in either trial and weight loss in the fall was most likely due to insufficient diet, as there was no weight loss recorded in the spring trial. Thus, it seems logical to conclude that either target substance was biotransformed with little or no repercussion as it passed through the system, as was suggested by Naf et al. (1992), or the PAHs were bound tightly within the matrix so that target fragments passed through the system without releasing any toxic compound or interrupting any gastrointestinal function (Baer at al., 1995). However, dietary inconsistencies detracted from overall results, indicating the need for further studies to support possible hypotheses that birds will not consistently choose target fragments as grit

The Dose Experiment

This experiment examined 3 major components: 1) weight change throughout the experiment, 2) liver weights and liver-to-body weight ratios at the end of the experiment, and 3) daily feed consumption as a response to target ingestion. The purpose of the dose experiment was to determine how these 3 components were affected by sporadic exposure to clay target. If a bird or mammal consumed lead pellets on an infrequent basis, it likely would be negatively affected as a result (Ma, 1989; Vyas et al., 2001). It is not known if this is the case with clay target fragments. This is pertinent information because of the large amount of target fragments available on shooting range grounds across the country (Peddicord et al., 1993).

In the summer experiment, weight of experimental birds did not change regardless of sex ($P>0.05$) or treatment ($P>0.05$), i.e., forced target ingestion invoked no weight loss. However,

there was a decrease ($P<0.01$) in daily feed consumption for the 0.5g, 1.0g, and 1.5g treatments, indicating that the birds were eating differently, perhaps in response to their treatment. No experimental birds died or displayed apparent physiological distress, suggesting that the change in feed consumption could be attributed to the differing quantities of target occupying space in the crops, dictating how much the birds would eat.

In the summer trials, liver-to-body weight ratios for female quail were greater than liver ratios for males ($F=12.56$, $P=0.0013$). Coturnix quail are sexually dimorphic and females are slightly larger than males (Fitzgerald, 1969), which, in part, explains the difference in liver weights. Because there was no difference ($P>0.05$) in final body weight between the two sexes (where females would typically have larger final body weights), it is possible that the difference in liver-to-body weight ratios is a reflection of the larger female livers. The change could also be attributed to activity in the liver as a result of the target material, since liver weights increased while body weight remained the same. However, this is merely speculation as there were no tests performed on the livers. Based on all these results, target ingestion likely had little effect on the birds; however, scarcity of empirical evidence prevents us from drawing a strong conclusion.

During the winter experiment, female quail weighed more than male quail at the beginning ($P<0.01$) and end ($P<0.01$) of the trials. I attributed the difference to egg production by females throughout the winter trial, making them substantially heavier than the males. Weights did not differ significantly ($P>0.05$) by sex in the summer, as females did not produce eggs. As was the case with the summer experiment, the liver-to-body weight ratios in winter were higher in females than males, explained by the females' overall larger body weight, and substantially larger livers. As in the summer, this may be attributed to liver response to target material. However, it is also likely that there was substantial lipid accumulation on the liver,

explaining the drastic increase in liver weight when compared to males. These factors may explain the difference ($P<0.01$) in liver-to-body weight ratios between sexes. Yet lack of liver tests prevents a firm conclusion.

The winter feed response trial was similar to the summer experiment, indicating different amounts of feed were consumed across treatments ($P<0.01$). Again, this was likely related to the amount of target material in the crop. An alternate explanation for both summer and winter trials is that the presence of a foreign substance, toxic or not, dissuaded the birds from eating because of lingering residues, aftertaste, and/or mild sickness. However, it did not appear that the effects were long-lasting or permanent, as the birds increased the amount of feed consumed with time. There did not appear to be any effect on the health of the birds, nor did the females cease egg production in the winter. It seems likely that forced target ingestion had little to no effect on the bird; however, as in the summer trial, these conclusions are tentative.

I compared the summer and winter trials and found significant differences in weight and weight changes by sex and season. The seasonal difference in weight between sexes was related to egg production by females in winter; females were heavier in the winter versus summer. Differences in weight between seasons can also be related to food intake. Because the weather was on average $5^{o}C$ cooler in the winter, the birds likely ingested more to compensate for declines in the temperature (Kelly et al., 2002). With increased ingestion, winter quail gained more weight than summer quail, causing a seasonal difference in weights.

Although the summer and winter seasons cannot be qualitatively compared, it is interesting to draw some comparisons based on the treatment similarities. There was a substantial difference in final weights between sex ($F=27.68$, $P<0.01$) and season ($F=56.32$, $P<0.01$) for the winter birds. However, there was no change in final weight ($F=0.36$, $P=0.78$) for the various

treatments. This could indicate that regardless of the amount of target ingested, either 0 grams or 1.5 grams, experimental birds were not adversely affected. Therefore, regardless of incomplete replication, it is possible that the sporadic exposure to target had little or no effect on the overall health of the birds. They ate and subsisted in what appeared to be a normal manner. However, Coon and Dieter (1981) found that ducks fed petroleum (a substance heavy in PAHs) in a controlled lab setting could consume considerable amounts without showing signs of illness. When stressed by lower temperatures than accustomed to, they died. Because of the inadequacy and scarcity of data, it would be prudent to conduct further tests on the nature of this trial. However, preliminary experiments have demonstrated that it is unlikely that wildlife, specifically birds, will suffer physiological distress if they consumed target fragments on an infrequent and sporadic basis.

The Incorporation Experiment

This experiment sought to identify the 3 main components of the dose experiment; weight change throughout the experiment, liver weights and liver-to-body weight ratios at the end of the experiment, and daily feed consumption as a response to target ingestion. The variation in this experiment examined how the 3 components were affected by continuous exposure to target fragments, thus repeated exposure to PAHs.

The summer and winter trials of this experiment were not analogous. The amounts of target incorporation in the summer trials were 10% and 20%. Four days into the experiment, the 20% experimental birds were emaciated and weak; the trial was terminated 4 days later. However, in both summer and winter trials, if the trial had continued, the birds would have likely

died of starvation with no human intervention. The birds likely were starving as a result of insufficient nutrition as opposed to target poisoning – it is probable there was not enough food mixed in with the target dust to satisfy daily nutritional requirements, ie. incorporation amounts were too high. However, if the target dust was an appetite suppressant, it is unlikely that wild birds would consume such high quantities of target material on a daily basis; if their nutritional needs were not being met, they would choose another location at which to forage.

In the summer experiment, both sexes lost weight in all treatments except the control group, 0%; males consistently lost more weight than females ($P<0.01$). Weight loss increased as incorporation amounts increased, and there were significant changes in final weights ($P<0.01$). However, the weight loss was most likely a result of the decreasing feed rather than increasing amount of target dust.

In the winter experiment, the circumstances were similar. Females were larger than males at the beginning of the experiment ($P<0.01$). However, they lost more weight than the males by the end ($P=0.0004$). It is possible that they consumed more to compensate for the extra amount of energy used to produce eggs. Treatment effects were the same as summer, i.e., there was no difference in initial mass ($P=0.13$); however, by the end of the experiment, a substantial decrease in weight had occurred ($P<0.01$) as a result of the increasing incorporation amounts (5%, 10%).

Liver-to-body weight ratios in the summer and winter trials increased as incorporation amount increased ($P<0.01$). It is probable that the change in liver ratios was a direct result of body weight loss for the varying treatments: 20% birds on average lost more weight than 10% birds and control birds. Liver weights were similar for both sexes; however final body weights decreased dramatically as incorporation amounts increased, causing a change in liver-to-body weight ratios. It is possible that liver ratios changed as a result of target poisoning: Patton and

Dieter (1980) observed enlarged livers in birds that were fed small doses of PAHs found in our sample of clay target. Since we did not perform biochemical and histological tests on individual livers, it is impossible to determine why the liver ratios were different across treatments. Further tests, including liver and kidney analyses, would help answer this question.

In the winter trial, I reduced the amount of target dust incorporated into the feed to 5% and 10%, theoretically allowing for sufficient nutrition and opportunity to detect the effects of target dust on sustainability. Similar to the summer experiment, there were significant differences in initial and final mass measurements between male and female quail. In this trial, the female group lost 17g and 21g for the 5% and 10% trials, respectively. This was likely related to egg production; females regularly laid eggs, thus using more energy and losing more weight if they were not receiving adequate nutrition. There was no apparent physiological distress nor was there mortality, possibly indicating that target ingestion had little to no effect on the regulatory functions of the birds. As in the summer trial, it is likely that birds still received inadequate nutrition, as indicated by increased weight loss with increased incorporation, yet similar liver weights between sexes; however, I could not conclude if this was due to insufficient feed or the target material.

In both the summer and winter incorporation feed trials, daily feed intake did not change. Overall, the birds ate comparable quantities daily, suggesting that the target material did not suppress their appetite, despite the amount incorporated in the feed. Additionally, they ingested large amounts of target daily that did not cause mortality, indicating that the dust was potentially not toxic to the birds. Reasons for weight loss were most likely a result of the small capacity for holding food in their crops; if most of that capacity was occupied by target dust, they would have been deprived of nutrition, resulting in weight loss.

The seasonal effect on feed intake, i.e. higher consumption in winter, detracted from the validity of a replication experiment. The lack of birds and time made seasonal replications impossible. However, these experiments created a sturdy foundation upon which other experiments can be based. Additionally, they have supported the idea that the PAHs bound within clay targets are in all likelihood tightly bound in the short-term, thus prohibiting escape from the petroleum matrix (Baer et al., 1995).

Clay Target Analysis

Analysis of the target dust revealed larger quantities of PAHs in this study than the clay target study completed by Baer et al (1995; Table 1.10); total PAH count for Baer et al. (1995) was 1,076,032 ng/dry g, in comparison to 1,962,766 ng/dry g for this study. Despite these larger numbers, there appeared to be no immediate or apparent effect of target ingestion on study birds, nor did the quail ingest the target when given the choice. All these factors indicate there is potentially no danger to wildlife that inadvertently ingest clay target fragments. However, due to small sample sizes in this study and incomplete replication, further studies are recommended.

Benzo(a)pyrene

Because there is no known standard of comparison for PAHs in animal tissues (Beyer et al, 1996), it is difficult to ascertain if the amounts present in this study are high, medium, or low. However, from the abundance of data available on PAHs and BaP, it is known that minute doses of BaP induce tumor formation, among other symptoms of sickness (Dipple, 1985). Our study

birds did not develop any gross or apparent abnormalities, nor did they appear to be adversely affected throughout the study. However, the longest study was only 25 days, not necessarily allowing for the growth of any apparent abnormalities. This could indicate that under the controlled circumstances in which they subsisted, the PAHs did not leach from the target substrate; if BaP had leaked into their system, it seems likely that physical indications of duress would have been apparent. However, further tests are needed to reinforce these findings.

SUMMARY AND CONCLUSIONS

Three experiments were conducted on captive Coturnix quail over the course of 2 years. The consumption experiment attempted to identify if quail would ingest target fragments and limestone when presented with the opportunity. Some target fragments were ingested; however, limestone fragments were consumed in much greater quantities as a grit component. The dose and incorporation experiments tested the effects of target dust ingestion when received on a sporadic (dose) and continuous (incorporation) basis. Quail in the dose experiment suffered minor weight losses and no apparent reaction to the forced dosing they received on a weekly basis. Quail in the incorporation experiment suffered substantial weight loss, most likely due to insufficient nutrition as opposed to target poisoning. However, because of lack of biochemical analyses for both dose and incorporation experiments, it is impossible to determine if the target had any effect on the physiological well-being of each bird. Several references suggest that PAHs bioaccumulate in tissues and organs while others suggest that they are readily biotransformed. This study does not shed light on this subject, but rather offers results which can

perhaps be used as the basis for further biochemical examinations dealing with clay target toxicity.

CHAPTER 2 – LEAD CONTAMINATION IN WILD AVIAN AND MAMMALIAN SPECIES RESIDING ON OR AROUND A SHOOTING RANGE

INTRODUCTION

Lead is a soft, metallic element occurring naturally in a variety of environments. Because of its malleability, low melting point, and low cost, lead is used as the main substrate in a multitude of products, including ammunition (Scheuhammer and Norris, 1996). Sediments and soils at trap and skeet ranges may contain extremely high quantities of spent lead shot (Jorgensen and Willems, 1987). Spent shot typically remains in the top 10 cm of soil throughout the shooting range habitat, making accidental ingestion possible for a wide scope of animals (Scheuhammer and Norris, 1996), including Passerine species and small mammals

Avian species, specifically Passeriformes, frequently mistake lead pellets for grit or seed because of similarities in size and color (Vyas, et al., 2000). Birds are especially susceptible to lead because of gizzard acidity and grinding activity, resulting in toxic and frequently lethal levels of ionic lead released into the bloodstream (VanCantfort, 1999). Lead has several detrimental effects on the body. It damages the nervous, excretory, hematopoietic, and skeletal systems (Kendall et al., 1996; Scheuhammer and Norris, 1996). Symptoms of lead poisoning include loss of appetite, emaciation, weakness, tremors, and paralysis; such poisoning often results in death (Losito and Mirarchi, 1991). While there is a substantial body of work

documenting the effects of lead on waterfowl, there have been few studies investigating lead toxicity in Passerine species.

For small mammals inhabiting shooting ranges, soil ingestion may be a significant path of lead consumption (Stansley and Roscoe, 1996). Particles of soil may adhere to dietary items and be consumed by a range of animals (EPA, 1993). Additionally, fragments of soil that stick to fur can be accidentally ingested during grooming activities, thus negatively affecting the animal (EPA, 1993). Typical symptoms of lead poisoning in mammals include weight loss, reduced growth, and changes in organ-to-body weight ratios (Goyer et al, 1970; Bankowka and Hine, 1985, Ma, 1989). The objective of this study was to test for lead poisoning in Passerine species and small mammals at the shooting range on the Blacksburg Ranger District of the Jefferson National Forest in western Virginia.

METHODS

Avian Sampling

I operated 8 mist-nets daily for 20 days in July, 2002 at the Jefferson National Forest shooting range in Montgomery County and for 5 days at a control area, approximately 13 km east of the shooting range. The nets were set up along the periphery of the range, as well as 5-10m away from the range perimeter. They were placed at different locations where foraging activity was apparent and remained there throughout the experiment for 20 consecutive days. The nets were opened at dawn and remained open for 3-4 hours, depending on weather conditions and the degree of foraging and territorial activity. I weighed each captured bird and measured tail and

wing length, recorded the sex, plucked breast feathers for chemical analysis, and banded each with a numbered USFWS legband. All birds that flew into the net were included in the sample; both resident and migratory birds were captured and then released after measurements and feathers were taken. Feather samples were rinsed with deionized water, stored in Nasco Whirlpak® bags, and sent to the Research Triangle Institute in the Research Triangle Park, NC for lead analysis using inductively coupled plasma spectroscopy.

Small Mammal Sampling

I trapped small mammals in Victor® snap traps at the shooting range and a control site 3 km north of the shooting range, during the first 2 weeks of May and September, 2002. Trap lines of approximately 150 traps spaced 5m apart, were set at night within 5m of the perimeter of the range and baited with peanut butter and oatmeal. I checked the traps at dawn each morning to minimize the occurrence of birds and larger animals becoming caught and/or springing the traps. The shooting range was trapped the first week of May and September and the control site the second week of May and September.

I returned captured specimens to the laboratory, where I weighed and necropsied them, and froze them at -20^{o}C. I weighed livers and kidneys and collected samples of each for lead analysis. Liver-to-body weight and kidney-to-body weight ratios were determined by dividing the fresh weight of the organ by the fresh weight of the body and multiplying by 100. Liver and kidney samples were sent to the Toxicology Laboratory at the Virginia-Maryland Regional College of Veterinary Medicine in Blacksburg, VA for lead analysis using atomic absorption spectrophotometry.

Statistical Analysis

I used Analysis of Variance (Statistical Analysis System, Cary, NC) to compare lead content in feathers between the shooting range and control site. PROC GLM was used for overall comparisons and species-specific comparisons. Additionally, I used PROC GLM to compare lead content in mammalian liver and kidney samples between the shooting range and control locations. All tests were considered significant at $\alpha=0.05$.

RESULTS

Avian Weights and Feather Samples

I captured 20 birds at the shooting range site (11 Passerine spp.), and 20 birds (7 Passerine spp.) at the control site. The average weight of birds captured at the shooting range (all spp. combined) was 32.88g, compared to 32.05g at the control site (Table 2.1). The average lead content in feathers from birds collected at the shooting range (26.29ppm) was higher ($F=5.21$, $P<0.028$) than the value at the control site (1.26ppm; Table 2.1; Appendix 2). Five species were common to both sites: Northern cardinal (C*ardinalis cardinalis)*, Eastern towhee (*Pipilo erythophthalmus)*, Carolina wren (*Thryothorus ludovicianus)*, Blue grosbeak (*Guiraca caerulea)*, and Carolina chickadee (*Poecile carolinensis)*. Blue grosbeaks and Carolina wrens had the highest average lead values at both the shooting range and control sites (Table 2.2). Lead content in feathers from these 5 species combined at the shooting range was higher ($F=14.91$, $P=0.0008$) than lead content in feathers of the 5 species combined at the control site. Lead values in ground

Table 2.1 – Average body weight (g) and lead concentration (ppm) values in Passerine species collected at the Blacksburg Ranger District, Jefferson National Forest shooting range and control site, Montgomery County, Virginia, 2002.

	Shooting Range			Control Site		
Variable	N	$\bar{x}$	SE	N	$\bar{x}$	SE
Body weight[a] (g)	20	32.88	4.06	20	32.05	2.55
Lead content in feathers[b] (ppm)	20	26.29	10.96	20	1.26	0.42

a. Body weight comparison between shooting range and control locations: P=0.86
b. Lead content comparison between shooting range and control locations: P=0.028

Table 2.2 – Average lead concentrations (ppm) for five Passerine species collected at the Blacksburg Ranger District, Jefferson National Forest shooting range and control site, Montgomery County, Virginia, 2002.

	Average Lead (ppm)					
	Shooting Range			Control Site		
Species	N	$\bar{x}$	SE	N	$\bar{x}$	SE
Blue grosbeak[a]	2	112.95	85.55	1	1.3	------
Carolina chickadee[b]	1	9.0	------	1	0.1	------
Carolina wren[c]	1	110.7	------	5	2.02	1.15
Eastern towhee[d]	2	15.8	2.60	5	0.3	0.20
Northern cardinal[e,f]	2	54.3	13.6	5	1.24	0.57

a. Lead comparison between shooting range and control locations: P=0.5
b. Lead comparison between shooting range and control locations: -------
c. Lead comparison between shooting range and control locations: P<0.01
d. Lead comparison between shooting range and control locations: P<0.01
e. Lead comparison between shooting range and control locations: P=0.0008
f. Overall lead comparison between five species on shooting range and control locations: P<0.01

vs. both ground and tree foragers at the shooting range was elevated for several species, including the blue grosbeak, Carolina wren, and northern cardinal (Table 2.3). Ground and tree foragers at the shooting range, including wood thrush, Carolina chickadees, and worm-eating warblers, had lower lead levels compared to only ground foragers at the range. Lead values for all foraging types at the control site were considerably lower ($P<0.01$) than the shooting range.

Peromyscous leucopus Samples

Resident species of small mammals in the area include the white-footed mouse (*Peromyscus leucopus)*, the house mouse *(Mus musculus)*, the meadow vole *(Microtus pennsylvanicus)*, the pine vole *(M. pinetorum)*, the Southern short-tailed shrew (*Blarina carolinensis*), and other shrew species *(Sorex spp)*. To maintain homogeneity, only *Peromyscus leucopus* samples were used because they constituted the majority of small mammals caught. The average weight for all white-footed mice captured at the shooting range (n=26; $\bar{x}$=21.33g) was similar ($F= 2.27$; $P=0.14$; Table 2.4) to the average weight of mice captured at the control site (n=29; $\bar{x}$=19.67g; Table 2.4). However, average liver weights (shooting range=1.2g; control=0.88g) and kidney weights (shooting range=0.27g; control=0.21g) of mice collected at the shooting range were heavier ($F=16.63$, $P=0.002$ and $F=18.09$, $P<0.01$, respectively) than for mice collected at the control site. Liver-to-body weight ratios were higher ($F=15.33$; $P=0.0003$) at the shooting range than the control site, as was the case with kidney-to-body weight ratios ($F=11.53$; $P=0.0013$).

Table 2.3 – Lead content (ppm) of ground foraging vs. ground and tree foraging Passerine species at the Blacksburg Ranger District, Jefferson National Forest shooting range and control site, Montgomery County, Virginia, 2002.

Shooting range			Control site		
Species	N	Lead content	Species	N	Lead content
Ground foragers		(ppm)	Ground foragers		(ppm)
Blue grosbeak	2	27.4, 198.5	Blue grosbeak	1	0.1
Carolina wren	1	110.7	Carolina wren	5	0.1. 0.1, 3.2 0.7, 6.0
Northern cardinal	2	67.9, 40.7	Northern cardinal	5	3.4, 0.8, 0.7 1.2, 0.1
Ground, tree foragers			Ground, tree foragers		
Wood thrush	6	0.5, 2.7, 0.2, 1.7, 2.5, 4.0	Gray catbird	1	0.1
Carolina chickadee	1	9.0	Carolina chickadee	1	0.1
Worm-eating warbler	1	0.2	Tufted titmouse	1	0.1

Table 2.4 – Average body weight (g), liver weight (g), liver ratio (g/100g body weight), kidney weight (g), and kidney ratio(g/100g body weight) values for *Peromyscous leucopus* specimens collected at the Blacksburg Ranger District, Jefferson National Forest shooting range and control site, Montgomery County, Virginia, 2002.

	Shooting Range			Control Site		
Variable	N	$\bar{x}$	SE	N	$\bar{x}$	SE
Body weight[a] (g)	26	21.33	0.66	29	19.67	0.86
Liver[b] (g)	26	1.2	0.067	29	0.88	0.042
Liver-to-body weight ratio[c] (g/100g)	26	5.63	0.27	29	4.48	0.13
Kidney[d] (g)	26	0.27	0.011	29	0.21	0.0093
Kidney-to-body weight ratio[e] (g/100g)	26	1.28	0.048	29	1.08	0.037

a. Body weight comparison between shooting range and control locations: P=0.14
b. Liver weight comparison between shooting range and control locations: P=0.002
c. Liver-to-body weight ratio comparison between shooting range and control locations: P=0.0003
d. Kidney weight comparison between shooting range and control locations: P<0.01
e. Kidney-to-body weight ratio comparison between shooting range and control locations: P=0.0013

Lead Content in Livers and Kidneys

Lead content in livers ($F=9.78$; $P=0.0029$) and kidneys ($F=22.49$; $P<0.01$) was higher at the shooting range than at the control site (Table 2.5; Appendix 3). The average lead concentration reported in livers at the shooting range was 0.20 ppm and 0.0091 ppm at the control site (Table 2.5). The average lead concentration in kidneys was 0.79 ppm at the shooting range and 0.0056 ppm at the control site. One mouse at the shooting range (n=26) had a liver level above 1.0 ppm and 13 had background lead levels (<1.0ppm). At the control site, 3 of 29 mice registered background lead levels; the remaining 26 specimens were below the minimum detection limit (MDL). Six mice from the shooting range had elevated lead levels in kidney tissue (>1.0 ppm) and 3 kidney samples registered levels greater than 2 ppm, indicative of potential subclinical or clinical exposure. Only one sample at the control site registered a background kidney lead level (<1.0ppm), while the remaining 28 were below the minimum detection limit (Appendix 3).

DISCUSSION

Lead Availability to Birds

It is reasonable to assume that birds at the shooting range would have higher lead content in their system than those on the control site. They had more opportunity to ingest lead due to unusually high presence of lead in their habitat. However, on both experimental and control sites, several species consistently had a high lead burden, likely resulting from dietary variation.

Table 2.5 – Average lead concentration (ppm) of liver and kidney samples from *Peromyscous leucopus* specimens collected at the Blacksburg Ranger District, Jefferson National Forest shooting range and control site, Montgomery County, Virginia, 2002.

	Shooting Range			Control Site		
Variable	N	$\bar{x}$	SE	N	$\bar{x}$	SE
Liver[a]	26	0.20	0.065	29	0.0091	0.0054
Kidney[b]	26	0.79	0.17	29	0.0056	0.0056

a. Lead comparison between shooting range and control locations: P=0.0020
b. Lead comparison between shooting range and control locations: P<0.01

The blue grosbeaks (n=2) and Carolina wren (n=1) captured on the shooting range had high lead levels (>25 ppm; Table 2.3) compared to control site specimens. Their diet consists primarily of insects, with occasional berries and seeds, all foraged from the ground, suggesting these birds mistook the shot for seed or grit (Vyas et al., 2001). Additionally, higher occurrence of grit intake is known to occur in birds with diets of plant material and hard-bodied insects (Stafford and Best, 1999), the main dietary components of these bird species. Northern cardinals also feed primarily on seeds and insects, and the 2 specimens netted at the range both had high lead levels ($P<0.01$; Table 2.3), when compared to the control site. It is likely they ingested lead pellets by mistaking them for grit or food, similar to the grosbeaks and wren. It is also possible they ingested some form of lead through other vectors, such as insects, earthworms, air, and soil.

A different foraging strategy, that may reduce lead intake, is gleaning insects from trees and collecting berries from shrubs and bushes. Bird species netted on the shooting range foraging in this manner included wood thrushes, Carolina chickadees, and worm-eating warblers. These birds (n=8) had a relatively low lead burden (<10ppm) based on feather analysis (Table 2.3). They do not consistently forage on the ground, thus reducing the opportunity to inadvertently ingest lead pellets found in soil and leaf litter. While most birds netted at the shooting range had elevated lead levels, the highest levels were observed in bird species that typically forage for insects and seed on the ground, thus increasing the risk of lead ingestion through lead pellets (Vyas et al., 2000) or insects, earthworms, and soil.

Birds at the control site had lower lead levels, relative to shooting range birds (Table 2.3). However, some control site specimens had detectable levels, explained only by random exposure events to lead in the environment. On average, most levels were below 1.0 ppm, considered a

relatively safe range based on a study completed by Dauwe et al (2000), in which great (*Parus major*) and blue tit (*P. caeruleus*) nestlings were studied using feathers as lead indicators.

Despite the likelihood that the lead levels found in birds at the shooting range were high, there is no direct comparison for Passerine species to determine mild to acute lead poisoning. Dauwe et al (2000) focused on great and blue tit nestlings, not adult birds. However, based on the numbers from this study, it is safe to conclude that birds at the shooting range ingested lead pellets, or some form of lead: 85% of the birds (n=17) had lead levels above 1.0 ppm and 60% of this group (n=10) had lead levels above 6.0 ppm. At the control site, only 35% of the birds (n=7) had levels above 1.0 ppm and none of these levels exceeded 6.0 ppm. Additionally, 50% of the control birds (n=10) had lead levels below the minimum detection limit, indicating they did not ingest lead with the same frequency as birds at the shooting range, likely due to differential exposure to lead in the environment.

Lead in Small Mammals

In this study, small mammals at the shooting range had higher lead levels than those from a control area. Previous studies investigating lead toxicity in small mammals have suggested that lead pellets may be inadvertently ingested (Ma, 1989). Liver-to-body weight ratios (5.63g/100g at the shooting range and 4.48g/100g at the control site) and kidney-to-body weight ratios, (1.28g/100g at the shooting range and 1.08g/100g at the control site) reflect differences in organ weight rather than bodyweight (Table 2.4). Body weight values at both sites were similar, yet there was a significant difference in organ-to-body weight ratios, a symptom of lead ingestion (Goyer et al., 1970). Franson et al.(1996), suggested tissue lead levels above 1.0 ppm are

considered elevated, and levels above 2.0 ppm are indicative of potential subclinical or clinical exposure. When organs were analyzed for lead content, specimens collected at the shooting range had elevated lead levels. Although 13 (50%) mice at the shooting range had liver and kidney lead levels above 0.1ppm, only 1 (4%) specimen had elevated liver levels (>1.0ppm); only 9 (35%) specimens had elevated kidney levels and 3 (12%) were possibly victims of lead poisoning (>2.0ppm). Despite the presence of these samples, the numbers are relatively small, indicating mice are not likely to ingest lead pellets on a frequent basis (Peddicord and LaKind, 2000). The risks for Passerine spp. are greater because they mistake lead pellets for grit, while small mammals seem to eat it incidentally and fairly sporadically (Vyas et al., 2000, Peddicord and LaKind, 2000).

SUMMARY AND CONCLUSIONS

Passerine species as well as small mammalian species that reside around shooting ranges are in danger of lead poisoning through mistaken consumption of lead pellets as grit and/or dietary items. Few studies have been conducted on the effects of lead on small mammals and fewer have been completed concerning Passerine species. It is apparent from this study that both birds and mammals on shooting ranges consume lead derived from lead pellets or insects, earthworms, soil, and water. Because many shooting ranges are constructed each year, it is important to investigate the occurrence of additional lead poisoning on shooting ranges, as lead pellets do not degrade; instead, they become more harmful as they age in the soil, thus adding to the increasing rate of mortality of numerous species of wildlife each year.

MANAGEMENT IMPLICATIONS AND CONCLUSIONS

Based on the limited findings from this study, I did not find overwhelming evidence to indicate that clay targets are harmful to species that ingest fragments. The quail from all treatments did not exhibit apparent signs of illness, nor was there mortality throughout the experiment. However, there were no physiological tests performed to reinforce the notion that clay targets are not harmful to species that ingest them. Before issuing any mandates on the toxicological status of clay targets, I would strongly recommend biochemical tests of livers, kidneys, and body fat of exposed animals, to determine if there is significant bioaccumulation of any PAHs, specifically the 16 PAHs of special concern. These tests were not possible in this study due to a lack in time and funding.

In addition to biochemical analyses , I would advocate further testing to observe if wild birds actually ingest target fragments in their natural habitat. A method to obtain such desired results would be the establishment of an outdoor aviary on a shooting range that is currently out of use. Observations could be made on a daily basis to record if birds would conceivably ingest target fragments. Vyas et al. (2002) conducted a similar study with lead pellets to observe if Passerine spp. in an outdoor aviary inadvertently consumed lead pellets on the shooting range grounds. This experimental set-up would be feasible for clay target ingestion as well.

If more laboratory tests were performed, it would be prudent to create stressful situations (such as weather-induced circumstances) to determine if this would have an effect on the physiological nature of any bird being fed target dust. My winter studies were conducted under conditions of meteorological duress; however, I attempted to control the cold temperatures with heat lamps in an attempt to create a similar situation to the summer experiments. Despite my

efforts, I inadvertently created stressful situations in the winter experiments. Without having information from biochemical analyses and histological tests, the birds did not appear to have any adverse reaction. However, it would be beneficial to create purposeful conditions that would stress the bird to determine if stress was the exact cause of a reaction.

Based on the preliminary results from my study, I do not foresee serious effects from clay target fragment ingestion. However, if further tests prove that there is bioaccumulation and potential negative effects, it would be logical to substitute a substance less rich in PAHs, or devoid or PAHs altogether for the petroleum pitch. This is important because of the large numbers of clay targets used on shooting ranges annually. If a substitution was not feasible, I would suggest improved methods of clean-up on shooting ranges to properly dispose of target fragments. However, because target fragments have a large trajectory when shot, a petroleum pitch substitution would be the most appropriate alternative.

It has been firmly established that lead is detrimental to the well-being of multiple wildlife species, including Passerine species and small mammals. Therefore, further tests are not necessary. My study was meant to identify the presence of lead contamination in certain wildlife species on the shooting range of the Blacksburg Ranger District of the Jefferson National Forest in Montgomery County, VA. The most feasible solution to the problem of lead contamination on shooting ranges would be either lead substitution or proper clean-up methods. Since the USFWS banned the use of lead shot for waterfowl hunting in 1991, steel shot has become the major alternative. However, in addition to the added expense, steel is approximately 30% lighter than lead, as well as significantly harder (Scheuhammer and Norris, 1996). Such qualities have impaired overall shooting effectiveness in most hunters and target shooters. Despite the development of modern steel ammunition that has overcome most deficiencies (Brister, 1992;

Coburn 1992), it would seem that shotgunners at shooting ranges prefer the use of lead, as reflected by the large amounts of lead pellets still present on shooting ranges across the country. A relatively new substitution is in the process of manufacture; tungsten/polymer is a substance that would have the same density as lead (Marchington, 1994). Based on the known toxicology of tungsten, this new polymer would be nontoxic to birds (Kazantzis, 1986); however, the price would be higher than both lead and steel pellets (Scheuhammer and Norris, 1996).

In addition to lead substitutions, clean-up methods should be used on all shooting ranges. In the United States, approximately 15 companies have equipment for lead shot recovery on range grounds; however, this machinery is designed for use on relatively dry, flat surfaces, as opposed to hilly, forested, and marsh areas (Scheuhammer and Norris, 1996). Many shooting ranges, including the range at the Jefferson National Forest in Craig County, are hilly and forested. Therefore, it would be prudent to develop new machinery that can accommodate various landscape-types. Additionally, it would be logical to avoid building future shooting ranges near wetland and marsh areas, where larger populations of birds are likely to congregate.

In the modern world, many species of wildlife often face insurmountable pressure and threats to their survival. Rachel Carson (1962) was among the first to draw attention to the dangers of chemicals in her celebrated manuscript, Silent Spring. She cited numerous cases in which herbicides and pesticides caused the demise of countless species of plants and animals. It is forty years later and there are still many instances where birds and mammals are victims of chemical poisoning. Polycylic aromatic hydrocarbons and lead are two of the countless contaminants that result in new fatalities every day. With luck, we can find a manner in which to diminish or alleviate the environment of such toxic components so as to lessen the dangers the planet's wildlife encounter each day.

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APPENDICES

Appendix 1 - PAH values (ng/dry g) for clay target analysis (Texas A&M University) for studies completed at the Virginia Tech Center Woods Research Station in October 2001, June-July 2002, and January-March 2003.

Target compounds	Number 1	Number 2	Number 3	Mean	SD
Decalin	<0.2	<0.2	<0.2		
C1 – Decalin	<0.3	<0.3	<0.3		
C2 – Decalin	<0.4	<0.4	<0.4		
C3 – Decalin	<0.4	<0.4	<0.4		
C4 – Decalin	<0.4	<0.4	<0.4		
Naphthalene	175	175	187	179	6.9
C1 – Naphthalenes	698	693	670	687	14.9
C2 – Naphthalenes	2080	1970	1840	1963	120
C3 – Naphthalenes	2120	2020	1940	2027	90.2
C4 – Naphthalenes	1220	1240	1110	1190	70.0
Benzothiophene	48.1	46.8	53.3	49.4	3.4
C1 – Benzothiophene	284	266	312	287	23.2
C2 – Benzothiophene	294	258	323	292	32.6
C3 – Benzothiophene	237	305	251	264	35.9
Biphenyl	151	126	107	128	22.1
Acenaphthylene	108	78.4	75.2	87.2	18.1
Acenaphthene	167	155	162	161	6.0
Dibenzofuran	67.6	57.6	57.7	61.0	5.7
Fluorene	421	420	388	410	18.8
C1 – Fluorenes	2370	2320	2230	2307	70.9
C2 – Fluorenes	5660	6100	5850	5870	221
C3 – Fluorenes	9350	10100	8620	9357	740
Carbazole	376	417	355	383	31.5
Anthracene	2220	2210	1990	2140	130
Phenanthrene	9730	9620	8700	9350	566
C1 – Phenanthrene/Anthracene	41300	41300	37600	40067	2136
C2 – Phenanthrene/Anthracene	66100	68200	59500	64600	4540
C3 – Phenanthrene/Anthracene	72500	70500	66600	69867	3001
C4 – Phenanthrene/Anthracene	60700	62500	55800	59667	3467
Dibenzothiophene	2110	2140	1900	2050	131
C1 – Dibenzothiophene	11800	11500	10800	11367	513
C2 – Dibenzothiophene	27200	27400	25300	26633	1159
C3 – Dibenzothiophene	29000	27100	28700	28267	1021
Fluoranthene	5380	5270	4850	5167	280
Pyrene	64000	65600	59600	63067	3107
C1 – Fluoranthenes/Pyrenes	308500	301900	278700	296367	15652
C2 – Fluoranthenes/Pyrenes	935200	922900	832100	896733	56311
C3 – Fluoranthenes/Pyrenes	795200	841000	750300	795500	45351
Naphthobenzothiophene	190000	187300	171600	182967	9936
C1 – Naphthobenzothiophene	909500	886400	818200	871367	47470
C2 – Naphthobenzothiophene	1437000	1425800	1285600	1382800	84364
C3 – Naphthobenzothiophene	879600	879500	773800	844300	61055
Benz(a)anthracene	223400	254900	215100	231133	20997
Chrysene	495800	488200	418200	467400	42778
C1 – Chrysenes	2398200	2392000	2219400	2336533	101488
C2 – Chrysenes	2408700	2490000	2089900	2329533	211472
C3 – Chrysenes	1204600	1164100	1085400	1151367	60612

Target compounds	Number 1	Number 2	Number 3	Mean	SD
C4 – Chrysenes	91800	91900	78600	87433	7650
Benzo(b)fluoranthene	114200	122500	120100	118933	4271
Benzo(k)fluoranthene	32500	35600	36100	34733	1950
Benzo(e)pyrene	334900	335600	330900	333800	2536
Benzo(a)pyrene	337000	341800	326900	335233	7605
Perylene	86200	88000	88300	87767	1380
Indeno(1,2,3-c,d)pyrene	36500	42900	40700	40033	325
Dibenzo(a,h)anthracene	61800	65600	67000	64800	2691
C1 – Dibenzo(a,h)anthracene	318700	301400	295100	305067	12220
C2 – Dibenzo(a,h)anthracene	174900	209100	186400	190133	17403
C3 – Dibenzo(a,h)anthracene	95700	87900	99300	94300	5828
Benzo(g,h,i)perylene	161400	160500	155400	159100	3236
TOTAL PAHs	14449328	14537781	113148942	14045350	777571

Appendix 2. Body weight (g) and lead content (ppm) in feathers of bird species collected at the Blacksburg Ranger District, Jefferson National Forest shooting range and control site, Montgomery County, Virginia, 2002.

Species	Location	Body Weight(g)	Lead Content(ppm)
Blue grosbeak	shooting range	13.0	27.4
Blue grosbeak	shooting range	40.0	198.5
Blue-headed vireo	shooting range	19.0	3.7
Carolina chickadee	shooting range	7.5	9.0
Carolina wren	shooting range	14.5	110.7
Eastern towhee	shooting range	40.0	18.4
Eastern towhee	shooting range	39.0	13.2
Hooded warbler	shooting range	10.0	2.9
Hooded warbler	shooting range	9.0	4.2
Northern cardinal	shooting range	44.0	67.9
Northern cardinal	shooting range	60.0	40.7
Ovenbird	shooting range	19.0	7.4
Scarlet tanager	shooting range	27.0	10.0
Wood thrush	shooting range	49.5	0.5
Wood thrush	shooting range	49.0	2.7
Wood thrush	shooting range	50.0	0.2
Wood thrush	shooting range	60.0	1.7
Wood thrush	shooting range	51.6	2.5
Wood thrush	shooting range	42.0	4.0
Worm-eating warbler	shooting range	13.5	0.2
Blue grosbeak	control site	34.0	1.3
Carolina chickadee	control site	10.0	<0.1
Carolina wren	control site	19.0	<0.1
Carolina wren	control site	15.5	<0.1
Carolina wren	control site	17.0	3.2
Carolina wren	control site	20.0	0.7
Carolina wren	control site	17.8	6.0
Eastern towhee	control site	42.0	<0.1
Eastern towhee	control site	41.5	<0.1
Eastern towhee	control site	41.5	1.1
Eastern towhee	control site	40.5	<0.1
Eastern towhee	control site	39.0	<0.1
Gray catbird	control site	38.0	5.8
Gray catbird	control site	34.5	<0.1
Northern cardinal	control site	43.5	3.4
Northern cardinal	control site	41.5	0.8
Northern cardinal	control site	38.3	0.7
Northern cardinal	control site	39.0	1.2
Northern cardinal	control site	44.5	<0.1
Tufted titmouse	control site	24.0	<0.1

Appendix 3 – Body weight (g) and lead content (ppm) of liver and kidney samples from *Peromyscous leucopus* samples collected at the Blacksburg Ranger District, Jefferson National Forest shooting range and control site, Montgomery County, Virginia, 2002.

Sample#	Location	Body Weight (g)	Liver (ppm)	Kidney (ppm)
2E	shooting range	22.43	0.47	0.32
3E	shooting range	22.81	<MDL	0.17
4E	shooting range	26.15	<MDL	0.14
5E	shooting range	19.65	0.17	0.19
6E	shooting range	21.83	0.16	1.30
7E	shooting range	21.93	<MDL	0.47
8E	shooting range	17.72	0.16	0.70
9E	shooting range	22.80	<MDL	<MDL
10E	shooting range	14.50	<MDL	0.14
11E	shooting range	19.24	<MDL	<MDL
12E	shooting range	27.05	0.49	3.06
13E	shooting range	17.48	0.28	2.00
14E	shooting range	21.14	<MDL	<MDL
15E	shooting range	21.12	<MDL	0.56
1*	shooting range	23.41	0.28	1.03
2*	shooting range	25.04	<MDL	0.28
3*	shooting range	19.22	0.10	0.33
4*	shooting range	25.62	1.56	2.93
5*	shooting range	28.83	0.10	0.37
6*	shooting range	17.24	0.48	1.94
7*	shooting range	21.30	<MDL	0.39
8*	shooting range	20.11	0.19	1.25
9*	shooting range	21.96	0.26	1.04
10*	shooting range	18.01	0.54	1.69
19*	shooting range	19.69	<MDL	0.13
20*	shooting range	18.22	<MDL	<MDL
1C	control site	21.69	0.05	<MDL
2C	control site	14.33	<MDL	<MDL
3C	control site	20.29	<MDL	<MDL
4C	control site	18.29	<MDL	<MDL
5C	control site	23.63	<MDL	<MDL
6C	control site	14.12	<MDL	<MDL
7C	control site	15.39	0.12	<MDL
8C	control site	16.17	<MDL	<MDL
9C	control site	9.88	<MDL	<MDL
10C	control site	26.86	<MDL	<MDL
11C	control site	17.21	0.09	<MDL
12C	control site	16.09	<MDL	<MDL
13C	control site	29.81	<MDL	0.16
14C	control site	22.56	<MDL	<MDL
15C	control site	26.97	<MDL	<MDL
16C	control site	27.79	<MDL	<MDL
17C	control site	18.70	<MDL	<MDL
18C	control site	16.15	<MDL	<MDL

Sample#	Location	Weight (g)	Liver (ppm)	Kidney (ppm)
1*	control site	17.00	<MDL	<MDL
2*	control site	19.84	<MDL	<MDL
3*	control site	17.21	<MDL	<MDL
4*	control site	18.02	<MDL	<MDL
5*	control site	22.06	<MDL	<MDL
6*	control site	20.29	<MDL	<MDL
7*	control site	24.56	<MDL	<MDL
8*	control site	18.79	<MDL	<MDL
9*	control site	18.48	<MDL	<MDL
10*	control site	15.57	<MDL	<MDL
11*	control site	22.60	<MDL	<MDL

VITA

Gabriela Rae Gonzalez was born to Gabriel and Carol Gonzalez on April 18, 1977 in Raleigh, North Carolina. She graduated from William G. Enloe High School in 1995. She continued her education at North Carolina State University, where she graduated magna cum laude with a Bachelor of Science degree in zoology and a double minor in forestry and French.

Shortly following graduation, she moved to Boston where she worked as an environmental instructor for the Massachusetts Audubon Society. Following this position, she accepted a biological position with the U.S. Fish and Wildlife Service in Manteo, North Carolina. When this term was complete, she worked as an avian biologist for the Smithsonian Environmental Research Center on the Chesapeake Bay in Edgewater, MD. Gabriela then proceeded to attend Virginia Polytechnic Institute and State University, where she started her M.S. program in Wildlife Science. During the past 2 years, she has sought to identify the toxicological effects of clay targets and lead pellets on avian and mammalian species.

After graduation in August, 2003, Gabriela plans to start work at Kiptopeke State Park, VA, banding and tracking migratory raptors, which will hopefully lead to a permanent position in raptor biology and conservation.