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Lead Exposure in Avian Species

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LEAD EXPOSURE IN AVIAN SPECIES

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by

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Abstract

Lead causes detrimental health effects such as weakness, weight loss and eventual death in avian species. Bone mineral density and the effects of dietary anti-oxidants were analyzed in relation to blood lead levels. Using post-mortem samples as a diagnostic tool for determining lead exposure was investigated. The results suggested that there is a correlation between bone density and bone lead content and that pre-mortem blood correlate with post-mortem fluid. Other results were inconclusive due to the small sample sizes.

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Chapter 1 Background

Lead as a Resource

Lead is a soft, malleable metal with a melting point of 327.5°C which dries quickly and has a high resistant to corrosion (Bentor, 2008). These desirable properties made lead a prime resource in construction, ceramic glazes and water pipes for over 7,000 years. Before the early 1900's, lead was found in ammunition, brass, burial vault liners, leaded glass and crystal, paints, pewter, toys, utensils, and water and sewage pipes. Since the advancement in technology, lead materials have been incorporated into everyday life as bearing metals, cable coverings, caulking, gasoline additives, radiation shielding in medical equipment, starting-lighting-ignition (SLI) batteries in vehicles, solders in food cans, and terne metal for gas tanks. During the 1980's, the Environmental Protection Agency discovered the harmful properties of lead as a neurotoxin and took action by removing lead from gasoline, soldering in food cans, paint and other common items (Callaghan, 2008). Despite the significant decrease of lead consumption within the past twenty years, lead is still used in over 900 different methods including mining, battery manufacturing, soldering, electrical wiring, ammunition molding, and radiation protection (Habal, 2006).

Lead is commonly obtained from galena, a lead sulfide ore found in igneous and sedimentary rocks (Galena Mineral Data, n.d.). Lead mines exist in thirty-eight countries around the world, with major contributors including China (35%), Australia (20%), the United States (12%), Peru (9%), and Mexico (3%). In 2006, 1,100 miners working at the ten mines in the United States produced 715 million dollars of lead. Industrial and SLI batteries accounted for 90% of the lead consumed in the U.S. (Carlin, 2006).

Lead Contamination

In the Environment

Lead exists in several forms in the soil, water, and air. Factors including weather, wind directions, soil surface features, and lead particle size influence the environmental uptake of lead. For example, rough or hairy leaf surfaces contain eight times more lead than smooth leaf surfaces. In dry conditions, atmospheric lead remains localized near the source while in wet conditions, precipitation transports lead globally and through many phases of the environment. Inorganic lead contaminates animals through inhalation, ingestion, and skin absorption. In contrast, organic lead is less prevalent in the environment and is generally absorbed through the skin. (Pattee, 2003).

Historically, lead contamination was common near bodies of water as a result of the rapid accumulation and disintegration of lead shot fired at waterfowl. Many states responded by making the use of lead shot on waterfowl illegal. In 1997, six years after banning the use of lead shot on waterfowl, officials estimated 1.4 million ducks were saved from lead poisoning (Pattee, 2003). Another use of lead, which is no longer allowed, is the addition of lead to gasoline to assist in combustion. Reactions occurring in the automobile engine released 70 to 75% of gasoline lead into the air though exhaust fumes and boat engine released 9 to 31% of gasoline lead into the water (Pattee, 2003). Ten years after the U.S. government began regulating lead in gasoline, lead content in the Mississippi decreased by 40% which shows the significant effect of government regulation and the considerable amount of time required to clear lead from the environment (Pattee, 2003).

In Birds

Ingestion of lead material is the most prevalent lead poisoning pathway in birds. In waterfowl, lead shot is commonly consumed due to its similar appearance to grit (e.g sand or small pebbles), which is required for grinding food in the gizzard. Once in the gizzard, the lead object disintegrates as the abdomen works to remove shells and to crush food. Although lead shot was banned in the U.S. in 1991, lead poisoning in waterfowl still exists. The winter of 2001 yielded 150 trumpeter swan deaths in Washington State (Swans, 2001). In 1999, the U.S. Geological Survey studied 30 different avian species from ten states which had swallowed lead sinkers used in fishing. This study indicated loons were the most affected by this method of lead poisoning, followed by brown pelicans (Nadis, 2008).

Birds of prey are also at risk for lead poisoning. These birds ingest lead bullets in the meat they consume, which is often the culprit in such situations (USGS, 2007). The most significant example of lead poisoning in a non-waterfowl species is in the California condor. The nearly extinct condor population has flourished from 22 birds in 1982 to nearly 300 today. However, environmentalists claim the population cannot be maintained in the wild due to lead poisoning, the most common cause of death for condors (Ritter, 2006).

Birds appear to be more affected by lead than other animals due to their small body masses, which cannot process the toxins, and their ability to consume entire objects, such as lead sinkers. All bird species can be impacted by lead, but species most affected include loons, swans, pelicans, geese, ducks, cranes, herons and eagles (Nadis, 2008). Although there are other potential sources of lead in the environment, ingesting a solid lead sinker or being shot by lead ammunition is the most common source of lead poisoning in birds.

In Mammals

Other animals, in addition to birds, are affected by lead. Lead poisoning has been documented in mammals such as horses, cattle, buffalo, humans, and zoo animals including primates, bats, foxes, ferrets, panthers, and bears (Pattee, 2003). In theses animals, the most common mode of lead contamination was ingestion of industrial lead, waste containing lead, or lead paint. Mammals can transmit lead to their offspring through the placenta or milk, which is not a concern in birds. Atmospheric lead can also be of concern in mammals. Approximately 50% of lead inhaled remains in lungs after exhalation and is subsequently absorbed into the bloodstream (Pattee, 2003). Lead accumulates in humans for at least 20 days in blood and 600 to 3000 days in bone. In humans, increased lead content has been correlated with increased violent behavior and lower IQs, likely a result lead neurotoxicity (Nevin, 2000).

Antioxidant Effect on Lead

Unstable atoms which lack one electron are known as free radicals. Limited amounts of free radicals are acceptable in biological systems but excessive amounts may occur after injury, disease, pollutant damage, UV radiation, or smoke contamination (Kane, 2008). A surplus of free radicals can cause oxidative stress and inhibit the ability to repair damaged cells. In addition, free radicals cause changes in cell structure, proteins, and DNA composition (Kane, 2008). Antioxidants include vitamins and amino acids, such as vitamin C and taurine, and assist in stabilizing free radicals, thereby minimizing cell damage (Kane, 2008).

Studies have shown that lead's toxicity is partially due to the toxin's ability to induce oxidative stress. Numerous studies show transition metals create reactive oxygen species (ROS) which contribute to DNA damage, enhance peroxidation, and decrease antioxidant defense systems (Gurer, 2000). Shafiq-ur-Rehman researched lead exposure and lipid peroxidation in fish. He concludes that fish chronically exposed to lead toxicity show an increase in lipid peroxidation and abnormal motor activity and suggests anti oxidants alpha-tocopherol may reduce lead neurotoxicity, while iron may aggravate toxicity. Lead also inhibits heme and hemoglobin synthesis and impacts red blood cell conformation (Gurer, 2000). Due to the effects of lead causing oxidative stress, scientists

suggest some antioxidants as a treatment with long-lasting effects. Antioxidants such as vitamins B, C, and E, as well as zinc, ethoxyquin, S-adenosyl-L-methionine (SAM), N-acetylcysteine (NAC), α -lipoic acid (LA), and captopril have been explored as treatments for lead toxicity (Gurer, 2000). Vitamin E was found to inhibit lead absorption in red blood cells. SAM has been shown to be useful in chelation therapy preventing lead accumulation in blood, liver, and brain. Another antioxidant therapy for lead poisoning is NAC which can be used without chelation (Gurer, 2000).

Effects of Lead

Lead is highly toxic and can produce a wide range of health effects in both adults and children. Lead poisoning can be difficult to diagnose, because there are no unique signs or symptoms. Some of the early symptoms of lead exposure in humans include reduced attention span, irritability, stomach discomfort and or constipation, persistent fatigue, and insomnia. If lead poisoning is not treated in the early stages, long-term or permanent health damage can occur (NSC, 2005). This is typically the case, because lead poisoning is difficult to diagnose in the early stages. In adults, lead poisoning can cause fertility problems, increased blood pressure, hearing and vision impairments, nerve disorders, or poor muscle coordination. Since young children are still developing, even a small amount of exposure to lead can result in anemia, hearing loss, brain damage and/or mental retardation, behavioral problems, liver and kidney damage, developmental delays, hyperactivity, or even death (EPA, 2008).

Lead toxicity is the result of lead's ability to mimic calcium in biological processes. Calcium-dependent channels and anion exchanges allow lead to penetrate cell membranes Lead's affinity for binding to calcium-dependent sites can be 1000 times greater than that of calcium. Due to this strong affinity, lead inhibits calcium binding in the cell, thereby disrupting the protein's ability to function. One example of the importance of calcium is seen in calmodulin, a protein which regulates neurotransmitters by detecting calcium concentrations. When lead binds to calmodulin, the protein increases the release of neurotransmitters, resulting in excessive chemical signals which are transformed into electrical impulses that control nervous system functions (Tufts, 2006).

Lead also affects many different species of animals including birds. Lead poisoning has affected every major species of waterfowl in North America as well as been reported in a variety of other species of birds. Clinical signs of lead poisoning in birds include a loss of appetite, gasping, tremors, weakness, drooping wings, weight loss, reduced breast muscle, green watery diarrhea, an inability to fly or flee from predators, or even death (USGS, 2007). Lead poisoning is not the primary cause of death, but rather the effects of lead poisoning such as an increased susceptibility to disease, reproductive problems, and an increase in predation due to their weakened state result in increased mortality. At necropsy, ducks, geese, and swans with lead poisoning typically have enlarged gallbladders, absent or reduced amounts of visceral fat, gizzard linings that are cracked, green-stained, and peeling, or an impacted esophagus or proventriculus containing mud, food, or sand(Department of Natural Resources, 2008) . These effects are evident whether lead shot is present or not. Loons with lead poisoning usually have dark green stained ventriculus lining, which is common if a lead object is present in the body. Birds that die from lead poisoning also exhibit slight anemia, and acid-fast inclusion bodies are evident in kidney epithelial cells under microscopic examination. Canada geese may have cephalic edema, while in waterfowl a low dose of lead can result in anemia and a lethal dose can result in death due to a heart attack or muscle paralysis (USGS, 2007).

Dual-Energy X-ray Absorptiometry (DEXA)

Dual-energy x-ray absorptiometry (DEXA), also known as bone densitometry, is a machine used to measure bone mineral density (BMD). The DEXA machine allows for regional and global measurements of the entire body and is used as a screening test for diagnosing osteoporosis, a disease in which a gradual loss of calcium occurs, causing bones to become brittle and often break easily if left untreated (Dartmouth-Hitchcock, 2008). A whole body scan takes about three minutes, while a scan of the AP lumbar spine, single hip or forearm takes about thirty seconds. In order to ensure accuracy among all patients, a spine phantom and a T-bar step phantom are measured regularly in order for calibration and quality control of the machine (ACR & RSNA, 2008).

There are two types of DEXA equipment, which include a central device and a peripheral device. The central device measures bone density in the hip and spine and the peripheral device measures bone density in the wrist, heel, and fingers. The central device is most commonly used in hospitals and doctor offices and the peripheral device is a mobile device available for sale in drugstores due to its small size. However, the peripheral DEXA test is not as sensitive as the central DEXA test and cannot be used to monitor response to treatment, because bone mass varies from location and measuring the BMD of the heel or finger is not as accurate as the spine or hip. Even though the central DEXA devices are much more sensitive than the peripheral DEXA devices, they are also much more expensive (ACR & RSNA, 2008).

The DEXA machine works by sending a very thin, invisible beam of low-dose-x-rays (less than one-tenth the dose of a normal chest x-ray) containing two distinct energy peaks through the bones. The soft tissue absorbs one of the peaks and the bone absorbs that other peak. The actual bone mineral density is determined by subtracting the soft tissue amount from the total. Special software is connected to the DEXA machine, which computes the final values in order for a physician to make a proper diagnosis (acr & rsna, 2008).

Lead Testing

There are many different analytical methods available to detect, measure, and monitor lead. Techniques are continuously modified to improve accuracy and precision and obtain lower detection limits. Some of the analytical methods available analyze the amount of lead present in biological samples, which include blood, urine, serum, and cerebrospinal fluid; however, blood is most commonly used. Some of the most common procedures include, anode stripping voltammetry (ASV), atomic absorption spectrometry (AAS), graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma-atomic emission spectroscopy (ICP/AES), and inductively coupled plasma mass spectrometry (ICP/MS) (FEDRIP, 2005).

ASV is an analytical technique that is used to detect heavy metals, which are usually toxic to humans, animals, and sometimes plants and include lead, mercury,

arsenic, and cadmium. This method works by electroplating a sample onto an electrode, which concentrates any metal present in the sample. The metal on the electrode are then sequentially stripped off, causing a current. This current can be measured, and is proportional to the amount of metal being stripped off. (MTI, 2008)

AAS measures the concentrations of specific elements. This technique is extremely sensitive and can measure parts per billion ($\mu g\ dm^{-3}$) in a sample. This method uses the concept that metals absorb light at varying wavelengths when heated. In order to detect lead, a UV light is focused through a flame, which contains an aspirated sample of interest. If lead is present, it will absorb a specific amount of UV light, which will reduce its intensity. The AAS device measures this change in intensity and converts it into absorbance. The wavelength absorbed is directly proportional to the amount of lead present in a sample. Unknown lead concentrations can be calculated by comparing to a standard curve made from known lead concentrations (RSC, 2008).

GFAAS is a technique that uses the concept that free atoms will absorb light at frequencies or wavelengths characteristic of the element of interest. The amount of light absorbed can be directly correlated to the concentration of the mineral present. In this procedure, samples are deposited in a small graphite tube, which is then heated to vaporize and atomize the mineral. GFAAS has a detection limit of 0.05ng/mL for lead in blood samples (UMEA University, 1996).

ICP/AES is an emission spectrophotometric technique, which uses the idea that excited electrons emit energy at a given wavelength as they return to ground state. In this technique, a single wavelength is selected for a given element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount of that element in the sample being analyzed. By determining which wavelengths are emitted by a particular sample and at what intensity, the composition of an unknown sample can be determined using a standard curve (University of Missouri, 2008).

ICP/MS is a technique in which a plasma or gas consisting of ions, electrons and neutral particles is formed from argon gas. The elements in a particular sample are atomized and ionized by the plasma. The resulting ions pass through a series of cones into a high vacuum mass analyzer. The mass-to-charge ratio (m/e) is used to identify the isotopes of the elements and the amount of the isotope (element) is directly proportional

to the intensity of a specific peak in a mass spectrum. (University of Missouri, 2008) ICP/MS is quite expensive compared to GFAAS; however, low detection limits, reliability, ability to analyze multiple metals from a single sample, and ease of use have made this a popular technique for tracing metal analysis. ICP/MS, ASV, AAS, and GFAAS all analyze samples with great accuracy and precision and sensitivity in the low to sub-ppb range (0.1-15ppb) (FEDRIP, 2005).

There are also other specialized methods for lead detection analysis, which include differential pulse anode stripping voltammetry, neutron activation analysis (NAA), x-ray fluorescence spectroscopy (XRFS), and isotope dilution mass spectrometry (IDMS). IDMS is considered one of the most reliable methods for lead detection at low concentrations, but due to the high cost of the equipment needed and the technical knowledge needed to run the equipment, this method is not very commonly used. Since it is a very reliable, this method is used as the standard in order to compare the accuracy of other methods. A comparison was performed between ASV, GFAAS, and IDMS, and it showed that all three methods are reliable and can be used in order to measure the level of lead in the blood accurately (FEDRIP, 2005).

There are also many different biomarkers available in order to monitor lead exposure. There are several biochemical assays that can be used to detect the presence and the extent of lead exposure. These assays include ALA (δ-aminolevulinic acid), coproporphyrin, ALAD (ALA dehydratase) activity, 1,25-dihydroxyvitamin D, and EP (erythrocyte protophyrin) concentrations (FEDRIP, 2005). All of these assays have become well known and are reliable and sensitive, but ALAD and erythrocyte protoporphyrin have been shown to be very useful and sensitive assays for determining lead exposure. It has been shown that EP concentration is proportional to blood lead over the range of 30-80 μg/dL. ALAD activity was also proportional to blood lead concentration ranging from 10 to 40 μg/dL (FEDRIP, 2005). High-performance liquid chromatography (HPLC) followed by quantification of a fluorescent end product can be used as well as a colorimetric method for detection of ALA in urine. However, urinary ALA is not proportional to blood lead until the blood concentrations reached 60-70 μg/dL, a concentration at which clinical symptoms would already be present (FEDRIP, 2005).

Lead can be measured and quantified in a variety of tissues, which include the testes, kidney, lungs, muscles, liver, heart, and brain. While the results are accurate and similar to those obtained using blood and urine, they are not commonly used unless lead detection is determined post mortem. However, since lead accumulates over a lifetime in bones and measurements of lead in blood only represent recent exposure, x-ray fluorescence has been used to measure lead concentration in bones. The lead level in bone has been shown to be a good representation of stored lead in body tissue (FEDRIP, 2005). The advantage of this technique is that no sample preparation is needed and it can be safely done in live subjects. The only downside to this technique is that its precision is dependent upon the mass of the bone analyzed, which means that measurements from thin bones have greater error compared to those from larger bones. Teeth have also been analyzed for lead using ASV and AAS, which both have great precision and accuracy. Hair has also been analyzed for lead using ICP/AES (FEDRIP, 2005)

Environmental Science Associates (ESA Inc) designed and produced the LeadCare® machine. The LeadCare® machine uses 50μL of blood and can provide a blood lead concentration within about three minutes. The machine uses anodic stripping voltammetry with a hydrochloric acid reagent in order to provide a lead concentration of the sample being analyzed. This machine is reliable and can provide accurate lead concentration results of any blood samples up to 65 μg/dL with a minimum level of 1.6 μg/dL. Any lead concentration above the maximum level of 65 μg/dL will be displayed as "high" by the LeadCare® machine (LeadCare® User Manual).

The LeadCare® machine has become a very common way of testing for lead. Due to its reasonably low cost and portability, veterinary clinics and pediatric offices are using this machine in order to test for lead. This machine is ideal when the amount of lead exposure needs to be determined in order for a proper diagnosis to be made and treatment to begin. This is why this method is preferred and used in these clinics rather than ALA, AAS, PP, or GFAAS.

Previous LeadCare® Testing

A previous Major Qualifying Project completed by Amanda McCullough and Amanda Tarbet investigated LeadCare® accuracy using pre-mortem blood and post-mortem body fluid. The primary objectives of their research were to, "determined the ability of the LeadCare® device to detect lead in post-mortem body fluids and to analyze archival data for trends in patients from the Tufts Wildlife Clinic tested positive for lead" (McCullough & Tarbet, 2007). Due to time constraints, this research was only able to evaluate a small sample size and further experiments needed to be done in order to make the results significant. Therefore, the pre and post mortem analysis of this project allowed for the opportunity for subsequent research, which was completed in the following study and incorporates results from McCullough and Tarbet.

Chapter 2 Project Description

Lead poisoning has affected every major species of waterfowl in North America as well as a variety of other species of birds. Therefore, the primary objectives of this Major Qualifying Project were to compare the pre-mortem blood and post-mortem body fluid lead content using LeadCare® testing and to measure bone mineral density and investigate its relationship to lead toxicology.

In order to fulfill the overall project goals, several specific objectives were accomplished, including:

- Compared the amount of lead present in pre-mortem avian blood samples and post-mortem avian body fluid samples collected during necropsy using LeadCare® analysis.
- Examined X-rays to see if lead shot was present in the birds and compared X-ray observations to blood lead levels.
- Tested bone mineral density using DEXA and compared the results to a lead toxicology report of the corresponding bones.
- Categorized birds into three separate dietary groups possibly containing different levels of antioxidants. Analyzed the relationship of diet and average blood lead levels from birds in the Wildlife Clinic.

Chapter 3 Methods

Lead Analysis in Pre-Mortem Blood

Pre-mortem blood samples were collected by Wildlife Clinic staff according to our protocol posted in the clinic's radiology room, freezer room, and laboratory (Appendix A). Blood was collected from any bird deemed clinically appropriate by Wildlife Clinic staff members, particularly if the bird's condition required it to be euthanized. All species were considered for collection and the amount of blood collected varied by bird size, with the optimal and preferred amount collected being 0.05 mL. Collected blood was deposited into a green top container with heparin in order to prevent coagulation. Green top tubes were labeled with case number, species, and date and placed in a Styrofoam holder in the refrigerator. Blood was stored for 1-19 days, although the majority (10 out of 14) was tested within 4 days of collection.

Blood was tested for lead using LeadCare® technology and following the Environmental Sciences Associates (ESA) Biosciences LeadCare® instructions (Appendix B). The LeadCare® device was calibrated using a CC4 calibration button. 50 µl of the stored blood sample was pipetted into a LeadCare® reagent container after wiping the pipette tip with a Kimwipe®. The solution in the container was mixed for approximately 1 minute. Using a new pipette tip, 50 µl of the sample was placed on a LeadCare® test strip which was then placed in the LeadCare® device for analysis. Results from the LeadCare® analysis were recorded in Tufts Wildlife Clinic LeadCare® records and in records kept for this research.

Lead Analysis in Post-Mortem Body Fluid

Post-mortem cadavers were labeled by the Wildlife clinic staff with the appropriate case number, date, and species which correlated with the pre-mortem blood samples taken. The cadavers were then placed in the freezer and stored at -20°F. Cadavers were placed in the refrigerator 2-3 days prior to necropsy. The birds were then dissected following the standard avian protocol. To prepare the birds for necropsy, the feathers were dampened and removed from the area of incision. The birds were then placed on the dissecting table in a supine position and a midline incision was made from below the jaw to the cloaca. The skin was peeled back from the tissues, exposing the pectoral muscles. The pectoral muscles were then incised from cranial to caudal just lateral to the keel. The pectoral muscles were then reflected back from the sternum, exposing the airsacs. The coracoid and the clavicle were then disarticulated or cut with bone scissors from the sternum. The abdominal musculature was then cut where it meets the sternum and ribs, while avoiding organs and structures in the abdominal cavity. Bone scissors were then used to cut through the ribs on both sides of the body, again avoiding the internal organs and structures. The caudal end of the sternum was then lifted and reflected back cranially, while cutting the fine connective tissue between the sternum and internal structures, exposing all the internal organs and structures including the heart. A cardiac stick was attempted first in order to obtain post-mortem body fluid. The primary source of body fluid came from the heart, however if no sample was obtained or if more sample was needed, fluid was gathered from the accumulated fluid behind the organs in the dorsal part of the body cavity. The post-mortem body fluid was tested for lead using the same protocol used for the pre-mortem blood samples. The lead levels in the pre-mortem blood and post-mortem body fluid were then compared for analysis.

Radiological Analysis

Before an x-ray was performed, the records were checked to see if a radiograph had been previously taken. If none were found in the records, an x-ray was taken. The cadavers were x-rayed after being thawed in the refrigerator for 2-3 days. The x-ray machine at the Tufts Wildlife clinic was used and their protocol for each species was followed. The birds were placed in a supine position with their wings pulled out on a film, which was on top of the x-ray table. The appropriate settings were adjusted including the kVp and the mA, which varied depending on the species. The x-ray field and the light field were properly aligned and the room was cleared before the x-ray was taken. The film was then developed in the small animal hospital and the x-ray was then observed for traces of lead, indicated by metallic areas, which are characterized by dense, bright white areas. Lead shot was characterized by metallic circular objects. After the x-rays were observed, the observational results were compared to the lead levels found in the blood.

Bone Analysis

Avian humerus bones were collected from nine birds during necropsy and stored in Whirl-zip plastic bags at -20°F. Dual-energy x-ray absorptiometry (DEXA) was performed on the bones at Tufts Large Animal bone laboratory. Each bone was placed in a plastic box and surrounded by GOYA white rice to simulate tissue surrounding the bone. Bones that were smaller in size (including a rock dove and two Cooper's hawks) were analyzed on the Hologic DEXA machine under high resolution. Larger bones (including a mallard, three herring gulls, a red tailed hawk, and a common loon) were examined as right forearms. Results from the analysis quantified bone mineral content (BMC), bone mineral density (BMD), and bone area. Appendices C and D show the raw DEXA data results and an example of a DEXA radiograph of a common loon humerus bone respectively. The nine avian bones were then packaged and sent to California Animal Health and Food Safety Laboratory System in Davis, California for toxicological analysis of lead. The BMD and results from the lead toxicology report were compared for bone analysis. The toxicology report is located in Appendix E.

BMD was also compared to bird age, weight, and health condition. This information was obtained from the Wildlife Clinic records for each bird. This raw data is located in Appendix F. Health condition was evaluated by severity of emaciation in comparison to BMD. A correlation between bone length and BMD was also explored. Bone length was determined by using the measurement feature on the KODAK radiograph software. This tool measured the length of the bone in centimeters as it appeared on the x-ray of the bird. An example of how the bone was measured is located in Appendix G.

Data Analysis for Antioxidant Correlations

The Tufts LeadCare® test results from 2002 to 2008 were collected, sorted and analyzed for a correlation between bird diets high in antioxidants to lead levels in the blood. Only avian species were used for this analysis, which was performed using Excel. The average blood lead levels for each species were calculated, plotted, and compared. The blood lead levels were also split up into eight ranges and the number of birds within each range for each species was determined and plotted. Lastly, the birds were divided into three different categories which included, birds that are typically meat eaters, birds that are typically plant eaters, and birds that are typically scavengers. The average blood lead levels of these three groups were also calculated, plotted, and compared. The distribution of birds within each pre-determined ranges of blood lead levels for these three categories was also determined, plotted, and compared.

Chapter 4 Results

Lead Analysis Results in Pre-Mortem Blood vs. Post Mortem Body Fluid

One of the primary goals of this project was to analyze and compare the pre-mortem blood lead content and the post-mortem body fluid content using the LeadCare® test. Twenty-three pre-mortem avian blood samples from varying species at Tufts Wildlife Clinic were analyzed and recorded. However, only twelve corresponding post-mortem body fluid samples were collected because some birds did not require euthanasia and were released into the wild. Samples 12-18 in Table 1 were collected by McCullough and Tarbet. Their data was incorporated with this analysis to maximize sample size. Detailed records of the data are located in Appendix H and Appendix I.

Sample #	Pre-Mortem Blood (µg/dL)	Post-Mortem Body Fluid (µg/dL)
1	6.3	6.3
2	2.7	4.7
3	4	5.1
4	1.6	1.6
5	1	3.3
6	1.1	2.8
7	9.4	6.8
8	6.2	7.6
9	3.9	1.3
10	19.5	2.6
11	65	65
12	3	5.7
13	9.2	4.7
14	30	9.4
15	9.4	10
16	5.8	4.7
17	1.5	2
18	65	65

Table 1: Results of pre-mortem and post-mortem lead content using LeadCare® analysis

Figure 1 displays a graphical comparison of the pre-mortem and post-mortem lead results from the LeadCare® analysis for each bird. The pre-mortem samples are indicated by the blue diamonds and the post-mortem samples are indicated by the pink squares. At each sample number, pre-mortem and post-mortem values correspond to one bird. Overall, the differences between the pre-mortem and post-mortem samples were relatively small. The statistical analysis of this data is located in Table 2.

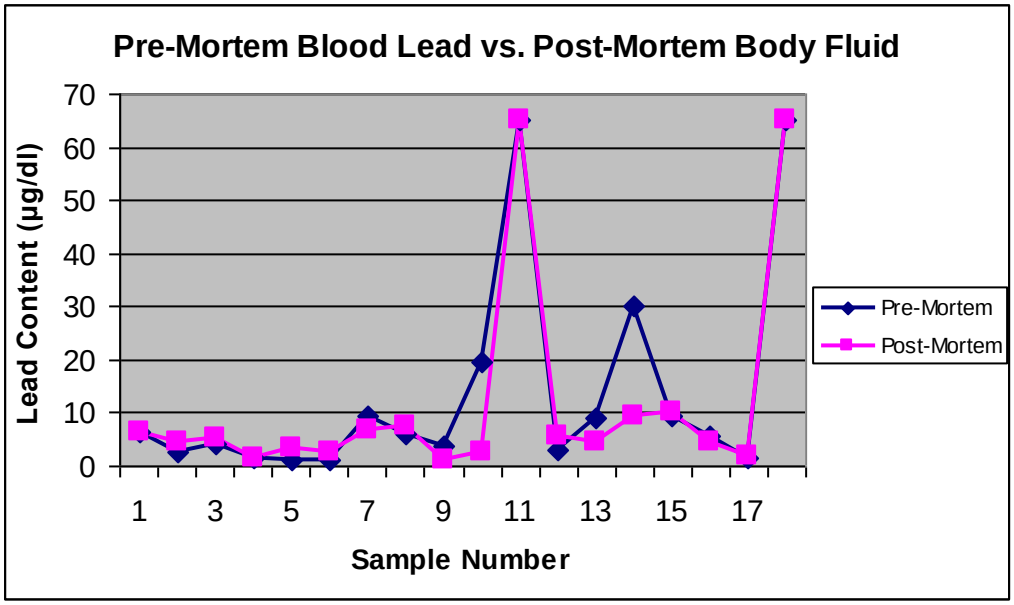

Figure 1: Pre-mortem and post-mortem lead content results using LeadCare® analysis

Table 2 lists the statistical analysis of the LeadCare® results. The p value was calculated by ANOVA analysis to be 3.6×10^{-6} (p value< 0.005), indicating that there is a significant correlation between pre and post-mortem values.

Sample Number	Difference	Variance from ANOVA	Standard Deviation
1	0	0	0
2	2	2	1.4142
3	1.1	0.605	0.77782
4	0	0	0
5	2.3	2.645	1.6263
6	1.7	1.445	1.2021
7	-2.6	3.38	1.8385
8	1.4	0.98	0.98995
9	-2.6	3.38	1.8385
10	-16.9	142.805	11.95
11	0	0	0
12	0	0	0
13	2.7	3.645	1.9092
14	-4.5	10.125	3.18198
15	-20.6	212.18	14.5664
16	0.6	0.18	0.42426
17	-1.1	0.605	0.77782
18	0.5	0.125	0.35356
Average:	-2	21.33888889	2.380588333

Table 2: Statistical analysis of the pre-mortem and post-mortem samples

Radiological Analysis Results

Figure 2 displays a radiograph of a Mallard duck with two metal objects, which are suspected to be lead shot. The suspected lead shots are indicated by red arrows. The mallard's blood lead level was 9.4µg/dL.

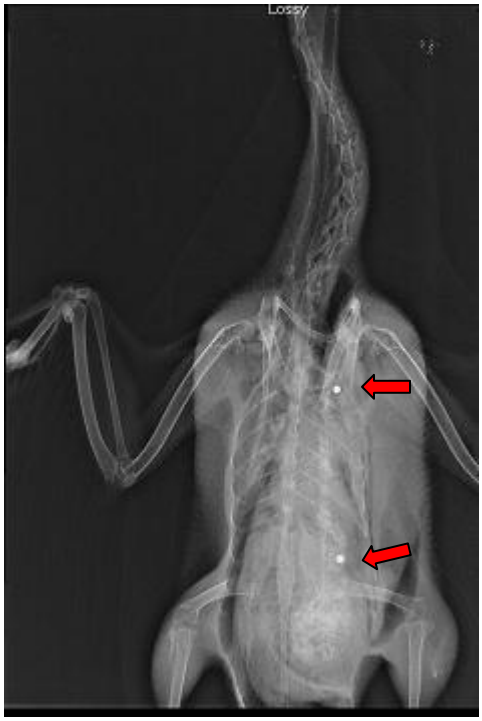

Figure 2: X-ray of Mallard Duck

Bone Analysis Results

The final objective of this research was to analyze the calcium inhibiting properties of lead on bone tissue. In order to achieve this goal, bone mineral density and bone toxicology were compared. Bone mineral density was evaluated by using DEXA technology while a toxicological analysis for lead was performed at the California Animal Health and Food Safety Laboratory System.

Figure 3 shows the correlation between bone lead levels and mineral density in avian humerus bones. The graph shows a slight correlation between these two measures.

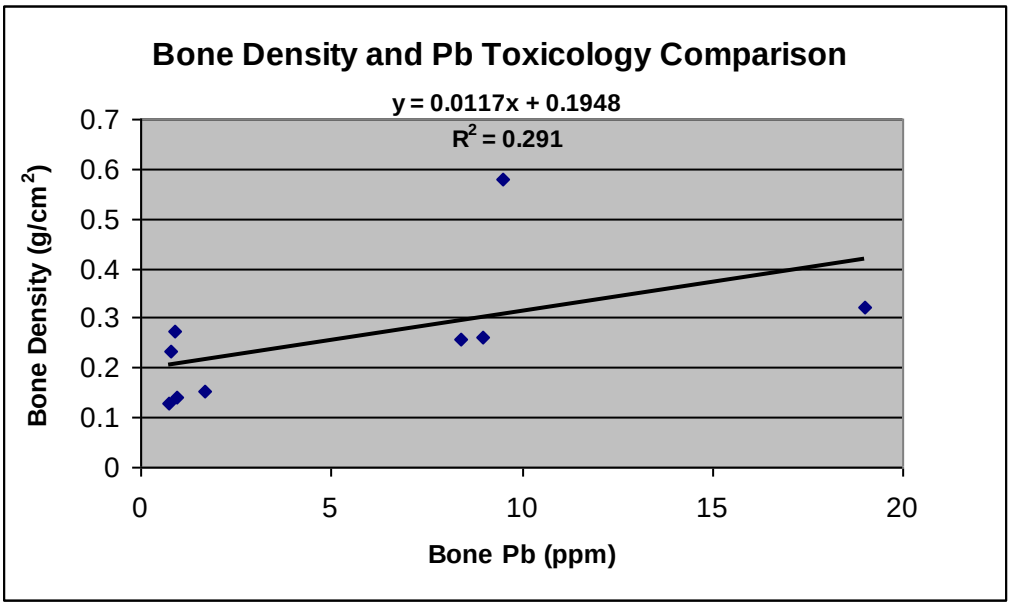

Figure 3: Bone Mineral Density compared to Bone Lead Toxicology in Avian Species

However, when the outlier at density 0.58 g/cm^2 and toxicology 9.5 ppm was removed, the graph showed a stronger correlation. Figure 4 shows the correlation increased from R^2= 0.291 to R^2=0.5159 (R^2=1 being optimal correlation).

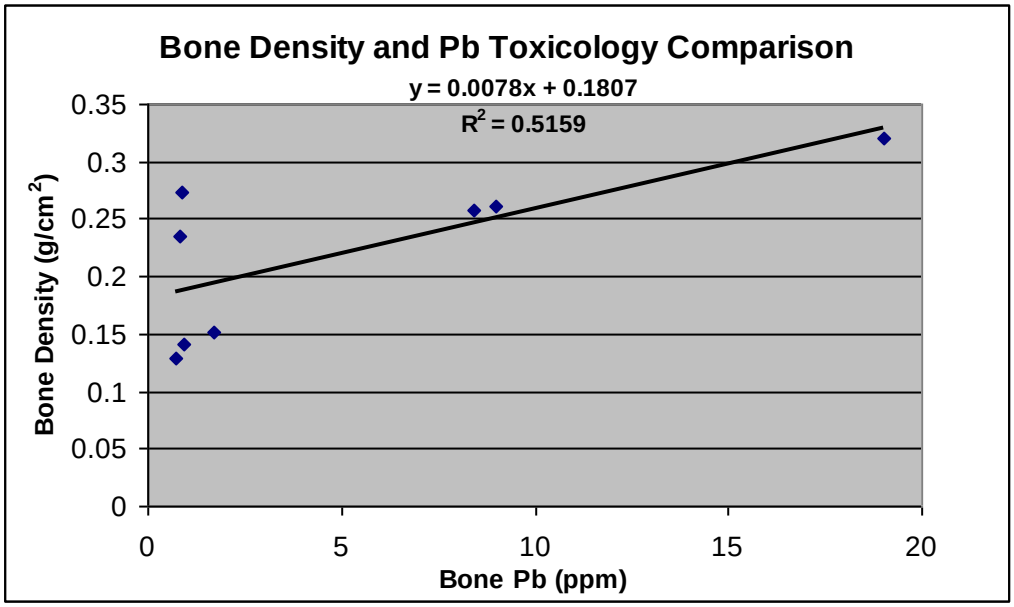

Figure 4: Bone Mineral Density and Lead Toxicology Compared with Outlier Removed

Lead tends to accumulate in bones over time. Therefore, bone mineral density was compared to the age of the bird in Figure 5. Adult birds appeared to have the lowest bone mineral density, although the highest bone mineral densities were from birds of unrecorded ages. Juvenile birds also appeared to have higher bone mineral densities (0.235, 0.261) than adults (0.273, 0.1517, and 0.1402) although the sample size is too small for statistical analysis. The graph represents a total of nine birds, which include three adults, two juvenile, and four unknowns.

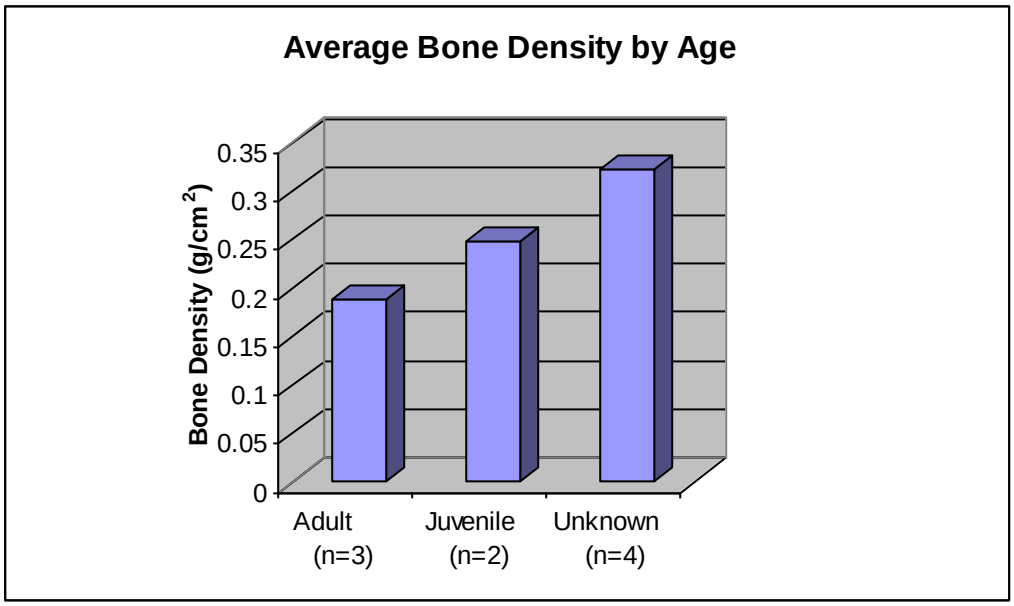

Figure 5: Average Bone Mineral Density Per Avian Age

Bone mineral density and bird body mass were compared as shown in Figure 6. A strong correlation was found between the data (R^2=0.7308), with larger birds having greater bone densities. Some records did not have weight information and were omitted. Therefore, this sample represents a smaller sample than other analyses (six birds in total).

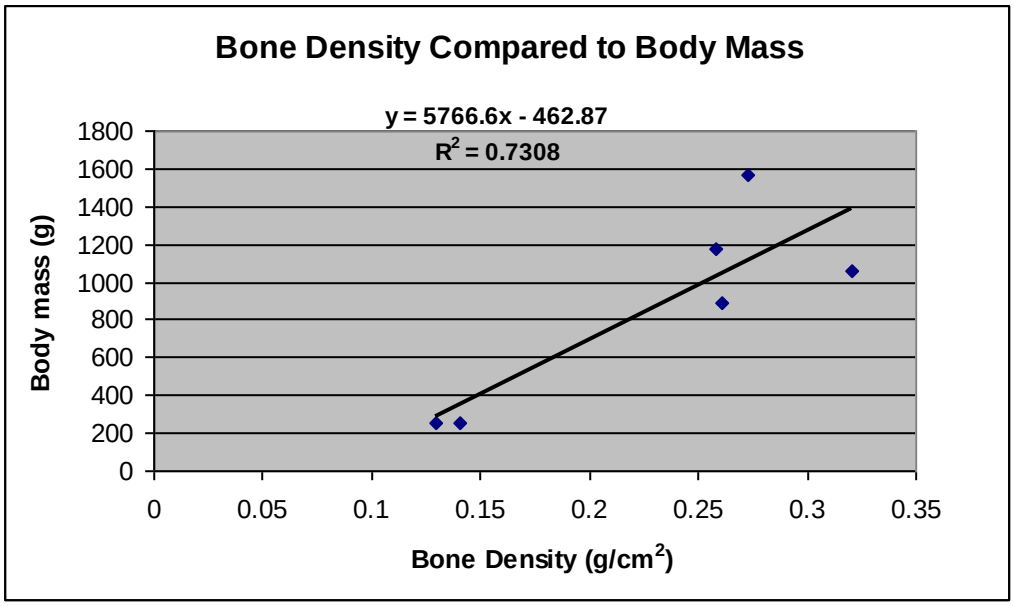

Figure 6: Bone Mineral Density Compared to Avian Body Mass (n=6)

Figure 7 depicts the relationship between bone mineral density and bone length. Seven samples which had radiographs on file were also analyzed by DEXA. These results indicate that an increase in length results in an increase in density, with a correlation coefficient of 0.7.

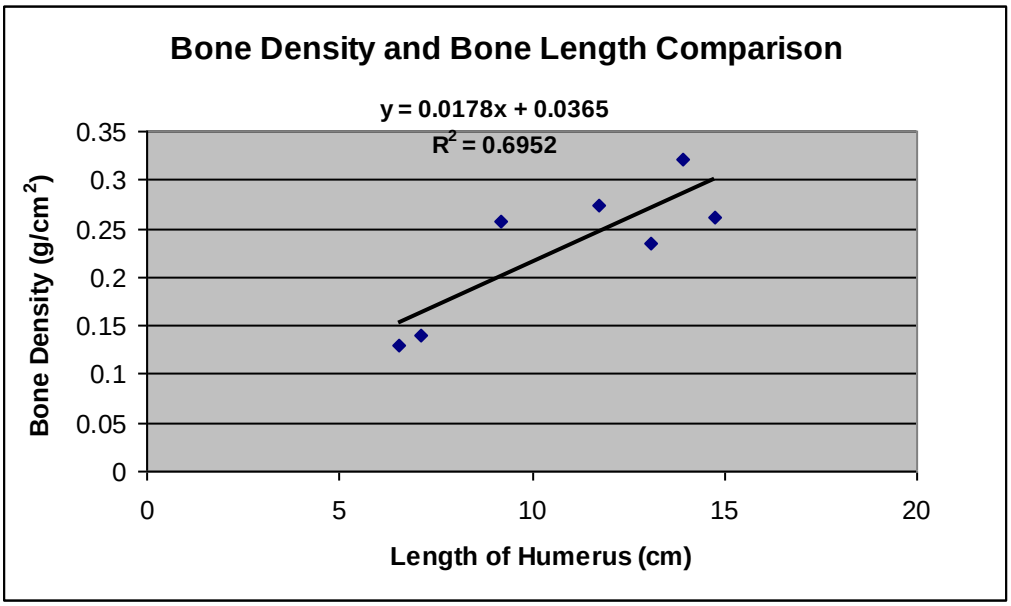

Figure 7: Bone Mineral Density Correlation to Bone Length in Avian Species

Lead is a toxin which can lead to serious health difficulties and the health condition of each bird is recorded in Wildlife Clinic files. This graph displays the relationship between health condition, specifically the severity of emaciation as recorded by Wildlife Clinic staff, and bone mineral density. The graph represents a total of eight birds, which include two normal, one moderately, and five severely emaciated specimens. Both the moderately and the severely emaciated birds appear to have lower bone densities than the birds who were not identified as emaciated, although the samples size is too small for statistical analysis.

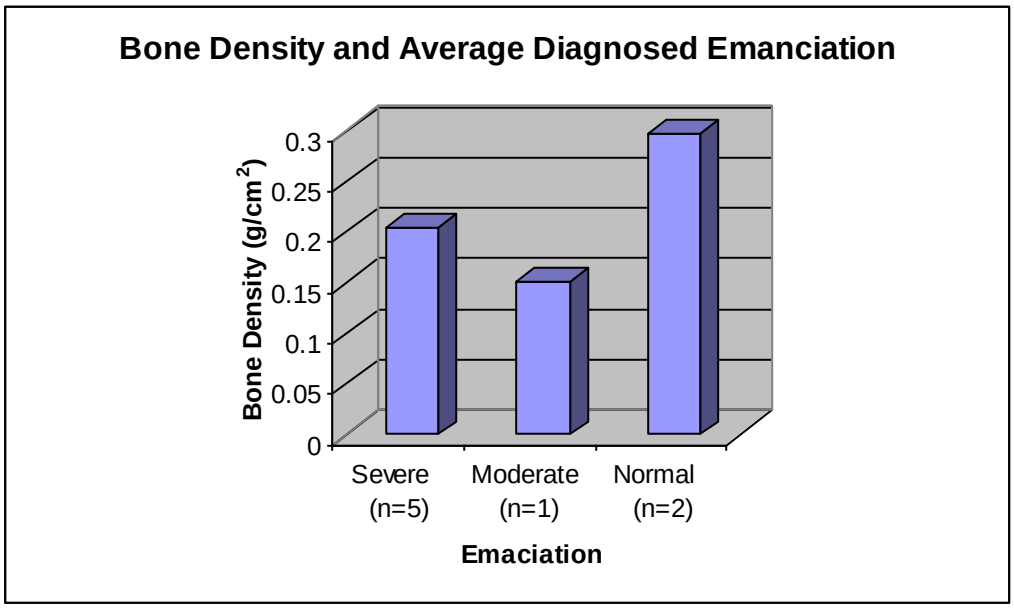

Figure 8: Bone Mineral Density Compared to Emaciation Severity as declared by Tufts Wildlife Clinic Staff

Antioxidant Correlation results

Figure 9 displays the average blood lead levels for 14 different avian species using the data obtained from the Tufts Wildlife clinic from 2002-2008. On average according to the data obtained, pigeons, swans, and vulture have the highest blood lead levels (20-25µg/dL) and loons, owls, gulls, and herons have the lowest blood lead levels (<5µg/dL).

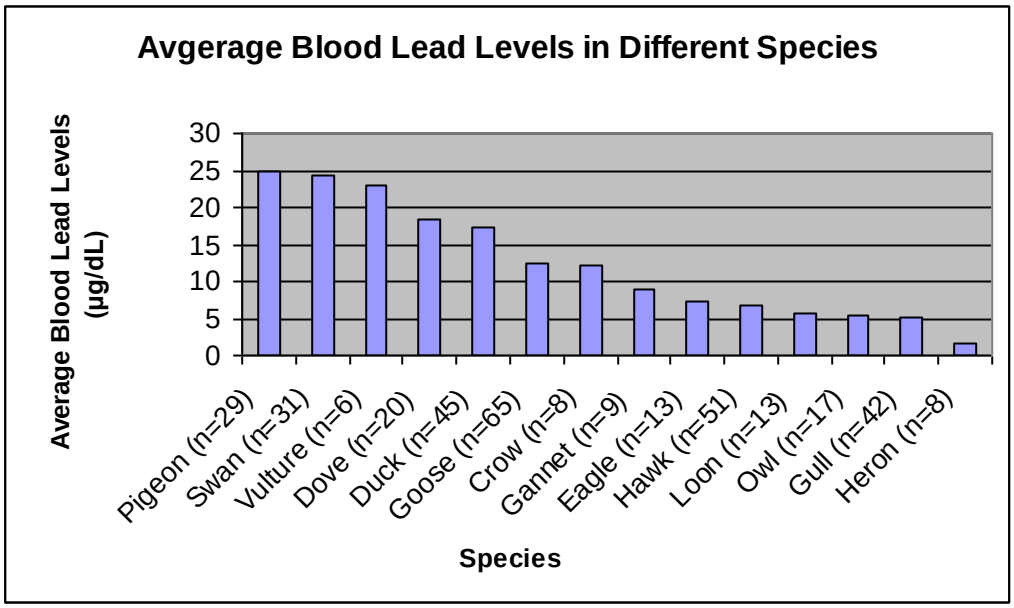

Figure 9: Average Blood Lead Levels of Varying Species

Figures 10-23 display the distribution of blood lead levels in the 14 different avian species displayed in Figure 9. The blood lead levels were divided into 8 different ranges in order to display the distributions. The majority of the birds fall below 20µg/dL, which is not considered a serious lead toxicity level.

Figure 10 demonstrates the low blood lead content of 7 crows, although one bird between 2002 and 2007 was tested to have a detrimental lead content.

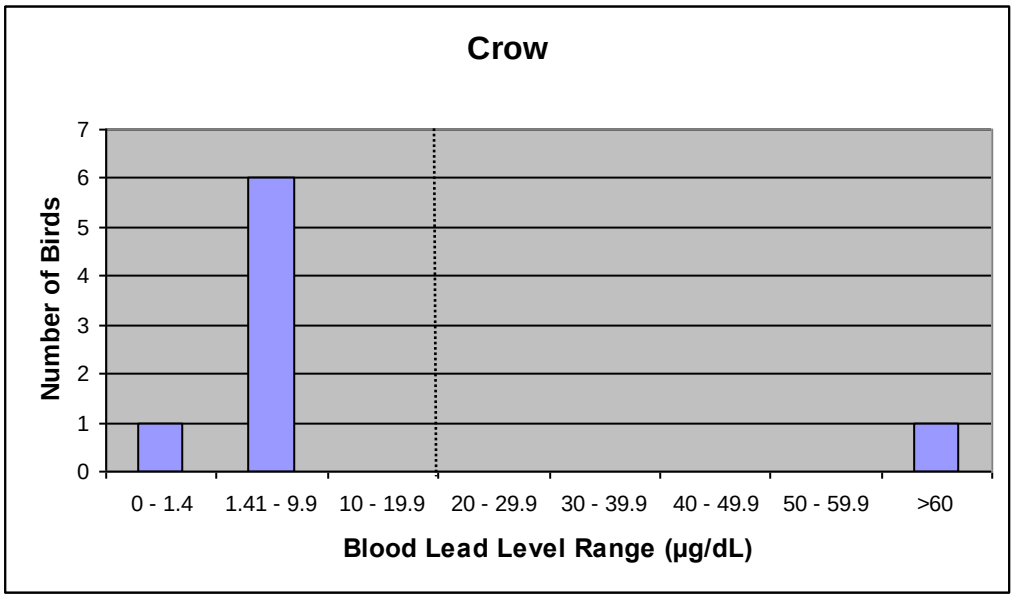

Figure 10: Distribution of Crow Blood Lead Levels

Figure 11 graphically analyzes the blood lead content of 20 doves. Although 6 birds may have been considered for treatment, a larger number did not appear to have lead levels of considerable concern.

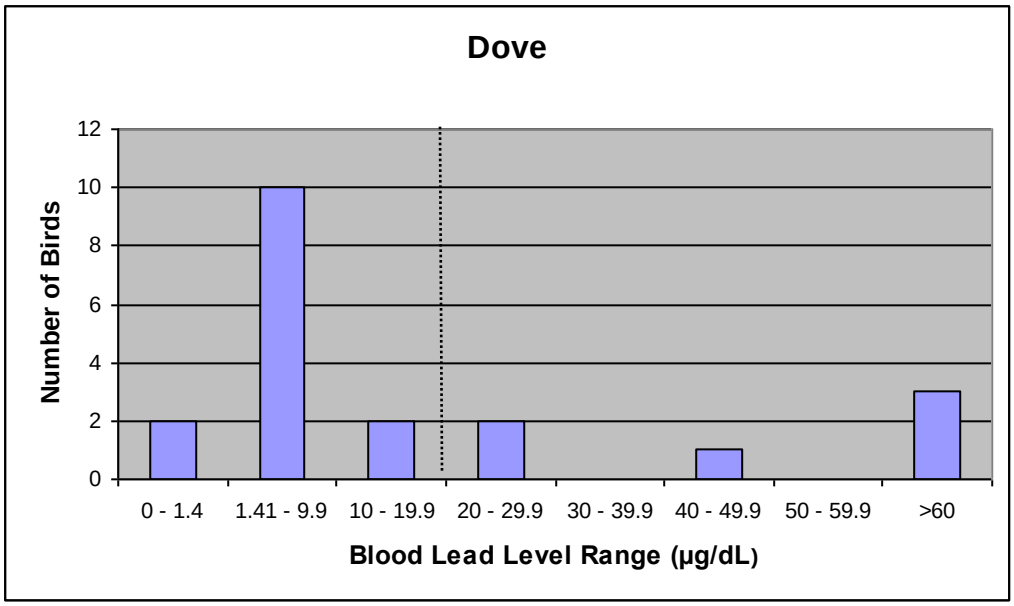

Figure 11: Distribution of Dove Blood Lead Levels

Figure 12 shows the majority of ducks tested for blood lead content were below 20µg/dL and therefore were not seriously intoxicated by lead.

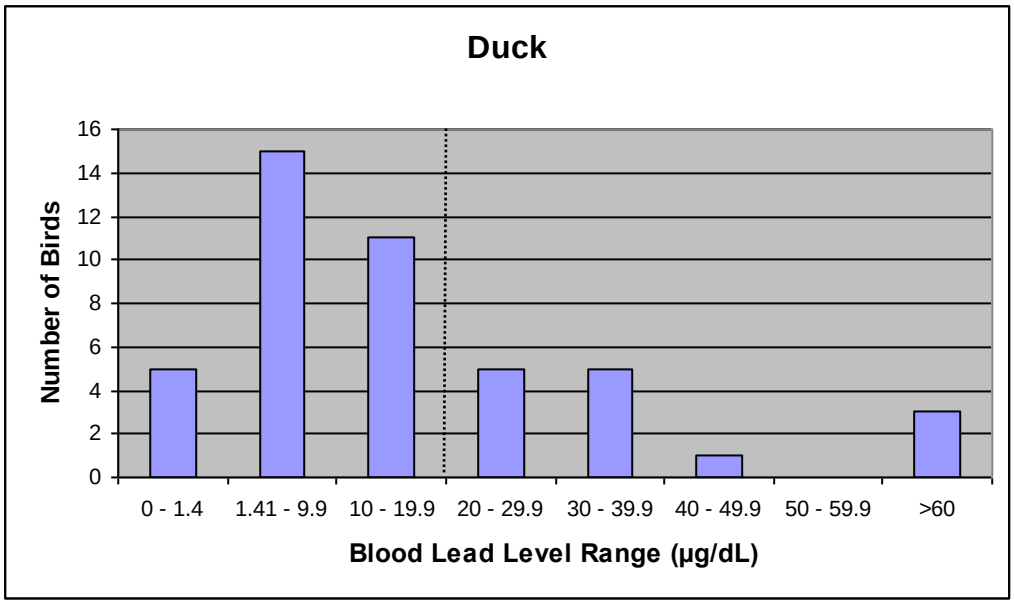

Figure 12: Distribution of Duck Blood Lead Levels

Figure 13 displays the lead blood content of 13 eagles from 2002 to February 2008. Only one eagle appeared to have levels of concern.

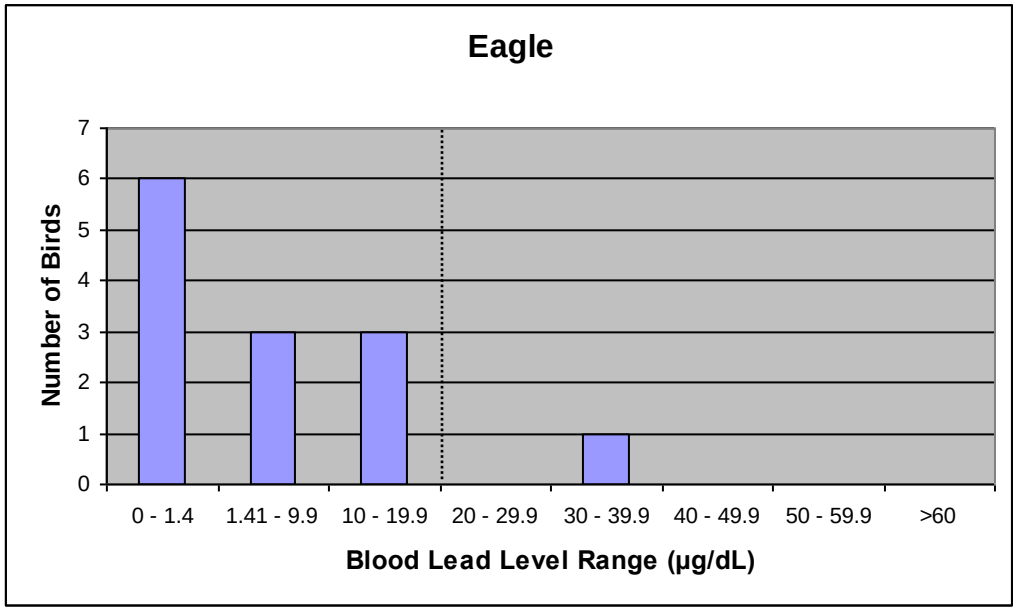

Figure 13: Distribution of Eagle Blood Lead Levels

Figure 14 shows the blood lead content of 9 gannets. All except one gannet had very low lead content.

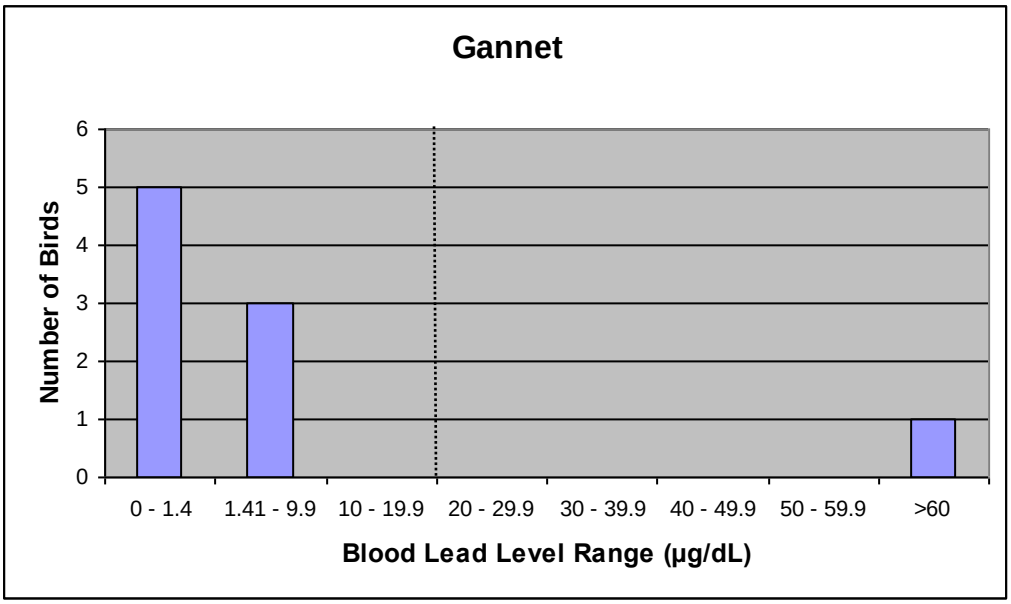

Figure 14: Distribution of Gannet Blood Lead Levels

Figure 15 demonstrates the blood lead content of geese. Most geese tested were in the 1.41-9.9 µg/dL range, although there were a quite a few with lead levels of concern.

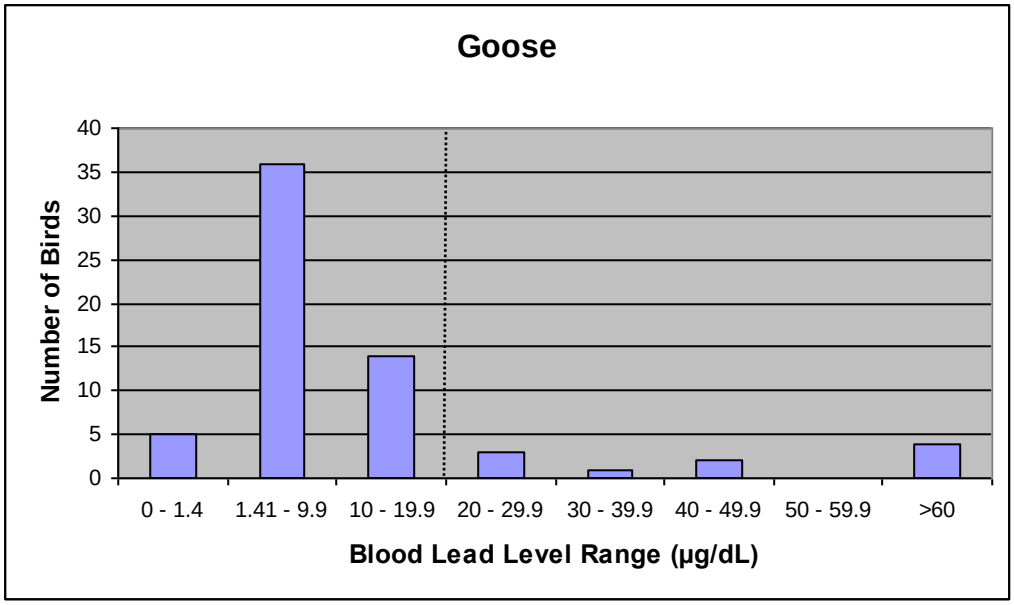

Figure 15: Distribution of Goose Blood Lead Levels

Figure 16 shows very few gulls to have concerning blood lead levels.

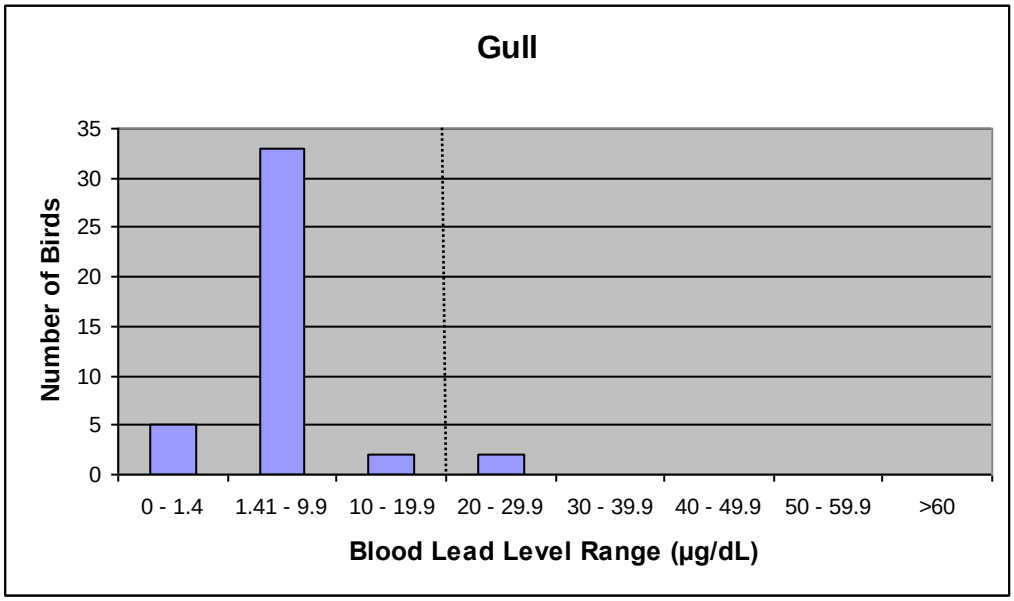

Figure 16: Distribution of Gull Blood Lead Levels

Figure 17 analyzed several hawks which were below 20 µg/dL. Some hawks did show considerable levels of lead.

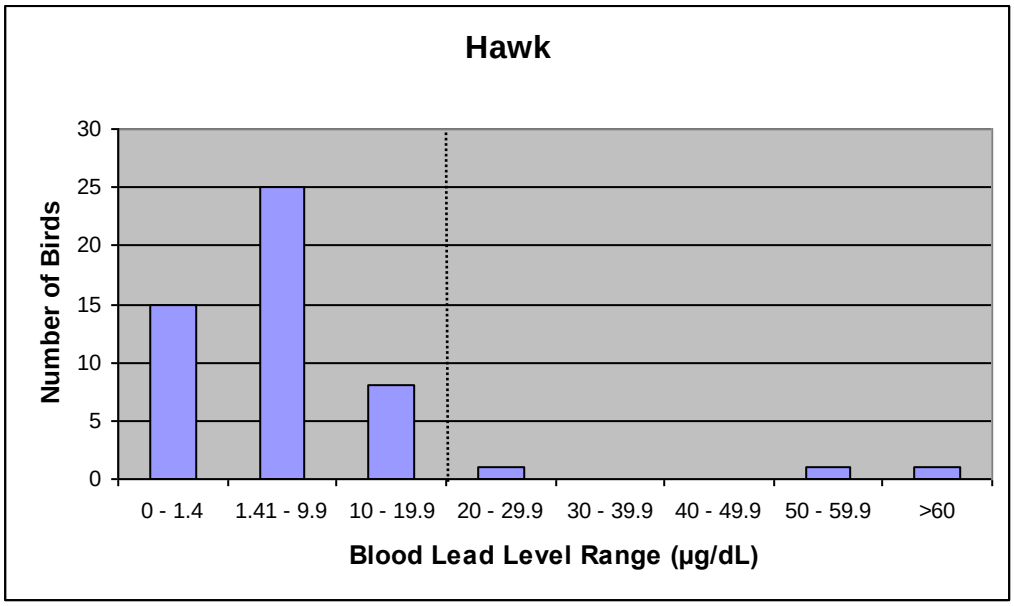

Figure 17: Distribution of Hawk Blood Lead Levels

Figure 18 displays the blood lead content of 8 Herons, all of which had very low blood lead levels.

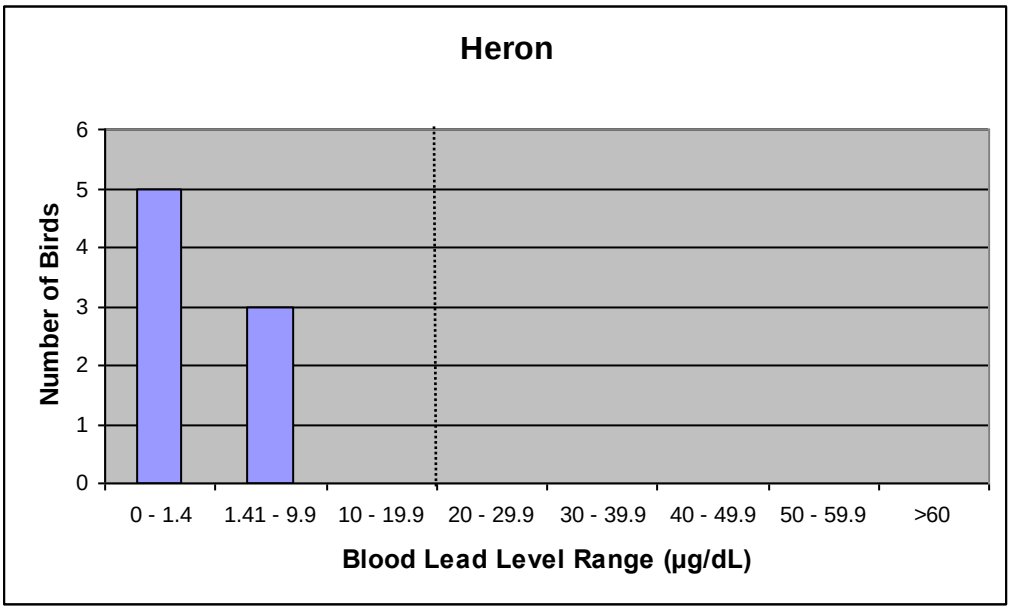

Figure 18: Distribution of Heron Blood Lead Levels

Figure 19 shows the blood lead content of 13 loons. All except one loon had very low lead content.

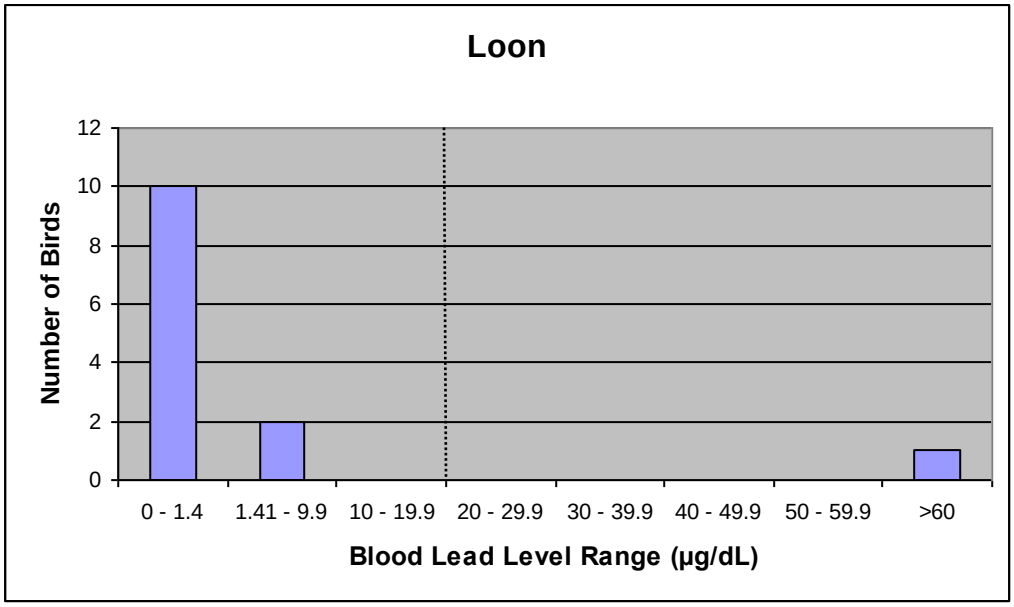

Figure 19: Distribution of Loon Blood Lead Levels

Figure 20 displays the blood lead content of 17 Owls. Only one owl had a significant blood lead level of greater than 60µg/dL.

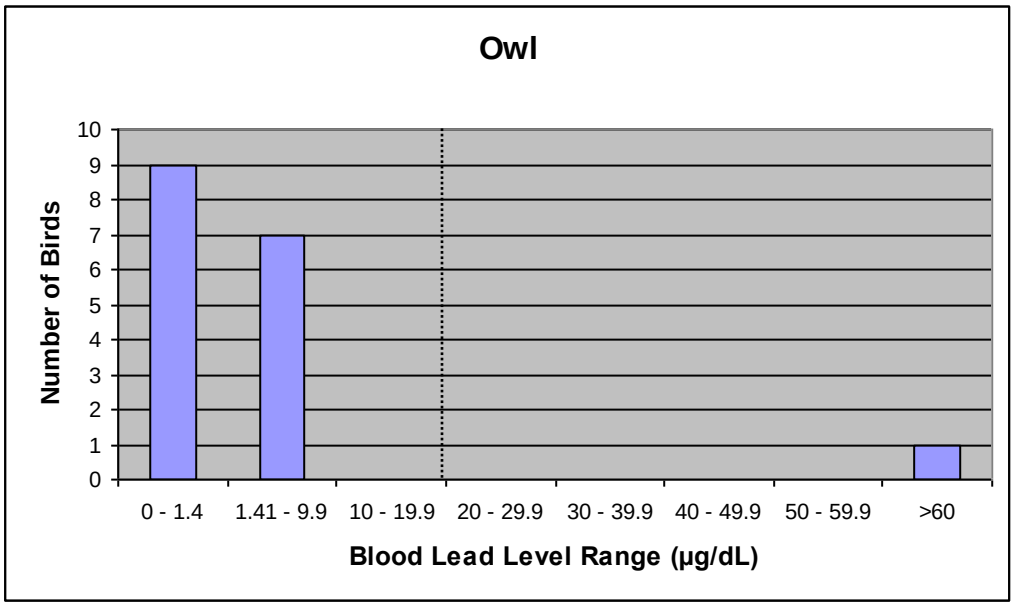

Figure 20: Distribution of Owl Blood Lead Levels

Figure 21 shows the distribution of blood lead levels of several pigeons. Even though several pigeons contain high blood lead levels, the majority are below 20 µg/dL.

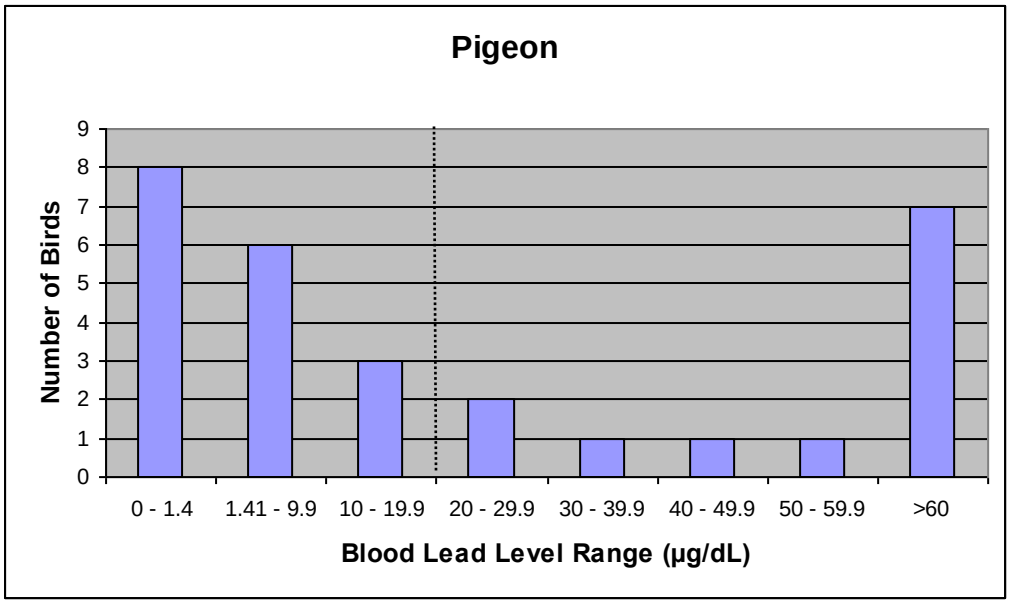

Figure 21: Distribution of Pigeon Blood Lead Levels

Figure 22 displays the distribution of blood lead levels of several swans. Some have a significant level of lead, however the majority fall below 20 µg/dL.

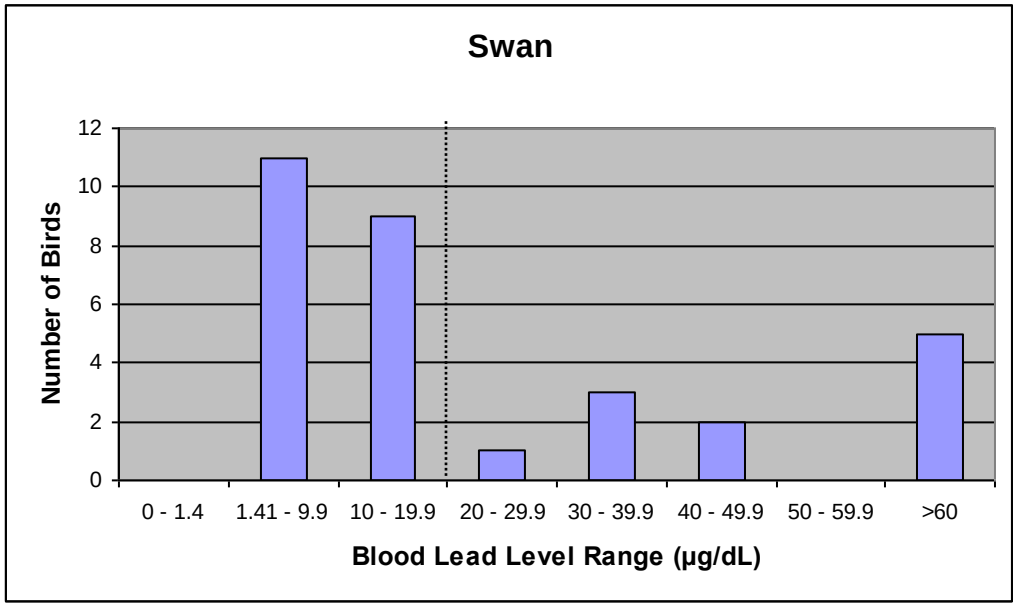

Figure 22: Distribution of Swan Blood Lead Levels

Figure 23 shows blood lead levels of 6 Vultures. Two of the six vultures have significant blood lead levels of greater than 20 µg/dL.

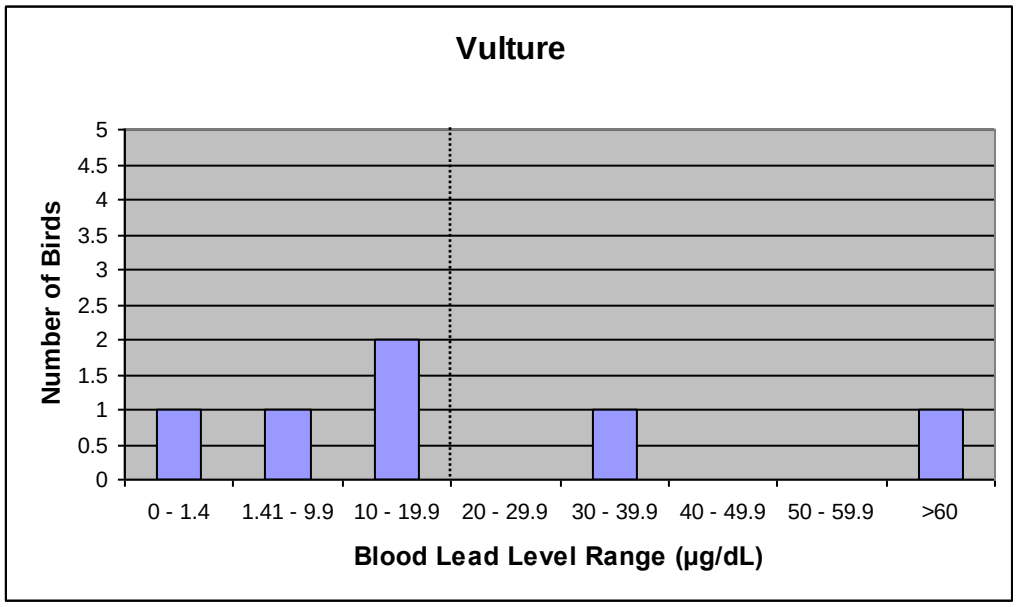

Figure 23: Distribution of Vulture Blood Lead Levels

Figures 24-26 display the distribution of blood lead levels for birds that typically follow particular diets. Figure 24 displays the distribution of blood lead levels for birds that typically follow carnivorous diets, Figure 25 displays the distribution of blood lead levels for birds that typically follow herbivorous diets, and Figure 26 displays the distribution of blood lead levels for birds that typically follow scavenger diets.

Figure 24 shows that the majority of birds that typically have carnivorous diets are below 20 µg/dL and that most of the birds fall between 0 µg/dL and 1.4 µg/dL.

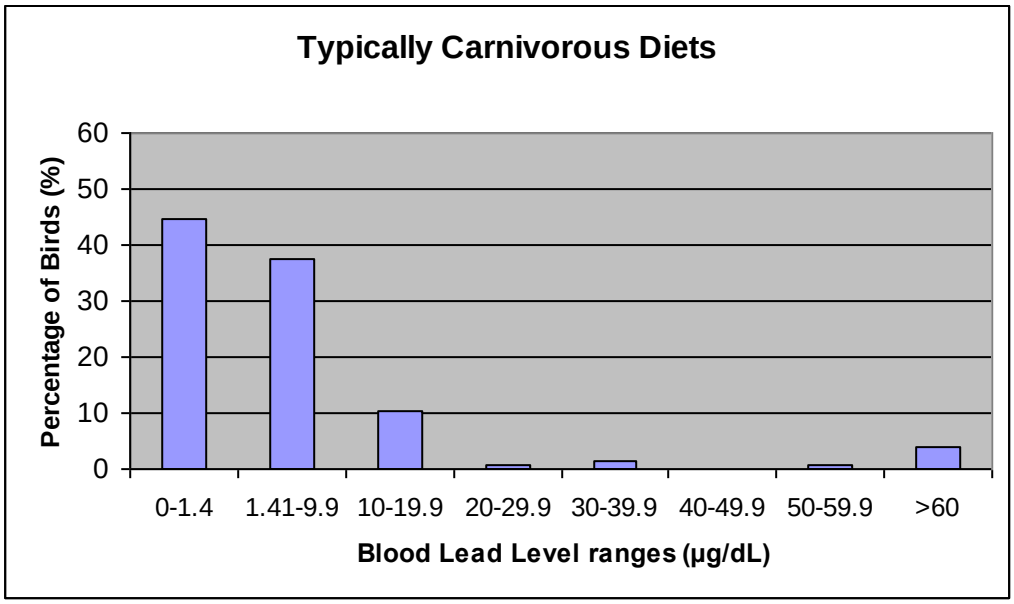

Figure 24: Distribution of Birds that typically have Carnivorous Diets

Figure 25 shows that the majority of birds that typically have herbivorous diets are also below 20 µg/dL and that most of the birds fall between 1.41 µg/dL and 9.9 µg/dL.

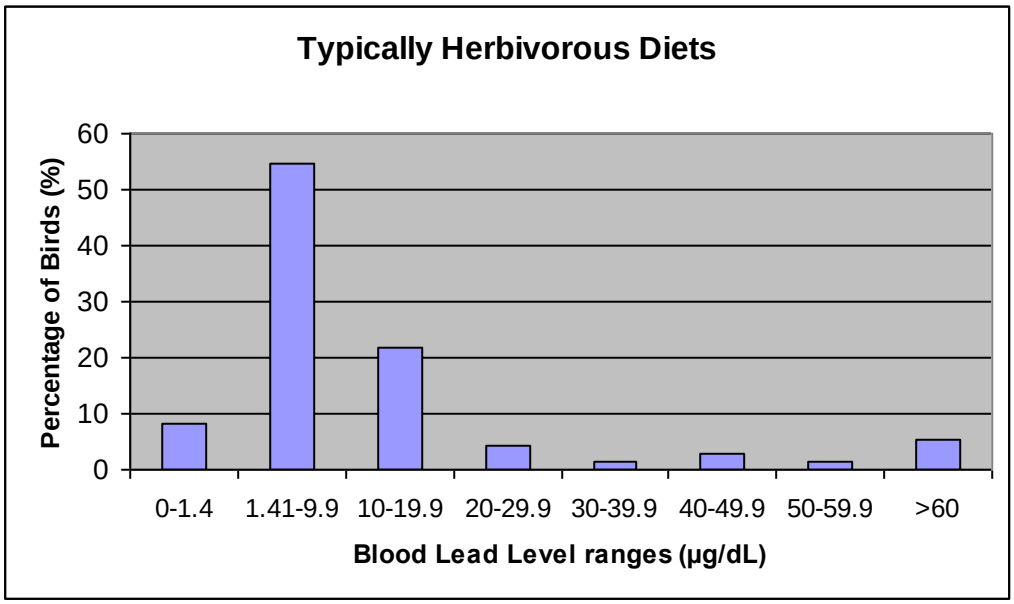

Figure 25: Distribution of Birds that typically have Herbivorous Diets

Figure 25 shows that the majority of birds that typically have scavenger diets are also below 20 µg/dL and that most of the birds fall between 1.41 µg/dL and 9.9 µg/dL.

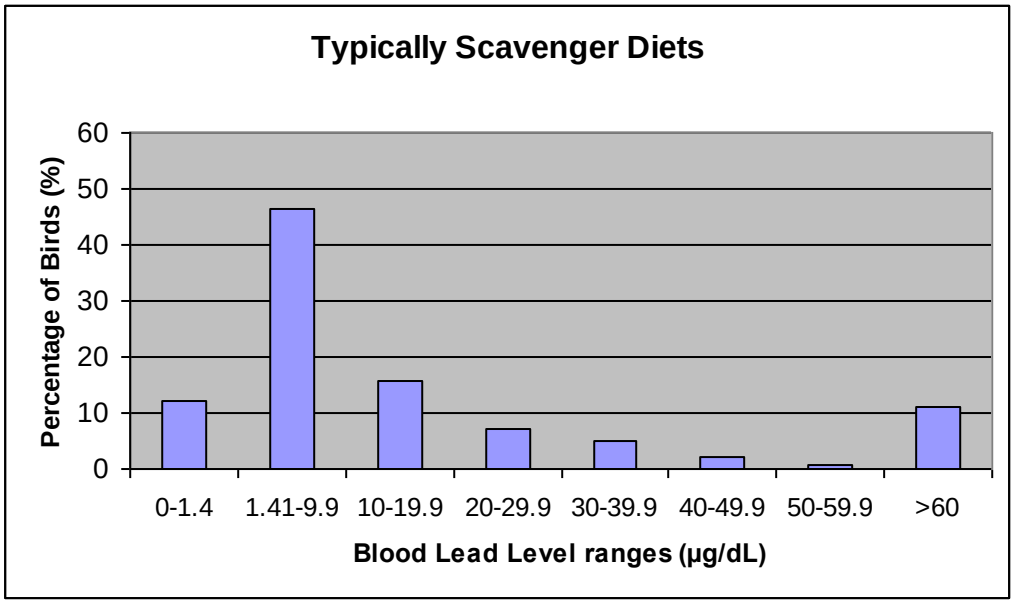

Figure 26: Distribution of Birds that typically have Scavenger Diets

Chapter 5 Discussion

I. Lead Analysis in Pre-Mortem Blood vs. Post Mortem Body Fluid

The analysis of pre-mortem blood and post-mortem body fluid samples demonstrates the similarity of their lead contents. Out of eighteen samples tested for pre-mortem and post-mortem lead content, fifteen were found to have differences less than three μg/dl. The research of McCullough and Tarbet showed that samples tested within seven days after collection were relatively accurate, but after seven days LeadCare® analysis tended to stray from the original lead content. Therefore, the differences between pre-mortem and post-mortem samples occurred, because the three other samples were tested outside of the seven day range. Other causes of variation may include variation in location from which the fluid was obtained or whether or not the bird was thoroughly thawed for necropsy.

Our research supports the hypothesis that pre-mortem and post-mortem lead content analysis will show similar results. Birds with elevated blood lead levels are considered for treatment if above 20 μg/dl. Furthermore, this research indicates that birds analyzed after death with body fluid levels above 20 μg/dl can be diagnosed as having had lead toxicity by using the LeadCare® technology post-mortem.

After including the data of McCullough and Tarbet, only eighteen samples were statistically analyzed. While the statistical analysis indicates that this is an adequate sample size, in the future, a larger number of samples would be desirable for more accurate analysis. Future studies of LeadCare® analysis should consider the project for an extended amount of time to collect a larger amount of samples. In addition, analyzing data specific to certain species would be ideal, although the sample size for that species would also need to be significantly large. Other studies could also include the effects of time after death to analysis, as well as effects of post-mortem storage conditions.

II. Radiological Analysis

It was predicted that birds with metal objects, suspected to be lead shot, present on their radiographs would have higher blood lead levels as well. The radiographs for every bird that was used to compare pre-mortem blood and post-mortem body fluid were observed. However, only two of the radiographs actually showed metal objects present. The radiographs with metal objects present were of a mallard duck (shown in Figure 2) and a turkey, which both had low blood lead levels of 9.4μg/dL and 1.6μg/dL respectively. These results could be due to the fact that if the bird was actually shot with lead shot it could have resulted in a rapid decline in the bird's health. This could have damaged major organ systems, not allowing sufficient time for the lead to be absorbed into the bloodstream before death. Although the sample number was small, it is expected that similar results will be found if this testing is repeated. Future experiments should compare lead absorption by ingestion versus being shot by lead in order to determine if this conclusion is accurate.

III. Mineral Density and Lead Toxicity in Bones

Bone mineral density (BMD) and lead toxicity reports from seven avian humerus bones revealed a slight correlation (R^2=0.291). This correlation increased to R^2=0.5159 after removing an outlier, indicating that a larger sample size of one specific species might show a stronger correlation. The outlier removed represented data from a common loon which is known to have more dense bones than other birds for diving purposes. This outlier is an example of the differences between avian species and demonstrates the importance in focusing the studies on individual or closely related species.

Age was also examined in relation to bone mineral density. Research has shown that lead in the blood is processed over 20 days in humans, but that lead in the bone accumulates over 600 to 3,000 days. Therefore, older birds were hypothesized to have more lead in their bones. However, the data collected is not supportive of this hypothesis. In fact, our results show juvenile birds to have higher bone mineral density. One reason this may be true is that bone releases lead into the bloodstream as it grows or changes. Since younger bones are more susceptible to change, lead content may be higher in the

blood. In addition, the sample size was extremely small due to some files without a recorded age.

Bone length, body mass, and health condition were also compared to the BMD. The relationship between bone length and BMD showed a positive trend indicating that longer bones are denser. It was predicted that lead would decrease the amount of body fat in an animal due to its highly toxic properties. However, our results did not support the prediction. The increase in body mass may be due to the size of the bird species, thus, it may not necessarily be affected by the lead. Therefore, we recommend a more comprehensive study be done in which only specific species are evaluated for a correlation between BMD and body mass. Emaciation is diagnosed by observing body mass. Birds that were described as severely emaciated had slightly higher BMD than moderately emaciated birds. However, birds with normal health conditions on average had the highest lead content. Again, the small sample size may have contributed to inaccurate results. In addition, several other variables could contribute to starvation in birds such as lack of food supply, other toxins, or traumatizing situations.

IV. Antioxidant Correlation

Literature has stated that antioxidants may be used a possible treatment for lead poisoning, which is why this study explored a possible correlation between diets high in antioxidants and low blood lead levels. The LeadCare® data from 2002-2008 from the Tufts Wildlife Clinic were collected, sorted, and analyzed in order to find a possible correlation. In order to analyze this data, the average blood lead levels for each species was calculated and plotted for comparison, which is shown in Figure 9. For further analysis, the species of birds were divided into three separate categories (shown in Figure11.1-11.3) which included birds that are typically meat eaters, birds that are typically plant eaters and birds that are typically scavengers. It was predicted that birds that are typically meat eaters or scavengers would show relatively high blood lead levels compared to birds that are typically plant eaters, whose diets typically contain higher antioxidant levels. However it was determined that there were too many variables affecting the data in order to make accurate conclusions. For example, only birds that

were suspected to have lead poisoning were tested with the LeadCare® test instead of testing every bird that entered the clinic. Also, since the comparison was on wild birds, it is impossible to control their diets and know exactly what each bird ate, so assumptions were made about what each species typically eats based on the literature. Environmental location could also cause inaccurate results. For example, if certain birds lived near a golf course, the chemicals from the course could have affected the results or if particular birds have a lot of human interaction; this could have affected their typical diets. Since there were so many variables that could have affected the outcome of these results, more accurate and controlled experiments would need to be performed in the future in order to determine if there is any correlation between antioxidant levels and lead levels and if antioxidants could be used as a possible treatment for lead poisoning.

V. Conclusion

Lead shot and lead sinkers and jigs contribute to the majority of lead poisoning in birds in the wildlife. Some countries and states have placed bans on them in order to protect the wildlife. In 1987 in the UK, lead weights were banned and in Canada small sinkers and jigs have been made illegal in national wildlife areas and in national parks. A ban on lead fishing gear is being considered by the US Fish and Wildlife Service where loons and trumpeter swans breed (Dawn, 2008).

In 1991, a total ban on the use of lead shot for waterfowl hunting was issued by the United States. Other lead shot bans have also been issued as well for other game hunting in national wildlife refuge wetlands. Currently, New York, New Hampshire, and Maine all ban small sinkers and other states such as Minnesota and Arizona are considering bans on lead shot. Australia, Finland, Mexico, Switzerland, Norway, Sweden, Denmark, Netherlands, and the United Kingdom have all established bans or restrictions on lead shot (University of Minnesota, 2008).

In order to protect the wildlife from lead exposure, a complete ban of lead bullets and fishing tackle is needed. Wildlife lead poisoning cases have immediately dropped in areas where lead has been banned. Lead-free fishing and hunting gear should be readily available, easy to purchase, and competitively priced in comparison to lead fishing and hunting gear. By banning fishing and hunting gear that contains lead, shops would be

forced to provide environmentally safe alternatives and people would be required to use them. One important aspect is that the non-lead alternatives must also be nontoxic. A zinc sinker can be just as toxic and harmful as lead. During 1999 and 2000 an educational campaign, more than 40,000 lead sinkers were traded for steel sinkers by fisherman in New Hampshire and Vermont. These lead sinker exchanges are continuing to expand as well in order to collect lead sinkers and keep them out of the environment (Nadis, 2008).

Currently people are focused on lead exposure in the home, however it is also important to ban lead in all forms to keep it out of the home and the environment. State and regional laws on lead shot and fishing tackle need to be uniform throughout in order to shift to environmental friendly alternatives. State and regional bans as well as restrictions are working in the right direction; however a complete nationwide ban is needed in fishing and hunting gear in order to protect the health and safety of the environment and the wildlife and people that live in it.

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Appendices

Appendix A: Lead Testing Protocol

WPI Blood Lead Project

- Draw blood from any bird* entering Clinic if clinically appropriate, especially if it is to be euthanized.
    - At least 50 µL (0.05 ml) preferably, more if possible depending on size of bird.
    - All species of birds* should be considered for blood collection.*
- Place blood in green top microtainers with heparin. These are located in the x-ray room in a Ziploc bag labeled '**WPI blood Pb study**.'
  NOTE: should you mistakenly put blood in EDTA it can still be used.
- Label tube using provided Sharpie with case number and date and store in Styrofoam tube holder in the refrigerator in the lab.
- In the event that the bird does die, label the cadaver with the pre-made labels (located in the Ziploc bag with the microtainers). Record case number, species, and date.
- Place labeled cadavers in the **purple bin** on the left side of the disposable freezer near the bathroom.
    - We will be visiting the clinic at least once a week to analyze samples.

Thank you all for your help in collecting blood samples for our WPI blood lead project and we look forward to working with everyone during the school year!

*One of the goals of this study will be to further identify in which species we may encounter Pb toxicosis. Although we are interested in sampling a wide variety of species, certain species are considered a higher priority because Pb poisoning has been previously documented. These include:
--waterfowl
--loons & grebes
--raptors
--cormorants & herons
--woodcock
--**squirrels** (not birds, but we've seen significant Pb in them)-other rodents like porcupines & beavers would also be interesting.
--wild turkeys
--pigeons & doves
--woodpeckers

Appendix B: LeadCare® Protocol

Retrieved from online manual at: http://www.woongbee.com/POCT/leadcare.htm

LeadCare® Childhood Blood Lead Testing

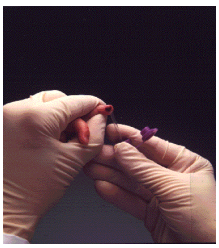
The LeadCare System is for the determination of lead in whole blood. When you test young patients for lead levels, you want fast, accurate, inexpensive results. You want the LeadCare system, a simple, foolproof way to perform blood lead measurements using a finger stick or venous sample. No more waiting days for expensive lab analyses. You get quantitative blood lead results equivalent to those reported by outside laboratories in just three minutes.

A LeadCare system analysis costs far less than you'd pay an outside laboratory, and it qualifies for reimbursement as a quantitative blood lead. You'll also cut your staff's result-tracking and administrative time. You'll save your patients days of possibly needless worry plus the time-consuming inconvenience and cost of a return visit. Blood lead measurement couldn't be easier.

LeadCare is easy and safe to use. The hand-held analyzer is portable and requires neither manual calibration nor refrigeration. Its unique gold electrode sensor contains no mercury or other toxic materials. The point-of-care LeadCare system was developed by ESA and Andcare with a grant from the CDC. It's the diagnostic tool which makes sense medically and economically.

Fast! *Easy as 1- 2- 3*

STEP ONE



Draw a capillary or venous blood sample using EDTA or heparin as anticoagulants.

STEP TWO

Using the pipette provided with the kit, dispense 50 µl, about two drops of blood, into the reagent and mix.

STEP THREE

Transfer it to the sensor strip. Press the button. Just three minutes later, you have your result.

Accuracy

LeadCare System vs. Atomic Absorption Spectroscopy performed at a major lead outreach and referral clinic/hospital
Number of Samples: 112
Slope: 1.07
Intercept: -0.57 µg/dl
Correlation coefficient: 0.97

Portable

Power source: 9V battery or AC adapter
Dimensions: 7.7 in x 4.2 in x 2.5 in. (19.6cm x 10.7cm x 6.4cm)
Weight: 14 oz

Specification

Test method: Electrochemical with disposable sensors
Blood lead level range: 1.4 - 65 µg/dl
Blood sample volume: 50 µl
Test time: 3 minutes
Calibration: Electronic calibration button
Classification: Moderately complex under CLIA guidelines. Suitable for use in physician's office laboratory.

Theory of Anodic Strippimg Voltammetry (ASV)

ASV Method

Anodic Stripping Voltammetry is a highly precise, virtually interference-free method.

1. Whole blood is added to the reagent solution (Fig. 1),
2. Any lead present is released from the blood components (Fig. 2).
3. Now any lead in the reagent solution is concentrated (plated) onto a thin-film electrode during the plating step of the analysis cycle (Fig. 3).
4. The plated lead is removed from the electrode by applying a stripping current (Fig. 4) and the amount of lead is measured by integration of the electrical current released during this rapid electrochemical step.

Anodic Stripping Voltammetry

Figure 1

Figure 2

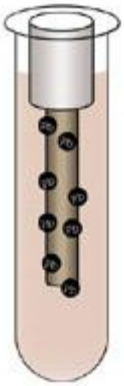


Figure 3

Figure 4

The current released during the stripping step, is a directly proportional to the amount of lead present in the blood sample.

Accurate Results

LeadCare® System vs. Atomic Absorption Spectroscopy performed at a major lead outreach and referral clinic/hospital

Number of Samples	**112**
Slope	**1.07**
Intercept	**-0.57 µg/dl**
Correlation Coefficient	**0.97**

Method Correlation

Results from the Model 3010B Lead Analyzer have shown close correlation with the widely accepted graphite furnace methodologies. This is further supported by results from numerous proficiency surveys.

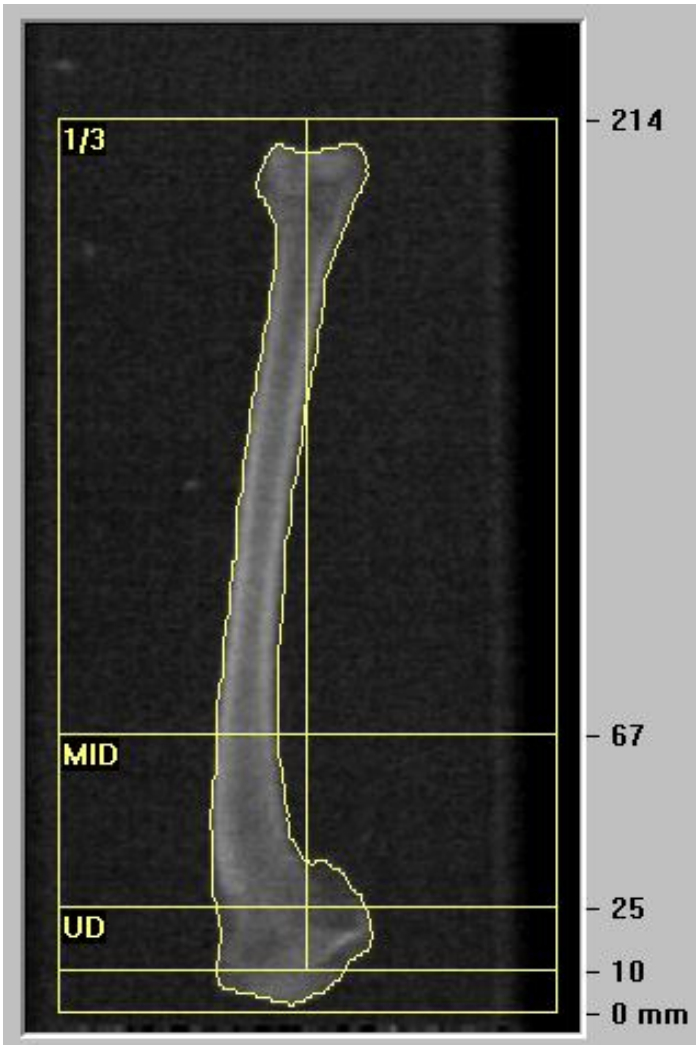
The Model 3010B provides the sensitivity you need for the detection of blood lead in childhood lead screening, industrial hygiene and occupational health monitoring programs.

The LeadCare system operates by fundamentally the same principal but uses a single-use electrode contained on a disposable slide.

Appendix C: DEXA results- raw data

Species	ID number	Area (cm^2)	Bone Mineral Content (g)	Bone Pb Toxicology (ppm)	Bone Density (g/cm^2)
Red Tail Hawk	W071542	9.22	2.52	0.89	0.273
Rock Dove	W0741544	3.9761	0.6033	1.7	0.1517
Cooper's Hawk	W071549	4.8054	0.6204	0.74	0.1291
Herring Gull	W080005	10.88	3.5	19	0.321
Mallard Duck	W071597	8.74	2.26	8.4	0.258
Gull	W080004	10.34	2.43	0.81	0.235
Cooper's Hawk	W071583	4.6507	0.6521	0.94	0.1402
Herring Gull	W080037	13.29	3.47	9	0.261
Common Loon	TV08-051	28.98	16.82	9.5	0.58

Appendix D: Example of a DEXA radiograph of common loon humerus bone.



Appendix E: Lead Toxicology Report

California Animal Health & Food Safety Laboratory System
PO Box 1770
Davis, CA 95617
(530) 752-8700

Final
Version 1
This report supersedes all previous reports for this case

CAHFS Case #: D0803979
Referral #: C. Casavant/S. Whi
Date Collected:
Date Received: 04/01/2008
Case Coordinator: Robert H. Poppenga, DVM, PhD, DABVT
Electronically Signed and Authorized By: Poppenga, Robert H. on 4/4/2008 11:55:51AM

Email To:
Pokras, Mark
mark.pokras@tufts.edu

Specimens Received: 9 Bone Tissue;

Case Contacts

Bill-To	TUFTS VET MED CLIN PATH LAB	508-887-4669	200 WESTBORO RD, N GRAFTON, MA 01536
Submitter	Pokras, Mark	508-887-4669	200 Westboro Road;Tufts Univ. School of Veterinary Medicine, North Grafton, MA 01536

Specimen Details

ID	ID Type	Taxonomy	Gender	Age
Common Loon	Anonymous Identifier	Common Loon		
Cooper's Hawk	Anonymous Identifier	Cooper's Hawk		
Cooper's Hawk	Anonymous Identifier	Cooper's Hawk		
Gull	Anonymous Identifier	Gull		
Herring Gull	Anonymous Identifier	Herring Gull		
Herring Gull	Band	Herring Gull		
Mallard Duck	Anonymous Identifier	Mallard		
Red Tail Hawk		Avian		
Rock Dove	Anonymous Identifier	Pigeon		

Case Summary

The lead analysis was performed on a subsample of bone from mid-shaft.

Reporting limit (Rep. Limit): The lowest routinely quantified concentration of an analyte in a sample. The analyte may be detected, but not quantified, at concentrations below the reporting limit.

Clinical History

Charge In-State

Toxicology

LEAD - TISSUE/OTHER

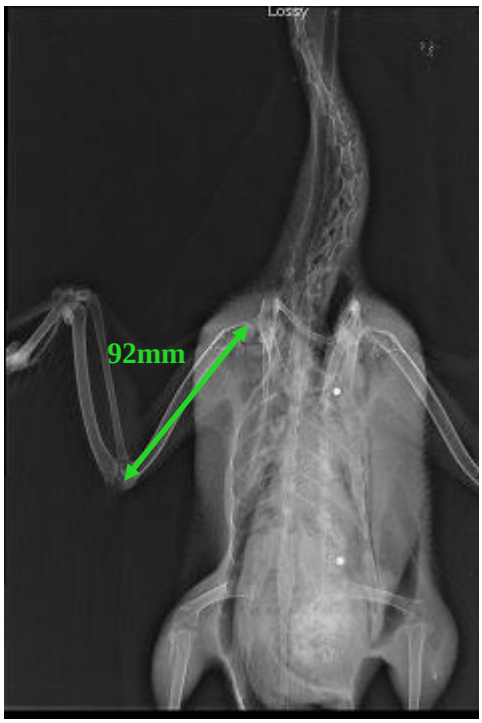
Animal/Source	Specimen	Specimen Type	Date Tested	Results	Units	Rep. Limit
Red Tail Hawk	W071542	Bone Tissue	04-Apr-2008	0.89	ppm	0.06ppm
Rock Dove	W0741544	Bone Tissue	04-Apr-2008	1.7	ppm	0.06ppm
Cooper's Hawk	W071549	Bone Tissue	04-Apr-2008	0.74	ppm	0.06ppm
Herring Gull	W080005	Bone Tissue	04-Apr-2008	19	ppm	0.6ppm

Mallard Duck	W071597	Bone Tissue	04-Apr-2008	8.4	ppm	0.6ppm
Gull	W080004	Bone Tissue	04-Apr-2008	0.81	ppm	0.06ppm
Cooper's Hawk	W071583	Bone Tissue	04-Apr-2008	0.94	ppm	0.06ppm
Herring Gull	W080037	Bone Tissue	04-Apr-2008	9.0	ppm	0.6ppm
Common Loon	TV08-051	Bone Tissue	04-Apr-2008	9.5	ppm	0.6ppm

Appendix F: Age, Weight, Bone Length, and Body Condition Data Analyzed with Bone Mineral Density Data

Species	ID number	Age	Weight (g)	Length of Humerus (mm)	Body Condition
Red Tail Hawk	W071542	Adult	1564	117.3	depressed, slight dehydration, not emaciated
Rock Dove	W0741544	Adult	no weight	no record (look under W071545)	moderate emaciation
Cooper's Hawk	W071549	no age	254	65.2	severe emaciation
Herring Gull	W080005	no age	1056	139	no abnormalties
Mallard Duck	W071597	no age	1174	92	moderate/severe emaciation with keel protrusion
Gull	W080004	Juvenile	no weight	130.8	severe emaciation
Cooper's Hawk	W071583	Adult	252	71.1	severe emaciation with keel protrusion
Herring Gull	W080037	Juvenile	894	147.6	severe emaciation with keel protrusion
Common Loon	TV08-051	no record	no record	no record	no record

Appendix G: Example of how Humerus Bones were measured.



Appendix H: Pre-Mortem Blood Lead Content, Storage, and Bird Radiograph Data

Date Collected	Date Tested	Days in refrigerator	Case Number	Species	Blood Pb Level (µg/dL)	X-ray Info
11/16/2007	11/19/2007	3	W071480	Rock Dove	6.3	Yes; no metal
11/12/2007	11/16/2007	4	W071500	Barred Owl	2.7	No; attempted x-ray but looked like a goose
11/12/2007	n/a		W071497	Cooper's Hawk	released	
11/27/2007	11/28/2007	1	W071531	Greater Black-Backed Gull	2.8	Yes; no metal
12/2/2007	12/5/2007	3	W071542	Red Tailed Hawk	4	Y; no metal
12/3/2007	12/5/2007	2	W071544	Rock Dove	1.6	Y (we x-rayed); no metal
12/5/2007	12/6/2007	1	W071549	Coopers's Hawk	1	Y; no metal
12/19/2007	1/7/2008	19	W071583	Cooper's hawk	1.1	Y; no metal
12/20/2007 *tufts also tested	1/7/2008	18	W071543	Red tailed hawk	5.2 *had trouble getting sample*	Y; no metal
12/27/2007	1/7/2008	11	W071597	Mallard	9.4	Y; 2 metal shots
12/21/2007 *tufts also tested	1/7/2008	17	W071582	Domestic goose	12.8	Y: no metal
1/3/2008	1/7/2008	4	W080004	Gull	6.2	Y (we x-rayed); no metal
1/3/2008	1/7/2008	4	W080005	Herring gull	3.9	Y (we x-rayed); no metal, but odd object
1/7/2008 *tufts also tested	1/7/2008	0	W080013	Canada goose	14.5	Y; no metal
1/7/2008	1/7/2008	0	n/a	Swan	18.2	n/a

n/a	1/23/2008	n/a	W080037	Herring gull	19.5	Y (we x-rayed); no metal
2/6/2008 *tufts also tested on 2/6 = 8.6 µg/dL	2/20/2008	14	W080061	Red tailed hawk	3.8	Y; no metal
2/23/2008	3/12/2008	18	W080097	Loon	0.9	Y; no metal
n/a	Tested in Maine	n/a	n/a	Bald eagle	>65	Y; no metal
3/19/2008	3/19/2008	0	W080142	Canadian Goose	20.1	Y; no metal
~2/21/08	3/20/2008	~28	W080090	Great Horned Owl	0.8	Y; no metal
3/27/2008	3/27/2008	0	W080158	Turkey	1.6	Y; one metal object
n/a	n/a	n/a	TV08-051	Common Loon	>65	
4/7/2008	4/7/2008	0	W080173	Canadian Goose	29	
4/3/2008	4/3/2008	0	BAOW 1-69	???	11.1	

Appendix I: Post-Mortem Body Fluid Lead Content Tested After Necropsy

Date Stored in fridge	Date Tested	Case Number	Species	Body Fluid Pb Level (µg/dL)	Cadaver Location
11/16/2007	11/19/2007	W071480	Rock Dove	6.3	Freezer, then fridge
11/12/2007	11/16/2007	W071500	Barred Owl	4.7	fridge
11/12/2007	n/a	W071497	Cooper's Hawk	released	n/a
11/27/2007	Attempted 11/29/07	W071531	Greater Black-Backed Gull	n/a	Freezer, then fridge, but was disposed of due to improper label
12/5/2007	12/6/2007	W071542	Red Tail Hawk	5.1 (**not thawed)	Freezer, then fridge
12/5/2007	12/6/2007	W071544	Rock Dove	1.6 (**not thawed)	Freezer, then fridge
12/6/2007	12/14/2007	W071549	Cooper's Hawk	3.3	Freezer, then fridge
1/9/2008	1/11/2008	W071597	Mallard	6.8	Freezer, then fridge
1/9/2008	1/11/2008	W080004	Gull	7.6	Freezer, then fridge
1/9/2008	1/11/2008	W080005	Herring gull	1.3	Freezer, then fridge
1/23/2008	1/25/2008	W071583	Cooper's hawk	2.8	Freezer, then fridge
1/23/2008	1/25/2008	W080037	Herring gull	2.6	Freezer, then fridge
3/13/2008	3/16/2008	n/a	Bald Eagle	64.5	From Maine
	~3/20/08	TV08-051	Common Loon	>65	
	4/9/2008	TV07-265	Common Loon	0.5	