Terrestrial Mammal Health and Bioaccumulation Studies for Evaluating Fly Ash Exposure and Effects

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Introduction

On December 22, 2008 an earthen dike broke at a 40-acre waste retention pond at the TVA's Kingston electric generating plant in Roane County, Tennessee. Fly ash spilled into the surrounding waterways. Numerous heavy metals can be found in fly ash and can lead to deleterious health effects in humans and animals. The short and long term effects of this event on human, animal and environmental health are being investigated. Studies evaluating contaminant levels in water, fish, amphibians and birds in the spill and control areas have been performed.

Terrestrial mammals, including raccoons (*Procyon lotor*), have also been included in the monitoring plan and represent another trophic level of potential exposure. Raccoons are omnivorous. Raccoons are plentiful and provide an appropriate population to examine the bioaccumulation of heavy metals and the potentially deleterious health effects that may result from exposure to fly ash. This study reports the health effects of fly ash exposure for 2 years after the initial accident.

Methods

During the first two years of this study, raccoons were captured in live traps (Have-a-heart®) using various food baits. Ten animals were captured from the area of the ash spill each year, 2009 and 2010. Five animals were also captured at control sites each year. Raccoons were sedated with a combination of xylazine and ketamine. Blood was collected for metal (and metalloid) concentrations, complete blood count and plasma biochemistry panel. The animals were then humanely euthanized with an overdose of pentobarbital. All animal procedures performed in this study were approved by the University of Tennessee, Knoxville Institutional Animal Care and Use Committee.

Raccoons then had full necropsies performed and gross lesions were noted. Numerous tissues were collected for metal analysis and histopathology. Specific tissues collected for analysis are shown in table 1. All metal analyses, including analysis of blood, were performed by Pace Analytical and chain of custody was maintained through collection, storage and analysis. Tissues were analyzed by inductively coupled plasma – mass spectrometry for the following metals or metalloids: aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, potassium, selenium, silver, sodium, strontium, thallium, vanadium, and zinc.

Control animals were analyzed separately by year because the primary site of capture was not the same for 2009 and 2010. Exposed animals were analyzed separately by year. Results of the metal analysis were analyzed with an analysis of variance (ANOVA) test and post-hoc testing if groups were normally distributed and had equal variance. A Bartlett's test for inequality of population variances was performed to determine if the data was normally distributed and had equal variance. If the data was not normally distributed or did not have equal variance, a Kruskal-Wallis test was used to analyze the data with Dunn's method for post hoc analysis. Statistical significance was set at $P > 0.05$. Because many samples did not have metal concentrations above the detectable limit, statistical analysis was only performed if 50% of the samples for that metal and tissue had concentrations above the minimum detectable limit; at least 15 samples of testicle needed measurable concentrations to be analyzed statistically.

Blood was analyzed for complete blood count and plasma biochemistry panel by the University of Tennessee College of Veterinary Medicine (UTCVM) Clinical Pathology Laboratory. Animals were split into four groups for analysis of blood values (control 2009, control 2010, exposed 2009, exposed 2010); results were analyzed as described for toxicology data. Any additional blood was stored frozen for possible future analysis. Histopathology was performed by a board certified veterinary pathologist. Because of the small number of animals with pathologic lesions, control animals were compared to exposed animals regardless of year. Prevalence of pathologic lesions was analyzed with a chi square test or Fischer's Exact where appropriate with significance set at $p > 0.05$.

Results

A total of 30 animals were collected and sampled during 2009 (n = 5 controls and 10 exposed) and 2010 (n = 5 controls and 10 exposed). Analysis of metal/metalloid concentrations was performed on tissue samples from 30 raccoons. Results are shown in table 2.

Complete blood counts were performed on 25 animals (n = 3, 2009 controls; n = 9, 2009 exposed; n = 4, 2010 controls; n = 9, 2010 exposed). Blood samples from 5 animals clotted and could not be analyzed. The hematocrit of exposed animals from 2010 (38.3%) was higher than that of exposed animals from 2009 (32.2 %; $P = 0.03$) No other significant differences were present.

A plasma biochemistry panel was performed on 30 animals (control = 10; 2009 = 10; 2010 = 10). Control animals from 2009 (7.44 mg/dL) had lower calcium concentrations than exposed animals from 2009 (8.3 mg/dL) and control (8.7 mg/dL) and exposed animals from 2010 (8.5 mg/dL; $P = 0.002$). The exposed animals from 2010 (150.8 mg/dL) had lower cholesterol than control animals from 2009 (198.2 mg/dL; $P = 0.05$). The exposed animals from 2010 (0.55 mg/dL) had higher creatinine levels than exposed animals from 2009 (0.2 mg/dL; $P = 0.002$). No other significant differences were present.

Tissues from 29 animals had histopathology performed to determine whether microscopic lesions were present. Tissue from one exposed 2010 animals was not analyzed. Lesions observed in the animals included: pulmonary anthracosis (n = 19), eosinophilic pneumonia (n = 11), portal fibrosis (n = 16), gonadal interstitial hypertrophy (n = 11), and pulmonary fibrosis/granuloma (n = 7). There was no significant difference in the occurrence of lesions between exposed animals from 2009 and 2010. The samples from exposed animals were

combined and then compared to the control animals. There were no differences in the occurrence of lesions between exposed and control animals.

Discussion

Previous research on concentrations of various metals in raccoons is scarce. Where available, species specific (raccoon) values for the various metals will be included in the discussion. However, when not available, research from other animals or humans will be provided for reference. Raccoons and humans are both omnivorous and have similar gastrointestinal anatomy (monogastric) and raccoons are being used as sentinels for human exposure to metals in this study.

Metal and Metalloid Analysis

Arsenic concentrations were higher in the hair of exposed animals than control animals, however, only the exposed animals from 2010 had significantly higher concentrations than control animals. Acute arsenic poisoning is associated with respiratory symptoms if inhaled and fever, anorexia, melanosis and possibly death when ingested (Friberg et al., 1979; Saha, 2003). Chronic poisoning typically leads to hyperkeratosis of plantar/palmar surfaces of the feet/hands in people. Other tissues affected by chronic arsenic exposure include mucous membranes, peripheral nervous system, liver, circulatory system; carcinogenic and teratogenic effects can also be seen. Reported arsenic concentrations found in the hair of healthy humans ranged from 0.174 to 0.460 mg/kg wet weight (Friberg et al, 1979). The highest mean of arsenic concentration (0.455 mg/kg) found in the exposed animals from 2010 was similar to the upper range of normal human concentrations. Although the concentrations of arsenic were higher in exposed animals compared to controls, they are likely still below levels that would indicate a risk of toxic effects. Additionally, no abnormalities were seen on necropsy or histopathology that would indicate arsenic toxicity.

Barium levels in the liver were higher in all animals collected in 2010 compared to 2009; additionally, concentrations were higher in the brains of exposed animals from 2010 compared to control animals from 2009. Barium concentrates in the skeleton and poisoning typically results in gastrointestinal, cardiac and skeletomuscular stimulation followed by paralysis (Friberg et al, 1979). No reference values for concentrations of barium in the liver or brain of raccoons is available, however, no clinical signs associated with toxicity (tremors, paralysis) were observed. Measured concentrations in the liver and brain were likely below the threshold associated with toxicity in all animals.

Control animals from 2010 had higher concentrations of boron in their hair than all animals from 2009; there was no difference between control and exposed animals from 2010. Boron is considered an essential metalloid of plants and animals, and likely humans (CDC, 2011). Most forms of boron are non-toxic to humans and animals. Most animal studies have found that increased boron exposure has the most profound effects on the developing fetus. No pregnant animals were captured in this study and therefore no feti could be examined for abnormalities. Other studies in animals have found that boron toxicity can lead to decreased hemoglobin, desquamation of foot pads, lung hemorrhage, hepatic inflammation and coagulative necrosis. No clinical signs or pathological lesions associated with any of these abnormalities associated with boron toxicity were observed in the animals in this study. Reference ranges for boron are not

available for raccoons, so determining what concentrations may be associated with disease is challenging. However, the exposed animals did not have higher concentrations of this metalloid and no animals had clinical signs or pathologic lesions associated with boron toxicity.

Calcium levels in the blood were significantly different, but when analyzed post hoc, no pairs were significantly different. This may indicate an additive effect of the different samples. Overall, the calcium concentrations were higher in 2010 than 2009. The calcium levels in gonads of exposed animals from 2010 were higher than exposed animals in 2009. Tissue concentrations of calcium for raccoons are not available, but when viewed in context with the plasma biochemistry panel results, it is unlikely that the animals in the study had elevated calcium levels (Fowler and Miller, 2003). No evidence of abnormalities associated with decreased or elevated calcium is present.

Chromium levels were higher in the hair of all animals from 2009 compared to control animals from 2010. Other tissues did not have concentrations of chromium that were high enough to quantify, but another study did examine concentrations in the liver and kidney of raccoons (Burger et al, 2002). In humans, the highest concentration of chromium is typically found in the hair. Human hair concentrations ranging from 0.2 to 2 mg/kg have been reported (Friberg et al, 1979). The highest concentration found in the control animals from 2010 was 1.2 mg/kg. In nature, most chromium is found in the divalent or trivalent states which have not been shown to be toxic. Hexavalent chromium can cause skin ulceration, dermatitis, mucous membrane ulceration, allergic reactions, and induce cancer. No evidence disease associated with exposure to hexavalent chromium was present on post mortem exam or histopathologic exam.

Copper concentrations were higher in the hair of all animals in 2010 compared to all animals in 2009. There was no difference in the copper concentrations in the liver or kidney, but a previous study did examine copper concentrations in the liver and kidney of raccoons (Burger et al, 202). Kidney and liver disease, and hemolysis will result from copper toxicity; acute gastrointestinal clinical signs can also occur following ingestion. Normal hair concentrations of copper are not available for raccoons, but liver concentrations in newborn humans are approximately 30 mg/kg wet weight and ranges from 5 to 10 mg/kg wet weight for adults (Friberg et al, 1979). There were no differences in liver copper concentrations between groups, but the mean liver copper concentration in all raccoons was 11.8 mg/kg. This value is slightly above the range reported for adults, but no evidence of toxicity was noted.

The muscle concentrations of iron were higher in exposed animals from 2010 compared to control animals from 2010. Ingestion of excess iron can lead to lesions in the gastrointestinal tract, metabolic acidosis, and liver disease. Iron typically concentrates in blood, liver and spleen and concentrations range from 500 to 800 mg/kg in humans (Friberg et al, 1979); muscle iron concentrations for raccoons are not available. The mean blood iron concentration for all raccoons was 354.7 mg/kg which is below the typical concentration found in humans. The differences in iron concentrations are unlikely to lead to toxic effects and no evidence of disease from iron exposure was present.

Magnesium concentrations in tissues (brain, kidney, liver, muscle) were generally higher in 2010 than in 2009 with varying significance. Magnesium toxicity is uncommon in humans but can lead to abdominal cramping and diarrhea; the likelihood of developing magnesium toxicity increases if kidney failure is present (NIH, 2011). If normal renal function is present, as was the

case in these raccoons, magnesium toxicity is unlikely to occur. Further research is needed to determine what levels of magnesium are considered normal in various tissues of humans or animals.

Mercury concentrations in the muscle were significantly different, but when pairs were compared with post hoc testing, none were different from another. Differences were not observed for other tissues, but mercury levels in kidney and liver were measured in other studies (Burger et al, 2002). Organic forms of mercury are the most likely to lead to toxic effects. Chronic exposure to methylmercury can lead to degeneration and atrophy of the cerebral cortex, ataxia and effects on a fetus. Blood mercury levels of 0.2 mg/kg in humans are not likely to lead to neurologic clinical signs (Friberg et al, 1979). Although only 9 animals had blood mercury levels above the detectable limit, the mean blood mercury concentration was 0.01 mg/kg. The measured concentrations of mercury are unlikely to lead to toxic effects. If raccoon muscle were consumed by humans, the amount of mercury in the muscle would need to be considered to avoid bioaccumulation and possible toxic effects.

Potassium levels in tissues (brain, hair, liver) were generally higher in 2010 animals compared to 2009 animals with varying significance. Hyperkalemia can lead to significant cardiac rhythm abnormalities and possibly death (Duncan et al., 1994). Significant differences were not noted in blood concentrations which would be the most likely to indicate a problem. No arrhythmias were noted on physical examination. Normal tissue concentrations of potassium need to be determined for raccoons.

Selenium concentrations in hair and muscle were higher in exposed animals than control animals in their respective years with varying significance. The main effects of chronic increased exposure to selenium include liver disease, anemia, reduced growth and shortening of life span. Effects on reproduction and teratogenesis have also been reported (Friberg et al, 1979). There were no differences in hematocrit or red blood cell count between exposed and control animals and no significant difference in prevalence of liver pathology was observed between control and exposed animals. The highest levels of selenium are typically found in the kidney followed by the liver. One study found that “normal” raccoons had selenium concentrations of 2.38 mg/kg wet weight and 2.01 mg/kg wet weight for the kidney and liver respectively (Burger et al, 2002). Although no significant differences were noted in kidney or liver selenium concentrations, the mean selenium concentrations of raccoons in this study were 1.89 mg/kg and 1.16 mg/kg respectively, both below the concentration of “normal” raccoons. Although reproductive success or evidence of teratogenesis has not been assessed in this study, the concentrations of selenium are unlikely to lead to toxic effects.

Sodium levels were significantly different in the blood of raccoons but when pairs were analyzed post hoc, no significant differences were noted. Normal blood (plasma) sodium levels are reported to range from 3270 to 3450 mg/kg (Fowler & Miller, 2003); the blood sodium mean concentrations reported for the raccoons ranged from 2,711 to 3,592 mg/kg. Variability in concentration may be due to differences in analysis methods; the normal values from Fowler & Miller were not obtained with mass spectrometry. Future analysis may include determining sodium levels as part of the plasma biochemistry panel. Sodium toxicity is uncommon but can lead to excessive thirst, muscle weakness and edema. These clinical signs were not observed in any of the raccoons and the measured levels are unlikely to cause toxicity.

Zinc concentrations in the brain were significantly different although when pairs were compared post hoc, no significant differences were found. Zinc toxicity is typically associated with nausea and diarrhea (Friberg et al, 1979). Zinc concentrations in humans are typically around about 1 mg/kg in serum or plasma and about 10 times that in blood. The mean concentration of zinc in the blood of all raccoons examined in this study was 8.01 mg/kg. No clinical signs or pathologic lesions associated with zinc exposure were observed in any of the animals. The observed levels of zinc are unlikely to lead to toxicity.

Histopathological Evaluation of Tissues

Overall, there were no significant differences in the prevalence pathologic lesions between control and exposed animals. The lesions noted were not considered fly ash related. The pulmonary anthracosis is attributed to the foraging behavior of this species. The pulmonary eosinophilic infiltrates and granulomas are typically associated with parasitism. The hepatic fibrosis is nonspecific and may be related to cumulative portal track insults from aging, or infectious or toxin exposure. The gonadal interstitial cell hypertrophy may be a seasonal variation.

Complete Blood Count and Plasma Biochemistry

The complete blood counts were unremarkable overall. The only difference observed was the hematocrit was higher in the 2010 exposed animals when compared to the 2009 exposed animals. The normal range for hematocrit is 31.4 to 42.2% (Fowler & Miller, 2003) and the mean values for all groups fell within this range. Although a difference was observed, this difference was likely clinically insignificant.

Control animals from 2009 had lower plasma calcium concentrations than all other animals. Normal plasma calcium levels for raccoons typically range from 8.3 to 9.7 mg/dL (Fowler & Miller, 2003). Hypocalcemia can be due to kidney disease, but there was no evidence of kidney disease on pathology and this below normal mean may represent normal variation in wild populations. Other common causes of hypocalcemia in dogs and cats include hypoparathyroidism, pancreatitis, puerperal tetany or intestinal malabsorption disorder, but none of these were evident on pre or post mortem examination (Duncan et al, 1994). Calcium levels in analyzed tissues were also lower in the control animals from 2009, although they were not significantly different from other groups of animals. The control animals from 2009 were caught in a different site from the control animals of 2010 and unknown environmental factors at the site may have played a role in the decreased calcium levels. Regardless, no evidence of disease associated with the hypocalcemia was observed.

The exposed animals from 2010 had lower cholesterol than control animals from 2009. The normal range of cholesterol levels for raccoons is 148 to 274 mg/dL (Fowler & Miller, 2003). The means of all groups fell within this reference range and likely represents variability in wild populations. These differences observed were unlikely to lead to disease.

The exposed animals from 2010 had higher creatinine levels, an indication of kidney function, than exposed animals from 2009. The reference range for creatinine in raccoons is 0.7 to 1.1 mg/dL (Fowler & Murray, 2003). Creatinine is typically evaluated in combination with blood urea nitrogen and elevations in both, referred to as azotemia, indicate renal disease. Renal disease is not indicated in these animals by blood results or histopathologic analysis. Reduced

levels of creatinine may be associated with smaller amounts of muscle mass, but the amount of muscle mass on animals was not analyzed in this study. Subjectively, there was not a difference between groups; all groups had a similar range of length and weight of animals included in the analysis.

Conclusion

Although differences were observed as a result of the various tests that were performed, there are no indications at this point that any significant accumulation of metals or metalloids is occurring in animals exposed to fly ash when compared to animals from control (unexposed) areas. The concentrations of various metals and metalloids are likely too low to lead to deleterious health effects. And although reproductive fitness was not specifically examined in this study, there was no evidence on pathology of gonads or concentrations of various toxins that reproductive health would be affected at the concentrations of various metals/metalloids observed in the exposed raccoons. Additionally, no clinically significant differences were observed on complete blood counts or plasma biochemistry analysis. Finally, although microscopic lesions were observed in numerous animals, there was no significant difference in the prevalence of these lesions between control and exposed animals and the lesions can be attributed to other mechanisms. With regard to the differences in various metal/metalloid concentrations between groups, consideration should be given to varying bioavailability of the various metals or metalloids in the environment or consumable material (plants). Bioavailability could potentially be affected by climate conditions such as rain and temperature. Continued monitoring of raccoons at the site will determine if long term accumulation of any metals or metalloids occurs or if clinically significant changes occur in blood (CBC, chemistry) analysis over the next 5 years.

Literature Cited

1. Burger, J., K.F. Gaines, C.G. Lord, et al., Metal levels in raccoon tissues: differences on and off the Department of Energy's Savannah River site in South Carolina. *Environmental Monitoring and Assessment*, 74:67-84, 2002.
2. Centers for Disease Control and Prevention (CDC). Boron relevance to public health, Accessed at: http://www.atsdr.cdc.gov/toxprofiles/tp26-c2.pdf , October 14, 2011.
3. Duncan, J.R., K.W. Prasse, E.A. Mahaffey. *Veterinary laboratory medicine clinical pathology, 3rd ed*. Iowa State University Press, Ames, 1994.
4. Fowler, M.E. and R.E. Miller, eds. *Zoo and Wild Animal Medcine*, 5th edition. Saunders, St. Louis, 2003.
5. Friberg, L., G.F. Nordberg, and V.B. Bouk, eds. *Handbook on the Toxicology of Metals*. Elsevier/North-Holland Biomedical Press, New York, 1979.
6. National Institutes of Health. Magnesium. Accessed at: http://ods.od.nih.gov/factsheets/magnesium/ , October 14, 2011.
7. Saha, K.C. Diagnosis of arsenicosis. *Journal of Environmental Science and Health*, A38(1):255-272, 2003.

Table 1: Tissues collected during necropsy of raccoons for analysis of metal concentrations and histopathology. Samples were collected from a total of 30 animals.

Tissues Collected for Metal Analysis	Tissues Collected for Histopathology
Blood	Liver
Hair	Kidney
Liver	Adrenal Glands
Kidney	Lungs
Brain	Brain
Gonad (Testicle)	Gonads (Testicles and Ovaries)
Skeletal Muscle	

Table 2: Tissue samples were analyzed with mass spectrometry for 26 metals and metalloids. Concentrations above the lower detectable limit were only found in some of the tissues. This table shows how many samples of 30 that were analyzed had concentrations above the lower detectable limit [Tissue (n)]. Ten samples were from control/unexposed animals and 20 samples were from exposed animals caught in the area of the ash spill. Only 19 samples of gonad (testicle) were analyzed (5 from control animals and 14 from exposed animals). For tissues that had at least 15 samples with concentrations above the lower detectable limit of a particular metal, statistics (ANOVA for parametric data and Kruskal-Wallis for non-parametric) were performed to determine if differences existed between groups where group 1 included control animals from 2009, group 2 included exposed animals from 2009, group 3 included control animals from 2010, and group 4 included exposed animals from 2010. P values are shown (significance set at $p < 0.05$) for groups that were analyzed with statistics. For significant results, the mean or median (M) and numbers in each group analyzed are shown. Additionally, groups with the same letter were determined to be significantly different from each other in post-hoc analysis. P values denoted with (*) showed overall significance but no pairs were shown to be significantly different in post-hoc analysis.

Metal	Tissue (n)	Group 1 (n)	Group 2 (n)	Group 3 (n)	Group 4 (n)	P
Aluminum						
	Blood (1)					NA
	Brain (1)					NA
	Gonad (0)					NA
	Hair (29)					0.19
	Kidney (2)					NA
	Liver (1)					NA
	Muscle (5)					NA
Antimony						
	Blood (0)					NA
	Brain (0)					NA
	Gonad (0)					NA
	Hair (14)					NA
	Kidney(1)					NA
	Liver (3)					NA
	Muscle(1)					NA
Arsenic						
	Blood (0)					NA
	Brain (0)					NA
	Gonad (0)					NA
	Hair (26)	0.106[a] (4)	0.403 (7)	0.094[b] (5)	0.455[a,b] (10)	0.001
	Kidney (8)					NA
	Liver (7)					NA
	Muscle (5)					NA
Barium						
	Blood (4)					NA
	Brain (15)	0.024[a] (3)	0.029 (7)	0.05 (2)	0.16[a] (3)	0.04
	Gonad (16)	0.0195 (1)	0.0309 (4)	0.0633 (3)	0.057 (8)	0.07
	Hair (29)	1.594 (4)	2.4054 (10)	3.96 (5)	2.2 (10)	0.5
	Kidney (27)	0.8805 (4)	0.614 (8)	0.788 (5)	0.806 (10)	0.17
	Liver (16)	0.026[a,d] (2)	0.031[b,c] (8)	0.058[a,b] (2)	0.051 [c,d] (4)	<0.001
	Muscle (16)	0.402 (2)	0.05 (8)	0.079 (3)	0.0567 (3)	0.63
Beryllium						
	Blood (0)					NA
	Brain (0)					NA
	Gonad (0)					NA
	Hair (0)					NA
	Kidney (0)					NA
	Liver (0)					NA
	Muscle (0)					NA
Boron						
	Blood (0)					NA
	Brain (1)					NA
	Gonad (0)					NA
	Hair (24)	0.475[a] (2)	0.678[b] (7)	1.7[a,b] (5)	1.24 (10)	0.008
	Kidney (0)					NA
	Liver (0)					NA
	Muscle (0)					NA
Cadmium						
	Blood (0)					NA
	Brain (5)					NA
	Gonad (5)					NA

	Hair (12)					NA
	Kidney (29)	1.4015 (4)	1.7482 (10)	3.102 (5)	1.251 (10)	0.41
	Liver (29)	0.3173 (5)	0.3072 (10)	0.4540 (5)	0.2733 (9)	0.73
	Muscle (1)					NA
Calcium						
	Blood (27)	55.4 (3)	56 (9)	69.2 (5)	61.25 (10)	0.012*
	Brain (26)	58.295 (4)	76.5229 (7)	59.96 (5)	64.74 (10)	0.11
	Gonad (18)	56.1 (2)	58.7[a] (5)	68.6 (3)	69.0[a] (8)	0.009
	Hair (29)	522.15 (4)	625.32 (10)	764 (5)	682.9 (10)	0.18
	Kidney (30)	158.384 (5)	86.682 (10)	96.42 (5)	79.41 (10)	0.83
	Liver (28)	72.9325 (4)	64.817 (10)	64.45 (4)	74.57 (10)	0.16
	Muscle (9)					NA
Chromium						
	Blood (0)					NA
	Brain (6)					NA
	Gonad (4)					NA
	Hair (20)	1.2[a] (2)	0.91[b] (5)	0.24[a,b] (5)	0.4 (8)	0.003
	Kidney (7)					NA
	Liver (1)					NA
	Muscle (6)					NA
Cobalt						
	Blood (1)					NA
	Brain (16)	(0)	0.0186 (1)	0.0198 (5)	0.0168 (10)	0.25
	Gonad (1)					NA
	Hair (22)	(0)	0.1063 (7)	0.1346 (5)	0.1497 (10)	0.78
	Kidney (19)	(0)	0.0394 (4)	0.0734 (5)	0.0424 (10)	0.18
	Liver (22)	0.0836 (2)	0.0713 (6)	0.086 (5)	0.0792 (9)	0.82
	Muscle (1)					NA
Copper						
	Blood (30)	1.398 (6)	1.588 (10)	1.4 (5)	1.47 (10)	0.51
	Brain (30)	3.4842 (5)	3.3098 (10)	3.9 (5)	3.99 (10)	0.4
	Gonad (18)	3.1495 (2)	1.0679 (5)	1.1 (3)	1.15 (9.2)	0.19
	Hair (30)	7.54[a,c] (5)	8.97[b,d] (10)	23.7[a,b] (5)	20.0[c,d] (10)	<0.001
	Kidney (30)	4.84 (5)	4.2212 (10)	5.6 (5)	4.42 (10)	0.61
	Liver (30)	7.3986 (5)	12.4236 (10)	16.56 (5)	11.12 (10)	0.68
	Muscle (30)					0.07
Iron						
	Blood (30)	322.86 (5)	306.48 (10)	397 (5)	394.8 (10)	0.18
	Brain (29)	18.798 (5)	18.0011 (9)	17.26 (5)	18.08 (10)	0.78
	Gonad (18)	379.685 (2)	36.256 (5)	24.2333 (3)	39.8625 (8)	0.17
	Hair (30)	94.436 (5)	149.048 (10)	53.06 (5)	198.17 (10)	0.14
	Kidney (30)	96.73 (5)	99.477 (10)	109.84 (5)	105.98 (10)	0.96
	Liver (30)	422.62 (5)	481.96 (10)	435.8 (5)	444.97 (10)	0.93
	Muscle (30)	32.5 (5)	38.6 (10)	28.9[a] (5)	41.2[a] (10)	0.01
Lead						
	Blood (7)					NA
	Brain (2)					NA
	Gonad (0)					NA
	Hair (29)	0.1623 (4)	0.2564 (10)	0.34 (5)	1.177 (10)	0.22
	Kidney (24)	0.186 (3)	0.072 (6)	0.1268 (5)	0.2714 (10)	0.42
	Liver (25)	0.2472 (3)	0.134 (8)	0.1958 (5)	0.2804 (9)	0.82
	Muscle (1)					NA
Magnesium						

	Blood (9)					NA
	Brain (29)	141.2[a,b] (5)	145.2[c,d] (9)	160.2[a,c] (5)	157.5[b,d] (10)	<0.001
	Gonad (18)	126.35 (2)	123 (5)	137 (3)	128.5125 (8)	0.74
	Hair (29)	109.6075 (4)	115.362 (10)	144.3 (5)	125.04 (10)	0.39
	Kidney (30)	128.8[a] (5)	135.7 (10)	154[a] (5)	150 (10)	0.004
	Liver (30)	152.1[a,b] (5)	157.9[c,d] (10)	185[a,c] (5)	175[b,d] (10)	<0.001
	Muscle (30)	242.3 (5)	231.8[a] (10)	265 (5)	274[a] (10)	0.02
Manganese						
	Blood (2)					NA
	Brain (30)	0.3114 (5)	0.3272 (10)	0.368 (5)	0.37 (10)	0.06
	Gonad (19)	1.2725 (2)	0.2843 (6)	0.3467 (3)	0.365 (8)	0.61
	Hair (29)	5.5905 (4)	21.5249 (10)	15.9 (5)	19.39 (10)	0.1
	Kidney (30)	4.2762 (5)	1.0959 (10)	1.3 (5)	1.207 (10)	0.06
	Liver (30)	2.4262 (5)	2.8804 (10)	3.06 (5)	2.191 (10)	0.06
	Muscle (27)	0.1604 (5)	0.2223 (10)	0.1925 (4)	0.5475 (8)	0.17
Mercury						
	Blood (9)					NA
	Brain (19)	0.0099 (3)	0.0225 (7)	0.034 (2)	0.0310 (7)	0.6
	Gonad (11)					NA
	Hair (28)	0.5587 (4)	0.463 (10)	0.855 (4)	0.927 (10)	0.74
	Kidney (30)	0.177 (5)	0.1532 (10)	0.1734 (5)	0.1921 (10)	0.96
	Liver (28)	0.156 (5)	0.6778 (10)	0.5408 (4)	0.6298 (9)	0.68
	Muscle (24)	0.0193 (3)	0.0195 (8)	0.025 (3)	0.365 (10)	0.05 (*)
Molybdenum						
	Blood (11)					NA
	Brain (7)					NA
	Gonad (10)					NA
	Hair (13)					NA
	Kidney (30)	0.3154 (5)	0.3887 (10)	0.444 (5)	0.423 (10)	0.17
	Liver (30)	0.8599 (5)	0.9448 (10)	1.118 (5)	0.8113 (10)	0.39
	Muscle (11)					NA
Nickel						
	Blood (1)					NA
	Brain (8)					NA
	Gonad (4)					NA
	Hair (19)	0.0714 (1)	0.2745 (2)	0.2018 (5)	0.2666 (10)	0.73
	Kidney (11)					NA
	Liver (9)					NA
	Muscle (9)					NA
Potassium						
	Blood (29)	944.8 (5)	910.3 (10)	809.5 (5)	703.6 (10)	0.09
	Brain (30)	3470 (5)	3499 (10)	3870 (5)	3635 (10)	0.05 (*)
	Gonad (19)	2233 (2)	2361.5 (6)	2820 (3)	2627.5 (8)	0.77
	Hair (30)	270.6[a] (5)	190.7[b,c] (10)	714[a,b] (5)	685[c] (10)	<0.001
	Kidney (30)	1476.6 (5)	1824.3 (10)	1896 (5)	1793 (10)	0.91
	Liver (30)	1956[a] (5)	2000[b] (10)	2300[a,b] (5)	2090 (10)	0.007
	Muscle (30)	3686.8 (5)	3617.3 (10)	4048 (5)	3728 (10)	0.42
Selenium						
	Blood (30)	0.454 (5)	0.477 (10)	0.434 (5)	0.459 (10)	0.84
	Brain (30)	0.2584 (5)	0.2796 (10)	0.266 (5)	0.304 (10)	0.6
	Gonad (18)	0.8123 (2)	0.542 (5)	0.52 (3)	0.5125 (8)	0.21
	Hair (30)	2.57 (5)	3.4 (10)	2.9 (5)	4.7 (10)	0.04 (*)
	Kidney (30)	2.0122 (5)	1.8136 (10)	1.84 (5)	1.94 (10)	0.34

	Liver (30)	1.1159 (5)	1.2654 (10)	1.026 (5)	1.148 (10)	0.8
	Muscle (30)	0.25[a] (5)	0.35 (10)	0.3 (5)	0.41[a] (10)	0.004
Silver						
	Blood (0)					NA
	Brain (7)					NA
	Gonad (0)					NA
	Hair (7)					NA
	Kidney (0)					NA
	Liver (8)					NA
	Muscle (1)					NA
Sodium						
	Blood (30)	3592 (5)	2711 (10)	3274 (5)	3029 (10)	0.05 (*)
	Brain (30)	1233.2 (5)	1207.66 (10)	1354 (5)	1306 (10)	0.11
	Gonad (19)	1572 (2)	1129.9 (6)	1443.3 (3)	1408.8 (8)	0.32
	Hair (17)	225.1 (1)	306.1 (1)	187.6 (5)	138.2 (10)	0.12
	Kidney (30)	1580.32 (5)	2039.5 (10)	2214 (5)	2124 (10)	0.07
	Liver (30)	1715.2 (5)	1653 (10)	1458 (5)	1535.4 (10)	0.38
	Muscle (30)	581.9 (5)	684.04 (10)	601 (5)	736.3 (10)	0.85
Strontium						
	Blood (13)					NA
	Brain (15)	0.0236 (2)	0.0303 (8)	0.071 (1)	0.0548 (4)	0.08
	Gonad (11)					NA
	Hair (25)	0.5582 (1)	1.4386 (9)	1.122 (5)	1.099 (10)	0.27
	Kidney (22)	0.3544 (4)	0.0417 (8)	0.0623 (3)	0.0511 (7)	0.27
	Liver (13)					NA
	Muscle (8)					NA
Thallium						
	Blood (0)					NA
	Brain (0)					NA
	Gonad (0)					NA
	Hair (0)					NA
	Kidney (0)					NA
	Liver (0)					NA
	Muscle (0)					NA
Vanadium						
	Blood (0)					NA
	Brain (0)					NA
	Gonad (1)					NA
	Hair (21)	(0)	0.2089 (10)	0.1717 (3)	0.3075 (8)	0.51
	Kidney (5)					NA
	Liver (23)	0.1997 (2)	0.2894 (10)	0.0803 (3)	0.2648 (8)	0.32
	Muscle (2)					NA
Zinc						
	Blood (30)	10.24 (5)	9.34 (10)	6.08 (5)	6.53 (10)	0.22
	Brain (30)	13.7 (5)	13.9 (10)	14.9 (5)	16.5 (10)	0.017 (*)
	Gonad (19)	22.2 (2)	13.3 (6)	15.6 (3)	16.0 (8)	0.21
	Hair (30)	161.0 (5)	250.4 (10)	225.6 (5)	232.8 (10)	0.23
	Kidney (30)	51.77 (5)	20.49 (10)	21.74 (5)	21.28 (10)	0.98
	Liver (30)	36.0 (5)	38.4 (10)	43.9 (5)	45.3 (10)	0.27
	Muscle (30)	62.3 (5)	50.99 (10)	60.96 (5)	57.76 (10)	0.23

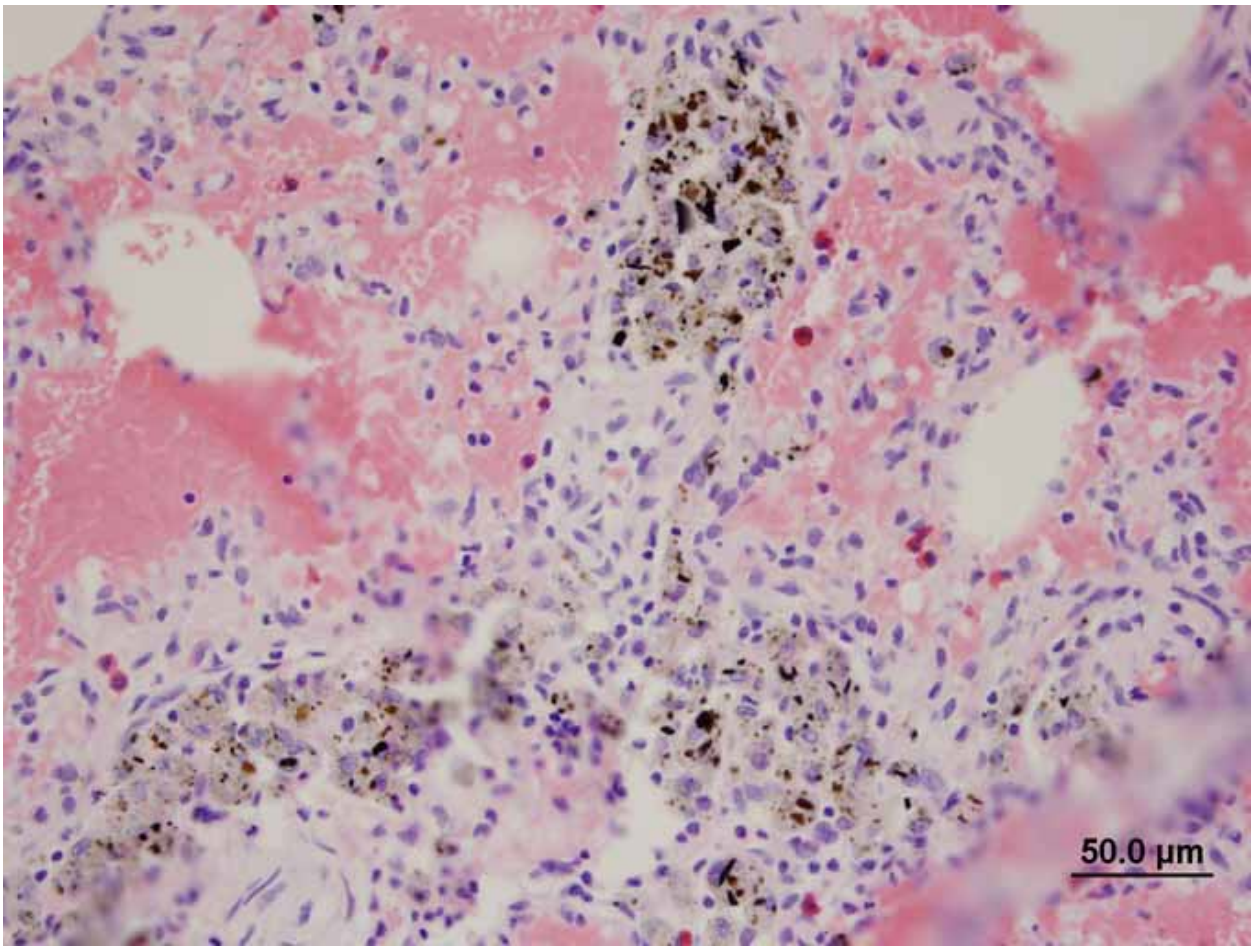

Figure 1: Pulmonary anthracosis was identified in 19 animals (7 control animals, 12 exposed animals). The dark staining in this figure is consistent with anthracosis. There were no significant differences between control and exposed animals in the prevalence of pulmonary anthracosis or any other pathologic lesions observed.