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Source: Journal of Wildlife Diseases, 16(2) : 175-182

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-16.2.175

BASELINE LEVELS OF SELECTED TRACE ELEMENTS IN COLORADO OIL SHALE REGION ANIMALS

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Abstract: Baseline levels of boron, fluorine, molybdenum, and copper are described for 18 mule deer *(Odocoileus hemionus)* and for 45 composite samples of deer mice *(Peromyscus maniculatus)* from the Piceance Creek Basin, Rio Blanco County, Colorado. These data were collected before oil shale mining took place, and can be used to compare with levels found after mining is initiated. The data can thus be used to monitor changes in levels in animal tissues and as a basis for mitigating possible harmful effects due to the mining.

Mean ppm (± S.D.) dry basis of each element is presented for selected tissues of each species. Results are also presented by habitat type for deer mice and by age for mule deer. Significant differences ($P < 0.05$) in molybdenum levels in deer mice were found between habitats. Significant differences ($P < 0.05$) were found between fawns and adult mule deer for boron levels, but not for the other elements. A need to standardize bone selection for analysis of fluorine was indicated. Kidneys appeared to be the organ of choice for baseline sampling of molybdenum and copper, and livers may be the organ of choice when toxic levels are suspected.

INTRODUCTION

A major concern about oil shale mining is the possibility of mining processes releasing toxic levels of trace elements into the ecosystem. Among those elements are boron, molybdenum, and fluorine. These elements could enter the biotic system by being carried by airborne particulates during the oil shale retorting process, or by the leaching, percolation, and runoff of water from above or below ground storage piles of overburden and spent shale.[4] Copper is also of concern because of its relationship with molybdenum and these two elements' effects on each other's toxicity in animal bodies.[14]

Two prototype oil shale tracts, C-a and C-b, will soon begin operation in the Piceance Creek Basin, Rio Blanco County, Colorado (Fig. 1). Since these are the first attempts at shale mining in Colorado, no information is available concerning normal trace element levels in the wild fauna. The specific objective of this study was to determine baseline levels of boron, molybdenum, fluorine and copper in deer mice *(Peromyscus maniculatus)* and mule deer *(Odocoileus hemionus)* from the Piceance Basin before the mining process began. These levels could be used to compare with levels found after mining is initiated to monitor changes in levels in animal tissues. These changes would be a basis for mitigating possible harmful effects due to the mining. Additional objectives were to determine if differences in levels of these elements occurred in deer mice living in different habitats, or if differences occurred in mule deer due to age.

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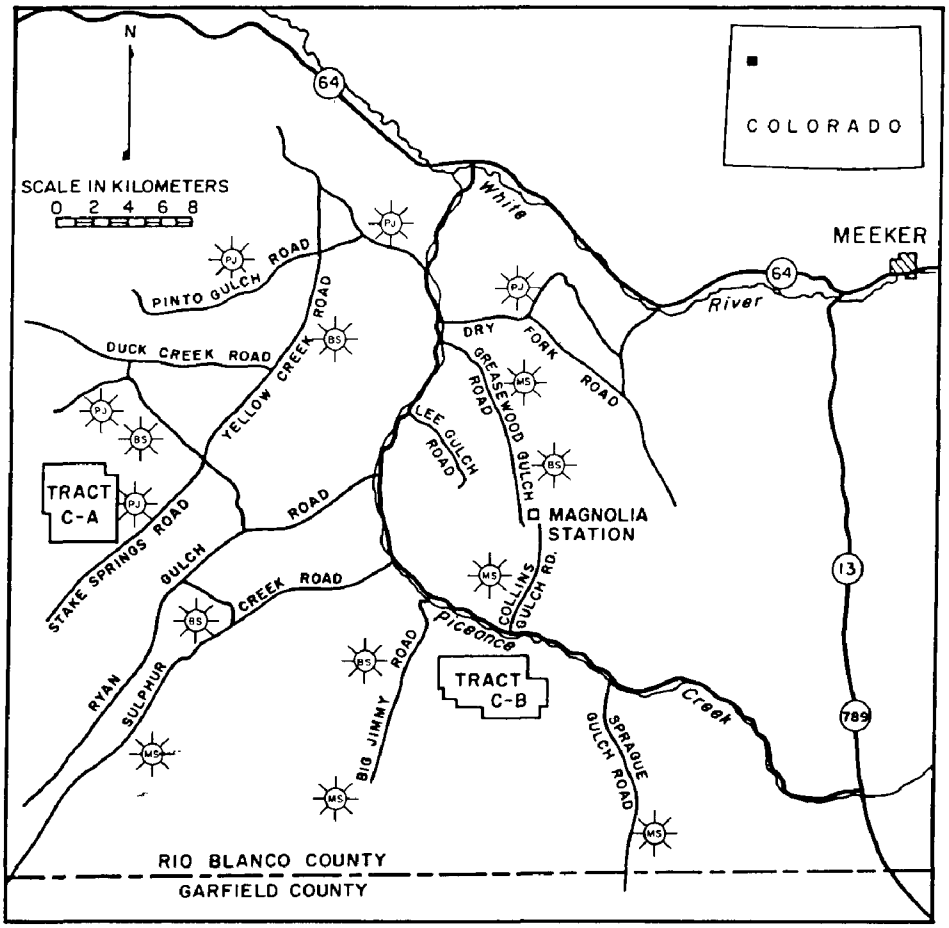

FIGURE 1. Locations of sampling points for deer mice *(Peromyscus maniculatus)* for baseline tissue trace element determinations in the Piceance Creek Basin, Colorado, during June, 1978. PJ = pinyon-juniper habitat, BS = big sagebrush habitat, MS = mountain shrub habitat. Locations of oil shale tracts C-a and C-b are also shown.

METHODS

Deer mice and mule deer were selected as species to be sampled because they were among the most ubiquitous and easily obtained animals in the Piceance Basin, in summer and winter, respectively. Their ubiquity was desirable because they could act as indicator species for trace element contamination over the entire basin. Deer mice, because they lacked the greater mobility of mule deer, could also act as an indicator species within specific habitats within the basin.

Deer mice were collected during June, 1978 from three habitats within the basin. The habitats were those dominated by either pinyon-juniper *(Pinus edulis-Juniperus osteosperma)*, big sagebrush *(Artemisia tridentata)*, or mixed mountain shrub [mixture of serviceberry *(Amelanchier* spp.), mountain mahogany *(Cercocarpus montanus)*,

oakbrush *(Quercus gambeli),* bitterbrush *(Purshia tridentata),* and big sagebrush]. Three composite samples of three mice each were live-trapped from five different locations within each habitat (Fig. 1). The captured animals were killed and carcasses were immediately frozen until they could be prepared for chemical analyses. Age comparisons for deer mice were not made because accurate methods for aging were not available.[12] Eighteen mule deer were shot during January, 1979. Specific organs were removed and frozen until analysis. Since mule deer are much more mobile than deer mice and are found in all habitat types, no attempt was made to collect from any particular points within the basin area. Mule deer were aged by tooth eruption[13] and were classified as either fawns (approximately 0.5 yr old) or adults (1.5 yr and older).

Livers and kidneys were selected for analysis of molybdenum[3,14] and copper,[14] and bones for boron[14] and fluorine.[1,3,14] Entire organs and skeletons from all three deer mice in each composite sample were analyzed. Skeletons were cleaned of other tissue as well as possible. Laboratory analysis of whole organs or skeletons for mule deer was impractical. Instead, a composite of several small pieces cut from the liver and each kidney of each animal, and a small section cut from the middle of the metacarpus of each animal, were analyzed.

All samples were weighed, dried, weighed again, ashed at 450 C for 12 h, and dissolved in hydrochloric acid. Boron levels were quantified by the spectrophotometric method using azomethine-H (Pierce Chemical Co., Rockford, Ill.) as a complexing agent.[5] Fluorine was separated from the ashed sample by gaseous diffusion and quantified by molecular absorption spectrophotometry.[10] A Sargent-Welch Model SD spectrophotometer was used for analysis of boron and fluorine. Because molybdenum was found in low quantities, it was preconcentrated by a thiocyanate complexing agent and submitted to solvent extraction using isoamyl alcohol. Quantification was by nitrous oxide atomic absorption spectrophotometry, or in the case of extremely low concentrations, graphite furnace atomic absorption spectrophotometry.[2] Copper was quantified by flame atomic absorption spectrophotometry.[2] A Perkin-Elmer Model 5000 spectrophotometer was used for analysis of molybdenum and copper. All data were expressed in ppm dry weight. Precision of laboratory procedures was determined by retesting a random sample of 10% of all samples, where enough sample material existed for retesting. Samples that yielded results that appeared spurious were also retested. Precision for all samples was within 10% of the mean sample value 90% of the time.

When exact levels of elements were obtained, statistics were employed to describe the levels of each element by species, for all animals of that species combined. T-tests ($P < 0.05$) were used to compare levels of each element between habitats for deer mice and levels of each element between fawns and adult mule deer.

RESULTS AND DISCUSSION

Baseline levels of the elements, mean sample weights of analyzed tissues, and statistical analyses are presented in Table 1 for deer mice and in Table 2 for mule deer.

Mule deer fawns had significantly more boron in the metacarpals than did adults. Kaufman and Kaufman[7] reported that immature old-field mice *(Peromyscus polionotus)* had significantly less boron than did adults, as determined by whole body analysis. No explanation can be given for these associations at this time, since boron has yet to be identified as essential for any physiological process in animals. The conflicting results indicate that boron may accumulate during different ages in

TABLE 1. Mean (± S.D., where applicable) trace element levels (ppm dry wt) in deer mice *(Peromyscus maniculatus)* collected from three habitats in the Piceance Creek Basin, Rio Blanco County, Colorado, during June, 1978. Sample size indicated is number of composite samples of three mice each. Numbers in parentheses are ranges in data values.

	Whole Skeleton Mean Sample Wt = 6.3g		Liver Mean Wet Sample Wt = 2.7g		Kidney Mean Wet Sample Wt = 0.7g	
Habitat	Boron	Fluorine	Molybdenum	Copper	Molybdenum	Copper
Pinyon-juniper N=15	≤2	51.3±17.6 (31-100)	6.5±1.4 (4.4-9.0)	23.3±2.4 (19-29)	5.7±0.7 (4.2-6.9)	22.0±4.4 (18-37)
Mt. Shrub N=15	≤2	56.3±32.9 (19-120)	8.0±1.8 (5.8-12.0)	75.4±146.9 (20-622)	6.9±1.2 (4.9-8.7)	22.7±2.3 (18-26)
Big Sagebrush N=15	≤2	38.2±22.9 (16-93)	7.6±1.3 (5.7-10.0)	40.1±56.0 (18-249)	7.8±0.7 (6.8-9.2)	22.6±2.1 (21-27)
Sig. Diff. Among Habitats[1]	—	None	MS,BS>PJ[2]	None	BS>MS>PJ[2]	None
All Habitats Combined N=45	≤2	48.8±26.5 (16-120)	7.4±1.6 (4.4-12.0)	46.3±93.3 (18-622)	6.8±1.2 (4.2-9.2)	22.4±3.2 (18-37)

[1]T-tests ($P < 0.05$) were used to compare levels among habitats.
[2]PJ = Pinyon-Juniper, MS = Mountain Shrub, BS = Big Sagebrush.

different species. In four species of wild, xeric rodents, Wiener *et al.*[16] reported levels of 4.3-6.3 ppm dry basis of boron. In the present study, skeletons of deer mice had no more than 2 ppm. The xeric rodents probably had accumulated much more boron in their skeletons than did the deer mice in this study. This would be reasonable since bones are the primary accumulation sites of boron[14] and by considering the diluting factor that the whole body analyses had on the skeletal boron concentrations of the xeric rodents.

Exact levels of boron were not presented for deer mice because there was not enough bone in each composite sample of three mice (mean sample weight = 6.3 g) to analyze more precisely. This was not a problem for mule deer, where mean sample weight of metacarpal used was 18.5 g. The necessary minimum amount of bone needed for colorimetric analysis obviously lies somewhere between these two weights, and more than three deer mice are necessary to obtain this amount of bone.

Fluorine levels in deer mice fluctuated between habitats. There was also great variability within habitats. Fluorine levels for mule deer were not reported precisely because levels were too low in the metacarpals for more exact quantification by current laboratory procedures. This also prevented age comparisons for mule deer. Based on the literature and the data from the present study, fluorine accumulates to different levels in different bones, and levels also vary by species. Karstad[6] and Newman and Yu[11] reported various levels of fluoride that they considered "normal" for white-tailed deer *(Odocoileus virginianus)* and black-tailed deer *(Odocoileus hemionus columbianus)*, respectively. Karstad used mandibles (167-465 ppm) and antlers (134-152 ppm), and Newman and Yu used ribs (157-465 ppm), metatarsals (89-442 ppm), and a digit (54 ppm). Kay *et al.*[8] used seven different bones of mule deer and white-tailed deer and found differences between species and high variations in the same bones within each species. The metacarpal samples from the present study do not compare well with any bones from the previously cited deer literature. In the present study entire skeletons of deer mice had many times the fluoride levels of the metacarpals of the mule deer. These data indicate a need for standardization of bone sampling for fluorine, at least within each species.

Molybdenum and copper results obtained in this study are consistent with generalizations made by Underwood[14] in that species differences were noticeable for molybdenum in both liver and kidney, and for copper in the liver. However, the present study shows no statistically significant differences in levels of either molybdenum or copper due to age in mule deer. Liver copper levels fluctuated much more than kidney copper levels both between and within most categories of comparison for both species. Kidneys may then be the organ of choice for sampling normal or baseline levels of copper and molybdenum in the future. Liver, because of its ability to accumulate higher levels of these elements, may be the organ of choice when sampling for suspected toxic levels.

Statistically significant differences between habitats were shown for molybdenum levels of both liver and kidney samples from deer mice, though which habitats were different depended on which organ was sampled (Table 1). No specific reason can be given for the habitat variation of this element. Differing amounts of molybdenum in the soil or in the food plants among deer mice habitats are the most likely reasons. Wiener *et al.*[16] reported whole body molybdenum levels in four species of wild xeric rodents (3.1-3.8 ppm dry weights).

Kienholz[9] found means of 1.7 ppm dry weight of molybdenum and 120 ppm dry weight of copper in livers from 10 mule deer collected near Rifle, Colorado,

TABLE 2. Mean (± S.D., where applicable) trace element levels (ppm dry wt) in mule deer *(Odocoileus hemionus)* tissues collected from Piceance Creek Basin area, Rio Blanco County, Colorado, during January, 1979. Numbers in parentheses are ranges in data values.

	Metacarpus		Liver		Kidney	
	Mean Sample Wt = 19g		Mean Wet Sample Wt = 28g		Mean Wet Sample Wt = 21g	
	Boron	Fluorine	Molybdenum	Copper	Molybdenum	Copper
Adults N=12	1.4±0.3 (0.8-1.8)	≤0.3	1.3±0.7 (0.6-2.8)	61.4±34.0 (19-114)	2.1±1.0 (0.9-4.0)	28.6±15.0 (15-65)
Fawns N=6	2.3±0.8 (1.2-3.6)	≤0.5	1.3±0.6 (0.7-2.4)	97.3±59.6 (45-227)	1.9±0.4 (1.3-2.4)	26.5±4.0 (23-32)
Sig. Diff. Between Age Groups[1]	Yes	—	No	No	No	No
All Deer Combined N=18	1.7±0.7 (0.8-3.6)	≤0.4	1.3±0.7 (0.6-2.8)	73.4±47.3 (19-227)	2.0±0.9 (0.9-4.0)	27.9±12.5 (15-65)

[1]T-tests ($P < 0.05$) were used to compare levels between adults (1.5 yr and older) and fawns (0.5 yr old).

located about 30 km south of the Piceance Basin. The mean molybdenum level was thus close to mean results obtained in the present study. However, Kienholz's mean copper level was considerably higher. Given the rather large variations in liver copper in the present study, Kienholz's mean copper level seems rather high, but not surprisingly so. Wiener *et al.*[15] found mean levels of 3.0 ppm molybdenum and 26.1 ppm copper in whole body samples of wild white-tailed deer in South Carolina.

Essentially nothing is known of toxic levels of any of these elements in these two species. Karstad[6] and Newman and Yu[11] reported levels of fluoride in various bones of white-tailed deer (4300 ppm) and black-tailed deer (2048 ppm), respectively, that were associated with fluorosis in the animals. Neither gave specific levels at which detrimental effects began to occur. To be able to list precise toxic levels for each species, other data would have to be compiled. These data include the chemical form in which the elements are found in the diet, the availability of specific chemical forms in a given species' diet, the absorption rate of those forms by the animal, and levels of various other elements in the diet that affect toxicity.[14] Research directed toward defining toxic levels or boron, molybdenum, fluorine, and copper for animals in the oil shale region, along with monitoring the levels throughout the mining process, is strongly recommended.

Acknowledgements

I am grateful to Dr. Robert Meglen and the staff of the Environmental Trace Substances Research Program of the University of Colorado, Boulder, for performing the chemical analyses. This project was funded by the Office of Biological Services, U.S. Fish and Wildlife Service through a contract to Dr. Julius Nagy, Dept. of Fishery and Wildlife Biology, Colorado State University, Fort Collins. I thank Jim Durr and Jon Haufler for aiding in the deer collections.

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Received for publication 5 October 1979