SELENIUM AND MINING IN THE POWDER RIVER BASIN, WYOMING: PHASE III - A PRELIMINARY SURVEY OF SELENIUM CONCENTRATIONS IN DEER MICE (*Peromyscus maniculatus*) LIVERS[1]

M.L. Raisbeck, D.G. Steward, Laurel E. Vicklund, George F. Vance, Lowell K. Spackman, and Jim G. Luther[2]

Abstract: Samples of liver tissue from deer mice trapped on not-yet-mined areas and reclaimed areas at five surface coal mines in the Powder River Basin of northeastern Wyoming were analyzed for selenium. The overall mean concentration of selenium in wet weight liver tissue was 1.685 ppm. The mean value from not-yet-mined areas was 1.437 ppm; the mean value from reclaimed areas was 1.910 ppm (significant at $p<0.1016$). When one not-yet-mined outlier was removed, significance rose to $p<0.0004$. Mine-to-mine comparison of samples stratified by type (that is, by not-yet-mined or reclaimed), showed average tissue concentrations from the reclaimed area of Mine 1 to be higher ($p<0.0143$) than reclaimed area samples from each of the other mines. Average tissue concentrations from the reclaimed area of Mine 1 were also higher ($p<0.0143$) then not-yet-mined area samples at Mine 1. No statistically significant differences were found between mines for samples from not-yet-mined areas, and no statistically significant differences were found between Mines 2, 3, 4, and 5 for samples from reclaimed areas. Multiple analysis of variance using the factors: site (mine) and type (not-yet-mined or reclaimed) was not statistically significant ($p<0.2115$). Simple linear regression showed that selenium concentrations in dry tissue could easily be predicted from wet tissue selenium ($r2=0.9775$), demonstrating that percent water in the samples was relatively constant. Animal body weight in general was not a predictor for either wet or dry tissue selenium concentrations, but was related to body weight at the higher tissue concentrations of selenium encountered in samples from the reclaimed area at Mine 1. Mouse body weights at Mine 1 were higher on the reclaimed area than mouse body weights from the not-yet-mined area.

Additional Key Words: surface coal mining, reclamation

Introduction

Surface coal mining in Wyoming is regulated by the Department of Environmental Quality. In 1991, that agency fostered the development of joint subcommittees, with members drawn from the regulatory agency, the surface coal mining industry, and the academic community, to investigate the relationship between selected environmental variables and the effects of selenium released or potentiated by surface coal mining. Interest in selenium was stimulated by reports of selenium toxicity in wildlife at various irrigation projects in the western United States. Concern was expressed by some that removal and replacement of overburden during mining might enhance the availability selenium in the postmining environment.

---

[1]Paper presented at the 1995 National Meeting of the American Society for Surface Mining and Reclamation, Gillette, Wyoming, June 5-8, 1995.

[2]Submitted by the Wyoming State Subcommittee on Selenium in Soils, Vegetation, Overburden, and Wildlife: M.L. Raisbeck, Department of Veterinary Toxicology, University of Wyoming, Laramie, WY; D.G. Steward, AMAX Coal West, Inc., Gillette, WY 82717; Laurel E. Vicklund, Cordero Mining Company, Gillette, WY 82717; George F. Vance, Plant, Soil and Insect Sciences, University of Wyoming, Laramie, WY 82071, Lowell K. Spackman and Jim G. Luther, WDEQ-LQD, Cheyenne, WY 82002

Proceedings America Society of Mining and Reclamation, 1995 pp 354-361
DOI: 10.21000/JASMR95010354

One subcommittee: The Joint Subcommittee on Selenium in Soils, Vegetation, Overburden, and Wildlife, developed a three-phase plan for studying selenium at surface mines in the Powder River Basin of Wyoming. Phase III of the study was designed to investigate the effects of selenium on selectedtarget organisms. The first stage of the third phase, presented here, is a preliminary survey of selenium concentrations in the livers of deer mice (*Peromyscus maniculatus*), a small mammal common on not-yet-mined areas and reclaimed areas at surface mines in the Powder River Basin. Phases I and II of this subcommittee's efforts are discussed elsewhere in this publication (see, for example, Vicklund et al. 1995).

Deer mice were selected for the study based on the size of their home range, their diet, and their abundance in the study area. Liver tissue was chosen for analysis because liver is one of the prime locations of bioaccumulation in the body (Osweiler, et al. 1986). Deer mice have small home ranges (typically less than an acre) and are somewhat omnivorous. Their diet includes insects, fungi, and carrion, but is composed primarily of seeds, fruits, and shoots (Clark and Stromberg 1987). It was assumed that anything consumed by the deer mouse would not travel much further than would the deer mouse itself, and the diet would thus reflect, to a certain degree, the availability of selenium in the soil, vegetation, and water in an area about the size of the deer mouse's home range. Deer mice may thus serve as useful of indicators selenium availability in relatively small areas. However, this inventory of selenium concentrations in deer mice livers does not presume to illuminate any cause and effect relationship between tissue concentrations and concentrations in the diet, or in the surrounding environment. Further study, including detailed laboratory study, is necessary to define a causal relationship.

No literature on tissue concentrations of selenium in wild deer mice could be found. However, some articles gave specific tissue concentrations on laboratory mice. For example, Toyoda et al. (1989) indicated that dietary concentrations of 0.4 ppm sodium selenite will result in liver tissue concentrations of approximately 1.3 ppm after three weeks of feeding. This level of dietary selenium is well below the 5 ppm often cited as the limit of dietary safety (Thatcher 1961). Data from the Wyoming State Veterinary Laboratory (personal communication) indicate that concentrations of 1.5 to 12 ppm can occur in mice after eight weeks of exposure to 7 ppm of selenium in their drinking water. The lack of tissue concentration and toxicological information precluded definitive conclusions about the meaning of the selenium tissue concentrations presented in this paper.

Methods

Sample Numbers

The original project design called for five animals from reclaimed areas and five animals from not-yet-mined areas to be taken from each of five mines in the Powder River Basin of northeastern Wyoming. These mines are located within 50 miles of Gillette, Wyoming. Additional animals were trapped on some of the mines, resulting in 63 samples (instead of the originally planned 50) for analysis.

Capture Methods and Handling of Samples

Mice were trapped on 01 and 02 November 1994, and identified in the field with the use of standard field guides (Burt and Grossenheider 1976, Clark and Stromberg 1987). All were captured in Museum Special snap traps, baited with a mixture of peanut butter and birdseed. The traps were initially set on 31 October; captures were collected and traps reset daily.

Disposable surgical gloves and face masks were used to protect the field technician from potential exposure to hanta virus. Each capture was weighed and bagged separately in a Ziploc storage bag, with all data recorded on the bag. Samples were immediately placed on ice in the field, and refrigerated within 12 hours of collection. Refrigerated samples were then shipped on ice in a cooler for next day arrival at the University of Wyoming Veterinary Toxicology Laboratory in Laramie, Wyoming.

Laboratory Analysis

Tissue samples were analyzed for selenium by a well-established fluorometric method (Dolan et al. 1985). In this method, samples are digested with nitric acid, magnesium nitrate, and gently increasing heat for 48 hours together with appropriate controls and standards. After digestion, selenium in the digest is reduced to Se^{+4} with HCl, reacted with diaminonapthalene, and quantitated fluorometrically using a Shimadzu RF-535 fluorescence detector (Shimadzu Instrument, Tokyo, Japan).

Data Analysis

Data were encoded in Lotus spreadsheet format, and transcribed to ASCII format. Descriptive statistics, analysis of variance, and regression analysis were then conducted using SAS PC.

Results and Discussion

Table 1 summarizes the results of the liver tissue inventory. The overall mean level of selenium in wet weight liver tissue was 1.685 ppm. The mean value from not-yet-mined areas was 1.437 ppm; the mean value from reclaimed areas was 1.910 ppm. The largest value encountered was from a not-yet-mined area; at 8.058 ppm (from Mine 3), this was twice the highest level encountered on any of the reclaimed areas. The overall difference between not-yet-mined areas and reclaimed areas was not significant at $p<0.1016$ ($F=2.76$).

However, as shown on Table 1, selenium concentrations were higher for mice from reclaimed areas than from not-yet-mined areas, except for samples taken at Mine 3. When the outlier of 8.058 ppm was removed from the data set, the Mine 3 average reclaimed concentrations rose slightly above the Mine 3 not-yet-mined concentrations (1.545 reclaimed versus 1.176 not-yet-mined, $F=1.57$; $p<0.2319$). Removal of the outlier from the data set in which all mines were included made the difference between average reclaimed concentrations and average not-yet-mined concentrations significant (1.910 ppm versus 1.208 ppm, $F=14.05$, $p<0.0004$).

Liver selenium concentrations were compared between mines with the samples were stratified by type (that is, by not-yet-mined or reclaimed). In this comparison, the samples from the reclaimed area of Mine 1 were statistically significantly different ($F=9.16$, $p<0.0143$) from the reclaimed areas of all other mines. No statistically significant differences were found between mines for not-yet-mined samples. Graphical analysis (Figure 1) shows the relationship between Mine 1 and the other four mines sampled.

Mine 1 exhibited a distinct pattern in the data, with all not-yet-mined samples having lower selenium concentrations than the reclaimed samples (Figure 1). Only Mine 3 (outlier removed) exhibited this same data pattern, although selenium concentrations at Mine 3 were not so high as at Mine 1. Overall selenium concentrations (both not-yet-mined and reclaimed) were highest at Mine 1. At Mines 2, 4, and 5, selenium concentrations in mouse liver samples were not stratified between not-yet-mined and reclaimed, but were intermingled. With the exception of Mine 3, the lowest tissue concentration found at each mine was on the not-yet-mined area and the highest tissue concentration was found on the reclaimed area. At Mine 3, the lowest tissue concentration was obtained from the reclaimed area and the highest tissue concentration was found on a not-yet-mined area.

Table 1.
Selenium Concentrations (ppm) in Wet Liver Tissue of Deer Mice

Overall Concentrations							
mean sd range n	1.685 1.146 0.343 - 8.058 63						
		Mine 1	**Mine 2**	**Mine 3**	**Mine 4**	**Mine 5**	**Pr>F**
mean sd range n		2.234 1.221 0.782 - 4.613 11 (a)	1.412 0.336 1.102 - 2.410 12 (a)	1.837 1.742 0.352 - 8.058 16 (a)	1.272 0.722 0.504 - 3.356 12 (a)	1.664 0.817 0.343 - 3.236 12 (a)	F: 1.29 0.2859

Not-yet-mined							Reclaimed						Pr>F
mean sd range n	1.437 1.322 0.343 - 8.058 30 (a)*						1.910 0.921 0.352 - 4.613 33 (a)						F: 2.76 0.1016
	Mine 1	**Mine 2**	**Mine 3**	**Mine 4**	**Mine 5**	**Pr>F**		**Mine 1**	**Mine 2**	**Mine 3**	**Mine 4**	**Mine 5**	**Pr>F**
mean sd min max n	1.328 0.406 0.782 1.774 5 (a)* (i)**	1.254 0.134 1.102 1.422 6 (a) (iii)	2.323 2.822 0.850 8.058 6 (a) (iv)	1.019 0.348 0.504 1.568 7 (a) (v)	1.311 0.793 0.343 2.593 6 (a) (vi)	F: 0.88 0.4874		2.989 1.160 1.844 4.613 6 (a) (ii)	1.571 0.414 1.360 2.410 6 (b) (iii)	1.545 0.617 0.352 2.303 10 (b) (iv)	1.627 0.991 0.845 3.356 5 (b) (v)	2.016 0.736 1.196 3.236 6 (b) (vi)	F: 3.76 0.0143

* Means followed by different letters are significantly different at $p<0.01$ within the not-yet-mined or reclaimed type.

** Roman numerals represent pairwise comparison between mines of not-yet-mined and reclaimed types. Different roman numerals between the pairs indicate significant difference at $p<0.0143$ (F:9.16).

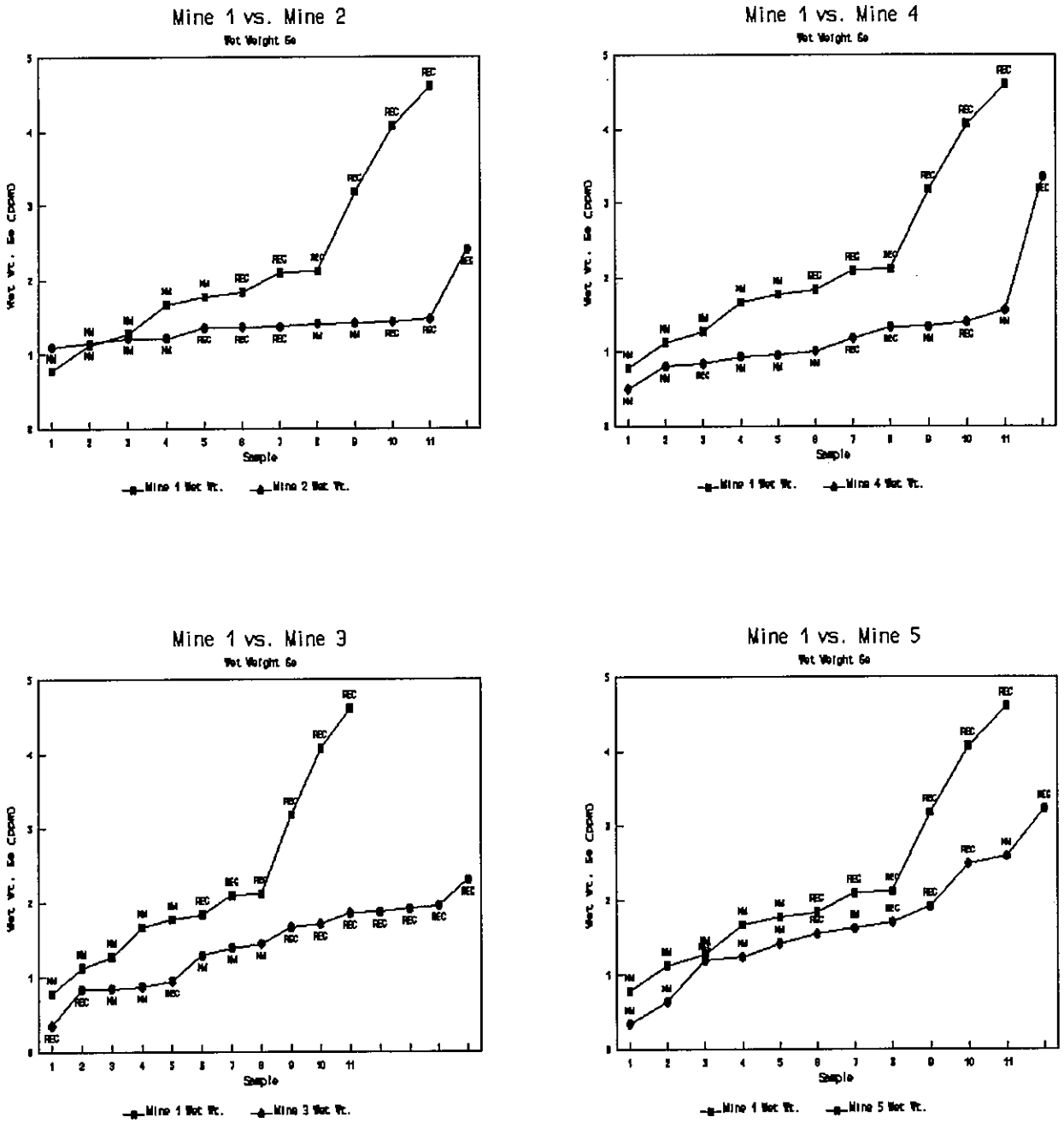

FIGURE 1.

Selenium concentration in liver tissue samples based on wet tissue weight. Mines 2, 3, and 4 graphed in comparison to Mine 1, the mine with the highest overall tissue concentrations. A sample is one mouse liver. There are at least five samples for each reclaimed area and each not-yet-mined area of each mine. Some mines have more than five samples in each of these areas.

NM: Not-yet-mined REC: Reclaimed

Figure 2 shows the relationship between tissue selenium concentration and body weight for Mine 1, where the heaviest mice and the highest tissue concentrations were found. Clearly, the heavier mice were found on the reclaimed area and their tissue concentrations of selenium were elevated above those of their lighter counterparts on the not-yet-mined area of the mine. This relationship was not evident for any of the other mine sample locations simply because neither the selenium concentrations nor the body weights were high enough to illustrate the relationship. Based on the graph of body weight versus tissue concentration, it is tempting to speculate that, within the range of concentrations investigated, increased tissue selenium is related to increased body weight. Because selenium is a micronutrient, this hints more at selenium deficiency than selenium toxicity as the norm for deer mice in the Powder River Basin.

Multiple analysis of variance using two factors: site (Mines 1, 2, 3, 4, 5) and type (not-yet-mined or reclaimed), was not statistically significant ($F=1.48$, $p<0.2115$). Simple linear regression showed that selenium concentrations in dry tissue could easily be predicted from wet tissue selenium ($r^2=0.9775$), demonstrating that percent water in the samples was relatively constant. Animal body weight was not a predictor for either wet or dry tissue selenium concentrations over the entire data set. However, body weight was related to wet tissue selenium concentration with an $r^2=0.50$ at Mine 1.

Conclusion

These results indicate that further investigation into the concentrations of selenium in the liver tissue of healthy deer mice would be fruitful. Because of the limited information available in the literature, it is not possible to definitively interpret the concentrations with respect to health effects in deer mice. However, there appears to be a clear trend toward liver tissue concentrations that are elevated in animals trapped on reclaimed areas when compared to concentrations on not-yet-mined areas. It appears that higher concentrations of selenium were often encountered in the liver tissue of higher body weight animals, or, conversely, the mice with higher concentrations of selenium in the liver were also heavier.

Acknowledgements

This study was cooperatively funded by members of the coal mining industry in the Powder River Basin of Wyoming. Their support is gratefully acknowledged. Powder River Eagle Studies of Gillette, Wyoming, collected the samples in a timely and efficient manner. Graphical analysis was facilitated by J. David Green of Gillette, Wyoming.

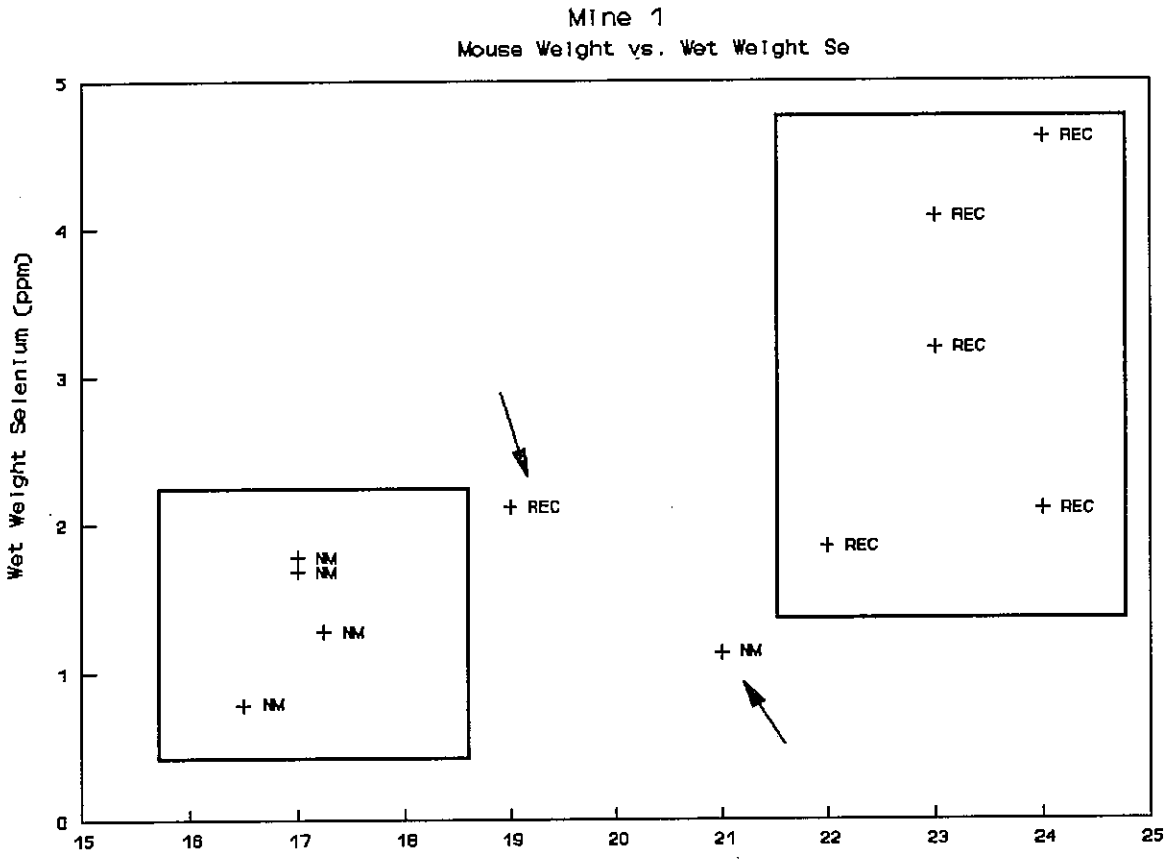

FIGURE 2

Mouse Body Weight Versus Selenium Concentration in Liver Tissue at Mine 1

Arrows Point to Outliers

NM - Sample from not-yet-mined area
REC - Sample from reclaimed area

Literature Cited

Burt, W.H. and R.P. Grossenheider. 1976. A Field Guide to the Mammals. Houghton-Mifflin, Publishers, Boston. 284 p.

Clark, T.W. and M.R. Stromberg. 1987. Mammals in Wyoming. University of Kansas Museum of Natural History, Lawrence. 314 p.

Dolan, M., K.R. Zinn, G.E. Rottinghaus. 1985. TLC, HPLC, GLC, and neutron activation determination of selenium. Proc Midwest Association of Official Analytical Chemists, 6/17-6/19, Chicago.

Osweiler, G.D., T.L. Carson, W.B. Buck, and G.A. Van Gelder. 1986. Clinical and diagnostic veterinary toxicology, 3rd edition. pg 132-142. Kendall-Hunt, Dubuque, Iowa. 494 p.

Thatcher. 1961. Selenium content of soils. In M.S. Anderson et al. (ed) Selenium in Agriculture. Agricultural Handbook No. 200. Agricultural Research Service, US Dept of Agriculture, Washington D.C. 53 pp.