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Correlative Analysis of Heavy Metal Bioconcentration and Genetic Damage in White-Footed Mice (*Peromyscus leucopus*) from a Hazardous Waste Site

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Heavy metals are common constituents of hazardous waste sites and may cause health problems in wildlife and nearby human residents. Previous studies have been conducted on the bioaccumulation/bioconcentration of metals in biological tissue using small mammals such as rodents, shrews, and mustelids (Smith and Rongstad 1981; Scanlon et al. 1983; Ogle et al. 1985); however, there have been few attempts to correlate tissue residues with other physiological or genetic biomarkers. This sort of correlative information may be critical in understanding the meaning of bioaccumulation/bioconcentration data in terms of actual physiological response and overall health of exposed organisms.

In this study, livers of Peromyscus leucopus (white footed mouse) from a hazardous waste site and a matched reference site were analyzed for the presence of selected metals. The white-footed mouse has an average total body length of 153.5 mm and prefers wooded, brushy habitat. The diet included seeds, nuts, plant material, fungi, and some invertebrates (Caire et al. 1989). Waste site animals were obtained from a facility in southcentral Texas, approximately 24 hectares in area, which has been used since the early 1960's as a fire fighting training facility. Until 1980, ignitants used on training structures included refinery waste products. Since then, only diesel fuel has been used (McBee et al. 1987; McBee and Bickham 1988). Two sludge retention ponds located on the training school grounds collect run-off such as ignitants, flame retardants, fire-fighting chemicals, and water from the practice structures. Chemical analysis of sludge and water from the ponds indicated the presence of a number of compounds including partially combusted hydrocarbons, PCB's, and several heavy metals (Brown 1980). Brown and Donnelly (1982) showed that water extracts from the retention ponds gave positive responses in the Salmonella /mammalian microsome assay and the Bacillus DNA Repair Assay, indicating that compounds present in waste water were mutagenic. McBee et al. (1987) and McBee and Bickham (1988) also found that Peromyscus trapped around the banks of the retention ponds showed significantly increased levels of somatic metaphase chromosome aberrations manifested as chromatid and chromosome breaks, acentric fragments, ring and dicentric chromosomes and translocation figures, and significantly increased variation in whole cell DNA content of splenocytes as measured by flow cytometry compared to animals from reference sites. The most likely route of exposure for these animals was through ingestion of soil particles during foraging and grooming (McBee et al. 1987).

Of metals found at the site, at least four (cadmium, chromium, lead, and zinc) have been shown to induce chromosome aberrations in mammalian cell cultures or have

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been implicated as clastogens in human worker exposure studies. Clastogenicity studies of cadmium have given conflicting reports of chromosomal damage. Bui et al. (1975) found no chromosomal aberrations in cultured human lymphocytes; whereas, Deknudt and Leonard (1975) found significant aberration increases in Japanese Itai-Itai patients. Natural chromium occurs in hexavalent (VI) and trivalent (III) states although chromium (III) is the more common form. Hexavalent chromium is considered a powerful mutagen. It causes DNA damage and gene mutation in bacterial and mammalian cells and has been shown to cause chromosomal aberrations in mammals (Levis and Bianchi 1982; Stella et al. 1982). Chromosomal aberration reports for lead have been contradictory. O'Riordan and Evans (1974) showed no significant increases in chromosomal aberrancy in humans continually exposed to lead via the workplace. Several other studies, however, indicate that an increase of chromosomal aberrancy as well as sister chromatid exchanges may occur in humans occupationally exposed to lead (Bauchinger et al. 1976; Nordenson et al. 1978; Grandjean et al. 1983). Zinc is not believed to be carcinogenic, however, it has been linked to chromosomal anomalies (Babich et al. 1985).

This study was undertaken to measure levels of these four metals in liver tissues of wild white-footed mice used in the previous genetic studies and to address possible correlations between heavy metal bioconcentration and three measures of genotoxicity.

MATERIALS AND METHODS

Liver tissue samples were obtained from 40 adult Peromyscus leucopus. Twenty-eight individuals (17 males, 11 females) were live-trapped at the hazardous waste site and 12 individuals (9 males, 3 females) were collected from a matched reference site approximately 1 km west of the contaminated site. All animals analyzed in this study were used in previous genetic toxicity tests (McBee et al. 1987; McBee and Bickham 1988). After animals were sacrificed, livers were removed and stored at a minimum of -70°C until analysis. EPA SW-846 method 3050 (Environmental Protection Agency 1986a) for acid digestion of sediments, sludges, and soils was used as modified by the Oklahoma State University Water Quality Research Laboratory for heavy metal analysis of animal tissue.

One gram samples were weighed to the nearest 0.01g . When whole livers weighed less than 1g, the weight was recorded and the entire liver was used. Ten ml of nitric acid:reagent grade water (1:1, v/v) using 70-71% concentrated nitric acid (Baker® instra-analyzed, heavy metal grade) were added to each sample and refluxed for 15 minutes at approximately 95°C in watch glass covered beakers. Beakers were cooled and 5 ml of concentrated nitric acid were added. Samples were refluxed for an additional 30 minutes. Samples were again refluxed with fresh nitric acid for 12-15 hours, until the solutions turned light lemon yellow. They were then allowed to evaporate without watch glasses until 5 ml of solution were left for each sample. The samples were cooled and 2 ml reagent grade water and 3 ml 30% hydrogen peroxide (Fisher) were added and then heated until effervescing stopped. Samples were alternately cooled and 1 ml hydrogen peroxide was added until samples remained unchanged or until the total amount of hydrogen peroxide added was 10 ml. After cooling, an additional 10 ml of reagent grade water were added and samples were allowed to reflux another 15 minutes or until no floating congealed fat was observed. Beakers were then cooled. Samples were individually poured into acid-washed 100 ml glass volumetric flasks. For each

sample, the watch glass and empty beaker were rinsed with reagent grade water. All rinse water from each sample was added to the appropriate volumetric flask. Samples were then brought to 100 ml with reagent grade water.

Analyses of cadmium, total chromium, lead, and zinc concentrations, using flame and graphite techniques, were performed using a Perkin-Elmer 5000 Atomic Absorption Spectrophotometer with a Perkin-Elmer HGA-500 Heated Graphite Atomization system and a Perkin-Elmer AS-40 Automatic Sampler. For flame analysis, acid-digested samples were introduced into the instrument via direct aspiration to an acetylene flame. Electrodeless discharge lamps were used in conjunction with Heated Graphite Atomization (HGA) for cadmium and lead analysis. Data were obtained as concentration values (mg/l). With the exception of the heat source and sample introduction, instrumentation was the same for HGA analysis. HGA analysis allows greater sensitivity for some metals such as lead and cadmium and increases precision and accuracy. Aliquots of sample (20 µl)were introduced via the automatic sampler into a small graphite tube which was heated to approximately 3000°C by electrical resistance.

Quality assurance/quality control (QA/QC) included analyzing blanks and duplicates every twenty samples. Blanks were prepared by the same acid digestion procedure as sample livers without the addition of biological tissue; whereas, duplicates were prepared from unused tissues. EPA reference standards and laboratory prepared standards were used as analytical quality controls and were analyzed to ensure proper instrument operation and calibration.

RESULTS AND DISCUSSION

Tissue metal concentration values for animals from the hazardous waste site and the reference site are given in Table 1. Several individuals had tissue residues below detection limits for chromium (0.01 ppm) and lead (0.002 ppm). For statistical analyses, these levels were recorded as 0.01 ppm and 0.002 ppm, respectively. The nonparametric Wilcoxon Rank Sum test was used to compare liver contaminant residues between waste site and reference site animals and between sexes within sites. No significant differences were found between sexes within sites. Mean lead and chromium levels were significantly higher in waste site animals compared to reference site animals but zinc and cadmium levels were not statistically different.

Cadmium and lead levels found in white-footed mice at these sites were comparable to those found in a study by Smith and Rongstad (1981) which examined eight species of mammals, including large carnivores, shrews, and small rodents such as Peromyscus maniculatus, obtained from two different natural copper-zinc deposits in Wisconsin. Zinc levels were approximately 10 mg/g higher at both sites in this study than those found by Smith and Rongstad (1981); however, their values were obtained from whole-body analysis and may not be directly comparable to those obtained from target tissue analysis.

The significantly elevated residue levels of lead and total chromium may be interpreted to suggest that these metals may be partially responsible for increased chromosomal aberrancy found in small mammals residing around the sludge retention ponds. If, however, these metals are primarily responsible for the observed genetic damage, there should be strong correlation between levels of these two compounds and the three measures of chromosomal aberrancy documented in

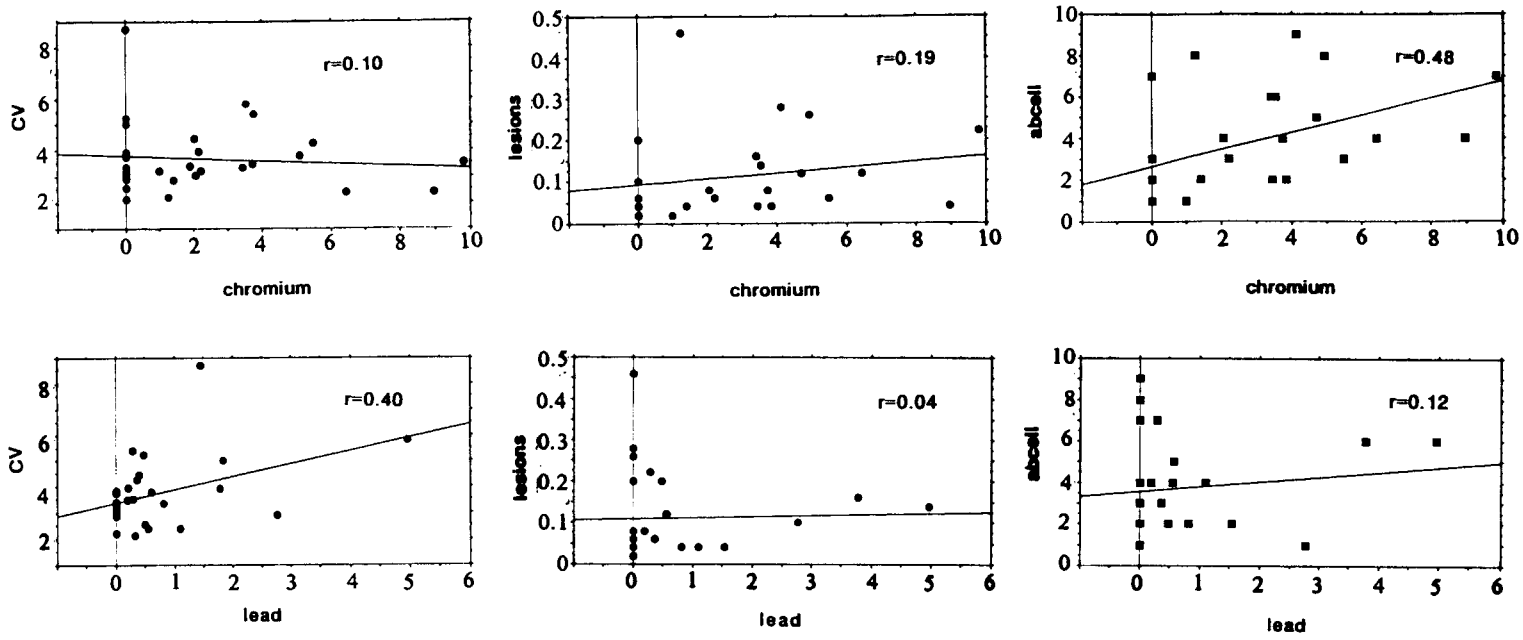

Figure 1. Correlations between tissue concentrations (μg/g ww) for chromium and lead and coefficient of variation around mean nuclear DNA content (CV), number of aberrant cells per individual (Abcell), and percent lesions per cell (Lesions) for rodents inhabiting a hazardous waste disposal site and a reference site.

Table 1. Mean concentrations and ranges (μg/g) of four metals in liver tissue of Peromyscus leucopus collected from a hazardous waste site (HWS, n = 28) and a matched reference site (RS, n = 12).

Metal	HWS	RS
Cadmium	2.009 (0.124 - 19.169)	2.206 (0.200 - 6.733)
Chromium	3.053** (0.010 - 9.790)	0.720 (0.010 - 2.227)
Lead	0.829** (0.002 - 4.953)	0.233 (0.002 - 2.772)
Zinc	40.699 (22.649 - 71.709)	46.012 (30.857 - 96.634)

**Significantly different ($P < 0.05$) by the Wilcoxon Rank Sum test.

earlier studies (McBee et al. 1987; McBee and Bickham 1988). For each animal in which data were available for all variables, the coefficient of variation (CV) around the mean nuclear DNA content, the number of lesions per 50 cells, and the number of aberrant cells per individual were plotted against metal concentrations (Fig. 1). No strong correlations were found among these data; however, strongest correlations were between CV and lead concentration ($r = 0.40$) and between aberrant cells per individual and chromium concentration ($r = 0.48$). Cadmium and zinc showed weakly positive to weakly negative correlations between all three genetic measures and tissue metal concentration.

In spite of the significantly increased levels of lead and chromium, these correlative data indicate that neither of these metals can be considered totally responsible for the increased levels of genetic aberrancy documented in those animals. A number of other compounds present at the site such as partially combusted hydrocarbons and polynuclear aromatic hydrocarbons may contribute to or cause induction of genetic damage and synergistic interactions may account for some observed clastogenicity. Although this study did not demonstrate a strong correlation between measures of genetic damage and residue levels of one group of presumed causative agents, it does indicate that additional investigations directed toward combined residue and biomarker analyses are needed to elucidate relationships among exposure, uptake, and measurable physiological response in members of endemic populations.

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