Bull. Environ. Contam. Toxicol. (1997) 59:279-284

Bulletin of Environmental Contamination and Toxicology

Assessment of Metal Uptake and Genetic Damage in Small Mammals Inhabiting a Fly Ash Basin

J. D. Peles,[1,2] G. W. Barrett[2]

[1]Savannah River Ecology Laboratory, Drawer E, Aiken. South Carolina 29802, USA
[2]1nstitute of Ecology, University of Georgia, Athens, Georgia 30602, USA

Received: 18 January 1997/Accepted: 22 April 1997

Coal burning for electrical power produces 67 million metric tons of solid waste per year (Adriano et al. 1980). Most of this solid waste is in the form of fly ash that often contains high concentrations of heavy metals (Adriano et al. 1980; Carlson and Adriano 1991). To date, however, no *in situ* investigations have been conducted to examine the effects of fly ash contaminants on species of small mammals.

Because many of the metals commonly found in fly ash are potentia1 mutagens (McMurphy et al. 1996), genetic biomarkers may be useful for assessing the biological consequences of exposure to fly ash. The development of techniques for the assessment of DNA damage has led to increased use of genetic biomarkers in recent years (Fisher et al., 1993). For example, flow cytometry (FCM) has been used to demonstrate genetic damage in a number of species from contaminated habitats (e.g., Bickham et al. 1988; McBee and Bickham 1988; Lamb et al. 1991; George et al. 1991).

Small mammals are a model group for examining contaminant uptake and assessing the effects of contaminants in natural systems (McBee and Bickham 1990). Therefore, the purpose of this investigation was to (a) examine metal uptake in cotton rats *(Sigmodon hispidus)* and rice rats *(Oryzomys palustris)* collected from a fly-ash contaminated site and a reference site, and (b) compare the extent of DNA damage in small mammals between the contaminated and the reference site as determined by flow cytometry.

MATERIALS AND METHODS

This study was conducted at the Savannah River Site (SRS) near Aiken, SC. Fly ash produced from coal burning at the D-Area power facility is sluiced with water and sent into two receiving basins. The sluiced ash consequently moves into primary and secondary sedimentation basins. Effluent from the secondary basin flows into an adjacent swamp before being released into a nearby stream (see Sandhu et al. 1993 for a detailed description of the study area). Habitat for small mammals is provided by successional vegetation within the receiving basins and

Correspondence to: J. D. Peles

by vegetation surrounding the sedimentation basins and swamp. The reference site (Ellenton Bay) is located approximately 2.5 km from the contaminated site. Composition and structure of vegetation surrounding the bay is similar to that found at the ash basin site. Both sites were dominated by panic grass *(Panicum hemitomon)* and broomsedge *(Andropogon virginicus).*

Adult *S. hispidus* (>80 g) and *O. palustris* (>50 g) were collected by live-trapping from 15 August - 15 September 1996. Individuals were sacrificed within 24 hrs of capture and the liver, kidneys, and spleen removed. Liver and kidney tissues were frozen at -70°C until used for analysis of heavy metals. Spleen tissue was processed immediately for flow cytometry.

Metals analysis was conducted at the University of Georgia Chemical Analysis Laboratory. Samples of liver and kidney tissues were freeze dried at -50°C and 0.28 mbar prior to analysis. Freeze-dried samples were digested with 10% HNO_3 and concentrations of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), lead (Pb), nickel (Ni), selenium (Se), and zinc (Zn) in liver and kidney tissues were determined using a Jarrell-Ash 965 inductively-coupled plasma spectrometer. A bovine liver reference standard (National Bureau of Standards No. 1577) was used for quality assurance purposes.

Spleen tissue was homogenized by extruding through a 50 mesh wire screen cloth to obtain a cell suspension in minimum essential medium (MEM). Cells were washed twice with MEM and filtered through a 35 pm nylon mesh filter. Following filtration, 2 ml aliquots were prepared for freezing by adding 1 ml of the cell suspension to 1 ml of a freezing buffer (MEM and fetal calf serum). Samples were stored at -70°C for less than one week.

Prior to flow cytometry, cell nuclei were stained with propidium iodide following the procedures outlined by Fisher (1994). Flow cytometry was conducted at the University of Georgia Cell Analysis Facility using a Coulter EPICS Elite flow cytometer. The system was aligned daily using fluorescent microsphere beads and alignment was checked throughout the day. Five replicates of 10,000 cells were analyzed for each individual. The fluorescence value, which is proportional to the amount of DNA, was plotted against the number of cells to obtain a DNA flow histogram for each replicate. This histogram was used to calculate coefficient of variation (CV) around the G1 peak. The mean CV of the five replicates was used to calculate a CV value for each individual.

Mann-Whitney Rank Tests were used to compare concentrations of each metal in liver and kidney tissues of *S. hispidus* and *O. palustris* between the contaminated and reference sites. Metal concentrations were below minimum detection limits for As, Cd, Cr, Ni, and Se in some individuals. Concentrations were recorded as the minimum detection value (0.08 ppm) for these metals. Mann-Whitney Rank Tests also were used to compare CV values between sites for each species.

Spearman-Rank Correlation Tests were used to test for a correlation between concentrations of each metal and CV in *S. hispidus.*

RESULTS AND DISCUSSION

Previous investigations have reported increased levels of heavy metals in both aquatic (Alberts et al. 1985) and terrestrial (Carlson and Adriano 1991) habitats at the Ash Basin site compared to reference sites. Results reported for aquatic habitats were based on metal concentrations in solution and suspended solids collected from the ash basins (Alberts et al. 1985) whereas those for terrestrial habitat were based on metal concentrations in substrate at an ash basin adjacent to the current study site (Carlson and Adriano 1991). No data exist regarding metal residues at Ellenton Bay. However, because of the location of this site and its status as a protected research area at SRS, there exists little potential for contamination of this site by heavy metals. Thus, it was hypothesized that metal concentrations would be greater in tissues of small mammals collected from the Ash Basin site compared to Ellenton Bay

Results generally supported this hypothesis concerning metal concentrations in small mammals. For example, concentrations of As, Cu, and Ni in liver tissue of *S. hispidus* collected from the Ash Basin were significantly greater compared to Ellenton Bay (Table 1). Likewise, concentrations of Cd, Cr, Cu, and Zn were significantly greater in kidneys of cotton rats from the Ash Basin. Interestingly, however, the concentrations of Cd and Cr in liver and Pb in kidneys were significantly higher in cotton rats from Ellenton Bay compared to the Ash Basin.

Increased metal uptake was observed for rice rats collected from the Ash Basin compared to Ellenton Bay. Concentrations of As, Cu, Mn, Pb, Se, and Zn were significantly greater in liver tissue of *O. palustris* collected from the Ash Basin compared to Ellenton Bay (Table 2). No significant differences were observed between sites regarding metal concentrations in kidney tissue of rice rats.

It was expected that the potential for metal uptake would likely differ between species as a result of dietary preferences. For example, *S. hispidus* is a terrestrial, herbivorous small mammal species, whereas *O. palustris* is a semi-aquatic, omnivorous species that often feeds on aquatic invertebrates (Cothran et al. 1991). However, increased metal concentrations were observed in tissues of both species collected from the Ash Basin compared to Ellenton Bay.

Small mammals are an important intermediate for the transfer of toxic metals to higher trophic levels (Laurinolli and Bendell-Young 1996). Thus, our results regarding increased metal uptake among small mammals have important implications regarding potential bioaccumulation of metals in the food chain (Anderson et al. 1982; Brewer and Barrett 1995; Brueske and Barrett 1991). This is especially important since there exists the potential for two species with

contrasting dietary and habitat preferences to accumulate and serve as intermediates for the transfer of heavy metals to higher trophic levels.

Table 1. Mean metal concentrations (ppm ± S.D.) in liver and kidney tissues of Sigmodon hispidus collected from fly-ash contaminated habitat (Ash Basin; n = 25) and a reference site (Ellenton Bay; n = 19).*

	Liver Tissue		Kidney Tissue	
Metal	Ash Basin	Ellenton Bay	Ash Basin	Ellenton Bay
As	2.77 ± 0.39a	1.32 ± 0.35b	2.00 ± 0.52a	1.37 ± 0.41a
Cd	0.28 ± 0.08a	0.86 ± 0.21b	1.57 ± 0.39a	0.41 ± 0.10b
Cr	1.81 ± 0.59a	5.04 ± 1.09b	4.52 ± 0.71a	2.23 ± 0.56b
Cu	14.3 ± 1.2a	9.82 ± 1.80b	23.0 ± 1.8a	17.4 ± 2.1b
Mn	10.6 ± 0.6a	9.12 ± 0.87a	11.5 ± 0.9a	12.0 ± 1.0a
Ni	1.55 ± 0.20a	0.56 ± 0.15b	1.58 ± 0.33a	1.29 ± 0.22a
Pb	4.53 ± 0.30a	2.58 ± 0.29a	3.91 ± 0.66a	5.30 ± 0.37b
Se	1.64 ± 0.36a	1.09 ± 0.36a	1.45 ± 2.54a	2.54 ± 0.93a
Zn	118.0 ± 5.0a	118.0 ± 8.6a	125.0 ± 4.0a	111.0 ± 4.0b

*Means with different superscripts indicate a significant difference ($P < 0.05$) between study sites (Mann-Whitney U-Test).

Table 2. Mean concentrations (ppm ± S.D.) in liver and kidney tissues of Oryzomys palustris collected from fly-ash contaminated habitat (Ash Basin; n = 9) and a reference site (Ellenton Bay; n = 10).*

	Liver Tissue		Kidney Tissue	
Metal	Ash Basin	Ellenton Bay	Ash Basin	Ellenton Bay
As	3.34 ± 1.31a	0.54 ± 0.32b	2.91 ± 0.57a	2.62 ± 0.48a
Cd	1.08 ± 0.68a	0.46 ± 0.20a	2.48 ± 1.02a	0.97 ± 0.29a
Cr	2.54 ± 0.78a	3.15 ± 0.90a	3.53 ± 1.00a	3.79 ± 1.08a
Cu	33.6 ± 10.2a	12.6 ± 2.5b	33.4 ± 5.1a	37.3 ± 3.3a
Mn	18.4 ± 4.7a	8.62 ± 2.17b	13.9 ± 3.3a	7.86 ± 1.12a
Ni	1.76 ± 0.44a	1.19 ± 0.32a	1.05 ± 0.39a	1.19 ± 0.30a
Pb	4.01 ± 0.68a	2.18 ± 0.56b	4.17 ± 0.59a	3.48 ± 0.30a
Se	2.14 ± 0.84a	0.080 ± 0.001b	2.15 ± 0.85a	2.13 ± 0.83a
Zn	193.0 ± 43.0a	106.0 ± 14.0b	131.0 ± 6.0a	125.0 ± 6.0a

*Means with different superscripts indicate a significant difference ($P < 0.05$) between study sites (Mann-Whitney U-Test).

No evidence of genetic damage as a result of exposure to fly ash contaminants was observed. Examination of flow DNA histograms revealed no evidence of aneuploidy among any individuals. CV values did not differ significantly ($P \geq 0.05$) between sites for cotton rats (Ash Basin mean ± S.D. = 2.89 k 0.34; Ellenton Bay = 2.81 ± 0.37) or rice rats (Ash Basin = 2.78 ± 0.25; Ellenton Bay = 3.03 ± 0.34). CV values of *S. hispidus* were not highly correlated with the concentration of any metal in liver or kidney tissue (range of Spearman Rank Correlation Coefficients = 0.04 - 0.33).

Although no evidence of genetic damage was observed in this investigation, the effects of exposure to fly ash contaminants on organisms at higher trophic levels at this site warrant further investigation. This is important since metals such as As, Cd, Cr, Mn, Ni, Pb, and Zn are known to have mutagenic, as well as toxic effects in many organisms (Kazantis and Lilly 1986). Future *in situ* investigations should (a) measure contaminant uptake in organisms at all trophic levels, and (b) use a variety of biomarkers (behavioral, physiological, genetic; McBee and Bickham 1990) to assess the effects of fly-ash contamination at this site.

Acknowledgments. We thank Steve Hilliard, Kevin Holloman, and John Kind for technical assistance. This research was supported by a grant from the Savannah River National Environmental Research Park and contract DE-FC09-96SR18546 between the U.S. Department of Energy and the University of Georgia's Savannah River Ecology Laboratory.

REFERENCES

Adriano DC, Page AL, Elseewi AA, Chang AC, Straughan I (1980) Utilization and disposal of fly ash and other coal residues in terrestrial ecosystems: A review. J Environ Qua1 9:333-344

Alberts JJ, Newman MC, Evans DW (1985) Seasonal variations of trace elements in dissolved and suspended loads for coal ash ponds and pond effluents. Water Air Soil Pollut 26: 111 - 128

Anderson TJ, Barrett GW, Clark CS, Elia VJ, Majeti VA (1982) Metal concentrations in tissues of meadow voles from sludge-treated fields. J Environ Qua1 11:272-277

Bickham JW, Hanks BG, Smolen MJ, Lamb T, Gibbons JW (1988) Flow cytometric analysis of the effects of low-level radiation exposure on natural populations of slider turtles *(Pseudemys scripta).* Arch Environ Contam Toxicol 17:837-841

Brewer SR, Barrett GW (1995) Heavy metal concentrations in earthworms following long-term nutrient enrichment. Bull Environ Contam Toxicol 54:120-127

Brueske CC, Barrett GW (1991) Dietary heavy metal uptake by the least shrew, *Cryptotis parva.* Bull Environ Contam Toxicol 47:845-849

Carlson CL, Adriano DC (1991) Growth and elemental content of two tree species growing on abandoned coal fly ash basins. J Environ Qua1 20:581-587

Cothran EG, Smith MH, Wolff JO, Gentry JB (1991) Mammals of the Savannah River Site. Savannah River Site - National Environmental Research Park Program, Publication 21

Fisher, SK (1994) Flow cytometric assessment of DNA damage in largemouth bass as a biomarker to evaluate genotoxicity related to environmental contaminant exposure. MS Thesis, Univ Georgia, Athens

Fisher SK, Jagoe CH, Dallas CE, Chesser RK, Smith MH, Brisbin IL (1993) Assessment of genetic damage as a biomarker in the remediation of contaminated or polluted sites. In: Proceedings of the United States Department of Energy Environmental Remediation Conference. US-DOE, Washington, DC, pp 629-634

George LS, Dallas CE, Brisbin IL, Evans DL (1991) Flow cytometric DNA analysis of ducks accumulating ^{137}Cs on a reactor resevoir. Ecotoxicol Environ Safety 21:337-347.

Kazantis G, Lilly LJ (1986) Mutagenic and carcinogenic effects of metals. In: Friberg L, Nordberg GF, Vouk VB (ed) Handbook on the toxicology of metals. Elsevier, Amsterdam, pp 319-390

Lamb T, Bickham JW, Gibbons JW, Smolen MJ, McDowell S (1991) Genetic damage in a population of slider turtles *(Traechemys scripta)* inhabiting a radioactive reservoir. Arch Environ Contam Toxicol 20: 138-142

Laurinolli M, Bendell-Young LI (1996) Copper, zinc, and cadmium concentrations in *Peromyscus maniculatus* sampled near an abandoned copper mine. Arch Environ Contam Toxicol 30:481-486

McMurphy LM, Biradar C, Taets C, Rayburn AL (1996) Differential effects of weathered coal fly ash and fly ash leachate on the maize genome. Arch Environ Contam Toxicol 31:166- 169

McBee K, Bickham JW (1988) Petrochemical-related DNA damage in wild rodents detected by flow cytometry. Arch Environ Contam Toxicol 40:343-349

McBee K, Bickham JW (1990) Mammals as bioindicators of environmental toxicity. In: Genoways HH (ed) Current mammalogy, vol 2. Plenum, New York, pp 37-88.

Sandhu SS, Mills GL, Sajwan KS (1993) Leachability of Ni, Cd, Cr, and As from coal ash impoundments of different ages on the Savannah River Site. In: Keefer, RF, Sajwan KS (ed) Trace elements in coal and coal combustion residues. Lewis Publ, Boca Raton, FL, pp 165-182