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Heavy Metals in the Hair of Opossum From Palo Verde, Costa Rica

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Abstract. Levels of lead, cadmium, mercury, chromium, and manganese were measured in the hair of opossum (*Didelphis virginiana*) from Palo Verde, Guanacaste, Costa Rica. This area has some agriculture, but is slated for extensive water development that will increase ecosystem exposure to a variety of agricultural chemicals. Metal levels were generally not intercorrelated except for chromium and lead, chromium and cadmium, and cadmium and lead. There were significant gender differences only for lead and chromium, with the significantly smaller females having higher levels. It is suggested that hair from mammals, particularly abundant ominivores may be useful, bioindicators of environmental quality over a long time period.

The bioaccumulation of heavy metals in tissues of animals has received considerable attention (Klaverkamp *et al.* 1983), and animals are frequently used for environmental biomonitoring (NRC 1991a). The objectives of using animal sentinels include the estimation of human health risks, evaluation of food chain contamination, monitoring of environmental quality, and identification of adverse effects on the organisms themselves (NRC 1991b). Heavy metals enter the food chain from industrial sources, natural erosion, and geochemical cycles. Once in the animal, the heavy metals can be stored in tissues, or they can be eliminated through excretion or by deposition in feathers or hair (Phelps *et al.* 1980; Goede and deBruin 1984, 1986).

Both mammalian hair and avian feathers can be used as indicators of heavy metal levels in other tissues and are thus reflective of exposure (Goede and deBruin 1986; Phelps *et al.* 1980). Feathers of birds have been used widely as bioindicators of environmental contamination (Goede and deBruin 1986; Furness *et al.* 1986; Burger and Gochfeld 1991). Peakall (1992) noted that although most laboratory studies of chemical hazards have used mammals, most field studies have used birds or fish. In general, levels of contaminants in hair or fur of wild animals have not been studied. Hair, however, has primarily been used to indicate individual exposure, although, in some circumstances, hair has been used to assess human population exposure in general (Katz and Katz 1992).

Hair can be used as an indicator of heavy metal levels in other tissues, particularly for mercury where there is little opportunity for external contamination. Metal levels in hair are correlated with internal tissue levels (Al-Shahristani *et al.* 1974; Francis *et al.* 1982), and can be used as bioindicators. For some metals, levels in hair are many times higher than other tissues, and for mercury the hair:blood ratio is in the 200:1–300:1 (Kershaw *et al.* 1980; Katz and Katz 1992). Thus, metal levels in hair can serve an early warning function. For example, there is a high correlation between mercury levels in hair and blood for humans, particularly early in exposure (Phelps *et al.* 1980). For lead, however, the correlation is low (Winneke *et al.* 1985). Despite the extensive use of human hair to assess exposure in humans, hair has not been used extensively with other mammals. However, Clark *et al.* (1989) did examine selenium levels in hair of raccoons (*Procyon lotor*) exposed at Kesterson National Wildlife Reserve. Selenium levels in hair provided a better separation among study sites than did liver, blood and feces (Clark *et al.* 1989).

In this paper, the levels of lead, cadmium, mercury, chromium, and manganese were examined in the hair of opossum (*Didelphis virginiana*) from a tropical dry forest in Palo Verde, Costa Rica. Palo Verde is a national park that is partially surrounded by agricultural land currently used mainly for grazing and for growing rice. However, the surrounding habitat is scheduled to be further developed for intensive agriculture (rice) with a massive irrigation system. The anticipated agricultural expansion will be accompanied by use of agricultural chemicals as well as other development.

Methods

Opossums are the largest marsupial in Costa Rica, and adults weigh from 0.8–2.5 kg (Gardner 1983). They forage along watercourses at night, and will eat almost anything (including fruit, small birds, small mammals, and insects).

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Table 1. Metal levels in hair of opossum in Costa Rica (mean ± SE, ppb in dry weight). Geometric means in parentheses

	Male	Female	Kruskal-Wallis X^2 (P)
Sample	12	12	
Weight (kg)	1.7 ± 0.1	1.4 ± 0.1	3.2 (0.06)
Metals (ppb)			
Lead	319 ± 46	524 ± 66	4.8 (0.02)
	(28)	(475)	
Cadium	36 ± 4	56 ± 13	NS
	(33)	(44)	
Mercury	228 ± 30	288 ± 51	NS
	(177)	(225)	
Chromium	1,569 ± 192	3,559 ± 638	7.7 (0.005)
	(1430)	(2980)	
Manganese	34,119 ± 7,867	29,202 ± 2,253	NS
	(27,200)	(28,200)	

As part of a study of population dynamics and sex ratio variation of opossums at Palo Verde National Park in Costa Rica (by MM), hair was clipped from trapped opossums. Palo Verde is a dry tropical forest in Guanacaste Province (Gill 1988) and has experienced a severe drought for the previous 3–4 years (1989–1992). The forest had scattered water holes, and many had dried up, causing opossums to concentrate near the ones with water. We collected samples of hair from the backs of 12 males and 12 females. Hair was stored in white, metal-free envelopes for later analysis. All opossums were weighed, and lactation stage was noted for females.

Mercury, lead, cadmium, chromium, and manganese were analyzed. For mercury, 0.2 g of hair was added to 5 ml redistilled nitric acid, and digested at 95°C for 1.5 h. This was cooled, made up to 100 ml with deionized water. Than 5 ml sulfuric acid, 15 ml potassium permanganate, and 8 ml potassium persulfate were added. This was digested at 95°C for 40 min, cooled, and cleared with 12% hydroxylamine hydrochloride, and analyzed with a cold vapor atomic absorption technique.

For the other metals, 0.2 g of hair was added to 5 ml ultrex nitric acid, and digested in Kjeldahl flasks until the volume was 0.5 ml. This was diluted with deionized water to 10 ml and analyzed by graphite furnace atomic absorption (EPA 1981). All concentrations were expressed in ng/g (ppb) on a dry weight basis using weights obtained from air-dried hair. Detection limits ranged from 0.3 ng/g for cadmium to 10 ng/g for lead. All specimens were run in batches which included known standards from the US National Bureau of Standards, method blanks, and spiked specimens. Recoveries ranged from 90% to 104%. Batches with recoveries less than 85% were rerun. The coefficient of variation on replicate, spiked samples ranged from 4 to 10%. Further quality control included periodic blind analyses of an aliquot from a large sample of known metal concentrations.

Data were analyzed with nonparametric procedures to compare concentrations between sexes, and Kendall tau correlation coefficients were used to compare the various metal concentrations and body weight. We report significance levels of $P < 0.07$ due to the small sample sizes. Both arithmetic and geometric means are given to facilitate comparisons with other studies.

Results

Males were significantly larger than females (Table 1). Metal levels varied, with generally lower levels of cadmium, lead, and mercury, intermediate levels of chromium, and relatively high levels of manganese (Table 1). Females had significantly higher levels of lead and chromium, despite their smaller size.

The correlations among metals is shown in Table 2. There were few significant correlations. However chromium was significantly correlated with lead and cadmium, and lead was significantly correlated with cadmium.

Table 2. Correlations among metals for opossum in Costa Rica. Kendall tau coefficient given above diagonal and P value (probability that tau = 0) is below diagonal. NS = tau not significantly different from 0

	Lead	Cadmium	Mercury	Chromium	Manganese
Lead	—	0.35	−0.01	0.52	−0.01
Cadmium	0.01	—	0.06	0.34	0.23
Mercury	NS	NS	—	0.18	−0.14
Chromium	0.0003	0.02	NS	—	0.17
Manganese	NS	NS	NS	NS	—

Discussion

Characteristics of a Sentinel

With increasing use of chemicals in Costa Rica, including pesticides, there is a need to develop bioindicators of exposure. The main attributes of bioindicators are listed below with some modifications (NRC 1991b). Notably, as analytic techniques have improved and detection limits have been lowered several orders of magnitude, the analytic constraints have changed.

1. The species should be relatively common and available.
2. The animal should be sufficiently large to provide adequate sample for analysis (this is decreasingly important as detection levels have dropped).
3. Age and gender should be determinable.
4. Life span should be appropriate (use short-lived or newborn animals to measure recent exposure and long-lived animals to integrate cumulative exposure).
5. The animal should accumulate the chemical (or have a biologic response) to an appropriate degree. It should occupy an appropriate trophic level. The levels it accumulates should be sufficient to measure (not close to detection levels), yet not so high that additional exposure will have little effect, nor should it approach toxic levels.

6. Home range must be appropriate (sessile animals to monitor point sources; mobile animals to integrate exposure over space).
7. The biology of the contaminant in the body should be understood in terms of special concentration by certain species, or deposition in certain organs.

Use of Metals in Hair of Mammals

Increasingly, environmental biomonitoring is seeking biomarkers of exposure or effect (Peakall 1992), and this is particularly important for ascribing significance to particular levels of exposure or contamination. However, there is still an important role for simply measuring the concentrations of contaminates in body tissues or fluids.

Hair has had a checkered history in biomonitoring exposures in humans. Hair is a keratinaceous substance, rich in sulfhydryl containing amino acids that avidly bind certain metals. As a readily available growing tissue it has received extensive study. Mercury, arsenic, and selenium, in particular, have an affinity for the sulfur-rich amino acids in hair, and tend to be highly concentrated there. Hair is a good indicator of mercury in humans, but because of external applications of selenium-containing products, it has not been useful for selenium (Katz and Katz 1992). Organic compounds do not accumulate in hair, but may be found in the oils secreted by hair follicles and may be measured when hair is analyzed (NRC 1991a). A controversial field of nutritional analysis and therapy based on human hair analysis has emerged.

Hair has been considered a poor indicator of lead exposure, because lead does not appear to have a strong affinity for keratin and because of the substantial contribution of external, airborne deposition. However, as air lead levels in the United States have declined since the elimination of leaded gasoline, there should be improvement in the utility of hair lead. Winneke *et al.* (1985) reported a mean hair lead concentration of 5.3 μg/g in a population of 114 children whose blood leads averaged 8.2 μg/dL. The correlation coefficient was 0.33.

The use of hair to assess heavy metal exposure in opossums is unique in three important ways: (1) It is one of the first uses of nonhuman hair to assess exposure in a mammal, (2) it is one of the first studies to examine metal levels in terrestrial mammals from uncontaminated sites, and (3) it is one of the first studies to examine metal levels in live, healthy mammals. Although there has been extensive work with marine mammals, there has been relatively little with terrestrial mammals. The work with marine mammals is not surprising since any die-off of large marine mammals attracts attention, and many marine mammals are endangered, making it imperative to determine if pollutants contributed to their demise (Honda *et al.* 1983; 1987; Skaare *et al.* 1990). The availability of marine mammal carcasses following a die-off has resulted in sufficient data on dead animals, but there is a lack of comparative data on healthy, intact animals. Since pollutant levels may have caused or contributed to their death, these dead animals should not be used as indicative of levels in the population as a whole.

Heavy metal levels in the internal tissues of terrestrial mammals have received little attention, perhaps because many species are not high on the food chain or because they are less visible (many are nocturnal). However, metal levels in the internal tissues have been examined in small mammals such as bats (Clark 1979), shrews and voles (Ma *et al.* 1991), and raccoons (*Procyon lotor*, Clark *et al.* 1989) that were exposed to heavily contaminated terrestrial ecosystems. In this study metal levels of opossums in hair were examined in a partially disturbed, but generally uncontaminated tropical forest.

Finally, metal levels usually have been examined in tissues other than hair. Examination of internal tissues requires collection of specimens, and is thus not as useful for long-term studies or for species of special concern. We suggest that hair might be useful as a noninvasive bioindicator of internal levels and of environmental exposure (see references in introduction), assuming the direct relationship between levels in hair and internal tissues, as occurs with humans (Phelps *et al.* 1980).

Small or abundant mammals will be particularly useful as bioindicators because they are relatively sedentary and will thus reflect local contamination. Further, since they are abundant, they will be useful for a long period of time. Moreover, there are well established techniques for trapping mammals, providing a representative sample of the population or habitat.

Hair has been viewed favorably as an indicator of blood levels for mercury, but there is considerable controversy for lead, at least for humans. However, hair would not be useful to monitor a population where airborne exposure was the main route of exposure.

Hair Versus Feathers

Hair is an integumentary structure which grows continuously and maintains a blood supply connection with the body. Growing from the base, each segment of hair contains a record of elements circulating in the blood at the time that segment of hair was formed. From base to tip a hair represents a continuous archive of blood levels of those elements that have an affinity for keratin. A feather, however, grows rapidly over a period of a few weeks, after which the blood supply shrivels and the feather remains on the bird for a period of months as a dead structure (Lanyon 1963). It represents an archive of metal levels to which the bird was exposed during the few weeks prior to feather formation.

Monitoring of metal levels in feathers has played a prominent role in understanding environmental contamination by mercury (Borg *et al.* 1969; NRC 1980). Several authors have used differential concentrations of metals in feathers formed on northern breeding grounds versus southern wintering grounds, to study exposure in species such as the osprey (*Pandion haliaetus*) (Berg *et al.* 1966) and the common tern (*Sterna hirundo*) (Burger *et al.* 1992). The use of hair and feathers is thus analogous, but it is not reasonable to expect them to be directly comparable. Nonetheless, in this paper we do make some comparisons.

Metal Levels in Opossums

Metal levels in terrestrial mammals are generally not examined, making comparisons difficult. Moreover, there are no data from mammals (any tissues) from Palo Verde or from other areas in Costa Rica. Animals can deposit heavy metals in both hair and feathers during development.

Comparable data are available from feathers of wood storks (*Mycteria americana*) from Palo Verde (Burger *et al.* 1993).

The levels of all metals in the opossum hair were lower than feather levels in either adult or young storks (except for manganese) (Burger *et al.* 1993). However mercury was the same order of magnitude whereas lead (5×), cadmium (5×), chromium (10×), and manganese (3×) were much lower in the hair of adult opossum compared to adult stork feathers. Young storks had half as much manganese as adults opossums.

The comparison between adult opossums and young storks is not unreasonable since both obtain all their food (and thus their heavy metals) from the Palo Verde environs. However, the opossums generally feed within the park whereas the wood storks also feed in the surrounding agricultural lards. This difference would suggest that metal levels in adult opossum hair might be significantly less than in young storks or that metals concentrate more in hair than in feathers. Opossums did not have lower levels of cadmium and manganese in their hair. It is thus of interest to speculate on where the opossums obtained these levels since the dry tropical forest itself might be expected to be low in heavy metals. Part of the refuge is a marshland that receives drainage water from surrounding areas, and metals may have come from this source. Moreover, metals can come from geologic processes including local rocks and soil, as well as from atmospheric distribution (Reilly 1990).

Female opossums had higher levels of lead and chromium than males, despite their smaller size. Larger animals are usually capable of catching larger prey, which in turn should have higher metal levels than smaller prey. Moreover, in other studies with mammals, females had lower levels of metals in their tissues, and levels further decreased with lactation (Honda *et al.* 1987). In this study, female opossums were lactating, but they still had higher levels of the two metals. This may reflect dietary or deposition differences.

There were few significant interactions among metals. However, cadmium and lead were positively correlated, a finding reported for feathers of several species of terns (*Sterna* sp) from Puerto Rico and Australia (Burger and Gochfeld 1991), and skimmers (*Rynchops niger*) from New York (Burger and Gochfeld 1992), but not for storks from Costa Rica (Burger *et al.* 1993).

Overall, this study found measurable levels of several metals in hair of opossum from a "protected" dry tropical forest in Costa Rica. Females had significantly higher levels of lead and chromium. It is suggested that hair of small mammals can be a useful bioindicator of local contamination.

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References

Al-Shahristani J, Shihab KM, Al-Haddod JK (1976) Mercury in hair as an indicator of total body burden. Bull Witt (Suppl) 53:105–112

Berg W, Johnsels A, Sjostrand B, Westermark T (1966) Mercury content in feathers of Swedish birds from the past 100 years. Oikos 17:71–83

Borg K, Wanntorp H, Erne MK, Hanko E (1968) Mercury poisoning in some Swedish wildlife. J Appl Ecol 3 (Suppl):171–172

———, Nisbet ICT, Gochfeld M (1992) Metal levels in regrown feathers: assessment of contamination on the wintering and breeding grounds in the same individuals. J Toxicol Environ Health 36:327–338

Burger J, Gochfeld M (1991a) Cadmium and lead in common terns (Aves *Sterna hirundo*): Relationship between parents and eggs. Environ Monit Assess 16:251–258

———, ———(1991b) Lead, mercury, and cadmium in feathers of tropical terns in Puerto Rico and Australia. Arch Environ Contam Toxicol 21:311–315

———, ———(1992) Heavy metal and selenium concentrations in black skimmers (*Rynchops niger*): Gender differences. Arch Environ Contam Toxicol 23:431–434

———, Rodgers JA Jr, Gochfeld M (1993) Heavy metal and selenium levels in endangered wood storks *Mycteria americana* from nesting colonies in Florida and Costa Rica. Arch Environ Contam Toxicol 24:417–420

Clark DR Jr (1979) Lead concentration: Bats vs. terrestrial small mammals collected near a major highway. Environ Sci Technol 1979:338–341

———, Ogasawana PA, Smith GJ, Ohlendorf HM (1989) Selenium accumulation by raccoons exposed to irrigation drain water at Kesterson National Wildlife Refuge, California, 1986. Arch Environ Contam Toxicol 18:789–794

EPA (1980) Ambient water quality criteria for arsenic. U.S. Environmental Protection Agency, Washington DC (NTIS 81-117327).

———(1981) Interim methods for sampling and analyses of priority pollutants in sediments and fish tissue. US Environmental Protection Agency, EPA 600/4-81-055, Cincinnati, OH

Furness RW, Muirhead SJ, Woodburn M (1986) Using bird feathers to measure mercury in the environment: Relationship between mercury content and moult. Mar Pollut Bull 17:27–30

Francis PC, Birge WJ, Roberts BL, Black JA (1982) Mercury content of human hair: A survey of dental personnel. Toxicol Environ Health 10:667–672

Gardner AL (1983) *Didelphis marsupialis.* In: Janzen DH (ed) Costa Rican natural history. Univ of Chicago Press, Chicago, IL, pp 468–469

Gill DE (1988) A naturalists' guide to the OTS Palo Verde Field Station. Organization for Tropical Studies, Durham, NC

Goede AA, deBruin M (1984) The use of bird feathers as a monitor for metal pollution. Environ Pollut A 37:287–308

———, ———(1986) The use of bird feathers for indicating heavy metal pollution. Environ Monit Assess 7:249–256

Honda K, Yamamoto Y, Kato H, Tatsukawa R (1987) Heavy metal accumulations and their recent charges in southern Minke Whales *Balaenoptera acutorostrata.* Arch Environ Contam Toxicol 16:206–216

———, Tatsukawa R, Itano K, Miyazaki N, Fajiyam T (1983) Heavy metal concentrations in muscle, liver and kidney tissue of striped dolphin, *Stenella coeruleoalba*, and their variations with body length, weight, age and sex. Agric Biol Chem 47:1219–1228

Katz SA, Katz RB (1992) Use of hair analysis for evaluating mercury intoxication of the human body: A review. Appl Toxicol 12:79–84

Kershaw TG, Clarkson TW, Dhahir PH (1980) The relationship between blood levels and dose of methylmercury in man. Archiv Environ Health 35:28–36

Klaverclamp JF, Hodgins DA, Lutz A (1983) Selenite toxicity and mercury-selenium interactions in juvenile fish. Arch Environ Contam Toxicol 12:413–442

Ma W, Denneman W, Faber J (1991) Hazardous exposure of ground-living small mammals to cadmium and lead in contaminated terrestrial ecosystems. Arch Environ Contam Toxicol 20:226–270

NRC (1978) An assessment of mercury in the environment, National Research Council, National Academy Press, Washington, DC

———(1991a) Monitoring human tissues for toxic substances. National Research Council, National Academy Press, Washington, DC

———(1991b) Animals as sentinels of environmental health. National Research Council, National Academy Press, Washington, DC

Phelps RW, Clarkson TW, Kershaw TG, Wheatley B (1990) Interrelationship of blood and hair mercury concentrations in a North American population exposed to methylmercury. Arch Environ Health 35:161–168

Reilly C (1990) Metal contamination of food. Elsevier Applied Science, London

Skaare JU, Markussen NH, Norheim G, Haugen S, Halt G (1990) Levels of polychlorinated biphenyls, organochlorine pesticides, mercury, cadmium, copper, selenium, arsenic and zinc in the harbour seal, *Phoca vitulina* in Norwegian waters. Environ Pollut 66:309–324

Snyder WS, Cook MJ, Nasset ES, Karhausen LR, Howells GP, Tipton IH (1975) International Commission of Radiological Protection: Report of the Task Group on Reference Manual. ICRP Publ 23, NY

Winneke G, Brockhaus A, Collet W, Kramer U, Krause C, Thron HL, Wagner HM (1985) Predictive value of different markers of lead-exposure for neuropsychological performance. In: Proc int conf on heavy metals in the environment, Athens—1985. World Health Organization, Geneva