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Synanthropic Primates in Asia: Potential Sentinels for Environmental Toxins

Gregory Engel,[1,2] Todd M. O'Hara,[3] Tamara Cardona-Marek,[3] John Heidrich,[4] Mukesh K. Chalise,[5] Randall Kyes,[2] and Lisa Jones-Engel[2]*

[1]*Swedish Cherry Hill, Family Medicine, Seattle, WA*
[2]*University of Washington, National Primate Research Center, Seattle, WA*
[3]*Institute of Arctic Biology, University of Alaska, Fairbanks, AK*
[4]*Quatros, LLC, Albuquerque, NM*
[5]*Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal*

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ABSTRACT Macaques are similar to humans both physiologically and behaviorally. In South and Southeast Asia they are also synanthropic, ecologically associated with humans. Synanthropy with humans raises the possibility that macaques come into contact with anthropogenic toxicants, such as lead and mercury, and might be appropriate sentinels for human exposures to certain toxic materials. We measured lead (Pb) and mercury (Hg) levels and characterized the stable isotopic compositions of $\delta^{15}N$ and $\delta^{13}C$ in hair from three groups of free-ranging macaques at the Swoyambhu temple in Kathmandu, Nepal, an urban population that has abundant contact with humans. Hair lead levels were significantly higher among young macaques and differed among the three groups of macaques that were sampled. Hair Hg levels were low. No statistical association was found between stable isotopic compositions ($\delta^{15}N$ and $\delta^{13}C$) and Pb and Hg levels. Our data did not find evidence that lead levels were associated with diet. We conclude that, in this population of macaques, behavioral and/or physiologic factors may play a significant role in determining exposure to lead. Chemical analysis of hair is a promising, noninvasive technique for determining exposure to toxic elements in free-ranging nonhuman primates. Am J Phys Anthropol 142:453–460, 2010. © 2009 Wiley-Liss, Inc.

Over the past decades, the public's increasing awareness of the potential adverse health effects of anthropogenic (human-derived) pollutants has prompted efforts to better detect environmental hazards and promote an understanding of the routes and mechanisms by which toxicants exert their adverse health effects. Ecotoxicologists have gained insight into these phenomena using wildlife as models for ecological and physiological effects (e.g., ecosystem health). Various animal populations have been proposed as "sentinels" for human exposure to toxicants present in the environment. (Aguirre et al., 2001). These "canaries in the coal mine" can potentially help to identify toxic threats to both public health and wildlife populations.

Not all animal species are relevant models for human toxicant exposures. The utility of an animal population as a sentinel depends on many factors, including its genetic and physiological characteristics and how closely its ecological niche mirrors that of sympatric human populations (Aguirre et al., 2001). In South and Southeast Asia, free-ranging macaques (genus Macaca), particularly rhesus (*M. mulatta*) and long-tailed macaques (*M. fascicularis*), are often synanthropic with humans, meaning that they flourish in ecological niches created when humans alter the environment (Southwick and Siddiqi, 1994; Engel et al., 2006; Fuentes, 2007; Jones-Engel et al., 2006, 2007, 2008; Sha et al., 2009). Macaques also share important immunological and physiological similarities with humans, particularly in the ways in which they respond to toxic exposures (Laughlin et al., 1987; Levin et al., 1987; Reuhl et al., 1989; Rice, 1992). All of these factors contribute to making macaques potentially valuable as sentinels for toxic exposures and predictors of physiologic responses to chemicals in humans.

Lead and mercury-potentially significant environmental toxicants

Lead occurs naturally in the earth's crust. However, in urban environments much higher concentrations of lead can accumulate. Common sources of lead include dust containing paint chips or lead released into the atmosphere from industrial or automotive emissions (during the era of leaded gasoline). Lead is an ingredient or byproduct of a variety of industrial processes, including welding, plumbing and construction as well as the manufacture of plastics, batteries, and electronics. Municipal waste incineration releases lead into the environment. Leaded pipes or metal pipes soldered with lead can

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*Correspondence to: Lisa Jones-Engel, University of Washington, Box 357330, Seattle, WA 98195. E-mail: jonesengel@wanprc.org

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introduce lead into drinking water. Containers sealed with lead solder and lead-glazed ceramics are other sources. Certain cosmetic products and ayurvedic medicines, used in South Asia, contain lead (Saper et al., 2008).

Lead toxicity (plumbism) remains a significant public health issue around the world (Mitra et al., 2009). Intense, acute exposure to lead is known to damage vertebrate nervous, renal, circulatory, hepatic, and reproductive systems. These consequences are well recognized and often clinically obvious in humans and animals with acute exposure (Goyer, 1990). The effects of lower levels of Pb exposure are more subtle, but may be significant. Research on the effects of lead in children continues to lower the levels of blood lead considered to be safe. Work by Lanphear et al. (2006) examined the correlation between lead levels and intellectual function in children and linked decrements in intellectual function to blood lead levels as they increased from 2.4 to 30 μg/dL. More recently, a large prospective study suggested that blood levels well below the 10 μg/dL threshold set by the Centers for Disease Control and Prevention are associated with decreased intellectual functioning in children (Jusko, 2008).

Several authors have addressed the issue of lead exposure in domestic and wildlife species. D'Havé et al. (2005) found that soil lead concentrations were positively correlated with lead levels in hair and spines of European hedgehogs living in proximity of a nonferrous metallurgic factory. Measurements of lead levels in dogs in India have shown that stray dogs in urban areas have higher blood levels than strays in unpolluted rural localities or pet dogs from either urban or rural areas (Swarup et al., 2000; Balagangatharathilagar et al., 2006) with the authors suggesting that these peridomestic species might be appropriate sentinels for human lead exposure. There is a dearth of research on toxic exposures among nonlaboratory primates (but see Zook et al., 1974; Fisher, 1954; Hindle and Stevenson, 1930). In the limited number of published studies, nearly all from zoo populations, the authors relied on postmortem samples because clinical signs of illness were difficult to detect or were of sudden onset and animal death followed quickly. Our literature search produced no publicly accessible articles on lead levels in free ranging primate populations.

Mercury has been shown to damage the immune and nervous systems, and cause birth defects (Ratcliffe et al., 1996). The toxic effects of mercury depend on its chemical form and the route of exposure. Methylmercury [CH_3Hg] is the most toxic form. Exposure to methylmercury is usually by ingestion, and it is absorbed more readily and excreted more slowly than other forms of mercury. Elemental mercury, Hg(0), the form released from broken thermometers, causes tremors, gingivitis, and excitability when vapors are inhaled over a long period of time (Ratcliffe et al., 1996). Although it is less toxic than methylmercury, elemental mercury may be found in higher concentrations in environments such as gold mine sites, where it has been used to extract gold (Palheta and Taylor, 1995). If elemental mercury is ingested, it is absorbed relatively slowly and may pass through the digestive system without causing damage.

More than half of the world's emissions of mercury occur in Asia (Pacyna and Pacyna, 2004). Mercury, which is a naturally occurring element, can be released into the environment through a variety of industrial and/or mineral extraction activities including the production of cement, the extraction of coal, copper, zinc, and gold. Mercury can also be released into the environment during oil and gas extractions because it is often associated with petroleum formation. Mercury that enters the environment can be transformed by aquatic bacteria into methylmercury, the form of mercury that is of greatest public health concern. Methylmercury is soluble and can be transmitted by surface waters and contaminate groundwater sources (Warner et al., 2008).

Using hair as a biomarker for lead and mercury exposure

While various tissues, including blood, hair, nails, saliva, bone and urine, feces, and exfoliated teeth have been proposed as matrices in which to detect lead exposure in mammals, some controversy exists regarding which are appropriate as biomarkers. Most research linking lead to physiologic effects in humans has been based on blood lead levels. Several authors, including Bergdahl et al. (2008) have provided evidence that hair lead levels parallel blood lead levels, and thus provide a potential alternative to blood as a matrix for sampling lead. Potential advantages of measuring lead levels in hair include the ability to obtain hair noninvasively and to store and transport it easily and with little infectious risk, important considerations when working with free ranging nonhuman primates. In addition, because lead is excreted in hair as it grows, each hair "captures" lead levels during an extended time frame—weeks to months. In comparison, blood lead levels may rise and fall relatively quickly following an acute exposure, decreasing the likelihood that a random sampling would detect an acute exposure. A potential disadvantage of measuring lead levels in hair is that most of the literature in this area focuses on blood lead levels; there is a dearth of published data specifically linking hair lead levels with physiologic effects.

Hair has been used to measure methylmercury in a variety of human and nonhuman populations in Asia (Sakai et al., 1995; Feng et al., 1998; Lee et al., 2000). Exposure and absorption of methylmercury occurs through diet and in turn is quickly distributed throughout the body. Although blood levels of mercury decline rapidly, the half-life of methylmercury is approximately 50 days. Hair provides an excellent biological record of previous exposures and is an ideal tool for field sampling (Foo et al., 1993).

Our study focuses on a specific population of macaques in Kathmandu, Nepal. Rhesus macaques have ranged on and around the religious temple at Swoyambhu for centuries (Chalise and Ghimire, 1998). Swoyambhu temple sits on a hilltop in Kathmandu, surrounded by the densely populated city (more than 13,000 people per sq.km). Massive population growth, industrialization, poverty, lack of infrastructure, and dearth of environmental protection have contributed to making the Kathmandu valley one of the world's most polluted areas (http://www.cleanairnet.org/caiasia). Measurements of lead levels in lichens growing along heavily traveled roads in the Kathmandu valley show high levels of lead (Chettri et al., 2001). Ground water samples from the Kathmandu Valley have been shown to contain iron, manganese and mercury concentrations that exceeded WHO recommendation (Khatlwada et al., 2002).

The ecological niche of the Swoyambhu macaques overlaps significantly with that of the surrounding

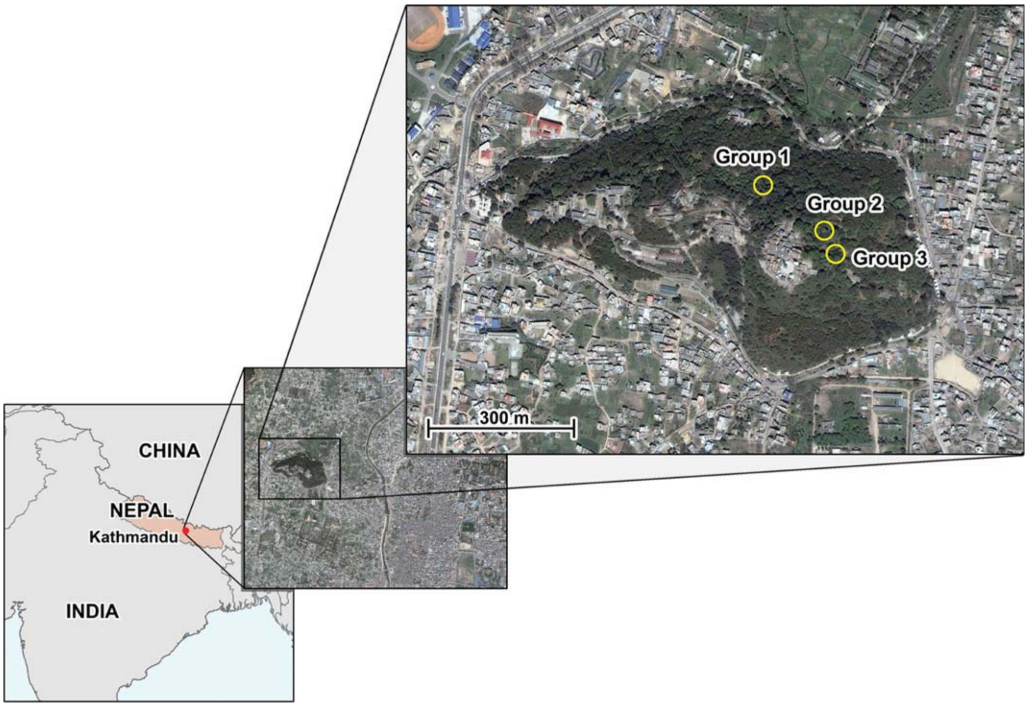

Fig. 1. Location of the Swoyambhu temple site in densely populated Kathmandu. The Swoyambhu sample populations and their trapping sites are indicated. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

human population. Considering this urban niche and synanthropic association with humans, we hypothesized that hair from the Swoyambhu macaques would contain lead and mercury, elements that have been shown to be toxic to humans and are commonly found in urban environments. We further hypothesized that the concentration of lead (Pb) and mercury (Hg) in macaque hair samples would vary among different demographic and ecologic groups, reflecting differences in physiology, feeding ecology, and behavior. We analyzed isotopic compositions of carbon and nitrogen in macaque hair to test the hypothesis that hair Hg and Pb levels are associated with differences in diet.

MATERIALS AND METHODS

Site and sample description

Swoyambhu temple, situated atop a hill approximately 3 km west of densely populated central Kathmandu (Fig. 1), is one of two temple sites in the Kathmandu valley with a large population of free-ranging rhesus monkeys (*Macaca mulatta*). This 2,000-year-old complex of Buddhist stupas, Hindu shrines, shops, and residences is a vibrant part of Kathmandu's cultural life. The most recent census estimated the total population of macaques at Swoyambhu to be between 308 and 387 (Chalise, 2006).

There are three principal water sources at Swoyambhu. Tap water, supplied by the Nepal Drinking Water Corporation and the Federation of Swoyambhu Management and Conservation Committee, is available intermittently in some homes. A stream flowing at the base of the hill is also commonly used by local residents as well as by the Swoyambhu macaques. This stream receives some sewage from local homes and runoff from a nearby factory that produces gunpowder. An open cement cistern near the shrine, fed by rainwater, provides a third water source used by both monkeys and humans. The greater part of the Swoyambhu macaques' diet comprises foods acquired directly or indirectly from the people who live and work on the site. This includes items scavenged from open refuse piles, raided from homes, snatched from food hawkers, or cajoled from visitors. Natural forage at Swoyambhu is very limited; macaques and humans share the seasonally available berries, nuts, and plants that grow in and around the wooded site.

Demographic profile of study population

Three groups of macaques share overlapping ranges within and around the Swoyambhu temple complex (Chalise and Ghimire, 1998) (Fig. 1). Hair from 37 rhesus macaques was collected during a 4-day period in May 2003 as part of a comprehensive health screening effort conducted at the request of the Federation of Swoyambhu Management and Conservation Committee. Trapping (catch and release), sedation and sampling methodologies have been reported elsewhere for this population (Jones-Engel et al., 2005). Groups were trapped on different days and in different locations. For statistical purposes, the study population was divided into young ($<$5 years, $N = 15$) and older ($\geq$5 years, $N = 22$) macaques. The number of males:females:juveniles:adults of animals sampled in Group 1 was 7:4:6:5. In Groups 2 and 3, the ratios were 5:5:4:6 and 4:12:5:11, respectively. There was no statistically significant difference in age composition ($\chi^2 = 0.4801$) or sex ($\chi^2 = 0.1144$) among the three groups.

Sample collection

Hair samples were obtained from sedated macaques by manually extracting 50–75 strands of hair from each macaque's shoulder. The hair from each animal was placed into an individual, small zip-lock bag and labeled with the animal's unique identification code. Hair growth rates are affected by several factors, including genetic variation, environment, season, nutritional state, infection, and even psychological stress. Studies on hair growth in macaques show significant variation by season. For purposes of estimation, if we assume an average sampled hair length of 50 mm, and a growth rate of 0.5 mm/day, an average hair in this study represents about 100 days, or 3 months' time frame. The hair samples were stored at room temperature. Each macaque's body weight was measured. Dental formulas were recorded and used to estimate age, based on observed dental eruption sequences and tooth wear.

Hair digestion protocol

Hair was rinsed with diluted RBS-35 (Pierce Biotechnology, contains 0.1–1% NaOH, manufacturer's directions were used for dilution) and ultrapure water to eliminate surface oils and other exogenous substances. After rinsing, hair was frozen and freeze-dried to remove water before weighing the individual samples. Hair (0.03–0.05 g) was weighed into a polytetrafluoroethylene-lined digestion vessel of a PerkinElmer Multiwave 3000, where the samples were digested with nitric acid: hydrogen peroxide (3:1, v/v). Digestates were diluted to 20 ml with ultrapure water (Barnstead International).

Analytical chemistry

Chemical analyses were performed at the University of Alaska Fairbanks (UAF). In general, our element analyses followed the hair digestion protocol described in Cardona-Marek et al. (2009). Measurements of Pb concentrations were made using methods described in Moses et al. (2009) and Woshner et al. (2008). Limited details are provided later for the sake of brevity.

Stable isotope analysis

Naturally occurring variations in the ratio of heavy to light isotopes of carbon ($^{13}C/^{12}C$) are useful indicators of feeding ecology as different kinds of plants fix carbon in characteristic patterns (Bentzen et al., 2008). Variations in nitrogen isotope concentrations ($^{15}N/^{14}N$) reflect protein sources and trophic relationships (Hoekstra et al., 2003). Freeze dried hair samples of 0.3−0.5 mg (weighed using a Sartorius M2P electronic microbalance) were loaded into small tin capsules and analyzed at the Alaska Stable Isotopes Facility at the University of Alaska Fairbanks. Nitrogen (N) and carbon (C) isotopic compositions were measured from means of duplicate samples by EA-IRMS using a Costech Elemental Analyzer (ESC 4010), and Finnigan MAT Conflo III interface with a Delta+XP Mass Spectrometer. Stable-isotopic compositions were expressed in δ notation as parts per thousand according to the following:

$$\delta X = [(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] \times 1000$$

where $X = {}^{15}N$ or ^{13}C, and R = the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The standards for ^{15}N and ^{13}C are those for atmospheric N_2 (air) and Pee Dee Belemnite (PDB) standard, respectively. Peptone ($\delta^{15}N = 7.0$, $\delta^{13}C = 15.8$; meat-based protein; Sigma Chemical Company) was used as a working laboratory standard to ensure appropriate quality control and assurance. Duplicate samples differed by less than 0.5‰ for $\delta^{15}N$ and $\delta^{13}C$. The means of duplicate values were reported for each tissue.

Measurement of lead concentrations

Hair was analyzed for Pb according to U.S. Environmental Protection Agency (US EPA) procedures as previously described by Dehn et al. (2005) and Moses et al. (2009) with minor modifications. Approximately between 0.01 and 0.05 g of hair was digested by a microwave procedure using nitric acid (HNO_3), hydrogen peroxide (H_2O_2), and hydrochloric acid (HCl). Element concentrations were analyzed at the University of Alaska Fairbanks using flame ionization Perkin Elmer A Analyst 800 AAS and reported as dry weight (dw). QA/QC followed standard laboratory procedures as described earlier and included method blanks, method duplicate, standard reference materials, spiked blanks, and spiked samples. Standard reference materials (SRMs) used for lead and copper analysis were the same ones as for mercury analysis. Recovery rates for lead in SRMs could not be calculated because the amount of SRM used was not enough for lead detection. Alternatively, blank spikes and matrix spikes recovery rates were within a range of 97–131%. Method detection limit for Pb was 0.05 ppm.

Measurement of mercury concentrations

Hair was rinsed with diluted RBS-35 and ultrapure water to eliminate surface oils and other exogenous substances as mentioned earlier. Hair (0.01−0.04 g) was loaded into a PTFE-lined digestion vessel of a Perkin Elmer Multiwave 3000, where the samples were digested with nitric acid and hydrogen peroxide. Digestates were diluted to 20 mL with ultrapure water. Mercury in a digestate volume of 0.05 mL was reduced with stannous chloride to form Hg^0, preconcentrated onto a gold trap, and detected by cold vapor atomic fluorescence spectrometry following a modified EPA Method 1631 for THg (all mercury species, organic and inorganic), on a Brooks Rand 1630 system (Model III Mercury Detector and Guru™ Software; Seattle, WA). QA/QC followed standard laboratory procedures as described in the study of Woshner et al. (2008), and included method blanks, method duplicate, standard reference materials, spiked blanks, and spiked samples. SRMs included a human hair (SRM IAEA-085 from the International Atomic Energy Agency) and bovine liver (SRM 1577b from the National Institute of Standards & Technology). Both SRMs were included in each batch of samples digested. Recovery rates for SRMs ranged from 69 to 141% for mercury in hair analysis. Method detection limit was approximately 47 pg, calculated as three times the standard deviation of the blanks. Data are presented on a dry weight basis for hair.

Statistical methods

Comparisons of Pb levels among population subgroups were conducted using the JMP-IN 4 statistical software package (SAS Institute, Cary, NC, version 4). Chi square tests contained in the SPSS statistics software package (release 11.0.1, Chicago, IL) were used to test for statistical significance of differences in Pb levels among population subgroups. Statistical significance of differences in average Pb levels and $\delta^{13}C$ and $\delta^{15}N$ between subgroups was determined using ANOVA (SPSS). Mercury concentrations were low, and no statistical assessments were conducted.

RESULTS

Lead and mercury concentrations in hair

Mean Pb, $\delta^{15}N$ and $\delta^{13}C$ by sex, age and trapping group are shown in Table 1. Lead concentrations in hair from the Swoyambhu macaques averaged approximately 4.5 ppm, with a maximum concentration of 10.2 ppm and minimum of 1.34 ppm. Lead concentrations in hair were significantly higher for young macaques (6.00 ± 1.54 ppm) compared with older animals (3.57 ± 1.36 ppm), $F(1,31) = 21.9$, $P < 0.0001$. There were no statistically significant differences in lead concentrations between males and females $F(1, 31) = 0.8058$, $P > 0.3763$. In both older ($F(2,17) = 0.0574$) and younger ($F(2,10) = 0.0314$) animals, lead concentrations differed statistically by trapping group, being highest in trapping Group 2 and lowest in trapping Group 3. Post-hoc Tukey comparison showed that macaques tested in trapping Group 3 had significantly lower mean Pb levels (3.46 ± 1.40 ppm) than group 1 (4.89 ± 1.50 ppm) and Group 2 (5.59 ± 2.13 ppm). No statistically significant relationship was detected between $\delta^{15}N$ and lead concentration $F(1,31) = 2.7457$, $P > 0.1076$ or between $\delta^{13}C$ and lead concentration $F(1,31) = 0.1114$, $P < 0.7408$. Mercury levels in hair samples were very low, ranging from 0.043 ppm to 0.594 ppm (Table 1).

Stable isotope analysis

Oneway analysis of variance of $\delta^{15}N$ showed a higher enrichment of the heavy isotope (^{15}N) in the youngest animals (<2 years old) $F(2,34) = 55.0774$, $P < 0.0001$, which is likely a lactational effect, as nursing individuals feed on milk protein produced by their mothers, and thus feed at a higher trophic level than their mothers. No differences in $\delta^{15}N$ were detected between the sexes, $F(1,35) = 0.001$, $P < 0.9935$ or among the trapping groups $F(2,34) = 0.2561$, $P < 0.7756$. No differences in $\delta^{13}C$ were detected between younger macaques and older animals, $F(1,35) = 1.01$, $P < 0.32$. However, $\delta^{13}C$ was higher among males than females $F(1,35) = 6.45$, $P < 0.016$ and lower in Group 3 than Groups 1 and 2 $F(2,34) = 13.433$, $P < 0.0001$.

DISCUSSION

Why study free-ranging rhesus macaques?

This study was performed in the context of ongoing multidisciplinary research on the human/primate interface in Southeast and South Asia. Our previous publications address issues such as bidirectional disease transmission and interspecies aggression between humans and synanthropic primate populations (Jones-Engel and Engel, 2009). We have focused our research on macaque populations at monkey temples (religious sites where monkeys, typically macaques, range freely, often provisioned by visitors and protected by local populations) and in urban areas precisely because human/macaque interactions in these contexts are often intense

TABLE 1. Mean Pb, Hg, $\boldsymbol{\delta^{15}N}$*, and* $\boldsymbol{\delta^{13}C}$ *by sex, age, and trapping group*

	Sex		Age class		Trapping group		
	Mean ± SD (*n*)		Mean ± SD (*n*)		Mean ± SD (*n*)		
	Males	Females	Juveniles	Adults	1	2	3
Pb (ppm)	4.82 ± 2.04 (16)	4.24 ± 1.65 (17)	6.00 ± 1.54 (13)	3.57 ± 1.36 (20)	4.89 ± 1.50 (11)	5.59 ± 2.13 (9)	3.46 ± 1.4 (13)
Hg (ppm)	0.141 ± 0.130 (16)	0.149 ± 0.074 (19)	0.143 ± 0.051 (13)	0.147 ± 0.124 (22)	0.106 ± 0.029 (11)	0.188 ± 0.159 (9)	0.150 ± 0.087 (15)
$\delta^{15}N$ (‰)	5.23 ± 0.66 (16)	5.23 ± 0.77 (21)	5.66 ± 0.89 (15)	4.94 ± 0.37 (22)	5.10 ± 0.61 (11)	5.27 ± 0.80 (10)	5.29 ± 0.76 (16)
$\delta^{13}C$ (‰)	−21.46 ± 0.50 (16)	−21.87 ± 0.47 (21)	−21.80 ± 0.45 (15)	−21.62 ± 0.56 (22)	−21.50 ± 0.54 (11)	−21.30 ± 0.30 (10)	−22.08 ± 0.35 (16)

(Southwick and Siddiqi, 1994; Singla et al., 1997; Southwick et al., 2005; Fuentes, 2006).

Although macaques and humans have lived commensally in Asia for centuries, relatively little is known about the complex ways that synathropic macaques interact with surrounding human populations and how living in human-shaped environments (urban, agricultural, etc.), impacts their health and ecology. Such information is significant for several reasons. These populations of macaques are important to local communities. They are cherished as a valuable natural heritage and considered to have inherent spiritual value relating to religious beliefs. In many communities macaques have become a tourist attraction, contributing significantly to the local economy (Jones-Engel et al., 2006). Finally, the Swoyambhu macaques illustrate important aspects of the human-primate interface. Data obtained from these animals, with their intense interaction and frequent contact with humans, provide a window on the multiple ways in which humans impact other nonhuman primate species at the primate/human interface, including endangered species.

Environmental sources of lead and mercury

Our research protocol did not include a search for possible sources of lead and mercury in the Swoyambhu area. However, other researchers have shown that lead is prevalent in the environment in the Kathmandu Valley (Chettri et al., 2001). The macaques of Swoyambhu could be exposed to lead in a variety of contexts including soils contaminated from lead emissions as a result of the continued use of leaded and adulterated fuels in Nepal as well as other potential sources of lead in the Swoyambhu site including dust containing lead paint, open refuse piles with point sources, such as batteries and tin cans that use lead solder. Ayurvedic medicines are common in South an SE Asia. Saper et al. (2008) showed that more than 20% of ayurvedic medicines that originated in South Asia contained toxic levels of lead, mercury, and arsenic. Discarded medicines and wrappers are a common sight in Swoyhambu (Jones-Engel, personal observation). The low levels of Hg in macaque hair detected in this study suggest that either Hg is not present to a significant degree around the microenvironment represented by Swoyambhu, or that it is present, but the macaques do not come into contact with or ingest it.

Juvenile macaques as sentinels for lead exposure

The data presented here suggest that demographic and/or behavioral variables are associated with lead exposure in the Swoyambhu macaques. Lead levels in younger Swoyambhu macaques were higher than those measured in older animals and an independent effect was seen with trapping location. A few hypotheses might explain these observations. First, lead absorption from the gastrointestinal tract has been shown to be greater among juvenile than among adult macaques (Pound et al., 1978). Second, behavior or ranging patterns may bring some animals into more frequent or intense contact with sources of lead. For example, it is possible that young macaques, often seen playing in a rough and tumble fashion, may come into more frequent contact with lead-containing soil and dust than older animals. Lead may also be ingested when the animal subsequently cleans itself. It is possible that point sources of lead are located in the ranges of one or more of the groups. Potential sample bias from nonrandom sampling of individuals' hair length could also influence results.

Differences in feeding behaviors offer another possible explanation. Juvenile macaques in general are less successful foragers than adults (Jansen and Van Schaik, 1993). They spend more time foraging but forage less efficiently. They may explore a variety of foods not eaten by adults and may mix foraging with play behavior. "Play" feeding may include ingestion or mouthing of items that adults do not consume. It is possible, though no research to date has examined this hypothesis, that this less selective play feeding, on less desirable foods such as batteries, wrappers, etc., might lead to increased consumption of contaminants, including lead. Less selective, more exploratory feeding is also observed in human juveniles, particularly toddlers, who spend more time on the ground and may place a variety of items (including paint chips) into their mouths (Agency for Toxic Substances and Disease Registry, 1988). Young macaques, in particular, may be good sentinels for human exposure to lead because their behavior and physiology are similar to those of human children, especially young children, who share a propensity for curiosity, and have a penchant for picking up objects and inserting them into their mouths. The activities of children often bring them into contact with soil. The Swoyambhu site may thus provide opportunities for both macaques and small children to come into contact with toxicants in the environment, including lead.

Although lead levels were associated with age and trapping location, our data revealed no statistical association between lead level and stable isotopic compositions. These data fail to support the hypothesis that lead levels are associated with dietary composition.

Future directions

Further research is needed to better appreciate whether and how behavior and feeding ecology influence lead exposure among the Swoyambhu macaques. More detailed data on feeding ecology and ranging behaviors should be acquired and a thorough search for sources of lead in and around Swoyambhu should be undertaken. Comparing lead levels in macaques to those in local human populations, including children, will help to test whether macaques are suitable sentinels for lead exposure in humans. Measurement of other environmental contaminants, such as arsenic and cadmium, could provide further insight into the ecotoxicology of these macaques. Refinements in the research protocol should include measurement of lead concentrations in both hair and blood, and could include other biomarkers for lead exposure, including porphyrins or aminolevulinate, δ-dehydratase (ALAD).

In this study we have shown that hair, which can be obtained noninvasively, is a viable means of detecting exposure to potential toxins such as lead and mercury. This technique could be applied to other species of primates, particularly endangered species, to monitor levels of toxins in those vulnerable populations.

CONCLUSIONS

Macaques are a potentially suitable sentinel for lead exposure in humans, and hair offers advantages for measuring lead levels in these animals. Here we report hair lead and mercury levels in macaques at the Swoyambhu temple in Kathmandhu, Nepal. Lead levels were higher in young macaques and differed among the three groups of macaques that range over and around the Swoyambhu site. Mercury was present in very small amounts. No association between lead and mercury level and the isotopic compositions of carbon/nitrogen was detected. Behavioral and/or physiologic factors may play a role in determining the lead levels in macaque hair.

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LITERATURE CITED

Agency for Toxic Substances and Disease Registry. 1988. The nature and extent of lead poisoning in children in the United States: a report to congress. (DHHS Publication No: 99–2926). Atlanta, GA.

Aguirre AA, Ostfeld RS, House CA, Tabor GM, Pearl MC, editors. 2001. Conservation medicine: ecological health in practice. New York: Oxford University Press.

Balagangatharathilagar M, Swarup D, Patra RC, Dwivedi SK. 2006. Blood lead level in dogs from urban and rural areas of India and its relation to animal and environmental variables. Sci Tot Environ 359:130–134.

Bentzen TW, Follmann EH, Amstrup SC, York GS, Wooller MJ, Muir DCG, O'Hara TM. 2008. Dietary biomagnifications of organochlorine contaminants in Alaskan polar bears. Can J Zool 86:177–191.

Bergdahl I, Skerfving S. 2008. Biomonitoring of lead exposure-alternatives to blood. J Toxic Environ Health, Part A 71:1235–1243.

Cardona-Marek T, Knott K, Meyer BE, O'Hara TM. 2009. Mercury concentrations in Southern Beaufort sea polar bears: variation based on stable isotopes of carbon and nitrogen. Environ Tox Chem 28:1416–1424.

Chalise MK. 2006. Primate census in different parts of Nepal. J Univ Campus TUTA, TU, Prospective on Higher Education 2:35–41.

Chalise MK, Ghimire M. 1998. Non-human primate census in different parts of Nepal. Bull Nat Hist Soc Nepal 8:11–15.

Chettri MK, Thapa KB, Paudel K, Acharya B. 2001. Biomonitoring of toxic heavy metals in Kathmandu valley using Lichens. Eco Print 8:69–75.

Dehn L, Sheffield F, Follmann E, Duffy L, Thomas D, Bratton G, Taylor R, O'Hara T. 2005. Trace elements in tissues of phocid seals harvested in the Alaskan and Canadian Arctic: influence of age and feeding ecology. Can J Zool 83:726–746.

D'Have H, Scheirs J, Mubiana V, Verhagen R, Blust R, De Coen W. 2005. Nondestructive pollution exposure assessment in the European hedgehog (*Erinaceus eropaeus*). I. Relationships between concentrations of metals and arsenic in hair, spines and soil. Environ Tox Chem 24:2356–2364.

Engel G, Hungerford L, Jones-Engel L, Travis D, Fuentes A, Schillaci M, Kyes R. 2006. Risk assessment: a model for predicting cross-species transmission of SFV from macaques (*M. fascicularis*) to humans at a monkey temple in Bali, Indonesia. Am J Primatol 68:934–948.

Feng Q, Suzuki Y, Hisashige A. 1998. Hair mercury levels of residents in China, Indonesia, and Japan. Arch Environ Health 53:36–43.

Fisher LE. 1954. Lead poisoning in a gorilla. J Amer Vet Med Assoc 125:478–479.

Fuentes A. 2006. Human culture and monkey behavior: assessing the contexts of potential pathogen transmission between macaques and humans. Am J Primatol 68:880–896.

Fuentes A. 2007. Human-nonhuman primate interconnections and their relevance to anthropology. Ecol Environ Anthropol 2:1–11.

Foo S, Khoo N, Heng A, Chua L, Chia S, Ong C, Ngim C, Jeyaratnam J. 1993. Metals in hair as biological indices for exposure. Int Arch Occ Environ Health 65:S83–S86.

Goyer RA. 1990. Lead toxicity: from overt to subclinical to subtle health effects. Environ Health Persp 86:177–181.

Hindle E, Stevenson AC. 1930. Hitherto undescribed intranuclear bodies in wild rats and monkeys, compared with known virus bodies in other animals. Trans Roy Soc Trop Med Hyg 23:327.

Hoekstra PF, Braune BM, O'Hara TM, Elkin B, Solomon KR, Muir DCG. 2003. Organochlorine contaminant and stable isotope profiles in Arctic fox (*Alopex lagopus*) from the Alaskan and Canadian Arctic. Environ Pollut 122:423–433.

Jansen CH, Van Schaik CP. 1993. Ecological risk aversion in juvenile primates: slow and steady wins the race. In: Pereira M, Fairbanks LA, editors. Juvenile primates-life history, development, and behavior. Chicago: University of Chicago Press. p 57–76.

Jones-Engel L, Engel G. 2009. The risks and contexts of emerging primate-borne zoonoses. In: Fuentes A, Panter-Brick C, editors. Health, risk and adversity. Oxford: Berghahn Books. p 52–77.

Jones-Engel L, Engel GA, Heidrich J, Chalise MK, Poudel N, Viscidi R, Barry P, Allan J, Grant R, Kyes R. 2006. Temple monkeys and health implications of commensalism, Kathmandu, Nepal. Emerg Infect Dis 12:900–906.

Jones-Engel L, Engel G, Schillaci M, Rompis A, Putra A, Suryana K, Fuentes A, Beer B, Hicks S, White R, Wilson B, Allan J. 2005. Primate to human retroviral transmission in Asia. Emerg Infect Dis 11:1028–1035.

Jones-Engel L, May C, Engel G, Steinkraus K, Schillaci MA, Fuentes A, Rompis A, Chalise MK, Aggimarangsee N, Feeroz MM, Grant R, Allan J, Putra A, Wandia IN, Watanabe R, Kuller L, Thongsawat S, Chaiwarith R, Kyes R, Linial M. 2008. Diverse contexts of zoonotic transmission of simian foamy viruses in Asia. Emerg Infect Dis 14:1200–1208.

Jones-Engel L, Steinkraus K, Murray S, Engel GA, Grant R, Aggimarangsee N, Lee BY-P, May C, Schillaci MA, Chutthapati T, Somgird C, Vojtech L, Zhao J, Linial M. 2007. Sensitive assays for simian foamy virus reveal a high prevalence of infection in commensal, free-ranging, Asian monkeys. J Virol 81:7330–7337.

Jusko TA, Henderson CR, Lanphear BP, Cory-Slechta DA, Parsons PJ, Canfield RL. 2008. Blood concentrations < 10 μg/dL and child intelligence at 6 years of age. Environ Health Persp 116:243–248.

Khatlwada NR, Takizawa S, Tran TV, Inoue NR. 2002. Groundwater contamination assessment for sustainable water supply in Kathmandu Valley, Nepal. Water Sci Technol 46:147–154.

Lanphear B, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger D, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J, Roberts R. 2006. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Persp 114:A85–A86.

Laughlin N, Bowman R, Franks P, Dierschke D. 1987. Altered menstrual cycles in rhesus monkeys induced by lead. Fund App Tox 9:722–729.

Lee WC, Lee SM, Kim JS, Bae CS, et al. 2000. An observation on the mercury contents of scalp hair in the urban residents of South Korea. Environ Toxicol Pharmacol 8:275–278.

Levin E, Schneider M, Ferguson S, Schantz S, Bowman R. 1988. Behavioral effects of developmental lead exposure in rhesus monkeys. Dev Psychbiol 21:371–382.

Mitra AK, Haque A, Islam M, Bashar SA. 2009. Lead poisoning: an alarming public health problem in Bangladesh. Int J Environ Res Pub Health 6:84–95.

Moses SK, Whiting AV, Bratton GR, Taylor RJ, O'Hara TM. 2009. Inorganic nutrients and contaminants in spotted seals (*Phoca largha*) and sheefish (*Stenodus leucicthys*) of NW Alaska: linking the health of arctic wildlife and subsistence users. Int J Circumpolar Health 68:53–74.

Pacyna E, Pacyna J. 2004. Global emission of mercury from anthropogenic sources in 1995. Water, Air, Soil 137:149–165.

Palheta D, Taylor A. 1995. Mercury in environmental and biological samples from a gold mining area in the Amazon region of Brazil. Sci Tot Environ 168:63–69.

Pound JG, Marlar RJ, Allen JR. 1978. Metabolism of lead-210 in juvenile and adult rhesus monkeys (*Macaca mulatta*). Bull Environ Contam Toxicol 19:684–691.

Ratcliffe HE, Swanson GM, Fischer LJ. 1996. Human exposure to mercury: a critical assessment of the evidence of adverse health effects. J Tox Environ Health 49:221–270.

Reuhl KR, Rice DC, Gilbert SG, Mallett JE. 1989. Effects of chronic developmental lead exposure on monkey neuroanatomy: visual system. Tox Appl Pharmacol 99:501–509.

Rice DC. 1992. Behavioral effects of lead in monkeys tested during infancy and adulthood. Neurotoxicol Teratol 14:235–245.

Sakai T, Ito M, Aoki H, Aimi K, Nitaya R. 1995. Hair mercury concentrations in cats and dogs in Central Japan. Brit Vet 151:215–219.

Saper RB, Phillips RB, Sehgal A, Khouri N, Davis RB, Paquin J, Thuppil V, Kales SN. 2008. Lead, mercury, and arsenic in US- and Indian-manufactured ayurvedic medicines sold via the Internet. JAMA 300:915–923.

Sha J, Gumert M, Lee B-P, Fuentes A, Rajathurai S, Chan S, Jones-Engel L. 2009. Status of the long-tailed macaque *Macaca fascicularis* in Singapore and implications for management. Biodiv Cons 18:2909–2926.

Singla SL, Kaur M, Lal S. 1997. Monkey bites: a public health problem in urban setting. Indian J Pub Health 41:3–5:24.

Southwick C, Malik I, Siddiqi MF. 2005. Rhesus commensalism in India: problems and prospects. In: Patterson J, Wallis J, editors. Commensalism and conflict: human-primate interface. Norman: American Society of Primatologists. p 240–257.

Southwick CH, Siddiqi MF. 1994. Population status of nonhuman-primates in Asia, with emphasis on rhesus macaques in India. Am J Primatol 34:51–59.

Swarup D, Patra R, Dwivedi S, Dey S. 2000. Blood lead and cadmium in dogs from urban India. Vet Hum Toxicol 42:232–233.

Warner N, Levy J, Harpp K, Farruggia F. 2008. Drinking water quality in Nepal's Kathmandu Valley: a survey and assessment of selected controlling site characteristics. Hydrogeo J 16:321–334.

Woshner V, Knott K, Wells R, Willetto C, Swor R, O'Hara T, 2008. Mercury and selenium in blood and epidermis of bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, FL: interaction and relevance to life history and hematologic parameters. Ecol Health 5.3:360–370.

Zook B, Sauer R, Garner F. 1974. Lead poisoning in captive wild animals. J Wildlife Dis 8:264–272.