American Journal of Primatology 71:998–1010 (2009)

RESEARCH ARTICLE

Assessment of Organochlorine Pesticides and Metals in Ring-Tailed Lemurs (*Lemur catta*) at Beza Mahafaly Special Reserve, Madagascar

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Like most of Madagascar's endemic primates, ring-tailed lemurs (*Lemur catta*) face a number of threats to their survival. Although habitat loss is of greatest concern, other anthropogenic factors including environmental contamination may also affect lemur health and survival. In this study, we examined ring-tailed lemurs from the Beza Mahafaly Special Reserve (BMSR), southern Madagascar for exposure to organochlorine (OC) pesticides and metals and examined differences in contaminant concentrations between sexes and among age groups, troops, and habitats. A total of 14 pesticides and 13 metals was detected in lemur blood (24 individuals) and hair (65 individuals) samples, respectively. *p,p′*-DDT, heptachlor, aldrin, heptachlor epoxide, endrin aldehyde, and endrin were among the most prevalent pesticides detected. Surprisingly, the persistent metabolite of *p,p′*-DDT, *p,p′*-DDE, was not detected. The most commonly detected metals were aluminum, zinc, boron, phosphorus, silicon, and copper, whereas metals considered more hazardous to wildlife (e.g. arsenic, cadmium, lead, selenium, vanadium) were not found above detection limits. Overall, concentrations of OC pesticides and metals were low and similar to those considered to be background concentrations in other studies examining the ecotoxicology of wild mammals. Few inter-sex, -age, -troop, and -habitat differences in contaminant concentrations were observed, suggesting a uniform distribution of contaminants within the reserve. Several statistically significant relationships between lemur body size and contaminant concentrations were observed, but owing to the lack of supportive data regarding contaminant exposure in wild primates, the biological significance of these findings remains uncertain. Results of this study document exposure of ring-tailed lemurs at BMSR to multiple OC pesticides and metals and provide essential baseline data for future health and toxicological evaluations of lemurs and other wild primates, especially those in regions with expanding agricultural and mining operations. Am. J. Primatol. 71:998–1010, 2009.

Key words: organochlorine pesticides; metals; pollution; toxicology; anthropogenic effects; conservation; primate

INTRODUCTION

Environmental contamination in tropical, developing countries occurs as a result of multiple anthropogenic activities and environmental processes [Castillo et al., 1997; Daly et al., 2007; Smith et al., 2007; Wandiga, 2001]. From an ecological perspective, this is of particular concern because of the potential impacts of contaminants on the highly valued and biologically diverse ecosystems characteristic of these regions [Daly et al., 2007]. Regulations governing chemical production, distribution, storage, use, and disposal in tropical countries are often scant

Contract grant sponsors: Primate Conservation Inc.; The Lindbergh Fund; The Geraldine R. Dodge Foundation; The Saint Louis Zoo; The John Ball Zoo Society; The National Geographic Society; The University of Colorado; The University of North Dakota; The University of California-Davis; Texas Tech University.

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Received 18 December 2008; revised 28 July 2009; revision accepted 28 July 2009

DOI 10.1002/ajp.20742
Published online 10 September 2009 in Wiley InterScience (www.interscience.wiley.com).

or inadequately enforced [Murray, 1994; Waichman et al., 2002; Wandiga, 2001]. As a result, large quantities of chemicals (primarily pesticides) are routinely used for agriculture, crop storage, and vector control at rates often comparable to or higher than those in more developed countries [Castillo et al., 1997; Lacher & Goldstein, 1997; Wandiga, 2001]. In addition, many pesticides banned in developed countries are still commonly used in tropical countries [Turusov et al., 2002; Wandiga, 2001]. For example, the organochlorine (OC) pesticide DDT is currently used for malaria control in many Asian, Latin American, and African countries [Curtis, 2002; Roberts et al., 2002; Romi et al., 2002; Turusov et al., 2002; Wandiga, 2001]. Metals are introduced into tropical systems through both natural (e.g. erosion, biogeochemical cycles) and anthropogenic (e.g. mining, industrial discharges) processes [Burger et al., 2006; El Khalil et al., 2008; Michel & Ludwig, 2005; Ogola et al., 2002; Ramirez et al., 2005]. Many metals categorized as trace elements or minerals (e.g. copper, iron, phosphorus, zinc) have specific physiological functions in wildlife and are internally regulated [Burger et al., 2006; Michel & Ludwig, 2005; Miller et al., 2007]. As such, these metals are not normally considered to be pollutants unless present in abnormally high concentrations. However, other metals such as lead, cadmium, and mercury have no known physiological function, are toxic to wildlife, and are therefore generally considered to be pollutants when found in biota or other environmental matrices [Eisler, 1985, 1988; Michel & Ludwig, 2005; Wolfe et al., 1998]. Atmospheric deposition is also a significant contributor of contamination in tropical ecosystems, particularly in remote and seemingly pristine areas far from agricultural and industrial activities [Daly et al., 2007; Shen et al., 2005; Smith et al., 2007]. As a result of these numerous chemical inputs, tropical wildlife are routinely exposed to a variety of environmental contaminants, many of which are known to accumulate in and adversely affect animals in both the laboratory and the wild [Shore & Rattner, 2001; Smith et al., 2007].

Lemurs are endemic to Madagascar, one of the world's most threatened ''biodiversity hotspots'' [Myers et al., 2000], and are among the world's most endangered primates [Mittermeier et al., 2006]. Ring-tailed lemurs (*Lemur catta*), with a current International Union for Conservation of Nature vulnerable rank [Mittermeier et al., 2006], are found in a wide variety of habitats in southwestern Madagascar including both spiny and riverine gallery forests, and range as far as the high altitude Andringitra Massif [Goodman et al., 2006; Sauther et al., 1999]. Currently, habitat loss is the primary threat to ring-tailed lemur populations, although other anthropogenic factors, including hunting in some areas, also affect this species [Mittermeier et al., 2006]. In addition, exposure to environmental contaminants may also impact the health and survival of these animals [Miller et al., 2007]. In recent years, multiple biomedical evaluations have been conducted on wild lemurs to assess the health of individuals and populations, and to identify potential risks of anthropogenic activities on lemur fitness [Dutton et al., 2003; Junge & Louis, 2005; Miller et al., 2007]. Although environmental pollution has been identified as a concern [Miller et al., 2007], no previous study has specifically examined the exposure of lemurs to environmental contaminants.

Substantial literature exists pertaining to contaminant exposure and accumulation in many groups of wild mammals [Shore & Rattner, 2001; Smith et al., 2007], but very little is known regarding the ecotoxicology of wild primates [Kilbourn et al., 2003; Wiktelius & Edwards, 1997]. Indeed, with the exception of certain trace metals reported in general health assessments [Dutton et al., 2003; Junge & Louis, 2005; Miller et al., 2007], no data exist regarding metal or pesticide exposure and accumulation in lemurs. However, given the critical conservation status of lemurs, the subsequent need for information on stressors affecting lemur populations, and the multiple sources of environmental contamination in tropical countries, data describing contaminant exposure in lemurs are clearly needed. Therefore, the primary objective of this study is to determine concentrations of OC pesticides and metals in ring-tailed lemurs within the Beza Mahafaly Special Reserve (BMSR), southern Madagascar. Different troops of lemurs in this population utilize a variety of habitats from intact gallery forest to highly altered environments [Cuozzo & Sauther, 2004; Sauther et al., 2006]. Thus, differences in pollutant concentrations between sexes and among age groups, troops, and habitat types are examined. In addition, potential relationships between lemur body mass and tissue contaminant concentrations by sex, age, troop, and habitat type are also examined. We hypothesize that older lemurs, and those utilizing agricultural fields and living in closer proximity to potential contaminant sources (e.g. the research camp, villages), will contain higher contaminant concentrations than conspecifics living in areas less impacted by anthropogenic influences (e.g. intact forest). This study presents baseline data regarding the exposure of wild lemurs to OC pesticides and metals and provides reference values essential for future investigations examining pollutant accumulation patterns and associated health risks in lemur populations.

METHODS

Study Site and Sample Collection

During June and July 2005, wild ring-tailed lemurs were captured and sampled in and around

the 80 ha gallery forest portion of BMSR, southern Madagascar (23°30′S, 44°40′E), as part of an ongoing assessment of this population's ecology, health, and conservation status [Cuozzo & Sauther, 2004, 2006a,b; Cuozzo et al., 2008; Miller et al., 2007; Sauther, 1998; Sauther et al., 1999, 2002, 2006]. The sample area includes an intact gallery forest that has not been affected by human disturbance for over 20 years, and a degraded habitat next to the intact forest. This adjoining area includes forest that has been highly impacted via anthropogenic alteration including removal of forest for planting of local crops and heavy grazing by livestock [Whitelaw et al., 2005]. The degraded habitat also includes a research camp with some lemur troops exploiting resources that include well water and human crops. In addition, lemurs using the camp commonly lick the walls of cement and painted structures (Fig. 1). Lemurs sampled were categorized by sex, age, habitat, and troop (Tables I and II). Individual lemurs ($n = 66$) were classified as subadults (2nd year individuals, $n = 10$), young adults (3rd and 4th year individuals, $n = 12$), adults ($n = 31$), or old adults (>10 years of age, $n = 12$). One individual included in our analysis was not assigned an age at the time of capture. Each lemur at BMSR is identified with a numbered tag and colored collar, which indicates the troop (black, blue, green, light blue, orange, red, teal, or yellow) in which each lemur was originally captured and identified. Troops sampled could be grouped into three categories depending on their major habitat use. Lemurs from habitats classified as Reserve used intact gallery forest only (teal troop). Lemurs from habitats classified as Reserve+Crop/Camp used intact gallery forest for most months, but also either used agricultural crops during the dry season of low food abundance, or came into the research camp on a regular basis (green, orange, red, and yellow troops). Lemurs from habitats classified as Degraded used gallery forest habitat that has been heavily grazed by domestic animals as well as both crop and camp environments (black, blue, and light blue troops).

Fig. 1. Ring-tailed Lemurs (*Lemur catta*) licking the walls of a camp structure at Beza Mahafaly Special Reserve in 2005.

Lemurs were captured using a Dan-Inject blow dart system (Dan-Inject, North America, Fort Collins, CO) and the drug Telazol® (Fort Dodge Laboratories, Fort Dodge, IA). Doses were determined based on protocols developed over 20 years and over 400 captures of ring-tailed lemurs at BMSR [e.g. Cuozzo & Sauther, 2004, 2006a,b; Cuozzo et al., 2008; Miller et al., 2007; Sauther et al., 2002, 2006; Sussman, 1991]. Captures primarily occurred as early as possible in the morning to allow each lemur adequate time to recover, and a trained veterinarian and veterinary students were onsite to monitor the health of each individual lemur [Cuozzo & Sauther, 2006a,b; Cuozzo et al., 2008; Miller et al., 2007]. Approximately 1 mL of whole blood was collected from each lemur via femoral venipuncture [Sondgeroth et al., 2007], transferred to a sterile collection tube, and frozen in liquid nitrogen. Frozen samples were later shipped to Texas Tech University (TTU) in September 2005 where they were stored at −80°C until analysis for OC pesticides. A hair sample was collected from each lemur by clipping a ~1.0 g tuft of hair from the distal end of the tail as close to the skin as possible. Each hair sample was placed in a sealed plastic bag, stored at ambient temperature, and shipped to TTU for metals analysis in September 2005. Following sample collection, lemurs were placed in covered mesh cages and/or dog kennels, and kept in a quiet place for recovery. Upon recovery, individuals were released in the area from which they were originally captured (normally within 6 hr, but in some cases following a full night's recovery). All methods and materials received approval and followed standard animal handling guidelines and protocols of the Institutional Animal Care and Use Committees of the University of Colorado and the University of North Dakota. Data collection in Madagascar was conducted with approval of Association Nationale pour la Gestion des Aires Protégées (ANGAP), the body governing research in Madagascar's protected areas, as well as CITES (05US040035/9).

TABLE I. Demographics of Ring-tailed Lemurs in the Beza Mahafaly Special Reserve in Southwestern Madagascar Sampled for Organochlorine Pesticides in 2005

	No. of individuals			Mean (±SE) mass (kg)		Age group				
Troop	Total	Females	Males	Females	Males	SA	YA	A	OA	Habitat
Black	11	4	7	2.2±0.1	2.2±0.1	1	3	6	1	Degraded
Green	2	0	2	NA	2.3±0.3	0	0	1	1	Reserve+Crop
Light Blue	1	1	0	2.2	NA	0	0	0	1	Degraded
Orange[a]	5	3	2	2.3±0.1	2.2±0.1	0	0	3	1	Reserve+Camp
Red	1	0	1	NA	2.3	0	0	0	1	Reserve+Crop
Yellow	4	2	2	2.4±0.1	1.9±0.2	1	0	1	2	Reserve+Camp
Total	24	10	14	NA	NA	2	3	11	7	NA

SA, Subadult; YA, Young adult; A, Adult; OA, Old adult; NA, not applicable.
[a]The age of one lemur in Orange troop was undetermined.

TABLE II. Demographics of Ring-tailed Lemurs in the Beza Mahafaly Special Reserve in Southwestern Madagascar Sampled for Metals in 2005

	No. of individuals			Mean (±SE) mass (kg)		Age group				
Troop	Total	Females	Males	Females	Males	SA	YA	A	OA	Habitat
Black	12	4	8	2.2±0.1	2.3±0.1	1	2	8	1	Degraded
Blue	4	2	2	1.8±0.2	2.0±0.1	2	2	0	0	Degraded
Green	12	5	7	2.3±0.1	2.2±0.1	1	2	5	4	Reserve+Crop/Camp
Light Blue	12	4	8	2.2±0.1	2.1±0.1	2	3	6	1	Degraded
Orange[a]	8	4	4	2.2±0.1	2.1±0.1	1	0	4	2	Reserve+Crop/Camp
Red	8	3	5	2.3±0.03	2.2±0.1	0	0	6	2	Reserve+Crop/Camp
Teal	4	1	3	2.1	2.2±0.1	1	2	1	0	Reserve
Yellow	5	2	3	2.4±0.1	1.8±0.1	2	0	1	2	Reserve+Crop/Camp
Total	65	25	40	NA	NA	10	11	31	12	NA

SA, Subadult; YA, Young adult; A, Adult; OA, Old adult; NA, not applicable.
[a]The age of one lemur in Orange troop was undetermined.

OC Pesticides Analysis

A certified OC pesticide mixture consisting of tetrachloro-meta-xylene (TCMX), heptachlor, gamma (γ)-BHC (lindane), alpha (α)-BHC, beta (β)-BHC, delta (δ)-BHC, endosulfan I, endosulfan II, dieldrin, endrin, *p,p′*-DDD, *p,p′*-DDT, *p,p′*-DDE, methoxychlor, aldrin, heptachlor epoxide, γ-chlordane, α-chlordane, endrin aldehyde, endosulfan sulfate, endrin ketone, and decachlorobiphenyl (DCBP) was obtained from Ultra Scientific (North Kingstown, RI). Organic solvents were either pesticide or gas chromatography/mass spectroscopy (GC/MS)-grade.

Lemur blood sample preparation, extraction, and OC analysis followed procedures previously developed for nitroaromatic compounds [Zhang et al., 2007]. Before extraction, each blood sample was thawed at room temperature, mixed thoroughly by vortexing, and a 0.2 mL aliquot was collected for analysis. The sample (aliquot) was then spiked with two internal standards (TCMX and DCBP). Liquid extraction with sonication was employed for extracting OCs from the blood samples. 1.2 mL of acetonitrile was added to the 0.2 mL sample, followed by mixing with a vortex-mixer for 1 min. Samples were then sonicated using an ultrasonic water bath (Branson, Danbury, CT) at 50°C. During sonication, samples were also mixed periodically with a vortex-mixer. After extraction for 2–3 hr, samples were centrifuged at 3500 rpm (Beckman Allegra 6R; Palo Alto, CA) for 10 min. Supernatants were collected and cleaned using Florisil solid-phase extraction cartridges, evaporated to 1 mL under nitrogen, and filtered (0.2 μm) before GC analysis.

Analyses were performed using an HP 6890 gas chromatograph equipped with an HP 6890 autosampler and an electron capture detector (ECD) (Agilent, Palo Alto, CA). Separation was performed with a 30 m × 0.25 mm i.d. (0.25 μm film thickness) HP-5 column from Hewlett-Packard (Wilmington, DE). Helium (99.999% purity) served as carrier gas at a constant linear velocity of 80 cm/sec. Argon:methane served as make-up gas for the detector. The oven temperature program began at 90°C, held for 2 min, increased to 130°C at a rate of 25°C/min, then made a 10°C/min ramp to 200°C, and finally increased to 250°C at a rate of 25°C/min. The injection port temperature

was 170°C, whereas the detector was 200°C. A 2 μL standard or sample was injected in splitless electronic pressure control mode. The ECD was operated in constant current mode. HP ChemStation software was used to control and monitor the chromatography.

Five-point standard curves with OC pesticide standards were developed to quantify OC residues in blood samples. Extraction efficiency, based on recovery of the two internal standards from the lemur blood samples, ranged from 83–117%. OC concentrations were not adjusted for extraction efficiency. The method detection limits were 0.05–0.6 ng/mL (dependent on OC analyte). A portion of the samples were analyzed using GC/MS to confirm the identity (not concentration) of the OC pesticides.

Metals Analysis

Lemur hair samples were analyzed for the presence of 20 metals (aluminum, antimony, barium, beryllium, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, phosphorus, selenium, silver, thallium, tin, vanadium, zinc) and three metalloids (arsenic, boron, silicon; hereafter referred to as metals) following the methods of Cobb et al. [2006] and Abel et al. [2007]. Briefly, hair samples were dried at room temperature, weighed, and then predigested for a minimum of 8 hr in acid-rinsed 250 mL Teflon® beakers (Fisher, Pittsburg, PA) using concentrated nitric acid in a 2:1 acid:sample mass ratio. Samples were heated to 120°C on hotplates to digest the remaining tissue and evaporated to approximately 20 mL before addition of 30% hydrogen peroxide. Digested samples were then filtered through glass fiber filter paper, quantitatively transferred to appropriately sized volumetric flasks, and diluted using ultra-pure water. Samples were analyzed within 72 hr by inductively coupled plasma-atomic emission spectrometry [Abel et al., 2007; Cobb et al., 2006]. Each sample was diluted to obtain metal concentrations in digests that would fall within the five-point calibration range of the instrument, and each element's five-point calibration had a multiple correlation coefficient (r value) of no less than 0.995. Accuracy and precision of sample digestion and analysis procedures were determined using National Institute of Standards and Technology (Gaithersburg, MD) standards and method blanks. Method detection limits were 0.05–10 μg/mL, depending on the metal. No methodological contamination was detected in the samples or the instrument according to quality-control data from each sample batch. An interference check was performed to establish a clean performance line for each metal constituent. Metal concentrations are reported on a dry-weight basis (μg/g).

Statistical Analyses

Statistical analyses were performed using program JMPin statistical software (Version 3.2, SAS Institute, Cary, NC, USA). Owing to failure of nontransformed and transformed data to achieve normality and homogeneity of variance, and in some cases owing to small samples sizes, nonparametric procedures were used to examine differences in contaminant concentrations in samples between sexes (Wilcoxon rank sums test) and among age groups, troops, and habitat types (Kruskal–Wallis test). In addition to examining differences among the three primary habitat types (Reserve, Reserve+Crop/Camp, Degraded), where possible we also categorized lemurs in the Reserve+Crop/Camp habitat into two separate groups, Reserve+Crop and Reserve+Camp, and repeated the analysis using four habitat types. Relationships between body mass and tissue contaminant concentrations were examined using linear regression analysis. Analyte/metal concentrations falling below detection limits were assigned values of one-half the detection limit for that particular analyte/metal [US EPA, 1998]. However, concentrations of several analytes/metals were below detection limits, limiting the extent and rigor of the statistical analyses that could be performed [Schmitt et al., 2005]. All statistical tests were considered significant when $P \leq 0.05$. Both arithmetic and geometric means were calculated to allow for comparisons with other studies.

RESULTS

OC Pesticides

A total of 14 OC pesticides was detected in blood samples collected from 24 individual lemurs: aldrin, α-BHC, β-BHC, γ-BHC, δ-BHC, α-chlordane, γ-chlordane, p,p'-DDD, p,p'-DDT, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, and methoxychlor (Table III). p,p'-DDE, endosulfan I, endosulfan II, endosulfan sulfate, endrin ketone, and dieldrin were not detected. All (100%) samples contained p,p'-DDT, whereas 22 (91.7%) contained heptachlor, 20 (83.3%) contained aldrin and heptachlor epoxide, 18 (75%) contained endrin aldehyde, 16 (66.7%) contained endrin, eight (33.3%) contained γ-BHC and α-chlordane, four (16.7%) contained δ-BHC, three (12.5%) contained γ-chlordane and methoxychlor, and one (4.2%) contained α-BHC, β-BHC, and p,p'-DDD. The relatively high frequencies of occurrence of detectable concentrations of p,p'-DDT, heptachlor, heptachlor epoxide, aldrin, endrin, endrin aldehyde, γ-BHC, and α-chlordane allowed comparisons between sexes (but not among age groups, troops, or habitats) and in some cases provided adequate sample sizes to examine relationships between lemur body size (mass) and OC pesticide residues in blood samples.

Overall, two significant differences in blood OC pesticide concentrations were observed in BMSR lemurs: the mean concentration of aldrin in female lemurs was higher than in males ($\chi^2 = 3.69$; df = 1; $P = 0.0547$) (Table III), and male lemurs from the

TABLE III. Mean (±SE) Organochlorine (OC) Pesticide Concentrations (ng/mL) Detected in Whole Blood of Ring-tailed Lemurs Sampled in the Beza Mahafaly Special Reserve in Southwestern Madagascar in 2005

	Mean OC pesticide concentration					
	Sex		Age group			
OC pesticide	Males (14)	Females (10)	Subadults (2)	Young adults (3)	Adults (11)[a]	Old adults (7)
Aldrin	3.0±0.6 (1.9) (ND-7)	5.2±0.9 (3.9)[b] (ND-9)	3.5±0.5 (3.5) (3–4)	4.1±2.0 (2.1) (ND-7)	4.0±0.9 (2.5) (ND-9)	3.5±0.9 (2.5) (ND-8)
α-BHC	1.0±0.8 (0.3) (ND-11)	ND	5.6±5.4 (1.7) (ND-11)	ND	ND	ND
β-BHC	1.0±0.6 (0.3) (ND-9)	ND	ND	ND	ND	1.5±1.3 (0.4) (ND-9)
γ-BHC	0.8±0.2 (0.5) (ND-2)	0.7±0.2 (0.4) (ND-2)	ND	0.8±0.6 (0.5) (ND-2)	0.8±0.2 (0.5) (ND-2)	0.6±0.3 (0.4) (ND-2)
δ-BHC	0.5±0.2 (0.3) (ND-2)	0.7±0.3 (0.4) (ND-3)	ND	1.2±0.9 (0.6) (ND-3)	0.4±0.2 (0.3) (ND-2)	0.8±0.3 (0.5) (ND-2)
α-chlordane	0.6±0.2 (0.4) (ND-3)	1.1±0.3 (0.7) (ND-3)	1.1±0.9 (0.7) (ND-2)	0.8±0.6 (0.5) (ND-2)	0.9±0.3 (0.5) (ND-3)	0.8±0.4 (0.4) (ND-3)
γ-chlordane	0.3±0.1 (0.3) (ND-1)	0.4±0.1 (0.3) (ND-1)	ND	ND	0.5±0.1 (0.4) (ND-1)	ND
p,p′-DDD	ND	0.3±0.1 (0.3) (ND-1)	ND	ND	0.3±0.1 (0.3) (ND-1)	ND
p,p′-DDT	31.9±3.5 (29.5) (16–57)	38.3±2.6 (37.4) (23–50)	22.5±1.5 (22.4) (21–24)	32.0±6.2 (30.6) (20–41)	37.3±3.1 (35.7) (17–50)	33.1±5.4 (30.6) (16–57)
Endrin	1.4±0.3 (0.9) (ND-3)	1.9±0.3 (1.4) (ND-3)	2.0±0.0 (2) (2)	1.4±0.6 (1.0) (ND-2)	1.7±0.4 (1.1) (ND-3)	1.5±0.4 (1.1) (ND-3)
Endrin aldehyde	1.7±0.3 (1.2) (ND-4)	2.1±0.4 (1.5) (ND-4)	1.1±0.9 (0.7) (ND-2)	1.4±0.6 (1.0) (ND-2)	2.1±0.4 (1.6) (ND-4)	1.8±0.4 (1.2) (ND-3)
Heptachlor	5.8±1.0 (4.4) (ND-13)	6.6±1.2 (4.8) (ND-13)	4.0±1.0 (3.9) (3–5)	5.1±2.49 (2.4) (ND-8)	6.0±1.0 (4.5) (ND-13)	6.7±1.6 (5.6) (2–13)
Heptachlor epoxide	16.6±4.0 (7.2) (ND-46)	32.9±7.8 (13.7) (ND-80)	19.0±5.0 (18.3) (14–24)	10.3±8.4 (4.3) (1–27)	29.7±7.4 (12.2) (ND-80)	21.4±7.6 (6.7) (ND-48)
Methoxychlor	5.2±4.8 (0.4) (ND-67)	9.2±9.0 (0.5) (ND-90)	ND	ND	ND	23.0±14.6 (1.8) (ND-90)

Numbers in parentheses to the right of column headings indicate the number of animals sampled in that corresponding group. Values in parentheses to the right of arithmetic means are geometric means. Values in parentheses below means are ranges. Detection limits range: 0.05–0.6 ng/mL. ND, not detected.
[a]The age of one of the 24 lemurs was undetermined.
[b]Mean aldrin concentration in females significantly higher than in males ($P \leq 0.05$).

Reserve+Crop/Camp habitat contained higher concentrations of heptachlor than males from the Degraded habitat ($\chi^2 = 4.01$; df = 1; $P = 0.0452$).

Linear regression analysis revealed multiple significant relationships between lemur body mass (kg) and OC pesticide concentrations (Table IV). When OC pesticide concentrations were examined by sex in all lemurs sampled, a significant negative relationship between body mass and endrin was observed for females. When pesticide concentrations were examined by troop, body mass was negatively correlated with endrin in black troop when sexes were combined. When OC pesticide concentrations in blood were examined by habitat type, body mass of lemurs from the Degraded habitat was negatively correlated with endrin when both sexes were combined. In the Reserve+Crop/Camp habitat, body mass was negatively correlated with α-BHC and α-chlordane in males, and with α-BHC when both sexes were combined. Also in the Reserve+Crop/Camp habitat, a positive relationship was observed between body mass and endrin aldehyde when both sexes were combined.

Metals

A total of 13 metals was detected in hair samples collected from 65 individual lemurs: aluminum, antimony, barium, boron, chromium, cobalt, copper, iron, manganese, nickel, phosphorus, silicon, and zinc (Table V). Arsenic, beryllium, cadmium, lead, molybdenum, selenium, silver, thallium, tin, and vanadium were not detected. All (100%) samples contained aluminum and zinc, whereas 64 (98.5%) contained boron, phosphorus, and silicon, 63 (96.9%) contained copper, 60 (92.3%) contained

TABLE IV. Summary of Significant ($P \leq 0.05$; denoted by asterisks) Relationships Observed from Linear Regression Analysis of Blood Organochlorine Pesticide Concentrations as a Function of Body Mass (kg) in Ring-tailed Lemurs Sampled in the Beza Mahafaly Special Reserve in Southwestern Madagascar, 2005

		Females		Males		All		
Grouping	OC pesticide	R^2	P	R^2	P	R^2	P	Relationship
Sex	Endrin	0.66	0.0043*	0.11	0.2523	0.16	0.0563	Negative
Troop								
Black	Endrin	0.90	0.0512[a]	0.21	0.3005	0.37	0.0457*	Negative
Habitat								
Degraded	Endrin	0.88	0.0179[a]	0.21	0.3005	0.36	0.0382*	Negative
Reserve+Crop/Camp	α-BHC	ND	ND	0.60	0.0407*	0.58	0.0040*	Negative
Reserve+Crop/Camp	α-chlordane	0.65	0.0994	0.62	0.0348*	0.22	0.1197	Negative
Reserve+Crop/Camp	Endrin aldehyde	0.12	0.5742	0.28	0.2223	0.35	0.0436*	Positive

ND, not detected.
[a]Not accepted as significant due to small sample size.

TABLE V. Mean (±SE) Metal Concentrations (μg/g, dry weight) Detected in Hair of Ring-tailed Lemurs Sampled in the Beza Mahafaly Special Reserve in Southwestern Madagascar in 2005

	Mean metal concentration					
	Sex		Age group			
Metal/ metalloid	Males (40)	Females (25)	Subadults (10)	Young adults (11)	Adults (31)[a]	Old adults (12)
Aluminum	42.1±4.1 (36.8) (15.9–126.0)	41.1±4.3 (36.9) (17.2–95.8)	42.1±5.9 (38.8) (19.8–83.4)	39.8±9.1 (34.0) (19.2–126.0)	40.2±4.2 (35.5) (17.2–104.0)	48.3±7.4 (42.7) (15.9–103.0)
Antimony	2.0±0.1 (1.9) (ND-7.4)	ND	2.2±0.1 (2.2) (ND-7.4)	ND	ND	ND
Barium	6.9±0.5 (6.3) (ND-16.5)	6.1±0.4 (5.7) (ND-11.5)	7.1±1.4 (6.0) (ND-16.5)	5.9±0.5 (5.7) (ND-9.8)	6.5±0.5 (6.0) (ND-12.8)	6.9±0.6 (6.6) (4.0–10.2)
Boron	17.6±1.1 (15.9) (ND-36.6)	22.1±1.6 (20.2)[b] (4.3–39.2)	16.3±2.5 (13.1) (ND-29.8)	20.4±2.5 (18.8) (7.1–39.2)	20.8±1.3 (19.5) (6.6–36.6)	16.9±2.1 (15.1) (4.3–32.1)
Chromium	1.3±0.3 (1.0) (ND-12.4)	ND	ND	ND	1.3±0.4 (1.0) (ND-12.4)	ND
Cobalt	1.9±0.3 (1.4) (ND-6.3)	2.8±0.5 (2.1) (ND-10.6)	2.0±0.5 (1.6) (ND-5.3)	2.3±0.9 (1.6) (ND-10.6)	2.6±0.4 (1.8) (ND-6.5)	1.8±0.5 (1.2) (ND-4.8)
Copper	12.2±0.7 (10.8) (ND-24.0)	11.3±0.4 (11.1) (8.2–17.7)	12.9±1.5 (12.3) (8.6–24.0)	11.9±0.9 (11.6) (8.2–18.5)	11.3±0.7 (9.9) (ND-17.7)	12.3±1.0 (11.9) (9.0–20.8)
Iron	16.9±4.6 (4.3) (ND-104.0)	11.2±4.4 (2.5) (ND-87.0)	10.3±4.0 (4.6) (ND-38.0)	9.6±8.6 (1.6) (ND-95.2)	15.6±5.0 (3.7) (ND-104.0)	21.8±9.2 (5.4) (ND-102.0)
Manganese	7.6±0.5 (6.9) (ND-15.6)	7.0±0.5 (6.5) (ND-12.5)	8.1±0.9 (7.5) (ND-12.2)	6.6±0.7 (6.2) (ND-11.3)	7.5±0.6 (6.8) (ND-15.6)	6.9±0.7 (6.4) (ND-10.2)
Nickel	3.2±0.5 (2.0) (ND-13.9)	4.7±0.9 (2.9) (ND-16.6)	2.1±0.8 (1.5) (ND-9.4)	3.7±1.4 (2.3) (ND-16.6)	4.5±0.7 (2.7) (ND-13.9)	3.1±0.9 (1.9) (ND-9.3)
Phosphorus	290.7±10.2 (263.6) (ND-395)	311.4±6.9 (309.6) (260–390)	320.3±15.9 (316.6) (245–395)	307.7±16.2 (303.2) (224–390)	288.4±11.5 (256.1) (ND-380)	298.3±9.9 (296.5) (250–340)
Silicon	81.9±7.5 (72.5) (38.8–220.0)	75.8±7.3 (64.5) (ND-148.0)	84.6±12.6 (77.7) (45.2–180.0)	77.4±15.2 (68.3) (39.9–220.0)	75.4±7.0 (68.5) (40.0–198.0)	90.0±15.3 (67.3) (ND-188.0)
Zinc	144.8±4.9 (142.1) (107–262)	140.4±3.8 (139.2) (114–195)	140.8±8.9 (138.3) (107–187)	136.1±4.6 (135.3) (115–166)	149.5±5.6 (146.9) (110–262)	136.7±6.1 (135.4) (115–195)

Numbers in parentheses to the right of column headings indicate the number of animals sampled in that corresponding group. Values in parentheses to the right of arithmetic means are geometric means. Values below means are ranges. Detection limits range: 0.05–2.0 μg/g. ND, not detected.
[a]The age of one of the 65 lemurs was undetermined.
[b]Mean boron concentration in females significantly higher than in males ($P \leq 0.05$).

barium, 37 (56.9%) contained manganese, 30 (46.2%) contained nickel, 27 (41.5%) contained iron, 26 contained cobalt (40%), and one (1.5%) contained antimony and chromium. The relatively high frequencies of occurrence of detectable concentrations of aluminum, zinc, boron, phosphorus, silicon,

copper, barium, manganese, nickel, iron, and cobalt allowed comparisons between sexes and among age groups, habitats, and troops, and in some cases provided adequate sample sizes to examine relationships between lemur body size and metal concentrations in hair samples.

One significant difference regarding metal concentrations in hair was observed between sexes: the mean concentration of boron in hair of female lemurs was higher than in males ($\chi^2 = 6.32$; df = 1; $P = 0.0119$) (Table V). Similarly, one significant difference regarding metal concentrations in hair was observed among the different lemur age groups: subadult males contained a higher mean antimony concentration than old adult males ($\chi^2 = 11.99$; df = 3; $P = 0.0074$). Five significant differences regarding metal concentrations in hair were observed in lemurs living in different habitats. Lemurs (sexes combined) from the Reserve+Crop/Camp habitat contained higher concentrations of cobalt than those from the Degraded habitat ($\chi^2 = 5.46$; df = 1; $P = 0.0194$). In addition, females from the Reserve+Crop/Camp habitat contained higher concentrations of cobalt ($\chi^2 = 6.05$; df = 1; $P = 0.0139$) and nickel ($\chi^2 = 4.57$; df = 1; $P = 0.0325$) than females from the Degraded habitat. When the Reserve+Crop/Camp habitat was separated into two categories (Reserve+Crop and Reserve+Camp), lemurs from the Reserve+Camp habitat were shown to contain higher concentrations of boron ($\chi^2 = 7.10$; df = 2; $P = 0.0287$) than lemurs from the Reserve+Crop habitat and higher concentrations of chromium ($\chi^2 = 11.40$; df = 2; $P = 0.0033$) than lemurs from the Reserve+Crop and Degraded habitats. No significant differences in metal concentrations were observed among troops.

Linear regression analysis revealed several significant relationships between lemur body mass and metal concentrations in hair (Table VI). When metal concentrations were examined by sex, a significant negative relationship between body mass and antimony was observed for males and both sexes combined. When metal concentrations were examined by age, a significant negative relationship between body mass and boron in hair of young adults was observed for females and for both sexes combined. In addition, body mass of adult lemurs was negatively correlated with concentrations of antimony in combined sexes. When metal concentrations were examined by troop, multiple significant relationships between body mass and hair metals were observed. In orange troop, both aluminum and iron were negatively correlated with body mass when sexes were combined. Zinc concentrations were negatively and positively correlated with body mass in red troop and light blue troop, respectively, when sexes were combined. In green troop, concentrations of barium were negatively correlated with body mass in males and combined sexes. When metal concentrations were examined by habitat type, a negative correlation was observed between body mass and antimony in males and combined sexes in the Degraded habitat.

DISCUSSION

In this study, ring-tailed lemurs from BMSR were examined for exposure to two classes of persistent environmental contaminants, OC pesticides and metals. Whole blood and hair were used as sample matrices, which will allow successive biomonitoring of the same populations and individuals in future studies [Smith et al., 2007]. Overall, 14 OC pesticides and 13 metals were detected in lemur blood and hair samples, respectively.

The detection of DDT in 100% of lemur blood samples was not surprising, as this OC pesticide is

TABLE VI. Summary of Significant ($P \leq 0.05$; denoted by asterisks) Relationships Observed from Linear Regression Analysis of Hair Metal Concentrations as a Function of Body Mass (kg) in Ring-tailed Lemurs Sampled in the Beza Mahafaly Special Reserve in Southwestern Madagascar, 2005

		Females		Males		All		
Grouping	Metal	R^2	P	R^2	P	R^2	P	Relationship
Sex	Antimony	0.001	0.9054	0.18	0.0062*	0.08	0.0253*	Negative
Age								
Adults	Antimony	0.12	0.2371	0.16	0.0953	0.15	0.0300*	Negative
Young adults	Boron	0.85	0.0247*	0.28	0.2830	0.43	0.0280*	Negative
Troop								
Green	Barium	0.27	0.3737	0.91	0.0008*	0.51	0.0096*	Negative
Light blue	Zinc	0.59	0.2337	0.37	0.1088	0.39	0.0293*	Positive
Orange	Aluminum	0.68	0.1731	0.69	0.1723	0.58	0.0288*	Negative
Orange	Iron	0.80	0.1069	0.43	0.3458	0.54	0.0373*	Negative
Red	Zinc	0.31	0.6223	0.68	0.0839	0.53	0.0419*	Negative
Habitat								
Degraded	Antimony	0.06	0.4882	0.24	0.0402*	0.18	0.0227*	Negative

ubiquitous in the environment and thought to be present in every living organism on the planet [Turusov et al., 2002]. Somewhat surprising, however, was the absence in samples of DDE, the more persistent breakdown product of DDT. The detection of higher DDT:DDE ratios in biological tissues is believed to indicate a more recent exposure, whereas lower ratios are thought to be more indicative of chronic (or previous) exposure [ATSDR, 2002; Luo et al., 1997]. DDT is currently used in Madagascar for malaria control [Curtis, 2002; Romi et al., 2002], lending support to the possibility of recent exposure in nontarget organisms. However, given the persistence and ubiquitous nature of DDE in the environment, it is likely that BMSR lemurs are exposed to and accumulate DDE but that this lipophillic DDT metabolite is sequestered in higher concentrations in body fat rather than circulating blood [ATSDR, 2002]. Few other studies have examined OC pesticide exposure and accumulation in wild primates, and those available indicate variation in exposure among species, locality, and tissue sampled. Although ring-tailed lemurs in this study contained 14 OC pesticides in blood, none were detected in blood plasma from orangutans (*Pongo pygmaeus pygmaeus*) in Malaysia [Kilbourn et al., 2003]. Conversely, although dieldrin was not detected in lemurs in this study, Koeman et al. [1978] reported moderately high concentrations (mean = 14.8 mg/kg) of this OC pesticide in livers of tantalus monkeys (*Cercopithecus aethiops*) in Nigeria. The presence of OC pesticides in liver compared to blood is expected, as these compounds readily accumulate in the former, often in high concentrations [Beyer et al., 1996; Yoshikane et al., 2006]. Overall, all OC pesticide concentrations detected in lemur blood samples in this study were in the low ng/mL range and are considered to be background concentrations.

Although several metals were detected in lemur hair samples, most metals considered hazardous to wildlife (e.g. arsenic, cadmium, lead, selenium, vanadium) were not found above detection limits. Metal concentrations in the hair of ring-tailed lemurs in this study were generally low but were higher than those previously detected in blood plasma of ring-tailed and other lemur species from multiple sites in Madagascar [Dutton et al., 2003; Junge & Louis, 2005; Miller et al., 2007; Table VII] and in serum from orangutans in Malaysia [Kilbourn et al., 2003]. These differences likely reflect the propensity of metals to more readily accumulate in hair than in blood [Burger et al., 1994; Kershaw et al., 1980] rather than greater overall metal exposure in lemurs sampled in this study. This is especially likely given the overlap between some individuals in the current sample with those reported in a previous study [Miller et al., 2007]. To our knowledge, no other study has reported metals in hair of wild primates; however, metal concentrations detected in lemur hair in BMSR are similar to those found in hair from other wild mammals from relatively unpolluted habitats and are considered to be low [e.g. Burger et al., 1994; Mora et al., 2000; O'Hara et al., 2001].

Vertebrates are exposed to environmental contaminants via four pathways: ingestion, dermal contact, inhalation, and maternal transfer [Smith et al., 2007]. Of these, ingestion and maternal transfer are likely the most significant routes of OC pesticide and metal exposure in ring-tailed lemurs at BMSR. Lemurs may ingest these pollutants through consumption of contaminated food items and water or while grooming following aerial deposition of contaminants on fur [Davis et al., 2007; Hariono et al., 1993]. Dietary intake is probably the primary route of contaminant ingestion in the BMSR lemurs, as these animals feed almost exclusively on leaves, stalks, flowers, and fruits of numerous plant species [e.g. Cuozzo et al., 2008; Sauther, 1998; Yamashita, 2002], and air-borne pollutants are known to be commonly deposited and retained on plant surfaces [Collins et al., 2006; Pfleeger et al., 1996]. However, in many plants only the roots accumulate significant concentrations of pollutants [Collins et al., 2006;

TABLE VII. Mean (±SD) Concentrations of Trace Metals Detected in Hair and Blood Plasma of Wild Lemurs in Madagascar

	Hair (μg/g)	Blood plasma (μg/mL)			
Metal	Ring-tailed lemur[a]	Ring-tailed lemur[b]	Ring-tailed lemur[c]	Decken's sifaka[d]	Red-fronted brown lemur[e]
Boron	19.4±7.7	<1.0	NA	<1.0	<1.0
Chromium	1.2±1.5	<0.1	NA	<0.1	<0.1
Copper	11.9±3.7	1.0±0.2	0.8±0.2	0.6±0.1	1.2±0.2
Iron	14.7±26.6	1.6±0.4	0.7±0.3	1.8±0.6	1.7±0.8
Zinc	143.1±26.9	0.9±0.2	0.7±0.2	1.5±0.3	1.4±0.4

[a]*Lemur catta* ($n = 65$), Beza Mahafaly Special Reserve [this study].
[b]*L. catta* ($n = 26$–27), Beza Mahafaly Special Reserve [Miller et al., 2007].
[c]*L. catta* ($n = 18$), Tsimanampetsotsa Strict Nature Reserve [Dutton et al., 2003].
[d]*Propithecus verreauxi deckeni* ($n = 20$), Tsiombokibo Classified Forest [Junge & Louis, 2005].
[e]*Eulemur fulvus rufus* ($n = 16$–20), Tsiombokibo Classified Forest [Junge & Louis, 2005].

Sims & Overcash, 1983]. If this is the case with the plants most commonly consumed by lemurs, the absence of roots in the diet may partially explain the low concentrations of OC pesticides and metals detected in this lemur population. In addition, ring-tailed lemurs at BMSR exhibit geophagy [e.g. Sauther, 1998]. Thus, the low concentrations of OC pesticides in these lemurs suggest a lack of significant soil contamination at BMSR, at least in areas where lemurs were sampled.

Miller et al. [2007] observed lemurs licking the walls of painted structures within the camp at BMSR (Fig. 1) and suggested this behavior may be a potential route of lead exposure. Indeed, lead poisoning has been reported in other wildlife from ingestion of lead-based paint chips [e.g. Finkelstein et al., 2003]. However, the absence of lead in lemur hair samples in this study suggests that lemurs licking building walls are not ingesting paint or that the paint on these structures is not lead-based. Finally, in utero and juvenile lemurs may also be exposed to OC pesticides and metals through placental and lactational transfer, respectively, from contaminated mothers [Addison & Brodie, 1977, 1987; Borrell et al., 1995]. Although to our knowledge maternal transfer of environmental contaminants has not been specifically examined in wild primates, it has been identified as a significant exposure route in other mammals [e.g. Addison & Brodie, 1977, 1987; Bernhoft et al., 1997; Borrell et al., 1995; Dip et al., 2003; Duinker & Hillebrand, 1979; Greig et al., 2007; Lahaye et al., 2007; McIlroy, 1981; Miranda et al., 2009].

Accumulation of environmental contaminants is related to duration and frequency of exposure and is generally expected to be greater in individuals and populations closer to contaminant sources [Smith et al., 2007]. Therefore, we hypothesized that older lemurs and those living in closer proximity to potential contaminant sources (i.e. the Degraded habitat) would contain higher contaminant concentrations than younger lemurs and those living farther from contaminant sources (i.e. the Reserve and Reserve+Crop/Camp habitats). However, this proved not to be the case, as metal concentrations were not higher in old adults compared with the younger age classes, and in the Degraded habitat compared with the Reserve and Reserve+Crop/Camp habitats (sample sizes were insufficient for inter-age and -habitat comparisons regarding OC pesticides). Given the low contaminant concentrations observed in this study, it is possible that the primary source of pollutant exposure in lemurs is atmospheric deposition, which would likely result in a more uniform distribution of contaminants throughout BMSR and its different habitats than would contamination from more localized sources (e.g. agricultural areas, villages).

The biological significance of the statistically significant relationships between lemur body mass and contaminant concentrations is unknown. Negative relationships between concentrations of persistent environmental contaminants and body mass or age may indicate the depuration of contaminants in females through maternal transfer [Addison & Brodie, 1987; Santos et al., 1999; Weis & Muir, 1997]; however, in this study few such relationships were observed and occurred more often in males than females. Two significant inter-sex differences in contaminant concentrations were observed, with aldrin and boron concentrations being higher in females than males. This could possibly reflect sex-specific differences in certain life history traits influencing exposure (e.g. microhabitat use, diet, grooming behavior) or in the toxicodynamics or toxicokinetics of OC pesticides and metals in lemurs following exposure [Smith et al., 2007]. Overall, as with results of previous biomedical evaluations of ring-tailed lemurs [Miller et al., 2007], the biological significance of statistical differences in several parameters examined in this study is uncertain. Owing to the paucity of data describing the ecotoxicology of ring-tailed lemurs and wild primates in general, it is most appropriate to defer discussion of these parameters until their biological significance is confirmed. Our intent in this study was to provide baseline data regarding contaminant exposure and associated demographic trends in BMSR ring-tailed lemurs for comparative purposes in future health and ecotoxicological investigations of this and other lemur populations.

Results of this study document that ring-tailed lemurs in BMSR are exposed to numerous OC pesticides and metals. Concentrations of these pollutants in lemur blood and hair are generally similar to or lower than those considered to be background levels in studies of other wildlife [e.g. Burger et al., 1994; Custer et al., 2000; Klemens et al., 2003; Mora et al., 2000; O'Hara et al., 2001]. However, wildlife are often under the influence of multiple stressors acting concurrently, affecting animals simultaneously through different mechanisms such that the impact of an individual stressor may be more pronounced than if acting alone [Gibbons et al., 2000; Peveling et al., 1999, 2003]. Thus, additional stressors such as disease, parasitism, invasive species, climate change, and environmental pollution may act synergistically on wildlife, resulting in a significant cumulative effect on populations already under stress from habitat loss or alteration [Gibbons et al., 2000; Peveling et al., 1999, 2003]. As such, the potential influence of contaminants on lemur health should not be dismissed. Information regarding exposure of lemurs to persistent environmental contaminants should prove especially important for conservation efforts, in light of continued and expanding mining operations in a number of areas in southern Madagascar [e.g. Gardner et al., 2009; Lowry et al., 2008]. This study provides baseline information for future conservation and health assessments of lemurs living

where these activities occur. It should be noted, however, that lemurs are likely exposed to numerous other environmental contaminants not examined in this study including mercury, polychlorinated biphenyls, and particularly phenylpyrazole, organophosphorus, pyrethroid, and benzoylurea insecticides used extensively in Madagascar for locust (*Locusta migratoria capito*, *Nomadacris septemfasciata*) control [Peveling et al., 1999, 2003; Smith et al., 2007]. These and other pollutants should also be considered in future ecotoxicological assessments and overall health evaluations of lemur and other endemic mammal populations.

ACKNOWLEDGMENTS

This project received approval by and followed standard animal handling guidelines and protocols of the Institutional Animal Care and Use Committees (IACUC) of the University of Colorado and the University of North Dakota. All data were collected in Madagascar with approval of CITES (permit no. 05US040035/9) and ANGAP (Association Nationale pour la Gestion des Aires Protégées), the body governing research in Madagascar's protected areas. Our appreciation goes to the Département des Eaux et Forêts, Ecole Supérieure des Sciences Agronomiques (ESSA) and ANGAP for permission to work at Beza Mahafaly. We thank Jeannin Ranaivanosy of ESSA for providing information on the use of pesticides at Beza Mahafaly. Our gratitude goes to the members of the Beza Mahafaly Ecological Monitoring Team for their capture and animal handling assistance, including Enafa and Edidy for providing their darting expertise, and Andry Randrianandrasana (ANGAP) for his support and facilitation of our project. Funding for this project was provided by Primate Conservation Inc., the Lindbergh Fund, the Geraldine R. Dodge Foundation, the Saint Louis Zoo, the John Ball Zoo Society, the National Geographic Society, the University of Colorado, Boulder, the University of North Dakota, the University of California-Davis, and Texas Tech University.

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