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Feces are Effective Biological Samples for Measuring Pesticides and Flame Retardants in Primates

Shaorui Wang, Tessa Steiniche, Jessica M. Rothman, Richard W. Wrangham, Colin A. Chapman, Richard Mutegeki, Rodolfo Quirós, Michael D. Wasserman, and Marta Venier*

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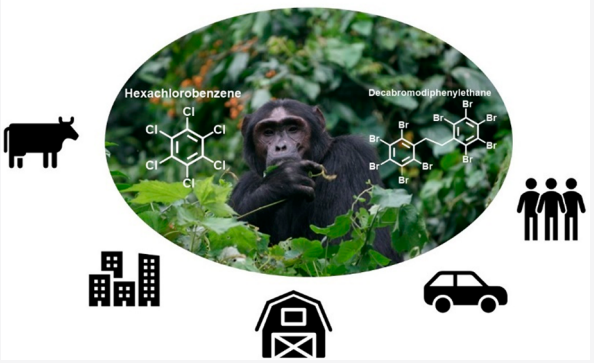
ABSTRACT: The habitats of wild primates are increasingly threatened by surrounding anthropogenic pressures, but little is known about primate exposure to frequently used chemicals. We applied a novel method to simultaneously measure 21 legacy pesticides (OCPs), 29 current use pesticides (CUPs), 47 halogenated flame retardants (HFRs), and 19 organophosphate flame retardants in feces from baboons in the U.S.A., howler monkeys in Costa Rica, and baboons, chimpanzees, red-tailed monkeys, and red colobus in Uganda. The most abundant chemicals were α-hexachlorocyclohexane (α-HCH), β-hexachlorocyclohexane (β-HCH), and hexachlorobenzene among OCPs across all sites, chlorpyrifos among CUPs in Costa Rica and Indiana, decabromodiphenylethane (DBDPE) in Costa Rica and Indiana and 2, 2', 4, 4'-tetrabromodiphenyl ether (BDE-47) in Uganda as HFRs, and tris(2-butoxyethyl) phosphate (TBOEP) as OPFRs across all sites. The detected chemical concentrations were generally higher in red-tailed monkeys and red colobus than in chimpanzees and baboons. Our methods can be used to examine the threat of chemical pollutants to wildlife, which is critical for endangered species where only noninvasive methods can be used.



INTRODUCTION

To ensure the viability of non-human primate (hereafter primate) populations that are threatened, biomonitoring is critical where increasing agricultural and industrial chemical pollutants pose significant toxicological threats. Costa Rica and Uganda are two examples of countries aiming to balance large-scale, pesticide-intensive agricultural approaches targeted to export crops, with small-scale subsistence farming and conservation of biodiversity.[1] We previously reported on four groups of chemicals, including legacy pesticides (OCPs), current use pesticides (CUPs), halogenated flame retardants (HFRs), and organophosphate flame retardants (OPFRs), in the air of forests in both countries.[2] Several of these chemicals are known endocrine-disruptors, interfering with normal hormone functioning and causing adverse developmental, immune, and reproductive effects.[3,4]

A major limitation to wildlife biomonitoring is obtaining biological samples. Sampling methods traditionally used for biomonitoring humans are prohibitive in many wild species (e.g., invasive blood draws) or difficult to collect (e.g., urine). Additionally, urine is not an appropriate matrix for persistent hydrophobic chemicals, such as legacy pesticides or brominated flame retardants (BFRs), because they are not sufficiently water-soluble for urine to be a major excretion route. Depending on their properties, chemicals entering the body can be quickly metabolized and excreted in urine or feces or absorbed, stored in adipose tissue, and slowly excreted in feces. Thus, feces can be an effective noninvasive matrix for biomonitoring, as they can be collected from wildlife with minimal disturbance and provide information regarding exposure. Long-term research on habituated primate groups throughout the tropics make sample collection and long-term monitoring of exposure feasible.

Few studies have used feces for toxicant analysis, especially with primates or other tropical animals.[5−14] In fact, for primates only one study we are aware of did so—Brockman et al. (2009) examined fecal tetrachlorodibenzo-*p*-dioxin (TCDD) concentrations in douc langurs (*Pygathrix nigripes*, *P. nemaeus*, *P. cinereal*) using an enzyme immunoassay.[15] In humans, digestive absorption of hydrophobic organic chemicals, such as dioxins, furans, polychlorinated biphenyls (PCBs), and hexachlorobenzene, is related to food intake,

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while fecal excretion is related to body burden or to the chemicals passing from the food into the feces directly.[16]

In this study, we optimized methods for examining large numbers of chemical pollutants in feces, including OCPs, CUPs, HFRs, and OPFRs in a captive setting and then applied the methods to fecal samples from wild primates in Costa Rica and Uganda. The goals of our study were: (1) to test the feasibility of the newly developed method; (2) to assess the amount and type of chemicals in the feces of wild primate populations living in landscapes with a mosaic of forest and agriculture; (3) to compare the levels of chemical pollutants in feces with those measured in previously collected air samples;[2] and (4) to preliminarily assess the source of chemicals present in feces by measuring them in food and soil.

MATERIALS AND METHODS

Chemicals and Reagents. The authentic standards, including 21 legacy pesticides, 29 CUPs, 47 HFRs, and 19 OPFRs, were purchased from Wellington Laboratories (Guelph, ON, Canada), Ultra Scientific (Santa Clara, CA, U.S.A.), AccuStandard (New Haven, CT, U.S.A.), Chem Service (West Chester, PA, U.S.A.), and Cambridge Isotope Laboratories (Andover, MA, U.S.A.). Specific details on standards manufacturers and a detailed list of target analytes, together with surrogate and internal standards are provided in Table S1 of the Supporting Information (SI). All standards were ≥95% purity. Silica gel (100−200 mesh, 75−150 μm, grade 644) and granular anhydrous sodium sulfate (Na_2SO_4) were purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.). Sand (20−30 mesh) was purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Florisil and alumina were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.) and MP Biomedicals (Santa Ana, CA, U.S.A.), respectively. Strata-X-AW cartridges (200 mg/3 mL) were purchased from Phenomenex (Torrance, CA, U.S.A.). HPLC or Optima grade solvents were purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.). Gases of 99.999% purity were purchased from Indiana Oxygen (Indianapolis, IN, U.S.A.).

Sample Collection. Fecal samples were obtained from captive baboons (*Papio anubis*, $n = 5$; *Papio hamadryas*, $n = 2$; *Papio papio*, $n = 3$) at a primate sanctuary in Indiana in 2017. Fecal samples from wild primates were collected between 2017 and 2019 from howler monkeys (*Alouatta palliata*) at a biological station in Costa Rica ($n = 12$)[2] and baboons (*Papio anubis*, $n = 10$), chimpanzees (*Pan troglodytes*, $n = 10$), red-tailed monkeys (*Cercopithecus ascanius*, $n = 10$), and red colobus monkeys (*Piliocolobus tephrosceles*, $n = 8$) from a national park in Uganda. All wild primate populations are part of long-term research projects, with many animals individually recognizable. Wild primate groups were observed throughout the day with fecal samples collected opportunistically upon defecation and placed immediately in sterilized amber glass vials. These samples were stored frozen at −20 °C in the field, shipped frozen at −20 °C to Indiana University (IU), and stored at −80 °C until processed. Commercial feed ($n = 5$) and soil samples ($n = 5$) were also collected in 2017 at the Indiana Sanctuary.

Chemical Analysis. Feces, commercial feed, and soil samples were freeze-dried for 24 h and pulverized using a mortar previously muffled at 500 °C for 8 h. For gas chromatographic (GC) and gas chromatographic mass spectrometry (GC/MS) analyses, approximately 0.5−1 g feces, 2 g commercial feed, and 2 g soil were weighed, spiked with surrogate standards (D-HCH and Epsilon-HCH for legacy pesticides, d_{10}-chlorpyrifos, d_{14}-trifulralin, and ^{13}C-*trans*-DCCA for CUPs, BDE-77, -166, and ^{13}C-BDE-209 for HFRs, d_{12}-TCEP, and MTPP for OPFRs), and extracted with hexane/dichloromethane (1:1, v/v) using an accelerated solvent extraction system (Dionex ASE 350, Sunnyvale, CA, U.S.A.). The extracts were concentrated and split into three subsamples. The first aliquot was used for gravimetrically measuring the lipid content. The extracts were transferred to preweighted aluminum saucers, covered with aluminum foil, and left in the hood overnight until constant weight was obtained. The lipid content for each species is included in Table S7. The second aliquot, used for legacy pesticides and HFRs, was cleaned using a column (glass, internal diameter [i.d.] 1 cm, length 25 cm) packed with neutral alumina (3 cm; 3% water activated), neutral silica (3 cm; 3% water activated), acid silica (6 cm; 3% water activated), and sodium sulfate (1 cm) from bottom to top. The samples were eluted with a 40 mL mixture of hexane and dichloromethane. The third aliquot, used for OPFRs and CUPs analyzed with GC/MS (Table S1), was cleaned using a column packed with neutral alumina (3 cm; 3% water activated), neutral silica (3 cm; 3% water activated), florisil (3 cm; 3% water activated), and sodium sulfate (1 cm) from bottom to top. The samples were first eluted with a 40 mL mixture of hexane and dichloromethane and then with 40 mL of ethyl acetate. All fractions were blown down to 1 mL and spiked with known amounts of internal standards (PCB155 for legacy pesticides, d_6-bifenthrin, d_{10}-diazinon, and ^{13}C-3-PBA for CUPs, BDE-118 and -181 for HFRs, and d_{15}-TEP, d_{15}-TPP, d_{21}-TPRP, d_{27}-TNBP, and d_{15}-TDCIPP for OPFRs).

For the liquid chromatography mass spectrometry (LC/MS/MS) analysis for remaining CUPs, 0.5−1 g of fecal samples were spiked with surrogate standards as mentioned above, sonicated with 5 mL acetonitrile/MeOH (1:1, v/v) for 15 min, and vortexed for 1 min. After sonication, the samples were centrifuged for 10 min at 4000 rpd and the supernatant transferred to a 15 mL tube. The sonication and centrifugation steps were repeated once more, and the extracts were combined and blown down to 1 mL with N_2. The extracts were diluted with 1 mL phosphate buffer (0.1 M, pH = 6.0), vortexed for 1 min and then loaded on the Strata-X-AW cartridge. The cartridge was conditioned with 5 mL of methanol, followed by 5 mL of a solution of phosphate buffer pH 6/methanol (90:10 v/v), and dried for 5 min. The cartridges were eluted with 5 mL of ethyl acetate, dried once again under vacuum for 5 min, and then eluted with 5 mL methanol/formic acid (90:10 v/v). The final extracts were evaporated to dryness and reconstituted in 100 mL of acetonitrile, spiked with internal standards (d_{10}-Diazinon and ^{13}C-3-PBA), and then run on LC−MS/MS.

Instrumental Analysis. Details on instrumental analyses can be found in the SI. The analytical instrument used for each individual compound measured here is provided in Table S1. Briefly, legacy pesticides in the second aliquot were analyzed using a gas chromatograph (GC) equipped with 63Ni electron capture detectors and a DB-5 column (250 μm i.d. and 0.1 μm film thickness, Agilent, Santa Clara, California). An Agilent 7890 GC coupled to an Agilent 5975C MS operating in the electron capture negative ionization (ECNI) mode with a Rtx-1614 capillary column (15 m × 250 μm × 0.10 μm film thickness; Restek Corp., Bellefonte, PA) was used for the analysis of HFRs and some CUPs, also in the second aliquot.

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Table 1. Median Concentration (Med, ng/g lipid) of the Most Abundant (DF > 50%) OCPs, CUPs, HFRs, and OPFRs in Feces from Indiana Sanctuary, Costa Rica, and Uganda[a]

	Indiana				Costa Rica				Uganda															
	Baboon (n = 10)				Howler monkey (n = 12)				Chimpanzee (n = 10)				Baboon (n = 10)				Red-tailed monkey (n = 10)				Redcolobus (n = 8)			
	Med	St. Dev	DF	*	Med	St. Dev	DF	*	Med	St. Dev	DF	*	Med	St. Dev	DF	*	Med	St. Dev	DF	*	Med	St. Dev	DF	*
α-HCH	2.4	0.9	100	bc	1.3	2.0	83	c	0.82	0.58	90	c	4.1	6.9	100	b	20	64	100	a	17	7.1	100	a
β-HCH	23	10	100	ab	2.8	29	100	c	5.3	3.7	100	bc	20	38	100	a	130	269	100	a	110	54	100	a
HCB	0.48	0.14	100	bc	1.0	1.3	100	c	0.42	0.28	100	c	1.1	2.1	100	b	6.9	18	100	a	6.0	3.2	100	a
α-chlordane					0.82	1.4	100	a									4.1	24	80	a				
γ-chlordane									1.9	1.2	100	c	8	13	90	b	40	96	100	a	36	16	100	a
p,p'-DDE	1.7	0.36	100	b																	260	155	100	a
o,p'-DDD									0.81	0.51	100	b	1.2	2.4	60	b	9.4	27	90	a	8.3	4.4	100	a
o,p'-DDT									0.83	0.75	40	b	7.0	6.0	80	a	25	53	100	a	25	15	50	a
heptachlor epoxide									0.83	0.52	60	b	0.69	2.0	100	b	8.0	28	60	a	5.6	1.9	63	a
HCHs[b]	25	10	100	b	4.8	31	100	c	5.7	4.1	100	c	23	45	100	b	150	325	100	a	125	61	100	a
chlordanes[c]					0.82	1.4	100	c	1.9	1.2	100	c	8.0	13	90	b	46	114	100	a	39	21	100	a
$\sum_9$OCPs	**27**	**11**	**100**	**bc**	**10**	**31**	**100**	**c**	**9.4**	**6.7**	**100**	**c**	**43**	**66**	**100**	**b**	**240**	**547**	**100**	**a**	**460**	**252**	**100**	**a**
chlorpyrifos	2.0	3.9	50	a	18	29	58	a																
γ-cyhalothrin	2.0	7.5	70																					
cypermethrin	0.56	1.5	30																					
$\sum_3$CUPs	**2.5**	**11**	**80**	**a**	**18**	**29**	**58**	**a**																
BDE-47									2.4	1.7	100	c	2.8	4.4	100	bc	21	64	90	a	10	10	100	ab
BDE-99									6.4	5.3	100	b					10	16	80	ab	16	7.6	88	ab
BDE-100									0.87	0.84	80	bc									11	3.5	63	a
BDE-209	0.59	0.76	100	a																				
DPs	1.2	1.5	100	b					0.23	0.46	70	c												
DBDPE	5.5	1.1	100	a	1.7	4.1	75	ab	4.0	25	100	a												
$\sum_6$HFRs	**7.6**	**22**	**100**	**bc**	**3.0**	**6.3**	**92**	**d**	**15**	**26**	**100**	**bc**	**8.0**	**25**	**100**	**cd**	**82**	**280**	**100**	**a**	**34**	**25**	**100**	**ab**
EHDP					43	150	92																	
TBOEP	52	20	60	bc	46	180	100	bc	46	25	100	c	320	350	100	ab	500	1600	100	a	460	1400	100	a
TCIPP	8.1	12	100	c	64	67	67	abc	44	32	100	bc	69	110	100	ab	450	1000	100	a	290	100	100	a
TDCIPP	4.5	1.4	100	b																	49	36	100	a
TEP	0.80	0.45	60	d	9.1	11	100	c	44	24	100	b	76	150	100	ab	220	650	100	a	120	48	100	ab
TNBP	2.6	2.8	100	c	2.7	26	67	c	9.6	7.3	100	c	18	23	70	bc	120	340	100	ab	170	120	100	a
$\sum_6$OPFRs	**54**	**33**	**100**	**c**	**78**	**240**	**100**	**c**	**140**	**83**	**100**	**bc**	**460**	**560**	**100**	**ab**	**1200**	**2900**	**100**	**a**	**1200**	**1500**	**100**	**a**

[a]The detection frequency (DF, in %) were also given. ANOVA results using logarithmically transformed concentrations are also shown in the columns headed by asterisks. Concentrations sharing the same letter are not significantly different at a $p < 0.05$ level. [b]Sum of α-H CH and β-HCH. [c]Sum of α-Chlordane and γ-Chlordane.

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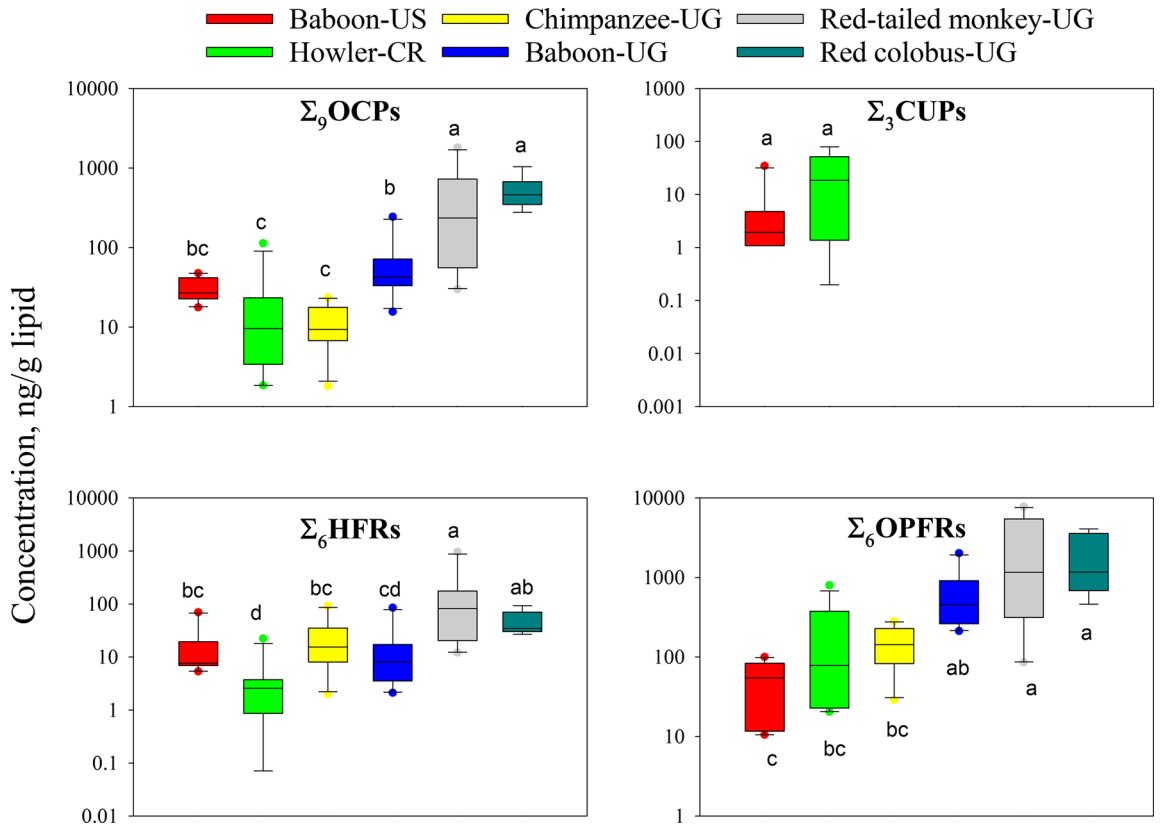

Figure 1. Total concentration (ng/g lipid) of OCPs, CUPs, HFRs, and OPFRs in feces from Indiana (US), Costa Rica (CR), and Uganda (UG). The boxes represent the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the black horizontal solid line inside each box represents the median. The letters represent the ANOVA results of the comparison of the logarithmically transformed concentrations among the species at Indiana, Costa Rica, and Uganda, respectively.

The remaining CUPs and their metabolites were analyzed by ultraperformance liquid chromatography coupled to a triple quadrupole tandem mass spectrometry (Agilent 1290 Infinity II UPLC- 6470 QQQ-MS). The system was equipped with a Synergi Polar-RP LC column (2.5 um; 150 × 2.0 mm, Phenomenex, Torrence, CA, U.S.A.). An Agilent 1290 infinity Π UPLC coupled to an Agilent 6470 triple quad MS operating in the electrospray ionization (ESI) mode with a C18 column (2.1 × 50 mm, 1.7 um, Waters, Milford, MA, U.S.A.) was used for OPFRs analysis.

Quality Assurance/Quality Control. Procedural blanks consisting of sand previously baked at 500 °C for 8 h were treated along with the feces to examine potential background contamination from laboratory operations. Blanks represented less than 10% of the chemicals measured in the actual samples. The reported concentrations were corrected by subtracting blanks on a mass basis (Table S2 for blank levels). Given that surrogate recoveries were generally within the range of 80–100% (Tables S3 and S4 for recoveries in feces, commercial feed, and soil), the reported data were not corrected for recoveries.

The accuracy and precision of the new analytical method were determined using fecal samples from the Indiana Sanctuary (n = 10), which were spiked with known amounts of the target analytes (Table S5 for percent recoveries). Overall, method validation results showed acceptable recoveries and precision for most of the analytes. The exceptions were β-HCH and λ-cyhalothrin, for which average recoveries were 42% and 148%, respectively. Concentrations for these two compounds are either underestimated or overestimated by this method and results should be interpreted cautiously. Median recoveries for the other legacy pesticides ranged between 60% for α-HCH to 107% for p,p'-DDD, with standard errors lower than 7%. For most CUPs, average recoveries were in the range 65–130%, with standard errors <15%. HFRs and OPFRs average recoveries ranged from 67 to 113% and 66 to 120%, with standard errors <12% and <10% respectively. The average recoveries of surrogates were generally in the range of 70–120% (Table S3) except for $^{13}C_{12}$–BDE209 for which recoveries were around 50%. BDE-209 was detected only in samples from the Indiana Sanctuary but the lack of detection in other fecal samples could be attributed to poor performance of this method for this compound.

Data Analysis. Data were examined for outliers with a Grubbs' test. Then, an analysis of variance (ANOVA) test with logarithmically transformed concentrations was used to compare concentrations across species. Statistics were calculated using Minitab 19 (State College, PA, U.S.A.) and Microsoft Excel 2016. Plots were generated using SigmaPlot 13 (Systat Software Inc., San Jose, CA, U.S.A.).

Data from the Indiana Sanctuary were used to calculate the daily excretion rates and daily intake rates for the most abundant chemicals, including β-HCH, chlorpyrifos, BDE-209, DBDPE, and TBOEP. The excretion rate (ng/kg/day) was calculated as follows:

$$\text{excretion} = \frac{C_{\text{feces}} \times \text{rate}_{\text{excr}}}{\text{body weight}} \quad (1)$$

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where C_{feces} is the median concentration in feces in the Indiana Sanctuary (ng/g) from this study, $rate_{excr}$ is the feces excretion rate (g/day), and body weight (kg) is the estimated weight range for baboons (9.2 to 26 kg).[17] An average feces excretion rate of 20 g/day was measured for baboons at the Indiana Sanctuary. The cumulative daily intake (ng/day) was calculated as follows:

$$\text{intake} = \sum (C_{food} \times \text{amount}_{food}) \quad (2)$$

where the C_{food} (ng/g) is the concentration of selected chemicals in each type of food and $amount_{food}$ is the average cumulative amount (g/day) of each food item consumed daily (Table S8). All dietary information is based on data provided for baboons at the Indiana Sanctuary. The standard errors of intake rates were calculated using a Monte Carlo method on 500 iterations.[18] Each variable included in these calculations was assumed to be normally distributed with a mean equal to the experimental value and its standard deviation equal to the experimental error, either measured as the variation of individual measurements or estimated based on other considerations.

RESULTS AND DISCUSSION

Legacy Pesticides. $\sum_9$OCPs (sum of α-HCH, β-HCH, hexachlorobenzene (HCB), α-chlordane, γ-chlordane, p,p'-DDE, o,p'-DDD, o,p'-DDT, heptachlor epoxide) were significantly higher in feces from red colobus and red-tailed monkeys than in the other species (Table 1, Figure 1; $p < 0.05$). Specifically, the median concentrations of $\sum_9$OCPs were 27, 10, 9.4, 43, 240, and 460 ng/g lipid for baboons from Indiana, howler monkeys from Costa Rica, and chimpanzee, baboon, red-tailed monkey, and red colobus from Uganda, respectively.

The most frequently measured OCPs at the three locations were α-HCH, β-HCH, and HCB, with detection frequencies higher than 80% (Table 1). Concentrations of α-HCH, β-HCH, and HCB were generally higher ($p < 0.05$) in feces from red colobus and red-tailed monkeys than in other species (Table 1). There is a very limited number of publications on the occurrence of pesticides in feces,[10,11,19,20] and a comparison with these studies is complicated because in some cases the results were reported for wet weight[19] or not normalized by lipid content.[11] Christensen et al. (2013) reported that the concentration of total HCHs (sum of α-HCH and β-HCH) in grizzly bear (*Ursus arctos*) feces from Canada ranged from 3.7 to 7.3 ng/g lipid,[10] which is within the range of our observations for howler monkeys and chimpanzees, but lower than for baboons at the Indiana Sanctuary and red-tailed monkeys and red colobus (Table 1 for total HCHs).

Interestingly, the ratio of α-HCH to β-HCH ($f_{\alpha/\beta}$) was generally <1 (Table S6) for all samples, which might be related to the longer half-life of β-HCH in biota than α-HCH.[21,22] Although HCHs were not frequently found in the air of Costa Rica and Uganda,[23,24] detectable HCH concentrations in the soil and water of Costa Rica and Uganda were observed in recent years.[23,25,26] For example, Daly et al. (2004) detected an α-HCH concentration in the soil of 7.5 pg/g at La Selva.[23] Similarly, HCHs were found in the air[27] sampled in 2010 in the Great Lakes Region of North America, even though the use of technical HCH mixture in North America was restricted in the 1970s.[27]

The median HCB concentrations in these samples ranged from 0.42 to 6.9 ng/g lipid. HCB concentrations in red-tailed monkeys and red colobus from Uganda (6.9 and 6.0 ng/g lipid, respectively) were significantly higher than those from the other species and locations, but similar to those observed in grizzly bear feces, ranging from 3.5 to 10 ng/g lipid.[10] Although the direct application of HCB has stopped globally, this compound is also a byproduct of other pesticides, including lindane, chlorothalonil, and pentachlorophenol, which are all chemicals that continue to be produced in high volume.[28,29]

α-Chlordane was detected in feces from howler monkeys, with detection frequencies higher than 90%. γ-Chlordane was only detected in feces from Uganda, also at high detection frequencies (>90%) and medians ranging from 1.9 to 40 ng/g lipid. Christensen et al. (2013) reported that the sum of the oxy-, α-, and γ-chlordane was 7.3 and 80 ng/g lipid (mean) in feces from grizzly bears that ate plants and salmon, respectively,[10] confirming that diet plays an important role in the total chemical load, likely due to bioaccumulation up the food chain. Differences in the chlordane isomer patterns can be explained in terms of chemical weathering. In chickens (*Gallus gallus domesticus*), α-chlordane and γ-chlordane shared similar absorption rates, but γ-chlordane exhibited a slightly more rapid elimination rate than α-chlordane, likely explaining why we mostly detected γ-chlordane.[30]

Heptachlor epoxide was detected in the range of 0.69 to 8.0 ng/g lipid in feces from Uganda, with detection frequencies higher than 60%. Heptachlor epoxide is a metabolite of heptachlor[31] and more toxic and persistent than the parent chemical.[32]

Among DDTs, o,p'-DDT, the parent chemical, and two metabolites, p,p' -DDE and o,p' -DDD, were detected in feces from Uganda. o,p'-DDT and o,p' -DDD concentrations were in the range of 0.83 to 25 ng/g lipid and 0.81 to 9.4 ng/g/lipid, respectively. p,p'-DDE was detected in red colobus at a median concentration of 260 ng/g and in baboons from Indiana at a much lower median concentration of 1.7 ng/g. p,p'-DDE is the main metabolite of DDT degradation, and it is considered a marker of past exposure to commercial DDT. It is not clear why p,p'-DDE was only detected in red colobus in Uganda, but we speculate that it might be related to interspecies differences in diet and subsequent metabolic pathways, leading to varying excretion patterns across species. The concentration of $\sum$DDTs in grizzly bear feces[10] were in the range of 0.55 and 220 ng/g lipid, while p,p' -DDE in feces from sea lions in Alaska was measured at an average concentration of 1300 ng/g lipid.[33] In general, o,p'-DDT was measured at higher concentrations than its metabolite o,p'-DDD, indicating that the direct excretion of o,p'-DDT in primates was higher than that of o,p'-DDD or that the biotransformation of o,p'-DDT in primates is slow. In a previous study in and around a national park in Uganda, o,p'-DDT and p,p'-DDT residues were detected in maize and p,p'-DDE was detected in fish from this park.[34] The authors hypothesized that the facial dysplasia observed in chimpanzee and baboons might be related to exposure to these chemicals,[34] but the dysplasia could also be due to infectious disease (e.g., yaws *T. pallidum* subsp. *pertenue*). Here, we confirm the internal exposure to these compounds in nearby primate populations.

CUPs. CUPs were detected only in feces from Indiana and Costa Rica, but not in any Ugandan samples (Figure 1). CUPs were not generally detected in air samples from the same

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locations in Uganda,[2] suggesting that these compounds are not widely used near the park. In addition, given the high polarity and water solubility of these chemicals, it is not surprising that these compounds were not found more extensively in the feces. However, the observed total CUPs concentration in wild primates in Costa Rica were generally comparable with that of OCPs.

Chlorpyrifos and λ-cyhalothrin were detected in more than 50% of feces from Indiana, while only chlorpyrifos was observed in 58% of feces from Costa Rica. This result is not surprising since chlorpyrifos contributed over 70% to the total CUPs concentration in the air samples collected in the same location as the feces.[2] Chlorpyrifos was also detected in all feces from the Indiana Sanctuary, with a median concentration of 2.0 ng/g lipid.

λ-Cyhalothrin and cypermethrin were detected in feces from the Indiana Sanctuary, but not in any other samples. λ-Cyhalothrin and cypermethrin, both pyrethroid insecticides, are intensively used both in households and by professionals to kill termites and in landscape applications in urban environments. These uses explain their detection in samples from the Indiana Sanctuary, but not in those from Costa Rica or Uganda.[35] However, the body burden of pyrethroids in terrestrial animals is not well studied,[36,37] making the comparison with other studies difficult.

HFRs. The most frequently detected HFRs were BDE-209 (DF, 100%), DPs (100%), and DBDPE (100%) in Indiana, DBDPE (75%) in Costa Rica, and BDE-47 (>90%) in Uganda (Table 1). $\sum_6$HFRs (sum of BDE-47, -99, -100, -209, DPs, and DBDPE) were generally the highest in feces from Uganda, especially for red-tailed monkeys (Figure 1). The relatively higher concentrations of HFRs in fecal samples collected from Uganda were not surprising, given that HFR concentrations, in particular pentaBDEs, in the air of the study areas in Uganda were comparable with those reported in the urban site of Chicago, U.S.A.

Penta BDE congeners (i.e., BDE-47, BDE-99, and BDE-100) were detected in samples from Uganda. Measured concentrations are comparable with those from a study on grizzly bear feces from Canada that reported medians for BDE-47, BDE-99, and BDE-100 of 0.90−4.6 ng/g lipid, 0.91−1.9 ng/g lipid, and 0.20−0.84 ng/g lipid.[10] BDE-209 was detected only in feces from baboons from the Indiana Sanctuary with a median concentration of 0.59 ng/g lipid, which is lower than that observed in grizzly bears from Canada (3.2 ng/g lipid).[10]

Among non-PBDEs flame retardants, only DPs and DBDPE were detected. DPs measured with median concentrations of 1.2 and 0.23 ng/g lipid in feces from baboons in the Indiana Sanctuary and chimpanzees in Uganda, respectively, with detection frequencies higher than 60%. Several studies have examined DPs occurrence in animals' feces, although the use of different reporting units complicate the comparison.[38,39]

DBDPE concentrations ranged from 1.7 to 5.5 ng/g lipid in feces from baboons in Indiana, howler monkeys in Costa Rica, and chimpanzees in Uganda. To our knowledge, there are no available data on the occurrence of DBDPE in feces, except for one laboratory study investigating the biological fate of DBDPE in rats.[40] Although DBDPE and BDE-209 were used in similar applications and there are speculations that DBDPE was introduced in the market as a replacement for BDE-209, we did not find a statistical association between the concentrations of these two chemicals in feces from Indiana. The significantly higher levels of DBDPE compared to BDE-209, combined with the lack of correlation between the two, point toward different sources or metabolic pathways for these compounds, despite structural similarities. The levels reported here for BDE-209 might be underestimated due to the relatively low recoveries of the associated surrogate standard.

These results can be interpreted in terms of how these compounds are metabolized in mammals. Low brominated PBDEs are preferentially retained in tissues, while high brominated PBDEs are mainly excreted in feces.[41] For example, Huwe et al. found that tetra- to hexa-BDEs were efficiently absorbed from the gastrointestinal tract, while a large fraction of BDE-209 dose was excreted in feces.[7] Morck et al. found that 90% of BDE-209 was excreted via feces after a single oral dose of ^{14}C-labeled BDE-209 in rats, resulting in 10% of the BDE-209 dose accumulated in the animals.[42] BDE-47 was preferentially retained in the adipose tissue and less than 15% was excreted through feces.[43,44] Therefore, the amounts of PBDEs we measured are mainly due to the endogenous excretion into the feces and they likely represent only a small fraction of the total load of HFRs in primates.

The presence of BDE-47, and in general of penta BDEs, in feces collected from primates from Uganda is not surprising to us, as we found that the atmospheric concentration of BDE-47 around the national park was unexpectedly high and comparable to that observed in urban Chicago.[2] Further studies are needed to determine the sources of HFRs around the national park.

OPFRs. The most frequently detected OPFRs in feces in all these locations were TBOEP, TCIPP, TEP, and TNBP, with detection frequencies higher than 60%. TBOEP was the most abundant chemical among OPFRs in all samples, followed by TCIPP. The median $\sum_6$OPFRs (EHDP, TBOEP, TCIPP, TDCIPP, TEP, and TNBP) of 54 and 78 ng/g lipid in feces from baboon in Indiana and from howler monkeys in Costa Rica were significantly lower than that in feces from other species (Table 1 and Figure 1). Four of the six observed OPFRs were nonchlorinated (e.g., EHDP, TBOEP, TEP, and TNBP), which have a lower bioaccumulation potential than chlorinated OPFRs[45] and hence, are easier to excrete. Our results support this finding.

Data on OPFRs in feces are scant and most are from in vivo laboratory studies.[46−48] The median TBOEP concentration in feces ranged from 46 ng/g lipid for chimpanzee to 500 ng/g lipid for red-tailed monkey. The predominance of TBOEP among OPFRs was also confirmed in air, house dust, and biota.[49,50] TEP in feces from Uganda was higher than in samples from Indiana and Costa Rica (Table 1). TEP is primarily used as an industrial catalyst and a plasticizer for resins, plastics, and gums, and in small amounts as a solvent, flame retardant, or antifoaming agent. It is also an intermediate in production of various chemicals. As an inert ingredient in pesticide products, TEP is used as a stabilizer in formulations applied before a crop emerges from the soil,[51] which is the likely source in Uganda.

EHDP was detected in 92% of the Costa Rican samples, but not in the other locations. EHDP is commonly used for several commercial applications including polyvynil chloride (PVC), rubber, and food packaging.[51] Not being chemically bound, it can easily leak into the environment, but we cannot speculate on possible sources of EHDP in Costa Rica.

TNBP and TCIPP were also relatively abundant in feces from red-tailed monkey and red colobus. The significant difference of TNBP and TCIPP among species can be

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attributed to differences in the biodegradation or metabolic pathway of OPFRs analogs in different species. For example, TNBP and TCIPP can biomagnify,[52,53] and they can also be biodegraded to form metabolites that can be readily excreted.[54]

Comparison of Chemical Patterns between Feces and Air in Uganda. In 2017, we collected air samples in the national park in Uganda.[2] As expected, most of the chemicals observed in the air, namely α-HCH, HCB, γ-chlordane, heptachlor epoxide, BDE-47, BDE-99, BDE-100, BDE-209, DPs, TNBP, and TCIPP, were also detected in the feces of primates (Figure 2).

Interestingly, some compounds (e.g., β-HCH, p,p'-DDE, o,p'-DDD, and o,p'-DDT) were found in feces but not in the air. Zhou et al. (2008) showed that higher levels of β-HCH and p,p'-DDE in biota than in the surrounding environment can be attributed to their resistance to microbial degradation and long half-life.[55] Historical residues of some persistent organic chemicals in environmental reservoirs, such as soil, are also potential sources of these chemicals in feces, as well as current uses, since DDT is still occasionally used for pest control. For instance, although DDTs were not detected in our air samples in Uganda, both parent DDTs and their metabolites (i.e., DDDs and DDEs) were observed in primate feces. Primates are either slowly excreting these chemicals, while lowering their body burden from dated exposure events, or they are still accumulating them from food sources rich with these pollutants like soil through geophagy or plants, potentially including crops.[34,56]

The HFR profiles for feces and air were generally similar, and they were both dominated by penta BDE congeners (e.g., BDE-47, -99, and -100). However, a higher proportion of DBDPE was observed in feces compared to air, while the opposite was true for BDE-209. The difference between these two structurally similar compounds can potentially relate to BDE-209 being more easily debrominated to lighter congeners by both abiotic and biotic processes,[57] resulting in a lower abundance in feces. In feces, TBOEP and TEP contributed to 45% and 21% to the total OPFRs, respectively, but they were not that abundant in air. Non-halogenated OPFRs, like TBOEP and TEP, are more difficult to be transformed in biota relative to halogenated OPFRs, which might explain their detection in feces but not in air.[58]

Relationship between Chemicals in Feces and Body Burden. The chemicals present in feces are coming from two possible sources: excretion via enterohepatic and intestinal circulation, which reflect xenobiotic body burden, or residual excretion after intestinal absorption, which is more directly associated with direct exposure to chemicals. The excretion time of these chemicals varies among chemical groups and primate species. Nonpersistent pesticides, like CUPs, are rapidly absorbed and eliminated with a biological half-life of 6−48 h,[59] while legacy pesticides have a longer biological half-life, reaching several years.[60] Similarly, HFRs have a longer biological half-life of up to 12 years[61] while OPEs, which are their replacements, have a relatively shorter half-life (e.g., hours to days) in biota.[45] To distinguish between these two mechanisms and gain preliminary insight into the source of chemicals measured in feces, we analyzed food items and soil. A complete survey of dietary habits for wild primates is complex and time-consuming and was outside the scope of this study. Rather, we collected soil, dietary information, and food items from the Indiana Sanctuary.

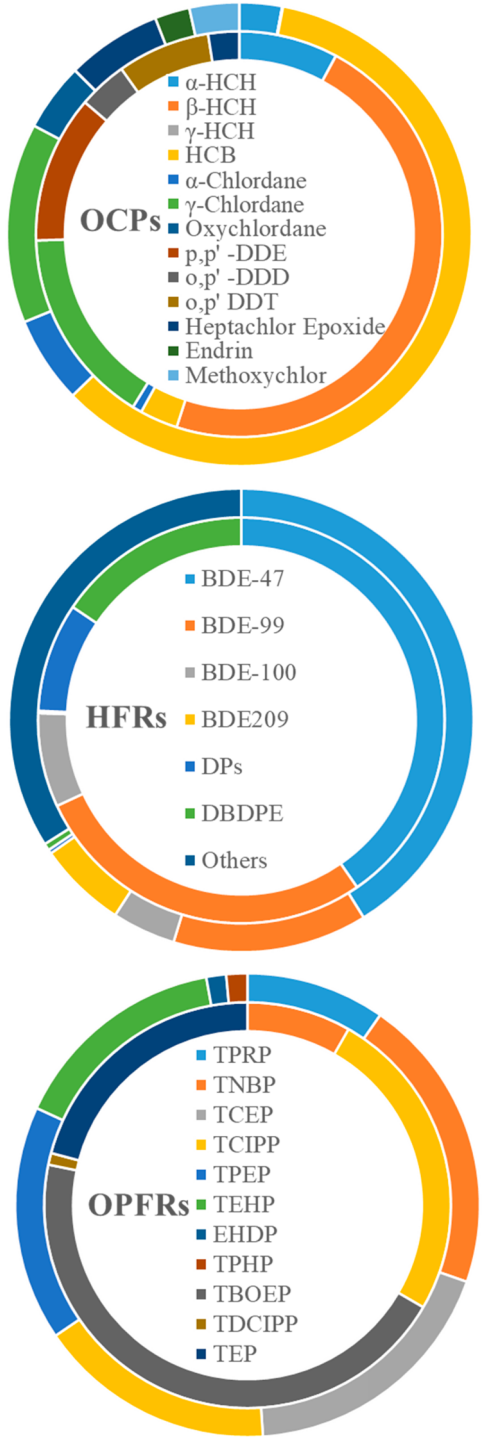

Figure 2. Patterns of target chemicals in feces (internal circle) and air (external circle) where the feces were collected in Uganda. Detailed information on the sample size and locations of air samples can be found in Figure S2.

The captive baboons' diet was comprised of fruit, vegetables, starchy items, and commercial feed (Table S8 for specific data on diet). Concurrent to the retrieval of fecal samples, we also collected soil samples and commercial feed samples used at the sanctuary and measured the same compounds that were targeted in feces (Tables S9 and S10). Commercial feed represents 10−15% of the total diet each day, indicating that target chemicals in feces may also come from other food items,

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Table 2. Calculation of Daily Excretion and Daily Intake Estimation for the Most Abundant Chemicals in Each Chemical Group Using Feces Obtained from Baboons at the Indiana Sanctuary

chemicals	excretion (ng/day)	daily excretion (ng/kg/day)	minimal risk levels (ng/kg/day)[a]	daily intake (ng/day)
β-HCH	460	18−50	70	28000 ± 34000
chlorpyrifos	40	1.5−4.3	1000	76000 ± 92000
BDE209	12.0	0.45−1.3	200	17 ± 13
DBDPE	110	4.2−12		6000 ± 7700
TBOEP	1000	40−113		200 ± 150

[a]The minimal risk levels was obtained from the Agency for Toxic Substances and Disease Registry's (ATSDR). Data on DBDPE and TBOEP were not available.

such as fruit and vegetables. Since samples from the fresh portion of the daily diet were not available, the concentration of β-HCH (14 ng/g),[62] chlorpyrifos (38 ng/g),[62] BDE-209 (0.005 ng/g),[63] DBDPE (3 ng/g),[64] and TBOEP (0.05 ng/g)[65] were obtained from the literature.

Interestingly, several chemicals from the three groups of compounds (i.e., β-HCH, p,p'-DDE, chlorpyrifos, λ-cyhalothrin, BDE-209, DBDPE, TBOEP, and TCIPP) were detected in feces, commercial feed, and soil (Figures S2 and S3 and Table S10). These similarities suggest that both feed and soil might be potential sources of these chemicals. Geophagy, or the behavior of eating soil, has been observed in wild and captive primates.[66,67] In this study, we did not record data on baboon geophagy, so soil was not included in the food intake estimates described further.

Daily excretion rates were highest for TBOEP and β-HCH and lowest for BDE-209, tracking measured concentrations in feces. All the cumulative daily excretion rates were below the minimal risk levels (MRL) obtained from the Agency for Toxic Substances and Disease Registry's (ASTDR), except for DBDPE and TBOEP, for which MRLs were not available. The comparison between excretion rates and MRLs could also provide information about the main elimination route for a specific chemical. For example, due to its high hydrophobicity, β-HCH is mostly eliminated via the feces, while chlorpyrifos, with much lower excretion rate and hydrophobicity, is eliminated via urine excretion. These results indicate that baboons in the Indiana Sanctuary may not be at risk from current exposure doses, with a few caveats: MRLs for primates are expected to be different from those of humans used here; these estimates are based on numerous assumptions and simplifications; and MRLs rely on the single chemical approach, while feces revealed that primates are exposed to a complex mixture of chemicals. Hence, our results should be interpreted cautiously.

The estimated daily intake chemical amounts were generally much higher than the excretion amounts (Table 2). This suggests that these chemicals could have been absorbed, accumulated, or degraded in the body, reducing the amount eliminated through the feces. Although the potential variations in diet and chemical concentration in food items have been taken into consideration in the Monte Carlo analysis, these values are clearly initial estimates for which we are unable to distinguish the effect of gender, age, and species, nor the other potential sources (e.g., soil). A systematic study of both captive and wild primates is needed to further understand the relationships between the amounts of chemicals ingested and measured in feces to estimate the body burden. In addition, measurements of these chemicals in other biological samples, like hair and blood, can provide a better understanding of body burden of these chemicals in primates, when possible.

Implications. The use of sentinel species for assessing environmental contamination that could impact people is a valuable early warning signal.[68] As humans and primates share a recent common ancestry, physiology, and diet, non-human primates are important sentinels for humans. The comparison between chemical patterns in air and feces in Uganda, as well as the results of the dietary analysis, both suggest that diet accounts for the observed differences in feces concentrations. The imbalance between excretion and intake also implies body circulation and possible accumulation of these chemicals in primates. The higher chemical concentrations generally observed in feces from red colobus and red-tailed monkeys, both highly herbivorous species, suggests that dietary sources such as crops, soil, or wild plants, rather than bioaccumulation across trophic levels, can shed light into exposure. Alternatively, excretion rates may vary phylogenetically and by body size, explaining differences in exposure. A more thorough analysis of interspecies variation is beyond the scope of this study, but future research will examine the role of primate traits, such as dietary niche and body size, as well as other phylogenetic trends and geography, to explain differences in exposure to and excretion of these chemicals.

The presence of numerous anthropogenic chemicals in primates living in protected areas warrants an evaluation of the possible biological effects resulting from exposure and a consideration of how exposure and susceptibility should influence conservation planning. To our knowledge, this is the first study to report on the occurrence of four groups of contaminants in feces of wild and captive primates. We suggest that the novel methods described here be used globally to examine the overlooked threat of chemical contamination on wild animals, especially primates, given their high risk of extinction and value as sentinel species for human health assessment.[69]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c02500.

Details on the information and instrumental analysis of the target chemicals; chemical mass in blanks; recoveries of the surrogates in matrix spike and feces from Indiana Sanctuary, Costa Rica, and Uganda; recoveries of the surrogates in commercial feed and soil samples; concentration of HCHs (sum of β-HCH and α-HCH) and ratio of α-HCH to β-HCH ($f_{\alpha/\beta}$) in feces; lipid content in feces of each species; amount of food item fed daily in Indiana Sanctuary; chemical concentration in commercial feed and soil collected in the Indiana Sanctuary; map of locations of air and feces samples in Uganda; and comparison of target chemicals between

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feces and commercial feed at the Indiana Sanctuary (PDF)

AUTHOR INFORMATION

Corresponding Author

Marta Venier − *O'Neill School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana 47405, United States;* orcid.org/0000-0002-2089-8992; Email: mvenier@indiana.edu

Authors

Shaorui Wang − *O'Neill School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana 47405, United States;* orcid.org/0000-0002-1445-4236

Tessa Steiniche − *Department of Anthropology, Indiana University, Bloomington, Indiana 47405, United States*

Jessica M. Rothman − *Department of Anthropology, Hunter College of the City University of New York, New York, New York 10021, United States*

Richard W. Wrangham − *Kibale Chimpanzee Project and Department of Human Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138, United States*

Colin A. Chapman − *Center for the Advanced Study of Human Paleobiology, Department of Anthropology, George Washington University, Washington, D.C. 20052, United States; Makerere University Biological Field Station, Kibale, Uganda; Shaanxi Key Laboratory for Animal Conservation, School of Life Sciences, Northwest University, Xi'an 712100, P. R. China; School of Life Sciences, University of KwaZulu-Natal, Scottsville, Pietermaritzburg 3629, South Africa*

Richard Mutegeki − *Makerere University Biological Field Station, Kibale, Uganda*

Rodolfo Quirós − *Organization for Tropical Studies, San Vito 60803, Costa Rica*

Michael D. Wasserman − *Department of Anthropology, Indiana University, Bloomington, Indiana 47405, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.0c02500

Notes

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