IPS - Research Grant Application

Please complete the following application form. Do not exceed the space provided. Font should be no less than 12pt. Please direct any questions regarding your application or this award to Prof Marina Cords (marina.cords@columbia.edu).

Application Due: March 1st

Last name: Steiniche **First name: Tessa**

Title of Proposed Project: Endocrine Effects of Anthropogenic Pollutants in Wild Primates

Affiliation (include the country): Indiana University, Bloomington, IN, USA

Email address: tsteinic@indiana.edu

I am not* a national of a non-human primate range-state

Career stage: doctoral candidate*

Provide a 200 word summary of your proposal:

Recent anthropogenic activity has drastically altered the chemical landscape, affecting the timing and types of chemicals primates encounter. In particular, accelerated land use change and landscape fragmentation associated with the expansion of agriculture and urbanization has resulted in the widespread introduction of agrochemical and industrial pollutants throughout primate habitats. This study will examine endocrine-disrupting effects of exposure to pollutants for primates inhabiting Kibale National Park, Uganda, a protected area providing habitat to one the world's most diverse and abundant primate communities. Exposure to pollutants and susceptibility to endocrine-disruption will be examined in two species of nonhuman primates, including olive baboons (*Papio anubis*) and red colobus monkeys (*Procolobus rufomitratus*). Primate exposure will be estimated by quantifying 101+ target compound concentrations in noninvasively collected fecal samples. Pollutants measured include halogenated flame retardants (HRFs), organophosphate esters (OPEs), current use pesticides (CUPs), and organochlorine pesticides (OCPs). Physiological effects of exposure will be assessed through endocrine biomarkers. We hypothesize fecal excretion patterns and susceptibility to toxicants will vary based on species' dietary strategy. Specifically, we predict adaptations which arose in response to dietary phytochemicals will influence the metabolism of chemical pollutants, having implications for primate ecology and evolution.

3 key words: pollution, endocrine-disruption, dietary niche

1. Describe the rationale and significance of your request and how it relates to theory in primatology (1 page maximum)

Interactions between primates and the chemicals in their environment play a major role in mediating physiology and behavior, having implications for fitness. Some current evidence of exogenous chemicals acting as a selective pressure in primates comes from studies examining interactions between primates and endocrine-active dietary phytochemicals. For example, Higham et al. (2007) demonstrated during periods of African black plum (*Vitex doniana*) consumption, a plant exhibiting progesterone-like compounds, baboons (Papio cynocephalus) exhibited higher levels of fecal progesterone and decreases in sexual swellings. Similarly, increased progesterone levels in wild female Phayre's leaf monkeys (*Trachypithecus phayrei*) and wild female chimpanzees (*Pan troglodytes schweinfurthii*) have been associated with seasonal availability and consumption of *Vitex* (Emery Thompson et al. 2008; Lu et al. 2011), and consumption of phytoestrogenic plants in wild red colobus (*Piliocolobus tephrosceles*) have shown correlations with elevated fecal estradiol and cortisol levels, increased aggressive and mating behaviors, and decreased social grooming behaviors (Wasserman et al. 2012). These studies suggest both physiological and social mechanisms of altered reproduction, indicating phytochemicals can potentially influence fitness.

Further evidence shows that primates exhibit adaptations to dietary phytochemicals, considered through examination of cytochrome P450s, functional enzymes utilized in the metabolism of xenobiotic chemicals (Chaney, et al. 2018; Kitanovic, et al. 2018). Within the primate lineage, CYP450s show phylogenic variation relating to dietary niche, indicating these genes have evolved under selective pressure from dietary toxins (Chaney, et al. 2018). Because all exogenous chemicals, including dietary phytochemicals and anthropogenic pollutants, are metabolized via similar physiological mechanisms (Ames, et al. 1990), an understanding of how primates have adapted to dietary phytochemicals may provide insight into species-specific responses to novel anthropogenic chemicals. Some species may possess relevant exaptations, or pre-adaptions that arose in response to dietary phytochemicals and are now utilized for pollutants. Consequently, primates that have adapted to regular interaction and higher loads of dietary phytochemical toxins may be better equipped to mitigate effects of novel anthropogenic chemical pollutants.

In this study we apply a novel methodology for noninvasive biomonitoring of 100+ anthropogenic pollutants and examine their effects on endocrine function in two species of nonhuman primates, olive baboons (*Papio anubis*) and red colobus monkeys (*Procolobus rufomitratus*). We hypothesize susceptibility to endocrine-disruption will differ based on species ecological niche, particularly relating to dietary adaptions. These two species represent diverse dietary strategies, expected to result in variation of exposure, metabolism, and excretion of pollutants as well as susceptibility to endocrine-disruption. Specifically, baboons are opportunistic omnivores, prioritizing fruits and seeds and frequently crop-raiding, increasing risk of exposure to current use pesticides. Red colobus rely heavily on a folivorous diet, having specialized foreguts for digesting plants high in fiber and phytochemicals, and minimally crop-raid, reducing risks of exposure to current use pesticides (Lambert and Rothman 2015).While organisms have been adapting to environmental chemicals since the beginning of life, this research explores new outlooks on the interactions between primates and their chemical environment, having implications for the theoretical underpinnings of primate evolution and applications in primate conservation.

2. What are your hypotheses and predictions? (1/2 page maximum)

H1. We hypothesize that primate exposure to anthropogenic pollutants will impact endocrine function. Halogenated flame retardants (HFRs) and organophosphate esters (OPEs) have demonstrated endocrine-disrupting potential as estrogen agonists, blocking endogenous estrogens (Song et al 2014; Zhang et al 2014), and as thyroid antagonists, decreasing production of thyroid hormones (Butt et al 2013). Chlorpyrifos, the most prevalent current use pesticide (CUP) found in the study area, has demonstrated potential to decrease circulating androgens, estrogens, and thyroid hormones (Juberg et al 2013). Organochlorine pesticides (OCPs) have demonstrated effects on thyroid receptor inhibition and increased estrogen receptor activation (Mnif et al 2011).
P1. We predict fecal concentrations of HRFs and OPEs will be negatively correlated with estrogen and thyroid hormone (T3).
P2. We predict fecal concentrations of current use pesticides (e.g. chlorpyrifos) will be negatively correlated with testosterone, estrogen, and thyroid hormone (T3).
P3. We predict of fecal concentrations of OCPs will be negatively correlated with thyroid hormone (T3) and positively correlated with estrogen.

H2. We hypothesize that endocrine-disrupting effects of pollutants will vary between species. Because red colobus possess relevant adaptions for metabolizing greater quantities of dietary phytochemical toxins, they may also have an increased capacity to mitigate effects of some anthropogenic pollutants.
P4. We predict red colobus monkeys will be less susceptible to endocrine-disrupting effects of pollutants compared to baboons.

3. What methods, data and statistics will you use to answer your question(s)? Please be specific. (1/2 page maximum)

Field sampling: This study will be conducted based in Kibale National Park, Uganda. Fecal samples will be collected weekly from identified individuals in both study populations (i.e. red colobus and olive baboons) over a period of 9 months. Concurrent observational data will be collected, including activity states, social interactions, reproductive state, mating behavior, and environmental and other external stressors, and used in data analyses to control for variables influencing hormone levels.

Pollutant analysis: Parent and metabolite compounds will be measured for 47 halogenated flame retardants (HFRs), 19 organophosphate esters (OPEs), 21 organochlorine pesticides (OCPs), and 29 current use pesticides (CUPs) in the Environmental Chemistry Lab at Indiana University. Briefly, samples are freeze-dried, extracted using an accelerated solvent extractor, cleaned on silica and florisil columns, and analyzed using GC/MS and LC/MS for target compounds.

Endocrine Biomarkers: Endocrine biomarkers will be quantified for estrogen (E2), testosterone (T), cortisol (GC), and thyroid hormones (T3). Fecal samples will be analyzed for endocrine biomarkers in the Primate Environmental Endocrinology Lab (PEEL) at Indiana University using enzyme immunoassays.

Statistical analysis: Hypotheses 1 will be tested with generalized linear mixed models (GLMMs), using hormone concentrations as our dependent variable and total fecal concentrations of each category of target pollutant as predictors. All models will include season, time of sample collection, social and environmental stressors as fixed variables, and individual primate IDs as a random effect. Hypothesis 2 will be tested using an analysis of variance (ANOVA) to compare fecal pollutant concentrations between species, and variation in endocrine-disrupting effects will be explored by establishing a baseline average of hormone concentrations for each species and evaluating deviation from this baseline using linear regression models.

4. Outline your plans for dissemination of your results (1/3 page maximum)

Results based on these data will be disseminated via manuscripts published in peer-reviewed journals, as well as talks and posters at professional conferences. Published work will note that all data were collected, handled, and archived according to the procedures described here. In Uganda, digital and hard copies of data, field reports, and/or manuscripts will be supplied to Makerere University, Uganda Wildlife Authority, and Uganda National Council for Science and Technology.

5. Provide a timeline for this project (2/3 page maximum)

September 2020 - April 2021 (Kibale National Park, Uganda): Training field assistants; collection of biological samples and behavioral data

May 2021- October 2021 (Indiana University): Laboratory analysis of fecal pollutants and hormones

November 2022 - May 2022 (Indiana University): Data analysis and dissertation writing and manuscript preparation/submission

6. Budget Provide detailed information for all expenditures not to exceed US$1500.00. List other funding sources for this project and amounts.

		Total (USD)
Travel Expenses		$2,600
Airfare	1500	
Baggage fees	500	
Hotel	300	
Car hire	300	
Materials and Supplies		$10,200
Amber glass vials	700	
Noldus Pocket Observer	1500	
Arbor Assay hormone kits	4000	
Pollutant analysis	4000	
Other		$6,700
Research fees	500	
Research assistants	4400	
Lodging	800	
Sample shipment	1000	
Total Direct Costs		$19,500
Highlighted costs requested from IPS, total $1500		

Research fees- $500
Fees to conduct research at Kibale National Park must be paid to UNCST and MUBFS, under the approved research permit NS605.

Sample shipment-$1,000
Environmental and fecal samples collected during field work will be shipped from Entebbe, Uganda to the United States. To preserve integrity of samples, samples will be packed in a cooler box and sent via expedited shipping. Rates are based on previous shipment costs via FedEx during pilot study.

*Additional support provided through Dr. Michael Wasserman's established field lab at Kibale National Park, Uganda, and through Dr. Marta Venier's Environmental Chemistry Lab, Indiana University. Pending funding includes NSF DDRIG Biological Anthropology, Ostrom Research Grant, ASP Small Research Grant, and Sigma Xi. Co-PIs pending funding includes NSF-CHN grant.

(Optional Section)
Conservation through Community Involvement (CCI)

If you plan to include CCI in your program you may be eligible for an additional award of US$500 to support these initiatives. Please describe your CCI plan below, addressing how these funds will be used and how this will impact conservation in your region. For more information on CCI and suggested CCI practices, please see the Guidelines for Conservation through Community Involvement posted in the publications section of the IPS website. (1/2 page maximum)

7. Literature cited (do not expand this section)

(1) Ames, BN et al (1990) *PNAS, 87*(19) **(2)** Butt, CM (2013) *Chem Res in Tox 26*(11), **(3)** Chaney, ME (2018) *Mol Phylogenetics and Evol 125* **(4)** Emery Thompson, M (2008) *Am J Primatology 70*(11) **(5)** Higham, J (2007) *Horm & Behavior 52*(3) **(6)** Juberg, D (2013) *Reg Tox Pharm 66*(3) **(7)** Kitanovic, S (2018) *Mol Ecology 27*(3) **(8)** Lambert, JE (2015) *Ann Rev Anth 44* **(9)** Lu, A (2011) *Horm & Behavior 59*(1) **(10)** Mnif, W (2011) *Int J of Envir Res and Pub Health, 8*(6) **(11)** Song, M (2014) *Chemosphere, 112* **(12)** Wasserman, MD (2012) *Horm & Behavior 62*(5) **(13)** Zhang, Q (2014) *Science & Technology, 48*(12)

8. Brief CV (principal investigator)

TESSA STEINICHE

EDUCATION

2016- present	PhD candidate, Biological Anthropology, Indiana University
2012	BSc, Biology, Truman State University

RECENT APPOINTMENTS

Fall 2019-2020	*Ostrom Fellow*, Ostrom Workshop, Indiana University Bloomington
Spring 2019	*Associate Instructor*, L376: Biology of Birds, Biology Department, Indiana University Bloomington
Fall 2018	*Associate Instructor*, L113: Biology Laboratory, Biology Department, Indiana University Bloomington
Spring 2018	*Associate Instructor*, B200: Science and Policy of Climate Change, Human Biology Department, Indiana University Bloomington

RELEVANT FIELDWORK AND LABORATORY EXPERIENCE

- May 2017- present: Kibale National Park, Uganda. Effects of anthropogenic pollutants on primate health with Dr. Michael Wasserman and Dr. Jessica Rothman
- January 2017-present: Las Cruces and La Selva Biological Stations and surrounding areas, Costa Rica. Effects of fragmentation on primate populations with Dr. Michael Wasserman
- January 2018-present: Environmental Chemistry Lab, Indiana University. Training in environmental pollutant analysis with Dr. Marta Venier and Dr. Shaorui Wang, School of Public and Environmental Affairs.
- August 2016-present: Primate Environmental Endocrinology Lab (PEEL), Indiana University. Training in hormone immunoassays and plant transfection assays with Dr. Michael Wasserman.
- July 2014-August 2015: Tokai Forest, Cape Town, South Africa. Tokai Baboon Project with Dr. Steffen Foerster and Dr. Larissa Swedell.
- August 2016-March 2017: Center for Conservation Biology, University of Washington. Non-invasive techniques for measuring wildlife physiology with Dr. Sam Wasser.

RELATED WORKS

- Wang S, **Steiniche T**, Romanak KA, Johnson E, Quirós R, Mutegeki R, Wasserman MD, Venier M. (2019) Atmospheric Occurrence of Legacy Pesticides, Current Use Pesticides, and Flame Retardants in and around Protected Areas in Costa Rica and Uganda. *Environmental Science & Technology 53*(11), 6171-6181.
- Benavidez KM, Iruri-Tucker A, **Steiniche T**, Wasserman MD. (2019) Primate microbial endocrinology: An uncharted frontier. *American Journal of Primatology 81*(10-11)
- Wang S*, **Steiniche T***, Rothman JM, Wrangham RW, Chapman CA, Mutegeki R, Quiros R, Wasserman MD, Venier M. Feces are effective biological samples for measuring pesticides and flame retardants in primates. *Environmental Science & Technology (in review).*
- **Steiniche T**, Wang S, Rothman JM, Wrangham RW, Chapman CA, Mutegeki R, Venier M, Wasserman MD. Endocrine effects of anthropogenic pollutants across the primate lineage with applications in Evolutionary Toxicology (*in prep, expected May 2020*)
- **Steiniche T**, Wang S, Romanak K, Rothman J, Wrangham R, Venier M, Wasserman MD. (2018) Methodology for quantifying anthropogenic chemical pollutants in primate feces. [Published abstract]. *American Journal of Physical Anthropology 165*(S66).

Send this application AS A SINGLE PDF DOCUMENT, named "LAST NAME, First name.pdf" to: Prof Marina Cords (marina.cords@columbia.edu)