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Conservation biology

# Associations between faecal chemical pollutants and hormones in primates inhabiting Kibale National Park, Uganda

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While anthropogenic pollutants are known to be a threat to primates, our understanding of exposure to pollutants *in situ* and their sub-lethal effects is still limited. We used non-invasive biomonitoring to examine associations between faecal concentrations of 97 chemical pollutants and faecal hormone metabolites of cortisol and oestradiol in four primate species inhabiting Kibale National Park, Uganda (chimpanzees—*Pan troglodytes*, olive baboons—*Papio anubis*, red colobus—*Piliocolobus tephrosceles* and red-tailed monkeys—*Cercopithecus ascanius*). Across all species ($n = 71$ samples), results demonstrated positive associations of organochlorine pesticides (OCPs) ($\beta = 0.143$, $p = 0.020$) and organophosphate esters ($\beta = 0.112$, $p = 0.003$) with cortisol in adult females. Additionally, we observed positive associations of OCPs ($\beta = 0.192$, $p = 0.013$) and brominated flame retardants ($\beta = 0.176$, $p = 0.004$) with cortisol in juveniles. Results suggest that cumulative pesticides and flame retardants are disruptive to endocrine function in these populations, which could have implications for development, metabolism and reproduction. Our study further demonstrates that faeces can be an important, non-invasive matrix for examining pollutant–hormone associations in wild primates and other critical wildlife populations.

## 1. Introduction

Although anthropogenic pollutants are an increasing threat to primates, biomonitoring assessments remain limited [1]. Much of our knowledge regarding toxic effects in wildlife has been acquired retrospectively in response to ecological disasters or observations of abnormal morphology or physiology [2,3]. As such, risk assessment models are generally based on chemical environmental fate,

persistence and laboratory toxicity testing [4,5]. This is problematic as toxicity tests historically focus on acute toxicity of single chemicals, whereas environmental exposure involves chronic exposure to mixtures of chemicals that can interact with one another. Chemical mixtures can contribute to additive, synergistic or other emergent physiological effects that cannot be predicted based on a series of single-chemical tests, even if exposure to single-chemical concentrations occurs below effective thresholds [6].

Wildlife biomonitoring provides an alternative to laboratory experiments for detecting adverse effects of chemical pollutants on the health of wildlife populations [7]. Since the early 1990s, most research on wildlife ecotoxicology has focused on fish and insects, and, to a lesser extent, birds and amphibians, whereas studies on mammals are comparatively scarce [8–10]. Studies that are non-destructive and do not involve experimental exposure in animals are also rare [11–13]. Mammalian wildlife, in particular primates, are valuable sentinel species to signal potential impacts in people, having physiological systems comparable to humans. As research on free-ranging wildlife can integrate ecological complexities unattainable in controlled laboratory settings, such studies can help identify environmental threats and inform practical solutions at both local and global scales [14].

Many pollutants adversely affect physiology by interfering with endocrine functioning [15]. Effects on two hormone axes, the hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal (HPG), are of particular interest due to their critical role in development, metabolism and reproduction [16,17]. Pesticides and flame retardants have been shown to influence HPA and HPG activity, acting as both oestrogen agonists or antagonists, inducing and inhibiting steroidogenic enzymes used in the conversion of testosterone to oestradiol, and catalysing the conversion of aldosterone to corticosteroids, having consequences for cortisol synthesis [18–21]. In the absence of adequate information on chemical interactions, current regulatory guidance suggests dose additive models, including dose addition of chemicals that act by the same mechanism. If mechanistic data are unknown, as with many chemicals, it is assumed that the impact of chemicals is cumulative but not interactive and involves adding concentrations [22].

Here, non-invasive biomonitoring was used to examine associations between faecal concentrations of 97 chemical pollutants, including organochlorine pesticides (OCPs), brominated flame retardants (BFRs) and organophosphate esters (OPEs), and faecal hormone metabolites of the HPA (i.e. cortisol) and HPG (i.e. oestradiol) axes across four species of primates inhabiting Kibale National Park in western Uganda: chimpanzees (*Pan troglodytes*), olive baboons (*Papio anubis*), red colobus (*Piliocolobus tephrosceles*) and red-tailed monkeys (*Cercopithecus ascanius*).

## 2. Materials and methods

### (a) Study site and species

Kibale National Park in western Uganda supports a high diversity and density of primates [23,24]. The park protects some of the last remaining expanses of sub-montane forest in eastern Africa, with surrounding land mostly used for small-scale subsistence agriculture and large-scale tea plantations. Faecal samples were collected from May to July 2017 from adult and juvenile chimpanzees ($N = 19$), olive baboons ($N = 28$), red colobus ($N = 12$) and red-tailed monkeys ($N = 12$) for a total of 71 samples. We defined juveniles as infants to subadults in life-history stages of pre-reproductive maturity, and adults as having reached reproductive viability.

### (b) Faecal sample collection

Fresh samples were collected from age-/sex-identified individuals upon defaecation using pre-cleaned spatulas and placed in sterilized 100 ml amber glass vials. Habituated groups were followed throughout the day and samples were collected opportunistically upon observation of defaecation. Samples were collected from at least 47 unique individuals, but individual IDs were not always available for every species. Collection from the same individual on the same day was possible. Samples exposed to urine were avoided, as well as collection of soil, leaves, or other debris with the sample. Freshly collected samples were kept in a small field cooler on ice for no longer than 6 h and stored frozen at −20°C until shipped frozen to Indiana University, Indiana, USA. Samples were then stored at −80°C until analyses.

### (c) Faecal pollutant and hormone analyses

We measured 22 OCPs, 50 BFRs and 25 OPEs following Wang *et al*. [11]. Details of pollutant methods are provided in the electronic supplementary material, tables S1–S3.

Details of enzyme immunoassay (EIA) methods are in the electronic supplementary material, table S4. Details of EIA validations, including parallelisms and accuracy tests, are in electronic supplementary material, table S5.

### (d) Data analysis

Pollutant concentrations for all congeners within a category were summed to calculate a total chemical load (i.e. ΣOCPs, ΣBFRs and ΣOPEs). Overall medians and detection frequencies of OCPs, BFRs and OPEs were calculated for each species (electronic supplementary material, table S6). To examine associations between pollutants and hormone metabolites, linear mixed effect models using restricted maximum-likelihood (package 'lmer' in R) were performed using log-transformed hormone concentration (i.e. oestradiol and cortisol) as the dependent variable, log-transformed pollutant concentration (i.e. ΣOCPs, ΣBFRs and ΣOPEs) as a predictor, and species category as a random effect (see electronic supplementary material for description of model selection). Because biological patterns and effects of hormones vary by sex and age, models were conducted after stratification by age/sex categories. Given limited sample size and because the endocrine system is highly conserved across vertebrates, with closely related species (i.e. primates) likely sharing similar endocrine responses to toxicants, models were not stratified by species, but species differences are considered as random intercepts. Descriptive data separated by species, age and sex are in the electronic supplementary material, figures S1–S3. Analyses were conducted using R v.4.0.3. Data are available in the Dryad Digital Repository [25].

## 3. Results

In total, 97 chemical pollutants were detected in faecal samples of the four primate species (electronic supplementary material, table S6). The most frequently detected OCPs were the insecticide beta-hexachlorocyclohexane (β-HCH) (i.e. lindane), fungicide hexachlorobenzene (HCB), chlordane pesticides (i.e. alpha- and gamma-chlordanes) and DDDs (i.e. p, p′-dichlorodiphenyldichloroethane and o, p′-dichlorodiphenyldichloro ethane), with detection frequencies greater than 60% for all species. Among BFRs, the most frequently detected compound was 2,2′,4,4′-tetrabromodiphenyl ether

**Table 1.** Summarized results of linear mixed models. For full model summaries, see electronic supplementary material, tables S7–S9. Significance at the 0.05(*) level is indicated.

| | | ΣOCPs | | | ΣBFRs | | | ΣOPEs | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | estimate | s.e. | *p* | estimate | s.e. | *p* | estimate | s.e. | *p* |
| adult males ($n = 20$) | cortisol | −0.053 | 0.105 | 0.619 | −0.018 | 0.069 | 0.799 | −0.068 | 0.043 | 0.136 |
| | oestradiol | −0.060 | 0.149 | 0.695 | 0.020 | 0.096 | 0.841 | −0.074 | 0.065 | 0.277 |
| adult females ($n = 29$) | cortisol | 0.143 | 0.057 | 0.020* | 0.055 | 0.054 | 0.320 | 0.112 | 0.033 | 0.003* |
| | oestradiol | −0.010 | 0.099 | 0.917 | −0.048 | 0.084 | 0.568 | −0.009 | 0.055 | 0.869 |
| juveniles ($n = 22$) | cortisol | 0.192 | 0.070 | 0.013* | 0.176 | 0.054 | 0.004* | 0.070 | 0.061 | 0.267 |
| | oestradiol | 0.133 | 0.076 | 0.095 | −0.109 | 0.071 | 0.140 | 0.082 | 0.054 | 0.156 |

(BDE-47), a widely used and abundant flame retardant, with detection frequencies greater than 70% in all species. Bromobenzenes (i.e. hexabromobenzene, pentabromobenzene, tetrabromo-*p*-xylene, pentabro moethyl benzene) were also widespread, with detection frequencies greater than 40% in all species. The most frequently detected OPEs were triethyl phosphate (TEP) and tris (2-butoxyethyl) phosphate (TBOEP), with detection frequencies greater than 50% in all species.

Positive associations of OCPs ($\beta = 0.143$, $p = 0.020$) and OPEs ($\beta = 0.112$, $p = 0.003$) with cortisol were observed in adult females. Additionally, positive associations of OCPs ($\beta = 0.192$, $p = 0.013$) and BFRs ($\beta = 0.176$, $p = 0.004$) with cortisol were demonstrated in juveniles (table 1; figure 1). While cortisol can exhibit diurnal patterns, there was no significant effect of the time samples were collected on cortisol concentrations ($\beta = 0.0000021 \pm 0.0000076$, $p = 0.79$) and we did not include this variable in our final models.

## 4. Discussion

### (a) Organochlorine pesticides

For pesticides, positive associations between ΣOCPs and cortisol were observed in adult females and juveniles. Similarly, a study in Greenland polar bears (*Ursus maritimus*) demonstrated a positive correlation between total organochlorines and cortisol measured from hair [26]. However, overall evidence regarding relationships between OCPs and glucocorticoids is conflicting. For example, an earlier study in free-ranging Svalbard polar bears showed that total pesticides were negatively correlated with plasma cortisol [27]. Mixed results are also seen throughout *in vitro* studies, with OCPs demonstrating both antagonistic and agonistic activity with glucocorticoid receptors [28]. While our results further support that OCPs do interact with HPA function, generalizations of overall outcomes are still unclear. As organochlorines are known to cause liver and adrenal damage in wildlife [29], the timing of exposures and overall pollutant load is likely to influence variation in cortisol synthesis. At our study site, the majority of exposure to OCPs is expected as a result of ingestion from crop-raiding of nearby subsistence farms [30], inhalation due to drift from both subsistence and commercial farming surrounding the park, and ingestion from contaminated dietary items within the park. As such, more detailed longitudinal monitoring will help elucidate relationships between exposure events and resulting adrenal function.

### (b) Brominated flame retardants

For BFRs, our results showed positive associations between ΣBFRs and cortisol in juveniles only. Accordingly, a study using human adrenocortical cell lines observed that BDE-47 exposure significantly increased cortisol production [31]. Although we found no significant relationships with oestradiol, previous studies have shown mixed results. While *in vivo* studies in fish species (*Oncorhynchus mykiss*, *Zoarces viviparus* and *Danio rerio*) have found a lack in oestrogenic activity related to BRFs [20], *in vitro* mechanistic studies have indicated varied outcomes, showing the stimulation of oestradiol secretion through the activation of aromatase expression [32] and inhibition of the hormone-metabolizing enzyme oestrogen sulfotransferase [33], as well as direct antagonistic effects [20]. Decreases in oestradiol could also be linked to disruptions in thyroid hormone production [34,35], a well-documented effect of BFRs. Although faecal BFRs were measured at the overall lowest concentrations compared to the other chemicals in our study, these excreted quantities likely represent only a small fraction of the total body burden. For example, rodent models have shown that developing mice have a reduced capacity to excrete BDE-47, creating greater risks of accumulation [36]. While atmospheric concentrations of BFRs at our study site indicate the potential for current exposure from potential sources such as old furniture, electronic devices and the open burning of plastics [37], maternal transfer from lactational depuration can put juveniles at additional risk for exposure and adverse health effects.

### (c) Organophosphate esters

For novel flame retardants, positive associations between ΣOPEs and cortisol were observed in adult females. While endocrine-disrupting effects of OPEs are not well characterized, a previous study showed that TBOEP, one of the most frequently detected chemicals in our samples, upregulated the expression of glucocorticoid receptors in zebrafish (*Danio rerio*) [38]. Additionally, other OPE congeners (i.e. BCIPP, BDCIPP and DPHP) have been shown to stimulate the transcription of CYP450s encoding for corticoid production in rodent models [39]. There is also mixed evidence from *in vitro* studies that

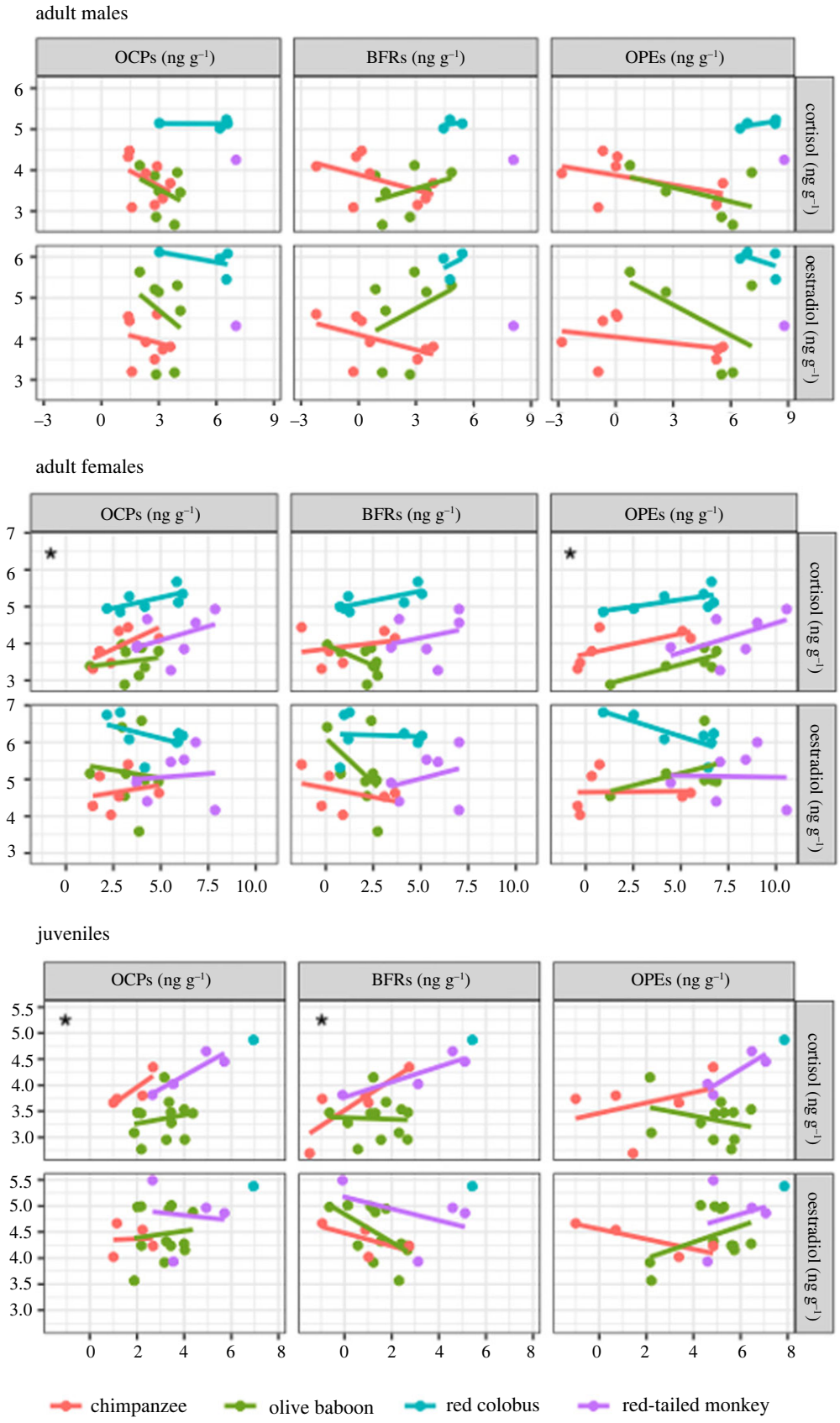


**Figure 1.** Associations between concentrations of (1) ΣOCPS, (2) ΣBFRs and (3) ΣOPEs and concentrations of cortisol and oestradiol hormone metabolites. Asterisks indicate significant relationships at the 0.05(*) level.

some OPEs (i.e. TMPP and TDBPP) are associated with increased glucocorticoids and others (i.e. TPHP and TDCIPP) decreased glucocorticoids [40]. While it is difficult to speculate on the sources of OPEs, TEP, one of the most frequently detected OPEs at our site, is used as a stabilizer in pesticide products [41], suggesting agrochemicals as a potential source.

## (d) Implications

Overall, our results demonstrated that cumulative pollutant concentrations were often associated with increased cortisol, showing positive correlations with OCPs and OPEs in females, and with OCPs and BFRs in juveniles. Other studies have also

demonstrated an association between cumulative pollutants and cortisol [42,43], despite the mixed evidence provided by tests on individual chemicals. A study examining steroidogenesis of oestradiol, progesterone, testosterone and cortisol in H295R human cell lines using burbot (*Lota lota*) liver samples containing mixtures of pesticides and flame retardants showed that only cortisol was increased at the highest mixture concentrations [42]. Similarly, exposure to a mixture of 29 persistent organic pollutants in female rodent models showed elevated corticosterone and a prolonged stress response [43].

Further, we observed that associations between pollutants and hormones were most prominent in females and juveniles, highlighting important age and sex differences in population susceptibility. Much of this variation may be influenced by body mass and lipid content, toxicokinetic traits which influence risk [44]. Exposure to even low doses of endocrine-disrupting chemicals during critical periods of development can result in profound organizational effects, affecting neurodevelopment, and immune and reproductive function [45,46]. Whereas activational effects of endocrine disruptors in adult primates are expected to be relatively more transient, disruption of steroid hormones in juveniles during development can result in permanent phenotypic changes in morphological, physiological and behavioural structure and function [47].

Our study was limited by a relatively small sample size, which may have influenced the ability to detect all associations between pollutants and hormones. Thus, statistical relationships due to chance cannot be ruled out, but it is also possible that biologically relevant associations were not statistically detected. Further, we note that relationships between faecal pollutants and hormones are not necessarily static and may fluctuate based on factors such as the timing and dose of chemical exposures, faecal excretion patterns, ecological factors (e.g. seasonality, climate), and social (e.g. hierarchical rank) and life-history factors (e.g. reproductive state). More detailed and longitudinal studies can explore how these variables shape the risks of endocrine disruption. Finally, while our study highlights similarities in responses across species, future studies can also examine potential interspecific variation in responses.

While studies using faeces to determine toxicant loads are rare, our results indicate an important opportunity to assess risks and outcomes of exposure to anthropogenic pollutants in vulnerable wildlife populations. Aside from endocrine disruption, exposure to pollutants can have cascading impacts, such as immune suppression [48,49], reproductive incapacity [50], behavioural toxicity [51] and decreased microbial diversity [52]. Non-invasive faecal biomonitoring can be especially useful in elucidating connections for improved human and wildlife health. Such research on exposure to anthropogenic chemicals and potential biological effects in primates and other tropical taxa is critically needed, given the paucity of studies in this region that contains the vast majority of the Earth's biodiversity [8,53,54].

Ethics. All primates in this study were observed without any invasive methods or contacts with researchers. Permissions to conduct this research were granted by the Uganda Wildlife Authority (UWA) (no. COD/96/06), Uganda National Council for Science and Technology (UNCST) (permit no. NS506) and Indiana University Institutional Biosafety Committee (no. 1229).

Data accessibility. Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.76hdr7t1s [25].

The data are provided in the electronic supplementary material [55].

Authors' contributions. T.S.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, writing—original draft and writing—review and editing; S.W.: data curation, formal analysis, methodology and writing—review and editing; E.C.: data curation, formal analysis, methodology, validation and writing—review and editing; R.M.: data curation, project administration, supervision and writing—review and editing; J.M.R.: project administration, resources, supervision and writing—review and editing; R.W.W.: project administration, resources and writing—review and editing; C.A.C.: project administration, resources and writing—review and editing; M.V.: conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation and writing—review and editing; M.D.W.: conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests

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