ANALYZING EUTHYROID & HYPERTHYROID INDOOR CAT EXPOSURE TO FLAME RETARDANTS

by

Mary-Catherine Osteen

Dr. Kate Hoffman and Dr. Heather Stapleton, Advisers

24 April 2020

Masters project submitted in partial fulfillment

of the requirements for the Master of Environmental Management degree in

the Nicholas School of the Environment of

Duke University

Executive Summary

Hyperthyroidism in cats has increased since its original description in the 1970s. Environmental exposures are suggested as a potential contributing factor. This research investigated pet cats' exposure to flame retardant chemicals in the home environment and associations with hyperthyroidism. Silicone collar tags were used as indicators of exposure to two classes of flame retardants: polybrominated diphenyl ethers (PBDEs) and organophosphate esters (OPEs). Though previous studies have documented PBDE exposure among house cats, less is known about exposure to OPEs. Thus, we first evaluated silicone tags as measures of internal exposure to OPEs. Cats wore silicone collar tags for 7 days in their home environment, after which tags were analyzed for flame retardants. Urine samples were collected from 9 cats and analyzed for OPE metabolites. Tris(2-chloroisopropyl) phosphate (TCIPP), was significantly and positively correlated with its urinary metabolites ($r \geq 0.73$; $p < 0.05$), and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) was significantly and positively correlated with its urinary metabolite ($r = 0.77$; $p < 0.05$). Several other OPEs from tags were correlated with their metabolites in urine, suggesting that tags capture information about cats' internal exposure; however, correlations were not statistically significant. To evaluate exposure differences by thyroid status, 12 hyperthyroid and 12 euthyroid cats (matched by age and sex) wore tags for 7 days. Tags were analyzed for PBDEs and OPEs. Two PBDEs, BDE-47 and BDE-99, were higher on tags worn by hyperthyroid compared to euthyroid cats ($p < 0.05$). Associations with thyroid status were not significant for OPEs; however, we caution against over-interpretation of these results given our limited sample size. Potential confounders, including diet and activity level, were evaluated; however, no significant differences were found between hyperthyroid and euthyroid cats ($p > 0.20$), suggesting these factors are not likely to confound associations with flame retardant exposures. Cumulatively, results suggest that exposure to PBDE flame retardants is higher among hyperthyroid cats, which is in agreement with previous studies that have reported differences in serum PBDE levels of hyperthyroid and euthyroid cats.

Introduction

Hyperthyroidism in cats (*Felis catus*), indicated by excessive circulation of thyroxine (T_4) and triiodothyronine (T_3) in the bloodstream, has been on the rise since its original diagnosis in 1979 (Peterson, 2012). In North America alone, an estimated two million cats, or approximately 1 in 10 pet cats, have been diagnosed with hyperthyroidism (Peterson, 2014). However, increases in the prevalence of hyperthyroidism among cats are widely debated, and the higher rates of disease have been attributed to better longevity, improved diagnostics, and more awareness of the disease (Peterson, 2014). Conversely, the underlying causes of hyperthyroidism in cats and the observed increases in prevalence are largely unknown.

Cat and human thyroids function very similarly in both species: hyperthyroid individuals experience: 1) presence of hyperplastic or adenomatous nodular additions to the thyroid; 2) higher incidence in middle and old age; 3) insidious onset (Wakeling et al., 2011). They also experience similar incidence of hyperthyroidism after development of nodular changes in the thyroid and subnormal thyroid-stimulating hormone (TSH) concentration, with an incidence of about 5-10% for both humans and cats in advancing age (Peterson, 2014). One of the most common symptoms of hyperthyroidism in both cats and humans is hypermetabolism, which causes excessive weight loss, even with increasing hunger (Kravetz, 2016; Peterson et al., 2016). Other symptoms in humans include impacts to cardiovascular (e.g. tachycardia), psychiatric (e.g. anxiety), and neuromuscular (e.g. muscle weakness) function (Kravetz, 2016). Humans have also experienced rapidly rising rates of hyperthyroidism (Harris and Pass, 2007) and papillary thyroid cancer (PTC) incidence (Lim et al., 2017). Like cats, humans are not typically diagnosed with hyperthyroidism until 50-70 years of age, and it is more common for women to be diagnosed than men (Edinboro et al., 2010). These similarities in diagnostic patterns and disease etiology

suggest that cats may provide a model organism for human development of hyperthyroidism. The underlying cause of increased hyperthyroidism is largely unknown in cats and humans; however, given the rapid changes in prevalence, some researchers have turned to environmental exposures as a potential causal factor. In particular, there has been interest in the potential for flame retardant chemicals to impact thyroid function, but data remain limited for both cats and humans.

Beginning in the 1970s, and due to a need to adhere to fire safety standards (e.g., California TB-117), polybrominated diphenyl ethers (PBDEs) were the dominant type of flame retardants used in residential furniture and some electronics to reduce flammability by slowing down the fire cycle (Blais and Carpenter, 2015). PBDEs are not chemically bound to the products that they are used in, and over time they can migrate out and enter the environment, leading to widespread exposure. Concerns over PBDEs' potential to bioaccumulate, persist in the environment, and have toxic effects has led to a ban on their use in Europe and to their removal from products sold in the U.S. (Fromme et al., 2016). However, because they are extremely environmentally persistent, PBDEs are still commonly found in household dust and exposure is expected to continue for decades (Hammel et al., 2018).

To continue to meet fire safety regulations, organophosphate esters (OPEs) have increasingly been used as PBDE substitutes. OPEs can be used alone as flame retardants but are also applied in commercial mixtures (e.g., Firemaster 550) (van der Veen and de Boer, 2012). As of 2011, Firemaster 550, a mixture of brominated and organophosphate compounds, was the second most commonly detected flame retardant in polyurethane foam in baby products (Stapleton et al., 2011). OPEs have a wide variety of uses outside of being used as flame retardants in polyurethane foam, including nail polishes (Mendelsohn et al., 2016), food

packaging (Li et al., 2019), outdoor equipment (Gomes et al., 2016), baby products (Hoffman et al., 2015), and other consumer and industrial products (Stapleton et al., 2009; Doherty et al., 2019).

Like PBDEs, OPEs are not bound to the products in which they are used (Stapleton et al., 2009). Indoor exposure occurs primarily through incidental ingestion of dust and inhalation, though dermal exposure and dietary exposure may also contribute to the body burden (Fraser et al., 2009; Mäkinen et al., 2009; Stapleton et al., 2009). Metabolites of tris(1,3-dichloroisopropyl) phosphate (TDCIPP), triphenyl phosphate (TPHP), tris(1-chloro-2-isopropyl) phosphate (TCIPP), and monosubstituted isopropylated triaryl phosphate (mono-ITP) have been observed ubiquitously in human urine samples (Hammel et al., 2016) and in urine representative of the United States general population, suggesting widespread exposure (Ospina et al., 2018). Previous research has also shown that cats are highly exposed to PBDEs, potentially due to grooming behavior (Peterson, 2012).

There are three proposed mechanisms of action by which PBDEs could disrupt thyroid hormone regulation in cats: 1) competitive binding to serum proteins that help transport thyroid hormones in the bloodstream, which could decrease thyroid hormone delivery to peripheral tissues (e.g. brain, liver); 2) altered transport across the cell membrane in the liver, which can affect intracellular regulation of hormones, and 3) antagonizing thyroid nuclear receptors in the brain, pituitary gland, and peripheral tissues, which alters the binding of T_3 to the thyroid hormone nuclear receptor (Peterson, 2012). In a study measuring serum PBDEs using 21 euthyroid, 41 hyperthyroid cats, and 10 feral cats, serum PBDEs were significantly lower in the feral cats tested, suggesting indoor exposure was a primary source of their exposure (Mensching

et al., 2012). Further, PBDEs in dust from homes of hyperthyroid cats were significantly higher than PBDEs in dust from homes of euthyroid cats (Mensching et al., 2012).

Data in humans suggest that these flame retardants are associated with changes in thyroid regulation and thyroid disease (Hoffman et al., 2017a). Exposure to BDE-209, for example, has been associated with higher odds of PTC (Hoffman et al., 2017a). PBDEs have also been associated with thyroid disease; a meta-analysis reported that relationships between PBDEs and thyroid hormones follow U-shaped patterns: low levels of exposure were inversely associated with thyroid hormones, while higher levels of exposure were associated with increases in thyroid hormones (Zhao et al., 2015). Though much less is known, exposure to OPEs has been associated with thyroid hormone alterations in humans (Meeker and Stapleton, 2010; Preston et al., 2017), thyroid disruption in fish (*Dario rerio*; Kim et al., 2015), and declines in circulating thyroid hormones in birds (*Gallus gallus domesticus*; Farhat et al., 2013). Additionally, an OPE compound, TCEP, has been associated with higher odds of PTC (Hoffman et al., 2017a). While these studies suggest there is a possible link between different classes of flame retardants and thyroid regulation and disease, prior research has been limited by small sample sizes (e.g. Zhao et al., 2015) and in many cases, spot measures of biomarkers for fast-metabolizing OPEs (e.g. Preston et al., 2017). In addition, varying doses of flame retardants and life-stage differences likely impede assessment of associations between flame retardants and thyroid function (Hoffman et al., 2017c).

With the rise in the use of OPEs due to the phase-out of PBDEs, it is important to evaluate this relationship, especially with previous research suggesting there may be human health consequences from exposure. Very few papers exist that examine relationships with OPE exposure and cats. One study with OPE exposures and cats found that the detection of different

types of OPEs in the blood serum of cats follows the distribution of exposure in the cat owners (Henríquez-Hernández et al., 2017). Another recently published paper using silicone pet tags as a method to measure flame retardant exposure with cats showed that the concentrations of an OPE compound, TDCIPP, were higher in the hyperthyroid group compared to the euthyroid group (Poutasse et al., 2019). Interestingly, Poutasse et al. found exposure to PBDEs was not associated with hyperthyroidism in cats, as the hyperthyroid and euthyroid silicone tag concentrations of PBDEs were largely similar in both groups (2019).

Further evaluations of links between exposure to OPEs and thyroid disease in humans is difficult due to the potential for early life exposure followed by a long latency to disease (e.g., thyroid malfunction) and the presence of numerous confounding factors. As such, household cats have been proposed as a model to investigate potential associations between flame retardants and thyroid issues. Cats share many of the same environmental factors as their owners and they have a compressed life cycle. In addition, many household cats spend the majority of their lives in a single environment, much unlike humans, and controlling for potential confounding variables (e.g., diet) is likely to be substantially easier for pet cats. As such, cats may provide a model to evaluate exposure to flame retardants (e.g., through indoor dust) and impacts on thyroid hormone regulation. In this study, further evidence to the use of cats as a model organism for flame retardant exposure and thyroid function was evaluated.

While measuring biomarkers for flame retardant exposure in urine, as well as blood, is appealing, individual variability in toxicokinetics and timing of exposure as well as the biological half-lives of chemicals, can lead to exposure misclassification (Anderson et al., 2017). OPEs are thought to be rapidly metabolized and excreted in urine within hours of exposure (Hoffman et al., 2017b). As such, biomarkers of exposure are variable over time and may not

capture information about long-term exposure. PBDEs are largely stored in adipose tissue but are in dynamic equilibrium with blood serum (Johnson-Restrepo and Villa, 2016). Biomarkers of exposure to PBDEs in blood serum may be influenced by weight loss (i.e. burning of adipose tissue), which is common with hyperthyroid cats (Peterson et al., 2016) and humans (Kravets, 2016). Researchers have identified silicone wristbands as a possible alternative to internal biomonitoring (Anderson et al., 2017). Bands are relatively low-cost and are thought to represent exposures from chemicals in the gas phase and attached to airborne particles. Wristbands also capture exposure over a longer time period, which may be preferable in assessing associations with health outcomes (Hammel et al., 2016). When evaluating silicone wristbands as indicators of internal exposure for OPEs, Hammel et al. found that levels of two OPEs, TDCIPP and TCIPP, on wristbands were significantly positively correlated with the levels of their metabolites found in urine collected from individuals wearing the bands (2016).

As an important step in determining relationships between flame retardants and thyroid disease in cats, the utility of silicone collar tag as measures of cats' OPE exposure will assist in verifying use of this external measure of exposure. Thus, the primary scientific objectices of this research were twofold: 1) To examine the urinary OPE metabolite concentrations in cats and compare them with measured parent OPE compounds extracted from the silicone tags, as has been done with humans (e.g. Hammel et al., 2016); and 2) To compare PBDE and OPE concentrations from silicone tags by hyperthyroid and euthyroid status to evaluate them as potential risk factors for hyperthyroidism.

Methods

Cat Participants

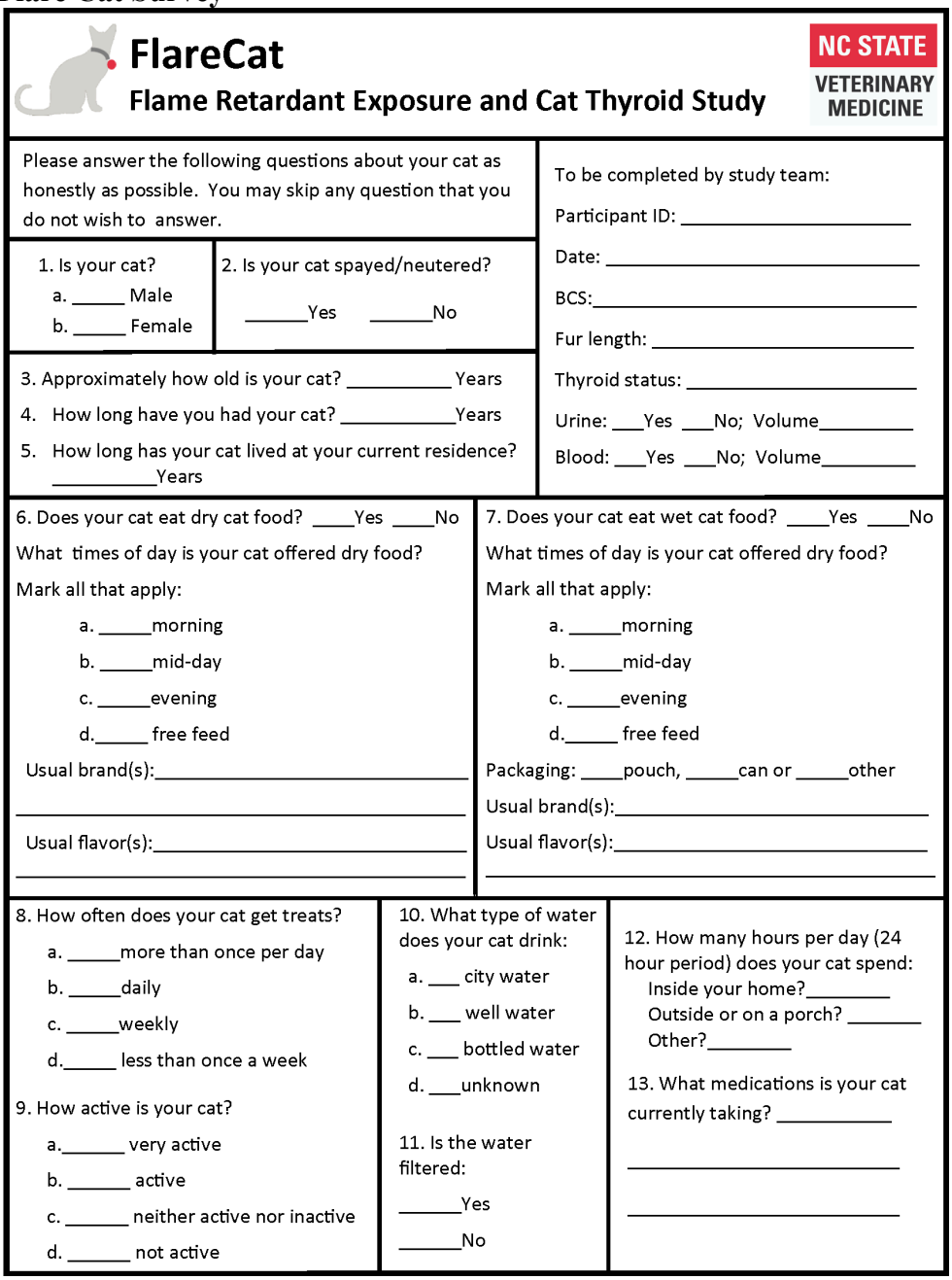
Owners were invited to enroll their indoor cats into the Flame Retardant Exposure and Cat Thyroid Study (FlareCat) study by the Clinical Studies Core at the Veterinary Medicine department at NC State University while there for routine vet care. Participation was restricted to cats that lived in the same house for >1 year to ensure that exposure measurements are representative of the recent past and to cats are inside for at least 22 hours per day. Between April 2019 and January 2020, **12** hyperthyroid cats were enrolled. As a control population, **12** euthyroid cats were age- and sex-matched to cats with hyperthyroidism to minimize potential confounding bias by these factors. At the time of enrollment, blood samples were obtained from all euthyroid and hyperthyroid cats and were analyzed for thyroid hormones by Michigan State University Veterinary Diagnostic Laboratory to verify each cats' thyroid status. As described below, owners were given a silicone tag attached to a cat collar and a survey to complete. At the end of the week, the silicone tag was wrapped in aluminum foil, and mailed to Duke University. Urine samples were also obtained from 9 cats by cystocentesis. These samples were used to evaluate silicone cat tags as measures of internal exposure to OPEs. The Institutional Animal Care and Use Committee (IACUC) at NC State University review and approved all study protocols.

Cat Survey

A short survey (**Figure 1**) was given to cat owners to assess factors related to cat livelihood, such as 1) outside time of cats, 2) diet (i.e., treats, dry vs. wet food, brands and flavors, and timing), and 3) cat energy. Cat owners were asked to fill out the survey while their cat was receiving

veterinary care at NC State University. Study staff were on hand to answer any questions about the survey.

Figure 1. Flare Cat Survey



FlareCat

Flame Retardant Exposure and Cat Thyroid Study

NC STATE VETERINARY MEDICINE

Please answer the following questions about your cat as honestly as possible. You may skip any question that you do not wish to answer.

1. Is your cat?
   a. _____ Male
   b. _____ Female

2. Is your cat spayed/neutered?
   _____Yes _____No

3. Approximately how old is your cat? _____ Years

4. How long have you had your cat? _____Years

5. How long has your cat lived at your current residence? _____Years

To be completed by study team:

Participant ID: _____

Date: _____

BCS:_____

Fur length: _____

Thyroid status: _____

Urine: ___Yes ___No; Volume_____

Blood: ___Yes ___No; Volume_____

6. Does your cat eat dry cat food? ____Yes ____No

What times of day is your cat offered dry food?

Mark all that apply:

a. _____morning
b. _____mid-day
c. _____evening
d._____ free feed

Usual brand(s):_____

_____

Usual flavor(s):_____

_____

7. Does your cat eat wet cat food? ____Yes ____No

What times of day is your cat offered dry food?

Mark all that apply:

a. _____morning
b. _____mid-day
c. _____evening
d._____ free feed

Packaging: ____pouch, _____can or _____other

Usual brand(s):_____

Usual flavor(s):_____

_____

8. How often does your cat get treats?
   a. _____more than once per day
   b. _____daily
   c. _____weekly
   d._____ less than once a week

9. How active is your cat?
   a._____ very active
   b. _____ active
   c. _____ neither active nor inactive
   d. _____ not active

10. What type of water does your cat drink:
   a. ___ city water
   b. ___ well water
   c. ___ bottled water
   d. ___unknown

11. Is the water filtered:
   _____Yes
   _____No

12. How many hours per day (24 hour period) does your cat spend:
   Inside your home?_____
   Outside or on a porch? _____
   Other?_____

13. What medications is your cat currently taking? _____

_____

_____

Cat Urine Sample Analysis

Urine samples were collected from a subset of nine pet cats via cystocentesis. Samples were transferred to standard polypropylene storage tubes and stored at -20°C until analysis. Six organophosphate ester (OPE) metabolites were analyzed in the urine samples, including: bis(1,3-dichloroisopropyl) phosphate (BDCIPP), the metabolite of TDCIPP; bis(1-chloro-2-isopropyl) phosphate (BCIPP) and bis(2-chloro-isopropyl) hydroxy-isopropyl phosphate (BCIPHIPP), metabolites of TCIPP; diphenyl phosphate (DPHP), a non-specific metabolite of TPHP, ITPs, and TBPPs; isopropylphenyl phenyl phosphate (ip-PPP), a metabolite of isopropylated triaryl phosphates (ITPs); tert-butyl phenyl phenyl phosphate (tb-PPP), a metabolite of tert-butylated triaryl phosphates (TBPPs). Previously established methods for analyzing OPEs in urine (human and canine) were used for the cat urine (Cooper et al., 2011; Butt et al., 2014; Wise et al. *in review*).

Briefly, each urine sample (~5.0 mL) was spiked with internal standards d_{10}-BDCIPP (10.0 ng), d_{10}-DPHP (10.0 ng), and d_{15}- ip-PPP (10.0 ng) in a 16 mL glass centrifuge tube. Additionally, 1.75 mL of 1.0 M sodium acetate (NaOAc) buffer (pH 5) and 100 µL of an enzyme solution comprised of 1000 units/mL β-glucoronidase and 33 units/mL sulfatase activity were added to each sample. The spiked samples were vortexed for about 20-30 seconds each, then they were incubated at 37°C for at least 12 hours.

To extract compounds from the urine samples, a solid-phase extraction (SPE) manifold, pre-rinsed with methanol (MeOH), using Strata X cartridges was employed. The cartridges were primed with 2 mL MeOH and 2 mL deionized H_2O before loading the urine samples. Sample tubes were rinsed with deionized water, and the rinse was added to the cartridges. Urine samples were allowed to elute through the columns to waste, then 2 mL of 5% trimethylamine in

acetronitrile was added to each column and eluted into a glass centrifuge tube. The cartridges were vacuumed to dryness, then the glass centrifuge tubes containing the samples were transferred from the SPE manifold to a nitrogen evaporator apparatus to be blown to dryness under a gentle stream of N_2 gas. They were reconstituted in 250 µL MeOH then transferred to an autosampler vial (ASV).

Samples were each spiked with $^{13}C_2$-DPHP (25.0 ng) to evaluate recovery and vortexed with the addition of liquid chromatography (LC)-grade water then filtered using nylon UniPrep syringeless filters. The urine OPE metabolites were analyzed using negative electrospray ionization LC-tandem mass spectrometry. Average recoveries for d_{10}-BDCIPP, d_{10}-DPHP, and d_{15}-ip-PPP in the cat urine were 414.1 ± 5.4%, 98.1 ± 0.6%, and 186.9 ± 1.6%, respectively. Lab blanks and a urine Standard Reference Material (SRM 3673; National Institute of Standards and Technology, Gaithersburg, MD) were extracted alongside the cat urine samples for quality assurance and control. The urine Standard Reference Material (SRM 3673) values here were higher than that of the mean reference values established in a previous study (Bastiaensen et al., 2019). Values below the method detection limits (MDLs) were replaced by multiplying 3 times the standard deviation and dividing by 5. MDLs here ranged from 0.003 ng/mL for BCIPHIPP to 0.194 ng/mL for DPHP. Specific gravity was measured using a hand-held refractometer (Atago) and measurements were used to correct for urine dilution.

Silicone Tag Collection and OPE Extraction

Silicone wristbands (24hourwristbands.com, Houston, TX) were prepared into cat tags and analyzed based on previously published methods (Hammel et al., 2016; Hammel et al., 2018; Wise et al. *in review*). To create the cat tags, the wristbands were cut to one-fourth the size and

attached to a metal ring for easy attachment on the cat's collar. Entire cat tags were cleaned with a consecutive 12-h Soxhlet extraction with 1:1 hexane:ethyl acetate and 1:1 ethyl acetate:methanol then dried in a vacuum oven prior to distribution to study participants. Owners received the cat tags wrapped in clean aluminum foil and affixed them to the cat's collar for the study period (i.e., one week). Field blanks were also prepared; however, they remained stored in foil at room temperature in the laboratory. At the conclusion of the study period, the cat tags from participants were wrapped in a clean sheet of aluminum foil and mailed back to Duke University, where they were stored at -20°C until extraction.

The cat tags were analyzed for a suite of common flame retardant chemicals. For extraction, each tag was cut into ~0.5 g pieces and weighed before being placed into a glass tube. Isotopically labeled compounds were added, including ^{13}C-TPHP for measuring TPHP, ITPs and TBPPs; dTCEP for measuring TCEP; dTDCIPP for measuring TCIPP and TDCIPP; and FBDE-69 for measuring PBDEs; and then they were extracted in a 15 minute sonication extraction with a 10 mL solution of 1:1 hexane:dichloromethane (v/v). This was repeated three times for a total volume of 30 mL. Nitrogen gas was used to concentrate the extracts to ~1.0 mL using a Savant SPD121P SpeedVac™ Concentrator (Thermo Scientific) before column chromatography. Extracts were then cleaned using 8.0 g of deactivated Florisil® (Acros Organics™ Florisil™, 100-200 mesh, FisherScientific) before two fractions were collected. The fractions, F1 and F2, were collected using hexane and ethyl acetate, respectively. Using an automated nitrogen evaporation system (TurboVap II, Zymark Inc.), F1 and F2 were combined and concentrated to 1.0 mL. Samples were concentrated to near dryness and reconstituted in 1.0 mL hexane. Then, isotopically labeled recovery standards were added to each sample before mass spectrometry analysis. Additionally, all samples were filtered prior to analysis using Chromafil Xtra nylon

filters (0.45µm pore size, 25 mm filter). The average percent recovery was 77.82 ± 6.2% for dTCEP, 113.68 ± 10.2% for dTDCIPP, 90.38 ± 5.9% for ^{13}C-TPHP, and 113.68 ± 1.8% for FBDE-69. A Q Exactive GC hybrid quadrupole-Orbitrap GC-MS/MS system (Thermo Scientific) was used to analyze the samples for many target compounds, which operated in full scan Electron Ionization (EI) mode. Brominated flame retardants were analyzed in the samples using a single quadrupole GC-MS (Agilent 6890N and 5975, respectively) operated in negative chemical ionization (NCI) mode. Field blanks (n = 3) and lab blanks (n = 3) were processed and analyzed among the cat tags for quality assurance and control. Values below the MDLs were replaced by dividing the MDL of 0.01 by 2 and the average mass of the silicone tags. MDLs here ranged from 0.01 ng/g-tag for 4tBPDPP to 0.13 ng/g-tag for 4IPPDPP.

Statistical Analyses

All statistical analyses were conducted in R Studio (v. 1.2.5019) using R v. 3.6.1 (R Project, 2019). Concentrations of flame retardants on silicone tags and their metabolites in urine samples were not normally distributed (assessed by Shapiro-Wilks tests). As such, Spearman's correlations were used to test the strength of the relationship between paired parent compounds from the tags (ng/g-tag) and urinary metabolites (ng/mL). Scatterplots were created to visualize relationships and identify any potential outliers. A Wilcoxon Rank Sum test was used to investigate the differences in the quantities of parent compounds on tags of hyperthyroid compared to euthyroid cats. We also considered multivariate regressions analyses to control for potential confounding by various covariates; however, our sample size was not sufficient to support these analyses. To evaluate the potential for confounding in associations between flame retardants and thyroid disease, Chi-Square tests were used to evaluate differences between

characteristics (e.g. food type) between hyperthyroid or euthyroid cats. Euthyroid cats were also examined separately to identify any differences parent compounds on tags by matching factors (i.e., sex and age 9-12 vs. 13-16 years); hyperthyroid cats were excluded from these analyses to minimize confounding.

Results and Discussion

Cat Participant Population

A total of 24 tags were distributed, worn for 7 days, and then returned to Duke University for analysis. All cat owners (100%) completed the survey while at NC State Veterinary Hospital. Hyperthyroid cats were age (within 2 years) and sex-matched to euthyroid cats with a mean age of 12.7 ± 2.49 years in hyperthyroid cats and a mean age of 11.9 ± 1.73 years in euthyroid cats with 5 males and 7 females in both groups (**Table 1**). Hyperthyroid cats lived in the residence longer on average (9.25 ± 3.44 years) than euthyroid cats (3.33 ± 2.20 years); however, they were owned by their current families for similar amounts of time (**Table 1**). By design, hyperthyroid and euthyroid cats were largely indoor cats with an average time indoors of 22.91 ± 1.93 and 24 ± 0 hours per day, respectively (**Table 1**). More than 80% of cats lived in housing described by their owners as a single-family home (**Table 1**).

Euthyroid cats were primarily fed dry food only (58%), with one eating wet food only and four eating a combination of wet and dry food (**Table 1**). Hyperthyroid cats were primarily fed both wet and dry food (67%), with one eating wet food only and three eating dry food only. A majority (54%) of both hyperthyroid and euthyroid cats were fed treats less than once a week, with only one cat in the hyperthyroid group being described as eating more than one treat per day. Additionally, hyperthyroid cats were evenly split on being described as active or not active

(50%); however, euthyroid cats were described more as not active (67%) then active (**Table 1**).

Further cat characteristics are summarized in **Table 1**.

Table 1. Selected Characteristics for Hyperthyroid and Euthyroid Cats

	Overall (n=24)	Hyperthyroid (n=12)	Euthyroid (n=12)	p-value (Chi-Square)
Sex				
Male	10 (42%)	5 (42%)	5 (42%)	-
Female	14 (58%)	7 (58%)	7 (58%)	
Spayed/Neutered (Yes)	24 (100%)	12 (100%)	12 (100%)	-
Mean Age ± SD (y)	12.3 ± 2.14	12.7 ± 2.49	11.9 ± 1.73	-
Mean Length Owned ± SD (y)	10.6 ± 3.78	11.71 ± 3.37	9.46 ± 3.97	-
Mean Length in Residence ± SD (y)	6.29 ± 4.14	9.25 ± 3.44	3.33 ± 2.20	-
Mean Hours Indoors ± SD	23.45 ± 1.44	22.91 ± 1.93	24 ± 0	-
Housing Type				0.27
Single Family	20 (83%)	11 (92%)	9 (75%)	
Apartment or Other	4 (17%)	1 (8%)	3 (25%)	
Fur Length				0.54
Short	21 (87.5%)	11 (92%)	10 (83%)	
Medium to Long	3 (12.5%)	1 (8%)	2 (17%)	
Food				0.23
Dry Only	10 (42%)	3 (25%)	7 (58%)	
Wet Only	2 (8%)	1 (8%)	1 (8%)	
Both	12 (50%)	8 (67%)	4 (33%)	
Treats				0.78
Daily	7 (30%)	4 (33%)	3 (27%)	
Weekly	3 (13%)	1 (8%)	2 (18%)	
Less than Once a Week	13 (57%)	7 (58%)	6 (55%)	
Activity Level				0.68
Active	10 (42%)	6 (50%)	4 (33%)	
Not Active	14 (58%)	6 (50%)	8 (67%)	

A dash (-) indicates N/A

Flame Retardant Concentrations in Urine

All OPEs were detected in at least one urine sample (**Table 2**). PBDEs were not evaluated in urine, as serum PBDE is the better biomarker of exposure (Genuis et al., 2017). Urine levels of BDCIPP, a metabolite of TDCIPP, were detected in the fewest samples at 66.7% (**Table 2**). Urine levels of BCIPHIPP and BCIPP, metabolites of TCIPP, were detected in 100% of samples, and BCIPP was detected at the highest median concentration in these samples (1.43 ng/mL; **Table 2**). DPHP, a metabolite of TPhP, ITPs, and TBPPs, was detected in 88.9% of samples (**Table 2**).

In humans, urine levels of BDCIPP, BCIPHIPP, and DPHP were all detected at 100% in the Hammel et al. study evaluating the efficacy of silicone wristbands vs. handwipes (2016). BCIPP was detected with the lowest frequency, at only 18% (Hammel et al., 2016). Interestingly, BCIPP was detected at the highest levels in cat urine samples, suggesting that it may be a more important TCIPP metabolite in cats relative to humans. Levels of other compounds in cat urine were generally lower than those reported for human populations (e.g. Hammel et al., 2016).

A notable difference between the human study and this cat study is the frequency of urine collection. The urine samples in the human study were collected on three days out of the five days of the experiment, then pooled together for analysis (Hammel et al., 2016). Here, cat urine was only collected as a spot sample, which may be a concern due to OPE compounds metabolizing rapidly in the body. However, OPE exposure may perhaps be stable and relatively chronic over time due to being in the same environment for many years.

Flame Retardant Concentrations on Cat Tags

All flame retardants investigated were detected on at least one tag (**Table 3**) and all tags had detectable levels of flame retardants. Two PBDEs, BDE 47 and BDE 99, were detected on >70% of the tags, with the other six PBDEs detected on < 60% of the tags (**Table 3**). Additionally, ten OPEs, including TCEP, TCIPP, TDCIPP, TPhP, EDHPP, 2IPPDPP, 4IPPDPP, B2IPPP, 4tBPDPP, and B4tBPPP, out of the eighteen evaluated, had a detection frequency >70% (**Table 3**).

The detection frequencies were similar to the study conducted by Poutasse et al. (2019). BDE-47 and BDE 99 were the only two PBDEs detected > 60% for both groups, with all other PBDEs detected < 50% (Poutasse et al., 2019). Interestingly, all MDLs were between 0.40 and 1.00 ng/g-tag (Poutasse et al., 2019) for all of the same PBDEs measured. Here, the MDL for all of the PBDEs was 0.02 ng/g-tag. It is possible that with a lower MDL, compound detection could have been higher for the Poutasse et al. study closer to the amounts seen here (2019). The authors did not evaluate any ITPs so those cannot be compared with this study; however, TPhP, TDCIPP, and TCIPP had similar detection frequencies to what was evaluated here (>90%; Poutasse et al., 2019). Here, TPhP, TDCIPP, and TCIPP all had MDLs of 0.02 ng/g-tag, whereas in the Poutasse et al. study, the MDLs were much higher at 0.43 ng/g-tag, 8.90 ng/g-tag, and 9.08 ng/g-tag, respectively (2019). TCEP was the only OPE that was detected less frequently, with a detection frequency < 60% (Poutasse et al., 2019), compared to the detection frequency of 80.8% seen here. This could be due to a difference in detection limits, with an MDL here for TCEP being 0.02 ng/g-tag and the MDL being much higher for the Poutasse et al. study at 5.82 ng/g-tag (2019).

Detection frequencies on silicone wristbands worn by humans have had vastly different detection frequencies compared to the silicone tags worn by cats. In a study evaluating PBDEs in humans, all PBDEs evaluated (sans BDE-181) were seen at $\geq$ 60% of wristbands, with 16 out of the 20 PBDEs having a detection frequency of 100% (n = 30; Hammel et al., 2018). Likewise, in a study evaluating the efficacy of handwipes and silicone wristbands with OPE exposure, all four OPEs evaluated, including TDCIPP, TCIPP, TPhP, and mono-ITP had a detection frequency of 100% on the wristbands (n = 40; Hammel et al., 2016). Additionally, in a study that evaluated both PBDEs and OPEs, the detection frequencies were higher for more PBDEs than just BDE-47 and BDE-99; however, detection frequencies of OPEs largely followed what was seen here (Romanak et al., 2019). The differences in detection frequencies seen between cats and humans could be due to cats living in a static environment while humans constantly change environments and potentially encounter a broader range of compounds.

Comparison Between Spot-Urine and Tags

This is the first study with cats that has evaluated urinary metabolites in order to validate the use of silicone tags as a passive sampling device. Here, concentrations of TCIPP on the silicone tags were correlated with both potential urinary metabolites, BCIPHIPP and BCIPP (r_s = 0.90 and r_s = 0.73, respectively; $p < 0.05$; **Figure 2** and **Table 2**). As may be expected since they are both metabolites of the same OPE, BCIPHIPP and BCIPP were significantly and positively associated with each other (r_s = 0.90; $p < 0.01$; **Table 2**). However, in studies conducted with humans, BCIPP was not detected frequently like BCIPHIPP, suggesting they are not correlated (Butt et al., 2014; Hammel et al., 2016). Additionally, in a study where BCIPP and BCIPHIPP were detected frequently, they were not associated with each other (r_s = 0.14; $p > 0.05$; Phillips et al.,

2018). Here, BCIPHIPP and BCIPP in cats may both be good measures of exposure to TCIPP since they are significantly positively associated with each other.

Concentrations of TDCIPP on the silicone tags were correlated with its potential metabolite, BDCIPP ($r_s = 0.77$, respectively; $p < 0.05$; **Figure 3** and **Table 2**). Interestingly, these correlations between parent compounds on silicone tags and urinary metabolites follow associations seen in humans (Hammel et al., 2016). Several other OPEs from tags were correlated with their metabolites in urine, although no other correlations were statistically significant. However, this suggests that tags capture information about cats' internal exposure. DPHP was the most variable in correlations with parent compounds, which is expected because DPHP is a non-specific metabolite (i.e. many compounds can metabolize to DPHP). Also, in a previous study investigating silicone wristbands and OPEs, DPHP was not significantly associated with parent compounds (Hammel et al., 2016). Additionally, ip-PPP, which was not evaluated here due to low detection, could be more associated with ITPs compared to DPHP due to the potential for different metabolism pathways.

Table 2. Detection Frequency and Distribution of OPE Metabolites in Urine with Spearman's Correlations for Urine and Tag Flame Retardant Concentrations

	Urine BCIPHIPP (ng/mL)	Urine BCIPP (ng/mL)	Urine BDCIPP (ng/mL)	Urine DPHP (ng/mL)
Urine % Detection	100.0%	100.0%	66.7%	88.9%
MDL (ng/mL)	0.003	0.004	0.03	0.194
Median (ng/mL)	0.25	1.43	0.11	0.76
Maximum (ng/mL)	3.04	5.86	1.50	2.75
Tag TCIPP (ng/g-tag)	0.90**	0.73*		
Tag TDCIPP (ng/g-tag)			0.77*	
Tag TPhP (ng/g-tag)				0.08
Tag EHDPP (ng/g-tag)				-0.33

	Urine BCIPHIPP (ng/mL)	Urine BCIPP (ng/mL)	Urine BDCIPP (ng/mL)	Urine DPHP (ng/mL)
Tag 2IPPDPP (ng/g-tag)				0.27
Tag 4IPPDPP (ng/g-tag)				0.07
Tag B2IPPPP (ng/g-tag)				0.08
Tag 4tBPDPP (ng/g-tag)				-0.48
Tag B4tBPPP (ng/g-tag)				-0.53
Urine BCIPHIPP (ng/mL)	1.00	0.90**	0.28	0.17
Urine BCIPP (ng/mL)		1.00	0.10	0.43
Urine BDCIPP (ng/mL)			1.00	-0.25
Urine DPHP (ng/mL)				1.00

*significant at the p = 0.05 level
**significant at the p = 0.01 level
***significant at the p = 0.0001 level
Gray cells indicate N/A

Figure 2. Correlation of TCIPP on silicone collar tags with its urinary metabolites, BCIPHIPP and BCIPP

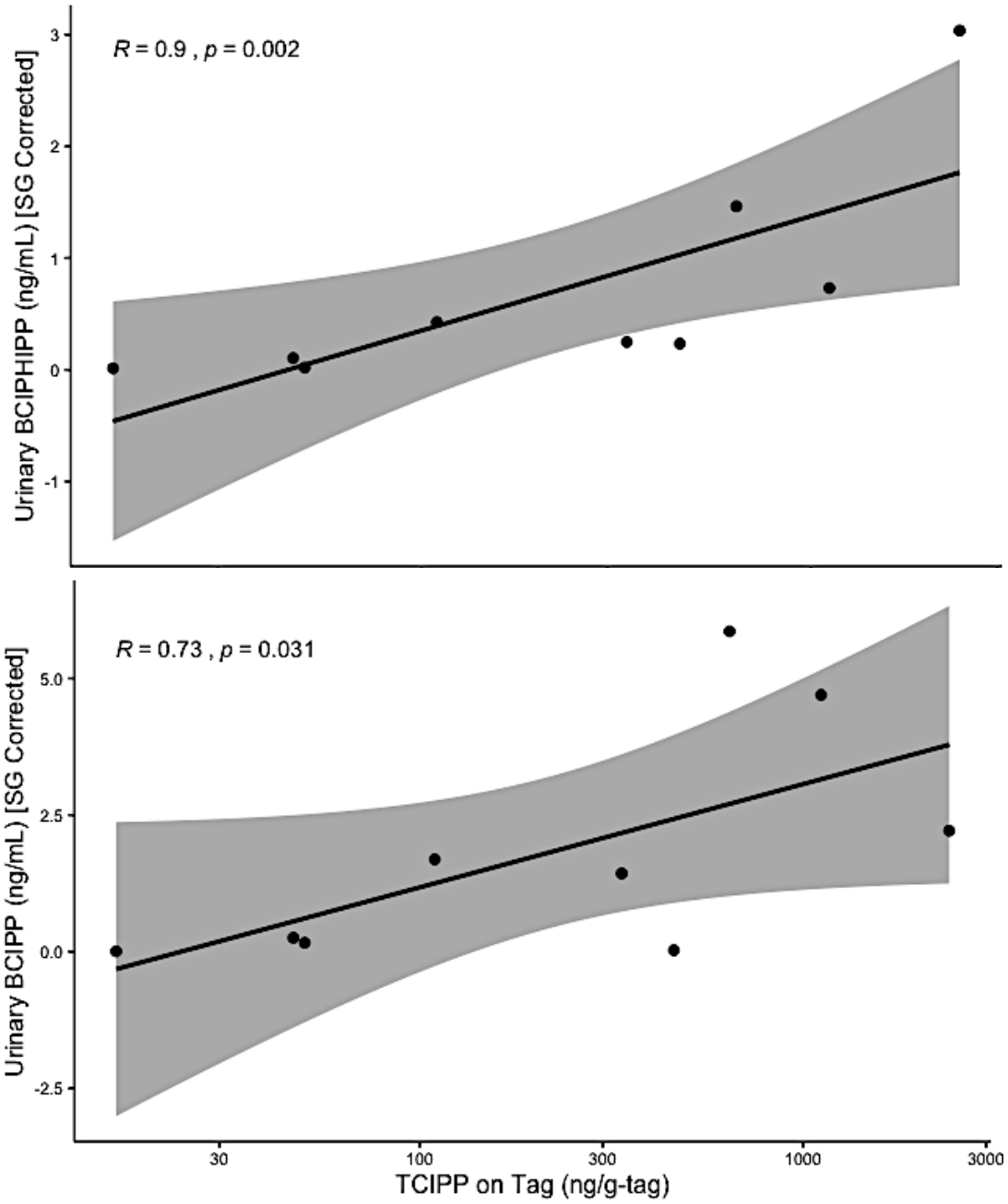
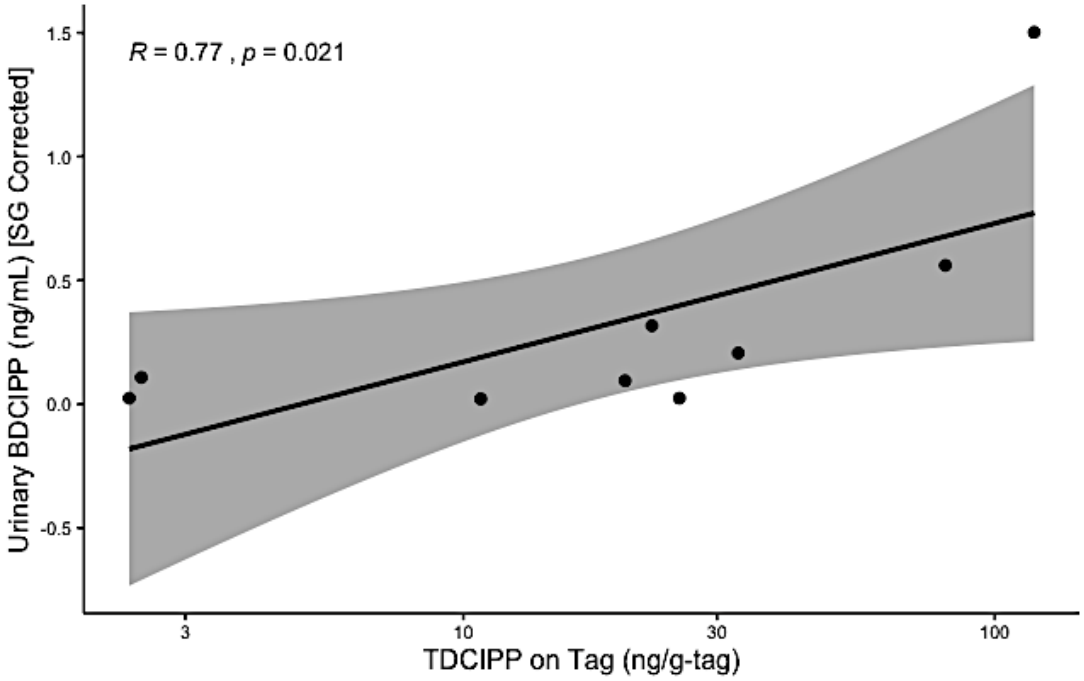


Figure 3. Correlation of TDCIPP on silicone collar tags with its urinary metabolite, BDCIPP



Flame Retardant Concentrations and Thyroid Disease

Both PBDEs evaluated, BDE 47 and BDE 99, were significantly greater on hyperthyroid tags compared to euthyroid tags (**Table 3**; p = 0.012 and p = 0.045, respectively). The geometric mean concentrations of BDE 47 were about 5X higher on tags worn by hyperthyroid cats than on tags worn by euthyroid cats (**Table 3**; GM = 11.85 ng/g-tag and 2.36 ng/g-tag, respectively). Similar results were seen with BDE 99, with tags worn by hyperthyroid cats being about 4.6X higher than tags worn by euthyroid cats (**Table 3**; GM = 4.87 ng/g-tag and 1.06 ng/g-tag, respectively).

In the similar study conducted by Poutasse et al., PBDE concentrations were not found to be different on tags worn by hyperthyroid and euthyroid cats; thus, the authors suggested that PBDE exposure was not associated with feline hyperthyroidism (2019). BDE 47 and BDE 99 were also the only two PBDEs evaluated with respect to thyroid status because of detection

frequency in samples (Poutasse et al., 2019). Interestingly, Poutasse et al. saw comparable geometric means to this study of 11.90 ng/g-tag and 7.00 ng/g-tag for BDE 47 and BDE 99, respectively, in the tags worn by hyperthyroid cats (**Table 3**; 2019). However, for the cats wearing euthyroid tags, the geometric means of this study were lower than the geometric means of 12.05 ng/g-tag and 6.83 ng/g-tag for BDE 47 and BDE 99, respectively, observed by Poutasse et al. (**Table 3**; 2019). The lower values seen here in the euthyroid group could be due to individual variability and/or differences between time in residence. Indeed, the cat participants in Poutasse et al. had comparable mean times in residence of 7.50 ± 4.61 years for hyperthyroid cats and 6.00 ± 4.56 years for euthyroid cats (2019). Hyperthyroid cats here on average were in residence for 9.25 ± 3.44 years while euthyroid cats on average were in residence much lower at 3.33 ± 2.20 years (**Table 1**).

BDE 47 and BDE 99 were the most abundant in a widely used flame retardant mixture, pentaBDE (Hale et al., 2003); both are highly persistent, which could explain their continued detection even though the use of pentaBDE was phased out in the 2000s (Fromme et al., 2016). The other PBDEs, related to the mixtures octaBDE and decaBDE (La Guardia et al., 2006), were not measured in this study, so no comparisons can be made. Additional previous research using serum PBDE concentrations showed no differences between euthyroid cats (n = 21) or hyperthyroid cats (n = 41); however, PBDE concentrations in house dust were significantly higher in homes of the hyperthyroid cats than the homes of the euthyroid cats (Mensching et al., 2012). The difference in sample size between the two groups in the Mensching et al. study could be driving the lack of a relationship between serum PBDE concentrations in the different groups. However, other previous research investigating differences in serum PBDE levels between hyperthyroid and euthyroid cats has shown that several PBDEs, including BDE 47 and BDE 99,

were higher in the serum of hyperthyroid cats (Norrgran et al., 2015; Walter et al., 2017). These studies are also plagued by small sample sizes and differing PBDE profiles (Norrgran et al., 2015; Walter et al., 2017). Walter et al. concludes the necessity of longitudinal studies to evaluate PBDE exposure and development of hyperthyroidism because of the potential for reverse causation; we would expect hyperthyroid cats to lose weight and as a result, have higher concentrations of PBDEs in their serum (2017). Importantly, because an external exposure measure was used, the analyses here are not subject to this potential bias of internal metabolism.

None of the 10 OPEs evaluated on cat tags was significantly different between the hyperthyroid and euthyroid cats. EHDPP trended towards being higher in the hyperthyroid group compared to the euthyroid group (**Table 3**; $p = 0.18$). The geometric mean (GM = 5.41 ng/g-tag) of EHDPP on euthyroid tags was lower than the geometric mean (GM = 7.85 ng/g-tag) on hyperthyroid tags (**Table 3**). Interestingly, TCIPP trended towards being higher in euthyroid groups compared to hyperthyroid groups; however, this difference was not statistically significant (**Table 3**; $p = 0.11$; GM = 300.31 ng/g-tag for the euthyroid group and GM = 159.43 ng/g-tag for the hyperthyroid group). Finally, TCEP appeared to have a large difference between the hyperthyroid and euthyroid groups, potentially due to the high outlier of 2999.24 ng/g-tag for a hyperthyroid cat; however, this difference was not statistically significant (**Table 3**; $p = 0.38$; GM = 1.27 ng/g-tag for the euthyroid group and GM = 9.45 ng/g-tag for the hyperthyroid group). In the only study to date that evaluated OPE exposure, Poutasse et al. identified one OPE, TDCIPP, that was significantly higher in hyperthyroid cats than euthyroid cats (2019). Here, the lack of differences found between hyperthyroid and euthyroid cats with OPEs is counter to their results. TDCIPP, with a detection frequency of 100%, was not statistically significantly different between hyperthyroid and euthyroid cats (**Table 3**; $p = 0.51$), though the GM level was slightly

higher in the hyperthyroid compared to the euthyroid cats (**Table 3**; 30.98 ng/g-tag and 23.03 ng/g-tag, respectively). It is possible that our sample size is too small to detect a difference of this magnitude between groups.

Table 3. Geometric Means and % Detection for PBDEs and OPEs

Compound	Detection Frequency (% Samples)	MDL (ng/g-tag)	Median (ng/g-tag)	Maximum (ng/g-tag)	Total Geometric Mean (ng/g-tag)	Hyperthyroid Geometric Mean (ng/g-tag)	Euthyroid Geometric Mean (ng/g-tag)	Wilcoxon Rank Sum (p-value)
PBDEs								
BDE 28	38.5%	-	-	-	-	-	-	-
BDE 47	100.0%	0.02	5.43	161.27	5.29	11.85	2.36	0.012**
BDE 66	23.1%	-	-	-	-	-	-	-
BDE 100	57.7%	-	-	-	-	-	-	-
BDE 99	92.3%	0.02	2.49	109.79	2.27	4.87	1.06	0.045*
BDE 85	19.2%	-	-	-	-	-	-	-
BDE 154	34.6%	-	-	-	-	-	-	-
BDE 153	26.9%	-	-	-	-	-	-	-
OPEs								
TCEP	80.8%	0.02	6.18	2999.24	3.46	9.45	1.27	0.38
TCIPP	100.0%	0.02	210.04	2406.13	218.81	159.43	300.31	0.11
TDCIPP	100.0%	0.02	28.38	1356.87	26.71	30.98	23.03	0.51
TPhP	100.0%	0.02	34.85	263.86	39.48	35.79	43.55	0.71
EHDPP	100.0%	0.02	7.40	16.91	6.52	7.85	5.41	0.18
2IPPDPP	100.0%	0.02	6.64	104.72	7.49	7.97	7.04	0.71
3IPPDPP	61.5%	-	-	-	-	-	-	-
4IPPDPP	96.2%	0.13	1.43	44.48	1.66	1.95	1.41	0.59
B2IPPPP	73.1%	0.02	0.38	18.27	0.27	0.35	0.21	0.67
24DIPPDPP	19.2%	-	-	-	-	-	-	-
B3IPPPP	7.7%	-	-	-	-	-	-	-
B4IPPPP	26.9%	-	-	-	-	-	-	-
B24DIPPPP	7.7%	-	-	-	-	-	-	-
2tBPDPP	7.7%	-	-	-	-	-	-	-

Compound	Detection Frequency (% Samples)	MDL (ng/g-tag)	Median (ng/g-tag)	Maximum (ng/g-tag)	Total Geometric Mean (ng/g-tag)	Hyperthyroid Geometric Mean (ng/g-tag)	Euthyroid Geometric Mean (ng/g-tag)	Wilcoxon Rank Sum (p-value)
4tBPDPP	100.0%	0.01	3.65	35.31	2.73	1.97	3.78	0.41
B2tBPPP	7.7%	-	-	-	-	-	-	-
B4tBPPP	88.5%	0.02	1.32	16.86	0.77	0.56	1.05	0.41
T4tBPP	11.5%	-	-	-	-	-	-	-

*significant at the p = 0.05 level
**significant at the p = 0.01 level
A dash (-) indicates N/A

Potential Confounding Variables

Iodine deficiency has been proposed as a reason for increased hyperthyroidism in cats. A study by Edinboro et al. showed that cats eating foods without iodine were more likely to develop hyperthyroidism compared to cats that ate iodine-supplemented foods (2004). However, as of 2010, cats with hyperthyroidism have not been documented to have iodine deficiency or an excess in their diet, leading researchers to question the potential for other factors to either work alone or synergistically with iodine deficiency to increase the risk of hyperthyroidism (Edinboro et al., 2010; Peterson, 2012). In a case-control study trying to identify risk factors for cat hyperthyroidism, researchers did not find significant relationships with some environmental exposures (e.g., herbicides and pesticides) but did find significant relationships with specific flavors of canned cat food (Martin et al., 2000). In one study, litterbox use and increasing age have also been associated with hyperthyroidism in cats (Wakeling et al., 2009). Here, litterbox use is not expected to be a confounding variable because all hyperthyroid and euthyroid cats lived indoors and were litterbox trained; this potential confounder would be more important when comparing outside (or feral) cats to indoor cats. Indeed, it could be more of a proxy indicator for living indoors instead of being an actual confounding variable.

Since increasing age has been evaluated as a potential risk factor for thyroid disease, euthyroid cats were examined for relationships between age and exposure. Hyperthyroid cats were not evaluated because age was a matching factor and therefore could not be evaluated with respect to thyroid status directly. While the sample sizes are small for each age class, three OPEs, EHDPP, 4tBPDPP, and B4tBPPP, are significantly different in younger cats aged 9-12 compared to older cats aged 13-16 (**Table 4**). The geometric mean concentrations of EHDPP were about 1.36X higher on tags worn by younger cats than on tags worn by older cats (**Table 4**;

GM = 6.14 ng/g-tag and 4.52 ng/g-tag, respectively). The geometric mean concentrations of 4tBPDPP were about 3.85X higher on tags worn by younger cats than on tags worn by older cats (**Table 4**; GM = 6.62 ng/g-tag and 1.72 ng/g-tag, respectively). The geometric mean concentrations of B4tBPPP were about 14.5X higher on tags worn by younger cats than on tags worn by older cats (**Table 4**; GM = 3.18 ng/g-tag and 0.22 ng/g-tag, respectively). For these three OPES, this suggests exposure is higher for younger cats than older cats, which could lead to higher body burden in younger cats compared to older cats. Evaluating sex differences in exposure to PBDEs and OPEs did not reveal any significant relationships (**Table 4**), suggesting similar exposure in both males and females. Although these factors are predictive of levels on the tags, these are likely to not confound associations seen here because hyperthyroid and euthyroid cats were age and sex-matched.

Whether or not euthyroid and hyperthyroid cats ate wet food and their activity levels were questioned to see if either were predictive of having hyperthyroidism. Cats' diets were not found to be predictive of hyperthyroidism (Chi-Square Test; $p = 0.23$), nor was just focusing on if wet food was a part of the diet at all. Further, whether or not a cat was described as active was not associated with thyroid status (Chi-Square Test; $p = 0.68$).

Table 4. Geometric Means and Wilcoxon Rank Sum P-Values for Age and Sex in Euthyroid Cats

Compound	Geometric Mean (Age Class 9-12)	Geometric Mean (Age Class 13-16)	Wilcoxon Rank Sum (p-value)		Geometric Mean (Males)	Geometric Mean (Females)	Wilcoxon Rank Sum (p-value)
PBDEs							
BDE 47	2.54	2.13	0.88		2.76	2.11	0.88
BDE 99	1.27	0.83	0.20		2.23	0.62	0.88
OPEs							

Compound	Geometric Mean (Age Class 9-12)	Geometric Mean (Age Class 13-16)	Wilcoxon Rank Sum (p-value)		Geometric Mean (Males)	Geometric Mean (Females)	Wilcoxon Rank Sum (p-value)
TCEP	6.57	0.13	0.34		8.38	0.33	0.07
TCIPP	419.79	187.90	0.15		518.08	203.42	0.20
TDCIPP	41.72	10.03	0.15		56.92	12.07	0.07
TPhP	42.75	44.70	0.88		49.35	39.84	0.88
EHDPP	6.14	4.52	0.02*		9.40	3.64	0.53
2IPPDPP	7.43	6.54	0.11		14.53	4.20	1.00
4IPPDPP	1.38	1.48	0.07		3.57	0.73	0.88
B2IPPPP	0.17	0.30	0.15		0.85	0.08	1.00
4tBPDPP	6.62	1.72	0.03*		9.69	1.93	0.15
B4tBPPP	3.18	0.22	0.01**		5.75	0.31	0.11
*significant at the p = 0.05 level **significant at the p = 0.01 level - not applicable							

Study Limitations

There were several limitations to this study. First, the small sample size limits the inferences that can be made from this research, as with a growing sample size some of the correlations found between tags and urine may change or disappear likewise with differences between hyperthyroid and euthyroid groups. With the addition of more samples, it would be advantageous to conduct a multiple linear regression model to see what effect (if any) the potential confounders play with hyperthyroid status. Here, potential confounders were examined one at a time. While our results suggest that the factors evaluated in surveys are not likely to confound observed associations, residual confounding is possible in these data.

Second, the biomarker of internal exposure collected can only shed light on the validity of OPEs measured on tags and urine, not PBDEs. A large number of studies have been conducted on PBDEs, while the data on OPEs is more limited. Because of this, more focus was

placed on evaluating OPEs here compared to PBDEs. Additionally, the urine sample collected was a spot-urine sample which is likely not representative of the cumulative internal exposure of OPEs. As stated previously, OPEs are likely rapidly metabolized and excreted within the span of hours (Hoffman et al., 2017b). Pooled-urine samples are preferred to create a better understanding of total exposure within a time period; however, with cats this is difficult and costly to perform as obtaining urine samples require veterinary care. Thus, a spot-sample was more realistic given the species model. Behaviors in cats in the home environment could also be variable from day to day (e.g. activity level), which could negatively reflect the concentrations of flame retardant metabolites in urine as well. However, these cats were all indoor cats by experimental design. Since their environment remains consistent over longer periods of time, it may indicate that OPE metabolites measured in urine have reached steady-state conditions.

Finally, it is impossible to know for sure whether or not the concentrations of flame retardants found on tags and in urine concentrations here in the present time are representative of the recent past and the cause of hyperthyroidism in cats. Development of hyperthyroidism is generally associated with insidious onset in middle to old age (Wakeling et al., 2011), which could indicate earlier exposures occurring that contribute to the development at those age points. Due to cats here not being in the same home their entire lives, the exposures could have been different and/or higher earlier in life leading to the development of hyperthyroidism. However, with pet cats, there is a unique opportunity to follow exposure through the development of hyperthyroidism due to their compressed life cycle. Indeed, cats could be followed throughout their life to the development of hyperthyroidism while indoor environment samples (e.g. dust) are taken to compare levels of exposure with cats that developed hyperthyroidism vs. cats that maintained their euthyroid.

Future Directions

A long-term goal of continuing this research is to evaluate the use of cats as a representative model for human exposure to flame retardants in relation to hyperthyroidism. As stated previously, hyperthyroidism in cats and humans is very similar, with both experiencing nodular changes in the thyroid, peak incidence in old age, and rapid, insidious onset (Wakeling et al., 2011). For a short-term future direction, it is imperative to expand the sample size in order to better validate exposure differences in hyperthyroid and euthyroid cats, as well as continuing to validate the use of silicone tags as a measure of exposure. Promising previous research has shown that serum levels of PBDEs and OPEs in cats and pet cat owners followed similar distributions; however, the results were also limited by small sample sizes (n = 20; Henríquez-Hernández et al., 2017). The authors concluded that cats were a good sentinel representative PBDEs and OPEs in humans (Henríquez-Hernández et al., 2017). By confirming the use of cats as a model species for hyperthyroidism, many of the unknowns and issues that occur with studying differences in humans (e.g. day-to-day variation) can be removed since cats largely are inside animals and can be modified more easily (e.g. changes in diet). Additionally, interventions in flame retardant exposure could be conducted in the home to assess how quickly changing these sources of exposure reflects on the silicone tags among other preventative health interventions. Finally, an important aspect to study to know if cats were a good model for human exposure is to design an longitudinal cohort study which enrolled pet cats early in life and followed them through development of hyperthyroidism. From there, environmental exposure measurements could be taken (e.g. dust samples), and owners could be followed as well to see what correlations in thyroid hormones become evident between pet cats and their owners.

Conclusion

The results of this silicone tag study with hyperthyroid cats case-control matched to euthyroid cats indicate that cats are exposed to numerous flame retardants in the home environment. Two PBDEs measured on the silicone tags, BDE-47 and BDE-99, were higher in hyperthyroid cats compared to their euthyroid counterpart. As the first study of its kind, OPE measurements on silicone tags (i.e. external measure) were compared to urinary OPE metabolites (i.e. internal measure) to validate the use of silicone tags on cats. The results of these comparisons suggest that silicone tags capture information about cats' internal exposure. The research conducted here supports the use of cats a model species. In future studies, it would be crucial to continue to enroll more cats to further validate the links between flame retardant exposure and thyroid status. Further, it would be imperative to begin a longitudinal cohort study with cats to further investigate whether cats can be used as a model species for human exposure as this research, and other previous research, suggests.

Literature Cited

Anderson, K.A., Points III, G.L., Donald, C.E., Dixon, H.M., Scott, R.P., Wilson, G., Tidwell, L.G., Hoffman, P.D., Herbstman, J.B. and O'connell, S.G. (2017). Preparation and performance features of wristband samplers and considerations for chemical exposure assessment. *Journal of Exposure Science and Environmental Epidemiology, 27*(6), 551.

Bastiaensen, M., Van den Eede, N., Su, G., Letcher, R.J., Stapleton, H.M. and Covaci, A. (2019). Towards establishing indicative values for metabolites of organophosphate ester contaminants in human urine. *Chemosphere, 236*, 124348.

Blais, M. and Carpenter, K. (2015). Flexible polyurethane foams: a comparative measurement of toxic vapors and other toxic emissions in controlled combustion environments of foams with and without fire retardants. *Fire Technology, 51*(1), 3-18.

Butt, C. M., Congleton, J., Hoffman, K., Fang, M., & Stapleton, H. M. (2014). Metabolites of organophosphate flame retardants and 2-ethylhexyl tetrabromobenzoate in urine from paired mothers and toddlers. *Environmental Science & Technology, 48*(17), 10432–10438.

Cooper, E. M., Covaci, A., van Nuijs, A. L. N., Webster, T. F., & Stapleton, H. M. (2011). Analysis of the flame retardant metabolites bis(1,3-dichloro-2-propyl) phosphate (BDCPP) and diphenyl phosphate (DPP) in urine using liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry, 401*(7), 2123–2132.

Doherty, B.T., Hammel, S.C., Daniels, J.L., Stapleton, H.M. and Hoffman, K. (2019). Organophosphate Esters: Are These Flame Retardants and Plasticizers Affecting Children's Health? *Current environmental health reports, 6*(4), 201-213.

Edinboro, C.H., Scott-Moncrieff, J.C., and Glickman, L.T. (2004). Review of iodine recommendations for commercial cat foods and potential impacts of proposed changes. *Thyroid, 14*, 722.

Edinboro, C.H., Scott-Moncrieff, J.C., and Glickman, L.T. (2010). Feline hyperthyroidism: potential relationship with iodine supplement requirements of commercial cat foods. *Journal of feline medicine and surgery*, *12*(9), 672-679.

Farhat, A., Crump, D., Chiu, S., Williams, K.L., Letcher, R.J., Gauthier, L.T. and Kennedy, S.W. (2013). In ovo effects of two organophosphate flame retardants—TCIPP and TDCIPP—on pipping success, development, mRNA expression, and thyroid hormone levels in chicken embryos. *Toxicological Sciences, 134*(1), 92-102.

Fraser, A.J., Webster, T.F. and McClean, M.D. (2009). Diet contributes significantly to the body burden of PBDEs in the general US population. *Environmental health perspectives, 117*(10), 1520-1525.

Fromme, H., Becher, G., Hilger, B., & Völkel, W. (2016). Brominated flame retardants–exposure and risk assessment for the general population. *International journal of hygiene and environmental health, 219*(1), 1-23.

Genuis, S.K., Birkholz, D. and Genuis, S.J. (2017). Human excretion of polybrominated diphenyl ether flame retardants: blood, urine, and sweat study. *BioMed research international*, 2017.

Gomes G, Ward P, Lorenzo A, Hoffman K, Stapleton HM. (2016). Characterizing flame retardant applications and potential human exposure in backpacking tents. *Environ Sci Technol., 50*(10), 5338–45.

Hale, R.C., Alaee, M., Manchester-Neesvig, J.B., Stapleton, H.M. and Ikonomou, M.G. (2003). Polybrominated diphenyl ether flame retardants in the North American environment. *Environment international*, *29*(6), 771-779.

Hammel, S.C., Hoffman, K., Webster, T.F., Anderson, K.A. and Stapleton, H.M. (2016). Measuring personal exposure to organophosphate flame retardants using silicone wristbands and hand wipes. *Environmental science & technology*, *50*(8), 4483-4491.

Hammel, S.C., Phillips, A.L., Hoffman, K. and Stapleton, H.M. (2018). Evaluating the use of silicone wristbands to measure personal exposure to brominated flame retardants. *Environmental science & technology, 52*(20), 11875-11885.

Harris, K.B. and Pass, K.A. (2007). Increase in congenital hypothyroidism in New York State and in the United States. *Molecular genetics and metabolism, 91*(3), 268-277.

Henríquez-Hernández, L.A., Carretón, E., Camacho, M., Montoya-Alonso, J.A., Boada, L.D., Bernal Martín, V., Falcón Cordón, Y., Falcón Cordón, S., Zumbado, M. and Luzardo, O.P. (2017). Potential role of pet cats as a sentinel species for human exposure to flame retardants. *Frontiers in veterinary science, 4*, 79.

Hoffman K, Butt CM, Chen A, Limkakeng AT Jr, Stapleton HM. (2015). High exposure to organophosphate flame retardants in infants: associations with baby products. *Environ Sci Technol., 49*(24), 14554–9.

Hoffman, K., Lorenzo, A., Butt, C. M., Hammel, S. C., Henderson, B. B., Roman, S. A., Scheri, R.P., Stapleton, H.M., and Sosa, J. A. (2017a). Exposure to flame retardant chemicals and occurrence and severity of papillary thyroid cancer: A case-control study. *Environment international, 107*, 235-242.

Hoffman, K., Butt, C.M., Webster, T.F., Preston, E.V., Hammel, S.C., Makey, C., Lorenzo, A.M., Cooper, E.M., Carignan, C., Meeker, J.D. and Hauser, R. (2017b). Temporal trends in exposure to organophosphate flame retardants in the United States. *Environmental science & technology letters, 4*(3), 112-118.

Hoffman, K., Sosa, J.A. and Stapleton, H.M. (2017c). Do flame retardant chemicals increase the risk for thyroid dysregulation and cancer? *Current opinion in oncology*, *29*(1), 7-13.

Johnson-Restrepo, B. and Villa, A.L. (2016). Human Exposure to Brominated Flame Retardants. In *Persistent Organic Chemicals in the Environment: Status and Trends in the Pacific Basin Countries I Contamination Status* (pp. 17-53). American Chemical Society.

Kim, S., Jung, J., Lee, I., Jung, D., Youn, H. and Choi, K. (2015). Thyroid disruption by triphenyl phosphate, an organophosphate flame retardant, in zebrafish (*Danio rerio*) embryos/larvae, and in GH3 and FRTL-5 cell lines. *Aquatic Toxicology, 160*, 188-196.

Kravets, I. (2016). Hyperthyroidism: diagnosis and treatment. *American family physician, 93*(5), 363-370.

La Guardia, M.J., Hale, R.C. and Harvey, E. (2006). Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. *Environmental science & technology, 40*(20), 6247-6254.

Li, J., Zhao, L., Letcher, R.J., Zhang, Y., Jian, K., Zhang, J. and Su, G. (2019). A review on organophosphate Ester (OPE) flame retardants and plasticizers in foodstuffs: Levels, distribution, human dietary exposure, and future directions. *Environment international, 127*, 35-51.

Lim, H., Devesa, S.S., Sosa, J.A., Check, D., and Kitahara, C.M. (2017). Trends in thyroid cancer incidence and mortality in the united states, 1974-2013. *JAMA*, *317*(13), 1338-1348.

Mäkinen, M.S., Mäkinen, M.R., Koistinen, J.T., Pasanen, A.L., Pasanen, P.O., Kalliokoski, P.J. and Korpi, A.M. (2009). Respiratory and dermal exposure to organophosphorus flame retardants and tetrabromobisphenol A at five work environments. *Environmental science & technology, 43*(3), 941-947.

Martin, K. M., Rossing, M. A., Ryland, L. M., DiGiacomo, R. F., and Freitag, W. A. (2000). Evaluation of dietary and environmental risk factors for hyperthyroidism in cats. *Journal of the American Veterinary Medical Association, 217*(6), 853-856.

Meeker, J.D. and Stapleton, H.M. (2010). House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. *Environmental health perspectives, 118*(3), 318-323.

Mendelsohn, E., Hagopian, A., Hoffman, K., Butt, C.M., Lorenzo, A., Congleton, J., Webster, T.F. and Stapleton, H.M. (2016). Nail polish as a source of exposure to triphenyl phosphate. *Environment international, 86*, 45-51.

Mensching, D. A., Slater, M., Scott, J. W., Ferguson, D. C., & Beasley, V. R. (2012). The feline thyroid gland: a model for endocrine disruption by polybrominated diphenyl ethers (PBDEs)? *Journal of Toxicology and Environmental Health, Part A*, *75*(4), 201-212.

Norrgran, J., Jones, B., Bignert, A., Athanassiadis, I. and Bergman, Å. (2015). Higher PBDE serum concentrations may be associated with feline hyperthyroidism in Swedish cats. *Environmental science & technology, 49*(8), 5107-5114.

Ospina, M., Jayatilaka, N.K., Wong, L.Y., Restrepo, P. and Calafat, A.M. (2018). Exposure to organophosphate flame retardant chemicals in the US general population: Data from the 2013–2014 National Health and Nutrition Examination Survey. *Environment international*, *110*, 32-41.

Peterson, M.E. (2012). Hyperthyroidism in cats: what's causing this epidemic of thyroid disease and can we prevent it? *Journal of feline medicine and surgery, 14*(11), 804-818.

Peterson, M.E. (2014). Animal models of disease: feline hyperthyroidism: an animal model for toxic nodular goiter. *Journal of Endocrinology, 223*(2), T97-T114.

Peterson, M.E., Castellano, C.A. and Rishniw, M. (2016). Evaluation of body weight, body condition, and muscle condition in cats with hyperthyroidism. *Journal of veterinary internal medicine, 30*(6), 1780-1789.

Phillips, A.L., Hammel, S.C., Hoffman, K., Lorenzo, A.M., Chen, A., Webster, T.F. and Stapleton, H.M. (2018). Children's residential exposure to organophosphate ester flame retardants and plasticizers: investigating exposure pathways in the TESIE study. *Environment international, 116*, 176-185.

Poutasse, C.M., Herbstman, J.B., Peterson, M.E., Gordon, J., Soboroff, P.H., Holmes, D., Gonzalez, D., Tidwell, L.G. and Anderson, K.A. (2019). Silicone pet tags associate tris (1, 3-dichloro-2-isopropyl) phosphate exposures with feline hyperthyroidism. *Environmental science & technology*, *53*(15), 9203-9213.

Preston, E.V., McClean, M.D., Henn, B.C., Stapleton, H.M., Braverman, L.E., Pearce, E.N., Makey, C.M. and Webster, T.F. (2017). Associations between urinary diphenyl phosphate and thyroid function. *Environment international, 101*, 158-164.

R Project. (2019). The R Project for Statistical Computing. Retrieved from: https://www.r-project.org/

Romanak, K.A., Wang, S., Stubbings, W.A., Hendryx, M., Venier, M. and Salamova, A. (2019). Analysis of brominated and chlorinated flame retardants, organophosphate esters, and polycyclic aromatic hydrocarbons in silicone wristbands used as personal passive samplers. *Journal of Chromatography A*, 1588, 41-47.

Stapleton, H. M., Klosterhaus, S., Eagle, S., Fuh, J., Meeker, J. D., Blum, A., and Webster, T. F. (2009). Detection of organophosphate flame retardants in furniture foam and US house dust. *Environ Sci Technol., 43*(19), 7490-7495.

Stapleton, H. M., Klosterhaus, S., Keller, A., Ferguson, P. L., van Bergen, S., Cooper, E., Webster, T. F., and Blum, A. (2011). Identification of flame retardants in polyurethane foam collected from baby products. *Environ Sci Technol., 45*(12), 5323–5331.

Van der Veen, I. and de Boer, J. (2012). Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. *Chemosphere, 88*(10), 1119-1153.

Wakeling, J., Everard, A., Brodbelt, D., Elliott, J., and Syme, H. (2009). Risk Factors for Feline Hyperthyroidism in the UK. *J. Small Anim. Pract.*, *50*(8), 406−414.

Wakeling, J., Elliott, J., and Syme, H. (2011). Evaluation of predictors for the diagnosis of hyperthyroidism in cats. *Journal of veterinary internal medicine, 25*(5), 1057-1065.

Walter, K.M., Lin, Y.P., Kass, P.H. and Puschner, B. (2017). Association of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) with hyperthyroidism in domestic felines, sentinels for thyroid hormone disruption. *BMC veterinary research, 13*(1), 120.

Wise, C. F., Hammel, S. C., Herkert, N., Ma, J., Motsinger-Reif, A., Stapleton, H. M., & Breen, M. (2020). Comparative exposure assessment using silicone passive samplers indicates domestic dogs are sentinels to support human health research. Environmental Science & Technology, *in review*.

Zhao, X., Wang, H., Li, J., Shan, Z., Teng, W. and Teng, X. (2015). The correlation between polybrominated diphenyl ethers (PBDEs) and thyroid hormones in the general population: a meta-analysis. *PLoS One, 10*(5), 0126989.