UNDERSTANDING PHTHALATE EXPOSURE IN DOMESTIC ANIMALS

By

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Abstract

Phthalates are a common plastic additive that is found in households everywhere. They have become a chemical of concern because they are potential endocrine disruptors in humans and animals. Research into the dangers of phthalates is still ongoing, and this paper serves to determine whether household pets can serve as sentinel species and model organisms in further research regarding phthalates. A thorough literature review of studies evaluating the potential use of canines and felines as model organisms for human diseases and for sentinel species for environmental pollutants was undertaken. We found that household pets are exposed to much of the same levels of phthalates as humans and have previously served as model organisms for other endocrine diseases such as diabetes mellitus. We believe that due to their comparable exposure levels and high degree of homology, canines and felines are good model organisms to be used in studies of the impact of phthalates and are good sentinel species that can be used to indicate phthalate metabolite levels in the humans they cohabitate with.

Introduction

Since their introduction into American manufacturing, phthalates have become a mainstay in the production of plastics. Used as an additive to increase the flexibility of plastic product, phthalates are most used to produce polyvinyl chloride (PVC). PVC plastics are found everywhere, from construction materials to food products, beauty products and even medical supplies. Exposure to phthalates is constant. In the U.S. alone, 470 million pounds of phthalates are produced or imported in a single year. (EPA source). Exposure to phthalates can occur in several forms; the phthalates found in food and drink packages can be consumed with the meal or beverage[10]. The phthalates found in beauty products can be absorbed through the skin (dermal toxicity source). Phthalates can also be inhaled – the compound can seep into the air of the surrounding environment[10]. Once in the human body phthalates are metabolized and excreted through the urine. Phthalates move quickly through the human body. When isotopically labelled DEHP was given to a healthy male volunteer, 65-70% of the isotopically labelled phthalates were excreted in the urine after 24 hours[6]. Even though phthalates are metabolized quickly, humans are constantly exposed to them and so phthalates are constantly entering the body. Every day that humans metabolize and excrete phthalates, they are ingesting, inhaling or absorbing more.

But what is the significance of phthalates? How are they relevant to everyday life? Phthalates have been a growing concern to human health because they are suspected endocrine disruptors[9]. In laboratory settings, male animals exposed to high levels of certain phthalates developed hormone-related issues such as infertility, change in anogenital distance, cryptorchidism, decreased sperm count, and hypospadias[9]. The impacts of phthalates on women are even more unclear; this proves to be a particular concern because adult women have higher level of metabolites in their urine then adult males[10]. One potential reason for this difference is the more prevalent use of cosmetics in women; beauty products such as make up[10]. Exposure to phthalates is not only a concern for adults. Phthalate metabolites have been found in breast milk and there is a growing concern that phthalate exposure to a fetus or newborn can lead to behavioral issues caused by hormonal issues[9]. Since much of phthalate exposure is environmental, it reasons that household pets are exposed to roughly the same number of plastics as their owners. In addition, they are also exposed to phthalates in the form of canned pet food, chew toys, food bowls, training devices and rubber collars.

Methodology

This study was performed as a literature review of published studies. To find suitable articles we searched for papers that were featured in veterinary journals and medical journals because we sought the intersection between human and animal research. Articles of interest covered studies in which canines and felines were used as sentinel species for environmental pollutants and delved into *why* they were suitable sentinel species. If we could determine a specific reason why dogs and cats served as good overall model organisms – not just case specific model organisms – then the logic could be applied towards our study. We also searched for specific research where phthalate metabolites are tested for in dogs and cats to determine whether household pets are exposed to and metabolize phthalates at all.

Keywords and phrases used in searches included: phthalates in pets, phthalates in dogs, phthalates in cats, phthalates, sentinel species, endocrine diseases in dogs/cats, domestic animals as model organisms. Many different databases were used, and many were accessed through the University of Arizona Library. Databases used include ScienceDirect, SpringerLink, Wiley Online, and Pubmed. Several journals featured articles of interest. The topics of these journals ranged widely, but all were relevant to our study. Journals used included: Chemosphere, Science in the Total Environment, Environmental Science and Pollution Research, Frontiers in Veterinary Science, Food and Chemical Toxicology, Hormone Research, Veterinary Sciences, International Journal of Andrology and The Journal of Steroid Biochemistry and Molecular Biology.

Results

In the paper written by Wooten and Smith (2013) regarding phthalates exposure in canine toys, significant exposure was discovered. In this study plastic ‘bumpers’ (devices used for dog training) and toys that commonly get chewed upon by dogs were used because of their chances of oral exposure. Bumpers and toys were purchased from commercialized large pet shops; shops that the general public can access, meaning that the general public can purchase these products. Synthetic saliva was used in the place of actual dog saliva. Wooten and Smith focused on ensuring the electrolyte concentration in the synthetic saliva was as close as possible to real dog saliva.

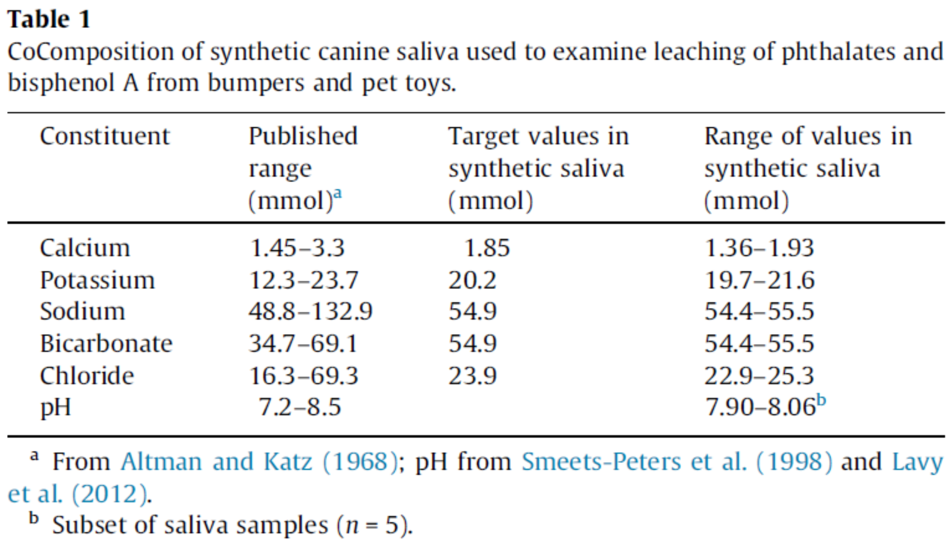

Table 1
CoComposition of synthetic canine saliva used to examine leaching of phthalates and bisphenol A from bumpers and pet toys.

Constituent	Published range (mmol)[a]	Target values in synthetic saliva (mmol)	Range of values in synthetic saliva (mmol)
Calcium	1.45–3.3	1.85	1.36–1.93
Potassium	12.3–23.7	20.2	19.7–21.6
Sodium	48.8–132.9	54.9	54.4–55.5
Bicarbonate	34.7–69.1	54.9	54.4–55.5
Chloride	16.3–69.3	23.9	22.9–25.3
pH	7.2–8.5		7.90–8.06[b]

[a] From Altman and Katz (1968); pH from Smeets-Peters et al. (1998) and Lavy et al. (2012).

[b] Subset of saliva samples ($n = 5$).

Table 1 from Wooten and Smith (2013) shows the values of electrolytes found in canine saliva, the target values for the synthetic saliva and the actual range of values achieved upon making the synthetic saliva. The published range of values differed widely between the electrolytes; the range of calcium values is smaller than the range of values for sodium. Target values for each electrolyte was achieved within the range of actual values found in the synthetic saliva.

Bumpers and toys were placed in 750mL of the synthetic saliva for 10 minutes. Toys that were brand new and toys that were chewed were tested. This was done to determine if chewing released more phthalates into the saliva. Transcriptional activation assays were used to test for *in vitro* endocrine activity. The study was statistically significant. A P value less than 0.05 is considered statistically significant and the P value of this experiment was p = 0.008. It was discovered that bumpers did release phthalates and aging and chewing increased the concentrations of DEP (diethyl phthalate), DBP (Di-n-butyl phthalate), and BBP (benzyl butyl phthalate) found in the synthetic saliva. Pet toys released less phthalates than bumpers except for one pet toy that leached comparable levels. There were no true differences between leachate levels for new and chewed toys.

DEP was the most abundant phthalate found in toy leachates. Toys made from felt over rubber had similar leachate levels to saliva blanks (which were the control). Toys made from just rubber leached DEP and BPA.

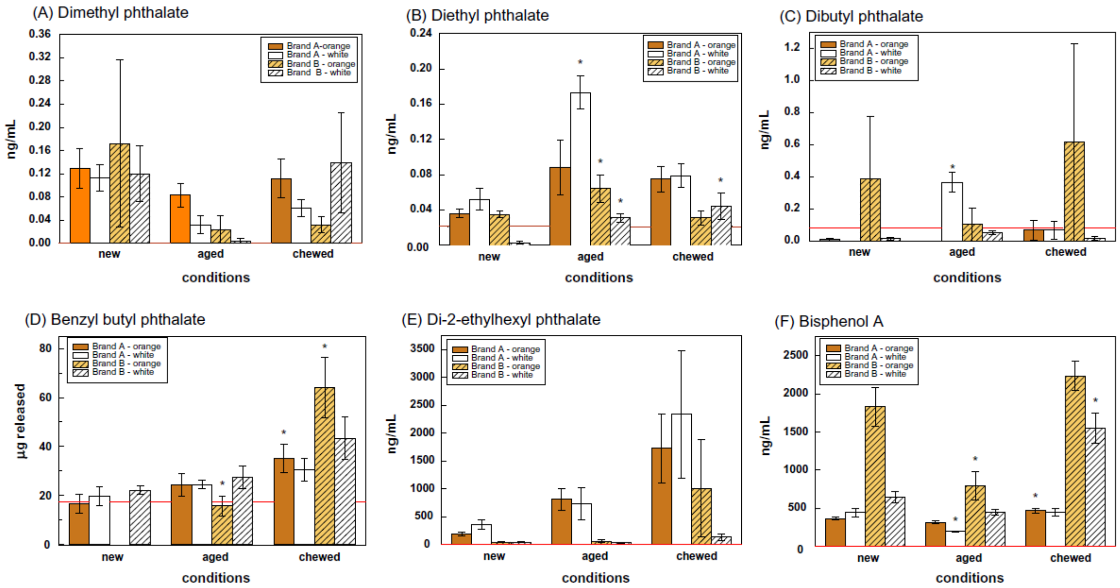

Fig. 1. Mean (±SE) concentrations, in ng/mL synthetic saliva, of dimethyl (a), diethyl (b), dibutyl (c), benzyl butyl (d), di-2-ethylhexyl (e) phthalate and bisphenol A (f) leached from plastic bumpers into synthetic canine saliva. An * indicates a significant difference ($p < 0.05$) in concentration between aged or chewed bumpers and the new bumpers of the same brand (A or B) and color (orange or white). Lines behind bars are the median saliva blank concentration for each chemical.

The different brands and colors of toys and bumpers leached different levels of phthalates. The leaching of DEP and BPA was significantly different among colors. The leaching of DEP, DEHP, and BPA was significant among different brands. Bumper leachates produced estrogenic and anti-androgenic activity in MVLN cells and MDA-kb2 cells, which are cells used for detection of anti or estrogenic activity[8]. Estrogenic activity and anti-androgenic was severely impacted by bumper color and conditions, and statistically significantly ($p < 0.001$). Estrogenic bioassay responses were significantly correlated with concentrations of DEP and BPA, and anti-androgenic activity was significantly correlated with concentrations of BPA. Pet toy leachate did not elicit anti-androgenic activity, but they did elicit estrogenic activity. Chewed toys produced more estrogenic activity than new. Estrogenic activity was not correlated with phthalate or BPA concentrations for toys.

In the paper written by Karthikraj et al. (2019) concerning urinary concentrations of phthalate metabolites in dogs and cats the levels of phthalate metabolites found in canines and felines were comparable if not higher than those in humans. In this study twenty-one phthalate metabolites were measured for in pet urine. Six of the twenty-one metabolites corresponded with low-molecular weight phthalates (LMW). These metabolites were monomethyl phthalate (mMP), monoeythyl phthalate (mEP), monoisopropyl phthalates (mIPrP), mono-n-butyl phthalate (mBP), monoisobutyl phthalate (mIBP), and mono-n-pentyl phthalate (mPeP). Ten of the metabolites corresponded with high-molecular weight phthalates (HMW). These metabolites were mono-3-carboxylpropyl phthalate (mCPP), monocylcohexyl phthalate (mCHP), monobenzyl phthalate (mBzP), mono-7-carboxyheptyl phthalate (mCHpP), monohexyl phthalate (mHxP), monoheptyl phthalate (mHpP), monooctyl phthalate (mOP), monoisonyl phthalate (mINP), monocarboxyisooctyl phthalate (mCIOP), and monocarboxyisonoyl phthalate (mCINP). Four of the twenty-one metabolites corresponded with diethylhexyl phthalates. These metabolites were mono-2-ethyl-5-carboxypentyl phthalate (mECPP), mono-2-carboxymethylhexyl phthalate (mCMHP), mono-2-ethyl-5-oxohexyl phthalate (mEOHP), and mono-2-ethyl-5-hydroxyhexyl phthalate (mEHHP). One metabolite corresponded with phthalic acid (PA).

Pet urine was collected from fifty cats and fifty dogs. The samples were collected from individual owners, veterinary hospitals and animal shelters. Four feline and seventeen canine urine samples were collected from individual owners. Eighteen feline and twenty canine urine samples were collected from veterinary hospitals. Twenty-eight feline and thirteen canine urine samples were collected from animal shelters. Samples kept in polypropylene (PP) containers. Canine samples were free-catch and cat samples collected by cystocentesis. Phthalate metabolites (PhMs) were found in all canine and feline urine samples.

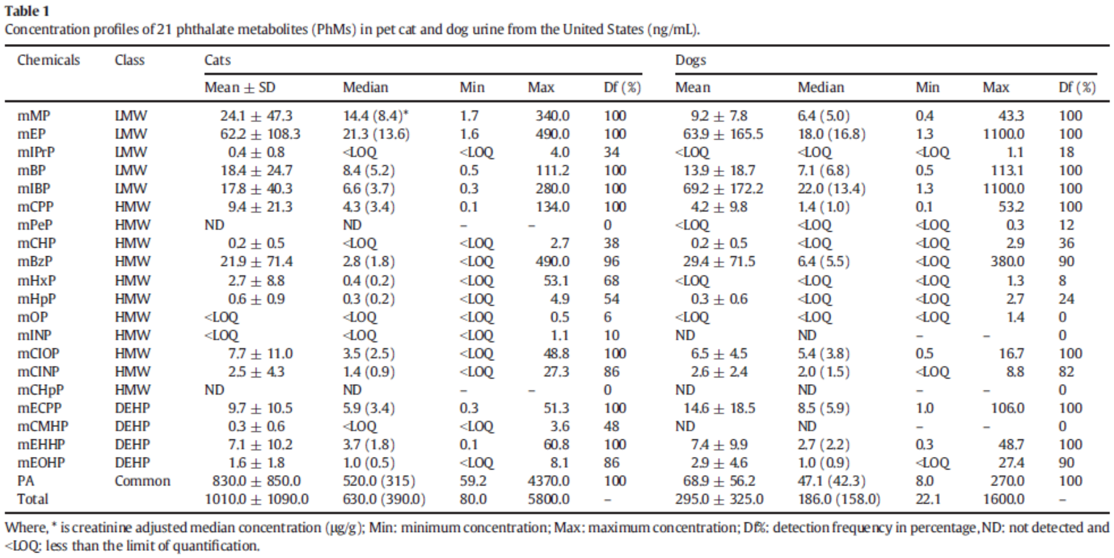

Table 1

Concentration profiles of 21 phthalate metabolites (PhMs) in pet cat and dog urine from the United States (ng/mL).

Chemicals	Class	Cats					Dogs				
		Mean ± SD	Median	Min	Max	Df (%)	Mean	Median	Min	Max	Df (%)
mMP	LMW	24.1 ± 47.3	14.4 (8.4)*	1.7	340.0	100	9.2 ± 7.8	6.4 (5.0)	0.4	43.3	100
mEP	LMW	62.2 ± 108.3	21.3 (13.6)	1.6	490.0	100	63.9 ± 165.5	18.0 (16.8)	1.3	1100.0	100
mIPrP	LMW	0.4 ± 0.8	<LOQ	<LOQ	4.0	34	<LOQ	<LOQ	<LOQ	1.1	18
mBP	LMW	18.4 ± 24.7	8.4 (5.2)	0.5	111.2	100	13.9 ± 18.7	7.1 (6.8)	0.5	113.1	100
mIBP	LMW	17.8 ± 40.3	6.6 (3.7)	0.3	280.0	100	69.2 ± 172.2	22.0 (13.4)	1.3	1100.0	100
mCPP	HMW	9.4 ± 21.3	4.3 (3.4)	0.1	134.0	100	4.2 ± 9.8	1.4 (1.0)	0.1	53.2	100
mPeP	HMW	ND	ND	–	–	0	<LOQ	<LOQ	<LOQ	0.3	12
mCHP	HMW	0.2 ± 0.5	<LOQ	<LOQ	2.7	38	0.2 ± 0.5	<LOQ	<LOQ	2.9	36
mBzP	HMW	21.9 ± 71.4	2.8 (1.8)	<LOQ	490.0	96	29.4 ± 71.5	6.4 (5.5)	<LOQ	380.0	90
mHxP	HMW	2.7 ± 8.8	0.4 (0.2)	<LOQ	53.1	68	<LOQ	<LOQ	<LOQ	1.3	8
mHpP	HMW	0.6 ± 0.9	0.3 (0.2)	<LOQ	4.9	54	0.3 ± 0.6	<LOQ	<LOQ	2.7	24
mOP	HMW	<LOQ	<LOQ	<LOQ	0.5	6	<LOQ	<LOQ	<LOQ	1.4	0
mINP	HMW	<LOQ	<LOQ	<LOQ	1.1	10	ND	ND	–	–	0
mCIOP	HMW	7.7 ± 11.0	3.5 (2.5)	<LOQ	48.8	100	6.5 ± 4.5	5.4 (3.8)	0.5	16.7	100
mCINP	HMW	2.5 ± 4.3	1.4 (0.9)	<LOQ	27.3	86	2.6 ± 2.4	2.0 (1.5)	<LOQ	8.8	82
mCHpP	HMW	ND	ND	–	–	0	ND	ND	–	–	0
mECPP	DEHP	9.7 ± 10.5	5.9 (3.4)	0.3	51.3	100	14.6 ± 18.5	8.5 (5.9)	1.0	106.0	100
mCMHP	DEHP	0.3 ± 0.6	<LOQ	<LOQ	3.6	48	ND	ND	–	–	0
mEHHP	DEHP	7.1 ± 10.2	3.7 (1.8)	0.1	60.8	100	7.4 ± 9.9	2.7 (2.2)	0.3	48.7	100
mEOHP	DEHP	1.6 ± 1.8	1.0 (0.5)	<LOQ	8.1	86	2.9 ± 4.6	1.0 (0.9)	<LOQ	27.4	90
PA	Common	830.0 ± 850.0	520.0 (315)	59.2	4370.0	100	68.9 ± 56.2	47.1 (42.3)	8.0	270.0	100
Total		1010.0 ± 1090.0	630.0 (390.0)	80.0	5800.0	–	295.0 ± 325.0	186.0 (158.0)	22.1	1600.0	–

Where, * is creatinine adjusted median concentration (μg/g); Min: minimum concentration; Max: maximum concentration; Df%: detection frequency in percentage, ND: not detected and <LOQ: less than the limit of quantification.

Table 1 from Karthikraj et al. (2019) depicts the mean, median, min and max ranges for the different phthalate metabolites in both cats and dogs. In felines PhMs were detected in all cat urine samples, ranging from 80 to 5800 ng/mL with a median concentration of 630 ng/mL. PA was the major metabolite found in 82% of samples. The median value for mEP was 21.3 ng/mL and was found in 3.4% of samples. The median value for mMP was 14.5 ng/mL and was found in 2.3% of all samples. The median value for mBP was 8.4 ng/mL and was found in 1.3% of all samples. The median value for mIBP was 6.6 ng/mL and was found in 1.0% of all samples. The median value for mECPP was 5.9 ng/mL and was found in 0.9% of all samples. For cats there was predominant exposure to LMW phthalates

In canines, metabolites were detected in all dog urine samples with concentrations ranging from 22 to 1600 ng/mL. The range of PhM values in canines was approximately 4 times lower than that in felines. The median concentration in canines was 186 ng/mL, which is approximately 3.5 times lower than felines. PA was a major metabolite found in urine samples with a median concentration of 47 ng/mL. The next most common metabolite was mIBP with a median of 22.1 ng/mL followed by mEP with a median value of 18.0 ng/mL.

Values in both species were compared. LMW metabolites accounted for 69% of PhMs in cats and 66% in dogs. HMW and DEHP metabolites accounted for 17% in cats and 19% in dogs and 14.5% in cats and 15% in dogs respectively. LMW (DEP and DBP in particular) are widely used in personal care products, dietary supplements and medication.

After the values of PhMs in dogs and cats were compared, the values were compared to that of the U.S. population.

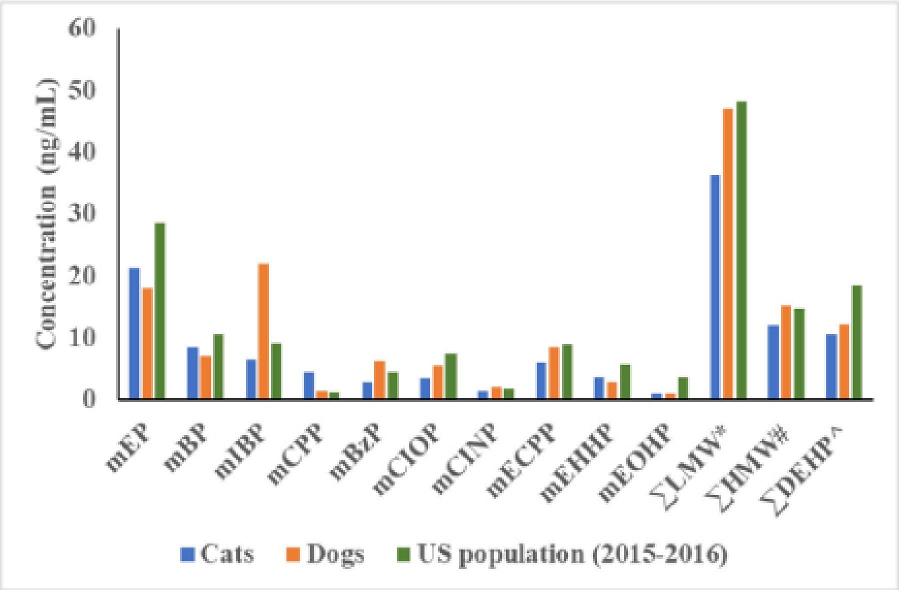

Fig. 2. Comparison of median concentrations of major phthalate metabolites in the urine of cats and dogs with those reported for the US population in 2015–2016 (CDC, 2019). * is the sum concentration of mEP, mBP, and mIBP; # is the sum concentration of mCPP, mBzP, mCIOP, and mCINP; and ^ is the sum concentration of mECPP, mEHHP, and mEOHP.

Figure 2 shows that the most abundant compounds in dogs and cats were also the most similar in people (the most abundant compounds being mECPP and mEHHP). In comparison, LMW and HMW phthalate metabolite levels were comparable or *higher* than in humans. The high levels of phthalic acid (PA) can perhaps be explained by a household pets' diet. Pet food is the probable and most direct source of exposure for PA. Higher concentration of PA in cats could be explained by the weak elimination of xenobiotics in cags due to low glucuronidation capacity. This is suspected but not exactly proven to be the reason.

Gender, age and habitat also played a role in PhM levels. Total concentrations of PhMs were higher in female cats and dogs although the values were not statistically significant. The mean concentration of PhMs in dogs decreased with increasing age. In dogs between ages 6 months and 2 years the median value of PhMs was 260 ng/mL, in dogs between ages 2 years and 5 years the median value was 240 ng/mL, and dogs between the ages of 5 years and 13 years the median value was 183 ng/mL. Inversely, the mean concentration of PhMs in cats increased with increasing age. In cats between the ages of 10 weeks and 2 years the median value was 865 ng/mL, in ages between 2 years and 5 years the median value was 1060 ng/mL and between the ages of 5 years and 20 years the median value was 1370 ng/mL. Urine samples taken from dogs and cats in shelters was found to have PhM values 1.3-2.4 times lower than those in samples collected from homes. There was a very large notable difference in mEP concentrations between homes and shelters. The median concentration for mEP in shelters was 2 ng/mL for dogs and 13.3 ng/mL for cats. The median concentration for mEP in homes was 33.4 ng/mL for dogs and 53.8 ng/mL for cats. There was an exception to this trend with mIBP in dogs, which was 4 times higher in shelters.

In the paper written by Henriquez-Hernandez et al. (2017), the potential role of pet cats as sentinel species for flame retardants was studied. For one type of flame retardant, organophosphate flame retardants (OPFRs), levels in cats and humans were comparable. We must note that this study focuses on flame retardants and not specifically phthalates, however phthalates and commercial chemical flame retardants are both concerning potential endocrine disruptors and so this study that indicates that cats could be a sentinel species for flame retardants is still relevant to phthalates. In this study twenty-two cats had blood samples taken via cephalic vein puncture, and twenty human blood samples were taken from volunteers. The twenty volunteers all met the requirements of being cat owners, but none owned the cats used in the study. Serum levels of 6 OPFR compounds were detected in 100% of the samples, and one other OPFR compound was found in 82% of cat samples and 92% of human samples. There was one compound, tricresyl phosphate, that was detected in 77.3% of cat samples and not detected in any of the human samples.

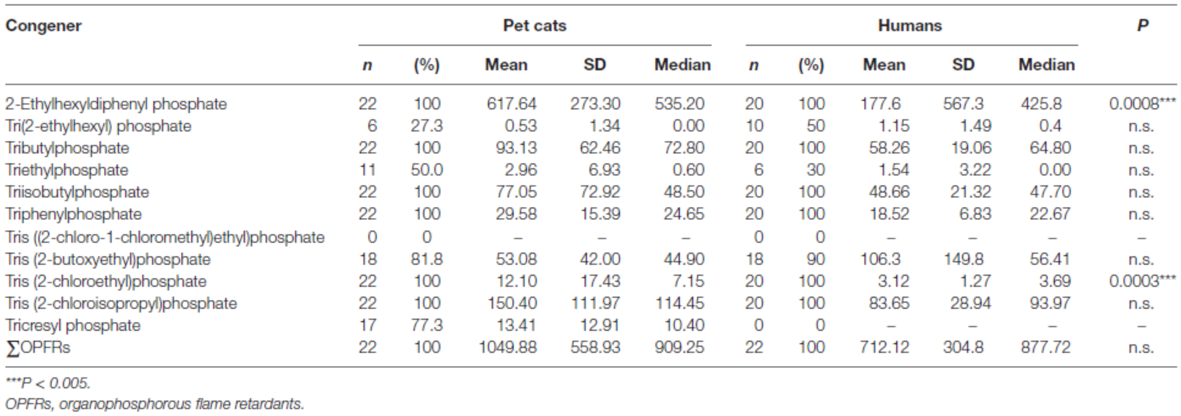

TABLE 3 | Concentrations of organophosphorous flame retardants (ng/g fat) in the whole series of cats ($n = 22$) and humans ($n = 20$).

Congener	Pet cats					Humans					*P*
	n	(%)	Mean	SD	Median	*n*	(%)	Mean	SD	Median	
2-Ethylhexyldiphenyl phosphate	22	100	617.64	273.30	535.20	20	100	177.6	567.3	425.8	0.0008***
Tri(2-ethylhexyl) phosphate	6	27.3	0.53	1.34	0.00	10	50	1.15	1.49	0.4	n.s.
Tributylphosphate	22	100	93.13	62.46	72.80	20	100	58.26	19.06	64.80	n.s.
Triethylphosphate	11	50.0	2.96	6.93	0.60	6	30	1.54	3.22	0.00	n.s.
Triisobutylphosphate	22	100	77.05	72.92	48.50	20	100	48.66	21.32	47.70	n.s.
Triphenylphosphate	22	100	29.58	15.39	24.65	20	100	18.52	6.83	22.67	n.s.
Tris ((2-chloro-1-chloromethyl)ethyl)phosphate	0	0	–	–	–	0	0	–	–	–	–
Tris (2-butoxyethyl)phosphate	18	81.8	53.08	42.00	44.90	18	90	106.3	149.8	56.41	n.s.
Tris (2-chloroethyl)phosphate	22	100	12.10	17.43	7.15	20	100	3.12	1.27	3.69	0.0003***
Tris (2-chloroisopropyl)phosphate	22	100	150.40	111.97	114.45	20	100	83.65	28.94	93.97	n.s.
Tricresyl phosphate	17	77.3	13.41	12.91	10.40	0	0	–	–	–	–
∑OPFRs	22	100	1049.88	558.93	909.25	22	100	712.12	304.8	877.72	n.s.

****P* < 0.005.*

OPFRs, organophosphorous flame retardants.

Table 3 from Henriquez-Hernandez et al. (2017) shows the breakdown of the OPFR levels found in cats and humans. There were some large differences between certain OPFRs, such as 2-ethylhexyldiphenyl phosphate. The mean value of 2-ethylhexyldiphenyl phosphate in cat samples was 617.64 and 177.6 ng/g fat in human samples. Over OPFRs had comparable levels between human samples and cat samples, for example triethyl phosphate. Triethyl phosphate has a mean value of 2.96 ng/g in cat samples and 1.54 ng/g in human samples. Overall, Henriquez-Hernandez and the cowriters determined that the distribution of OPFR among humans and cats were very similar.

Discussion

Studies focused on phthalates are very important because of the health risks phthalates pose to humans. In addition, phthalates could be a new potential concern in the veterinary community. While phthalates are a health concern in animals, they are also an important research opportunity. For many reasons, we can’t just humans as model organisms for testing phthalate impacts and this is where domestic animals come in. Household pets have several key characteristics that make them suitable model organisms and sentinel species. Household pets cohabitate with humans and are exposed to many of the same pollutants and have levels that are comparable or higher. This was shown in the paper written by Karthikraj et al. (2019). Household pets, such as canines and felines, also have a higher degree of homology with humans than mice and rats. Canines are already used as sentinel species for breast cancer[5], infectious diseases[7] and certain endocrine disorders[4]. Dogs, as well as cats, are good sentinel species for several reasons. There are similarities between the reproductive systems of humans and dogs/cats. Part of this is because of the similar reproductive systems shared by mammals. Another part of this is due to the high degree of homology. There are genetic similarities as well; studies done in dogs and cats can compare to humans because of our similarities as mammals. Dogs suffer from some of the same endocrine diseases as humans, and the mechanisms of those diseases in dogs is the same. For example, the type of diabetes mellitus seen in cats is similar to type 2 diabetes in humans[4]. When it comes to the differences between genetics in dogs, cats and humans those differences can be specifically clarified because both canine and feline genomes have been sequenced so we can accurately compare the genome similarity to that of people[4].

Future steps for this project would begin with measuring the phthalates found in cat and dog tissues. The easiest way to do so would be to measure the follicular fluid around the ovaries that have been removed in a routine ovariohysterectomy. We attempted to begin this experiment in fall of 2020 but ran into several roadblocks. One such roadblock was the COVID-19 pandemic, which prevented many in-person meetings and relegated all meetings to be online. Other roadblocks were administrative. The project required approval from IACUC because we would be working with animal tissues. We needed to gain permission from individual pet owners to use their animals in the experiment, and cooperation between us and the vet clinics that performed the ovariohysterectomy. Unfortunately, while we did secure IACUC approval in the fall of 2020, we ran out of time in the spring of 2021 to secure ovaries, vet clinic cooperation, and owner permission.

Conclusion

The overall impact of this study concerns human and animal health. There is a lot of concern regarding fertility in generations who have grown up surrounded by phthalates. Those generations include people who have already have children, and the children themselves. The fact that phthalates work as endocrine disrupters, and thus hormone disrupters is especially concerning because hormones play key roles in basic human functions. Hormones result in the reproductive maturation and maintain reproductive health. There is a concern for veterinarians because this could prove to cause endocrine issues in household pets. Despite being an animal lover, the main importance is how animals can be used as sentinel species, and I believe they can.

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