<u>SUPPORTING INFORMATION</u>

Silicone Pet Tags Associate Tris(1,3,-Dichloro-2-Isopropyl) Phosphate Exposures with Feline Hyperthyroidism

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Cat Recruitment

As stipulated by the inclusion criteria, all cats were over seven years old. Cat owners completed a consent form and three-page questionnaire about their cat's home environment. If multiple cats were recruited from the same home (n=10), owners completed a consent form and questionnaire for each cat individually.

The diagnosis of hyperthyroidism (n=39) was established on the basis of clinical signs consistent with the disease (e.g. weight loss despite good appetite), a palpable thyroid nodule on physical examination, high basal total thyroxine (TT_4) and free T_4 (fT_4) concentrations, and a good clinical response to treatment for hyperthyroidism. Hyperthyroid cats who had recently undergone treatment with radioiodine or were currently undergoing anti-thyroid drug treatment were eligible.

Non-hyperthyroid, or euthyroid, cats (n=39) were considered healthy on the basis of history, physical examination findings (e.g. lack of palpable thyroid tumors), and results of routine laboratory examinations (e.g. serum biochemical analysis) and serum thyroid profile. The serum thyroid profile included concentrations of fT_4, TT_4, total triiodothyronine (TT_3), and thyroid-stimulating hormone (TSH) (see next section).

If an enrolled cat did not regularly wear a collar, researchers provided a complimentary collar. Cats wore the pet tag for seven days before the owner removed the tag from the collar, resealed it in the PTFE bag, and returned it to the study coordinator.[1, 2]

Serum Thyroid Hormone Panel

Non-hyperthyroid cats were required to undergo a serum thyroid panel of tests, including free thyroxine (fT_4), total T_4 (TT_4), total triiodothyronine (TT_3), and thyroid-stimulating hormone (TSH), to assess his or her thyroid status and to determine eligibility for this study. After the cat owner completed the consent form and questionnaire, the recruiting veterinarian examined the cat for clinical findings of feline hyperthyroidism (e.g. palpable goiter). If no clinical features of feline hyperthyroidism were detected, then the veterinarian drew two to three mL of blood, and the sample was shipped to IDEXX Laboratories for the analysis of serum fT_4, TT_4, TT_3, and TSH, conducted by assays validated for cats as previously reported.[3-5] If fT_4 and TT_4 concentrations were within the respective reference intervals (Table S1), then the cat was eligible to be a non-hyperthyroid participant for the study.

Flame Retardant Extraction

The pet tags underwent post-deployment cleaning to remove particulate matter with two rinses of 18 MΩ·cm water and one of isopropanol.[2] The tags were stored in amber glass jars at -20 °C, and then extracted and analyzed as previously reported.[2, 6] Briefly, FBDE-118 and 2-bromobiphenyl were added as a recovery surrogates, with respective average recoveries of 91±18% (median=92%) and 90±19% (median=91%). Pet tags were extracted with two 100 mL volumes of ethyl acetate at ambient temperature. Sample extracts were combined and quantitatively reduced to one mL under nitrogen (Turbo-Vap L, Biotage, Charlotte, NC, USA; RapidVap, LabConco, Kansas City, MO, USA; N-EVAP 111, Organomation Associates, Berlin, MA, USA). Sample extracts were stored at 4 °C prior to instrument analysis.

The sample extract aliquots were combined with FBDE-126 as the internal standard. Targeted analysis of 44 FRs occurred using an Agilent 7890A gas chromatograph coupled with an Agilent

5975C mass spectrometer (Santa Clara, CA). The gas chromatograph was operated in electron impact mode (70 eV) and select ion monitoring.

Instrument Parameters

The instrument parameters were configured as previously reported.[2] Briefly, an Agilent 7890A gas chromatograph was coupled with an Agilent 5975C mass spectrometer (Santa Clara, CA) for analysis of 44 flame retardant analytes. An Agilent DB-5MS column (30 m × 0.25 mm × 0.25 µm) was operated in electron impact mode (70 eV) and select ion monitoring. Samples were loaded using an Agilent 2 mm dimpled liner and pulsed splitless injection. The temperatures of the MS source, quadruple, and detector transfer line were set to 250°C, 150°C, and 300°C respectively. The pulse pressure was 30 psi (0.5 min) at a 3 mL/min purge and a 35 mL/min purge after 1 minute. The temperature profile started at 90°C (1.25 min), ramped to 240°C (10 °C/min), ramped to 310°C (20 °C/min), and held at 310°C (10 min).

The limits of detection (LODs) and limits of quantitation (LOQs) were determined as previously reported.[2] Briefly, for each analyte, the lowest standard with a 15:1 signal-to-noise ratio was run seven times. The resulting standard deviation was used to calculate a 99% confidence interval with the Student's *t*-value and appropriate degree of freedom. LOQs were five times higher than the LODs. The method LODs and LOQs for all analytes, surrogate standards, and internal standard are reported in Table S2.

Quality Control

To ensure pet tags met the data quality objectives, QC samples[1, 7] accounted for 47% of the total samples analyzed. QC samples included cat tag conditioning verifications (n=4), trip blanks (n=1), laboratory control blanks (n=4), sample duplicates (n=1), sample overspikes (n=2), instrument solvent blanks (n=43), and continuing calibration verifications (n=13). All target analytes were below their respective LODs in all blank QC. All calibration verifications were within data quality objectives at ±30% of the true value for 70% of the target analytes.

A “cat collar” QC sample was included because two cat tags were returned with the collars still attached. Only TCIPP was detected in this QC, below the LOQ. Because the TCIPP LOQ was over 10-fold lower than either pet tag TCIPP concentration, no correction was made to the samplers returned with the collars.

Particulate-Bound Fraction

A measure of bioavailability is the octanol-air partition coefficients (K_{oa}) of individual chemicals.[8] Because LMW PBDE congeners have lower log K_{oa} values (e.g. 2 to 13),[1] they partition more readily into the air than particulate matter.[9, 10] Consequently, the LMW congeners also partition more readily into the silicone pet tags than particulate matter. In contrast, HMW PBDE congeners have higher log K_{oa} values and are more frequently detected in house dust than in air.[8, 9]

For this study, any particulate matter on the silicone pet tags was removed during the post-deployment cleaning process (Section 2.4).[1] Some previous studies did not include this step prior to laboratory extractions.[8] In general, particulate-bound FRs are “biologically unavailable” for uptake by silicone PSDs.[11] Washing the samplers prior to extraction enabled this study to focus only on FRs sequestered by the polymer matrix.

Table S1. Reference ranges and summary statistics are reported for hormones included in the serum thyroid profile for the 39 non-hyperthyroid cats recruited for the study. Out of free thyroxine (fT_4), total T4 (TT_4), total triiodothyronine (TT_3), and thyroid-stimulating hormone (TSH) concentrations, a cat was eligible to be a non-hyperthyroid participant if the fT_4 and TT_4 concentrations were within the respective reference intervals.

Thyroid Hormone	Reference Range	Geometric Mean	Standard Deviation	Median	Cat Study Range
fT_4 (ng/dL)	0.7-2.6	1.15	0.41	1.10	0.50-2.10
TT_4 (ug/dL)	0.8-4.7	2.27	0.47	2.20	1.70-3.50
TT_3 (ng/dL)	52-182	34.0	7.12	35.0	4.1-48.0
TSH (ng/mL)	0.05-0.42	0.04	0.07	0.05	0.01-0.41

Table S2. Target analytes, CAS numbers, and method limits of detection and quantification are reported.

Target Analyte	Abbreviation	CAS	MW	Method LOD (pmol/g)[a]	Method LOQ (pmol/g)
Polybrominated diphenyl ethers					
2-bromodiphenyl ether	BDE-1	7025-06-1	249.1	3.01	15.1
3-bromodiphenyl ether	BDE-2	6976-00-2	249.1	2.46	12.3
4-bromodiphenyl ether	BDE-3	101-55-3	249.1	2.76	13.8
2,4-dibromodiphenyl ether	BDE-7	53592-10-2	328.0	1.76	8.81
2,4’-dibromodiphenyl ether	BDE-8	49602-91-7	328.0	1.70	8.51
3,2’-dibromodiphenyl ether	BDE-10	2050-47-7	328.0	2.16	10.8
3,3’-dibromodiphenyl ether	BDE-11	6903-63-5	328.0	1.83	9.15
3,4-dibromodiphenyl ether	BDE-12	189084-59-1	328.0	1.80	8.99
3,4’-dibromodiphenyl ether	BDE-13	57186-90-0	328.0	1.19	5.91
4,4’-dibromodiphenyl ether	BDE-15	2050-47-7	328.0	1.05	5.24
2,2’,4-tribromodiphenyl ether	BDE-17	147217-75-2	406.9	1.51	7.52
2,3’,4-tribromodiphenyl ether	BDE-25	147217-77-4	406.9	1.12	5.58
2,4,4’-tribromodiphenyl ether & 2’,3,4-tribromodiphenyl ether	BDE-28 & BDE-33	41318-75-6 & 337513-67-4	406.9	1.02	5.11
2,4,6-tribromodiphenyl ether	BDE-30	49690-94-0	406.9	1.39	6.96
2,4’,6-tribromodiphenyl ether	BDE-32	189084-60-4	406.9	1.52	7.62
3,3’,4-tribromodiphenyl ether	BDE-35	147217-80-9	406.9	2.90	14.5
3,4,4’-tribromodiphenyl ether	BDE-37	147217-81-0	406.9	0.654	3.27
2,2’,4,4’-tetrabromodiphenyl ether	BDE-47	5436-43-1	485.8	1.59	7.93
2,2’,4,5’-tetrabromodiphenyl ether	BDE-49	243982-82-3	485.8	1.46	7.29
2,3’,4,4’-tetrabromodiphenyl ether	BDE-66	189084-61-5	485.8	1.94	9.70
2,3’,4’,6-tetrabromodiphenyl ether	BDE-71	189084-62-6	485.8	1.04	5.23
2,4,4’,6-tetrabromodiphenyl ether	BDE-75	189084-63-7	485.8	1.43	7.14
3,3’,4,4’-tetrabromodiphenyl ether	BDE-77	93703-48-1	485.8	0.642	3.21
2,2’,4,4’,5-pentabromodiphenyl ether	BDE-99	60348-60-9	564.7	1.52	7.61
2,2’,4,4’,6-pentabromodiphenyl ether	BDE-100	189084-64-8	564.7	1.57	7.84
2,3,4,5,6-pentabromodiphenyl ether	BDE-116	189084-65-9	564.7	1.42	7.10

2,3’,4,4’,5-pentabromodiphenyl ether	BDE-118	446254-80-4	564.7	1.51	7.54
2,3’,4,4’,6-pentabromodiphenyl ether	BDE-119	189084-66-0	564.7	1.08	5.38
2,2’,3,4,4’,5’-hexabromodiphenyl ether	BDE-138	182677-30-1	643.6	1.17	5.87
2,2’,4,4’,5,5’-hexabromodiphenyl ether	BDE-153	68631-49-2	643.6	0.766	3.82
2,2’,4,4’,5,6’-hexabromodiphenyl ether	BDE-154	207122-15-4	643.6	0.928	4.63
2,3,4,4’,5,6-hexabromodiphenyl ether	BDE-166	189084-58-0	643.6	0.771	3.85
2,2’,3,4,4’,5,6-heptabromodiphenyl ether	BDE-181	189084-67-1	715.5	12.5	62.8
2,2’,3,4,4’,5’,6-heptabromodiphenyl ether	BDE-183	207122-16-5	715.5	10.8	53.9
2,3,3’,4,4’,5,6-heptabromodiphenyl ether	BDE-190	189084-68-2	715.5	7.10	35.5
Organophosphate flame retardants					
Tri-n-butyl phosphate	TNBP	126-73-8	266.3	4.43	22.3
Tri-n-ethyl phosphate	TNEP	78-40-0	182.2	10.8	53.9
Triphenyl phosphate	TPHP	115-86-6	326.3	1.31	6.53
Tris(2-chloroethyl) phosphate	TCEP	115-96-8	285.5	20.4	102
Tris(1-chloro-2-isopropyl) phosphate	TCIPP	13674-84-5	327.6	27.7	139
Tris(1,3-dichloro-2-isopropyl) phosphate	TDCIPP	13674-87-8	427.9	20.8	104
Brominated flame retardants					
2-ethylhexyl-2,3,4,5-tetrabromobenzoate	EH-TBB	183658-27-7	549.9	8.37	41.8
Di(2-ethylhexyl)tetrabromophthalate	TBPH	26040-51-7	706.1	1.46	7.29
Reference Standards					
2-Bromobiphenyl	2-BBP (SS)	2052-07-5	233.1	3.54	17.7
5’-Fluoro-3,3’,4,4’,5-pentabromodiphenyl ether	FBDE-126 (IS)	N/A	583.7	N/A	N/A
5’-Fluoro-2,3’,4,4’,5-pentabromodiphenyl ether	FBDE-118 (SS)	N/A	583.7	1.78	8.89

LOD – Limit of detection; LOQ – Limit of quantitation; SS – Surrogate standard; IS – Internal standard; N/A – Not applicable.

Table S3. Unadjusted odds ratios are reported for flame retardants detected in at least one tag.

Target Analyte	Unadjusted Odds Ratio (95% CI)	P-value (odds ratio)
TNBP[a]	1.61 (0.313, 8.29)	0.566
TNEP[a]	0.852 (0.117, 6.23)	0.874
TCEP[a]	0.278 (0.027, 2.91)	0.279
TCIPP[a]	1.03 (0.952, 1.12)	0.409
TDCIPP[a]	1.36 (0.923, 2.02)	**0.059***
TPHP[a]	1.09 (0.469, 2.53)	0.840
Σ_6OPEs[a]	*1.03 (0.955, 1.12)*	*0.415*
BDE-8	--	--
BDE-12	--	--
BDE-15	--	--
BDE-17	--	--
BDE-25	--	--
BDE-28& BDE-33	0.390 (0.076, 1.99)	0.258
BDE-47	0.940 (0.447, 1.98)	0.870
BDE-49	0.702 (0.177, 2.78)	0.614
BDE-66	0.759 (0.045, 12.7)	0.848
BDE-99	0.987 (0.508, 1.92)	0.970
BDE-100	0.641 (0.329, 1.25)	0.190
BDE-138	--	--
BDE-153	0.689 (0.291, 1.63)	0.396
BDE-154	0.501 (0.156, 1.61)	0.246
Σ_{36}BDEs	*0.859 (0.564, 1.01)*	*0.326*
EH-TBB[a]	0.489 (0.061, 3.93)	0.492
Σ_2BFRs[a]	*0.490 (0.061, 3.93)*	*0.492*

Bold*: p<0.10

[a]Odds ratio calculated using nmol/g tag concentrations

Table S4. Spearman's rho correlation coefficients are reported for OPEs detected in over 10% of matched cat tag samples (n=78). Correlation coefficients were calculated from concentrations in units of picomole of target analyte per gram of pet tag.

		TPHP	TCIPP	TDCIPP	TNBP	TCEP	TNEP
TPHP	r_s	1	0.461	0.305	0.296	0.238	0.131
	p-value	--	**<0.001***	**0.007***	**0.009***	**0.036***	0.253
TCIPP	r_s		1	0.394	0.111	0.082	0.263
	p-value		--	**<0.001***	0.335	0.478	**0.020***
TDCIPP	r_s			1	-0.053	0.271	0.155
	p-value			--	0.648	**0.016***	0.176
TNBP	r_s				1	0.129	0.033
	p-value				--	0.259	0.773
TCEP	r_s					1	0.047
	p-value					--	0.684
TNEP	r_s						1
	p-value						--

Bold*: $p<0.05$

Table S5. Spearman's correlation coefficients are reported for PBDE congeners detected in over 10% of match cat tag samples (n=78). Correlation coefficients were calculated from concentrations in units of picomole of target analyte per gram of pet tag.

		BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-49
BDE-47	r_s	1	0.577	0.491	0.519	0.592	0.462
	p-value	--	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
BDE-99	r_s		1	0.542	0.632	0.675	0.439
	p-value		--	**<0.001***	**<0.001***	**<0.001***	**<0.001***
BDE-100	r_s			1	0.620	0.537	0.426
	p-value			--	**<0.001***	**<0.001***	**<0.001***
BDE-153	r_s				1	0.830	0.445
	p-value				--	**<0.001***	**<0.001***
BDE-154	r_s					1	0.543
	p-value					--	**<0.001***
BDE-49	r_s						1
	p-value						--

Bold*: p<0.05.

Figure S1. The Mantel-Cox non-parametric test for comparing survival curves indicated that hyperthyroid and non-hyperthyroid TDCIPP tag concentrations were statistically different.

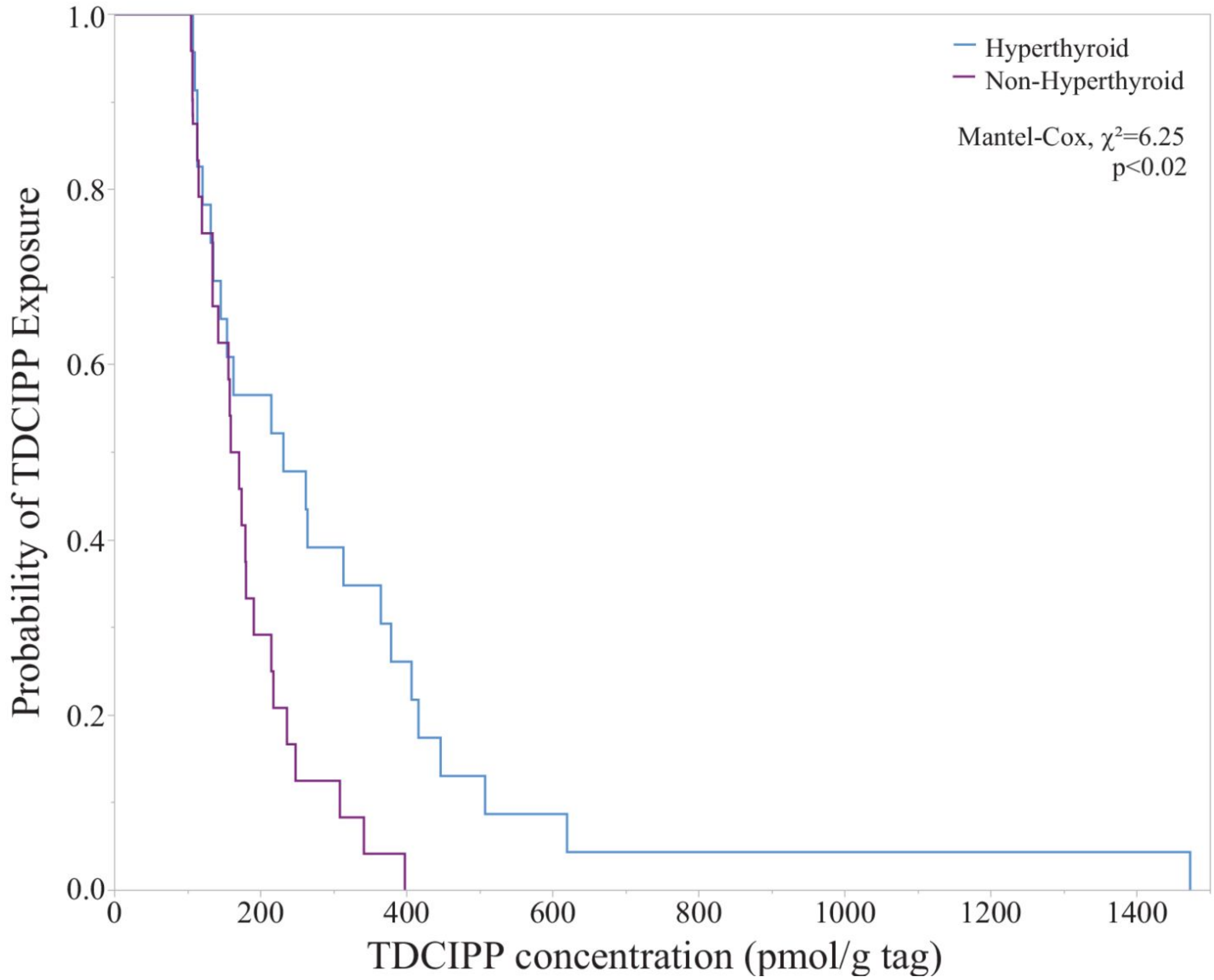

Figure S2. Cat owners appreciated the opportunity to share photos of their cats participating in the study.

REFERENCES

1. 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.; O'Connell, S. G. Preparation and Performance Features of Wristband Samplers and Considerations for Chemical Exposure Assessment. *J. Expo. Sci. Environ. Epidemiol.* **2017**, *27* (6), 551.

2. Kile, M. L.; Scott, R. P.; O'Connell, S. G.; Lipscomb, S.; MacDonald, M.; McClelland, M.; Anderson, K. A. Using Silicone Wristbands to Evaluate Preschool Children's Exposure to Flame Retardants. *Environ. Res.* **2016**, *147*, 365-372.

3. Peterson, M. E.; Rishniw, M.; Bilbrough, G. E.; Cote, K. B. Comparison of in-Clinic Point-of-Care and Reference Laboratory Total Thyroxine Immunoassays for Diagnosis and Post-Treatment Monitoring of Hyperthyroid Cats. *J. Feline Med. Surg.* **2018**, *20* (4), 319-324.

4. Lucy, J. M.; Peterson, M. E.; Randolph, J. F.; Scrivani, P. V.; Rishniw, M.; Davignon, D. L.; Thompson, M. S.; Scarlett, J. M. Efficacy of Low-Dose (2 Millicurie) Versus Standard-Dose (4 Millicurie) Radioiodine Treatment for Cats with Mild-to-Moderate Hyperthyroidism. *J. Vet. Intern. Med.* **2017**, *31* (2), 326-334.

5. Peterson, M. E. More Than Just T4: Diagnostic Testing for Hyperthyroidism in Cats. *J Feline Med. Surg.* **2013**, *15* (9), 765-777.

6. O'Connell, S. G.; Kincl, L. D.; Anderson, K. A. Silicone Wristbands as Personal Passive Samplers. *Environ. Sci. Technol.* **2014**, *48* (6), 3327-3335.

7. Project Quality Assurance and Quality Control. In *Epa Publication Sw-846: Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*, Final Update V to Ed 3 ed.; 2015.

8. Hammel, S. C.; Phillips, A. L.; Hoffman, K.; Stapleton, H. M. Evaluating the Use of Silicone Wristbands to Measure Personal Exposure to Brominated Flame Retardants. *Environ. Sci. Technol.* **2018**, *52* (20), 11875-11885.

9. Liagkouridis, L.; Cousins, A. P.; Cousins, I. T. Physical-Chemical Properties and Evaluative Fate Modelling of 'Emerging' and 'Novel' Brominated and Organophosphorus Flame Retardants in the Indoor and Outdoor Environment. *Sci. Total Environ.* **2015**, *524*, 416-426.

10. Weschler, C. J.; Nazaroff, W. W. SVOC Exposure Indoors: Fresh Look at Dermal Pathways. *Indoor Air* **2012**, *22* (5), 356-377.

11. Anderson, K.; Hillwalker, W. Bioavailability. In *Ecotoxicology*, Jorgensen, S. E.; Fath, B. D., Eds. Elsevier: Oxford, 2008; Vol. 1, 348-357.