THE FELINE THYROID GLAND: A MODEL FOR ENDOCRINE DISRUPTION BY POLYBROMINATED DIPHENYL ETHERS?

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Abstract*

Polybrominated diphenyl ethers (PBDEs) are ubiquitous flame retardants and known endocrine disruptors used in many household products since the 1970s. They are lipophilic and bioaccumulate, with tissue residues in humans and wildlife exponentially increasing since their inception. Feline hyperthyroidism (FH) is a common disease of geriatric domestic cats first recognized only after PBDE production began. FH has been associated with a variety of risk factors and the associated thyroid abnormalities are morphologically and functionally analogous to human toxic (multi)nodular goiter (TNG), but its etiology remains unknown. Thyroid hyperplasia, a prelude of TNG, was linked with experimental exposure of rats to PBDEs in 1975. Structurally similar to thyroxine (T4) and triiodothyronine (T3), hydroxylated metabolites of PBDEs have been shown to bind to transthyretin, the dominant high-affinity serum binding protein in cats, and to thyroid hormone receptors (TRs), and also to inhibit 5’-deiodinases. Experimental exposures to PBDEs have resulted in decreased T4 levels via uridinediphosphoglucuronosyl transferase (UDPGT) induction.

To test the hypothesis that PBDE exposure is associated with endocrine disruption of the feline thyroid gland and hyperthyroidism, we analyzed serum from 62 client-owned domestic and 9 feral cats (2 of which were pooled) for 15 PBDE congeners with gas chromatography/high resolution mass spectrometry (GC/HRMS). To evaluate potential sources of exposure, dust samples from 19 homes and 10 commercial canned cat food samples were extracted using a modified EPA Method 3545 and analyzed via GC/HRMS. Total lipid-adjusted serum PBDE burdens ranged from 373 ng/g lipid to 51,063 ng/g lipid (mean: 5,865 ng/g lipid) for client-owned cats and 457-3712 ng/g lipid (mean: 1,203 ng/g lipid) for feral cats. This difference was statistically significant and may be attributable to environment, diet, and/or age. Among the client-owned cats, total lipid-adjusted PBDEs are extremely high compared to published values for human beings and represent the highest documented PBDE burdens in any species on the planet to date. Total lipid-adjusted PBDEs did not correlate with age or thyroid status. BDE-153 had a higher percent composition in serum of euthyroid cats than in hyperthyroid cats. The percent composition of BDE-183 was significantly higher in feral cats versus client-owned cats of either thyroid status. Elevated TSH was not detected in any subject. In fact, all of the hyperthyroid cats and 79% of the euthyroid cats, including all of the euthyroid feral cats, had
TSH concentrations below the detection limit of 0.03 ng/ml on the canine DPC IMMULITE®
assay standardized with recombinant feline TSH. Concentrations of PBDEs in dust ranged from
510 ng/g to 95,448 ng/g (mean: 8,098 ng/g) and were significantly higher in dust samples from
homes of hyperthyroid cats as compared to those of euthyroid cats. PBDEs in canned foods
ranged from 0.42-3.09 ng/g (mean: 1.79 ng/g). We estimated that total daily ingestion of PBDEs
for domestic cats ranges from 32 to 3,906 ng/kg BW (mean: 375 ng/kg BW). Backward
calculation from the feline serum body burden suggested daily exposure to 1,038
ng/kg/day (range: 66 -9,038 ng/kg/day). At the high end, forward and backward calculation
estimates equated to 6.5 and 15%, respectively, of the lowest single acutely toxic dose known to
disrupt neurodevelopment in laboratory animals. These estimates also equated to 1.3 and 3.0%,
respectively, of the lowest single acutely toxic dose known to disrupt thyroid function. Body
burdens of PBDEs in cats chronically exposed to them may reach endocrine disruptive
concentrations over time, a possible explanation as to why FHS is predominantly a
geriatric disease.

We concluded that domestic cats are highly exposed to PBDEs, largely through ingestion of
household dust during grooming. If PBDEs play a role in hyperplasia leading to thyroid
autonomy, they apparently do not do so by markedly increasing TSH. It seems more plausible
that PBDEs may act directly on thyroid nuclear receptors, at pituitary TRs, and/or at an
earlier, potentially developmental, time point.

*[This report is based on the research described in greater detail in:
Mensching D (2008). The Feline Thyroid Gland: A Model for Endocrine Disruption by
Polybrominated Diphenyl Ethers? Master’s Thesis, University of Illinois at Urbana-Champaign,
Spring 2008.]*
Introduction

Polybrominated diphenyl ethers (PBDEs) are ubiquitous flame retardants and known endocrine disruptors used in many household products since the 1970s. They are lipophilic and bioaccumulate, such that tissue residues in humans and wildlife have exponentially increased since their inception (Darnerud et al., 2001). Feline hyperthyroidism (FH) is a common disease of geriatric domestic cats first recognized only after PBDE use became widespread. FH has been associated with a variety of risk factors and is analogous morphologically and functionally to human toxic nodular goiter (TNG) (Scarlett et al., 1988; Scarlett, 1994; Martin et al., 2000; Edinboro et al., 2004; Olczak et al., 2005), but its etiology remains unknown. Thyroid hyperplasia, a prelude of TNG, was linked with experimental exposure of rats to PBDEs in 1975 (Norris et al.) and again in recent years (NTP, 2001; Stoker et al., 2004). Structurally similar to thyroxine (T4) and triiodothyronine (T3), hydroxylated metabolites of PBDEs have been shown to bind to thyroid hormone receptors (TRs) (Marsh et al., 1998) and to transthyretin (Meerts et al., 2000), the dominant high-affinity serum binding protein in cats, and also to inhibit 5’-deiodinases (Szabo et al., 2007). Experimental exposures to PBDEs have resulted in decreased serum T4 via induction of uridine diphosphoglucuronosyltransferase (UDPGT) (Zhou et al., 2001; Hallgren and Darnerud, 2002; Stoker et al., 2004). Few studies to date have investigated the association of PBDEs and the development of feline hyperthyroidism (Dye et al., 2007). The goals of this project included documenting PBDE burdens in the serum and adipose tissue of domestic cats and correlating these burdens with the presence or absence of hyperthyroidism, identifying a subclinical stage in the pathophysiology of the disease in which thyroid stimulating hormone (TSH) is elevated and correlating TSH elevation with PBDE burdens, and examining both household dust and commercial canned cat foods to determine the likely sources of exposure for domestic cats.
Research Objectives

Based on the temporal association of the arrival of PBDEs and the recognition of FH, the known epidemiologic risk factors for FH, the global and especially the North American ubiquity of PBDEs both in food and household dust, the propensity for domestic cats to groom themselves, and the known endocrine disruptive properties of PBDEs on the HPT axis, we hypothesized that PBDEs play a role in the development of FH with ingestion of canned cat food and household dust serving as sources of exposure. We further hypothesized that PBDEs competitively inhibit the action of thyroxine in cats to prevent negative feedback at the level of the pituitary, increasing TSH, and we theorized that such an increase might account for the epizootic of adenomatous hyperplasia, which ultimately results in autonomous function and thyrotoxicosis.

Specific research objectives included the following:

1) To compare PBDE burdens in serum and adipose tissue in age-matched euthyroid versus hyperthyroid cats to determine if hyperthyroidism correlates with heavier contaminant loads.

2) To standardize a TSH assay for cats to identify a subset of euthyroid cats with elevated TSH concentrations and to correlate TSH concentrations with PBDE burdens in serum.

3) To evaluate the PBDE content of commercial canned cat foods and household dust to identify predominant exposure sources for domestic cats.

The inclusion of samples from a population of feral cats in California was serendipitous and not part of the original study design. These data enabled a comparison between serum PBDE burdens of geriatric, predominantly indoor cats with cats of a presumptively non-geriatric, outdoor population.
Materials and Methods

Recruitment of participants, sample collection, and T4 determination

Predominantly indoor, client-owned cats ≥ 7 years of age were enrolled in the study at the Cat Hospital of Chicago (CHOC; Chicago, IL), Arboretum View Animal Hospital (AVAH; Downers Grove, IL), Veterinary Clinics of America (VCA; Albany, CA), and the Veterinary Teaching Hospital of the University of Illinois, College of Veterinary Medicine (VTH; Urbana, IL). VCA was recruited to the study through the Veterinary Information Network (VIN; www.vin.com). A small population of feral cats in northern California was also included in the study. Animal use was approved by the University of Illinois Institutional Animal Care and Use Committee (IACUC Protocol #05173). Serum T4 was analyzed by radio- or chemiluminescent immunoassay by commercial veterinary laboratories chosen by the veterinary practices involved. Hyperthyroid cats were actively hyperthyroid at the time of sample collection. Euthyroid cats were clinically normal and either had normal thyroxine ranges at the time of sample collection or had their status confirmed as euthyroid by nuclear scintigraphy.

TSH standardization and determination

Using recombinant feline TSH (rfTSH) generously donated to the project by Dr. Duncan Ferguson (Rayalam et al., 2006a and b), the DPC IMMULITE® chemiluminescent canine TSH assay was calibrated for feline samples (DPC®, Los Angeles, CA). Calibration standards, a control zero calibrator, and study participant samples were submitted to the Clinical Pathology Laboratory at the University of Illinois, College of Veterinary Medicine.

Sample extraction and clean-up

Serum samples were extracted by liquid-liquid extraction using methanol, hexane, and anhydrous ethyl ether. Dust, canned food, and adipose tissue samples were extracted by pressurized fluid extraction (PFE), a WMRC-modified version of EPA Method 3545 (U.S. EPA, 1998). Silica gel fractionation, modified U.S. EPA method 3630, was performed to clean up the samples. Gel permeation (size exclusion) chromatography (GPC) was performed as a clean up step to remove the majority of lipids from the samples. The method is a WMRC-modified version of EPA Method 3640.
Lipid determination

Lipid percentage was determined for all serum samples using the sulfo-phospho-vanillin reaction method of Frings et al. (1972). For adipose tissue samples, lipid percentage was determined using the Labconco® Freeze Dryer 4.5 (Kansas City, MO).

Analysis of sample extracts

A modification of U.S. EPA draft method 1614 (U.S. EPA, 2003) was used in the analytical process. Instrumentation included a Micromass Autospec NT High Resolution (>10,000) mass spectrometer (HRMS) equipped with a HP 6890 gas chromatograph (GC) outfitted with a single Stx®-500 column (15 m x 0.25 mm internal diameter x 0.15 μm df). Peak retention time and the abundance ratio of selected ion fragments were used to identify and confirm individual PBDE congeners 17, 28, 47, 49, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190, and 209.

Quality Assurance/Quality Control

Blanks, reagent blanks, spiked samples (with PBDE reference standards), duplicates, surrogates, and internal standards were employed to assure and control for the quality of the analytical results.

PBDE exposure calculations

Forward calculation of PBDE intake for domestic cats was performed using the following estimates: 1) the average cat weighs 5 kg, 2) the average volume of canned food ingested per day for cats eating exclusively canned food is 5 ounces, and 3) the average amount of dust ingested per day per cat is 200 mg, a weight-adjusted estimate derived from U.S. EPA estimates of dust ingestion by toddlers. The total amount of PBDEs ingested/day in food was determined by multiplying the measured PBDE concentrations in canned cat food by 142 grams (5 ounces). The total amount of PBDEs ingested/day in dust was determined by multiplying measured PBDE concentrations in house dust by 0.2 g (200 mg). Total PBDEs ingested/day in canned food and dust were added together and divided by the average body weight (BW) of 5 kg to determine the total ng of PBDEs ingested/kg/day.
PBDE intake for domestic cats was calculated from measured lipid-adjusted serum residues in a backward fashion by converting each 1 ng/g of PBDE body burden to an intake of 0.177 ng/kg/day from the following formula from McDonald (2005):

\[
\text{Intake (ng/kg/d)} = [(\ln 2) \times (\text{PBDE } \mu\text{g/kg}_\text{lipid measured})] \times \frac{\text{[(fraction adipose) x (1,000 ng/μg)]}}{\text{[(half life d) x (fraction absorbed)]}}
\]

where fraction adipose = proportion of body fat (0.3 used for adult women)

half life d = half life of the congener in days

fraction absorbed = bioavailability of the congener (ranged from 0.78-0.94)

**Statistical analyses**

Statistical analyses (ANOVA/t-tests, Spearman’s rank correlation, linear regression, Fisher Exact test, and Cluster analyses) were performed using Excel® (Microsoft, Seattle, WA), SYSTAT®12 software (SPPS, Chicago, IL), and SAS (SAS Institute Inc., Cary, NC). Where possible, data with non-normal distributions were log transformed to normality. Non-parametric analyses were conducted thereafter if log transformation did not achieve normality. A p value < 0.05 was chosen to detect significant differences.

**Client communication and questionnaire**

Several documents were prepared for veterinarians recruiting clients to the study. The documents included an introductory letter, a client consent form, and a questionnaire. The clients were asked to contribute a dust sample from their homes from their vacuum bag. The clients were also asked to complete a questionnaire regarding their cat’s health history, diet, and environmental factors.

**Results**

**T4 and TSH**

A total of 62 client-owned cats were included in our study, including 21 euthyroid and 41 hyperthyroid cats. Serum samples from 10 feral cats were also collected. One hyperthyroid cat, one euthyroid cat, and all feral cats were from California. All other cats were from Illinois. Thyroid status was determined for all cats as sample volumes allowed using serum total T4 and TSH. Serum T4 was determined for all client-owned cats and for 6 feral cats. Thyroid status was not determined in those feral cats (n = 4) from whom a separate aliquot of serum was not
provided (distinct from PBDE serum tube) due to separate laboratory and storage conditions required for the two analyses. Of those feral cats from which two separate samples of serum were obtained (n = 6), two cats had subnormal thyroxine values, such that they could not be considered euthyroid. Of the remaining 4 samples, 2 were pooled for PBDE analysis to meet the minimum required volume, resulting in 3 samples for which a complete dataset was obtained. TSH was determined for 18 client-owned euthyroid, 36 client-owned hyperthyroid, and 9 feral cat samples (n = 10, 2 pooled samples). In statistical analyses, values below the detection limit of 0.03 ng/ml were assigned a value of 0.015 ng/ml (midpoint between detection limit and zero). Table 1 summarizes these findings.

Table 1: Summary of serum T4 and TSH results in study groups.

<table>
<thead>
<tr>
<th>Environment and Thyroid Status</th>
<th>Mean T4 (μg/dl)</th>
<th>Range of T4 (μg/dl)</th>
<th>TSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Client-owned euthyroid cats</td>
<td>2.29 (n = 21)</td>
<td>1.5-4.8</td>
<td>Mean = 0.038 (n = 5; range 0.030-0.060) All others &lt; 0.03 (n = 13)</td>
</tr>
<tr>
<td>Client-owned hyperthyroid cats</td>
<td>7.96 (n = 41)</td>
<td>2.8-24</td>
<td>All &lt; 0.03 (n = 36)</td>
</tr>
<tr>
<td>Feral euthyroid cats</td>
<td>1.62 (n = 6)</td>
<td>1.21-2.49</td>
<td>0.11 (n = 1) All others &lt; 0.03 (n = 8)</td>
</tr>
</tbody>
</table>

Regarding TSH determination, the rFTSH standards showed good correlation ($r^2 = 0.992$, p < 0.001) with the results from the DPC IMMULITE® canine TSH assay, correcting for the 35.7% cross-reactivity of the assay for feline TSH (Rayalam et al., 2006a, b). All of the hyperthyroid cats for which serum was available for TSH testing (n = 36) had undetectable TSH values, noted as <0.03 ng/ml, the limit of detection for the assay. Of 24 euthyroid samples tested, 79% were undetectable. The difference between TSH concentrations of euthyroid versus hyperthyroid cats was statistically significant (Fisher Exact test, two-tailed, p < 0.01). Of the five (5) euthyroid samples at or above the detection limit, the highest measured TSH was 0.06 ng/ml (corrected:
0.17 ng/ml). The mean of these results was 0.038 ng/ml (corrected: 0.11 ng/ml). Incidentally, the highest TSH concentration measured, 0.11 ng/ml (corrected: 0.31 ng/ml), came from a feral cat sample for which insufficient serum was available for T4 analysis but which had the highest PBDE burden of the feral cats (3,712 ng/g lipid).

**PBDEs in serum and adipose tissue**

PBDEs were found in every sample tested. Results for the range of total lipid-adjusted PBDEs in serum with the mean and median for each group are listed in Table 2. One of the euthyroid serum samples was lost during sample preparation. Results from feral cat sample #5 were unreliable based on surrogate recovery. The serum sample for one hyperthyroid cat appeared to have been contaminated with ~0.62 ng/g of each congener (presumably inadvertently spiked). This value was subtracted from the raw data. Due to small sample volumes and a similar estimated age class, serum samples from feral cats 1 and 4 were pooled for analysis. Lipid percentages in serum ranged from 0.025 to 0.74 with a mean of 0.25% and were not significantly different between euthyroid and hyperthyroid cats. Spike and surrogate recoveries from adipose tissue were extremely variable with relative percent differences (RPDs) of 59 and 58%, respectively. This was attributed to sample heterogeneity.

Table 2: Range of total serum PBDEs in ng/g lipid with mean and median for total client-owned cats, client-owned euthyroid, client-owned hyperthyroid, and feral cats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Range of total serum PBDEs</th>
<th>Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total client-owned (n = 62)</td>
<td>373-51,063</td>
<td>5,865</td>
<td>2,615</td>
</tr>
<tr>
<td>Client-owned euthyroid (n = 21)</td>
<td>467-15,949</td>
<td>5,263</td>
<td>2,851</td>
</tr>
<tr>
<td>Client-owned hyperthyroid (n = 41)</td>
<td>373-51,063</td>
<td>6,173</td>
<td>2,517</td>
</tr>
<tr>
<td>Feral cats (n = 8)</td>
<td>457-3,712</td>
<td>1,203</td>
<td>759</td>
</tr>
</tbody>
</table>
Total lipid-adjusted serum PBDE concentrations did not differ significantly between euthyroid and hyperthyroid groups. Those of feral cats, however, were significantly lower than the client-owned cats (p < 0.01). When the lipid-adjusted serum PBDE concentrations were normalized with a log transformation, there was no correlation between the log of the total PBDE concentrations in the serum with the age of client-owned cats. Figure 1 plots these values, distinguishing data points from euthyroid cats as “a” and those from hyperthyroid cats as “b.” The inner ellipse encompasses the central 50% of the data. All data points considered in the statistical comparison between euthyroid and hyperthyroid cats are within or on the outer ellipse. There are no outliers in this analysis, as designated by the lack of data points outside the outer ellipse.

**Figure 1:** Log ΣPBDEs (ng/g lipid) versus age in euthyroid (a) and hyperthyroid (b) cats.

The mean serum total PBDE concentration in cats with quantifiable TSH was 5,477 ng/g lipid (range 800-15,949; median 2,851 ng/g lipid). The mean serum total PBDE concentration in
euthyroid cats with TSH concentrations below the detection limit was 5100 ng/g lipid (range 1,316-15,026 ng/g lipid; median 2,680 ng/g lipid). There was no significant difference between the two groups.

Client-owned cats ranged from 7.7 to 17 years of age. Euthyroid cats had an average age of 13.0 years, and hyperthyroid cats averaged 12.8 years. No significant correlation was found between serum total PBDE concentrations and serum T4. Among the serum samples, congeners 47, 99, 153, 154, 183, and 209 predominated (Figure 2). Two cats, both hyperthyroid, had extremely high serum BDE-209 concentrations which measured 14,837 and 48,783 ng/g lipid, equating to 41.1 and 95.5%, respectively, of the total serum PBDEs. Of all the congeners assayed, BDE-190 was detected least often, with small amounts measured in just two cats.

**Figure 2:** Percent composition of prominent P BDE congeners in serum of client-owned euthyroid, client-owned hyperthyroid, and feral cats. “Other” represents the sum of the concentrations of minor PBDEs also identified in serum.
Table 3: Statistically significant results obtained from PBDE percent composition profiles of feral and client-owned euthyroid and hyperthyroid cats (Figure 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study Group</th>
<th>Statistical Relationship</th>
<th>Study Group</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Profile</td>
<td>Euthyroid Cats</td>
<td>≠</td>
<td>Feral Cats</td>
<td>0.03</td>
</tr>
<tr>
<td>Overall Profile</td>
<td>Hyperthyroid Cats</td>
<td>≠</td>
<td>Feral Cats</td>
<td>0.03</td>
</tr>
<tr>
<td>BDE-153</td>
<td>Euthyroid Cats</td>
<td>&gt;</td>
<td>Hyperthyroid Cats</td>
<td>0.049</td>
</tr>
<tr>
<td>BDE-153</td>
<td>Feral Cats</td>
<td>&gt;</td>
<td>Hyperthyroid Cats</td>
<td>0.04</td>
</tr>
<tr>
<td>BDE-183</td>
<td>Feral Cats</td>
<td>&gt;</td>
<td>Euthyroid Cats</td>
<td>0.0007</td>
</tr>
<tr>
<td>BDE-183</td>
<td>Feral Cats</td>
<td>&gt;</td>
<td>Hyperthyroid Cats</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

The overall congener profiles (i.e. relative percentages among PBDEs detected in serum) were not significantly different between the client-owned hyperthyroid and euthyroid cats. However, BDE-153 showed a marginal but significant difference between these groups (p = 0.049). The euthyroid cats had 4.9% BDE-153 versus 2.6% in the hyperthyroid group. The overall congener profile for the feral cats differed significantly between the euthyroid (p = 0.03) and hyperthyroid groups (p = 0.03). Feral cats had a significantly higher (p = 0.0007) percent composition of BDE-183 than euthyroid cats. The percent compositions of both BDE-153 (p = 0.04) and BDE-183 (p = 0.0006) were significantly higher in the feral group as compared with the hyperthyroid cats.

The average ratio of BDE-47 to 99 in client-owned cats was 0.74:1. Euthyroid cats had an average ratio of 0.80:1, and hyperthyroid cats had an average ratio of 0.71:1. Feral cats had an average BDE-47 to 99 ratio of 0.70:1. The ratio of BDE-209 to 183, the next higher brominated congener, was calculable for just 5 samples because of lack of detection in one or both parameters in most study participants. The mean ratio of BDE-209 to 183 was 67.6:1 with a wide range of 10.7:1 to 244:1.
Adipose tissue was collected from just five (5) cats, only one of which was hyperthyroid (indicated by *). Total adipose tissue PBDEs measured 79, 295, 417, 860*, and 4753 ng/g lipid with a mean of 1,281 and a median of 417 ng/g lipid. Lipid percentage ranged from 90 to 96% and was 93% for the sample from the hyperthyroid cat.

**PBDEs in canned food**

PBDEs were detected in all 10 samples of commercial canned cat food analyzed, and concentrations ranged from 0.42 to 3.09 ng/g, uncorrected for lipid, with a mean and median equal to 1.79 ng/g (Table 4). Figure 3 shows a cluster analysis of the PBDEs within the tested samples. A lesser distance between foods in the analysis indicates a more similar PBDE congener profile. This represented just a small number of commercial foods and only fish-based foods. However, it is interesting to note the relative clustering according to labeled content of fish, indicating that fish within a species are similarly burdened by specific congener profiles.

**Table 4:** Total PBDEs of 10 commercial canned cat foods.

<table>
<thead>
<tr>
<th>Brand of Commercial Canned Cat Food</th>
<th>Total PBDEs (ng/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fancy Feast Cod, Sole, &amp; Shrimp Feast</td>
<td>3.09</td>
</tr>
<tr>
<td>Meijer Main Choice Salmon Dinner</td>
<td>3.01</td>
</tr>
<tr>
<td>Purina Friskies Pacific Salmon Dinner in Sauce</td>
<td>1.89</td>
</tr>
<tr>
<td>9 Lives Ocean Whitefish Dinner #1</td>
<td>2.01</td>
</tr>
<tr>
<td>9 Lives Ocean Whitefish Dinner #2</td>
<td>1.50</td>
</tr>
<tr>
<td>Fancy Feast Ocean Whitefish &amp; Tuna Feast</td>
<td>1.65</td>
</tr>
<tr>
<td>Fancy Feast Trout Feast</td>
<td>1.90</td>
</tr>
<tr>
<td>Purina Friskies Ocean Whitefish &amp; Tuna Dinner</td>
<td>0.42</td>
</tr>
<tr>
<td>Meijer Main Choice Ocean Whitefish &amp; Tuna Dinner</td>
<td>0.75</td>
</tr>
<tr>
<td>Fancy Feast Tuna &amp; Gravy</td>
<td>1.69</td>
</tr>
</tbody>
</table>
Only one cat in the study ate dry food exclusively, and only one cat in the study ate canned food exclusively. The remainder ate a combination of the two. Attempting to estimate real-world exposure of the study cats to PBDEs through client recollection of specific foods fed over the lifetime of a cat and subsequent analysis of said foods would have been fraught with assumptions and prohibitively expensive, respectively. Since the PBDE levels in canned foods were comparatively low, since the diets are not necessarily made with ingredients from single locations, and because we had a limited budget, further definition of which fish flavors revealed which levels was not a top priority. However, the contribution of dietary PBDEs to the total body burdens in domestic cats was estimated using two methods of calculation (see PBDE exposure calculations below).

**PBDEs in dust**

A total of 19 samples of dust were received, including two from one household submitted independently for each of two cats included in the study, and one that represented the sole sample for three cats sharing the same residence. In calculations of mean and median, the analytical results for the latter cats were triplicated to reflect this representation. The range of

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**Figure 3:** Cluster analysis of PBDE content of 10 commercial canned cat foods.
total PBDEs measured in dust was 510-95,448 ng/g with a mean of 8,098 ng/g and a median of 1,959 ng/g. The range for total PBDE concentrations in dust for euthyroid cats (n=14) was 510-4,911 ng/g with a mean of 1,698 ng/g and a median of 1,489 ng/g. In the dust from homes of hyperthyroid cats (n=7), total PBDE concentrations ranged from 1,060 to 95,448 ng/g, with a mean of 19,372 ng/g and a median of 3,137 ng/g. A log (log) transformation of the dust data revealed a significant elevation (p = 0.01) in the total PBDE concentration of dust from ‘hyperthyroid’ versus ‘euthyroid’ homes. Figure 4 shows theoretical distributions of these differences calculated from these data. The significant elevation of total PBDEs in dust from hyperthyroid homes was retained after removal of the highest value (95,448 ppb) from the data set of the dust from hyperthyroid homes (p = 0.04). Figure 5 provides a further graphic depiction of the significant difference between PBDEs in dust from homes of cats that were hyperthyroid versus euthyroid.

**Figure 4:** Theoretical distributions of dust concentrations in homes of euthyroid versus hyperthyroid cats. Count (y-axis) represents numbers of cats, and x-axis is the log (log) of dust total PBDE concentration.
Dust samples for three cats, all hyperthyroid, had surrogate BDE-209R recoveries well above 100%. These same samples all had extreme levels of measured BDE-209 measuring 14,000, 9,000, and 36,000 ng/g. Respectively, surrogate BDE-209R recoveries were 334, 320, and 150%. The BDE-209 values were corrected downward by the percent recoveries of the surrogates but, as such, are suspected to be an underrepresentation of the actual BDE-209 content of the dust. Since the BDE-209R internal standard was added pre-extraction, additional samples were not available for re-examination. An explanation proposed by the project chemist for the high surrogate recoveries from these samples is that the extreme BDE-209 and “spillover” to the later retention time of BDE-209 overwhelmed the detector (John Scott, W MRC, personal communication). Furthermore, this “spillover” of BDE-209 was detected as BDE-209R, the percent recovery of which became falsely elevated. When correcting BDE-209 results for the falsely elevated recovery of BDE-209R, the BDE-209 results were decreased erroneously. If this explanation is accurate, the measured BDE-209 and after correction for surrogate recovery is an underestimate of the true amount in the sample. Thus, the difference between dust from euthyroid and hyperthyroid cats could be even greater if the BDE-209 could be more accurately assessed.

**PBDEs in serum versus dust**

The sum of PBDEs in serum was not significantly correlated with the sum of PBDEs in dust samples. Comparisons of PBDEs in serum and dust by congener were limited by the small number of cats for which each sample was collected and further compromised by inability to normalize the data due to undetected congeners. Figure 5 depicts the sum of lipid-adjusted PBDEs in serum, sum of PBDEs in dust, and the T4 for each cat with all data points. Each variable was standardized to its own mean and plotted according to its variance from that mean (vertical dotted line, as viewed) so that the three variables could be graphically compared to each other. Euthyroid and hyperthyroid cats are designated and separated graphically by the horizontal dotted line.
Figure 5: Comparison of ΣPBDEs lipid in serum, ΣPBDEs in dust, and T4 standardized to the mean (0) of each variable in hyperthyroid versus euthyroid client-owned cats.
**PBDE exposure calculations**

Forward calculation of PBDE intake by domestic cats equated to 375 ng PBDEs/kg/day (range of 32-3906 ng/kg/day). Backward calculation of PBDE intake by domestic cats equated to 1,038 ng/kg/day (range of 66-9,038 ng/kg/day).

**Client questionnaires**

A total of 19 client questionnaires were completed (n = 9, hyperthyroid; n = 10, euthyroid). Associations investigated included percentage of time indoors, number of cats in the home, number of litter boxes, percentage of canned food in diet, number of televisions in the home, number of computers in the home, and frequency of vacuuming. None of the factors had significantly different distributions between the euthyroid and hyperthyroid groups.

**Conclusions**

This study served as a pilot investigation into the role of PBDEs in the development of feline thyroid adenomas and associated hyperthyroidism. We have documented that domestic cats are highly-exposed to PBDEs, and our data strongly suggest that the main source is ingestion of household dust. Given that our study sampled a small number of canned cat foods, however, we recommend an exhaustive analysis of the wide array of both canned and dry cat foods on the market to further define the contribution of dietary PBDEs to total body burdens in domestic cats. Such a study will be expensive and will need to be repeated over time, as sources of food ingredients and concentrations of contaminants that reach those foodstuffs are in flux.

Our study did not support a difference in contaminant load between client-owned domestic cats that were euthyroid and hyperthyroid or a correlation between T4 and PBDEs in serum. However, our findings do not rule out PBDEs as an etiologic factor for FH. The finding of significantly increased total PBDEs in dust from homes with hyperthyroid cats compels further study. We also add to the literature the fact that elevated TSH was not detected as hypothesized, but instead TSH was potentially decreased in response to PBDEs, although the insensitivity of the assay below the current detection limit precludes definitive conclusions. Among client-owned cats in this study (≥ 7 years), age was not correlated with serum PBDE burdens. With the exception of BDE-153, congener profiles of euthyroid and hyperthyroid cats were comparable.
Feral cats were burdened with significantly lower PBDE concentrations in serum than client-owned cats, a difference likely to be attributable to age, diet, and environment. The overall congener profile of feral cats differed significantly from client-owned cats, suggesting differences in exposure.

Domestic cats may very well be the proverbial ‘canary in the coal mine’ for PBDE-induced disease. Species differences notwithstanding, with an average lifespan of 15 years and an existence even more restricted to the home environment than most cat owners, domestic cats may serve as sentinels for PBDE-induced geriatric disease in humans who have only been exposed to PBDEs for half of a lifetime to date. Further studies are necessary to define the role of PBDEs in the development of FH. These research data may arm us against an unnecessarily prolonged legacy of a PBDE-contaminated world.


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