Environmental Research 151 (2016) 145–153

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

U.S. domestic cats as sentinels for perfluoroalkyl substances: Possible linkages with housing, obesity, and disease

Phillip C. Bost [a], Mark J. Strynar [b,1], Jessica L. Reiner [c], Jerry A. Zweigenbaum [d], Patricia L. Secoura [e], Andrew B. Lindstrom [b,1], Janice A. Dye [f,*,1]

[a] Student Contractor to the U.S. Environmental Protection Agency, United States
[b] U.S. Environmental Protection Agency, National Exposure Research Laboratory, Exposure Methods and Measurement Division, Research Triangle Park, NC 27711, United States
[c] National Institute of Standards and Technology, Hollings Marine Laboratory, Charleston, SC 29412, United States
[d] Agilent Technologies, Wilmington, DE 19808, United States
[e] North Carolina State University, Veterinary Teaching Hospital, Raleigh, NC 27606, United States
[f] U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Environmental Public Health Division, Research Triangle Park, NC 27711, United States

ARTICLE INFO

Article history:
Received 17 June 2016
Received in revised form
14 July 2016
Accepted 18 July 2016

Keywords:
Cats
Obesity
Perfluoroalkyl substances (PFAS)
Sentinels

ABSTRACT

Perfluoroalkyl substances (PFAS), such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are persistent, globally distributed, anthropogenic compounds. The primary source(s) for human exposure are not well understood although within home exposure is likely important since many consumer products have been treated with different PFAS, and people spend much of their lives indoors. Herein, domestic cats were used as sentinels to investigate potential exposure and health linkages. PFAS in serum samples of 72 pet and feral cats, including 11 healthy and 61 with one or more primary disease diagnoses, were quantitated using high-resolution time-of-flight mass spectroscopy. All but one sample had detectable PFAS, with PFOS and perfluorohexane sulfonate (PFHxS) ranging from $<$LOQ to 121 and $<$LOQ to 235 ng/mL, respectively. PFAS prevalence and geometric means in cats were very similar to contemporary NHANES reports of human sera in the U. S. population. The highest PFAS serum concentrations detected were in indoor cats due to disproportionately elevated PFHxS levels. Ranked by quartile, contingency testing indicated that total PFAS levels were positively associated with living indoors and with higher body weight and body condition scores. Individual PFAS quartile rankings suggested positive associations with respiratory effusion, thyroid, liver, and possibly chronic kidney disease. Domestic cats appear to be useful sentinels for assessing primary PFAS exposure routes, especially indoor sources of relevance to children. Additional case-control studies in pet cats are warranted to better define the potential health associations observed herein. A "One Health" approach assessing humans, pets, and their common environment may improve our understanding of chronic low-level, largely indoor, PFAS exposure and effects in humans and animals alike.

Published by Elsevier Inc.

1. Introduction

Perfluoroalkyl substances (PFAS) are ubiquitous chemicals of major concern due to their global distribution, association with a range of adverse health outcomes in human epidemiologic studies (Melzer et al., 2010; Lopez-Espinosa et al., 2012; Barry et al., 2013; Timmermann et al., 2014; Gleason et al., 2015) and evidence of toxicity in laboratory animals (Lau et al., 2007). Owing to their high-energy carbon-fluorine bonds, many PFAS are thermally stable, resistant to biodegradation processes, and uniquely able to repel both water and oils (Lehmler et al., 2005). Manufactured since the late 1940 s, production sharply increased in the 1980 s as their utility for industrial and commercial applications expanded (Prevedouros et al., 2006). Personal and household product applications have included nonstick cookware, food contact surface coatings (Begley et al., 2008), and anti-stain products for textiles, furniture and carpeting (Lewandowski et al., 2006).

Subsequent accumulation of PFAS in aquatic environments, biota, wildlife, and humans led to the addition of perfluorooctane sulfonate (PFOS) to the list of restricted use compounds in Annex B of the Stockholm Convention on Persistent Organic Pollutants

* Correspondence to: U.S. Environmental Protection Agency, ORD/NHEERL/EPHD, Mail Drop: B-105–02, Research Triangle Park, NC, 27711, United States.
E-mail address: dye.janice@epa.gov (J.A. Dye).
[1] Indicates joint senior authorship.

http://dx.doi.org/10.1016/j.envres.2016.07.027
0013-9351/Published by Elsevier Inc.

(POPs) (Stockholm 2009). Additionally, the Office of Water of the United States Environmental Protection Agency (USEPA) has issued health advisories for PFOS (70 ng/L) and perfluorooctanoic acid (PFOA, 70 ng/L) in drinking water, respectively in 2016 (USEPA, 2016). Although some manufacturers voluntarily phased-out specific long-chain PFAS (USEPA, 2010), in other parts of the world their importation and manufacturing continues to increase and remains largely unregulated.

Despite routine detection of PFAS in human serum in the ng/mL range (Calafat et al., 2007; Kato et al., 2011), routes of human exposure are not well understood and corresponding health risks remain unclear. Oral ingestion is likely important as many PFAS are frequently detected in food (Tittlemier et al., 2007), food packaging (Begley et al., 2008), surface water (Saito et al., 2004), and contaminated well (Lindstrom et al., 2011) or municipal drinking water (Gyllenhammar et al., 2015). PFAS can also slough or volatilize from household products, thus accumulating within house dust. We (Strynar et al., 2008) and others (Björklund et al., 2009; Beesoon et al., 2012) have shown house dust to contain PFAS in the ng/g range. In the U.S., for example, total PFAS median (917 ng/g) and maximum (52,900 ng/g) levels were measured in house dust from Ohio and North Carolina (Strynar et al., 2008). It is estimated that for two-year-olds, 36% of PFOS exposure is attributed to house dust ingestion, where as in adults, only 6% is (Egeghy et al., 2011).

Sentinel species can be defined as animals who share a common environment with humans and can be used to measure the extent of exposure when human measurement is impractical or unethical. The canary in the mineshaft is a classic and probably the best known example of a sentinel species whose extraordinary sensitivity to toxic gases alerted miners to dangerous occupational conditions. An emerging variation on the theme of animal sentinels relates to an increasing appreciation that animals and humans often share risk to health from exposure to environmental agents. As proposed by the interdisciplinary “One Health” concept, animals may provide more direct information on environmental stressors, food safety, and thus potential risks for human health (Frazzoli et al., 2015). Accordingly, a “One Health” approach to assessing humans, animals, and ecosystems may provide improved understanding for appropriate regulatory and public health response (Zinsstag et al., 2011). In this context, domestic cats may be acutely or chronically exposed to many of the same agents as their human companions — sharing the same tap water, commercial food (protein) sources, and, depending on their housing status — comparable indoor or outdoor spaces. Consequently, cats may serve as “*situational*” sentinels. As an example, recognition of neurobehavioral changes in so-called “dancing cats” of Minamata Bay, Japan in the 1950 s preceded the episode of severe neurologic disease among local residents. Maladies in both species were due to consumption of methylmercury-contaminated seafood (Tsuchiya et al., 1992). Likewise, humans and cats can be similarly exposed to and affected by lead ingestion (Dowsett et al., 1994).

Cats may also serve as “*health*” sentinels because they develop a number of chronic conditions that are closely analogous to that of humans (e.g., asthma (Dye et al., 1996), pulmonary fibrosis (Cohn et al., 2004), hyperthyroidism (Dye et al., 2007), urolithiasis (Robinson et al., 2008), chronic kidney disease (Callaway, 2015; Brown et al., 2016), obesity (Van de Velde et al., 2013), type 2 diabetes mellitus (Nelson et al., 2014), and cardiomyopathies (Fox et al., 2014). Recent advances into sequencing the feline genome are anticipated to benefit both humans and cats by mapping the mutations underlying conditions that afflict both species (Callaway, 2015). Their lifespan, while longer than laboratory rodents, is considerably shorter than that of people, providing a distinct advantage where prolonged latency periods associated with the human life span precludes early detection of, or linkage to, specific environmental agents. In case control studies of pet cats, tobacco smoke exposure is associated with increased risk of lymphoma (Bertone et al., 2002) and oral squamous cell carcinoma (Snyder et al., 2004). Risk appears to reflect inhalation and oral (via grooming) routes of exposure. Thus, cats are a naturally-occurring animal model for head and neck neoplasia in humans (Wypij et al., 2013).

For these reasons, we had previously hypothesized that pet cats may be useful sentinels for humans to assess potential relationships between chronic exposure to persistent organic pollutants (POPs) via indoor environments and adverse health outcomes (Dye et al., 2007). We investigated serum polybrominated diphenyl ethers (PBDEs) levels in cats and showed: (1) many cats had BDE-47/BDE-99 congener ratios similar to house dust samples, and (2) estimated dust ingestion in cats was comparable to that of toddlers — because indoor cats likewise have increased contact with PBDE-containing upholstery and carpeting and engage in dust ingestion via frequent grooming (Dye et al., 2007).

In the present investigation, we used a *one health*, multidisciplinary approach – with collaboration across environmental analytical, exposure, and health scientists – to investigate serum levels of PFAS across a cross-section of domestic cats. In so doing we used domestic cats as sentinels to (1) better assess primary exposure routes of PFAS and to (2) evaluate potential associations with specific conditions or disease in cats, and by inference - analogous conditions in people. As a cross-sectional study, this was intended to be a hypothesis-generating effort by which to guide future, more focused, research. By design, serum from cats presenting to local clinics, shelters, and a veterinary teaching hospital were included to allow comparisons of outdoor and indoor housing status and across a broad spectrum of disease conditions. We hypothesized that: (1) the majority of cats would show evidence of PFAS exposure; (2) PFAS serum levels would increase with increasing time spent indoors; (3) that levels in primarily outdoor (including feral) cats would be lower and profiles would mirror that of water or food (i.e., with PFOS more prevalent and perfluorohexane sulfonate (PFHxS) lower or absent) (Egeghy et al., 2011; Begley et al., 2008); and conversely (4) levels in cats housed mainly indoors would be higher and profiles would reflect that of house dust (i.e., dominated by PFOS + PFOA + PFHxS) (Strynar et al., 2008; Björklund et al., 2009).

2. Materials and methods

2.1. Cats and serum

Over a 4 month period in 2008, serum was obtained from 72 cats presenting to clinics and shelters in the Raleigh, NC area, including the Veterinary Teaching Hospital at North Carolina State University. In all cases, serum was obtained for clinical purposes (e.g., serology, geriatric screening, or case diagnostics). With permission, once all clinical testing was complete, remaining serum was stored at −80 °C until analysis. Samples were assigned unique numbers to which subject and case information was linked via a unique code for each cat (i.e., age, breed, sex, neuter status, weight, housing, primary complaint(s) and current clinical diagnoses). Seven cats were re-evaluated during this period, allowing assessment of replicate samples 2–30 days later. While collating case summaries, the coauthors were blinded to the results of PFAS analysis, and likewise, while performing PFAS analysis, the analysts were blinded to clinical case details.

2.2. Sample analysis and instrumentation

More detailed information on reagents, instrument settings (Table S1), procedures, and quality control (QC) steps are provided

in the supplemental section. In brief, 50 µL subsamples were prepared using a modified method for rodents (Reiner et al., 2009) and analyzed using an Agilent 1100 series HPLC interfaced with a 6200 series Accurate-Mass LC-TOF system (Agilent Technologies, Palo Alto, CA). The following PFAS were included in the analysis: perfluorobutane sulfonate (PFBS), PFHxS, PFOS, perfluorohexanoic acid (C6), perfluoroheptanoic acid (C7), PFOA, perfluorononanoic acid (C9), perfluorodecanoic acid (C10), perfluoroundecanoic acid (C11), and perfluorododecanoic acid (C12). In total, 72 case (serum) samples, one serum collection tube blank, seven analyst-blinded replicates, nine batch duplicates, and six analyst-blinded duplicates were performed across three batches. Duplicates were defined as split aliquots of the same cat's sample obtained on the same day. Each analytical batch included method and matrix blanks, 11 calibration curve standards, duplicates, and QC samples. In each batch, the limit of quantitation (LOQ) for each analyte was defined as the lowest standard curve point within $\pm 30\%$ of its theoretical value.

2.3. Quality control samples

Matrix and collection tube blanks were below each assay's LOD for all target analytes. The mean recovery of all QC analytes in the 25–100 ng/mL range was within $\pm 30\%$ of the theoretical spiked concentration (Table S2). QC samples ≤ 10 ng/mL were also generally good except for C7 which was outside of this range at 10 ng/mL (187%) and 2 ng/mL (211%), along with C6 (160%) and PFHxS (137%) also at the 2 ng/mL level. Overall performance of blanks, QC and duplicate samples indicated acceptable data quality. Table S3 shows LOQs for each analyte across analytical batches. Within each batch, when acceptable performance could not be verified at the lowest levels, LOQs shifted up slightly. Replicate samples and analyst-blinded duplicates had mixtures of analytes at variable concentrations. Most duplicates in the middle range of the calibration curves had relative percent differences of $<20\%$, with performance declining only at the very low portion of the calibration curve (Table S4).

2.4. Statistical analysis

Summary statistics were calculated using Microsoft Office Excel (version 2007, Microsoft Corporation, Redmond, WA). For duplicate samples, the average was used. Pearson correlations, linear regressions, *t*-tests, and one-way analyses of variance (ANOVA with Bonferroni post hoc correction) were performed on natural log-transformed sample measurements. Pearson correlations were run to elucidate associations between individual PFAS concentrations. Where necessary, compound concentrations less than the LOQ were assigned a value of LOQ/$\sqrt{2}$ prior to analyses. Linear regression was used to examine potential relationships between PFAS concentration and age and body weight. Fisher's exact testing (one-sided) was used to assess 2×2 contingencies, while the Chi Square Test for Trend was used to assess 2×4 assignments. All group comparisons were performed using GraphPad Prism 4.03 (GraphPad Software, La Jolla, CA). Results were considered significant when $p \leq 0.05$.

3. Results

3.1. Prevalence of PFAS in cat serum

All but one serum sample contained at least one quantifiable target analyte, with PFOS and PFHxS being the most abundant compounds present, together representing $>75\%$ of the total mass ($\sum$PFAS) measured in the cats (Fig. 1). PFOS, the most

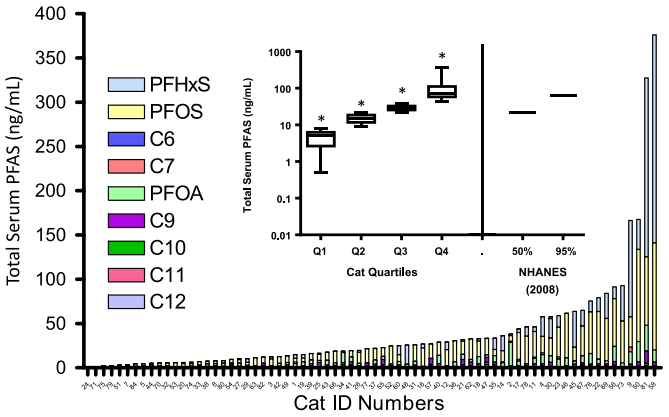

Fig. 1. Cat serum values arranged by increasing concentration of total PFAS. Despite wide variability in total PFAS concentrations in cat serum, PFOS [(white) (yellow on-line version) and PFHxS [(medium gray) (light blue on-line version) were the predominant constituents. The insert shows a box whiskers plot distribution of cats by quartile based on serum total PFAS levels. *indicates significant difference from other quartiles. For comparison, ΣPFAS levels reported in NHANES for human serum from 2008 are depicted based on the GM of the 4 dominant analytes (GM Σ [PFOS + PFOA + C9 + PFHxS]). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

prevalent compound, was detected in 97% of the cats (70/72). Other prevalent analytes included, in decreasing order, perfluorononanoic acid (PFNA or C9, 93%), PFOA (or C8, 82%), perfluorodecanoic acid (PFDA or C10, 65%), and PFHxS (47%) (Table 1). Across all cats, the concentrations of PFOA, PFOS, and PFHxS ranged from < LOQ to 26.4, 121, and 235 ng/mL, respectively (Table 1). It is interesting and potentially informative to note that geometric means (GM) of PFOS, PFOA, C9, C10, and perfluoroundecanoic acid (C11) in cats from this study were very similar to GM reported in humans in the U.S., aged 12 and older, as part of the 2007–08 National Health and Nutrition Examination Survey (NHANES) (Kato et al., 2011) (Table 1). As in humans, the short-chain perfluorinated carboxylic acids, C6 and C7, and PFBS were infrequently detected ($<12\%$). Consistent with these findings, the shorter-chain compounds (C6, C7, PFBS) are relatively more water soluble and thus somewhat less likely to bind plasma proteins. They are therefore eliminated more readily than the longer chain analytes (e.g., C8 and above). We also noted that the presence of PFOS was correlated with PFOA, C9, and C10 and C11; while PFHxS was only correlated with PFOS and PFOA. PFOA was correlated with every compound except C7, C11 and C12 (Table S5). These associations may be an indication of similar sources and/or that these analytes were eliminated in a similar manner.

3.2. PFAS quartiles in cats

Based on the sum of all analytes (ΣPFAS) measured in each subject, cats were grouped into quartiles as Q_1, Q_2, Q_3, and Q_4 ($n = 18$/quartile). Serum ΣPFAS concentrations for each quartile were significantly different than the other quartiles (Fig. 1 insert), consistent with differential exposure (i.e., different sources and intensities within the cat's living conditions) across the four groups. Approximately half of Q_4 cats had ΣPFAS concentrations $\geq 95\%$ of the U.S. population (Kato et al., 2011). In the sections that follow, we examined factors that may have contributed to elevated ΣPFAS levels observed.

3.3. Subject characteristics

In this cross-sectional study, subjects included 66 client-owned pets, both indoor and outdoor cats, 5 feral cats, and one resident shelter cat. Ages ranged from 0.5 to 19 years. By sex, 40% were

Table 1
Analyte summary statistics in cats.

	% of Total PFAS	% > LOQ	Median (ng/mL)	Range (ng/mL)	Geometric Mean (ng/mL)[a]	NHANES Geometric Mean (ng/mL)[b]
PFBS	0.08	1.39	< LOQ	< LOQ-2.35	–	< LOQ
PFHxS	32.6	47.2	< LOQ	< LOQ-235	6.91	1.96
PFOS	45.3	97.2	9.05	< LOQ-121	8.89	13.2
C6	0.00	0.00	< LOQ	–	–	–
C7	0.62	11.1	< LOQ	< LOQ-5.31	–	< LOQ
PFOA	12.5	81.9	3.28	< LOQ-29.2	3.28	4.13
C9	5.91	93.1	1.77	< LOQ-13.8	1.72	1.49
C10	1.59	65.3	0.39	< LOQ-2.76	0.67	0.29
C11	1.21	59.7	0.21	< LOQ-2.88	0.5	0.20
C12	0.21	34.7	< LOQ	< LOQ-0.69	–	–

[a] Calculation based on values below the LOQ being assigned the value of LOQ/$\sqrt{2}$.
[b] 2008 NHANES data not available for either C6 or C12.

female, with 26 (of 28) neutered; and 60% were males, with 42 (of 44) neutered. Breeds included domestic short-haired cats ($n=48$), domestic long-haired cats ($n=8$), Siamese ($n=5$), Himalayans ($n=2$), one each representing eight other pure breeds, and one unknown (Table S6).

3.4. PFAS concentrations and profiles compared to housing status

In Fig. 2, we depict ΣPFAS serum concentrations in cats with known housing status ($n=50$). As hypothesized, levels in feral cats ($n=5$) were relatively low (≤ 25 ng/mL). Likewise, ΣPFAS levels of the largely outdoor pet cats ($n=8$) were <50 ng/mL, as were 26 (of 36; 72%) of cats housed mainly indoors. Moreover, 8 (of 36; 22%) mainly indoor cats had ΣPFAS levels between 50–100 ng/mL, while two (6%), both exclusively indoor cats, had levels >300 ng/mL.

We further evaluated composite PFAS analyte profiles based on housing status (Fig. S1). As hypothesized, profiles of largely outdoor cats revealed that PFOS was prevalent while PFHxS was low or absent. Analyte profiles for indoor cats with higher (50–100 ng/mL) levels ($n=8$) had prevalent PFHxS and PFOS, closely resembling that of the 95th percentile ΣGM (Σ[PFOS + PFOA + C9+ PFHxS]) in the NHANES report (Kato et al., 2011). The two

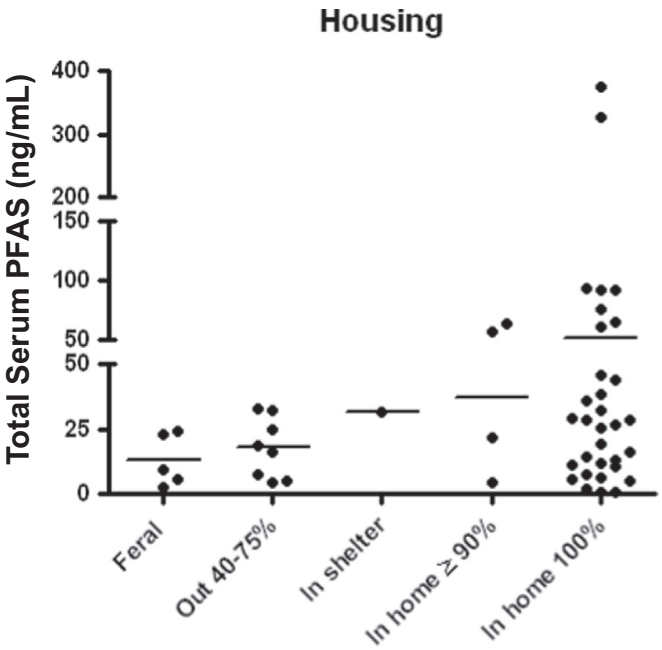

Fig. 2. Total serum PFAS levels in cats are depicted based on housing status. Line indicates the mean concentration per group.

cats with levels >300 ng/mL (and PFHxS ≫ PFOS > PFOA) were remarkably similar to serum profiles of the three youngest teenagers evaluated in a Canadian home in 2008 (Fig. S1) (Beesoon et al., 2012). Dust and carpeting samples from the Canadian home's family room likewise showed exceptionally high PFHxS and moderate PFOS levels. This analyte profile was consistent with the known content of a carpet anti-stain product that had been applied repeatedly in their household over a 15-year-period (Beesoon et al., 2012).

3.5. Associations of cat characteristics with PFAS quartiles

Excluding the resident shelter cat, contingency testing was used to evaluate whether indoor cats were disproportionately in Q_4 compared to Q_{1-3}. Based on ΣPFAS quartiles, cats were further subdivided into a predominately indoor group (living 90% or exclusively indoors; $n=36$) and an outdoor group (pet cats living 40–75% outdoors and feral cats; $n=13$). Fisher's Testing revealed that Q_4 cats were significantly more likely to be indoor cats ($p=0.014$). In fact, based on ΣPFAS, no outdoor cats were present in Q_4 (Fig. S2). A Test for Trend examined whether the proportion of indoor cats increased across the Q_1 through Q_4 rankings and showed a suggestive association ($p=0.06$) (Table S6).

We also used contingency testing to assess associations between ΣPFAS or individual analyte quartiles with cat characteristics such as age, sex, or hair coat length. Results did not indicate that male, long-haired, or older cats were more likely to be in the highest quartile (Fig. S2; Table S6); although 8 (of 9) cats with the highest PFOA and PFHxS levels measured were in cats ≥ 10 years-of-age (the median for this group).

A marginally significant relationship between ΣPFAS concentration and body weight ($r^2=0.054$, $p=0.052$) was determined (Fig. S3). Accordingly, when cats were subdivided by body weight, Q_4 cats were disproportionately more likely to weigh ≥ 4.5 kg (more than the median for this group) (Odds Ratio of 5.3; 95% CI 1.54–18.44) (Fig. S2; Table S6). Trend testing similarly revealed that "heavier" cats were disproportionately present in the upper quartiles (Fig. 3A). Furthermore, Fisher's Exact Testing of body weight revealed significant associations with PFOS ($p=0.0008$) and PFHxS ($p=0.012$); but not PFOA or C9. A Test for Trend showed robust associations with body weight and PFOS ($p=0.0006$) and PFHxS ($p=0.001$), and possibly PFOA ($p=0.05$) but not C9.

Analogous to human body mass index (BMI) assessments, in cats, body condition scores (BCS) are used clinically to assess relative adiposity (1=very thin, 5=optimal, 9=very obese). Contingency testing across BCS likewise showed that Q_4 cats were disproportionately more likely to have scores ≥ 5 (the median for this group) (Odds Ratio of 4.4; 95% CI 0.96–19.8) (Fig. S2; Table S6). Significant associations with BCS and PFOS were also observed with Fisher's Exact Test ($p=0.05$) and Test for Trend ($p=0.03$)

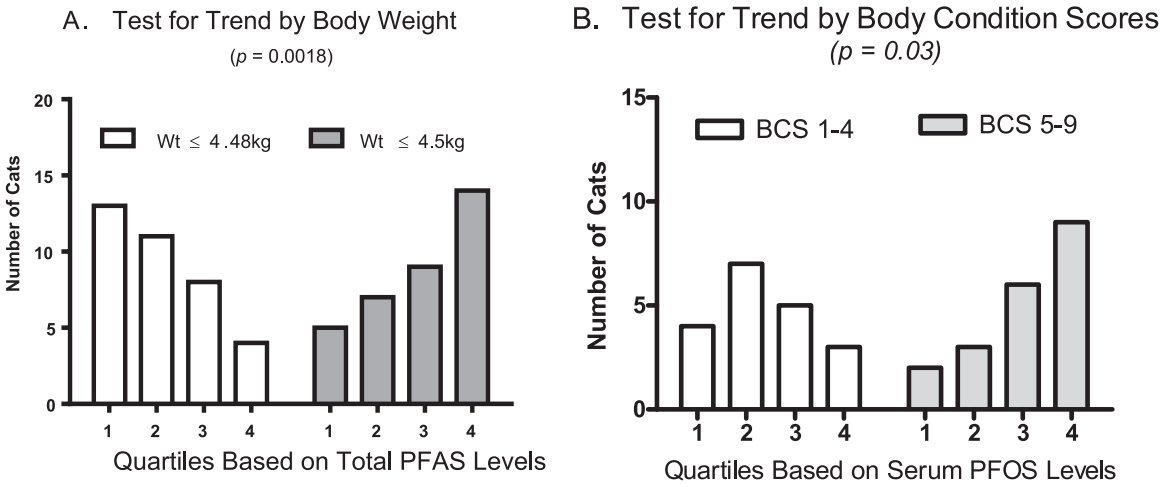

Fig. 3. A & B. Contingency Test for Trend based on (A) associations of body weight (Wt) with quartile rankings (Q_1 lowest; Q_4 highest) based on serum total PFAS concentrations, and (B) body condition scores (BCS) with quartile rankings based on serum PFOS concentrations.

(Fig. 3B).

Sources of PFAS exposure for the cats in the present study are not known, but one hypothesis is that diet could play an important role. As obligate carnivores, domestic cats may consume fish, a food known to contain PFAS (Tittlemier et al., 2007; Haug et al., 2010). Alternatively, other foods and food packaging could contribute to the exposures made evident in this study (Begley et al., 2008). Future studies are needed to assess PFAS levels present in cat food. For example, in a similar study involving PBDEs, we showed that BDE-47 content of fish- or seafood-based canned cat food was significantly correlated with relative risk of developing feline hyperthyroidism (Dye et al., 2007). Herein, when we regressed serum PFAS levels against body weight, a borderline significant relationship between ΣPFAS concentration and body weight was determined. Given this marginal association, in addition to food intake, other factors likely play significant roles for PFAS exposure in cats.

Additional exposure routes related to the indoor environment are interesting to consider because they are common for both cats and humans. Shared sources likely include water (Egeghy et al., 2011), ingestion of house dust (Strynar et al., 2008; Björklund et al., 2009; Wu et al., 2015), contact with PFAS-treated textiles (floors, carpets, furniture) and inhalation of volatile PFAS (Martin et al., 2010). It is noteworthy the extent to which toddlers and indoor cats may share similar exposure pathways. Children spend time crawling on floors, sitting on furniture, and engaging in hand-to-mouth activity which exposes them to house dust, dirt, and other materials found on indoor surfaces. Due to their grooming behavior, cats share similar exposures to materials on indoor surfaces, which pet dogs do not share to the same extent.

Accordingly, our data showed that cats in the highest PFAS quartile were significantly more likely to live indoors. Total, ΣPFAS, serum levels increased in proportion to time spent indoors. Moreover, the highest concentrations detected in pet cats were due to disproportionately greater PFHxS levels. As previously reported, the most contaminated house dust samples also contain disproportionately greater amounts of PFHxS (Strynar et al., 2008; Björklund et al., 2009; Beesoon et al., 2012). PFHxS was used in post-market carpet treatment applications (Beesoon et al., 2012). The wide variation in ΣPFAS serum levels in these indoor cats further suggests that some (e.g., those with chronic regurgitation) necessitated greater use of carpet and furniture stain-resistant products. Of possible relevance, PFHxS levels were 30–40% higher in adolescent and elderly humans compared to middle-aged adults (Kato et al., 2011). Taken together — the higher serum levels observed for PFHxS in certain aged humans or in individual cats may reflect increased anti-stain product usage for those family members (and pets) prone to generating stains.

3.6. Associations of disease sub-classifications with PFAS quartiles

Eleven (of 72; 15%) of cats were healthy and 61 (85%) had one or more primary disease diagnoses. Based on ΣPFAS levels, contingency testing did not indicate that diseased cats were generally more likely to be in Q_4 vs. $Q_{1–3}$; nor was there any trend that diseased cats were increasingly present in the higher quartiles (Fig. 4). Therefore, cats were subdivided into groupings based on standard veterinary sub-classifications of disease. In decreasing order, these included: cardiovascular disease (40%; $n=25$ of 61 affected cats), neoplasia (21%; $n=13$), kidney/urinary disease (21%; $n=13$), alimentary disease (16%; $n=10$), infectious/immune-mediated disease (15%; $n=9$), endocrine disease (11%, including diabetes, Cushing's disease, and hyperthyroidism; $n=7$), respiratory disease (8%; $n=5$), and primary liver disease (3%; $n=2$).

Based on ΣPFAS levels, there were no cats with neoplasia in Q_4. Thus, contingency testing revealed a significant, but negative, association with neoplasia ($n=13$) and cats in Q_4 vs. $Q_{1–3}$ ($p=0.016$) (Fig. 4). However, trend testing across quartiles failed to show any association with cats having (or not having) neoplasia ($p=0.54$). Furthermore, based on ΣPFAS levels, no significant associations were observed with cats in Q_4 vs. $Q_{1–3}$ and cardiovascular, alimentary, infectious/immune, or endocrine disease categories

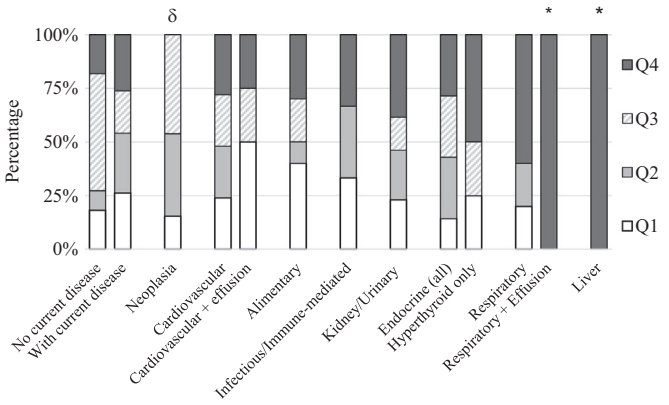

Fig. 4. Cat disease sub-classifications across quartile ranking (Q_1 lowest; Q_4 highest) based on total serum PFAS levels. Indicates disproportionately fewer (δ) and greater (*) numbers of cats with this condition in Q_4 vs $Q_{1–3}$.

(Fig. 4). Likewise, trend testing across quartiles did not show associations with these categories (data not shown). Results are consistent with a recent human epidemiology report that also failed to find associations between PFAS exposure and cardiac disease (Mattsson et al., 2015). Furthermore, because these cats were largely neutered adults, it is beyond the scope of this study to assess potential associations between PFAS levels and developmental or reproductive outcomes (Itoh et al., 2016), including testicular cancer (Barry et al., 2013). We acknowledge that this is a limitation of using adult pet cats for these assessments, however, their neutered status may have contributed to the negative association observed between ΣPFAS serum levels and cases of neoplasia.

Again based on ΣPFAS rankings, weak associations were observed for cats having liver ($p=0.06$), respiratory ($p=0.10$), and possibly kidney/urinary disease ($p=0.19$) (Fig. 4). Of possible relevance, post-mortem studies in animals (Olivero-Verbel et al., 2006; Cui et al., 2009) and humans (Maestri et al., 2006) show high PFAS tissue distribution to the liver, lungs, and kidneys. Pharmacokinetic studies in pigs (Numata et al., 2014) and micro-minipigs (Guruge et al., 2016) also show high distribution of PFOS and longer chain perfluorinated carboxylates to the liver, with corresponding longer half-lives for these compounds. Such findings can be explained by the fact that, unlike classic POPs, PFAS do not bioaccumulate in storage lipids, but instead partition within membrane phospholipids that have a high affinity for charged species, and interact with proteins like albumin, liver-fatty acid binding proteins, and organic anion transporters (OATs) (Ng et al., 2014). This results in ongoing enterohepatic recirculation and in renal tubular reabsorption, especially of C6-C8 species (Weaver et al., 2010); thus contributing to the persistency of PFOS and PFOA in people.

Lastly, we examined associations between specific disease entities and individual analyte quartile rankings. Three (of 4) cats with the highest PFHxS levels detected had chronic kidney disease (CKD), although contingency testing did not show significant associations with PFHxS or other analytes (Table S7). There were, however, marginally significant differences in serum PFOA ($p=0.07$) and PFHxS ($p=0.07$) concentrations in cats with CKD ($n=13$) compared to those without (Fig. 5).

Like humans, cats commonly develop urolithiasis and CKD. The frequency of the diagnosis of CKD in cats has increased in recent decades and the prevalence of CKD in cats now exceeds that of dogs (Brown et al., 2016). For this reason, it was notable that cat #9 (4th highest ΣPFAS levels including PFHxS >100 ng/mL) had CKD related to idiopathic hypercalcemia. Unexplained hypercalcemia has been increasingly recognized in cats since 1990 (Midkiff et al., 2000). High PFAS serum levels may impair renal tubular secretion of fixed acids (via interference with OATs) (Ng et al., 2014). We hypothesize that high PFAA levels could therefore exacerbate metabolic acidosis in cats, especially those consuming acidifying diets for management of urolithiasis. As such, high PFAS levels may promote calcium mobilization from bone and in turn, moderate hypercalcemia, thus augmenting calcium renal excretion — which on a chronic basis would increase the risk of developing CKD.

Closer inspection of cats with respiratory disease noted that all three cats afflicted with effusive disease (one with idiopathic pleural effusion and two with chylothorax) were in Q_4. Despite the uncommon occurrence of these conditions, both Fisher's ($p=0.014$) and Trend Testing ($p=0.018$) indicated significant associations (Fig. 4). Furthermore, there was a significant association between pulmonary effusive disease and cats in Q_4 based on PFHxS levels (Table S7). Likewise, cats with effusive disease had

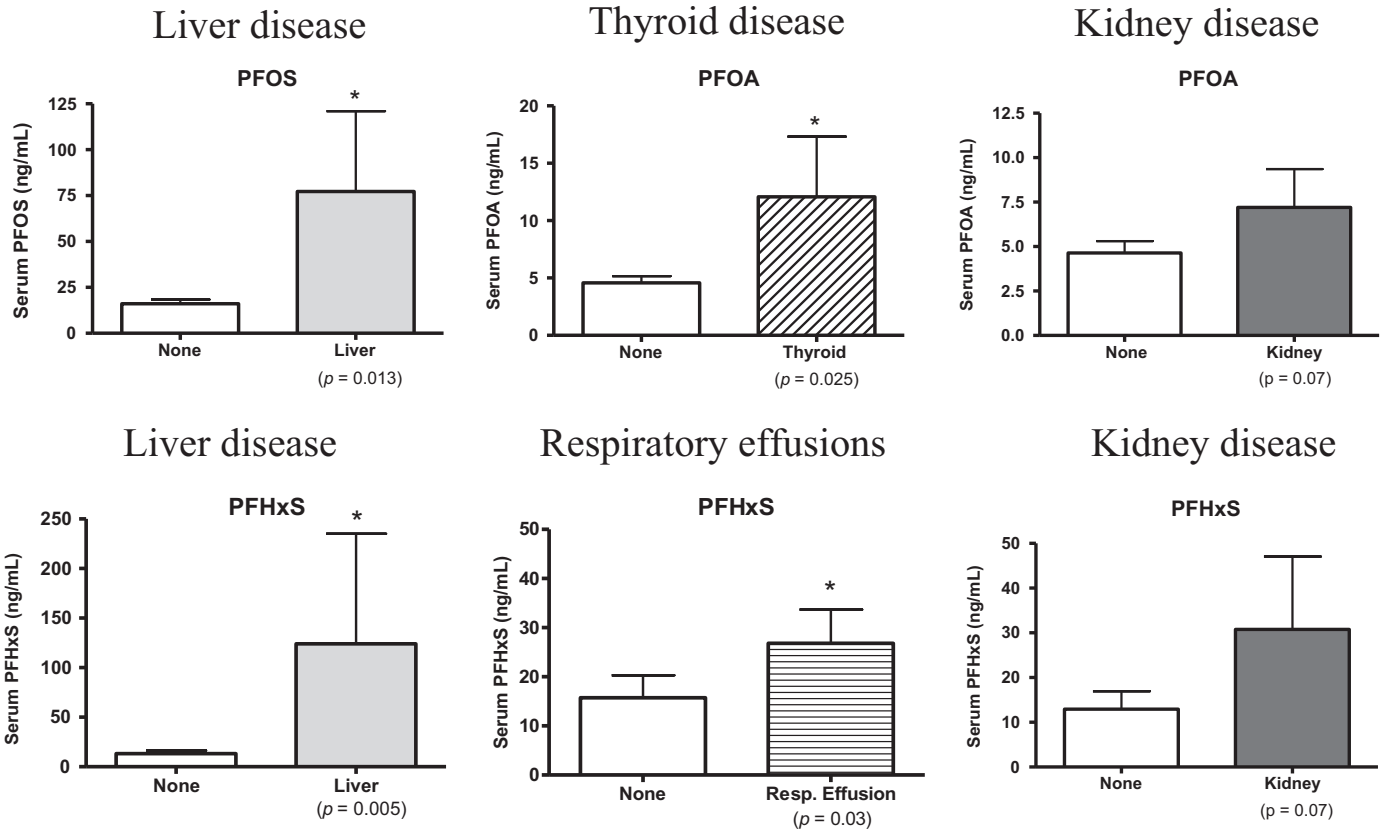

Fig. 5. Comparison of serum PFAS levels (mean ± SE) in cats with or without certain disease conditions including primary liver disease ($n=2$), feline hyperthyroidism ($n=4$), respiratory effusive disease ($n=3$), or kidney/urinary tract disease ($n=13$).

significantly greater PFHxS serum levels than cats without (Fig. 5). Although we are not aware of any similar reports in humans, PFAS have a high tendency to partition into lipid bilayers including the saturated phospholipid, dipalmitoylphosphatidylcholine (DPPC), found in lung surfactant (Lau et al., 2007; Ng et al., 2014). Even at low concentrations, PFAS have been shown to disrupt surfactant surface tension (Gordon et al., 2007). In cats, DPPC is the main phospholipid in the alveolar space, while the unsaturated phospholipid, stearoyl-linoleoyl-phosphatidylcholine (SLPC), dominates the pleural space (Mills et al., 2006). We postulate that, similar to known PFAS-DPPC physical interactions contributing to surfactant dysfunction, PFAS-SLPC interactions could disrupt pleural fluid surface tension – and therefore contribute to excess pleural fluid accumulation and collapse of affected lung lobes. Relatedly, prenatal PFOS exposure in rats has been shown to result in neonatal mortality due to labored breathing and inability to fully inflate the lungs after birth, possibly due to surfactant dysfunction (Grasty et al., 2005).

Among cats with endocrine disease, there was a significant association between hyperthyroidism ($n=4$) and Q_4 status based on PFOA ($p=0.05$) and a weak association with ∑PFAS ($p=0.26$) (Fig. 4; Table S7). Since 2 (of 3) of the highest PFOA values measured were in cats with hyperthyroidism, PFOA serum levels were significantly greater in hyperthyroid cats than in cats without this condition (Fig. 5). Notably, serum PFOA concentrations have been associated with thyroid disease in U.S. adults (Melzer et al., 2010) and children (Lopez-Espinosa et al., 2012).

Finally, based on PFOS levels, we observed that both cats with primary liver disease were in Q_4. One cat, #58, had the highest PFOS and PFHxS levels measured, thus equivalent, weak associations were noted across quartile rankings whether by PFOS, PFOA, or PFHxS (Table S7). Significantly increased PFOS and PFHxS levels were detected in the cats with primary liver disease compared to those without (Fig. 5). Cat #58 was evaluated for chronic regurgitation and had clinically significant elevations in serum cholesterol and liver enzymes (e.g., alanine transaminase, ALT). ALT is a marker of hepatocellular damage. In humans, levels of PFOS (Gallo et al., 2012) and PFHxS (Gleason et al., 2015) have been positively associated with ALT levels; while PFOS and PFOA levels have been positively associated with serum cholesterol (Nelson et al., 2010).

Because serum bile acids are commonly increased in cholestatic liver disease, and knowing that PFAS have natural interferences in serum [e.g., the bile acid, taurodeoxycholic acid (TCDA)], we used TOF mass spectrometry to ensure adequate discrimination of PFOS from TDCA. Analysis of replicate samples showed that PFOS levels remained relatively constant over the elapsed time while TDCA levels were quite variable (Fig. S4). Upon re-evaluation of cat #58 (28 days later), the replicate sample (#58-R) still showed high PFOS and PFHxS levels, and the cat continued to exhibit elevated TDCA, cholesterol, and ALT levels owing to its liver disease. As discussed earlier, enterohepatic recirculation of PFAS, similar to that of bile acids, appears to contribute to the persistence of PFAS in exposed people and animals. In the liver disease cases herein, it is not possible to discern whether increased PFAS levels in these cats contributed to their observed liver disease, or conversely, whether existing liver disease in some manner reduced PFAS elimination, and thus elevating PFAS serum concentrations in these cats, or both. Limited evidence in humans with liver cancer or cirrhosis suggests that hepatic pathology may alter the distribution of PFAS between serum and the liver (Yeung et al., 2013).

4. Discussion

In summary, to our knowledge, this is the first published report of PFAS in individual domestic cats. Results clearly show that cats are exposed to PFAS and appear to be useful sentinels, especially for assessing indoor exposures of children. The fact that all but one cat had measureable serum levels of PFAS parallels the finding that virtually all human serum samples collected from the industrialized world contain PFAS in the ng/mL range. The fact that one cat did not have detectable levels suggests that the levels detected do not simply represent contamination during collection of or processing of serum. Importantly, concentrations and analyte profiles of PFOS, PFHxS, PFOA, and C9 in cat sera largely paralleled that of the contemporary NHANES report (2007–2008) in serum of U.S. adults; while in some instances, the levels in cats closely matched that of the 95% percentile, including the exceptionally high levels noted in teenagers in the Canadian home case report (Kato et al., 2001; Beesoon et al., 2012). PFAS have also been measured in captive tigers and lions in China with mean PFOS serum concentrations of 1.18 and 2.69 ng/mL, respectively (Li et al., 2008); while in dogs in Japan, serum PFOS ranged from 12 to 57 ng/g and PFHxS from 0.89 to 32 ng/g (Guruge et al., 2008). Clearly, none of these species reflect exposure conditions for humans, in particular toddlers and teens, as closely as the domestic cat. While serum half-lives of PFOS and PFOA in humans and rodents are fairly well established, such information is unknown in the cat. However, the observed consistency of PFOS concentrations across the replicate sampling periods (Fig. S4) suggests that PFAS half-lives in the cat may be relatively prolonged; hence, more similar to humans than rodents. In humans the half-life of PFOS is approximately 4.8 years while in certain rodent species it is 1–2 months (Olsen et al., 2007; Chang et al., 2012). For PFOA, the half-life is 3.5 years in humans, days in male rats, and only hours in female rats (Olsen et al., 2007).

The associations observed herein between increased ∑PFAS in cats with living indoors and with obesity is quite intriguing. In the U.S., obesity is a growing problem in both pets and people. In the past 30 years, obesity has more than doubled in children and quadrupled in adolescents (Ogden et al., 2014). A recent survey of U.S. pets revealed that the number of overweight pet cats is at an all-time high, with nearly 60% considered overweight or obese by their veterinarian (Sourcebook, 2012). It may be that indoor cats simply get less exercise and are more likely to participate in boredom-related excessive eating and grooming, thus increasing their exposure to PFAS via these routes. On the other hand, experimental studies have shown that mice exposed *in utero* to PFOA had significantly increased body mass by 10 weeks-of-age which persisted through mid-life (Hines et al., 2009). Similarly in children, higher prenatal serum PFOA concentrations were associated with greater adiposity at 8 years of age and a more rapid increase in BMI between 2 and 8 years (Braun et al., 2016). In overweight children, PFAS levels have been associated with insulin resistance and with higher triglyceride concentrations (Timmermann et al., 2014); while in adults, PFAS have been associated with increased BMI and waist circumference (Lin et al., 2009; Ng et al., 2014). Mechanistically, PFAS appear to activate peroxisome proliferator-activated receptors (PPARs) (Lau et al., 2007). In turn, PPAR activity appears to play a critical role in adipocyte differentiation and metabolism and can influence cellular insulin sensitivity (Watkins et al., 2015) and may promote adipogenesis (Xu et al., 2016). PFAS are purportedly one of a group of chemicals that may act as obesogens (Holtcamp et al., 2012; Janesick and Blumberg, 2016).

In terms of the utility of domestic cats as *health* sentinels for PFAS exposure, it is important to reiterate that as a cross-sectional study, we used contingency testing simply to explore potential associations between increased PFAS serum levels and various subject factors and disease conditions. Any associations noted herein should in no way be considered causal. Thus, more focused, case-control studies in cats with better defined dietary and indoor source information are warranted to further examine relationships

between PFAS serum and/or organ specific PFAS levels with adiposity, respiratory, liver, thyroid, or renal health outcomes. Studies to investigate relationships between serum levels in co-habitating cats and humans (across toddlers, teens, and adults) would assist in establishing the role of house dust ingestion as a predominant route of exposure to PFAS and other POPs (e.g., PDBEs). With 74 million domestic cats occupying 36 million homes in the U.S. (APOP, 2014), pet cats represent an underutilized resource by which to explore these relationships and gain improved understanding of shared environmental exposures and "one health" risks for humans and pets alike.

Funding sources

This work was conducted with assistance from Cooperative Research and Development Agreements (CRADA) with Agilent Technologies (#437–07). It was partially supported under Student Service Contract # EP10D000071.

Disclosure

The authors have no disclosures to report regarding this publication.

Disclaimer

This paper has been reviewed by the National Exposure Research Laboratory and the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that contents necessarily reflect the views and polices of the EPA, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

Acknowledgements

The authors thank Drs. Gary Hatch, Erin Hines, and Elin Ulrich for critical review of this manuscript. They are especially grateful to Dr. Karyn Harrell and colleagues at the Veterinary Teaching Hospital, NCSU, and to all cat owners and referring veterinarians in the Raleigh metropolitan area who made this study possible.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2016.07.027.

References

Association for Pet Obesity Prevention (APOP), 2014. Pet Obesity (Statistics) (⟨http://www.petobesityprevention.org/pet-obesity-fact-risks/⟩).
Barry, V., Winquist, A., Steenland, K., 2013. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environ. Health Perspect. 121 (11–12), 1313–1318.
Begley, T.H., Hsu, W., Noonan, G., Diachenko, G., 2008. Migration of fluorochemical paper additives from food-contact paper into foods and food simulants. Food Addit. Contam. 25, 384–390.
Beesoon, S., Genuis, S.J., Benskin, J.P., Martin, J.W., 2012. Exceptionally high serum concentrations of perfluorohexanesulfonate in a Canadian family are linked to home carpet treatment applications. Environ. Sci. Technol. 46 (23), 12960–12967.
Bertone, E.R., Snyder, L.A., Moore, A.S., 2002. Environmental tobacco smoke and risk of malignant lymphoma in pet cats. Am. J. Epidemiol. 156 (3), 268–273.
Björklund, J.A., Thuresson, K., De Wit, C.A., 2009. Perluoroalkyl compounds (PFCs) in indoor dust: concentrations, human exposure estimates, and sources. Environ. Sci. Technol. 43 (7), 2276–2281.
Braun, J.M., Chen, A., Romano, M.E., Calafat, A.M., Webster, G.M., Yolton, K., Lanphear, B.P., 2016. Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: the HOME study. Obesity 24 (1), 231–237.
Brown, C.A., Elliott, J., Schmiedt, C.W., Brown, S.A., 2016. Chronic kidney disease in aged cats: clinical features, morphology, and proposed pathogeneses. Vet. Pathol. 53 (2), 309–326.
Calafat, A.M., Wong, L.Y., Kuklenyik, Z., Reidy, J.A., Needham, L.L., 2007. Polyfluoroalkyl chemicals in the U.S. population: data from the national health and nutrition examination survey (NHANES) 2003–2004 and comparisons with NHANES 1999–2000. Environ. Health Perspect. 115 (11), 1596–1602.
Callaway, E., 2015. 'I can haz genomes': cats claw their way into genetics. Nature 517 (7534), 252–253. http://dx.doi.org/10.1038/517252a.
Chang, S.C., Noker, P.E., Gorman, G.S., Gibson, S.J., Hart, J.A., Ehresman, D.J., Butenhoff, J.L., 2012. Comparative pharmacokinetics of perfluorooctanesulfonate (PFOS) in rats, mice, and monkeys. Reprod. Toxicol. 33 (4), 428–440.
Cohn, L.A., Norris, C.R., Hawkins, E.C., Dye, J.A., Johnson, C.A., Williams, K.J., 2004. Identification and characterization of an idiopathic pulmonary fibrosis-like condition in cats. J. Vet. Intern Med. 18 (5), 632–641.
Dowsett, R., Shannon, M., 1994. Childhood plumbism identified after lead poisoning in household pets. N. Engl. J. Med. 331 (24), 1661–1662.
Egeghy, P.P., Lorber, M., 2011. An assessment of the exposure of Americans to perfluorooctane sulfonate: a comparison of estimated Intake with values inferred from NHANES data. J. Exp. Sci. Environ. Epid 21 (2), 150–168.
Dye, J.A., McKiernan, B.C., Rozanski, E.A., Hoffmann, W.E., Losonsky, J.M., Homco, L. D., Weisiger, R.M., Kakoma, I., 1996. Bronchopulmonary disease in the cat: Historical, physical, radiographic, clinicopathologic, and pulmonary functional evaluation of 24 affected and 15 healthy cats. J. Vet. Intern. Med. 10 (6), 385–400.
Dye, J.A., Venier, M., Zhu, L., Ward, C.R., Hites, R.A., Birnbaum, L.S., 2007. Elevated PBDE levels in pet cats: sentinels for humans? Environ. Sci. Technol. 41 (18), 6350–6356.
Fox, P.R., Basso, C., Thiene, G., Maron, B.J., 2014. Spontaneously occurring restrictive nonhypertrophied cardiomyopathy in domestic cats: a new animal model of human disease. Cardiovasc. Pathol. 23 (1), 28–34.
Frazzoli, C., Bocca, B., Mantovani, A., 2015. The one health perspective in trace elements biomonitoring. J. Toxicol. Environ. Health B. Crit. Rev. 18 (7–8), 344–370.
Gallo, V., Leonardi, G., Genser, B., Lopez-Espinosa, M.J., Frisbee, S.J., Karlsson, L., Ducatman, A.M., Fletcher, T., 2012. Serum perfluorooctanoate (PFO)A) (and perfluorooctane sulfonate (PFO)S) (concentrations and liver function biomarkers in a population with elevated PFOA exposure). Environ. Health Perspect. 120 (5), 655–660.
Gleason, J.A., Post, G.B., Fagliano, J.A., 2015. Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US populations (NHANES), 2007–2010. Environ. Res. 136, 8–14.
Gordon, S.C., Schurch, S., Amrein, M., Schoel, M., 2007. Effects of perrfluorinated acids on pulmonary surfactant properties in vitro. Toxicologist 96, 91.
Grasty, R.C., Bjork, J.A., Wallace, K.B., Wolf, D.C., Lau, C.S., Rogers, J.M., 2005. Effects of prenatal perfluorooctane sulfonate (PFOS) exposure on lung maturation in the perinatal rat. Birth Defects Res. B Dev. Reprod. Toxicol. 74 (5), 405–416.
Guruge, K.S., Manage, P.M., Yamanaka, N., Miyazaki, S., Taniyasu, S., Yamashita, N., 2008. Species-specific concentrations of perfluoroalkyl contaminants in farm and pet animals in Japan. Chemosphere 73 (Supplement 1), S210–S215.
Guruge, K.S., Noguchi, M., Yoshioka, K., Yamazaki, E., Taniyasu, S., Yoshioka, M., Yamanaka, N., Ikezawa, M., Tanimura, N., Sato, M., Yamashita, N., Kawaguchi, H., 2016. Microminipigs as a new experimental animal model for toxicological studies: comparative pharmacokinetics of perfluoroalkyl acids. J. Appl. Toxicol. 36 (1), 68–75.
Gyllenhammar, I., Berger, U., Sundström, M., McCleaf, P., Eurén, K., Eriksson, S., Ahlgren, S., Lignell, S., Aune, M., Kotova, N., Glynn, A., 2015. Influence of contaminated drinking water on perfluoroalkyl acid levels in human serum – a case study from Uppsala, Sweden. Environ. Res. 140, 673–683.
Haug, L.S., Thomsen, C., Brantsaeter, A.L., Kvalem, H.E., Haugen, M., Becher, G., Alexander, J., Meltzer, H.M., Knutsen, H.K., 2010. Diet and particularly seafood are major sources of perfluorinated compounds in humans. Environ. Int. 36 (7), 772–778.
Hines, E.P., White, S.S., Stanko, J.P., Gibbs-Flournoy, E.A., Lau, C., Fenton, S.E., 2009. Phenotypic dichotomy following developmental exposure to perfluorooctanoic acid (PFOA) in female CD-1 mice: low doses induce elevated serum leptin and insulin, and overweight in mid-life. Mol. Cell. Endocrinol. 304 (1–2), 97–105.
Holtcamp, W., 2012. Obesogens: an environmental link to obesity. Environ. Health Perspect. 120 (2), a62–a68.
Itoh, S., Araki, A., Mitsui, T., Miyashita, C., Goudarzi, H., Sasaki, S., Cho, K., Nakazawa, H., Iwasaki, Y., Shinohara, N., Nonomura, K., Kishi, R., 2016. Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido study on environment and children's health. Environ. Int. 19 (94), 51–59.
Janesick, A.S., Blumberg, B., 2016. Obesogens: an emerging threat to public health. Am. J. Obstet. Gynecol. 214 (5), 559–565.
Kato, K., Wong, L.Y., Jia, L.T., Kuklenyik, Z., Calafat, A.M., 2011. Trends in exposure to polyfluoroalkyl chemicals in the U.S. population: 1999–2008. Environ. Sci. Technol. 45 (19), 8037–8045.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–394.
Lehmler, H.J., 2005. Synthesis of environmentally relevant fluorinated surfactants–a review. Chemosphere. 58 (11), 1471–1496.
Lewandowski, G., Meissner, E., Milchert, E., 2006. Special applications of fluorinated organic compounds. J. Hazard Mater. 136 (3), 385–391.
Li, X., Yeung, L.W., Taniyasu, S., Lam, P.K., Yamashita, N., Xu, M., Dai, J., 2008. Accumulation of perfluorinated compounds in captive Bengal tigers (*Panthera tigris tigris*) (and African lions (*Panthera leo Linnaeus*) in China. Chemosphere 73 (10), 1649–1653.
Lin, C.Y., Chen, P.C., Lin, Y.C., Lin, L.Y., 2009. Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults. Diabetes Care. 32 (4), 702–707.
Lindstrom, A.B., Strynar, M.J., Delinsky, A.D., Nakayama, S.F., McMillan, L., Libelo, E. L., Neill, M., Thomas, L., 2011. Application of WWTP biosolids and resulting perfluorinated compound contamination of surface and well water in Decatur, Alabama, USA. Environ. Sci. Technol. 45 (19), 8015–8021.
Lopez-Espinosa, M.J., Mondal, D., Armstrong, B., Bloom, M.S., Fletcher, T., 2012. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environ. Health Perspect. 120 (7), 1036–1041.
Maestri, L., Negri, S., Ferrari, M., Ghittori, S., Fabris, F., Danesino, P., Imbriani, M., 2006. Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. Rapid Commun. Mass. Spectrom. 20 (18), 2728–2734.
Martin, J.W., Asher, B.J., Beesoon, S., Benskin, J.P., Ross, M.S., 2010. PFOS or PreFOS? Are perfluorooctane sulfonate precursors (PreFOS) important determinants of human and environmental perfluorooctane sulfonate (PFOS) exposure?. J. Environ. Monit. 12 (11), 1979–2004.
Mattsson, K., Rignell-Hydbom, A., Holmberg, S., Thelin, A., Jönsson, B.A., Lindh, C.H., Sehlstedt, A., Rylander, L., 2015. Levels of perfluoroalkyl substances and risk of coronary heart disease: findings from a population-based longitudinal study. Environ. Res. 142, 148–154.
Melzer, D., Rice, N., Depledge, M.H., Henley, W.E., Galloway, T.S., 2010. Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. national health and nutrition examination survey. Environ. Health Perspect. 118 (5), 686–692.
Midkiff, A.M., Chew, D.J., Randolph, J.F., Center, S.A., DiBartola, S.P., 2000. Idiopathic hypercalcemia in cats. J. Vet. Intern. Med. 14 (6), 619–626.
Mills, P.C., Chen, Y., Hills, Y.C., Hills, B.A., 2006. Comparison of surfactant lipids between pleural and pulmonary lining fluids. Pulm. Pharmacol. Ther. 19 (4), 292–296.
Nelson, J.W., Hatch, E.E., Webster, T.F., 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ. Health Perspect. 118 (2), 197–202.
Nelson, R.W., Reusch, C.E., 2014. Animal models of disease: classification and etiology of diabetes in dogs and cats. J. Endocrinol. 222 (3), T1–T9.
Ng, C.A., Hungerbühler, K., 2014. Bioaccumulation of perfluorinated alkyl acids: observations and models. Environ. Sci. Technol. 48 (9), 4637–4648.
Numata, J., Kowalczyk, J., Adolphs, J., Ehlers, S., Schafft, H., Fuerst, P., Müller-Graf, C., Lahrssen-Wiederholt, M., Greiner, M., 2014. Toxicokinetics of seven perfluoroalkyl sulfonic and carboxylic acids in pigs fed a contaminated diet. J. Agric. Food Chem. 62 (28), 6861–6870.
Ogden, C.L., Carroll, M.D., Kit, B.K., Flegal, K.M., 2014. Prevalence of childhood and adult obesity in the United States, 2011–2012. J. Am. Med. Assoc. 311 (8), 806–814.
Olivero-Verbel, J., Tao, L., Johnson-Restrepo, B., Guette-Fernández, J., Baldiris-Avila, R., O'byrne-Hoyos, I., Kannan, K., 2006. Perfluorooctanesulfonate and related fluorochemicals in biological samples from the north coast of Colombia. Environ. Pollut. 142 (2), 367–372.
Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R., 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluoro-hexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 115 (9), 1298–1305.
Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H., 2006. Sources, fate and transport of perfluorocarboxylates. Environ. Sci. Technol. 40 (1), 32–44.
Reiner, J.L., Nakayama, S.F., Delinsky, A.D., Stanko, J.P., Fenton, S.E., Lindstrom, A.B., Strynar, M.J., 2009. Analysis of PFOA in dosed CD1 mice. Part 1. Methods development for the analysis of tissues and fluids from pregnant and lactating mice and their pups. Reprod. Toxicol. 27 (3–4), 360–364.
Robinson, M.R., Norris, R.D., Sur, R.L., Preminger, G.M., 2008. Urolithiasis: not just a 2-legged animal disease. J. Urol. 179 (1), 46–52.
Saito, N., Harada, K., Inoue, K., Sasaki, K., Yoshinaga, T., Koizumi, A., 2004. Perfluorooctanoate and perfluorooctane sulfonate concentrations in surface water in Japan. J. Occup. Health 46 (1), 49–59.
Snyder, L.A., Bertone, E.R., Jakowski, R.M., Dooner, M.S., Jennings-Ritchie, J., Moore, A.S., 2004. p53 expression and environmental tobacco smoke exposure in feline oral squamous cell carcinoma. Vet. Pathol. 41 (3), 209–214.
Sourcebook, U.S. Pet Ownership and Demographics, 2012 edition. Stockholm ⟨http://chm.pops.int/Convention/Pressrelease/COP4Geneva8May 2009/tabid/542/language/en-US/Default.aspx⟩.
Strynar, M.J., Lindstrom, A.B., 2008. Perfluorinated compounds in house dust from Ohio and North Carolina, USA. Environ. Sci. Technol. 42 (10), 3751–3756.
Timmermann, C.A., Rossing, L.I., Grøntved, A., Ried-Larsen, M., Dalgård, C., Andersen, L.B., Grandjean, P., Nielsen, F., Svendsen, K.D., Scheike, T., Jensen, T.K., 2014. Adiposity and glycemic control in children exposed to perfluorinated compounds. J. Clin. Endocrinol. Metab. 99 (4), E608–E614.
Tittlemier, S.A., Pepper, K., Seymour, C., Moisey, J., Bronson, R., Cao, X.L., Dabeka, R. W., 2007. Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. J. Agric. Food Chem. 55 (8), 3203–3210.
Tsuchiya, K., 1992. The discovery of the causal agent of Minamata disease. Am. J. Ind. Med. 21 (2), 275–280.
USEPA 2010/15 PFOA Stewardship Program. ⟨http://www.epa.gov/oppt/pfoa/pubs/stewardship/index.html⟩ (December 20, 2006).
U.S. Environmental Protection Agency Advisories for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS), 2016. Washington DC. ⟨https://www.epa.gov/ground-water-and-drinking-water/drinking-water-health-advisories-pfoa-and-pfos⟩.
Van de Velde, H., Janssens, G.P., de Rooster, H., Polis, I., Peters, I., Ducatelle, R., Nguyen, P., Buyse, J., Rochus, K., Xu, J., Verbrugghe, A., Hesta, M., 2013. The cat as a model for human obesity: Insights into depot-specific inflammation associated with feline obesity. Br. J. Nutr. 110 (7), 1326–1335.
Watkins, A.M., Wood, C.R., Lin, M.T., Abbott, B.D., 2015. The effects of perfluorinated chemicals on adipocyte differentiation in vitro. Mol. Cell Endocrinol. 400, 90–101.
Weaver, Y.M., Ehresman, D.J., Butenhoff, J.L., Hagenbuch, B., 2010. Roles of rat renal organic anion transporters in transporting perfluorinated carboxylates with different chain lengths. Toxicol. Sci. 113 (2), 305–314.
Wu, X.M., Bennett, D.H., Calafat, A.M., Kato, K., Strynar, M., Andersen, E., Moran, R.E., Tancredi, D.J., Tulve, N.S., Hertz-Picciotto, I., 2015. Serum concentrations of perfluorinated compounds (PFC) among selected populations of children and adults in California. Environ. Res. 136, 264–273.
Wypij, J.M., 2013a. A naturally occurring feline model of head and neck squamous cell carcinoma. Pathol. Res. Int., 502197.
Xu, J., Shimpi, P., Armstrong, L., Salter, D., Slitt, A.L., 2016. PFOS induces adipogenesis and glucose uptake in association with activation of Nrf2 signaling pathway. Toxicol. Appl. Pharmacol. 1 (290), 21–30.
Yeung, L.W., Guruge, K.S., Taniyasu, S., Yamashita, N., Angus, P.W., Herath, C.B., 2013. Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicol. Environ. Saf. 96, 139–146.
Zinsstag, J., Schelling, E., Waltner-Toews, D., Tanner, M., 2011. From "one medicine" to "one health" and systemic approaches to health and well-being. Prev. Vet. Med. 101 (3-4), 148–156.