ARE SERUM LEVELS OF BROMINATED FLAME RETARDANTS AND THYROID STATUS CORRELATED IN CATS?

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Introduction

Brominated flame retardants (BFRs) such as polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), their hydroxylated metabolites (OH-PBDEs) and brominated phenols, are known as endocrine disrupting compounds (EDCs)[1,2]. This is likely due to structural resemblance to endogenous steroid hormones and their ability to influence the hormone homeostasis in the body[3]. *In vitro* studies have shown that some brominated phenols and OH-PBDEs have even stronger affinity than thyroxine (T_4) itself in binding to human transtyretin (TTR), which is the blood transport protein for the tyroxine hormone in vertebrate species[4,5]. The thyroid hormones triidothyronine (T_3) and thyroxine (T_4) plays an important role by regulating the body metabolism.

In cats, there has been an steady increase of the feline hyperthyroidism (FH), an endocrine disease first described in 1979[6]. In contrast to humans, the etiology of FH has not been proven and remains controversial. Veterinary epidemiological studies have reported association between indoor living and consumption of canned cat food and an increased risk for developing FH[7]. Further, Dye et al.[7] hypothesized that hyperthyroidism in cats could be due to exposure of PBDEs through diet and house dust. Studies from Sweden and the US shows that cats have a 50 and 20-100 times greater serum concentrations of ∑PBDE compared to humans living in the same country[7,8,9]. This is probably due to the cats licking behavior, cleaning their fur. Dust, which serves as a sink for BFRs in the home environment[10], sticks to the fur and ingestion on BFRs becomes an important source of exposure.

The work presented here builds upon an earlier published work were PBDEs, DecaBB (i.e. BB-209) and brominated phenolic compounds were identified in a pooled cat serum sample[11]. Our aim with this study was to explore any correlation between FH and PBDEs, DecaBB, OH-PBDEs or brominated phenols. First, PBDEs, DecaBB, OH-PBDEs and brominated phenols were quantified in cat serum from 40 Swedish pet cats (6-17 years of age), all with known thyroid status (hyperthyroid or non-hyperthyroid), second, principal component analysis (PCA) were applied to explore associations between selected compounds (BDE-47, -99, -153, -209, BB-209, 2,4,6-tribromophenol and 6-OH-BDE47) and thyroid status (total tyroxine, T_4).

Materials and methods

Materials: Serum samples (0.5-1.5 mL) from 40 Swedish pet cats with hyperthyroid or non-hyperthyroid status were collected and analysed. Hyperthyroid status was determined by their total T_4 levels in plasma. Some of the sick cats were treated with the drug Felimazole (active substance is tiamazole). Samples were drawn during 2010-2011 at the laboratory of University Animal Hospital at the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. After collection and before analysis, the serum was stored in plastic tubes at -20°C.

Method: The individual serum samples were extracted and cleaned-up according to a modified version of the Hovander method[12] and validated for OH-PBDEs, as described by Norrgran et al.[11]. In short, the sample preparation method includes several steps; denaturation of proteins and extraction of analytes, separation of phenolic and neutral compounds, lipid removal and last purification on a sulfuric acid treated silica gel column.

Analysis: The brominated compounds were analyzed by GC (ECNI)-MS in selected ion monitoring (SIM) mode (m/z: 79, 81) and the chlorinated compounds by GC/ECD. PCB, BB-209, PBDEs, OH-PBDEs and simple brominated phenols were identified by retention time using authentic reference standards. The phenolic compounds were methylated during sample preparation and accordingly identified as their methyl ether derivatives.

The PCA analysis: The percentage of BDE-47, -99, -153, -209, BB-209, -6-OH-BDE47 and 2,4,6-tribromophenol relative to the sum was calculated and log-transformed prior to the PCA analysis. Before the PCA scores were plotted, they were centered and scaled to 100%. The eigenvector loadings were added to the PCA plot as vectors. Hotellings 95% confidence ellipses were calculated for the two groups of cats.

Results and discussion

The box-and-whisker-diagrams below (Fig. 1 and Fig. 2) summaries the brominated burden of eleven flame retardants and their potential metabolites (2'-OH-BDE68, 6-OH-BDE47, 5-OH-BDE47, 4'-OH-BDE49, 5'-OH-BDE99, 4'-OH-BDE101) in serum from 40 individual Swedish pet cats. The diagrams also include data of two persistent PCBs (CB-153 and CB-138; Fig. 1) and several brominated phenols (Fig. 2). In a cohort of young Swedish men from 2006, a median serum concentration of 94 pmol/g lipid (n=200) for CB-153 was reported[13]. That is similar to the median concentration of 122 pmol/g fat found in the cats. Median for CB-138 was 117 pmol/g fat as shown in Fig. 2.

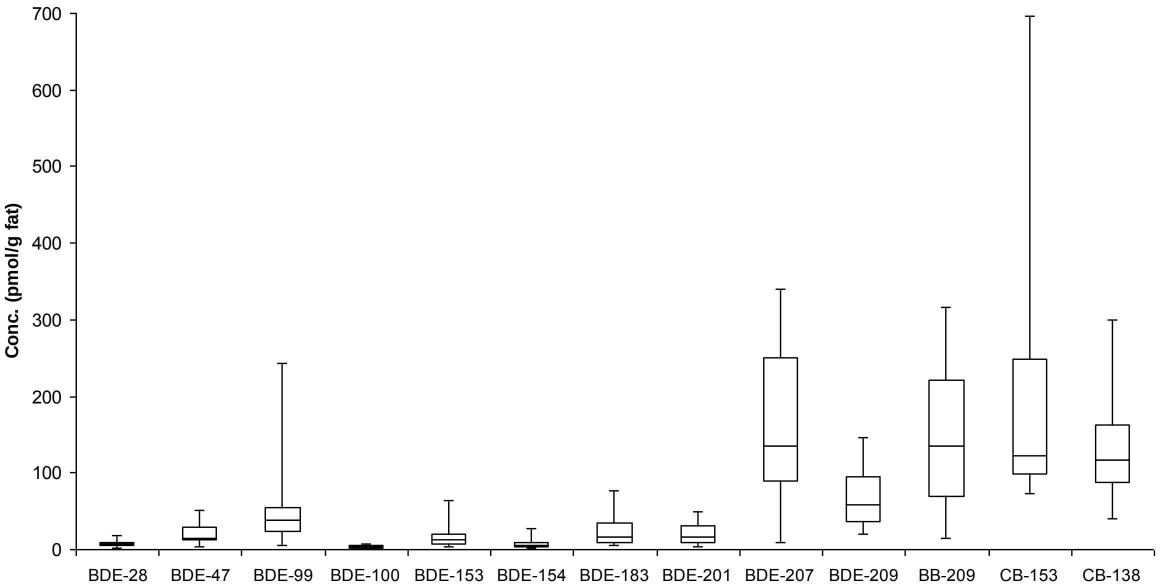

Figure 1. Box-and-whisker diagram of [PBDEs], [BB-209] and [PCBs] in cat serum (n=40)
Lower and upper whisker showing the 10th and 90th percentile

The present data from individual cat serum samples brings out valuable information about the concentration of the compounds in individual cats. The distribution of PBDE congener among the individual cats, as shown by the box-and-whisker diagram (Fig. 1), is similar to that of the pooled serum sample[11]. The pattern resembles that of what has been reported in dust (except for the abundance of BB-209) which suggests dust being a contributing source for exposure of PBDEs. Median value of BDE-47 and BDE-99 was 15 and 38 pmol/g fat. That is more than five times the median concentrations of 2.7 pmol/g fat for 18 year old men signing in for the Swedish army[13]. Further, the relatively high abundance of BDE-99 in comparison to the other congeners suggests a recent or continues exposure, alternatively slow PBDE metabolism in the cat. The most remarkable result is the occurrence of BB-209 in all of the analyzed samples. The median concentration of BB-209 is 135 pmol/g fat which is more than twice the median of BDE-209 (59 pmol/g fat). The result is surprising since the DecaBB production ceased more than a decade ago. The presence of BB-209 can not be explained by dust exposure since it is has not been reported nor discussed as a BFR component in house dust samples. BB-209 has however, been reported in air as a minor pollutant at a recycling plant for electronics in the south of Sweden[14].

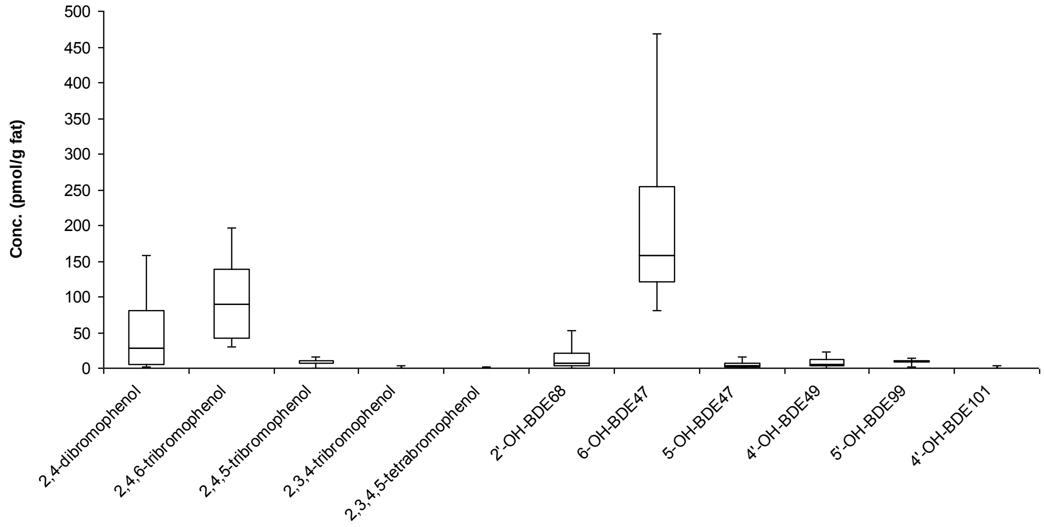

Figure 2. Box-and-whisker diagram of [OH-PBDEs] and [brominated phenols] in cat serum (n=40)
Lower and upper whisker showing the 10^{th} and 90^{th} percentile

The most abundant phenolic compound, 6-OH-BDE47 was present in all of the cats with a median of 160 pmol/g fat followed by 2,4,6-tribromophenol and 2,4-dibromophenol with a median of 90 and 30 pmol/g fat, respectively. All of these three brominated phenols may be metabolites of PBDEs and polybrominated benzenes, at the same time, they are also well-known natural products[15]. This is complicating the issue of metabolic transformations of PBDEs in the cats. However, it is intriguing to see very low concentrations of all other OH-PBDEs, than the natural product 6-OH-BDE47. Considering the relatively high body burden of PBDEs in cats, there might be other important metabolic routes (other than hydroxylation of PBDEs) for elimination of PBDEs in the cat.

In the second step, PCA was performed on selected PBDEs (BDE-47, -99, -153, -209), BB-209, 6-OH-BDE47 and 2,4,6-tribromophenol concentrations, relative to the sum of these substances, to study possible differences in patterns between healthy and hyperthyroid cats. The result from the PCA analysis is shown in Fig. 4. The PCA plot shows a modest, not very defined, but a tendency towards hyperthyroid cats having high concentrations of 6-OH-BDE47 (shown as 6-OH47 in Fig. 3) and 2,4,6-tribromophenol (shown as TBA in Fig. 3). This plot is based on a rather small dataset (8 healthy vs. 19 hyperthyroid cats) and is the first samples to be analyzed as a part of a larger study that is ongoing, including 100 cats (50 healthy and 50 hyperthyroid cats). The remaining 13 cats in this first data set were hyperthyroid cats on medication with normal T_4 levels (medically healthy cats). They were divided into a third group, which is not dealt with herein.

T-test (after normalization of the dataset) was also performed on all of the compounds to explore if there was a statistical significant difference in concentration between the two groups. So far, BB-209 is the only compound to prove a significant difference in concentration between healthy and sick cats ($p< 0.025$) where healthy cats had higher serum concentrations of BB-209 than the sick cats. This may be explained by the increased body metabolism of the hyperthyroid cats which is a direct effect of the disease.

In conclusion, we believe that the reason why we do not see a better distinction between healthy and hyperthyroid cats is that the data set is yet too small. However, it is possible to see a tendency towards hyperthyroid cats having higher concentrations of 6-OH-BDE47 and 2,4,6-tribromophenol. This might explain why earlier studies[7,9] did not find a significant correlation between ∑PBDE and thyroid status, simply because the OH-PBDEs are more potent as endocrine disruptors and of considerable importance for the association with FH. Finally, it is of key interest to determine the source of BB-209.

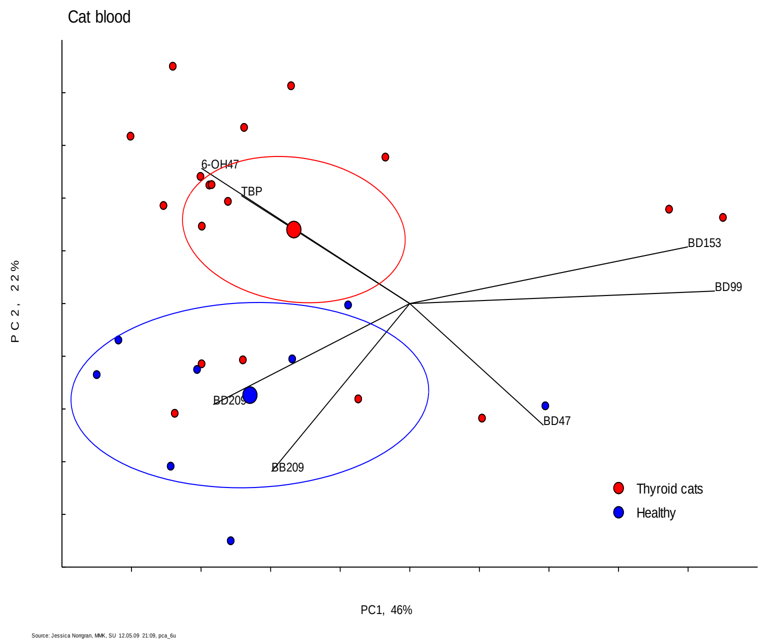

Figure 3. PCA plot: Scatter graph of scores on principal components 1 and 2

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