Cats as a biomarker for exposure to POPs in home environments - with focus on brominated chemicals and associations to feline hyperthyroidism

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Dubium apientiae initium

(Doubt is the origin of wisdom)

René Descartes

List of publications

This thesis is based on the following articles and manuscript, referred to in the text by their roman numerals (I-IV). The published articles are printed with permission from the publisher. Shared first authorship (**Paper IV**) is indicated with an †.

I **Decabromobiphenyl, Polybrominated Diphenyl Ethers, and Brominated Phenolic Compounds in Serum of Cats Diagnosed With the Endocrine Disease Feline Hyperthyroidism**
J. Norrgran, B. Jones, N-G. Lindquist, Å. Bergman
Arch Environ Contam Toxicol. 2012, **63** (1), 161-168

II **Higher PBDE Serum Concentrations Indicate Link to Feline Hyperthyroidism in Swedish Cats**
J. Norrgran, B. Jones, A. Bignert, I. Athanassiadis and Å. Bergman
Environ. Sci. Technol. 2015, **49** (8), 5107-5114

III **Cats' Internal Exposure to Selected BFRs and Organochlorines Correlated to House Dust and Cat Food**
J. Norrgran Engdahl, B. Jones, I. Athanassiadis, Å Bergman, A. Bignert, J.M. Weiss
(Manuscript)

IV **Recovery Discrepancies of OH-PBDEs and Polybromophenols in Human Plasma and Cat Serum versus Herring and Long-Tailed Duck Plasma**
A-K. Dahlberg†, J. Norrgran†, L. Hovander, Å. Bergman, L. Asplund
Chemosphere 2014, **94**, 97-103

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List of abbreviations

POPs	Persistent organic pollutants
OHCs	Organohalogen compounds
OCPs	Organochlorine pesticides
OPs	Organophosphate esters
BFRs	Brominated flame retardants
PBDEs	Polybrominated diphenyl ethers
OH-PBDEs	Hydroxylated PBDEs
MeO-PBDEs	Methoxylated PBDEs
BPs	Brominated phenols
PBBs	Polybrominated biphenyls
HBCDD	Hexabromocyclododecane
TBBPA	Tetrabromobisphenol A
DBDPE	Decabromodiphenyl ethane
PCBs	Polychlorinated biphenyls
PCP	Pentachlorophenol
PCDEs	Polychlorinated diphenyl ethers
HCB	Hexachlorobenzene
4,4'-DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
4,4'-DDE	1,1-dichlorodiphenyldichloroethene
PCDFs	Polychlorinated dibenzofurans
PCDDs	Polychlorinated dibenzo-p-dioxins
PBDFs	Polybrominated dibenzofurans
PBDDs	Polybrominated dibenzo-p-dioxins
PFAS	Per- and polyfluoroalkyl substances
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
EEE	Electrical and electronic equipment
GI tract	Gastrointestinal tract
CSF	Cerebrospinal fluid
ADHD	Attention deficit hyperactivity disorder
TNG	Human toxic nodular goiter
FH	Feline hyperthyroidism
DM	Diabetes mellitus
THs	Thyroid hormones
T_4	3,3',5,5'-tetraiodo-L-thyronine
T_3	3,3',5-triiodo-L-thyronine

TSH	Thyroid-stimulating hormone
TRH	Thyrotropin-releasing hormone
HPT	Hypothalamus-pituitary-thyroid
TTR	Transthyretin
TBG	Thyroxin binding globulin
ALB	Albumin
CYP	Cytochrome P450
THDCs	Thyroid hormone disrupting compounds
TG	Triglycerides
CHOL	Cholesterol
PL	Phospholipids
TL	Total lipids
SRM	Standard reference material
PCA	Principal component analysis
LLE	Liquid-liquid extraction
GC/MS	Chromatography/ mass spectrometry
ECNI	Electron capture negative ionization
ECD	Electron capture detector
SIM	Selected ion mode
LOD	Limit of dectection
LOQ	Limit of quantification
PFBCl	Pentafluorobenzoyl chloride
CDC	Centers for Disease Control and Prevention
EFSA	European food safety authority
EPA	Environmental protection agency
IUPAC	International union of pure and applied chemistry
UNEP	United Nations Environmental Programme
WHO	World Health Organization

1 Introduction

We live in a world with numerous man-made chemicals, such as herbicides, pesticides, pharmaceuticals, preservatives, plasticizers, detergents and flame retardants (FRs) among many other chemicals. Their use in everyday living has improved our lives since the industrial revolution started. However there are two sides of the coin, and slowly, it became apparent that these industrial chemicals may exert undesirable adverse health effects in humans, domestic animals and wildlife. An adverse health effect is defined as "the potential of lowering the quality of life, contributing to a disabling illness, or leading to a premature death"[1].

The discovery of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (4,4'-DDT) as a potent insecticide for controlling vector diseases such as malaria and yellow fever was at first considered revolutionary. Paul Hermann Müller, the researcher that discovered the strong insecticidal property of 4,4'-DDT, was later awarded the Nobel Prize in Physiology or Medicine in 1948 for his discovery. After using the insecticide for two decades, it became evident that one of the major metabolites of 4,4'-DDT, 1,1-dichlorodiphenyldichloro-ethene (4,4'-DDE) affected the eggshell thickness in birds of prey, leading to dramatic population declines. The white-tailed sea eagle (*Haliaeetus albicilla*) were, due to the 4,4'-DDT contamination, close to extinction in e.g. Sweden [2]. The trend luckily changed direction due to the banning of 4,4'-DDT in most industrialized countries in the early 1970s. Today, 4,4'-DDT together with 25 other persistent organic pollutants (POPs), or classes of compounds, are regulated by the Stockholm Convention, a global treaty. The treaty has to date been ratified by 179 parties [3].

The focus within environmental research has been on the classical POPs which are chemicals that have shown toxic and persistent properties which means they degrade slowly in biota and in the environment. They are also subject for long-range transport [4]. Even though the POPs were manu-factured for specific aims e.g. polybrominated diphenyl ethers (PBDEs) as FRs in textiles and electrical equipment, they manage to spread into the environment. This is due to occasional discharges from e.g. release of FR treated consumer products, manufacturing plants, factories using FR in the production line or from waste disposal sites. POPs are ubiquitous, and are found even in remote environments such as the Artic [4]. PBDEs has for

instance been found in polar bears (*Ursus maritimus*) [5] and Greenland shark (*Somniosus microcephalus*) [6], which has not been close to a densely populated areas or industry.
The commercial production of PBDEs started in the 1970s [7] and one of the first report on PBDEs in the environment came from a study conducted by the U.S Environmental Protection Agency on sludge, soil and air close to manufacturing plants in New Jersey and Arkansas [8]. The first report on PBDEs found in the environment in Europe, was in fish from river Viskan and Klosterfjorden in Sweden [9]. Because of low reactivity and high lipophilicity, PBDEs are persistent and bioaccumulate in wildlife and enrich in food webs. The first scientific reports on PBDEs in human blood and mothers' milk were reported in 1997 and 1998, respectively [10,11]. A time-trend study of PBDEs in Swedish human milk, in 1972-1997, showed a dramatic increase in levels, more than 50 times over the time period [12]. It became evident that these industrial chemicals had found their way in to humans.

Over the last 20 years, focus has been on the hypothesis that environmental contaminants may interact with hormones in wildlife and humans, disturbing the hormone homeostasis, leading to various endocrine disease related endpoints such as diabetes, endocrine related cancer (breast, endometrial, ovarian, prostate, testicular and thyroid), immune-related and thyroid-related disorder (hypo- and hyperthyroidism) and neurodevelopmental disorders (ADHD, autism) [13].

The two most important exposure sources of BFRs such as PBDEs today are via food and dust intake in the indoor environment. This thesis will make use of the cat as a model to assess exposure to BFRs and other POPs in the home environment. Cats' exposure to dust in the home environment is larger than to adult humans due to their grooming behavior. This high exposure to dust is somewhat similar to that of small children because of their hand-to-mouth behavior, exploring by putting anything to their mouth. Further, cats have since the late 1970s showed an increasing incidence of hyperthyroidism, which is a common disorder in humans and today the most common endocrine disease in pet cats.

1.1 Aim of thesis

The aim of this thesis was firsthand to assess the body burden of brominated chemicals present in serum from cats to explore potential associations between contaminant concentrations and feline hyperthyroidism (FH) in cats. Analysis included neutral and phenolic compounds, foremost in respect to PBDEs. The aim included cats' external exposure, from commercial food and house dust, to brominated compounds.

In **Paper I**, the aim was to screen for brominated compounds in a pooled serum sample of hyperthyroid cats. Neutral compounds such as PBDEs and polybrominated biphenyls (PBBs), as well as phenolic substances such as OH-PBDEs and polybrominated phenols were identified in order to gain understanding of cats' metabolism.

In **Paper II**, the aim was to quantify the previous identified compounds (PBDEs, BB-209, OH-PBDEs and polybrominated phenols) in individual cat serum samples (n=82). The body burden of these brominated compounds was then compared between cats with normal thyroid status and those diagnosed with hyperthyroidism to explore a possible link between internal exposure to brominated substances and FH.

In **Paper III**, the aim was to explore cats' external exposure of brominated compounds from commercial cat food and house dust. Serum sample from pet cats (n=29) were matched with cat food (n=28) and dust samples (n=17) collected from their homes to explore possible correlations between external and internal exposure.

In **Paper IV**, the aim was to validate the analytical method used for serum extractions to be applicable for analysis of OH-PBDEs and polybrominated phenols in various blood matrixes, including cat serum. Recoveries of four bromophenols and nine OH-PBDEs were investigated in serum or plasma from human, cat, bird (long-tailed-duck) and fish (herring).

2 Flame retardants

Flame retardants are chemicals used in goods and materials such as textiles, polymers (i.e. plastics), electrical and electronic equipment, building and construction materials. They are aimed to slow down the development of fires by prolonging the time from ignition to a developed fire. Fire as a hazard, is an old threat to mankind, and already the ancient Egypt in 450 BC, alum was used to make wood more resistible toward fire [7]. The FRs used today emerged from needs due to increased production of flammable polymeric materials in the beginning of the 20th century. These new materials were cheap to produce but much more flammable compared to natural materials, such as wood and natural fibers (e.g. silk, wool and cotton). To compensate for this, these new polymer materials were treated with FRs.

FR are divided into subgroups depending on chemical composition; *inorganic*, *halogenated*, *phosphorus* and *nitrogen-based* chemicals, which are divided into reactive or additive FRs, depending on how they are in-corporated into the polymer. A reactive FR is chemically bound into the structure of the material during production whilst an additive is molded into the material. The additive FR may leak out of the goods over time.

Inorganic FR composes the largest group of FRs based on production volume. Inorganic salts such as aluminum and magnesium hydroxide ($Al(OH)_3$, $Mg(OH)_2$) and zinc borate ($4ZnO(B_2O_3 \cdot H_2O$) are commonly used. This group also includes FR synergists which are chemicals used together with some another chemical with the purpose to enhance its efficiency, e.g. antimony trioxide (Sb_2O_3). The group of halogenated FRs includes chlorinated paraffins, aliphatic and aromatic compounds and polymeric materials. The phosphorus FRs constitutes about 20% of the total use globally. Both inorganic and organic phosphorous compounds are included in this group. A recent article lists all present brominated, chlorinated and phosphorus containing FRs [14]. Nitrogen based FR includes mainly melamine and melamine derivatives and are often used together with phosphorous containing FRs [15].

This thesis addresses BFRs, i.e. PBBs, PBDEs, hexabromocyclododecane (HBCDD) and tetrabromobisphenol A (TBBPA) (Figure 1). Emerging or novel BFRs replacing the previously applied technical PBDE mixtures and HBCDD are not to be considered within the scope of thesis.

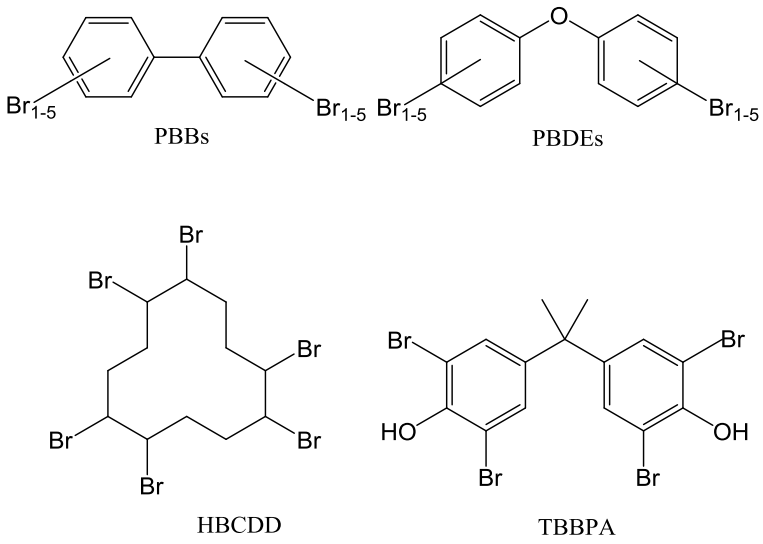


Figure 1. General chemical structures of polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs), both with a theoretical number of 209 congeners (upper row). Hexabromocyclododecane (HBCDD) consisting of three main isomers (α-, β- and γ-HBCDD) and tetrabromobisphenol A (TBBPA) (lower row).

2.1 Brominated flame retardants (BFRs)

The BFRs constitute about 25% of the global FR production by volume and there are 55 different products registered commercially at present [14]. TBBPA, HBCDD, Penta-, Octa-, and DecaBDE together constitute the highest production volumes among the BFRs [16]. BFRs are cost-effective to use in products. The loadings, i.e. how much are needed to meet flammability standards in weight are 2- 25% which is lower in comparison to other groups of FR [15].

For a fire to evolve, the material needs to come to vaporization and create a flame that can be ignited. Halogenated FRs' mode of action is by preventing free radical formation during this initial step of fire. Vaporization of the polymer creates reactive radicals (OH^*, H^*) that starts a chain reaction and

more radicals are formed. In the presence of heat, in the vapor phase, the halogen bonds of the flame retardant breaks to produce halogen radicals (X^*) which reacts with the polymer (R-H) to produce R-X and halide gas H-X. These halide radicals react with the reactive radicals [OH^*/H^* + H-X → X^* + H_2/ H_2O] to make them less reactive. However, the smoke and corrosive gases (e.g. hydrochloric and hydrobromic acid) formed in a fire, is a drawback when using halogenated FRs [17]. Another disadvantage of using additive FRs in a product is that they are prone to leak and migrate, out of the product leading to environmental contamination and less fire protection.

TBBPA is used both as an additive and a reactive FR. It is produced in the highest production volume of the BFRs. It is primarily used as a reactive FR in production of epoxy and polycarbonate resins for printed circuit boards. The molecule, despite having two hydroxyl substituents, is fairly insoluble in water (0.72 mg/L). pKa is 7.7 and 8.5 for the first and second hydroxyl respectively. TBBPA is highly lipophilic with log K_{ow} of 4.5. The half-life of TBBPA is short and estimated to about 2 days in humans [18].

HBCDD is a cyclic aliphatic compound containing six bromines. There are 16 possible stereoisomers of HBCDD. Technical HBCDD consists mainly of the γ- isomer (75-89%) and further also of α- (10-13%) and β-HBCDD (1-12%) and some impurities [19,20]. The main application for HBCDD is in polystyrene foam used in building construction (e.g. house walls, cellars). Another area of application is in textiles such as upholstered furniture, interior textiles, cushions and insulation in cars [21]. HBCDD has low vapor pressure, it is highly lipophilic (log K_{ow} is 5.8) and has low water solubility (0.0034 mg/L). HBCDD are effectively absorbed by the gastrointestinal (GI) tract upon dosing and are highly bioaccumulative and persistent. The biological half-life in humans has been calculated to about 64 days [22]. HBCDD was recently recognized as a POP and added to the list of regulated substances under the Stockholm Convention under Annex A, substances to be eliminated [3].

2.2 Polybrominated biphenyls (PBBs)

PBBs were one of the early BFRs produced commercially, with known manufacturing from at least around 1970. The PBBs were mainly used in thermoplastics (acrylonitrile-butadiene-styrene, ABS), but also in textiles and electronic equipment. They were produced in three technical mixtures; Hexabromobiphenyl (HxBB), Octabromobiphenyl (OBB), and Decabromobiphenyl (DBB). PBBs are persistent, highly lipophilic compounds with low vapor pressure and water solubility. Average Log K_{ow} for tetra-, hexa- and decabrominated PBBs are 6.5, 8.8 and 9.4, respectively [7,21].

PBBs are perpetually associated to the "Michigan farm incident" in 1973 when the toxicity of PBBs reached public awareness. Magnesium oxide, used as supplement in livestock and poultry feed with the trade name "Nutrimaster" was mixed up with "Firemaster", which was the trade name for FireMaster BP-6. The major PBB congener present in BP-6 were 2,2',4,4',5,5'-hexabromobiphenyl, BB-153. In addition to poisoning of farm animals, consumption of contaminated milk, beef and eggs were estimated to affect over nine million people. This fatal exposure went on for almost a year before the source of contamination was identified and measures could be taken [18,21,23].

The Michigan farmer residents demonstrated similar symptoms as those shown by PCB intoxicated humans from the "Yusho" incident in Japan in 1968 [24]. Symptoms such as tiredness, fatigue, loss of appetite, weight loss, pain and swelling of joints, abdominal pain and cutaneous effects such as halogen acne, diffuse alopecia i.e. hair loss were reported among the exposed farmers [18,23]. Chronic effects of PCBs and PBBs include thyroid hormone disruption, lowered immune response, impaired reproduction, and adverse neurodevelopmental effects and possibly liver cancer. Exposure to PBBs in adults in Europe today occurs mainly through consumption of fish and other seafood whilst the main source of exposure for infants and children are through mothers' milk and dairy products [25].

PBBs are readily absorbed by the intestines following ingestion and are distributed to adipose tissue, liver, kidney, heart, skin in the rat. Hydroxylated metabolites and debrominated products of the HexaBB are found mainly in feces [25]. Half-lives of BB153 in exposed woman from "the Michigan farm incident" was calculated to 13 and 29 years, for women with an initial serum concentration of >10 ppb and < 10 ppb, respectively [26].

HxBB was banned in the U.S 1974, the year after the Michigan farm incident occurred but production of OBB and DBB continued until 1979. In Europe, the last production of DBB was terminated in 2000 [7,27]. PBBs as a group of chemicals are now regulated under the EU Restriction of Hazardous Substances (RoHS) directive [28].

2.3 Polybrominated diphenyl ethers (PBDEs)

PBDEs have been produced since the 1970s to serve as FRs in a variety of products such as thermoplastics, electrical and electronic equipment (e.g. TV-screens, computers), textiles, and in building and construction materials. They were sold commercially under different trade names in three technical mixtures; Penta-, Octa- and DecaBDE depending on the average bromine content. The backbone consists of two aromatic rings connected with an ether bridge, leaving 10 available positions for bromine in the molecule (Figure 1). Structurally, there are 209 possible congers of PBDEs, numbered similarly as for PCBs and PBBs [29]. PBDEs are produced from bromination of diphenyl ether in the presence of a catalyst. This synthetic pathway is unspecific and therefore several congeners are formed in the process. The number of existing PBDE congeners is however less than for the PCB congeners. The average content of various congeners present in technical mixture are given in Table 1 [30].

Table 1. Average relative content of PBDE congeners present in technical PBDE mixtures (WHO/ICPS, 1994)

Technical	**Congener %**					
Product	**tetraBDEs**	**pentaBDEs**	**hexaBDEs**	**heptaBDEs**	**nonaBDEs**	**decaBDE**
PentaBDE	24-38	50-60	4-8			
OctaBDE			10-12	44	10-11	> 1
DecaBDE					< 3	97-98

The congener composition of commonly used PentaBDE, OctaBDE and DecaBDE has been determined by for instance La Guardia et al. (2006) [31]. In PentaBDE, the tetra- and heptabrominated congeners BDE-47 and BDE-99 were found to be the most abundant components in DE-71 and Bromkal 70-5DE. BDE-47 was present in 38.2% and 42.8% and BDE-99 in 48.6% and 44.8%, respectively. In OctaBDE, the hepta- and octabrominated congeners, BDE-183 and BDE-197, were the major components in DE-79 and Bromkal 79-8DE. BDE-183 was present in 42% and 12.6% and BDE-197 in 22.2% and 10.5%, respectively. In addition, in Bromkal 70-5DE, BDE-209 was also present in 49.6 % and BDE-206 and BDE-207 in 7.66% and 11.2%. The fully brominated diphenyl ether was found in 96.8 % and 91.6 % in Saytex 102E and Bromkal 82-0DE, two common DecaBDE formulations. BDE-206, the most abundant nonaBDE, present in 2.19 and 5.13%, respectively, in the technical formulations [31].

The physiochemical properties of PBDEs are low vapor pressures and high lipophilicity that increases with increasing number of bromine in the molecule. Log K_{ow} for tetraBDEs is between 5.9 - 6.2, pentaBDEs, 6.5 – 7.0,

octaBDEs 8.4 - 8.9 and for decaBDE 10 [32]. The lower brominated PBDEs, associated with PentaBDE, are more persistent, bioaccumulative and are more prone to biomagnify in biota than the higher brominated diphenyl ethers, associated with DecaBDE, due to slow degradation and being highly lipophilic.

Because of insight about their persistency, bioaccumulation and toxicity (chapter 2.6.2), the use of PentaBDE and OctaBDE were banned in the European Union in 2004 [33,34]. Concurrently, the Unites states producer phased out the two PBDE products on the U.S market. The commercial production of PentaBDE in China was also discontinued in 2004 whereas the OctaBDE formulation was never manufactured in China [35].

PentaBDE has been used extensively in the State of California to meet the flammability standard (TB117) on polyurethane foam to meet the smolder and open-flame tests. As of January 1st, 2015, California implemented a new legislation, making it mandatory to declare if and then which FRs is used in polyurethane foam containing products. The original flammability standard TB117 was also replaced with TB117-2013, which only require products to pass an updated smolder test to diminish the usage of FRs [36].

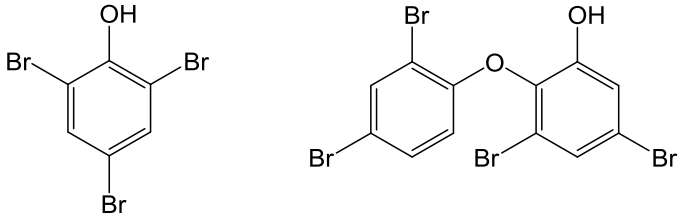
In Europe, DecaBDE is regulated under the EU Restriction of Hazardous Substances (RoHS) directive since 2008 [28] but has been suggested as a candidate for listing on Stockholm Convention [37]. The phase out of DecaBDE in the United States started in the end of 2013 [15]. Currently, there are no restrictions the production and use of DecaBDE/BDE-209 in China. The DecaBDE production plants are all situated in Laizhou Bay of Shandong provinces, in the northeast of China by the Yellow Sea. It is estimated that Asian-Pacific countries accounts for the largest turnover of FRs in electrical appliances globally, of which China plays a key role [35].

2.4 Hydroxylated-PBDEs (OH-PBDEs)

2.4.1 Natural production

Hydroxylated PBDEs (OH-PBDEs) and simple polybrominated phenols (Figure 2) are produced naturally in the marine environment through various marine plants and animals [38]. They have been found in the Baltic Sea [39,40] as well as in tropical waters e.g. outside the coast of Australia [38]. The main producers include seaweed, sponges, algae, corals, tunicates, bacteria and other marine life of the oceans. The identified naturally produced organohalogens are many and include molecules such as halogenated

alkanes and simple phenols to complex phenols such as tyrosines or hydroxylated and methoxylated diphenyl ethers [41]. Naturally produced OH-PBDEs are formed through oxidative dimerization of phenols. One characteristics of OH-PBDEs is related to position of the –OH or –MeO group in the molecule, i.e. substituted in the *ortho*-position [38,42,43]. Humans are typically exposed to these naturally produced compounds via seafood.



2,4,6-TBP 6-OH-BDE47

Figure 2. Examples of two naturally produced phenolic brominated compounds; 2,4,6-tribromophenol (2,4,6-TBP) and 3,5-dibromo-2-(2,4-dibromophenoxy)phenol (6-OH-BDE47).

2.4.2 Anthropogenic sources

Brominated phenols (BPs) are also manufactured industrially, whereof 2,4,6-TBP in largest production volumes. 2,4,6-TBP is used in production of BFR, as a reactive flame retardant intermediate and as a chemical in preserving wood. 2-BP, 2,4-DBP, 2,6-DBP, and 2,4,6-TBP have also been identified in vehicle emissions of gasoline containing lead [44].

In vivo metabolism of anthropogentic PBDEs is another source for internal exposure to OH-PBDEs in humans and rats [45,46]. In contrast to naturally produced compounds, metabolically formed OH-PBDEs are most commonly substituted in the *meta*,- and *para*-positions of PBDE [45,46]. Metabolism of PBDEs is further described in chapter 2.6.1.2.

2.5 Exposure sources of PBDEs, levels and trends

2.5.1 Human exposure

The internal exposure of PBDEs measured in blood (serum), milk and adipose tissue from humans varies between countries but are in general

about 10 times higher in North Americans compared to Europeans and Asians [18,47,48]. The higher concentrations measured in North Americans is a result of heavier usage of PBDEs to meet flammability standards [36,49].

The general human exposure to PBDEs in Europe and North America comes via dietary intake and inhalation/ingestion of indoor dust [18,47,48,50-52]. The main dietary contributor varies between countries and depends of the diet of choice. There are also differences in body burden in-between individuals. In general animalistic food products tend to contain higher levels of PBDEs due to their lipophilic content and vegetarians have lower PBDE burdens [51]. In general, the highest PBDE containing food items are fish> meat > dairy ≤vegetables [47]. Amongst the PBDEs, BDE-47 and BDE-209 constitute for the highest levels dietary exposure [47,51].

In Sweden for instance, the largest exposure to PBDEs in food is estimated to come from consumption of fatty fish and dairy products, whereas meat is considered the major contributing food source in the United States. For an breastfeed infants, mothers' milk will be an important source of exposure [47,51,53]. Other food items reported high in PBDEs besides fish are egg and poultry (if grown close to point sources of discharges) [54]. In other parts of the world e.g. in developing countries, point sources of discharges, as living or dwelling on landfill or close to a contaminated city dump, may be significant exposure sources.

Unlike traditional POPs, the general and probably the most important source of PBDE exposure comes from the indoor environment via inhalation, ingestion and dermal contact to dust or consumer products [48,50,54,55]. The indoor environment includes e.g. homes, offices, daycares, public buildings and cars. In a review article by Lorber et al. (2008), the daily intake of PBDEs from food and house dust were calculated and compared with body burden found in human serum and milk. It was found that inhalation and ingestion of PBDEs, via water and food, only represented 18% of the total exposure, whereas ingestion and dermal contact to house dust accounted for 82% of the total exposure of PBDEs [48]. Dust as an exposure pathway has also been suggested as the major contributor to the higher PBDE levels found in young children (2-5 years) compared to adults because of their pronounced hand-to-mouth activity and spending a lot of time on the floor [55-58].

2.5.1 Occupational exposure

Occupational exposure is an important source of exposure for workers of the flame retardant manufacturing plants or production line of materials and products impregnated with these chemicals [59]. Also personnel working with dismantling of e-waste and recycling of flame retardant containing goods have additional exposure compared to the general population [60-63].

2.5.2 Non-sound E-waste managing

Non-sound managing of e-waste is a growing environmental contamination source of concern for PBDEs and other hazardous compounds. China, Ghana, India, and Thailand are some of the countries engaged in this business. Enormous volumes of electronic scrap are shipped from economically strong countries for processing [35]. As an example, Ghana imports over 200 000 tons of second-hand or end of life EEE, that are turned over to e-waste once in the country to bypass national regulations such as the Basel convention [64].

United States and China are the two largest countries to produce e-waste followed by India [65]. E-waste which includes all types of electrical and electronic equipment (EEE) is a profitable business since there is potential to extract elements such as iron, aluminum, copper or valuable metals such as gold and silver [65]. On the downside, e-waste contains several hazardous compounds such as lead, mercury, chromium, various chemicals present in plastics and FRs which may pose a threat to human health and the environment if not handled in a sound way.

2.5.3 Levels and trends in humans and the environment

The environmental concentrations (in soil and sewage sludge) for the typical PentaBDE congeners, BDE-47 and BDE-99, are now decreasing in Europe. In Sweden, an annual decrease of about 20 % for BDE-154 and BDE-183 were seen in sewage sludge 2004-2010 [54]. During the same time period an annually increase by 16% was recorded for BDE-209. The time trend for HBCDD were studied in herring gull eggs from the North and Baltic Sea costs in 1998-2008 [54]. An increasing trend were observed until 2000, before concentrations levelled off and started to decline. From North America, a study of archived sewage sludge 1974-2008 from Chicago (IL), USA, were reporting declining concentrations of pentaBDE congeners from about 2000. Simultaneously, from the same study decaBDE concentrations were increasing, with a doubling every 5 years during 1995-2008 [54]. In the Artic, the temporal trends for PBDE and HBCDD are ambiguous, with indications on concentrations both increasing and levelling off depending on

matrix studied [19]. Temporal trends for PBDEs and HBCDD in Asia was unclear [54].

In serum from Swedish young men, signing in for military training, significantly declining levels of BDE-47 and BDE-99 have been shown between 2000 and 2009/2010 [66]. The median concentrations decreased from 1.32 to 0.63 ng/g (2.7 to 1.3 pmol/g) lipid and from 0.38 to < LOD ng/g (0.67 to < LOD pmol/g) lipid for BDE-47 and BDE-99, respectively [66]. In a temporal trend study of HBCDD and PBDEs in Swedish mothers milk 1980- 2004 it was concluded that the levels of BDE-47 and -99 peaked in the mid-1990s at around 4 and 1.5 pmol/g lipid respectively and was declining to around 2 and 0.5 pmol/g lipid respectively in 2004 [53,67]. During the same time period, the concentrations of HBCDD were increasing, nearly a fivefold, from about 0.1 pmol/g lipid to 0.6 pmol/g lipid [53,67].

2.6 Toxicokinetics of PBDEs

PBDE risks are related to uptake, distribution, metabolism and excretion, in short ADME. The toxicokinetics is discussed in some further detail, below.

2.6.1 Adsorption, Distribution, Metabolism and Excretion

2.6.1.1 Adsorption

PBDEs may enter the body via the lungs, through oral ingestion, skin diffusion and via placental transfer. All routes involve passive diffusion through biological cell membranes. Size, pKa and lipophilicity, are important factors influencing absorption of a chemical into the body. Since PBDEs span over a wide range of molecular masses, the bioavailability and toxicity amongst them will vary. It is notable however, that even with its high molecular mass, BDE-209 is bioavailable, as shown in numerous exposure assessment studies. Much of the knowledge regarding the effects of PBDEs has been learned from animal studies on rat and mice fed with commercial PBDE mixtures and/or single PBDE congeners [46,68,69].

Lower brominated PBDEs (e.g. PentaBDE congeners) are effectively taken up by the gastrointestinal (GI) tract upon an oral administration. From the GI tract, the PBDEs enter the bloodstream via the liver and are distributed to other tissues in the body, mainly lipid rich tissues (e.g. liver and adipose tissue), due to the lipophilic characteristics of the PBDEs. In a study, ^{14}C-BDE-99, -100, and -154 was given to rats as a single dose and the gastro-intestinal absorption was measured to 50, 73 and 77%, respectively [51].

Uptake of BDE-209 from the GI tract is lower than for medium brominated diphenyl ethers because of its higher molecular mass, but still taken up making the congener bioavailable. In mice and rats, the uptake after an oral administration of ^{13}C-BDE-209 was measured to between 1-10% [70].

2.6.1.2 Distribution and excretion

In the very first metabolism study of BDE-47, both rat and mouse were given an oral dose of the ^{14}C-labeled compound [69]. The study showed that 86% of the dose in rat remained in the body 5 days after exposure mainly stored in adipose tissue as the parent compound. Fourteen percent of the dose was excreted via feces as parent compound and non-conjugated metabolites, and less than 0.5% was found in the urine. It was estimated that approximately 3% of the dose was excreted as metabolites. In the mouse, 47% of the administrated dose remained in the body after 5 days, 20 % was excreted via feces, as parent compound and non-conjugated metabolites, and 33% via urine. In total, 39% of the dose was excreted as metabolites. Five days after dosing adipose tissue contained the highest concentrations of radioactivity followed by lung, kidney, liver and brain. Further, presence of hydroxylated metabolites of BDE-47 was indicated in the rat plasma in low amounts. The occurrence of OH-PBDE metabolites were confirmed and identified in rat plasma [46] and later in human plasma from highly exposed children in Nicaragua [45] and non-occupationally exposed pregnant women in Indiana, USA [71]. OH-PBDEs, metabolites of PBDEs, are accumulated in blood, bound to blood proteins and found in tissues with high blood perfusion e.g. liver and kidneys.

In humans, serum concentrations of BDE-47, -99, -100 and -153 showed positive correlations between mothers and their toddlers demonstrating similar exposures, which is an indication of placental and/or mothers' milk transfer [72]. No correlation was found for BDE-209 between mothers and their toddlers. However, in rats, BDE-209 were shown to cross the placenta and mothers' milk to exposure fetuses and lactating pups [73]

2.6.1.2 Metabolism

Exposures to technical PBDE mixtures and individual congeners have shown to induce both phase I and phase II enzymes present in hepatic microsomes from human, rat and rainbow trout [74-76]. DecaBDE was shown to have lower enzyme-inducting capacity than Penta- and OctaBDE. In addition, some studies of rats exposed to commercial PBDE formulations have shown increased activities of CYP1A1 and 1A2. This conclusion suggests presence of dioxin-like impurities in the technical products since induction of these enzymes are established responses of halogenated dioxin-like compounds [21].

Metabolism of the lower brominated PBDEs in humans occur through the same mechanisms as for PCBs i.e. via oxidation to form a phenol which in general are conjugated with a hydrophilic group, glucuronic acid or sulfate, to increase the hydrophilicity and to promote elimination. The oxidation is mediated through the CYP450 enzyme system either by direct insertion of a hydroxyl group or through an arene oxide intermediate. This metabolic pathway implies formation of two isomeric mono-hydroxylated OH-PBDEs, where one possibility is a result of a 1,2-bromine shift, i.e. when one bromine atom move position in the aromatic ring. In total, six hydroxylated tetraBDEs may be formed from BDE-47; 5-OH-BDE47, 6-OH-BDE47, 3-OH-BDE47, 4-OH-BDE42, 4'-OH-BDE49 and 2'-OH-BDE66 (Figure 3). Further, these tetraBDEs may be subject for debromination to triBDEs [71]. In an *in vitro* study from incubations of BDE-47 in human hepatic microsomes 5-OH-BDE47, 6-OH-BDE47 and 2,4-DBP was determined as the major metabolites [75].

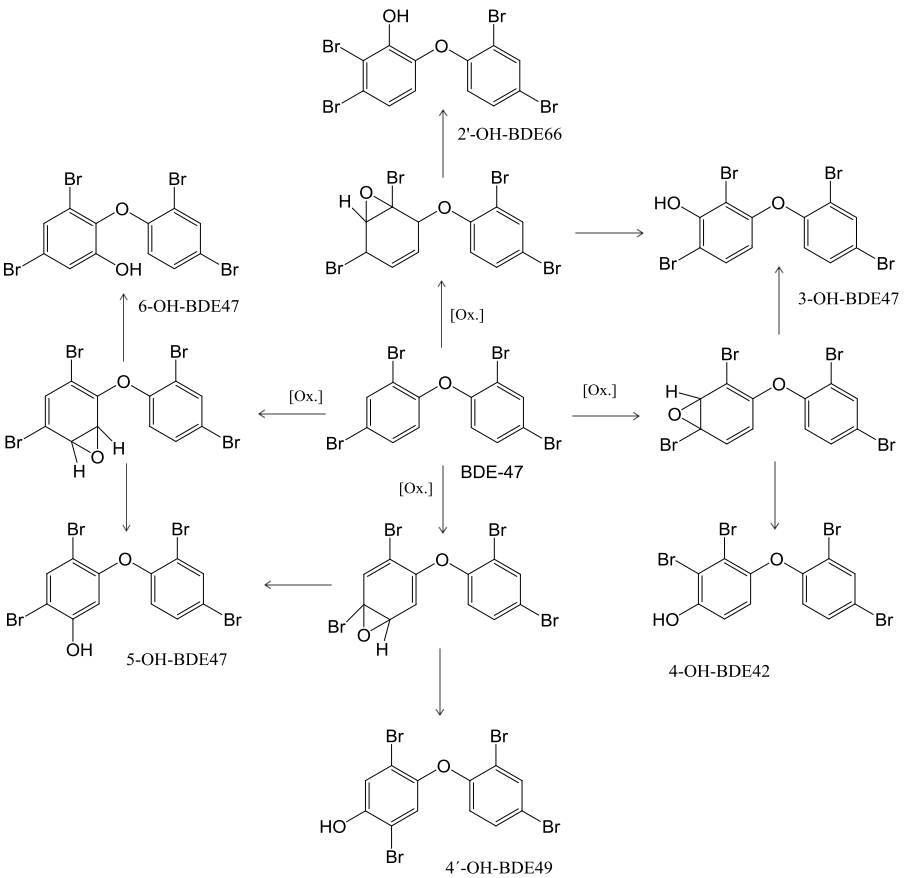


Figure 3. Oxidative metabolism of 2,2',4,4' tetrabromodiphenyl ether (BDE-47) in humans (Qiu et al. 2009).

In human plasma, 5-OH-BDE47 followed by 6-OH-BDE47 was found to be the major hydroxylated tetraBDEs present [45,71]. Similarly as for BDE-47, 11 hydroxylated pentaBDEs may theoretically be formed from BDE-99, all of which are subject to undergo debromination [77]. In an *in vitro* study of human liver microsomes six metabolites of BDE-99 were identified; 2,4,5-TBP, 4-OH-BDE90, 5'-OH-BDE99, 6'-OH-BDE99, 4'-OH-BDE101 and 2-OH-BDE123 [78]. The main OH-PBDEs metabolites of BDE-99 reported from plasma in non-occupationally exposed pregnant woman in Indiana, USA, was 5'-OH-BDE99 and 6'-OH-BDE99 accounting for 90% of the metabolites [71]. Further, has dihydroxylated metabolites of BDE-47- and BDE-99 been identified in incubation of BDE-47 and BDE-99 in human liver microsomes [79]. In addition, was CYP2B6 identified as the major enzyme involved biotransformation of BDE-47 and BDE-99 [75,78,79]. Feline metabolism of PBDEs is further discussed in Chapter 6.3.

The second proposed phase I metabolism pathway for PBDEs, is via cleavage of the ether bond, i.e. hydrolysis to form a simple polybrominated phenol and a brominated catechol [80] (Figure 4). Presence of 2,4-DBP and 2,4,5-TBP reported from incubation studies of BDE-47 and BDE-99 in human liver microsomes studies are indicatives that cleavage of the ether bond occurs [79]. This metabolic pathway has been described as a minor route in metabolism of polychlorinated diphenyl ethers (PCDEs) in rats [81]. Cleavage of CDE-74 resulted in formation of 4-chlorophenol and 2,4,5-trichlorophenol.

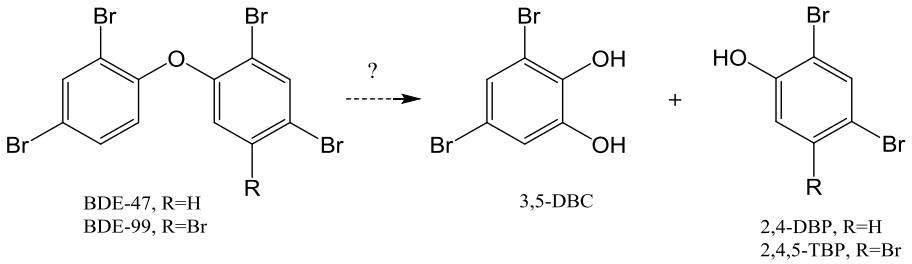


Figure 4. Suggested metabolic pathway for PBDEs includes cleavage of the ether bridge, leading to formation of a polybrominated catechol (3,5-dibromocatechol [3,5-DBC]) and phenols (2,4-dibromophenol [2,4-DBP] and 2,4,5-tribromophenol [2,4,5-TBP]).

Metabolism of the higher brominated PBDEs such as BDE-209 has mainly shown to occur through reductive debromination in biota similarly as in sediment or photolytically [54,76,82]. Debrominations of BDE-209 yields lower brominated congeners, firsthand nonaBDE isomers, which are metabolites of greater bioavailability potencies.

The PBDEs containing 4 to 6 bromines are the most persistent congeners. Lower or higher brominated congeners have shorter half-lives and/or lower bioavailability. The half-life of the fully brominated diphenyl ether, BDE-209, was measured in occupationally exposed workers to 15 days, the three nonaBDEs to between 18-39 days, BDE-203 to 37 days and BDE-183 to 94 days in humans [83]. Applying a pharmacokinetic model on non-occupational exposed humans based on estimated daily intake (DI) and measured body concentration of these compounds, half-lives of BDE-47 and BDE-99 was estimated to 644 and 1040 days. Similarly was the half-lives for BDE-100, BDE-153 and BDE-154 estimated to 573, 2380 and 1214 days, respectively [84].

2.6.2 Toxicity

PBDEs have low acute toxicity. Nevertheless, they are of concern since they have shown to affect the endocrine system at multiple sites. Main targets of PBDEs were thyroid hormone system and the reproductive and nervous system as well as the liver [51]. PBDEs have also shown to induce hyper-plasia in the thyroid gland and to alter the thyroid hormone homeostasis [51]. Following exposure to PBDEs (e.g. BDE-47, -99, -209, DE-71 or Bromkal 70-5DE), the thyroid hormones were lowered in rat, mice and fish, i.e. early signs of hypothyroidism [51,85]. Much of the concern regarding the toxicity of PBDEs involves the transformation products, the OH-PBDEs. There are several suggested mechanisms on how parent compounds/phenols may interfere with the thyroid hormone system decreasing the totl T_4 levels in plasma [16,86]. It has been shown that technical mixture of PBDEs (Penta- and OctaBDE) could induce liver UDP-glucuronosyltransferases, which are involved in the conjugation of T_4, thereby increasing the plasma clearance of the hormone [16,86]. The second proposed mechanism is by mimicking the structure of the native hormone acting competitively in binding to any of the thyroxin (T_4) transporting plasma proteins (thyroxin binding globulin (TBG), transthyretin (TTR) and albumin (ALB)) [16]. Special attention has been directed to the *para*-substituted diphenyl ethers with one or two bromines substituted next to the hydroxyl group that is structurally similar to the native thyroxine hormone (Figure 5). Phenolic compounds such as 2,4,6-TBP, TBBPA and 6-OH-BDE47 have shown TH-disrupting potencies by competitively binding to human TTR. 2,4,6-TBP, TBBPA and 6-OH-BDE47 were 10.2, 1.6 and 0.26 times as potent in binding to TTR as the hormone itself [85,87,88]. A third proposed mechanism by which PBDEs may interact with the thyroid hormone homeostasis is by direct contact with thyroid hormone receptors in the nucleus [89].

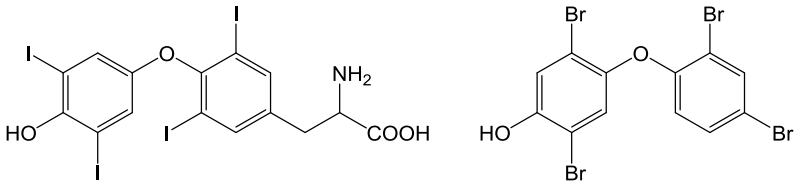


Figure 5. The structure of the thyroid hormone thyroxine (3,3',5,5'-tetraiodo-L-thyronine) [T_4] and one example of a *para*-substituted OH-PBDE, (3,6-dibromo-4-(2,4-dibromophenoxy)phenol [4'-OH-BDE49].

Another toxicological endpoint of PBDEs is impaired reproduction and high levels of PBDEs in humans have been associated with longer time to conceive [90] .

Neurotoxicity effects of PBDEs affecting the brain and nervous system is of particular concern regarding *in utero* exposure and exposure to small children. Thyroid hormones play an essential role in normal brain development e.g. neuronal proliferation, migration, synaptogenesis, synaptic plasticity and myelation processes [91]. Of great importance is the timing of the exposure since an individual is more susceptible towards hazardous chemicals during certain time periods in life e.g. during brain growth spurt (BGS) period. In humans, this intervenes the first trimester (neonatal period) and during the first 2 years of living. In prenatal exposed rat and mice, general alterations in spontaneous behavior were observed, which in practice affects their ability to adapt to new environments. Further, hyperactivity was along with attention difficulties found in the young animals while impaired ability to learn and impulsive behavior was found in the adult animals [51,85].

The target organ dealing with detoxification of PBDEs is the liver. Increased liver weight due to hepatocellular hypertrophy e.g. enlargement of hepatic cells was the observed histopathological changes [51].

PBDEs do not possess the dioxin-like toxic properties as non-*ortho* substituted polychlorinated biphenyls (PCBs), PBBs, polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDFs) do. The ether bond in PBDEs makes it energetically unfavorable for the molecule to adopt a co-planar conformation. However, it cannot be excluded that polybrominated dibenzofurans (PBDFs) or dibenzo-p-dioxins (PBDDs) may be present as trace contaminants in the technical PBDE mixtures.

3 Feline metabolism and thyroid disruption

3.1 Feline metabolism

The family of Felidae in which domestic cats are included, have a detoxification system of both endogenous and exogenous compounds, which differs from many other mammals. These metabolic differences include enzymes involved in both phase I and phase II reactions. A few studies have explored the activity of the feline cytochrome P450 (CYP) enzymes. In one study, the activity of CYP enzymes commonly involved in drug metabolism, were compared in human and cat liver microsomes. The study found that the activity of CYP1A, CYP2C, CYP2E and CYP3A were generally lower in cats than in humans while higher enzyme activities of CYP2B and CYP2D were reported in cats than in humans [92]. The highest activities of the isoenzymes in cats were found for CYP2B6 and CYP1A [92]. Another study concluded that hydroxylation of tolbutamide, as a response to CYP2C activities, was negligible in both male and female cats [93].

Further, cats have reduced activity of some phase II conjugation enzymes, i.e. glucuronyl transferases and sulphotransferases, responsible for catalyzing conjugation with glucuronic acid and sulfate [94,95]. Glucuronidation accounts for detoxification of a great variety of phenolic substances e.g. drugs such as acetaminophen and acetylsalicylic acid [96]. As conjugation with glucuronic acid and sulfate is limited in cats, clearances of phenolic substances are slower. Cats exhibit acute life threatening effects of e.g. acetaminophen (metabolized by CYP 2E), i.e. at much lower doses than is toxic to dogs and humans [96]. It is speculated that the Felidae family, referred to as hypercarnivores (i.e. meat makes up >70% of the diet), evolutionary have been less exposed to plant toxicants and consequently not subjected to an evolutionary similar to e.g. herbivores [94].

3.2 Thyroid hormones

The primary task of thyroid hormones (THs), i.e. thyroxine (T_4) (3,3',5,5'-tetraiodo-L-thyronine) and triiodothyronine (T_3), (3,3',5-triiodo-L-thyronine) in higher vertebrates is to regulate body metabolism, body growth, body weight, cholesterol metabolism, gluconeogenesis, maturation, reproduction

and brain development [97,98]. The THs are produced by the thyroid gland which is situated in front of the trachea below the larynx in cats and humans. In mammals, most of THs are produced as T_4, which has higher affinity for plasma thyroxine binding proteins necessary to transport THs from the production site in the thyroid gland(s) to target cells in the body. More than 99% of the THs are bound to any of the thyroxine binding plasma proteins; albumin, transthyretin (TTR) and thyroxine-binding globulin (TBG) [99]. Only prealbumin acts as a THs binding protein in cats and TBG has, to the best of my knowledge, not been demonstrated in cat plasma [100]. The unbound free T_4/T_3 is in rapid equilibrium with bound T_4/T_3, which is the form THs are available for uptake by the cells [86]. TTR, as a TH transporter protein, is unique in that it is, besides being synthesized in the liver, also is produced in the brain, by epithelial cells of the choroid plexus. These cells are also responsible for producing most of the cerebrospinal fluid (CSF). Thus, THs are transported over the blood brain barrier bound to TTR in CSF for further distribution in the brain [99]. Inside the cell, T_4 is converted by deiodinases to T_3, which is the active form binding to various TH cytosolic and nuclear receptors [99]. THs are cleared from plasma through conjugation with glucuronic acid or sulfate [86].

TH production in the thyroid glands is regulated by thyroid-stimulating hormone (TSH) released from the pituitary gland located below the brain (Figure 6). The pituitary, is controlled by the thyrotropin-releasing hormone (TRH) secreted from hypothalamus in the brain. The thyroid hormones control the secretion of TSH and TRH by negative feedback mechanisms. Hypothalamus-pituitary-thyroid glands are also referred to as the HPT-axis, which main purpose is to maintain normal or euthyroid hormone balance in the body [97].

Thyroid hormone interruption may have a large number of consequences on health and development. This is reviewed in quite some detail by UNEP/WHO 2012 [13]. Thyroid hormone disruption in cats is also the base for the work pursued in this thesis (c.f. below).

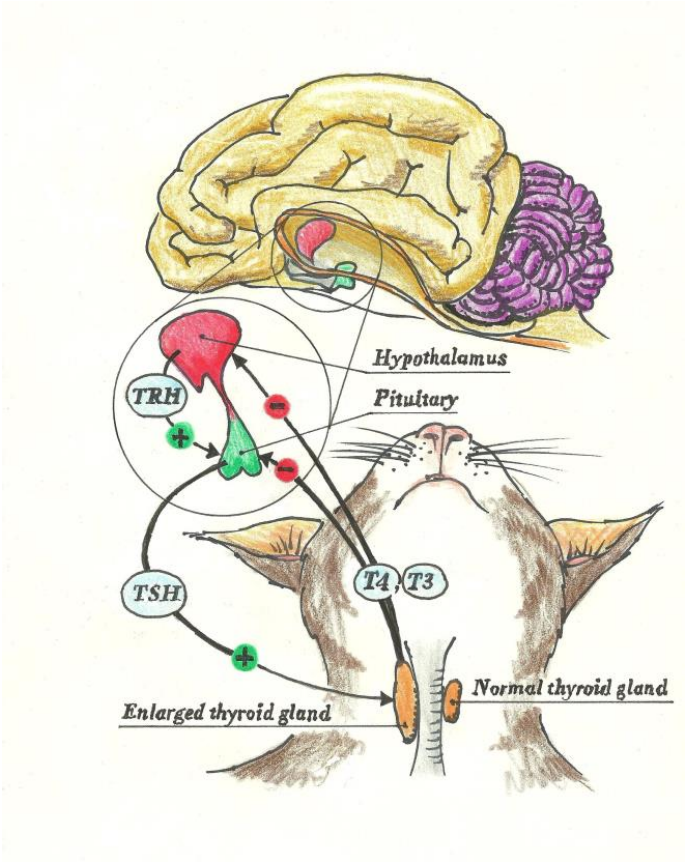


Figure 6. A Cat brain and the thyroid glands demonstrating regulation of the thyroid hormone homeostasis in mammals by the hypothalamus-pituitary-thyroid (HPT) axis.

3.3 Feline hyperthyroidism

Spontaneous hyperthyroidism in the cat, due to functional thyroid adenomas was first recognized in the late 1970s and reported in 1979 by M.E. Peterson at the Animal Medical center, in New York, USA [101] and ever since has the number of reported cases increased substantially worldwide over the last three decades.

Increased age in cats, like in humans, is associated with various age-related diseases such as endocrine disorders were hyperthyroidism and diabetes mellitus (DM) are common. In cats, the incidence of hyperthyroidism worldwide is 1.5-12 % [102] and DM 0.5 % [103]. Hypothyroidism is rarely occurring in cats while hyperthyroidism is recognized as the number one

endocrine disorder in senior pet cats [103]. An endocrine disorder is a result of imbalance i.e. over or underproduction of hormones within the endocrine system. Hyperthyroidism is associated with elevated plasma concentrations of T_4 (sometimes also T_3), and decreased levels of thyroid-stimulating hormone (TSH) [104].

Cats and humans are the only mammalians in which hyperthyroidism is known to occur frequently [105]. In contrast to humans, the etiology of feline hyperthyroidism (FH) is debated and the cause(s) still remains uncertain but it is likely to be a multifactorial disease [102,106]. FH is not regarded gender specific as in humans where there it is an order of magnitude higher incidence in women than in men [98,107]. In addition, purebred cats related to the Siamese and Himalayan breeds (two genetically similar breeds) show a decreased risk at developing FH suggesting hereditary to play a role [104]. However, there are epidemiological studies suggesting several other risk factors for developing FH e.g. indoor lifestyle (e.g. use of litterbox), a diet based on canned food, eating fish and environmental factors such as exposure to herbicides or regular use of flea powders/sprays [108-110].

In humans, in most cases, hyperthyroidism evolves from Graves' disease which is an autoimmune disease where antibodies bind to the TSH receptor to upregulate thyroxine production. Hyperthyroidism may also be derived from human toxic nodular goiter (TNG), which occurs more commonly in developing countries where iodine deficiency is a health issue. A long-gone progressed TNG, i.e. thyroid enlargement may be seen visually as a bulge by the neck. The pathophysiology of FH is similar to that of human TNG with clinical symptoms such as; weight loss, polyphagia, polydipsia, hyperactivity, aggression, diarrhea, vomiting and tachycardia [104].

The morphologic changes of the thyroid gland associated with the hyperthyroidism in cats are clear. In more than 95 %, cats with FH, have functional thyroid adenomatous hyperplasia i.e. thyroid enlargement of a benign kind. A majority (70%) of the hyperthyroid cats have an enlargement of both thyroid lobes, resulting in hyperactivity of the gland [102,104].

Based on the thyroid pathologic changes in hyperthyroid cats, several possible causes or combinations of these have been discussed including immunologic factors (e.g. immunoglobulins), nutritional factors (e.g. inadequate iodine intake, goitrogens in food or water), an infectious agent or environmental factors (e.g. toxins or thyroid hormone disrupting compounds) [102]. Even though, the initial effect of exposure to environmental contaminants such as PBDEs is lowered THs levels, this will have secondary effects on the TSH. Low plasma concentrations of THs increase the secretion of TSH, which induce hyperplasia (i.e. proliferation of follicular epithelial cells) as a response to the need for increased TH production. Over time, the thyroid glands may become adenomatous [102].

However, evidence against an autoimmune etiology in cats was shown and the hypothesis was rejected [105]. The hypothesis regarding inadequate (insufficient/excessive) iodine intake, have also been considered, but not verified. Iodine is essential for the thyroid gland to produce THs, but a high iodine intake may be a risk factor for developing FH [102,106].

Hyperthyroidism in cats is treated in similar ways as in humans; by the antithyroid substance tiamazole (metimazole) to reduce serum T_4 and T_3 or by surgical removal of the thyroid glands alternatively by radio therapy, administrating radioactive iodine to reduce the number of hyperactive cells in the gland.

4 POPs in cats

Pets, especially pet cats of today, spend a significant time indoors. That in combination with their grooming behavior i.e. licking their fur several times a day makes them highly exposed to house dust and chemicals accumulated in dust. Cats are also exposed to dust through inhalation but ingestion is a more important way of exposure. Recently it has become evident that dust is a significant source of exposure to POPs such as PBDEs, much more important than exposure via diet which traditionally has been considered the main exposure source for POPs [48]. In addition, cats in relation to dogs accumulate organohalogen compounds (OHCs) to a greater extent in their bodies, i.e. the bioconcentration factors seem to be higher in cats [111]. For these reasons, cats may accordingly be a suitable biomarker for exposure of indoor pollutants.

4.1 POPs in cats (*Felis catus*)

A review of the current literature on POPs reported in cat serum/plasma or blood is summarized in Table A2, presented in the Appendix to this thesis. Median concentrations (ng/g lipid weight) of PBDEs, OH-PBDEs, MeO-PBDEs, brominated phenols such as pentachlorophenol (PCP) and 2,4,6-TBP, PCBs, OH-PCBs and organochloride pesticides such as 4,4'-DDT, 4,4'-DDE and hexachlorobenzene (HCB) were reported from Australia, Japan, Pakistan, Sweden, UK and USA in the states of CA, GA and IL. If the results are given on wet weight basis and no lipid data were available (valid for Japanese, UK and GA, USA cats), the concentrations on pg/mL serum were recalculated to ng/g lipid weight to promote comparisons of contaminants in the cats. Calculations to lipid weight based concentrations were made assuming a cat blood density of 1.042 g/mL and a relative lipid content of 0.6 % [112].

4.1.1 PBDEs

The overall highest median serum concentrations of ∑PBDEs were reported in pet cats from USA, in CA (3742 ng/g l.w.) [113], IL (2850 ng/g l.w.) [114] and GA (944 ng/g l.w.) [115] (Figure 7). Australian pet cats showed the second highest ∑PBDE levels (174 ng/g l.w.) after American cats [116].

Median of BDE-47 and BDE-99 of Californian pet cats were (567 and 2239 ng/g l.w., respectively) [113]. The lowest concentrations were reported in Japanese stray cats (1.5 and 0.7 ng/g l.w.) and Pakistani indoor pet cats (1.9 and 2 ng/g l.w.) [117,118]. Euthyroid Swedish and UK healthy cats had similar levels of BDE47 (6.0 and 5.0 ng/g l.w.) and BDE-99 (14 and 11 ng/g l.w.) (**Paper II**) [119]. BDE-183 was reported from two Swedish studies (**Papers II**, **III**) and one from CA, USA. Californian cats had about 6-10 times higher concentrations than levels in Swedish pet cats. Median concentration for BDE-209 was the same in Japanese stray cats and Swedish euthyroid indoor pet cats (62 ng/g l.w.). The highest levels of BDE-209 were also reported for Californian pet cats, in concentrations 4.5 times higher than the Swedish euthyroid and Japanese cats (279 ng/g l.w.).

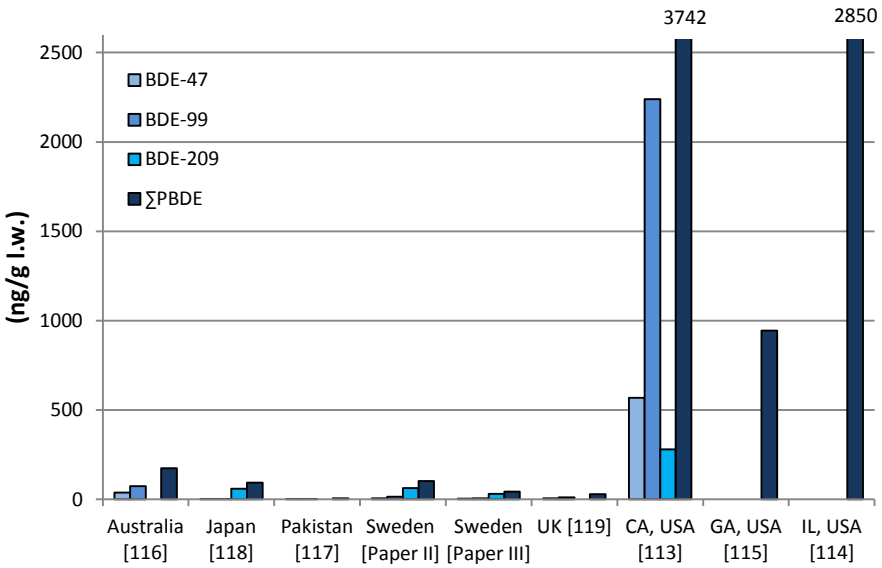


Figure 7. Median concentrations of BDE-47, -99, -209 and ∑PBDE) (ng/g l.w.) in cats as reported from different countries (Table A2, Appendix).

4.1.2 OH-PBDEs/MeO-PBDEs

Among reported OH-PBDEs in cats, 6-OH-BDE47 is by far the dominating compound, followed by 2'-OH-BDE68 (Figure 8). The highest levels of 6-OH-BDE47 were reported in Japanese stray cats (107 ng/g l.w.) followed by Swedish pet cats reported in two studies (68 and 42 ng/g l.w., respectively) and UK cats (1.8 ng/g l.w.) (**Papers II**, **III**) [118,119]. The median concentrations of 2'-OH-BDE68 were similar in Swedish euthyroid and UK control cats (1.0 and 1.3 ng/g l.w., respectively) and highest in

Japanese stray cats (6.7 ng/g l.w.). Three abundant human BDE-47 metabolites, i.e. 3-OH-BDE47, 5-OH-BDE47 and 4'-OH-BDE49, were reported under limit of quantification (<LOQ) from the study of UK pet cats [119]. Metabolism of PBDEs in cats is further discussed in Chapter 6.3.

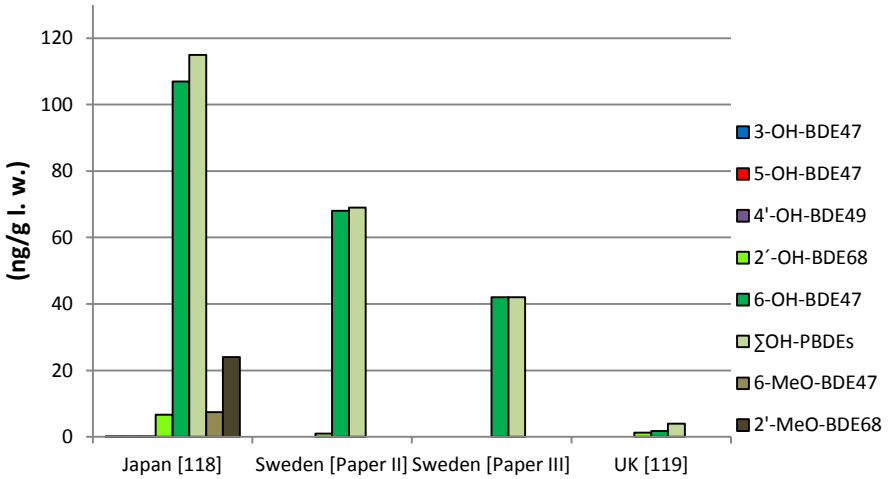


Figure 8. Median concentrations of OH-PBDEs and MeO-PBDE (ng/g l.w.) in cats as reported from different countries (Table A2, Appendix).

4.1.3 PCBs/OH-PCBs

For comparison of the occurrences of PCBs in cats from different countries, median concentrations of CB-153 were used and data are presented in Figure 9. The concentrations of CB-153 from five countries were in the same order of magnitude. Highest levels were found in euthyroid Californian (48 ng/g l.w.) and Swedish euthyroid pet cats (41 ng/g l.w.) followed by Japanese stray cats and UK control pet cats (35 and 33 ng/g l.w.) (**Paper II**) [113,118,119]. Lowest CB-153 concentrations were measured in Pakistani pet cats (5.6 ng/g l.w.) and from the second study of Swedish pet cats (10 ng/g l.w) (**Paper III**) [117]. Amongst the reported OH-PCBs, 4-OH-CB107 was found in highest concentrations in Japanese stray cats from two studies (16 and 9.3 ng/ g l.w.) [118,120]. The second highest abundant OH-PCB present in cats was 4-OH-CB162 found in Japanese and UK pet cats (4.3 and 1.3 ng/g l.w.) [118-120] (Figure 10).

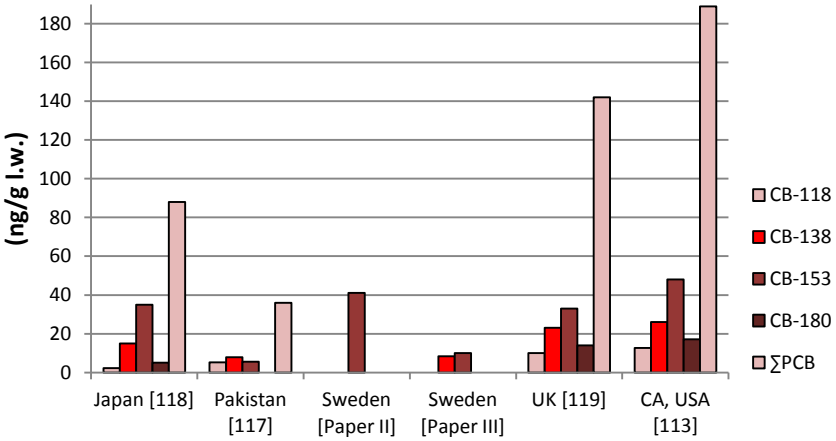


Figure 9. Median concentrations of CB-118, -138, -153, -180 and ∑PCB (ng/g l.w.) in cats as reported from different countries (Table A2, Appendix).

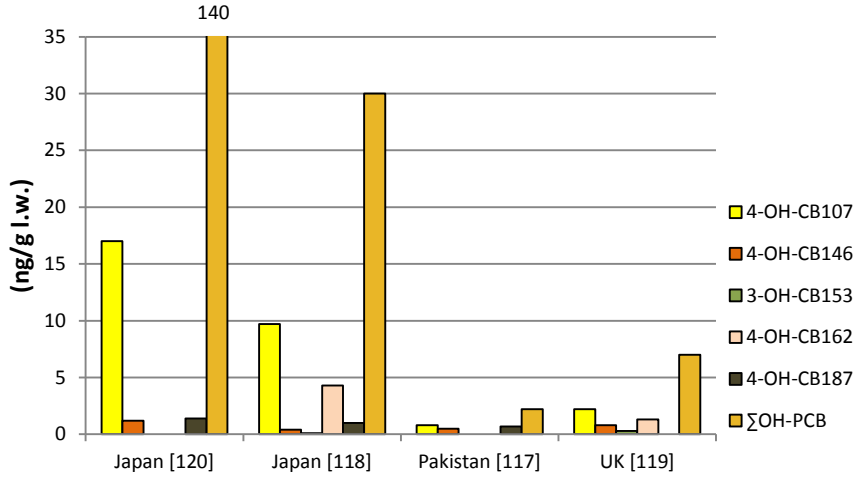


Figure 10. Median concentrations of OH-PCBs (ng/g l.w.) in cats as reported from Japan, Pakistan and the UK (Table A2, Appendix).

4.1.4 Organochlorine pesticides

Concentrations of a few pesticides (PCP, HCB, 4,4'-DDT and 4,4'-DDE) was reported in cats from five countries (Figure 11). The levels of PCP in Japanese cats (352 ng/g l.w.) were higher than in UK cats (217 ng/g l.w.) and Pakistani cats (97 ng/g l.w.) [117,119,120]. Swedish pet cats reported the lowest median concentration (18 ng/g l.w.) (**Paper III**). Concentrations of HCB were similar in Pakistani and UK cats (3.5 and 2.4 ng/g l.w.,

respectively). The highest concentration of 4,4'-DDT, reported as ∑DDT was found in Pakistani pet cats (111 ng/g l.w.) and Californian hyperthyroid cats (57 ng/ g l.w.) followed by Swedish pet cats (11 ng/ g l.w.) (**Paper III**) whilst being <LOQ in UK cats and reported as 0 ng/g l.w. in euthyroid California pet cats [113,117,119,120]. However, the highest median concentrations of 4,4'-DDE was reported in both euthyroid and hyperthyroid Californian pet cats (472 and 368 ng/g l.w.), which is much higher compared to Pakistani, UK control and Swedish pet cats (95, 83 and 13 ng/g l.w., respectively).

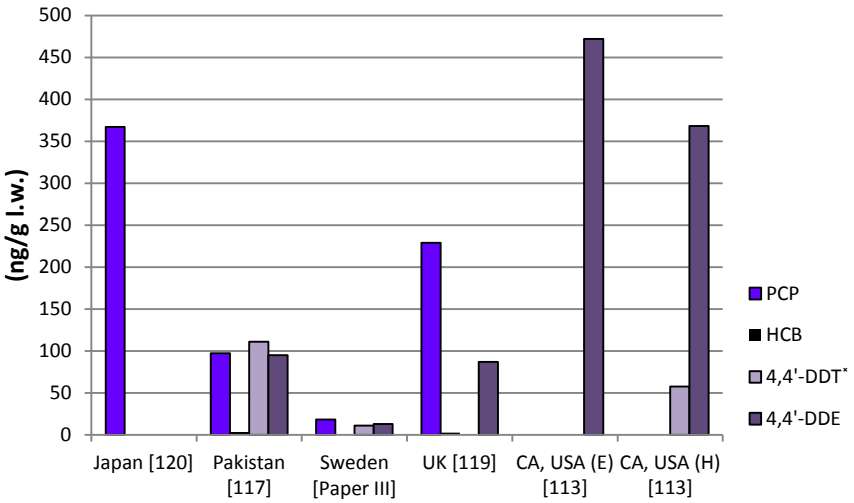


Figure 11. Median concentrations of some organochlorine pesticides (PCP, HCB and 4,4'-DDT (*∑DDT for Pakistan cats)) (ng/g l.w.) in cats as reported from different countries (Table A2, Appendix).

4.1.5 Perfluoroalkyl substances (PFAS)

Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been reported from two studies (data not included in Table A2 in the thesis appendix) on Swedish pet cats (n=26) (unpublished data) and Californian cats collected at animal shelters (n=26) [121]. Median concentrations for PFOS and PFOA in Swedish cats were 2.1 and 1.2 ng/mL. Maximum values of PFOS and PFOA were 4.6 and 13 ng/mL, respectively. Median serum concentrations in Californian cats for PFOS and PFOA was 11 and 2.3 ng/mL, respectively which is similar to that of Californians (n=46) reported in the same study (10 and 2.6 ng/mL respectively). However, total PFAS (∑12) was greater in cats in comparison to humans in California.

5 Chemical analysis

5.1 Samples

5.1.2 Cat serum

Blood samples (**Papers I, II, IV)** from client owned pet cats submitted to the laboratory of the university animal hospital at the Swedish University of Agricultural Sciences (SLU) in Uppsala were obtained during 2010. Blood samples of cats from the seventeen family homes (**Paper III)** were taken between autumn of 2013 to early 2014, concurrently as house dust was sampled.

Whole blood from cats consists of about 40% blood cells (i.e. white and red cells, platelets) and 60% extra cellular fluid (i.e. plasma) by volume. The plasma portion of the blood holds clotting factors, antibodies and other proteins like enzymes, lipids, vitamins, and hormones. Environmental contaminants will be associated to blood transport proteins in the plasma portion of the blood. Plasma is obtained as the supernatant when whole blood is treated with an anticoagulant (e.g. heparin) and centrifuged to remove the blood cells. Serum is the supernatant received after centrifugation of coagulated whole blood without an anticoagulant. With an anticoagulant present in the whole blood, clotting factors (e.g. fibrinogen) stay in the plasma portion, hence plasma is more protein rich compared to serum [122]. Whole blood samples were collected in plain glass tubes and were left for coagulation at ambient temperature for about 30 min. After centrifugation at 3000 G for 5 min, the supernatant i.e. blood serum was collected. Serum samples were then stored at -20°C prior to analysis.

The density of cat serum was determined experimentally by weighing aliquots of 1 mL serum (n=20) with a carefully calibrated pipette and balance. Average concentration was determined at 1.042 g/mL (SD=0.01 and CV =0.77%).

Serum levels of total cholesterol and triglycerides were determined enzymatically at the laboratory of the university animal hospital using evaluated standard methods [123,124]. The serum levels of total T4 and TSH

were determined with a solid-phase, chemiluminiscent competitive immuno-assay (Canine Total T4) and solid-phase, enzyme labeled chemiluminiscent immunometric assay (Canine TSH), respectively, evaluated for feline samples. Both assays were performed on an Immulite 2000 (Siemens Healthcare Diagnostics Ltd., Llanberies, Gwynedd LL55 4EL, UK) according to the manufactures instructions.

5.1.3 Cat food

Cat food, wet and dry, (**Papers II, III**) was purchased from varies grocery stores and pet shops in Stockholm or gathered from practicing veterinarians during 2013-2014.

Normal procedure in cat food manufacturing is to make up a common batch, in which the flavor is added to. It is required by law that the content of the flavor needs to constitute for at least 4% by weight. Most commonly, one or two flavors are added, hence 92-96% of the cat food from the same producer has the same composition across the different flavors. These basic batches of cat food contain abattoir offal like lung, heart, liver, kidney, and throat, foremost from chicken (which has neutral taste) but also from pig and cattle. The wet cat food are canned and preserved in an autoclave. Dry food is prepared in similar ways with a common batch, boiled and then dried to prepare pellets.

The lipid content of dry cat food (10-20%) is higher than for wet canned food (4-5.5%). Therefore, in order to extract similar amounts of fat (400-700 mg) from wet and dry food samples, approximately 10 g of wet food and 15 g of reconstituted dry food (pellets: water, 1:2) were used.

5.1.4 House dust

Dust samples (**Paper III**) were collected from 17 households around the Stockholm region in Sweden. The households were selected because they had at least one cat and children < 10 years of age. The living rooms were vacuumed with a Dustream™ dust collector (Indoor Biotechnologies Ltd., Wiltshire, United Kingdom) containing a disposable filter (mesh size 40 µm). The dust collected was so-called still standing dust, from surfaces little influenced by daily life such as walking and not containing bread crumbs and other larger particulate debris. Sampling of human and cat hair was avoided, as well as large assembly of gravel or other debris from the outside. The living rooms had not been vacuumed for at least 3 days prior to sampling. Dust samples were stored in aluminum packages at -20°C prior to analysis.

5.2 Sample preparation

All sample preparation except for dust samples was carried out in a clean room, a separate laboratory adapted for low level analysis of PBDEs with careful cleaning routines. Solvents used during clean-up and analysis were either distillated prior to usage or of highest possible quality grade available. All glassware including pipettes, were cleaned and heated at 300°C in an oven overnight prior to usage.

5.2.1 Extraction methods

A commonly applied liquid liquid extraction (LLE) method published by Hovander and co-workers [125], were applied for analysis of PCB, OCPs, BFRs and phenolic brominated compounds in cat serum in **Papers I**, **II**, **III** and **VI.** Serum sample (0.5-1 mL) was diluted to 5 g with 1% KCl (aq) (w/v) prior to extraction.

For the analysis of PCB, OCPs, BFRs and phenolic brominated compounds in dry and wet cat food, a LLE method published by Jensen and co-workers [126] were used to extract the lipids from the cat food samples in **Papers II**, **III**. After lipid determination, part of the lipids (250-300 mg) were weighed out and transferred to a test tube and surrogate standards were added. From here on the sample preparation of the cat food pursued according to the Hovander method [125] beginning at *step 2*, with separation of phenolic and neutral compounds. Approximately 10 g of wet canned food or 15 g of reconstituted dry food were weighed out for each extraction.

For the analysis of PBDEs, BB-209 and DBDPE in house dust samples (**Paper III**) a newly established LLE method by Van den Eede and co-workers [127] was applied. The method includes extraction on ultrasonic bath followed by Fractionation on a florisil® column to separate organophosphate ester flame retardants (OPFRs) from other BFRs such as PBDEs and decabromodiphenyl ethane (DBDPE). Approximately, samples of (50 mg) were weighed out for each extraction.

5.2.2 Lipid determination

Lipophilic compounds such as POPs are bound to lipophilic proteins (e.g. blood transport proteins) in serum or plasma, i.e. they are not dissolving in the hydrophilic portion of the matrix. To accurately state concentrations of POPs in blood it is crucial to do that both on lipid weight (ng/g lipid) and fresh weight (pg/g serum) to give the true picture of the contamination load of POPs. In addition it is also of importance to give the amounts on molar basis (pmol) rather than on weight basis (ng) when reporting contaminant

data. This is especially valid regarding comparison of brominated contaminants, where the number of bromine in the molecule greatly influences the molecular weight. For instance, 1 ng/g lipid of BDE-47 equals 2 pmol/g lipid whereas 1 ng/g lipid BDE-209 equals 1 pmol/g lipid. This means that on molar basis the amount of BDE-47 is actually twice the amount of BDE-209. In other words, the amounts of the higher brominated compounds tend to be overestimated if reported on ng basis in comparison to a lower brominated compound. With this, we strongly encourage the scientific community to report POP concentrations on molar (pmol) basis instead of mass (ng) basis.

It was shown that total lipid serum concentrations, i.e. the sum of triglycerides (TG), cholesterol (CHOL) (free and bound), and phospholipids (PL), were 20% higher after a meal compared to levels at fasting [128]. Serum concentrations of POPs (PCBs, HCB, 4,4'-DDE) measured on fresh weight increased by 20% as well after a meal, following the same trend as the blood lipids. This change in concentration, was most likely a rapid response of the POPs to the new equilibrium of lipids in the blood [128]. Consequently, reporting POPs based on serum concentrations alone will differ depending on when samples are taken. However, when comparing concentration of POPs on a lipid basis, there were no significant difference between serum from fasting and non-fasting humans [128].

Traditionally, lipid determination of blood was done gravimetrically by measuring the lipid content left after evaporation post extraction with an organic solvent. This procedure is tedious and requires careful handling of the samples. In the end, the result may still vary due to parameters such as low lipid contents (mg), temperature and humidity or organic solvent used in the extraction. In addition, there is a concern for potential loss of compounds with low vapor pressure (e.g. simple halogenated phenols). Therefore enzymatic lipid determination is nowadays, commonly applied when human serum and plasma samples are analysed. Moreover, only small amounts (<100 μL) is needed for enzymatic measurements of lipid [129].

Total lipid (TL) is calculated by adding the different classes of blood lipids together i.e. triglycerides (TG), cholesterol (CHOL), and phospholipids (PL). Further cholesterol is, divided into free and esterified cholesterol, i.e. bound to fatty acids. The average molecular weight of TG, CHOL and PL is estimated to 807, 571 and 714 g/mol respectively [130].

There are several suggested formulas for estimating total serum lipids (g/L) enzymatically, which are presented in Equation (Eq.) 1 through 4. Eq. 1, described by Philips and co-workers [128] is basically a shorter version of Eq. 2 described by Akins and co-workers [131] at the CDC, both of which

are on measurements of TG and total CHOL (TC), only. The formula itself adjusts for PLs, which is practical since PLs are not routinely measured in blood samples. The compensation of PL is built upon linear relationship between PL and TC. Further, formula in Eq. 1 & 2 assumes free cholesterol to be about 27% and esterified cholesterol, bound to fatty acids around 73% in serum.

(Eq. 1) TL (g/L) = 2.27*TC + TG +0.623
(Eq. 2) TL (g/L) = 1.677*(0.73TC) + (0.27*TC) +TG+ (0.766*TC) + 0.623
(Eq. 3) TL (g/L) = 1.31*CHOL + 1.31 TG + 0.92
(Eq. 4) TL (g/L) = 1.33*TG+ 1.12*CHOL + 1.48

In Eq. 3 Rylander and co-workers describes another formula for determining total lipids in human blood [132]. This formula makes use of a linear relationship between the sum of cholesterol and triglycerides to the total lipid, which was found to explain 97.2% of the variation. Measurements of PL were omitted in the formula. Eq. 4 was developed by Covaci and co-workers [129]. The formula is based on individual measurements of TG, CHOL and PL from Belgian, Swedish and Norwegian populations. Further, the ratio between free and esterified cholesterol is assumed to be 1:2 in Eq. 3 & 4. It was later shown that both the latter methods underestimate total lipids. In the Covaci equation, total lipid is underestimated because free cholesterol was not measured for and also omitted in the calculation [130]. The free cholesterol accounts for between 1and 8% of total lipids (pers. comm. Prof. Adrian Covaci). In calculations of total lipids, the Covaci formula in Eq. 4, in comparison to Akins formula, Eq. 2, yield 10% lower total lipids values [130].

As the Covaci formula was used within this thesis for lipid determination in cat serum, the reported concentrations on lipid weight might be slightly overestimated in comparison to the true value. Another important aspect to highlight here is that the formula is based on human serum and not cat serum. There may be a difference in lipid composition between human and cat serum. Unfortunately, little information is available on feline blood lipids, partly due to the fact that blood lipid diseases are rare in cats and little research has been performed. Density of cat serum was experimentally determined to 1.042 g/mL. This is within the range for humans were the density in whole blood and serum approximately is 1.05 g/mL and 1.02 g/mL, respectively [133].

Lipid percent in blood varies over a day and is dependent on how recent food has been ingested as blood lipid concentration increases after a meal [128]. In the study of euthyroid and hyperthyroid cats no information was available on when last meal was ingested prior to blood sampling. However,

it is recommended that cats have free access to food which will limit the diurnal variation in serum lipid content. Serum lipid content from the study varied between 0.2 and 0.7% with a mean lipid concentration of 0.47%. This is similar to what has been reported in human serum from occupational studies in Sweden where the average lipid value was approximately 0.64% (with some low values measuring 0.29%) [134]. Blood lipid content determined gravimetrically in a pooled sample (n=6) was compared to enzymatically calculated total lipids (n=6). Lipid content determined gravimetrically was 0.61% (RSD=0.18) and enzymatically 0.58% (RSD 0.07) [112]. The two methods were in agreement with each other. However, the enzymatically determined lipid values were 5% lower than that obtained gravimetrically. Normal concentrations of cholesterol in cats (75-220 mg/dL, 2.0 – 3.6 mmol/L) are slightly lower than in humans (150-200 mg/dL). Also the normal range of triglycerides is lower in cats (25-160 mg/dL, 0.6 – 1.1 mmol/L) compared to humans (145-250 mg/dL) which also could be a reasonable explanation for the lower lipid content results obtained enzymatically.

Assuming cat serum to have similar blood lipid constituents as human serum in enzymatic lipid determination most likely involves some systematic error. However, determination of 3-5 mg of lipids gravimetrically in a serum sample is not a better choice and will probably result in even greater uncertainties and inconsistencies in the lipid determination. When performing enzymatic lipid determinations, at least the systematic error will remain constant amongst all samples.

Within this thesis, lipid weights were determined gravimetrically in the food samples and enzymatically in the serum samples using the Covaci formula [129].

5.2.4 Derivatisation

Dervatisation of phenolic compounds (e.g. simple BPs and OH-PBDEs) to yield a less polar analogue is commonly performed to enhance chromatographic performance in gas chromatographic (GC) analysis. Within this thesis diazomethane was used as methylating agent to obtain methyl ether derivatives of the phenolic compounds. These methyl ether derivatives are stable and do not require immediate instrumental analysis. Diazomethane (CH_2N_2) was synthesized in house from N-methyl-N-nitroso-p-toluene sulfonamide [135] with permission from Swedish authorities. Diazomethane is classified as highly toxic and a known carcinogen, and is present as a gas at room temperature, thus all handling were carried out in a particularly designated fume hood, with great care wearing appropriate protection.

Other derivatisation agents used for alkylation (or methylation) are e.g. trimethylsilyl diazomethane [119], trimethylsilyl dimethylamine [117] and methyl iodide [136]. Acetylation with pentaflurobenzoyl chloride (PFBCl) [126] forming a PFB ester, is simple and fast if carried out in the presence of a catalysts. The limit of detection is also lowered for analysis on GC-ECD which is beneficial in quantification of halogenated compounds with lower grade of bromination. The larger PFB esters are less stable than methyl esters and samples may only be kept in a freezer for a couple of months [126].

5.2.3 Lipid removal

Lipid removal, as a clean-up step, was carried out with concentrated sulfuric acid and a sulfuric acid treated silica gel column. Partitioning with sulfuric acid is a rough lipid removal technique that destroys the lipids by oxidation and/or partitioning to sulfuric acid phase. Compounds suitable for this treatment need to be inert towards degradation or partitioning to sulfuric acid such as PCBs, PBDEs, MeO-PBDEs or bromoanisoles.

5.3 Instrument analysis, identification, quantification

All samples were analysed by gas chromatography and compounds were identified on retention time using authentic reference standards. The methylated phenolic compounds were identified as their corresponding methyl ether analogues.

Analysis of brominated compounds (neutral and phenolic) was performed by gas chromatography/ mass spectrometry (GC/MS) operating in electron-capture/negative ionization (ECNI) mode (GC/MS-ECNI). ECNI utilizes a reagent gas (e.g. CH_4, NH_3) to produce low energy electrons that are captured by molecules with high electronegativity such as the BFRs. These low energy electrons are absorbed up by brominated molecules to form negative halide anions [M-] and/or bromine anions [Br^-]. In ionization of polybrominated compounds, bromine anions are formed in considerable amounts producing high signals for Br^{79}, Br^{81} to monitor in selected ion mode (SIM) [137]. The limit of detection (LOD) with ECNI for analysis of brominated compounds is low, in the picogram (pg) range, which is crucial in biological/environmental samples were contaminants are present in very amounts. However, the selectivity is compromised with GC/MS-ECNI and co-eluting compounds may be an issue for identification/quantification purposes. In GC/MS-electron ionization (EI), the molecules are bombarded with electrons at 70 eV, to produce [M^+] ions. This ionization technique could give structural information if a molecular ion is achieved. In addition,

depending on number of bromines present in the molecule, a distinct isotope pattern will show. Hence, structural information from GC/MS-EI is important for identification purposes of yet unknown compounds. On the downside, the LODs in GC/MS-EI are much higher than for GC/MS-ECNI, which is a disadvantage for quantification purposes.

Analysis of PCBs and OCPs was performed by GC coupled to and electron capture detector (GC-ECD).

The limit of detection (LOD) of an environmental or biological sample is generally determined by a peak corresponding to 3 times the background noise of the sample. The limit of quantification (LOQ) for an analyte is generally defined as 10 times the background noise of the sample.

Within this thesis, the LOQ was determined as the smallest quantifiable peak for the set of samples analysed and sample the LOD was then set to LOQ/3. However, if the analyte was present in the blank, LOD was set to the average amount determined in the blanks and LOQ was set to three times the value of the LOD. The average amount of the blanks was also subtracted from the quantified amount in the sample.

There are other ways to determine the LOQ and among other things, have three times the standard deviation of the blank, been applied for the LOQ determinations in PBDE analysis of human serum [138].

The big challenge in this work has been to deal with small sample volumes holding the analytes of interest in pg amounts. Although laboratory background contamination was kept to a minimum due to working in a clean room and using chemicals and solvent of highest grade, some samples were >LOD but <LOQ. These samples concentrations were set to LOQ/$\sqrt{2}$.

5.4 Quality Assurance/Quality Control (QA/QC).

Cat serum

Along with each set of cat serum samples (n=20), two solvent blanks and two QCs (human plasma) samples were analysed for quality control. Surrogate standards (SS), one for each class of compounds (e.g. PCBs, PBDEs, OH-PBDEs and brominated simple phenols), were used to control the recoveries of the quantified compounds.

The LLE method used for analysis of organohalogens in human plasma, developed by Hovander and co-workers [125], was carefully validated to be

applicable for analysis of OH-PBDEs in in cat serum (**Paper IV)**. In addition, the accuracy in determining PBDE with our analytical method was evaluated by use of standard reference material (SRM 1957), Organic contaminants in non-fortified human serum, from the National Institute of Standards and Technology (NIST) (**Paper II)**.

In **Paper IV**, a method validation, including nine OH-PBDEs, four polybromophenols and three MeO-PBDEs in four matrices (human plasma, cat serum, herring- and long-tailed duck plasma) was carried out. The investigated OH-PBDEs included those of natural origin as well as typical PBDE metabolites. Matrix was spiked at two levels, low (0.5 ng) and high (5 ng) dose, in five replicates. The result of the recovery study showed high and reproducible results for OH-PBDEs, 2,3,4,6-tetraBP and 2,3,6-TBP, at both spiking levels, for human plasma and cat serum. Recoveries for the more volatile 2,4-DBP was not optimal but regarded as acceptable. The analyte recoveries for herring and long-tailed duck plasma were low and insufficient with great variability for the investigated phenolic compounds. Hence, this method was not suitable for analysis of OH-PBDEs or BPs without further method development. However, this important analytical issue with low recovery in herring and long-tailed duck plasma, was further extensively investigated and described in the thesis work of Dahlberg [139].

The MeO-PBDEs was added to the method validation to confirm that no MeO-PBDEs partitioned over with the phenolic compounds in the potassium hydroxide treatment step during clean-up. Thus, possible contribution of MeO-PBDEs would result in incorrect reported OH-PBDE concentrations since OH-PBDEs are analysed as their methyl-ether analogues (MeO-PBDEs). Recoveries of MeO-PBDEs in cat serum at low (93%-95%) and high (91%-93 %) dose were high and confirms that an almost complete separation between MeO- and OH-PBDEs is achieved during clean-up.

In **PaperII**, the accuracy of the PBDE analysis in cat serum was tested through analysis of standard reference material (SRM 1957) from NIST (Figure 12). The four first congeners (BDE-47, -99, -100, -153) are by NIST certified values which means that their values are based on the agreement of results obtained at NIST (using one to three analytical techniques), the Centers for Disease Control and Prevention (CDC) and from an inter-laboratory study using different analytical techniques. BDE-28, BDE-66, BDE-85, BDE-154, and BDE-183 are reference values given by NIST. They are not certified values, but estimates of the true value.

The mean values (ng/kg reconstituted serum) measured in our laboratory for the NIST certified PBDE congeners: BDE-47, BDE-99, BDE-100 and BDE-153 were 81%, 97%, 99% and 97 % respectively of those specified by NIST

(Figure 12.). Notably, for BDE-47, our method underestimated the "true" concentration with about 20% which the authors do not have an explanation for. The coefficient of variation (CV) varied between 14-24 % for certified values (n=10 samples).

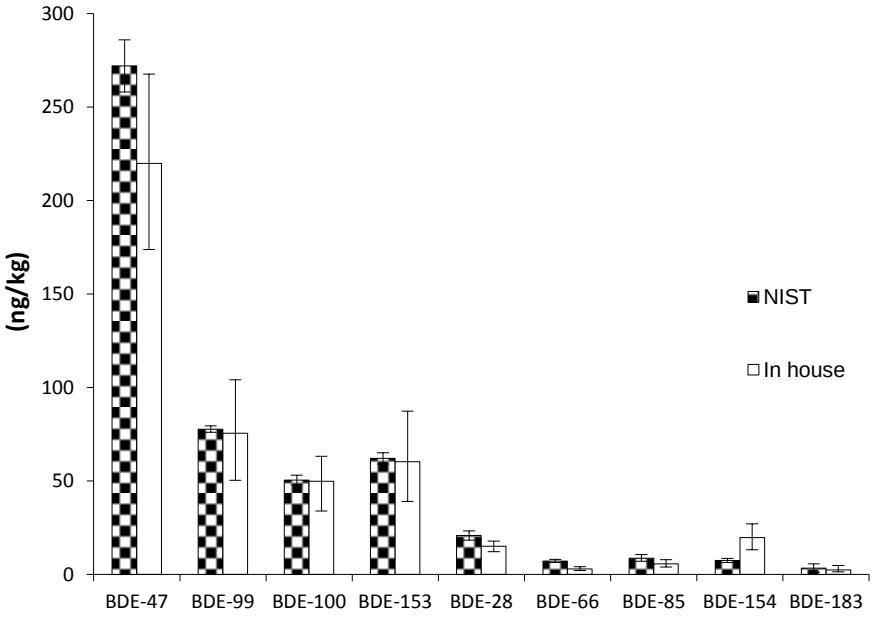


Figure 12. Mean concentration (ng/kg reconstituted serum) of PBDE congeners determined by NIST (SRM 1957) versus measured in house (n=10). Whiskers demonstrate measured min and max values.

The values obtained for the NIST reference PBDE congeners: BDE-28, BDE-66, BDE-85, BDE-154, and BDE-183 were 73%, 40%, 64%, 263% and 68% respectively of those by NIST determined values. Comparing the result with NIST reference values indicate all congeners but BDE-154 to be underestimated. BDE-183, a congener reported within this thesis, was underestimated with about 32%. BDE-154 was overestimated with about 160%. This is most likely due to chromatographic issues. BB-153 co-elutes with BDE-154 and since detecting on bromine anions, there is no way to distinguish the two. BDE-154 is therefore not reported herein. However, our measured value for BDE-154 was 85% of the sum of the certified value for BB-153 and reference value for BDE-154. The coefficient of variation (CV) varied between 10-40 % for reference values (n=10 samples).

PBDE congeners reported with in this thesis are in accordance with those of true and estimated values specified by NIST demonstrating that our analytical method is adequate.

Cat food

Along with each set of extracted cat food samples (n=6), one solvent blank and one QC (Whiskas wet food in pouch with salmon taste) were analysed for quality control. Surrogate standards (SS), one for each class of compounds (i.e. PCBs, PBDEs, OH-PBDEs and polybrominated simple phenols), were used to control the recoveries of the quantified compounds.

House dust

Along with each batch dust samples (n=17), five solvent blanks and two samples of standard reference material (SRM 2528), Organic contaminants in house dust, were analysis to ensure quality of the analysis. BDE-128 was used as surrogate standard (SS) to control the recoveries of the quantified compounds. The in house measured values for the nine PBDEs ranged between 91-105% of the by NIST given values (Figure 13).

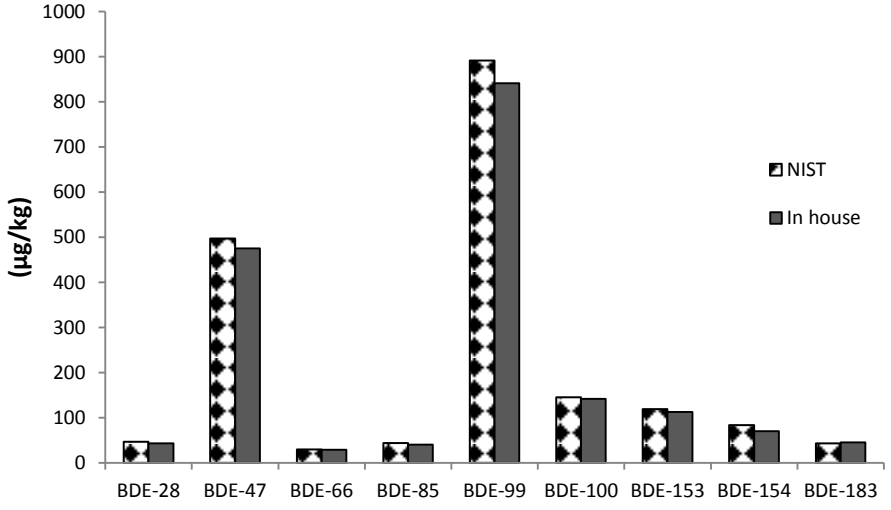


Figure 13. Mean concentration (µg/kg dust) of PBDE congeners determined by NIST (SRM 2528) values measured in house (n=2).

5.5 Statistics

Within this thesis, an integrated peak that was >LOD but <LOQ, was given the value LOQ/√2 in the statistical calculations. To set a peak that is <LOQ but >LOD to zero would give an overestimation of the true value.

Linear regression analysis on log-transformed data was used to test for linear association between age and compound concentrations in **Paper II**. The non-parametric Mann-Kendall trend test [140] based on the Kendall's tau (τ) coefficient, was applied to measure the association between serum concentrations in cats and matched food and dust samples in **Paper III**. The p-value gives the strength of the correlation and $p<0.05$ shows that the result is significant [141]. Only positive correlations between serum and food/dust concentrations were assumed, thus one-tailed tests and a significance level of 5% were applied in the statistical analysis.

Mann-Whitney U-test was applied to test for statistical differences in BFR load between healthy (euthyroid) and sick (hyperthyroid) cats in **Paper II**. The test is non-parametric and does not require data to be normally distributed, only similar distributions of the populations to be compared. Mann-Whitney *U*-test tries out if median concentrations of two populations are significantly different or not. A p-value <0.05, show a significant result, i.e. there is a significant difference in median concentration between the two groups [141].

Box-and-whisker plots were used to illustrate the distribution of the data in **Paper II**. The first quartile or 25th percentile, indicate that ¼ of the samples is below that limit. The second percentile or 50th percentile point out the median of the data set, whereas the third percentile or 75th percentile, represent ¾ of the data lies below this level. Whiskers point out extremes in the data such as minimum and maximum values or 10th and 90th percentile if non-normally distributed data [141].

Principal component analysis (PCA) is a multivariate method that is used to analyse datasets were several measures (e.g. brominated compounds) have been made on each individual. In PCA possible pattern within the data will be made visible. Further, PCA also identifies which characteristics that vary the most between individuals [141]. PCA was performed on the data from healthy and sick cats in **Paper II**.

6 Results and discussion

This chapter aims to highlight major findings from my research (**Paper I-IV**). The detailed background, methods applied and results of the specific studies are presented and discussed within each of the three published articles (**Papers I, II and IV**) and the manuscript (**Paper III**).

6.1 BFRs in cats and their sources of exposures (Paper I, II, III)

In **Paper I**, a pooled serum sample (16 mL) from 30 Swedish pet cats, diagnosed with FH, was analysed for BFRs. PBDEs, BB-209 and one natural product, 2'-MeO-BDE68 were tentatively identified. The dominating PBDE congeners of Penta-, Octa- and DecaBDE were represented in the cat serum, demonstrating exposure to all the technical mixtures (Figure 1A and 1B, **Paper I**). BDE-99 and BDE-47 were the dominating neutral compounds found in the pooled cat serum.

In **Paper II**, serum from 82 individual cats were analysed for the previous identified compounds in **Paper I**. Six PBDEs (BDE-47, -99, -153, -183, -201 and -209), BB-209 and CB-153 were frequently detected and quantified in the individual samples (Figure 1, **Paper II**). The detection rates of the neutral compounds were 89-100%. The box and whisker plot clearly shows that the data is skewed to the right. Therefore the 10^{th} and 90^{th} percentile was used instead of min and max values to illustrate the distribution of the extremes in the plot. This is a common approach when handling non-normal distributed data with extreme values (that are not outliers).

CB-153 was added to the list of analysed compounds for comparison to PBDE levels. Median concentration of CB-153 was 211 pmol/g l.w. (76 ng/g l.w.). The general congener pattern of Swedish pet cats from Stockholm/Uppsala region was BDE-209 > BDE-99 > BDE-201 > BDE-153 ≈ BDE-183 ≈ BDE-47 > BDE-28/-100, ranging from 56 pmol/g l.w. (54 ng/g l.w.) for BDE-209 to 11 pmol/g l.w (6 ng/g l.w.) for BDE-47 (Table S1, **Paper II**). The perbrominated biphenyl, BB-209, was the most abundant BFR, present in all of the analysed samples, in a median concentration of 87

pmol/g l.w. (82 ng/g l.w.). That is 40% of median concentration of the highly persistent and bioaccumulative CB-153, a surprisingly high level. The measured concentrations of BDE-209 were also elevated, but somewhat lower than for BB-209.

Concentrations of brominated compounds in euthyroid and hyperthyroid cats were compared in **Paper II** to explore possible associations between PBDE concentrations and thyroid status. From the study of individual cat serum samples (n=82), cats with euthyroid or normal thyroid status (n=23) and hyperthyroid cats (n=37) were selected (Figure 2, **Paper II**). Thus, cats on medication (n=22) were not included in the statistical comparison to strengthen the study. Age was identified as a cofounder in the study, thus higher PBDE concentrations of some PBDE were associated with increasing age. Since, the average age differed for the two groups of euthyroid and hyperthyroid cats, the serum concentrations were adjusted to the average age of 13 years by means of log-linear regression. Age-adjusted concentrations were then used for BDE-99, BDE-153, BDE-183 and CB-153 when comparing serum concentrations between euthyroid and hyperthyroid cats on lipid and fresh weight basis. Mann-Whitney U-test, a nonparametric statistical test was used to compare median concentrations between the two groups. Significant differences on both lipid and fresh weight (pmol/g l.w., pmol/g serum) for the eight analysed compounds were shown for BDE-99 ($p=0.0013$, $p=0.0010$), BDE-153 ($p=0.0007$, $p=0.0005$), BDE-183 ($p=0.0141$, $p=0.0124$) and CB-153 ($p=0.0040$, $p=0.0031$).

Paper II, when published, was the first study to report significant differences in median concentrations for some PBDE congeners (BDE-99, BDE-153 and BDE-183) and CB-153 between euthyroid and hyperthyroid cats. This could be an indication that an environmental factor such as PBDEs may be of importance for the etiology of feline hyperthyroidism.

The concentrations of individual PBDE congeners were similar or higher in Swedish cats than in Swedes. Concentration of BDE-47, -99 ,-153 and -209 in cat serum (**Paper II**) were compared to a cohort of young Swedish men signing in for the army, representing the general public [66] and to Swedish air craft maintenance workers [63], representing a highly exposed group of occupational workers (Figure 14). The levels of BDE-47 were similar in cats and highly exposed workers whereas the concentrations of BDE-99 were ten times higher in the cats compared to exposed workers and approximately 55 times higher than in non-exposed Swedes. Median concentrations of BDE-209 in Swedish pet cats were 14 and >300 times higher than exposed and non-exposed humans, respectively. This is in line with what has been reported from other studies were PBDE levels in pet cats were 10-100 times higher compared to humans from the same country [48,115,116,142].

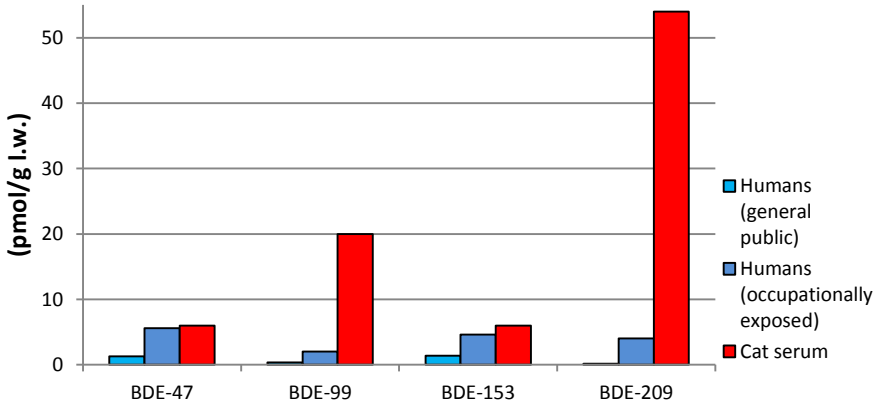


Figure 14. Median serum concentration of four PBDE congeners in Swedes representing the general public [66], occupationally exposed aircraft maintenance workers [63] and Swedish pet cats (n=83) (**Paper II**).

Beyond differences in serum levels between cats and human, the ratio of BDE-47/BDE-99, the two dominating of the lower brominated PBDE congeners, differs (Figure 15). The BDE-47/-99 ratio has frequently been used to demonstrate an exposure of the PentaBDE mixture (e.g. DE-71 and Bromkal 70-5DE). The ratio of BDE-47/BDE-99 in DE-71 and Bromkal 70-5DE are 0.8 and 1.0, respectively [31]. In Swedish house dust (**Paper III**) a ratio of 1.1 was measured.

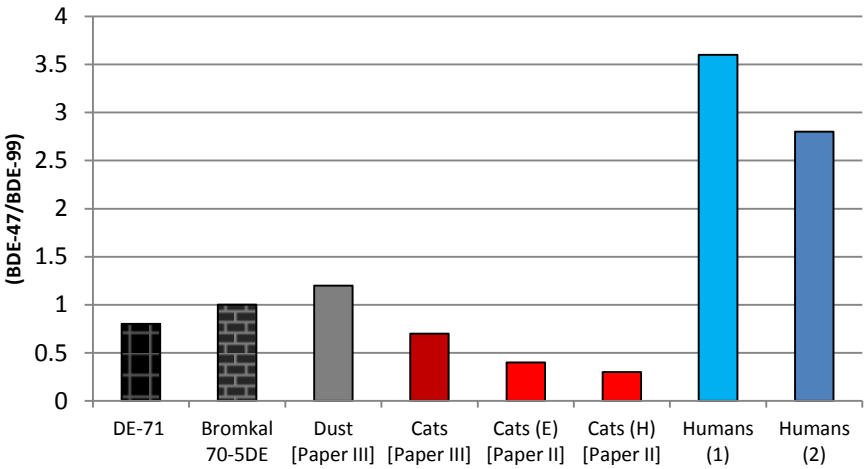


Figure 15. Ratio of BDE-47/BDE-99 in two technical PentaBDE mixtures (DE-71 and Bromkal 70-5DE) [31], house dust (**Paper III**), cats (**Paper III**), euthyroid (E) and hyperthyroid (H) cats (**Paper II**) and humans, (1) the general public [66] and (2) occupationally exposed [63,66].

In humans, BDE-47 is the more abundant congener of the two, giving ratios typically >1, and in the range of 3-10 [119]. In one study from the general public in Sweden, a ratio of 3.6 was measured and in occupationally exposed Swedes, 2.8 [63,66]. The BDE-47/BDE-99 ratio in Swedish pet cats from two studies were 0.7 (**Paper III**) and 0.4 and 0.3 for euthyroid and hyperthyroid cats, respectively (**Paper II**), i.e. <1. Thus, the ratio found in serum from Swedish pet cats, is more similar to a technical PentaBDE mixture and house dust than humans. In addition, median concentration of BDE-99 (0.12 pmol/g l.w.) in commercial cat food was not higher than for BDE-47 (0.25 pmol/ g l.w) (Table 4A, **Paper III**). Thus, the higher levels of BDE-99 in respect to BDE-47 observed in cat serum may not be explained by a higher exposure from food.

A BDE-47/BDE-99 ratio of <1 was also reported in Australian (euthyroid) (0.5), Pakistani (0.9), UK (control) (0.4) and in CA, USA (euthyroid) pet cats (0.25) (Table A2 in Appendix). Interestingly, Japanese stray cats, which spend no time indoors, having a low exposures to house dust, showed a ratio >2. At the same time, these cats also showed the lowest median levels of BDE-47 and BDE-99 (1.5 and 0.7 ng/g l.w., respectively). This is implies dust being a significant exposure source to PBDEs for indoor pet cats.

In **Paper III,** serum from Swedish pet cats were matched or paired with their preferred food and house dust from their homes to further explore cats' exposure sources to BFRs and some organochlorines (PCBs, HCB, 4,4'-DDT, 4,4'-DDE and PCP) (Table 3A, 3B, 4A, 4B and 5, **Paper III**). The profile of PBDEs, BB-209 and DBDPE found in house dust and food was similar (Figure 16A and 16C) whereas the profile in serum differed (Figure 16A).

In the matched serum samples**,** BDE-209 was abundant in highest median concentrations of analysed BFRs. This compound was also found in highest concentrations in dust and food samples (median of 320 pmol/g d.w. and 1.9 pmol/g lipid) (Figure 16B and 16C) explaining the high concentration of BDE-209 measured in serum. However, no correlations between serum concentrations of BDE-209 and its content in dust or food were observed, which is likely explained by the short half-life of BDE-209 in mammals, e.g. humans [83].

Serum concentrations of BDE-47 were found to correlate with dust but not at all with food samples (Figure 2, **Paper III**), further supporting dust as an important exposure source. The levels of PBDEs in dust, as reported in **Paper III,** are in agreement with what has been reported from Stockholm houses and apartments [143].

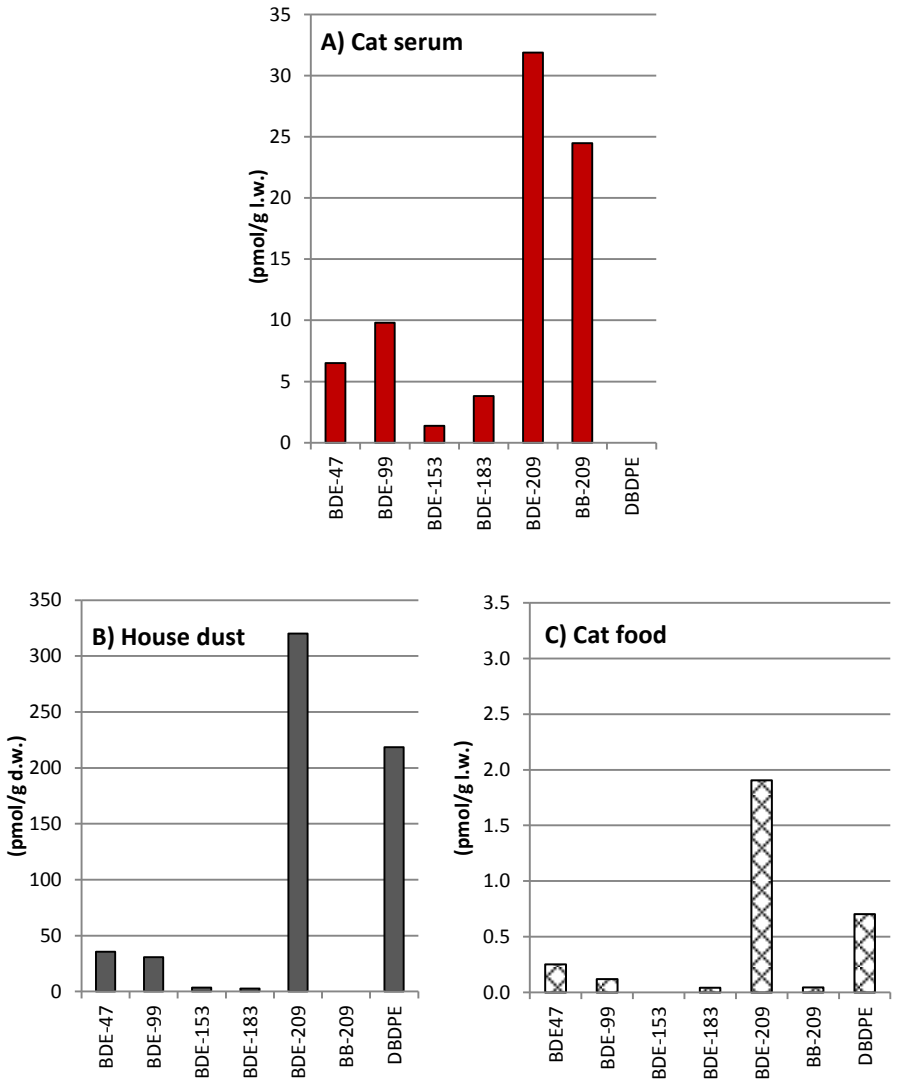


Figure 16. Profile of the median concentrations of five PBDE congeners, BB-209 and DBDPE in matched/paired samples of **A)** cat serum **B)** house dust and **C)** commercial cat food (wet/dry) (**Paper III**).

The elevated BDE-99 levels could also result from higher bioaccumulation due to lower metabolism in the cat, or contribution from debromination products originating from higher brominated congeners.

An overestimation of the true concentration is possible if co-eluting brominated compounds are present in the sample. It is known that BDE-99 from a technical product, may co-elute with some naturally occurring

compounds such as 5-Cl-6MeO-BDE47, 6'-Cl-2'-MeO-BDE68, 2',6-diMeO-BDE68 and degradation products of HBCDD found in environmental samples [144]. Thus, further studies may be required in this context.

Similarly as reported for the cats in **Paper II**, BB-209 was quantified in 89% of sampled cats in median serum concentration of 25 pmol/g serum. Further, a significant correlation was found between serum concentrations of BB-209 and cat food. The flame retardant was however, not detected in all cat food (43% >LOQ) indicating some additional exposure source(s). BB-209 could only be determined in a fraction of the dust samples (35%) and levels were low (median <LOD). It is worth to mention that BB-209 was identified, as a minor component, in settled dust from Boliden Rönnskär, a Swedish smelter facility, which is one of the world's largest recycles of electronic scrap [145]. The electronics handled here are primarily shipped from Europe. The occurrence of BB-209 in dust from this facility demonstrates that the flame retardant is still being circulated.

Decabromodiphenyl ethane (DBDPE) was identified in the second highest median concentration (218 pmol/g dust) in both dust and cat food, but not detected in any of the matched serum samples, indicating low bioavailability of the DBDPE in cats.

6.2 Brominated phenolic compounds in cats and their sources of exposures (Paper I, II, III)

In **Paper I**, phenolic brominated compounds such as OH-PBDEs and poly-bromophenols were analysed in a pooled serum (16 mL) sample from 30 Swedish pet cats diagnosed with FH. This was the first study, at the time, to report OH-PBDEs in cat serum, hence exploring the PBDE metabolism in cats. Five OH-PBDEs were identified in the pooled sample, of which 6-OH-BDE47 was by far the most abundant compound. 2'-OH-BDE68, 5-OH-BDE47 and 4'-OH-BDE49 were shown to be present as minor components. Further, three BPs; 2,4-DBP, 2,4,5-TBP and 2,4,6-TBP were identified in cat serum. Moreover, three peaks related to yet unknown chemicals (U4, U5 and U6) were found in the pooled cat serum sample at seemingly high concentrations (Figure 2A and 2B, **Paper I**). The hypothesis, that the unknowns, with similar retention times a TBP, actually were TBP isomers, was rejected. Retention times of authentic reference standards, including all six TBP isomers, were compared with the unknowns (U4, U5 and U6) only to confirm that they were indeed not TBPs. The second hypothesis, that the unknowns are chatecols, has not yet been explored.

Interestingly, in **Paper III**, in serum from cats with normal thyroid status, the unknown peak U4 and U5 were present in all of the individual serum samples. The same peaks were also present in the paired cat food samples, indicating food might be the source.

The phenolic compounds reported from individual serum samples in **Paper II** were 2,4,6-TBP, 2'-OH-BDE68 and 6-OH-BDE47. They were detected in 89%, 60% and 96% of the serum samples, respectively (Figure 1, **Paper II**). 6-OH-BDE47 was abundant in highest concentration in the individual samples, in a median concentration of 136 pmol/g l.w. (69 ng/g l.w.). Hence, CB-153 was only 1.55 times more abundant than 6-OH-BDE47. The polybrominated phenol, 2,4,6-TBP, was also among the compounds with high abundance, present in median concentration of 89 pmol/g l.w. (29 ng/g l.w.) in the individual cats.

No differences in median concentrations between euthyroid and hyper-thyroid cats were shown for any of the phenolic compounds neither on lipid nor on fresh weight basis.

In **Paper III**, concentrations of 2,4,6-TBP, 2'-OH-BDE68 and 6-OH-BDE47 and PCP in matched serum and cat food samples were compared. A significant correlation between serum concentration of 6-OH-BDE47 and food was found (Figure 17). The p-value of the non-parametric correlation test, Kendall's tau, showed statistical significance. On the basis of this finding, it may be speculated that food could be the source for other OH-PBDE found in serum from cats, although this needs confirmation.

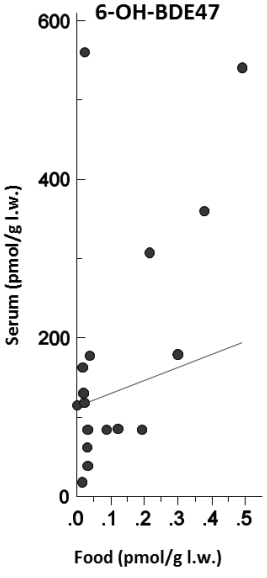


Figure 17. Correlation between serum concentration of 6-OH-BDE47 (y-axis) and cat food (x-axis) in paired samples. (n=17, b= 166, Kendall's tau=0.32, p< 0.037, one-tailed test)

6.3 Feline metabolism of PBDEs (Paper I, II, III)

The general PBDE profile in cats varies between countries (Figure 7, Chapter 4.1.1) due to different exposure patterns.

Few studies have reported OH-PBDE in cat serum, but from those who have, the profile was similar, i.e. 6-OH-BDE47 was the by far the dominating compound followed by 2'-OH-BDE68, 4-OH-BDE49 and 5-OH-BDE47 (**Paper II**) [118,146]. In fact, the two most commonly reported OH-PBDEs in cats (i.e. 6-OH-BDE47 and 2'-OH-BDE68) are of natural origin rather than being typical metabolites of BDE-47 (such as 4'-OH-BDE49 and 5-OH-BDE47) (**Paper II**), [117-119,146].

In the review of POPs reported in cats (Table A2, Appendix), Japanese stray cats showed the highest serum concentrations of 6-OH-BDE47, despite having the lowest levels of BDE-47 amongst all studies. In comparison to Swedish cats (**Paper II**), Japanese stray cats have about 1.4 times higher serum concentrations of 6-OH-BDE47 and 5 times lower concentrations of BDE-47 than Swedish pet cats. The ratio of 6-OH-BDE47/BDE-47 in Swedish pet cats was 12 (**Paper II**). These findings suggest additional source(s) of contribution for 6-OH-BDE47 other than oxidative metabolism of BDE-47, e.g. food. This was investigated in **Paper III**, and a significant correlation between serum concentrations of 6-OH-BDE47 and matched food samples were indeed found. Further, 6-MeO-BDE47 was present in almost four times the levels of BDE47 and 40 times the levels of 6-OH-BDE47 in cat food. If 6-MeO-BDE47 is demethylated to 6-OH-BDE47 [147] in cats, this could be an important source of 6-OH-BDE47.

Surprisingly low concentrations (mostly <LOD) of the naturally produced 2'-MeO-BDE68 and 6-MeO-BDE47 were found (Table 3A, **Paper III**). In **Paper I**, at least detectable levels of 2'-MeO-BDE68 were indicated in the pooled cat serum. Further, it is remarkable that no hydroxylated metabolites of BDE-99 was found in serum from cats since it is present in more than 1.5- 3.5 times of BDE-47 (**Paper II, III**).

PBDEs have been shown to be metabolically transformed to OH-PBDEs in exposed rats [46,77] and mouse [80] and in *in vitro* incubations of BDE-47 in human and rat hepatic microsomes [74,75] but not in cats. In polar bears with a high capacity to metabolize PCBs, it was concluded that the only detected OH-PBDE congener i.e. 6-OH-BDE47, was most likely accumulated from seals rather than being metabolically formed [148,149]. In **Paper III**, a significant correlation between matched serum concentrations of 6-OH-BDE47 and cat food was we demonstrated in paired samples, which supports that theory for cats.

Taken together, there is no evidence showing that PBDEs are metabolically transformed to OH-PBDEs to any major extent, in cats. Considering cats' unique metabolism and the finding of 6-OH-BDE47 as the dominating OH-PBDE congener, we suggest biotransformation of PBDEs to OH-PBDEs not to occur to any great extent in cats. Further, we postulate that the larger contribution of 6-OH-BDE47 found in cat serum is of natural origin, ingested via their food.

7 Conclusions and future perspectives

Several important findings are highlighted in this thesis. Swedish pet cats have higher serum levels of PBDEs than the general public in Sweden. The PBDE pattern in cats (Penta-BDE, OctaBDE and DecaBDE) is similar to the pattern found in house dust. BDE-209, the major component of DecaBDE was the most abundant congener, followed by BDE-99, the dominating PentaBDE congener found in cats, present in 10-55 times higher concentrations than in humans. The serum concentrations of BDE-47 in cats were shown to correlate with dust collected from their living rooms. These findings suggest house dust to be an important exposure source of PBDEs to cats.

The concentrations of BDE-99, BDE-153 and BDE-183 in hyperthyroid cats show associations to FH in the cats. Further, presences of the long-time ceased flame retardant decabromobiphenyl (BB-209) were detected in 100% of the sampled pet cats whilst it was only detected in 35% and 43 % of the match dust and food samples. A significant correlation was shown between matched serum and food samples for BB-209. However, in a few cats, BB-209 was detected in serum, but not in food or dust collected from their homes, suggesting other, yet non-identified, source(s) of exposure.

The feline metabolism detoxification system (including phase I and phase II enzymes) seem to be less developed in comparison to humans. No evidence was found to prove that metabolism of PBDEs to OH-PBDEs is taken place. No hydroxylated metabolites of BDE-99 were identified and the major OH-PBDE (6-OH-BDE47) measured in cats is a natural product and shown to correlate with their food.

In summary, this thesis demonstrates how indoor pet cats may be used as a biomarker for exposure to chemicals accumulated in dust in home environments.

For the future, it would be of particular interest to perform *in vitro* incubation studies with single PBDE congeners and technical mixtures in feline liver microsomes, to assess cat metabolism and possibly confirm the findings presented in this thesis, i.e. no or very limited formation of

OH-PBDEs. Analysis of whole tissue homogenates of liver, kidney and adipose tissue from cat may give valuable knowledge about of the distribution and retention of parent PBDEs and their possible metabolites. On the whole, very little is known on how POPs are metabolized in cats.

In addition, presence of BB-209 in cat litter needs further investigation. Cat litter, commonly used for indoor pet cats, has been associated with FH in epidemiological studies and are the next interesting exposure source for pet cats to be investigated.

8 Svensk sammanfattning

Vi lever i en värld där vi dagligen omges av kemikalier som exempelvis läkemedel, bekämpningsmedel, konserveringsmedel och flamskyddsmedel samt i skönhetsprodukter, målarfärg och plaster som har framställts på syntetisk väg. Samtidigt som kemikalierna har hjälpt oss till bättre hälsa och underlättat våra liv, har vi också fått betala ett pris för detta då vissa av dessa kemikalier har visat sig ha negativa effekter på människa och djurliv. Exempelvis belönades Paul Hermann Müller Nobelpriset i fysiologi/medicin 1948 för sin upptäckt att DDT kunde användas som bekämpningsmedel mot insekter, något som då ansågs revolutionerande. Efter två decenniers användande var det uppenbart att DDT bidrog till nedgång i rovfågel-populationer runt om i världen. Förklaringen låg i att äggskalen hade förtunnats och äggen torkades ut under ruvningen vilket ledde till att ungarna dog innan de hunnit kläckas. Beståndet av svenska havsörnar (*Haliaeetus albicilla*) sjönk drastiskt och var nära utrotning. Idag är DDT och 25 andra s.k. organiska miljögifter förbjudna och regleras genom den s.k. Stockholmskonventionen, en global konvention undertecknad av 179 parter/länder runt om i världen.

Kännetecknande för dessa organiska miljögifter är att de är långlivade (persistenta), tar lång tid att brytas ner i människa, djur och natur, och att de kan ge upphov till negativa effekter som cancer, reproduktions- och utvecklingsstörningar.

Polybromerade difenyletrar (PBDEer) tillhör en grupp av bromerade organiska ämnen som har använts för att flamskydda bland annat möbler, textilier, plaster och elektronik (t.ex. TV-apparater och datorer) sedan början på 1970-talet. De producerades och såldes som tre tekniska produkter (PentaBDE, OktaBDE och DekaBDE). Penta- och OktaBDE-produkterna är numera förbjudna och lyder under Stockholmskonventionen sedan 2004 då de har uppvisat persistens och toxiska effekter som bland annat sköldkörtel-hormonstörning. Den tekniska produkten DekaBDE är inte reglerad ännu, men är föreslagen som kandidat till Stockholmskonventionens lista.

Användningen av PBDEer har varit global och omfattande och därför hittas dessa kemikalier numera överallt i miljön, även i områden långt ifrån kemikalieindustrierna såsom i Antarktis, där användning av flamskydds-

medel dessutom har varit obefintlig. Exempelvis har PBDEer påvisats i isbjörn (*Ursus maritimus*) och i Håkäring/Grönlandshaj (*Somniosus microcephalus*). PBDE är fettlösliga substanser som kan analyseras i blod, modersmjölk och fettrika vävnader (t.ex. fett och lever) i människa och djur. De har identifierats i såväl människa som i vårt älskade husdjur katten.

Den här avhandlingen handlar om bromerade organiska miljögifter som har analyserats i katt (Felis catus) och undersöker huruvida det finns någon koppling mellan dessa och feline hypertyreos (FH) som är vanligt förekommande i katt. FH är en endokrin sjukdom som förekommer hos tamkatter världen över och det har skett en stark ökning av antalet rapporterade fall under de senaste 30 åren. Denna trend sammanfaller även med tidstrenden för ökad användning av flamskyddsmedel i hemmen. Fortfarande vet man inte säkert vad som ligger bakom denna ökning av FH, men miljöfaktorer såsom PBDE har föreslagits som en möjlig orsak.

I avhandlingens **Artikel I** identifierades bromerade organiska ämnen i serum från katt. Även fenolära substanser identifierades, d.v.s. metaboliter eller omvandlingsprodukter av dessa miljögifter för att få en bild av kattens metabolism. Då denna artikel publicerades, var detta helt ny och unik information. Serum från ett 30-tal katter, alla med diagnosen FH, polades ihop. Förutom de förbjudna produkterna PentaBDE och OktaBDE identifierades även BDE-209 (huvudkomponenten i DekaBDE) och dekabrombifenyl (BB-209) i relativt höga halter. Fyndet av BB-209, ett av de tidigare använda flamskyddsmedlen, är intressant. Trots att produktionen av kemikalien upphörde för 15 år sedan finns den fortfarande kvar i vår miljö.

I **Artikel II** genomfördes en studie där bromerade substanser som tidigare identifierats i Artikel I, kvantifierades i serum från enskilda prover från 82 katter. Målet var att undersöka om det var någon skillnad i halter mellan friska katter och katter med FH. Vi kunde visa att de sjuka katterna hade högre halter av BDE-99, BDE-153 och BDE-183 jämfört med de friska katterna. Vidare bekräftades det att BB-209 faktiskt var vanligt förekommande i katt, då alla individuella prov innehöll detta flamskyddsmedel. Dessutom förekom BB-209 i nästan lika höga halter som BDE-209.
Vi kunde även konstatera att det inte verkar som katten metaboliserar PBDE till fenolära metaboliter. Endast ett fåtal hydroxylerade PBDE (OH-PBDE) kvantifierades varav den dominerande, 6-OH-BDE47, också förekommer naturligt i den marina miljön där den produceras av t.ex. alger och svampar. Detta betyder att katten kan få i sig den via mat (t.ex. fisk och skaldjur) eller har den bildas via metabolism av BDE-47. Vidare identifierades inte heller några hydroxylerade metaboliter av BDE-99 i kattserum.

I **Artikel III** genomfördes en studie av 29 katters exponering för bromerade substanser via kattmat och damm från deras hem. När katter slickar sin päls för att hålla den ren får de även i sig dammpartiklar som fastnat i pälsen. På dammpartiklarna ansamlas kemikaler som är fettlösliga och persistenta såsom halogenerade organiska föreningar. Damm är således en viktig exponeringsväg för kemikalier i vår hemmiljö. Vidare kan man tänka sig att katters dammexponering liknar små barns (1-3 år), vilka har en högre dammexponering jämfört med vuxna. Detta beror på små barns beteende av att smaka/suga på leksaker och annat de kommer åt när de kryper på golvet. Uppskattning av inomhuskatters exponering kan därmed ge information om små barns exponering i hemmen. Halterna av BDE-47, en av de två dominerande kemikalierna i PentaBDE, visade sig korrelera med de uppmätta halterna i dammprover från vardagsrummen. Vidare korrelerade serumkoncentrationerna av BB-209 och 6-OH-BDE47 med vad katterna åt.

I **Artikel IV** utvärderades en tidigare publicerad extraktionsmetod för analys av bromfenoler och OH-PBDE i blod från människa, katt, fågel (alfågel) och fisk (strömming). Återvinningen av fyra bromfenoler, nio OH-PBDE och tre MeO-PBDE på två koncentrationsnivåer; låg (0.5 ng) och hög (5 ng), bestämdes i de fyra blodmatriserna. Metoden som ursprungligen tagits fram för bl.a. PBDE-analyser i human blodplasma visade sig vara applicerbar och ge tillförlitliga resultat även för analys av bromfenoler och OH-PBDE i humanplasma och kattserum. Återvinningen av dessa bromerade substanser i alfågel och strömming visade sig däremot vara otillräcklig och metoden är således inte tillförlitlig för blodanalyser av OH-PBDE i fisk och fågel.

Sammanfattningsvis har vi visat att våra svenska inomhuskatter har mycket högre halter av flamskyddsmedel såsom PBDE i deras blod jämfört med människor i Sverige. Katter är exponerade för Penta-, Okta- och DekaBDE. BDE-209 är den dominerande kongenen i kattblod följt av BDE-99. Den finns i 10-55 gånger högre halter i katt jämför med människa. Serumhalterna av BDE-47 i katter korrelerade med koncentrationen i damm från deras hem, vilket styrker hypotesen att damm är en viktig exponeringskälla för katter. Detta gör katten lämplig att använda som en biomarkör för exponering av kemikalier som finns i damm från våra hem.

Koncentrationen av BDE-99, BDE-153 och BDE-183 var högre i katter med giftstruma jämfört med katter med normal tyreoidastatus vilket skulle kunna vara en indikation på att det kan finnas en koppling mellan PBDE och FH.

Katters metabolism av miljögifter skiljer sig från människans. Det verkar inte som metabolism av PBDE till OH-PBDE sker i någon större utsträckning i katter och den dominerande OH-PBDEn i serum, 6-OH-BDE47, visade sig korrelera med vad katterna åt.

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10 Appendix

Contribution to **Paper I-VI**

I. Performed all laboratory work and was responsible for writing the publication.

II. Performed all laboratory work and was responsible for the data evaluation and publication writing.

III. Performed the laboratory work on cat serum and cat food, was responsible for evaluating all the data and took part in manuscript writing.

IV. Performed laboratory work on cat serum, evaluated all the data together with co-author Anna-Karin Dahlberg who I shared writing responsibilities with for the publication.

Table A1, Appendix. Numbering of PCB and PBDE congeners according to Ballschmitter, et al. (1993), Long chain alkyl-polysiloxanes as non-polar stationary phases in capillary gas chromatography, *Fresenius' J. of Anal. Chem.*, 346, 396-402.

	2'	3'	4'	2'3'	2'4'	2'5'	2'6'	3'4'	3'5'	2'3'4'	2'3'5'	2'3'6'	2'4'5'	2'4'6'	3'4'5'	2'3'4'5'	2'3'4'6'	2'3'5'6'	2'3'4'5'6'
2	1	4	6	8															
3	2		11	13															
4	3			15															
23	5	16	20	22	40	42	44	46	56	58									
24	7	17	25	28		47	49	51	66	68									
25	9	18	26	31			52	53	70	72									
26	10	19	27	32				54	71	73									
34	12	33	35	37					77	79									
35	14	34	36	39						80									
234	21	41	55	60	82	85	87	89	105	108	128	130	132	138	140	157			
235	23	43	57	63	83	90	92	94	107	111		133	135	146	148	162			
236	24	45	59	64	84	91	95	96	110	113			136	149	150	164			
245	29	48	67	74	97	99	101	102	118	120				153	154	167			
246	30	50	69	75	98	100	103	104	119	121					155	168			
345	38	76	78	81	122	123	124	125	126	127						169			
2345	61	86	106	114	129	137	141	143	156	159	170	172	174	180	182	189	194	196	199
2346	62	88	109	115	131	139	144	145	158	161	171	175	176	183	184	191		197	201
2356	65	93	112	117	134	147	151	152	163	165	177	178	179	187	188	193			202
23456	116	142	160	166	173	181	185	186	190	192	195	198	200	203	204	205	206	207	208	209
	2'	**3'**	**4'**	**2'3'**	**2'4'**	**2'5'**	**2'6'**	**3'4'**	**3'5'**	**2'3'4'**	**2'3'5'**	**2'3'6'**	**2'4'5'**	**2'4'6'**	**3'4'5'**	**2'3'4'5'**	**2'3'4'6'**	**2'3'5'6'**	**2'3'4'5'6'**

Table A2, Appendix. A global review of POPs reported in cat serum/plasma or whole blood (ng/g l.w.).

Country		Australia [116]		Japan [120]	Japan [118]		Pakistan [117]		Sweden [Paper II]		Sweden [Paper III]		UK [119]		USA (CA) [113]		USA (GA) [115]		USA (IL) [114]	
Matrix		serum		whole blood	whole blood		serum		serum		serum		plasma		serum		serum		serum	
Sampling year		2012		2009	2008-2011		2012		2010		2012-2014		2010-2011		2008-2010		2005-2006		2006-2007	
Sampling size (n)		65		5	10		20		60		19		61		22		18		61	
Health status[a]		E (30)	H (35)						E (23)	H (37)			C(21)	T2DM(20) ADM(20)	E (6)	H (16)	E (7)	H (11)	E (21)	H (41)
Age (median yrs)		5.9	6.2						10.6	14.5			12	12.4 11.9	13.5	14	5.9	6.2	13.4	13.7
		(ng/g l.w.)		(ng/g l.w.)[b]	(ng/g l.w.)[b]		(ng/g l.w.)		(ng/g l.w.)		(ng/g l.w.)		(ng/g l.w.)[b,c]		(ng/g l.w.)		(ng/g l.w.)[b]		(ng/g l.w.)	
		median	(min-max)		median	(min-max)	median	(min-max)	median	(min-max)	median	(min-max)	median		median	(min-max)	median	(min-max)	median	(min-max)
PBDEs																				
BDE-47	E/C	37	(4-2783)		1.5	(<0.7-5.6)	1.9	(<0.5-525)	6.0	(0-34)	3.2	(0-21)	5.0		567	(162-4941)				
	H	28	(0-697)						7.0	(0-33)					604	(49-3360)				
	T2DM												6.0							
	ADM												7.3							
BDE-99	E/C	74	(11-2119)		0.67	(<0.67-3.5)	2.0	(<0.4-665)	14	(4-306)	5.5	(1.3-57)	11		2239	(207-13554)				
	H	47	(22-1647)						24	(5-1280)					1513	(176-4483)				
	T2DM												14							
	ADM												37							
BDE-183	E/C	nd							5	(2-60)	2.8	(1.1-6.0)			33	(4.5-74)				
	H	nd							6	(2-136)					18	(3.6-43)				
BDE-209	E/C				59	(<0.67-3359)			62	(26-508)	31	(14-58)			279	(91-495)				
	H								47	(0-403)					220	(23-3373)				
∑PBDE	E/C	174 $\sum_5$	(5-5260)		93 $\sum_{11}$	(0.99-4159)	6.1	(1-1280)	102 $\sum_6$	(33-1029)	43 $\sum_5$	(17-151)	28 $\sum_7$		3742 ($\sum_9$)	(874-22537)	944	(357-4351)	2850	(470-16000)
	H	82 $\sum_5$	(5-3429)						99 $\sum_6$	(11-2784)					3412 $\sum_9$	(631-9491)	992	(480-6318)	2517	(370-51000)
	T2DM												31 $\sum_7$							
	ADM												59 $\sum_7$							
PBBs																				
BB-209	E								81	(13-653)	23	(4.0-237)								
	H								65	(8-288)										
OH-PBDEs																				
3-OH-BDE47					<0.16	(<0.16-4.5)														
5-OH-BDE47	C				<0.16	(<0.16-2.3)							<LOQ							
	T2DM												<LOQ							
	ADM												<LOQ							
6-OH-BDE47	E/C				107	(40-960)			68	(4-268)	42	(0-281)	1.8							
	H								85	(0-756)										
	T2DM												3.6							
	ADM												5.5							
4'-OH-BDE49	C				<0.16	(<0.16-4.0)							<LOQ							
	T2DM												1.3							
	ADM												3.1							
2'-OH-BDE68	E/C				6.7	(2.2-22)			1	(0-72)	0	(0-13)	1.3							
	H								3	(0-117)										
	T2DM												<LOQ							
	ADM												0.91							
∑OH-PBDEs	C				115 ∑23	(42-992)							3.8 $\sum_6$							
	T2DM												6.3 $\sum_6$							
	ADM												7.6 $\sum_6$							

Table A2 (cont.), Appendix. A global review of POPs reported in cat serum/plasma or whole blood (ng/g l.w.).

Country		Australia [116]	Japan [120]	Japan [118]	Pakistan [117]	Sweden [Paper II]	Sweden [Paper III]	UK [119]	USA (CA) [113]	USA (GA) [115]	USA (IL) [114]
Matrix		serum	whole blood	whole blood	serum	serum	serum	plasma	serum	serum	serum
Sampling year		2012	2009	2008-2011	2012	2010	2012-2014	2010-2011	2008-2010	2005-2006	2006-2007
Sampling size (n)		65	5	10	20	60	19	61	22	18	61
Health status[a]		E (30) H (35)				E (23) H (37)		C(21 T2DM(20) ADM(20)	E (6) H (16)	E (7) H (11)	E (21) H (41)
Age (median yrs)		5.9 6.2				10.6 14.5		12 12.4 11.9	13.5 14	5.9 6.2	13.4 13.7
		(ng/g l.w.)	(ng/g l.w.)[b]	(ng/g l.w.)[b]	(ng/g l.w.)	(ng/g l.w.)	(ng/g l.w.)	(ng/g l.w.)[b,c]	(ng/g l.w.)	(ng/g l.w.)[b]	(ng/g l.w.)
		median (min-max)		median (min-max)	median (min-max)	median (min-max)	median (min-max)	median	median (min-max)	median (min-max)	median (min-max)
MeO-PBDEs											
6-MeO-BDE47				7.4 (0.16-35)			0 (0-0)				
2'-MeO-BDE68				24 (5.1-288)			0 (0-1.7)				
Phenols											
2.4.6-TBP	E/C				12 (<5-85)	34 (1-72)					
	H					29 (3-117)					
OCPs											
PCP	C		352		97 (<10-555)		18 (4.1-186)	217			
	T2DM							258			
	ADM							342			
HCB	C				3.5 (<2-6.9)			2.4			
	T2DM							2.9			
	ADM							1.8			
4,4'-DDT	E/C				111 $\sum_{DDTs}$ (<1-2175)		11 (0-50)	<LOQ	0 (0-180)		
	H								57 (0-365)		
	T2DM							<LOQ			
	ADM							<LOQ			
4,4'-DDE	E/C				95 (<1-2150)		13 (0-120)	83	472 (106-1143)		
	H								368 (160-1746)		
	T2DM							79			
	ADM							137			
PCBs											
CB-105	C							1.8			
	T2DM							2.3			
	ADM							4.2			
CB-118	E/C			2.2 (<1.2-154)	5.3 (1.7-26)			10	13 (0-27)		
	H								22 (6.1-43)		
	T2DM							8			
	ADM							20			
CB-138	E/C			15 (<1.2-240)	7.8 (3.5-33)		8.4 (0-77)	23	26 (0-64)		
	H								23 (0-91)		
	T2DM							23			
	ADM							46			
CB-153	E/C			35 (4.8-496)	5.6 (<5-27)	41 (9-983)	10 (0-92)	33	48 (8-124)		
	H					73 (9-326)			46 (7.9-158)		
	T2DM							39			
	ADM							78			

Table A2 (cont.), Appendix. A global review of POPs reported in cat serum/plasma or whole blood (ng/g l.w.).

Country		Australia [116]	Japan [120]	Japan [118]	Pakistan [117]	Sweden [Paper II]	Sweden [Paper III]	UK [119]	USA (CA) [113]	USA (GA) [115]	USA (IL) [114]
Matrix		serum	whole blood	whole blood	serum	serum	serum	plasma	serum	serum	serum
Sampling year		2012	2009	2008-2011	2012	2010	2012-2014	2010-2011	2008-2010	2005-2006	2006-2007
Sampling size (n)		65	5	10	20	60	19	61	22	18	61
Health status[a]		E (30) H (35)				E (23) H (37)		C(21 T2DM(20) ADM(20)	E (6) H (16)	E (7) H (11)	E (21) H (41)
Age (median yrs)		5.9 6.2				10.6 14.5		12 12.4 11.9	13.5 14	5.9 6.2	13.4 13.7
		(ng/g l.w.) median (min-max)	(ng/g l.w.)[b]	(ng/g l.w.)[b] median (min-max)	(ng/g l.w.) median (min-max)	(ng/g l.w.) median (min-max)	(ng/g l.w.) median (min-max)	(ng/g l.w.)[b,c] median	(ng/g l.w.) median (min-max)	(ng/g l.w.)[b] median (min-max)	(ng/g l.w.) median (min-max)
PCBs (cont.)											
CB-180	E/C			5 (1.6-208)				14	17 (8.0-56)		
	H								18 (0-78		
	T2DM							15			
	ADM							31			
∑PCB	E/C		26	88 $\sum_{56}$ (6.4-2559)	36 (16-132)			142 $\sum_{29}$	189 $\sum_{8}$ (75-336)		
	H								211 $\sum_{8}$ (58-566)		
	T2DM							154 $\sum_{29}$			
	ADM							302 $\sum_{29}$			
OH-PCBs											
4-OH-CB107	C		16	9.3 (<0.10-30)	0.8 (<0.5-6.0)			2.1			
	T2DM							3.1			
	ADM							6.6			
4-OH-CB-146	C		1.1	0.4 (<0.10-4.5)	<0.5 (<0.5-7.2)			0.8			
	T2DM							1.8			
	ADM							3.8			
3-OH-CB153	C			<0.1 (<0.10-4.5)				0.3			
	T2DM							0.5			
	ADM							0.7			
4-OH-CB162	C			4.2 (<0.10-18)				1.3			
	T2DM							1.4			
	ADM							1.8			
4-OH-CB187	C		1.4	0.96 (<0.10-2.9	0.7 (<0.5-5.5)			<LOQ			
	T2DM							<LOQ			
	ADM							<LOQ			
∑OH-PCB	C		134	29 $\sum_{52}$ (2.4-176)	2.2 (<0.5-21)			6.7 $\sum_{18}$			
	T2DM							11 $\sum_{18}$			
	ADM							17 $\sum_{18}$			

[116] Chow et al. 2015, [120] Kunisue et al. 2009, [118] Mizukawa et al. 2013, [117] Ali et al. 2013, [Paper II], [Paper III], [119] Dirtu et al. 2013, [113] Gou et al. 2012, [115] Dye et al. 2007, [114] Menching et al. 2012

[a] E=euthyroid, C=control, H=hyperthyroid, T2DM=type 2 diabetes mellitus, ADM= acromegaly induced diabetes mellitus

[b] Concentrations were calculated from pg/mL serum according to following assumption; density of cat blood 1.042 g/mL and lipid % = 0.6 (Norrgran et al. 2012)

[c] Total lipids were calculated enzymatically when TG and CHOL measurements were available according to TL=1,33*TG+1,12*CHOL+148 (mg/dL) (Covaci et al. 2006)

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