University of Veterinary Medicine Budapest

Department of Physiology and Biochemistry
Division of Physiology

Indoor cats' exposure to organic flame retardants and its association with the incidence of spontaneous feline hyperthyroidism

By:

Scicluna Kurt

Supervisor:

Dávid Sándor Kiss PhD

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List of Abbreviations

ABS - Acrylonitrile Butadiene Styrene

BDEs – Brominateddiphenylethers87t

BFRs – Brominated Flame Retardants

BPA- Bisphenol-A

CFRs – Chlorinated Flame Retardants

CKD – Chronic Kidney Disease

CONTAM – Contaminants in the Food Chain

DDT - Dichlorodiphenyltrichloroethane

EFSA - European Food Safety Authority

EPA – Environmental Protection Agency

FDA – Food and Drug Administration

FH – Feline Hyperthyroidism

FRs – Flame Retardants

fT4 – free T4

GFR – Glomerular Filtration Rate

HBCD - Hexabromocyclododecane

INC – International Committee

MEO-PBDEs – Methoxylated-PBDEs

NFRs – Nitrogen Flame Retardants

NIS – Sodium/Iodide Symporter

OFRs – Organic Flame Retardants

PFRs – Phosphorus Flame Retardants

PBDEs - Polybrominateddiphenylethers

PCBs - Polychlorinatedbiphenyls

POPs – Persistent Organic Pollutants

OH-PBDEs – Hydroxylated PBDEs

RBP - Retinol Binding Protein

T3 - Triiodothyronine

T4 - Thyroxine

TBBPA – Tetrabromobisphenol-A

TBGs – Thyroxine Binding Globulin

TCBPA – Tetrachlorinatedbisphenol-A

TDICPP - Tris(1,3-dichloroisopropyl) phosphate

Tg - Thyroglobulin

TH(s) – Thyroid Hormone(s)

TR – Thyroid Receptor

TSH – Thyorid Stimulating Hormone

tT4 – total T4

TTR - Transthyretin

UNEP – United Nations Environment Programme

Introduction

Flame retardants are chemicals which are incorporated into raw materials that are intended for use in a final product. These raw materials include various types of plastics, textiles, and foams for a myriad of possible applications. This means that virtually all mattresses, carpets, paints, curtains, computers, phones, televisions, wirings, insulations, upholstery, seats etc. contain flame retardants.

These chemicals are released into the environment primarily during manufacture or by leaching out of the final product. Depending on the way they are incorporated into the polymer matrix of the raw material, flame retardants can leach out of the final product in the indoor setting owing to the chemical's occurrence in indoor dust.

Most of the organic flame retardants that were in heavy industrial use have been classified as persistent organic pollutants and their manufacture is no longer allowed, however novel OFRs are constantly emerging to replace the banned ones. Furthermore, the ban of manufacture of certain pollutants does not tackle the problemlj with retained, relatively old consumer appliances containing these banned pollutants. These old products represent an important source of indoor pollution and route of exposure,

Human and indoor animals' exposure to these chemicals can be quantified through levels in their blood. Significant exposure through dust inhalation and diet in humans and pet animals has been recorded and its possible adverse effects on the thyroid gland has been the focus of some scientific research in these past decades.

These recent studies have pointed out the seemingly coinciding novel use and the increasing demand of certain flame retardants in the 70s to the emergence and increasing prevalence of the disease. Furthermore, structural similarities between certain organic flame retardants and Thyroid hormones have analogous implications at the hormones' sites of action.

This literature review examines the role flame retardant chemicals have in the development of Spontaneous Feline Hyperthyroidism, as well as their presence in the environment and the cats' routes of exposure to these chemicals.

Chapter 1. Overview of Feline Hyperthyroidism

1.1 Anatomy and Physiology of the Thyroid Gland

The healthy cat's thyroid gland (Figure1) is made up of two lobes which are flat and elongated, ~2 cm long and ~0.3cm wide (Volkaert et al., 2016). There is one on each side of the neck, at the level of the 7th to 10th tracheal rings, lateral to trachea and caudal to the larynx (Konig and Liebich, 2009). Connective tissue septa divide the thyroid lobes into lobules which in turn are made up of thyroid follicles. These follicles are spherical and lined with epithelial cuboidal cells called the follicular cells, also known as thyrocytes or thyroid epithelial cells.

Sodium iodide symporters (NIS) are intrinsic membrane proteins of the follicular cells which allow for intracellular transport of Iodide from the blood. The follicular lumen contains a colloid that functions as a storage for Thyroid Hormones (THs), iodide and thyroglobulin (Tg), a precursor for the TH (Khan and Farhana, 2021). Synthesis of the THs occurs at the level of the follicular cells under the regulation of the pituitary gland, mediated by Thyrotropin, also called Thyroid Stimulating Hormone (TSH). TSH additionally functions in regulating the release of TH from storage in their colloid follicle (Burrow, Preedy and Watson, 2009).

In most mammals, thyroid hormones are transported in the blood via Thyroid Hormone Globulins (TBGs), transthyretin (TTR)and albumin. Cats, however, appear to lack TBGs and rely on TTR and Albumin for thyroid hormone transport (Tóthová and Nagy, 2018). Transthyretin is synthesized in the liver and is found in the blood and cerebrospinal fluid and is a known transporter of thyroxine and Retinol Binding Protein (RBP) (Liz et al., 2020).

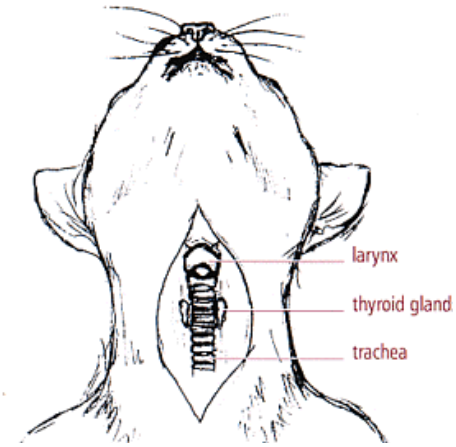


Figure 1: Schematic illustration of a healthy cat's thyroid gland

1.2 Spontaneous Feline Hyperthyroidism

Peterson was the first to document Feline Hyperthyroidism (FH) in 1979 (Peterson, Johnson and Andrews, 1979) and then in 1980(Peterson, Becker, Hurley and Ferguson, 1980). Since then, the disease's prevalence increased from 0.3% in 1979 to 4.5% in 1985 in North America (Scarlett, Sydney Moise and Rayl, 1988). In Germany the prevalence has increased from 0.2% in 1987 to 2.6% in 1998 (Bree, Gallagher, Shiel and Mooney, 2018). The steady increase in prevalence suggests a true increase of an unknown etiological factor(s) is responsible, rather than there being more diagnoses because of increased awareness of veterinary practitioners (Mooney, 2002).

The diagnoses are generally made in older cats. A clinical study in 1983 by Peterson involving 131 cats diagnosed with hyperthyroidism were found to be between the ages of 6-20 years (Peterson et al., 1983). More recent analytical studies from New Zealand in 2005 show that 81% of 125 hyperthyroid cats were over 12 years old (Olczak et al., 2005); another from South Africa in 2016 identically shows that cats older than 12 years and on a canned food diet are more likely to be diagnosed with the condition (McLean et al., 2016).

Cats exhibiting characteristic hyperthyroid clinical signs (weight loss with normal appetite, tachycardia, diarrhea, vomiting etc.) and presenting with palpable thyroidal mass while fitting the profile of a cat older than 6 years, warrant laboratory investigation for hyperthyroidism. Total T4 levels will be increased and diagnostic in 90% of hyperthyroid cats exhibiting clinical signs and presenting with a palpable thyroid nodule. Normal T4 values in a cat with clinical signs of FH and/or a palpable thyroidal nodule should be repeated (Peterson, Graves and Cavanagh, 1987).

It is important to distinguish between primary hyperthyroidism, which is the result of pathologic changes to the thyroid as previously described, and secondary hyperthyroidism, which can happen rarely through the classic example of a TSH-secreting pituitary tumor. A more common secondary compensatory form of hyperthyroidism occurs in some cats with Chronic Kidney Disease (CKD). In these cats, a functional hyperthyroid state with increased circulating T4, arises as a compensatory mechanism to decrease azotemia (circulating BUN and Creatinine) by increasing the Glomerular Filtration Rate (GFR) (Daminet, 2006). In 2008, Wakeling and her team demonstrated the clinical decline of serum Creatinine concentration in cats with CKD at the onset of the hyperthyroidism and its increase after treatment for

hyperthyroidism (Wakeling, Moore, Elliott and Syme, 2008). Practitioners should be aware of this compensatory mechanism as therapeutic management of hyperthyroidism may unmask a preexisting CKD.

Asymptomatic cats with normal to high baseline T4 cats with or without a small goiter can represent the early subclinical state of hyperthyroidism, in most cases, it precedes the clinical state and patients present with a small thyroidal (non-functioning) palpable mass, normal to high baseline tT4 and low TSH (Wakeling et al., 2007). TSH measurements in cats have been determined to be highly sensitive but poorly specific. This means that the rate of false negatives is low and the rate of true positives is high; the rates of false positive results is high, and the rates of true negatives is low. A high measurement is considered to be a negative result since TSH should theoretically be decreased in the clinical state of THs oversecretion. A high reading can be useful for excluding hyperthyroidism however using TSH low measurements as sole indicators for diagnosing FH should not be done. (Peterson, Guterl, Nichols and Rishniw, 2015) (Peterson, 2013).

Thyroid scintigraphy is a thyroidal nuclear imaging technique that entails the intravenous administration of pertechnetate, a pure gamma emitter (safer than the beta particle emitting radioactive iodine traditionally used) and its subsequent uptake by the follicular cell's NIS. Traditionally radioactive iodine is administered, however because of its radiotoxic beta particle emission pertechnetate is preferred (Peremans, 2012).

A thyroid scintigraphy study of 2096 cats in 2014 by Peterson and Broome showed that up to 90% of hyperthyroid cats have an enlarged thyroid mass on one or both of their thyroid's lobes (Peterson and Broome, 2014). Bilateral involvement was reported separately to be as high as 70% (Peterson and Ward, 2007). A palpable mass at the level of thyroid gland is indicative of feline hyperthyroidism, even in asymptomatic euthyroid cats (Boretti et al., 2009). Histopathological examination of these palpable masses reveals that in most cases (>90%), benign hyperplastic foci of abnormal follicular cells which can form multiple goitrous nodules ranging from 0.1-3mm are present (Peterson, 2012). These hyperplastic nodules of follicular cells display growth and TH secretion autonomous to pituitary TSH secretion. This was proved by transplanting normal thyroid tissue and goitrous nodular tissue onto athymic nude's mice where it was observed that this tissue retained its autonomous character (Peter et al., 1987). *In-vitro* culturing of goitrous nodular tissue in TSH-free medium also confirms the autonomous character of these nodules (Peterson, 2014).

The autonomous proliferation and secretion, and the hyperplastic foci of epithelial cells and (multi)nodular presentation are characteristics of neoplastic features. In the past a wide array of terms was used to describe these nodules, such as: hyperplastic goiter, thyrotoxic goiter, adenomatoid nodules, thyroid adenomas, adenomatous hyperplasia etc. The most common terms surviving today are benign adenomatous hyperplasia and adenoma, which represent the same fundamental benign neoplastic process of the epithelial follicular cells. In contrast, the presentation of a (simple hyperplastic) diffuse thyroidal enlargement, e.g. secondary to functioning pituitary neoplasia, shows different characteristics due to the thyroidal mass being TSH-dependent and presents as a diffuse non-neoplastic enlargement of the thyroid gland, which is not characteristic of a neoplastic process but rather a typical endocrine gland presentation of simple hyperplasia (Derwahl and Studer, 2002) (Peterson, Broome and Rishniw, 2015).

Malignant thyroid carcinoma is much rarer as studies have noted its prevalence to be between 1-3%. The risk of malignant transformation increases with time and is seen particularly in patients who undergo long-term symptomatic thyroid hormone antagonist (Methimazole and Carbimazole) therapy as their sole therapy, instead of being used concomitantly with thyroidectomy or radioiodine treatment (Peterson, 2011). The risk of metastasis increases with time, lymphatic drainage of the thyroid occurs towards the retropharyngeal lymph node for cranial third and the cervical lymph nodes for the caudal two thirds of the thyroid (Daniel and Neelis, 2014). In 2009 Hibbert and her team questioned the traditional thyroidal scintigraphy array's use in differentiating between thyroid adenoma and carcinoma and they found no scintigraphical features adequate to reliably distinguish thyroid adenoma from carcinoma (Hibbert et al., 2009).

Ectopic thyroid tissue can occasionally be found, albeit of low occurrence. Their predilection sites are at the base of the tongue (lingual), along the trachea (cervical), at the thoracic inlet and occasionally along the thoracic parts of the aorta (intrathoracic) (Noussios et al., 2011). During fetal development, a part of the oropharyngeal epithelium thickens and differentiates into the thyroidal primordium. Subsequently the thyroidal primordium will divide into the 2 lobes and concurrently descend to each lobe's characteristic position. During these processes the cells of the thyroidal primordium can defectively separate and dissociate from the thyroidal primordium. They become lodged in the surrounding developing tissues, the pharyngeal epithelium and the aortic sac, which gives rise to the thoracic parts of the aorta (Volkaert` et al., 2016) (Patnaik, Peterson and Hidgon, 2000). In the study involving the thyroid

scintigraphy of 2096 hyperthyroid cats, 3.9% of were reported having ectopic thyroid tissue with adenomatous hyperplasia. Thyroid cysts arising from the thyroidal epithelium can be classified as functional or non-functional on the basis of hyperthyroid or euthyroid state respectively. They are recognized as rare manifestation of long-term neoplastic changes (Miller et al., 2017) (Phillips, Radlinsky, Fischer and Biller, 2003).

The underlying etiological factors of these neoplastic changes have been under investigation for the last 30 years and multiple incriminating substances and hypothesizes have been proposed. Dietary exposure to Bisphenol-A, soy isoflavones, excess and deficiency of Iodine, Selenium, and exposure to OFRs have all been studied. (Peterson, 2012).

Soybeans are widely used to meet the dietary protein requirement of commercial cat foods. The polyphenolic soy isoflavone contents of soybeans, specifically genistein and daidzein, are known to be goitrogenic substances whose mechanism involves inhibition of thyroid peroxidase involved in synthesis of THs, they are also known for inhibiting the conversion of tT4 to T3, the active form. By blocking TH synthesis and the bioconversion to the active form, TSH concentrations are increased which lead to thyroid hyperplasia. Reports of their goitrogenic effects date back to the 30s, in 1976 it was reported that rats which were fed soy developed malignant hyperplastic goiter if iodine deficient. Detoxification via the UDP-glucuronosyltransferase pathway has been determined to be the preferred pathway for elimination in humans. Since this is absent in cats it is sensible to assume that the elimination of these compounds is less efficient in cats which predisposes them to higher exposure to these chemicals. Studies have found significant isoflavone content in dry food more consistently than in wet food (Court and Freeman, 2002) (Doerge and Sheehan, 2002) (Fitzpatrick, 2000) (Peterson, 2012). A study on 18 healthy cats demonstrated how cats fed a soy diet had a slightly higher tT4 and fT4 than the control group which were fed a soy-free diet (White et al., 2004).

Bisphenol-A (BPA) is globally produced for use in the production of polycarbonate plastic resin and epoxy resin. Plastic resins are used as raw materials for building and construction material, electrical material and for motor vehicle applications, while epoxy resins are used as protective coatings because of their adhesive properties and chemical and thermal resistance which protect against corrosion. Epoxy resin is popularly used in lining of metal products of food and beverage products, including canned pet food which represents an important route of exposure for cats. In a study involving 69 cats, serum levels of BPA were

positively correlated with indoor cats and in cats fed canned food (Kovaříková, Maršálek, Habánová and Konvalinová, 2020) (Tsai, 2006) (Pham and Marks, 2005).

Studies have shown BPA can bind to TR and displays the THs from it. It has been hypothesized that this antagonistic behavior could disrupt the negative feedback on the pituitary gland associated with cellular TH action. The absence or reduction of this negative feedback would cause elevated levels of TSH to remain elevated which in turn could cause thyroidal hyperplasia (Peterson, 2012).

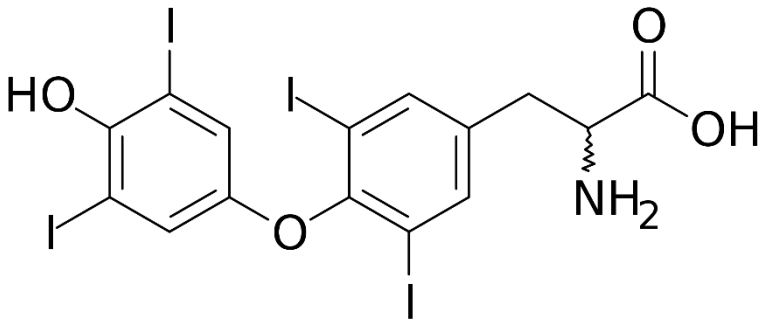


Figure 2: Structure of Thyroxine (T4)

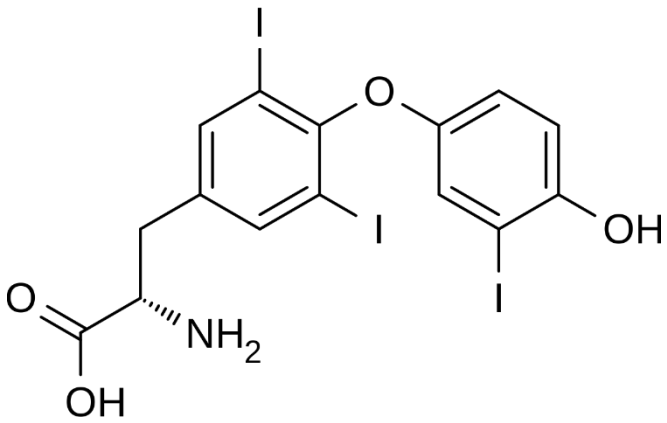


Figure 3: Structure of triiodothyronine (T3)

Chapter 2: Introducing Organic Flame Retardants

2.1 Defining Flame Retardants

Flame Retardants (FRs) are chemicals used in the industrial manufacturing of a wide array of products such as plastics, textiles (synthetic fibers), rubbers, electronics, building materials, furniture, paper, and other flammable materials. Their purpose, as their name suggests, = is to slow down and prevent combustion of the item they are added to. (Oh., 2020) (Sakai et al., 2001). Reactive FRs are incorporated into repeating oligomers or polymers of a material while additive FRs are molded into the material (Bergman et al., 2012).

FRs can be broadly categorized as organic or inorganic. Inorganic FRs are behind 50% of today's global production of FRs. They are mainly composed of metal hydroxides such as aluminum hydroxide ($AlOH_3$) which breaks down into aluminum oxide (Al_2O_3) and water when exposed to temperatures above 200°C. Aluminum oxide hydrate and magnesium hydroxide are other examples of metal hydroxides. They work similarly to aluminum hydroxide but decompose at around 350°C, making them more suitable for use in industrial thermoplastics (Brown, 1998). Zinc compounds such as zinc borate are commonly used synergistically with halogenated FRs (Stockholm Convention, 2009).

Organic Flame Retardants (OFRs) are made up of a carbon backbone and are further subcategorized depending on their other chemical component. Organophosphorus Flame Retardants (PFRs), Nitrogen-based FRs (NFRs) and Halogenated Flame Retardants are the

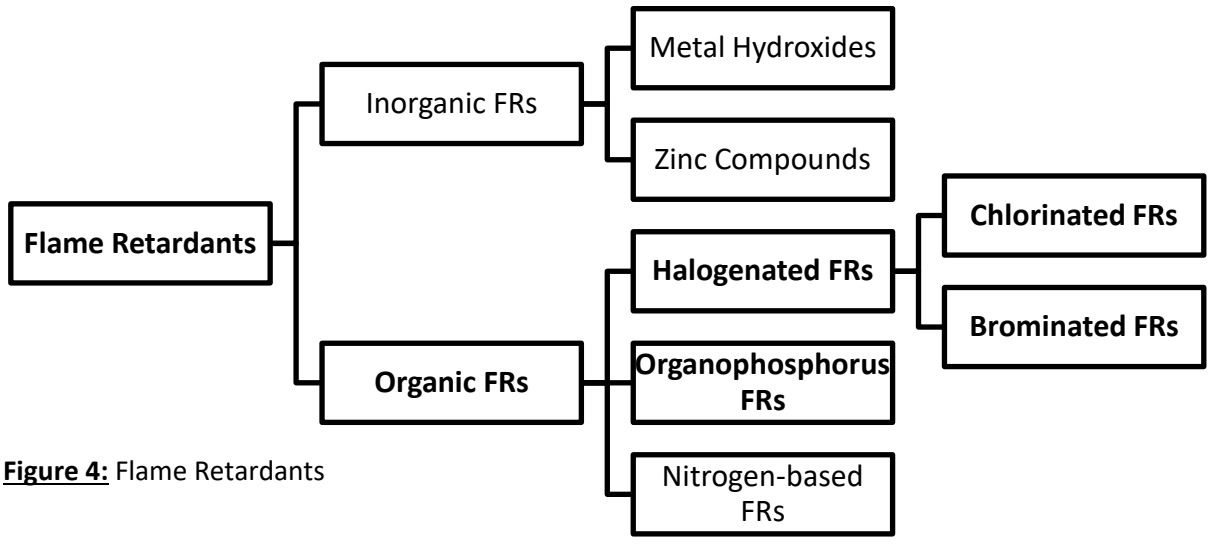


Figure 4: Flame Retardants

three main groups, the latter being made up of Brominated Flame Retardants (BFRs) and Chlorinated Flame Retardants (CFRs) (Bergman et al., 2012). Halogenated Flame Retardants work primarily by interfering with the combustion cycle at gas phase by the halogen incorporating the key combustion radicals $^{\bullet}OH$ and $H^{\bullet}$ (Salmeia, Fage, Liang and Gaan, 2015).

2.2 PCBs

The use of OFRs in industrial production started in 1929 with the introduction of Polychlorinated Biphenyls (PCBs). PCBs were used because they increase the flame retardant, insulative properties and chemical and thermal stability of the product that they are applied to. They are a group of CFRs made up of 209 congeners (structural isomers). Occupational exposure has been known to cause sever acute chloracne since the 1930s, however apart from worker safety guidelines in the 40s, the global industrial use of PCBs went on for more than 30 years until accidental findings of PCBs in 1966 in Sweden lead to their investigation which led to the global cessation of their use by the mid-1980s. 1.5 million tons of PCBs had been produced globally between 1929 and 1989 (Harremoës, 2008) (Ross, 2004) (Chatel, Naffrechoux and Draye, 2017).

The accidental finding of PCBs in the environment and their potential for bioaccumulation was made by Jensen in 1964. Jensen was a Swedish chemist at the department of analytical chemistry in the University of Stockholm who was appointed by the Royal Swedish Commission on Natural Resources to carry out analytical studies on human and wildlife fat tissue for chlorinated pesticides with special focus on DDT (Jensen, 1972).

DDT (DichloroDiphenylTrichloroethane) is an organochlorine compound first synthesized in 1874 by Zeilder, in Germany. Its insecticidal properties were reported first by

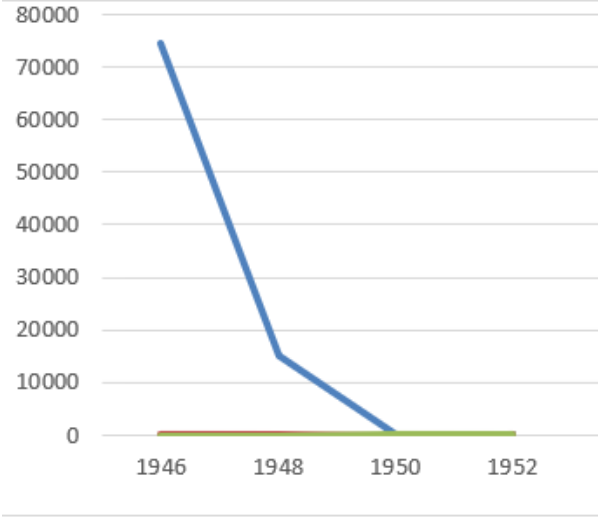


Figure 5: Total Number of Malaria cases (y-axis) & Years (x-axis), showing the decline of Malaria cases in Sardinia after the use of DDT.

Paul Muller around 1940 and it was made commercially available by the end of 1942. The Rockefeller Foundation, pioneered the use of DDT for the control of Malaria. They had seen previous successes when they launched national anti-hookworm campaigns which eventually globalized (Stapleton, 2004) (Storer, 1946). In 1938 Max Theiler from the Rockefeller Foundation held initial trials for a vaccine he developed for yellow fever. Theiler went on to win the Nobel Prize for this vaccine in 1951 (Norrby, 2007). In the early 1940s, the Rockefeller foundation ran it's first trials on Malaria control through repelling mosquito populations with the application of DDT on the skin in North Africa. They went on to use Sardinia as a test-site where from 1946 to 1950 they sprayed 267 metric ton of DDT onto the island via outdoor larvicidal spraying targeting the vector's breeding centers and indoor spraying. The results, as illustrated in Figure 3 were promising and showed a rapid decline of Malaria cases in Sardinia to 0 in just 4 years, while the vector breeding centers were reduced by 99.93% (Tognotti, 2009).

In the meantime, DDT's effect on the wildlife and environment were becoming of increasing concern and debate. An American investigation in the late 40s into DDT's adverse ecological effects on different species showed that DDT at moderate amounts was only lethally toxic in fish. Different forms of DDT were used for this investigation, the most toxic in fish was the DDT emulsion which killed all species of fish in the enclosure to which it was applied (Cottam and Higgins, 1946). In the late 1960s, an increased incidence of broken eggs of wild birds of prey was noted in England by Ratcliffe. He examined eggshell thickness, weight and breakage records dating as far back as 1906 and concluded that a dynamic trend of increased eggshell thinning can be traced to 1946 (Ratcliffe, 1967). Then in 1970 eggshell thinning was experimentally correlated to high acute doses of DDT (Tucker and Haegele, 1970). Various studies at that time were showing that DDT accumulated in the soil and was persistent in the environment and animal fats (Ginsburg, 1955) (Woodard, Ofner and Montgomery, 1945) (Smith, 1948). This led to an increased awareness of DDT in the 1950s which advanced the development of analytical equipment sensitive enough to detect $1x10^{-9}$ of a milligram that inadvertently led to the Jensen's finding of PCBs (Jensen, 1972).

Jensen was carrying out gas chromatography from ether extracts of dried homogenized muscle samples obtained from white-tailed eagles, young salmon, pike, and elk. DDT levels could be measured this way since bio-accumulation in animal fats had been well established. The gas chromatograms of these samples were found to have various peaks. Two of these correlated with the retention time of DDT and DDE, however, the rest were representing unknown chemicals. It would be two years from Jensen's discovery of these unknown

chemicals in 1964, until his deduction in 1966 that these compounds are in fact PCBs. He found exponentially higher levels of PCBs in fish-eating eagles than in their fish prey which led him to conclude that PCBs accumulate in the animal fats (Jensen 1972).

As figures 4 and 5 show, PCBs are composed of two biphenyl rings with the incorporation of chlorine atoms at different points of the biphenyl ring. The number of chlorine atoms and their placement dictates the nomenclature of the PCB. PCBs are resistant to hydrolysis, alkali, acids and corrosive chemicals. They are more stable than DDT and its metabolites because they lack the fundamental ethane bridge between the two chlorinated biphenyl rings, as seen in Figure 6 (Erickson, 2018).

The toxicity of PCBs was extensively studied in the following years. One study in 1976 found a positive correlation between PCBs and DDT levels in blubber samples taken from wild seals from the Baltics and Swedish west coast to the reproductive pathologic changes described as stenotic uterine horns causing obstruction and subsequent reproductive failures due to the inability of ovulated oocyte to travel down the oviduct for fertilization and implantation in the uterine body (Helle, Olsson and Jensen, 1976). A Dutch study by Brouwer in 1989 fed PCB-contaminated fish to seals in a controlled experiment. They reported a drastic reduction in plasma retinol concentrations compared to their control seal which were fed fish with low PCB levels. They also reported a significant reduction of plasma THs (Brouwer, Reijnders and Koeman, 1989).

Another study in 1977, involving the acute and chronic feeding of PCBs in different concentrations to experimental rats and histological examination of their thyroid gland and their serum TH levels, found accumulation of lysosomal bodies and colloid droplets in follicular cells as well as abnormal luminal microvilli with an increase in severity in the animals fed high doses and those fed for longer periods of 12 weeks. These pathologic changes were also associated with low serum thyroxine in both acute and chronic feedings and a compensatory hypertrophy and hyperplasia in the ones who underwent chronic feeding of PCBs. It was also noted that 35 weeks post the termination of PCB feeding, the histological morphology of their follicular cells correlated to the control's group and their serum thyroxine was within the normal parameters (Collins et al., 1977). In 1989, Brouwer also injected radioactively labelled Thyroxine (^{125}I-T_4) Intraperitoneally to rats which had been pretreated with 10mg/kg TCB (Tetrachlorobiphenyl, a PCB) and a group of control rats. Rats pretreated with TCB showed a 90% reduction in ^{125}I-T_4 binding to Transthyretin compared to the control

group. This drastic and selective binding inhibition appeared to be caused by monohydroxy metabolite of PCB and gave an explanation to the PCB-induced hypothyroidic state that the studies had been demonstrating (Brouwer, 1989).

FDA studies in the US from 1969 to 1971 found high levels of PCB in fish and their feed and significant amounts also in milk, cheeses, poultry and various food packaging material and their contents (Kolbye, 1972). A Japanese study in 1977 focused on human milk and found methyl sulfonated PCB metabolites found average concentrations of 0.2-0.5ppm. However, one mother who had previous occupational exposure through a capacitor factory had much higher levels of 5-10ppm (Yoshida and Nakamaru, 1977). Another Japanese study in 1979 found high PCB accumulation levels in dace and mackerel obtained from the cities of Niigata and Akita (Minagawa, 1979).

Contamination of areas where PCBs were manufactured and industrially applied had been found with values as high as 18ppm in soils in the vicinity of manufacturers and as high as 13 ppm in soils of the vicinity of user facilities (Stratton and Sosebee, 1976). In 1980, a study examined the sludge of Bloomington Indiana's sewage plant. They found that the municipal sewage system had been contaminated with PCBs by an electrical capacitor manufacturing plant. The sludge from this plant had been used by local residents as an alternative to traditional agricultural fertilizers since the mid-60s, the study examined the serum PCB levels of sludge users, occupationally exposed workers and their families, and non-exposed people. The conductors of the study negatively correlated PCB levels of sludge users with glove use suggesting a route of exposure. However, the levels themselves were not higher than the non-exposed participants. The moderately high PCB levels in family members of occupationally exposed people suggested that PCBs were transported home by occupationally exposed individuals on their person (Baker et al., 1980).

By the late 80s PCB contaminations had been found not only in urban and heavily industrialized areas such as the Great lakes in North America, the Baltic Sea in Europe and Tokyo Bay in Japan, but also in open ocean atmospheres, surface water, sediment and biota. By then the cumulative world production of PCB was estimated to be 1.2×10^6 tons, 31% of this was estimated to be present in the environment as global contamination, while 65% was estimated to be still in use in older equipment or in landfills or storage (Tanabe, 1988).

By the mid-70s public concern and awareness regarding PCB had grown enough for Monsanto, the sole producer of PCB in the US from the 1930s to 1977, to discontinue its use

of PCBs in open-ended applications (such as in caulks, sealants, plasticizers, surface coatings, ink, adhesive, and carbonless paper) but continued the use of PCBs in closed applications until in 1979. The US Environmental Protection Agency (EPA) issued a press release which legally banned the manufacturing and processing of PCBs in open applications and closed applications (such as in transformers and capacitors), and cited them as toxic and persistent chemicals (Archive.epa.gov, 2021) (Hamper, 2020) (Markowitz and Rosner, 2018).

In the late 1990s the United Nations Environment Programme (UNEP) organized an InterNational Committee (INC) the purpose of which was to implement international legally binding action beginning with the 12 Persistent Organic Pollutants (POPs) which were identified at the time. Their collaboration became known as the Stockholm Convention on POPs, a convention which is still globally active at present with 181 parties (Stockholm Convention website). DDT and PCBs were included in the first 12 POPs of the Stockholm Convention in its first meeting in 1998, the results of which came into force in 2001, for PCB the parties agreed to eliminate its production, use, import and export (except for waste management). The Stockholm Convention also obliged its parties to proper waste management of the POPs listed for elimination and this included the setting up- of appropriate stockpiles and proper disposal techniques (Lallas, 2001).

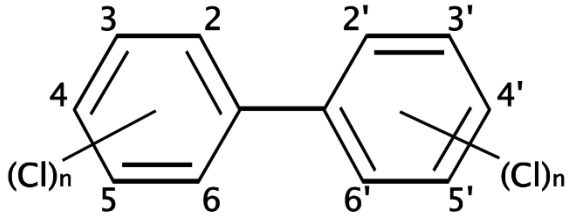
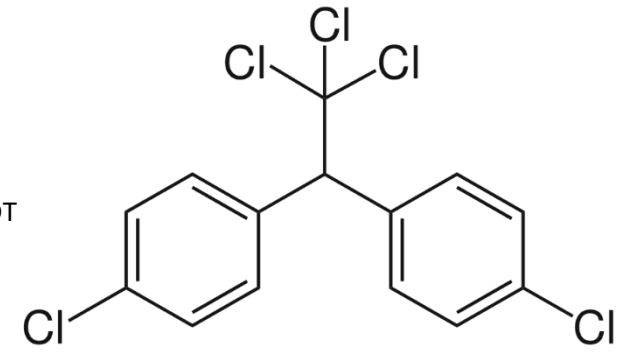


Figure 6: The Parent Compound Structure of PCBs

Figure 7: Chemical Structures of DDT



2.3 Brominated Flame Retardants

2.3.1 PBBs

PBBs (PolyBrominated Biphenyls) production started in the early 1970s and was halted in the same decade, PBBs are chemically similar to PCBs but are brominated instead of chlorinated, as seen in Figure 7. Three of the 209 possible congener were used for commercial mixtures, hexabromobiphenyl (Hexa-BB), octabromobiphenyl (Octa-BB) and decabromobiphenyl (Deca-BB). They were used as Brominated Flame Retardants for incorporation with acrylonitrile-butadiene-styrene (ABS) plastics intended for small appliances and automotive application (WHO HSG 83, 1993).

In Michigan in 1973, bags of Firemaster (the commercial name for a predominantly hexa-BB mixture of PBBs) were accidentally dispensed to animal farms in place of Nutrimaster bags, which contained Magnesium Oxide as a nutritional supplement intended for animal feed. By June 1975, 500 Michigan farms were quarantined and around 30,000 cattle, 4500 swine, 1500 sheep and 1.5 million chickens were culled. However around 50% of the PBBs mixed with feed was estimated to be present as a soil contaminant on farms through animal manure. Around 90% of Michigan people had detectable PBBs in their blood by 1978 (Saghir, 2018) (WHO HSG 83, 2010). 10 years after their exposure, these residents showed no clinical signs of illness. However, similar to PCB, chloracne was reported by some people who were occupationally exposed to PBBs (Kimbrough, 1987). Hexa-BBs were discontinued for use in North America after the Michigan incident in 1974. Octa-BBs and Deca-BBs however remained in use until 1979. The last producer of Deca-BBs in Europe was terminated in 2000 (Alaee, 2003).

In 2010 the EFSA's (European Food Safety Authority) CONTAM (Contaminants in the Food Chain) panel concluded that since PBBs are not presently produced or used, and since their environmental concentrations are decreasing, they should be thought of as a low concern for future research and monitoring evaluations (EFSA, 2010).

2.3.2 TBBPA

TetraBromoBisphenol-A (TBBPA) makes up 60% of the total global BFR market. China, the USA and the Middle East are the largest producers, and it is estimated that 241,352 tons were produced in 2016. Between 70-90% of TBBPA production is estimated to be used as a reactive flame retardant in resins and printed circuit boards. In essence, incorporation of TBBPA as a reactive FR means it is incorporated into the polymer matrix via covalent bonds. In this way, it does not readily leach out of the substance it is incorporated into. 10-20% of TBBPA production is estimated to be added as an additive FR to resins and acrylonitrile-butadiene-styrene (ABS) plastics, as an additive FR it can readily be released into the environment. TBBPA is produced by brominating BPA. (Liu et al., 2016).

TBBPA was first detected in 1983 in the sediment and mussels from Neya River in Japan (Watanabe, Kashimoto and Tatsukawa, 1983). Since then various studies have demonstrated it in multiple environments in the atmosphere: dust, soil, water phases from lakes and rivers, humans, and animals (Parsons et al., 2019) (Liu et al., 2016). High levels have been found in soils of former open waste burning sites, e-waste dismantling sites, vegetable soils, paddy soils and pond sediments in respective decreasing magnitudes, suggesting TBBPA from the burning and dismantling sites had diffused into the soil and had been transported through the soil profiles of the sites studied (Huang, Zhao, Liu and Sun, 2014). In Sweden studies have also demonstrated TBBPA and PBDEs as an airborne pollutant in offices containing computers (Sjödin et al., 2000). TBBPA and HBCDs are also present in human adipose tissue and blubber samples from dolphins and sharks. In the USA, HBCDs were found to be of 5-10 fold higher than TBBPA in all samples analyzed (Johnson-Restrepo, Adams and Kannan, 2008).

2.3.3 PBDEs

PBDEs (PolyBrominated Diphenyl Ethers) are another group of Brominated Flame Retardants, the use of which began in the 1960s. BDEs (Brominated Diphenyl Ethers) are similar to PCBs and PBBs but contain an ether bridge between the two phenyl rings, as seen in Figure 9, the group consists of another 209 theoretically possible congeners. The three most common commercially available mixtures contain a mixture of the following three compounds with one being the dominant PBDE of the mixture, Penta-BDEs, Octa-BDEs and Deca-BDEs (each comprised of multiple congeners of PBDEs). In 2003, the annual global production was estimated to be 67,125 metric tons. 82% of this amount is estimated to be Deca-BDEs. PBDEs

are reactive FRs, making them susceptible to leaching out of the products to which they applied (Siddiqi, Laessig and Reed, 2003). Penta-BDEs are more persistent in nature than Deca-PBDEs and Octa-BDEs. However, debromination of these highly brominated congeners has been demonstrated to occur in the common carp, anaerobic and aerobic bacteria. This represents an important environmental source of Penta-BDEs which are recognized as being more toxic (He, Robrock and Alvarez-Cohen, 2006) (Noyes, Kelly, Mitchelmore and Stapleton, 2010) (Daley, Shaw, Birnbaum and Blum, 2010). PBDEs were incorporation inro polyurethane foam, polystyrene, acrylonitrile-butadiene-styrene resins and plastics, cotton and textiles with polyester (Brits et al., 2019).

PBDEs are released into the environment during their production, incorporation and leaching from appliances into indoor air and at their disposal site (Watanabe, 2003). In 2009, Xu recorded relatively high concentrations of the octa-BDE technical mixture in the Nongkang River in Jinhu, China which surrounds a PBDE manufacturing plant (Xu et al., 2009). In 2006, Streets demonstrated the presence of PBDE congeners in waters of Lake Michigan in the USA. He also analyzed the PBDE levels of fish from all the Great lakes which includes Lake Michigan. Using these data, he demonstrated the positive relationship to the bio-accumulation factor. Another study in the Baltic Sea had also reached the same conclusion regarding bioaccumulation of PBDEs (Streets et al., 2006).

One study found higher levels of PBDEs from soils of farms which are prone to flooding by the River Trent in the UK compared to the control farms. The River Trent had been previously identified as a source for industrial contamination (Lake et al., 2011). Atmospheric deposition of PBDEs on leaves of maize was tested in 2015 by Brambilla, and he found that PBDEs are present on the leaves and that silage made from these leaves would contribute to the PBDEs found in dairy products (Brambilla et al., 2015). In Sweden, PBDE intake was found to be 0.7ng kg bw^{-1} d^{-1} in 2001. The Swedish study analyzed food items from the five food groups and found that fish and dairy products contained the highest proportions of PBDE congeners (Törnkvist et al., 2011). Another study correlated the concentration of PBDES in human milk to PBDE concentrations in meat, dairy and to a lesser extent fish consumption. They also found a positive association between PBDE levels in house dust and human milk (Wu et al., 2007). It is worth noting the variability of PBDE levels in dust and how such value are dependent on the atmospheric PBDE levels and exposure to dust. Dietary composition also varies from one country to another. This should be kept in mind when interpreting these results (Lake et al., 2011). An American study in 2010 found higher PBDE levels in young children

compared to their mothers who share the same household. They attributed these findings to the fact that children aged younger than 6 are more exposed to indoor pollutants in the dust through playing on the floor, increased hand-to-mouth activity, and the variation of the parent and child's diet-taking into consideration the presence of PBDEs in breast milk and the child's exposure (Lunder, Hovander, Athanassiadis and Bergman, 2010).

In 1986 a 2 year study experimentally fed Deca-BDE to 300 rats. The rats were grouped into 6 groups of 50 rats, 3 groups were compromised of male rats and the other 3 female rats. 2 groups (male and female) were fed the low dose of 25,000 ppm PBDE, another 2 groups (male and female) were fed the high dose of 50,000 ppm, and the remaining 2 groups were used as a control. Follicular cell hyperplasia of the thyroid gland was noted in 10/50 of the male mice receiving a low dose, and 19/50 of the male mice receiving a high dose. Follicular cell adenomas/carcinomas were also increased in male mice, 4/50 for the low dosed mice and 3/50 for the high dosed mice (Bucher, Huff and Kluwe, 1986). Studies have shown that hydroxylated metabolites of PBDE have a high affinity to Transthyretin and some studies have suggested that this could be due to the structural similarities between PBDEs and THs thus competitively binding to TTR and TRs (Zhang et al., 2008).

In 2006 a study recorded PBDE levels in the Baltic Sea to be as high as 40 times that of PCBs. They identified PBDEs, HBCD and TBBPA in sewage sludge samples from sewage treatment facilities. Samples were taken from 50 different sewage treatment plants and were traced to major cities and towns. Sewage sludge from areas with textile industry companies, producer of polystyrene or car upholster companies were found to have two to eight times higher BFR levels (Law et al., 2006).

Both Penta-BDE and Octa-BDE manufacturing were banned by the European Union in 2004 by the EU Council Directive 76/769/ECC (EU Memo/03/219, 2003). The European Union banned PBBs and PBDEs from new products in the EU Directive 2002/95/EC which gave producers a three-year transitional period to prepare for the ban until it came into force in 2006 (Directive 2002/95/EC). In 2009 Tetra-, Penta-, Hexa- and Hepta-BDEs were added to the Stockholm Convention's list of POPs further decreasing their use globally (Stockholm Convention, 2010). In Europe in 2017, Regulation (EU) 2017/227 banned Deca-BDEs future manufacture and their use in production (EU Commission Regulation 2017/227) (Brits et al., 2019). The EPA banned the manufacture, import, processing, and distribution of deca-BDEs in 2021 (EPA, 2021).

As is the case with most POPs, these bans will stop future PBDEs from entering the environment however they will not reduce the PBDEs leaching out from already-made products. Also, the sediments and soil which contain PBDEs will remain durable reservoirs for PBDE release (La Guardia, Hale and Harvey, 2006).

Methoxylated-PBDEs (MeO-PBDEs) are a naturally occurring chemically similar to industrial PBDEs. They have been found in polar bears, whales, dolphins, cetaceans, pinnipeds, porpoises, and seals from around the word. They have been found in preserved whale oil that was taken in 1921, confirming their natural origin. They likely stem from different marine species such as marine sponges in Australia and red algae or cyanobacteria in the Baltic Sea (Weijs et al., 2009).

2.3.4 HBCDs

Hexabromocyclodecanes (HBCD)s are a group of lipophilic BFRs comprised of alpha(α)-HBCD, Beta(β)-HBCD, and Gamma(γ)-HBCD. They have been in use since the early 80s and are used as additive FRs, incorporated primarily into polystyrene foam, upholstery and building materials (Kakimoto, Akutsu, Konishi and Tanaka, 2008).

Occupational exposure was detected in 10 male workers of an industrial plant, where their mean serum concentration was of 190ng/g lipid. For reference, the HBCD serum concentration of non-exposed personnel did not exceed 1ng/g lipid. β-HBCD was found in 2 of the serum samples while α-HBCD and γ-HBCD were found in all the serum samples (Thomsen et al., 2007). HBCD emissions into the environment are possible during its manufacture, use and disposal. Diffuse leaching of HBCDs during use is possible because of the additive technique with which it is incorporated into the product. This type of emission is expected to be higher in condensed urban areas. Since HBCD readily degrades at 240°C incineration plants are not thought to be a major source of emission. Landfills containing materials with HBCD incorporated into them could be sources of HBCD emissions into the air, surface waters and groundwater (Remberger et al., 2004).

A study in China in 2018 on the coast of Bohai sea examined fish and shellfish for HBCD levels and found evidence of biomagnification trends in predatory fish as compared to their prey. They estimated human HBCD dietary intake to be 5.22ng/kg/day for the local adult population (Zhang, Lu, Wang and Shi, 2018). A study on Japanese women in 2008 involving the chemical analysis of breast milk from 1988 to 2008 found no detectable levels of HCBDs

in samples collected before 1983, after that HBCDs were found in breast milk with increasing concentrations to present. The time trend of HBCD concentration in the women's breast milk was found to correlate to the time trend of HBCD consumption in Japan (Kakimoto, Akutsu, Konishi and Tanaka, 2008). In Korea, HBCD concentration in surface dusts of living rooms and sleeping rooms was found to be negligible while TBBPA concentrations in surface dusts from living rooms was found to be much higher than that of sleeping rooms (Barghi et al., 2017).

Another study found that humans' serum levels of HBCD correlated to dust exposure and ingestion rather than dietary intake, suggesting that people living in houses with high HBCD concentrations in dust are at more risk for exposure (Roosens et al., 2009). In 2008, Palace et al held feed trials involving 4 groups of Juvenile Rainbow trouts. Fish exposed to γ-HBCD had significantly higher free T3 plasma values and an increased Thyroid epithelial cell height, while all fish exposed to HBCDs had a lower free T4 on day 56 (Palace et al., 2008).

A study in 2019 exposed zebrafish to 3 different HBCD concentration to monitor it's toxic effect. The study involved 260 zebrafish which were split into 4 equal groups. They recorded that long term exposure to high concentrations of HBCD inhibits the secretion and synthesis of THs. Exposure to higher concentrations of HBCD was also correlated with an inhibitory effect on TRβ, a thyroid hormone receptor gene, and Crh (Corticotropin-releasing hormone) which are related to thyroid hormone production (Guo et al., 2019).

2.4 Organophosphorus Flame Retardants

Organophosphorus flame retardants (PFRs) were adopted by the industrial manufacturers to replace the use of PBDEs after the various bans that were imposed on it in the early 2000s. PFRs are further grouped into halogenated PFRs and non-halogenated PFRs (Wang et al., 2020). Tris(1,3-dichloroisopropyl) phosphate (TDICPP) and Tris(1,3-dichloroisopropyl)-phosphate (TDCPP) both refer to same chlorinated PFR, TDICPP is used as an additive FR. In the USA, 22,000 metric tons were estimated to have been manufactured or imported per year in 2010 and 2011. Recent studies have demonstrated that zebrafish embryos exposed to TDCIPP manifested developmental neurotoxicity, abnormal tail fins and severely disrupted the muscular and vascular development of these fish (Rhyu, Lee, Tanguay and Kim, 2019) (Li et al., 2018). In 2016, Feng demonstrated the metabolites of TDCIPP in

the urine of women from Shanghai, China. This suggests that these women were exposed from TDCIPP in their environment (Feng et al., 2016). A significant reduction of fT4 was detected in chick embryos exposed to TDCIPP (Farhat et al., 2013). One study analysed the serum of 50 men and their household dust and found a negative association for TDCIPP dust concentration and fT4 in the blood (Meeker and Stapleton, 2010).

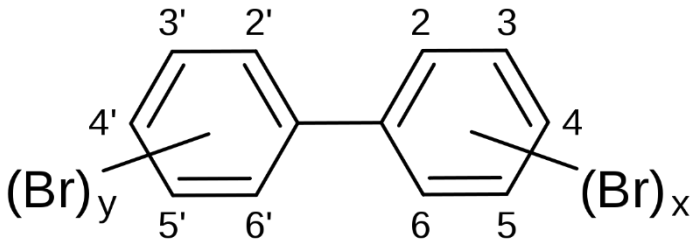


Figure 8: The Parent Compound structure of PBBs

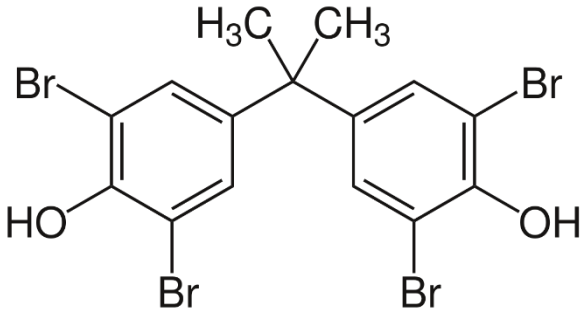


Figure 9: The Chemical structure of TBBPA

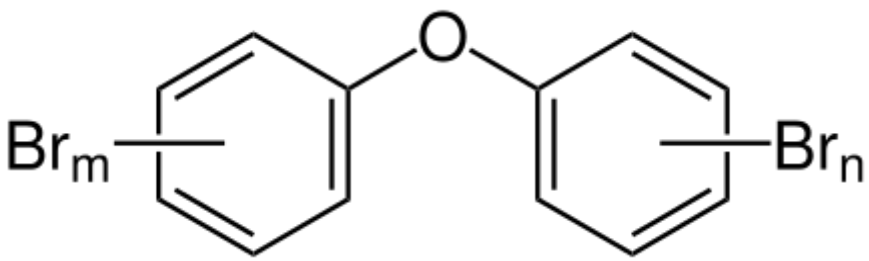


Figure 10: The Parent Chemical structure of PBDEs

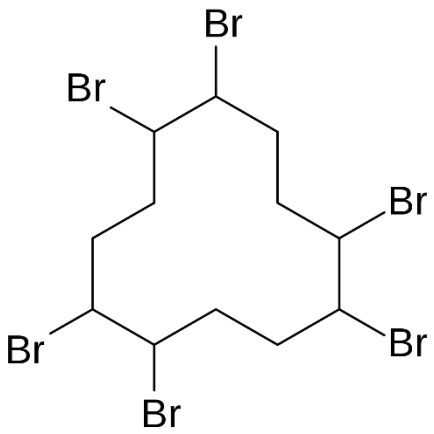


Figure 11 The Chemical structure of HBCD

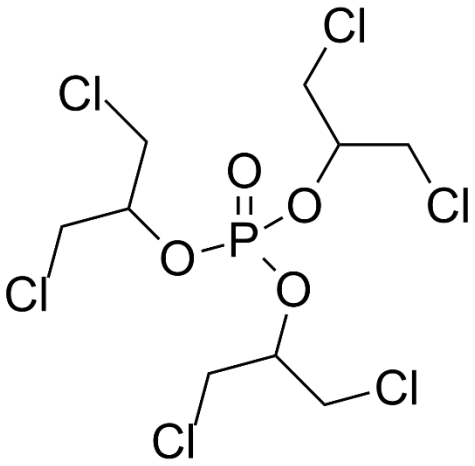


Figure 12: Chemical structure of TDICPP

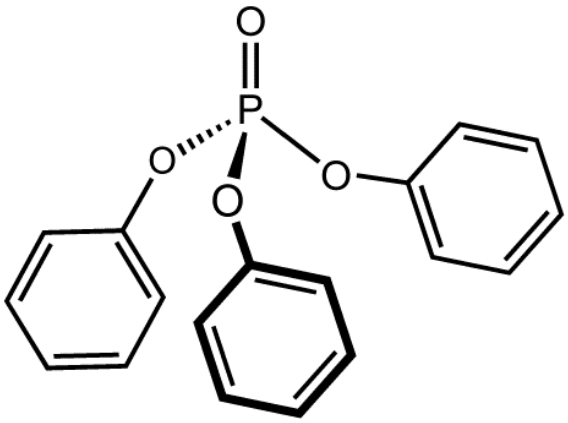


Figure 13: triphenyl phosphate TPHP

Chapter 3: Feline Hyperthyroidism's and Flame Retardants Relation

The emergence of Spontaneous Feline Hyperthyroidism in the 1980s and its increased prevalence coupled with the emergence of new OFRs in the late 20th century have led some to hypothesize and investigate if there is a link between the two (Jones, Engdahl and Weiss, 2019). The studies are motivated by the persistent nature of OFRs in the environment, human and animal tissues and their toxicological effects on the endocrine system.

In 2007, Venier collected sera from Hyperthyroid cats, healthy middle-aged cats and healthy young cats and compared their PBDE levels. She found PBDEs in all of the cats and established a positive relationship (characteristic of a POP) between concentrations of PBDEs and age (Venier et al., 2007).

Another study in 2017, analyzed the blood concentration of 51 cats, 20 of which had been diagnosed with feline hyperthyroidism for PBDEs and PCBs. The mean and median value of for the 13 measured PBDE congeners were higher for the hyperthyroid cats. For the 11 measured PCB congeners the mean value was similar in both groups, however, the median value was higher in hyperthyroid cats (Walter, Lin, Kass and Puschner, 2017).

3.1 The association of OFRs with thyroid disruption

3.1.1 Structural Similarities

The chemical structure of PCBs, TBBPA, PBDEs and especially their metabolites is strikingly similar to that of the THs as seen in Figure 10. They are all composed of a halogen and two biphenyl rings, PBDEs and its metabolites have an ether functional group connecting the biphenyl rings like in the THs, and metabolites of PBDE and PCB are hydroxylated similarly to the THs (McDonald, 2002).

In humans PBDEs have been shown to exhibit a high affinity to transthyretin, the thyroid hormones' transporter in the blood along with albumin. Quantitative analysis has demonstrated that hydroxylated PBDE metabolites have stronger affinity to TTR then regular PBDE congeners and an even higher affinity than T4. This mechanism allows transport of

PBDEs and its metabolites through the blood brain barrier, into fetal tissue and into the cerebrospinal fluid (Cao et al., 2017). A study in 2019 examined the interactions of BDE-47, OH-BDE-47 and HBBPA with transthyretin, they reported weak affinity for BDE-47 to TTR but high affinity for its hydroxylated counterpart and HBBPA. Both were demonstrated to have higher affinities than T4 (Chi et al., 2020).

In humans hydroxylated PBDEs have also been shown to bind with high affinity to Thyroid receptors. Thuhis was shown in *in-vitro* and *in-silico* investigations to produce weak hydrogen bonds with the receptor and consequently disrupt the normal thyroid hormone physiology (Li et al., 2010). It was also shown in *in-vitro* studies that hydroxylated metabolites of PCB suppress Thyroid hormone-receptor (ligand-receptor complex) induced transcription (Amano et al., 2010).

3.1.2 Studies with Rats

Fluctuation of the THs and developmental neurotoxicity post-exposure to Flame Retardants have been well-documented. In 1977 PCB feeding trials held on rats resulted in the observation of low plasma thyroxine and a histopathological accumulation of lysosomal bodies and colloid droplets in thyroid follicular cells in all rats and a compensatory hyperplasia and an increase of histopathological severity in those rats fed higher PCB concentration (Collins et al., 1977). In 1989 seals which were fed fish that were contaminated with PCBs showed a decrease in their plasma retinol and Thyroid Hormones (Brouwer, Reijnders and Koeman, 1989). It was also shown that after intraperitoneal injection of radiolabeled ^{125}I-T_4, the rats that were exposed to PCBs displayed a 90% reduction of ^{125}I-T_4 binding to Transthyretin (Brouwer, 1989).

In 2002 TBBPA and TCBPA (TetraChloroBisphenol-A) were demonstrated to competitively bind to thyroid receptors thus exerting an inhibitory influence on T3. Their activity was investigated using rat pituitary cell line GH3 cells, which are dependent on THs for GH secretion. It was shown that after exposure to TBBPA AND TCBPA, the GH levels increased, and GH3 cell line growth was not inhibited. These results implicated these OFRs as TH agonists as opposed to antagonists (Kitamura et al., 2002).

In 2004 a PCB feeding trial on rats showed that T4 levels were dynamically reduced until detectable levels were no longer measured by the third week. T3 was also reduced to a lesser extent than T4, and TSH was increased after day 7. Histopathological examination of their thyroid gland showed focal proliferative lesions of mild diffuse hyperplasia which were classified as: cystic hyperplasia, focal hyperplasia, follicular adenoma, and follicular carcinoma. Focal hyperplasia was observed in 12/22 rats receiving PCB feed and 6/24 of the control rats. Follicular adenomas were observed in 9/22 rats receiving PCB feed and 5/24 of the control rats, while a complete (not mixed) carcinoma was observed only in 4 rats which had received PCB feed (Vansell, 2004).

In 2012, a 70 day feeding trial investigated chronic exposure of PBDEs and HBCD on 75 rats which were split into five groups, four of them receiving varying concentrations of the BFRs in the diet and one control. Histological examination of the thyroid gland revealed a significant increase in epithelial thickness of the inner follicles of the rats exposed to higher BFRs concentrations. They also reported hepatic increased gene expression of lipogenic enzyme Me1 which is produced in response to the THs, however other TH induced expressions in the liver and and heart did not indicate that the BFRs were behaving as agonists to the THs' sites of action. (Ernest et al., 2012).

3.1.3 Studies with Fish

Different fish species have been used as experimental models for Brominated Flame Retardant toxicity with special focus on the thyroid gland. It is important to note that thyroid function in fish is also responsible for metamorphosis and osmoregulation, and that the thyroid function is not completely understood. Furthermore, the fish's thyroid system is not identical to mammals in all cases, however when compared to mammals, analogous genetic mechanism and identical chemical structures of THs are present (Deal and Volkoff, 2020). Exposure of different fish species to PBDEs has shown similar results with some notable contradictory bodies. Most investigations have demonstrated a decline in thyroid hormones and variable alterations in mRNA transcripts for TSHβ (more often than not increased for TSHβ) and for TRα and TRβ. Zebrafish larvae exposed to PBDEs have been demonstrated to display neurodevelopmental abnormalities, including altered motor behavior, inhibition of neuronal growth and also morphological abnormalities (Noyes and Stapleton, 2014).

Several studies have used Zebrafish as a model to assess TBBPA toxicity, there it was demonstrated that there was a clear effect on growth and development, an increase in oxidative stress and an altered expression of matrix metalloproteinases important for wound healing (Zhou, Yin and Faiola, 2020). In 2019 Parsons investigated the TRs' tissue specific expression in developing Zebrafish embryo-larva and found that after being exposed to THBBPA, TR-A expression was increased in the brain and branchial arches while TR-B expression was increased in the liver, brain, jaw cartilage and swim bladder. These results were also seen with BDE-47, a PBDE. The underlying mechanism could be the result of a true BFR-alteration, a reflection of a Thyroid hormone disruption or direct interaction with the TRs (Parsons et al., 2019).

In another study it was found that zebra fish embryo exposed to different concentrations of TBBPA would subsequently as larvae have increased T4 and decreased T3 contents accompanied with the following altered gene expressions; up regulated TSHβ and TG mRNA, and down regulated ttr and trβ mRNA. Zhu hypothesizes that the mechanism behind the neurodevelopmental toxicity of TBBPA is at least in part attributed to the disruption of TH (Zhu, Zhao, Yang and Zhou, 2018).

3.2 Cats' Exposure to OFRs

3.2.1 Exposure through indoor dust

Indoor cats are exposed to OFR through their diet and contact with indoor dust. Similarly, to young children, cats are exposed to indoor dust and its contaminants at a higher level than adult humans. In cats this is due to spending a lot of time on floors and their self-grooming (hand-to-mouth) behavior. An American study in California in 2011 examined the blood concentrations of PBDEs and PCB in 26 cats aged between 3 and 20 years, 16 of these cats were hyperthyroid while the other 10 where healthy cats. They found a mean PBDE concentration of 4,505 ng/g. They noted this as higher feline blood PBDE levels than in other places and they also noted that the PBDE levels were higher than the mean levels of California pregnant women which were 78.3 ng/g. They reported that levels of PCBs were lower in younger cats and increased with age. This correlated with dietary exposure being the primary source of PCB contamination, however age did not appear to be a factor for PBDE levels in

the cats. This suggested indoor PBDE contaminated dust to be the primary source of contamination for cats and correlated with research done on toddlers which approximated around 85% of daily PBDE intake in this group to be from household dust. They accredited these findings to the groups spending more time on the floor and increased hand to mouth behavior. They found no evidence correlating serum PBDE levels and feline hyperthyroidism (Guo et al., 2011).

In 2012, an American study examined the PBDE concentrations in 21 healthy owned cats, 41 hyperthyroid owned cats and 10 stray cats. They found that the serum PBDE concentration of the stray cats (which live outdoors and are in theory exposed to less indoor pollutants) was significantly lower than that of the healthy and hyperthyroid cats. They also took house dust samples and samples of the food from the participants. With these samples they were able to demonstrate that dust samples from hyperthyroid cats' households were significantly higher in PBDE levels and this value correlated with the Tt4 concentrations of these cats (Mensching et al., 2012).

An Australian study in 2015 analyzed the household dust and serum of hyperthyroid and euthyroid cats for PBDE levels. They were able to correlate ratios of PBDE congeners BDE47 and BDE99 in the dust samples to the samples from the cats, however, they did not find significant differences between the hyperthyroid and euthyroid cats (Chow et al., 2015).

A Swedish study in 2017 examined 17 paired samples of serum of indoor cats and samples of their households' dust from different rooms for PBDEs. They found correlations between the levels of PBDEs in cats and the levels of PBDEs in dust samples obtained from living rooms (Norrgran Engdahl et al., 2017).

Silicone passive sampling devices are worn by the participants for a period of time and have the ability to sequester volatile compounds (such as those in indoor dust) via diffusion. When collected these devices can provide personal exposure data. In 2019, they were made into dog tag shapes and fitted to the collars of 78 cats to monitor their exposure to OFRs after being worn for 7 days. Half (39) of the cat participants were hyperthyroid, and the other half were not. The tags from hyperthyroid cats had higher concentration of TDCIPP than the tags of the cats that were not hyperthyroid, and there was a positive association between serum fT4 and TDCIPP concentration in the tags. The results also showed that there was no significant difference between hyperthyroid cats' exposure to PBDEs and non-hyperthyroid cats' exposure to PBDEs (Poutasse et al., 2019).

A South African study by Brits and his team in 2019, examined eleven cat hair samples and twenty dust samples from indoor homes for BFRs and PFRs. Nine of the dust samples were taken from the floor and the other eleven were taken from vacuum cleaner bags. BDE-47, -99, -209 and BEH-TEBP (Bis(2-EthylHexyl)-TEtraBromoPhthalate, a BFR) were found in all dust and hair samples. BEH-TEBP and EH-TBB (2-Ethylhexyl-2,3,4,5-tetrabromobenzoate, a BFR) comprised 48% of the BFR profile in cat hair samples. They noted how animal studies have demonstrated the ability of EH-TBB and BEH-TBB to diffuse through the skin, due to their great lipophilic character. Brits hypothesized that cutaneous absorption of these chemicals following entrapments of dust in the hair and skin could be significant. The same study found TCIPP to be the dominant chlorinated PFR in all samples. (Brits et al., 2019).

3.2.2 Dietary exposure

A case-control study in 2000 involving 100 hyperthyroid and 163 healthy control cats reported that cats with preferences for fish flavor and liver and giblets flavors of canned food were more likely to be suffering from hyperthyroidism. 70% of cats with canned fish flavor, 62% of the cats with canned tuna flavor, 57% of cats with canned salmon flavor and 49% of cats with liver and giblet flavor preferences were hyperthyroid (Martin et al., 2000).

In 2007 Venier analyzed serum from 23 cats and over 30 cat foods for PBDE congeners and found relatively higher concentrations in dry foods and sera of cats with dry food preferences (Venier et al, 2007).

In 2015 Mizukawa measured PCBs in dry cat food and wet cat food, The median values for PCBs in dry cat food was 350pg/g and the value for wet cat food was 72pg/g. The author of this study concluded that the PCBs in the raw materials used to make dry cat food are concentrated during its manufacture. Hydroxylated PCBs were measured in the blood of cats and very low levels were found in their diet. This implies that biotransformation of PCBs occurs in the cat. Mizukawa also analyzed the PBDE levels of pet foods, and he found no significant difference between PBDE levels in dry and wet food. He noted how the increased BDE-209 in the cat's blood correlated with the levels in the food, however the author also acknowledged the analytical studies confirming BDE-209 in house dust samples. The congener composition of PBDEs found in wet cat food is similar to that found in fish which are processed into wet cat food (Mizukawa et al., 2015).

In 2015 Norrgran and her team in Sweden, found a PBB congener (BB-209) in all 82 cat serum samples analyzed. She measured 0.2-8pmol/g fat of BB-209 in three of four dry cat food brands. The concentrations in the upper range were found in fish flavored brands. The same study found the highest concentration of BDE congeners in fish flavored wet food. They also correlated the phenolic metabolites congeners found in the highest amounts in dry and wet food to the same phenolic metabolite pattern in the cat sera analyzed (Norrgran et al., 2015).

Elevated levels of the naturally occurring MEO-PBDE congeners were found in wet cat food that are predominantly made from fish. These compounds are known to bioaccumulate in fish. High concentration of the hydroxylated metabolites could be explained from the biotransformation of hydroxylated metabolites from methoxylated metabolites of PBDE, this has been shown in studies with various species' hepatic microsome. Mizukawa performed an *in-vitro* study with cat liver microsomes, in which he demonstrated that the Cytochrome P450 enzyme system is catalytically active in the demethylation of methoxylated PBDE metabolites to hydroxylated PBDE metabolites in cats. This correlated with his finding of 300pg/g for total OH-PBDEs as compared to <1pg/g for Total MEO-PBDE in cat sera (Mizukawa et al., 2015) (Nomiyama et al., 2017).

3.3 Feline Specific OFR Metabolism

The fate of FRs in the body is determined by Phase I cytochrome P450 monooxygenase (CYP) enzymes and Phase II conjugation enzymes. Cats have significantly differing xenobiotic metabolic pathways, and this represents an important interspecies difference that has implications on the species-specific congener pattern and their potential toxicity (Hakk, 2003). Cats' xenobiotic metabolism classically differs from that of other species because they have significantly lower phase 2 conjugation enzymes specifically UDP-glucuronic transferase and sulfotransferases (Hill and Shaw, 2014).

In dogs, THs are excreted via conjugation in the liver to glucuronic acid and sulfates and subsequent biliary excretion and entry to the enterohepatic recirculation system (Kaptein, Hays and Ferguson, 1994). It has been proposed that in animals with significant metabolic glucuronidation, microsomal enzyme inducing chemicals could facilitate the excretion of THs (Darnerud, 2008). Studies have shown the importance of glucuronide conjugation in human and rat liver preparations exposed to TCP (Tris(2-chloroethyl)phosphate), a halogenated PFR

(Chapman, 1991). That said, newer *in-vitro* studies on chicken embryos exposed to TPhP (triphenyl phosphate), a non-halogenated PFR have highlighted the importance of phase I hydroxylation metabolic detoxifying pathways in animals that are capable of phase II glucuronidation (Su et al., 2015).

In 2016, Zheng from China assessed the *in-vitro* Oxidative metabolism of BDE-47, BDE-99 and HBCDs in cats using cat liver microsomes. They noted that BDE-47 produced 6 hydroxy Terra-BDE metabolites while BDE-99 produced five hydroxy Penta-BDEs. When these metabolites are compared to the ones produced with human liver microsomes it can be seen that the major metabolite of BDE-47 in humans (6-OH-BDE-47) is only a minor constituent of the metabolites produced with cat liver microsomes. A lesser, unquantifiable amount of metabolites was observed with BDE-99, which suggested a relatively slower rate of oxidation for BDE-99 compared to BDE-47. This difference in rate of oxidation between the 2 PBDEs is not observed with human liver microsomes. HBCD oxidation in cat liver microsomes were observed to produce similar metabolites to those metabolized with chicken and rat liver microsomes (Zheng et al., 2016).

Recent studies have focused on exposing cats to FRs in anticipation of stimulating and quantifying mRNA expression of specific CYP subfamilies in an attempt to identify the specific xenobiotic metabolic pathways these chemicals undergo. One study carried out on eight male cats found that significant hepatic expression of CYP3A was evident post PCB exposure. They reported that extrahepatic expression of CYP1A1 in the kidneys and pulmonary expression of CYP2B11 was significant (Khidkhan et al., 2019). Another recent investigation sought to characterize feline hepatic CYP expression following a yearlong exposure to BDE-209 in their food. They recorded no significant difference between the mRNA expression of total CYP isoforms between the exposed and the control group, however specifically CYP2A12 was increased by 1.8-1.9-fold, and CYP3A131 was increased by two-fold compared to that of the control group (Khidkhan et al., 2020).

3.4 Summary

To summarize there are a number of reasons one could suspect a link between Feline Hyperthyroidism and OFRs;

The emergence of Feline Hyperthyroidism in the 80s coincides with the emergence of BFRs in the global industry, the increased prevalence of FH could also be explained by the growing use of OFRs in the industry, the emerging novel OFRs and the fact that they are persistent and bioaccumulate in nature. (Dye et al., 2007)

Indoor cats are particularly predisposed to exposure of OFRs in popular fish-derived cat food and indoor dust. Leaching out of FRs from indoor appliances and subsequent exposure through dust represents an important route of exposure for these chemicals both for cats and for humans, in fact it has been suggested that cats can be used as sentinels for humans as regards indoor contaminant exposure. Naturally occurring BDEs in fish products, albeit not anthropogenic substances, are still bio accumulative and as previously discussed they are converted to hydroxylated BDEs which have been shown to be structurally analogous to the THs (Henríquez-Hernández et al., 2017). In cats, species-specific xenobiotic metabolism in cats leads to different bio-accumulated metabolites (Nomiyama et al., 2017).

OFRs are further incriminated when considering the structural similarities between them and THs. This similarity is responsible for the experimentally proven interactions some OFRs and their metabolites display with Thyroid receptors and transthyretin, which due to lack of TBGs in cats is physiologically more important for the THs transport. Animal models on fish and rodents have demonstrated how these effects the hypothalamic-pituitary (HPT) axis. And a couple of studies in mice even reported adenomatous hyperplasia and carcinoma of the follicular cells of the thyroid gland (Chi et al., 2020) (Amano et al., 2010) (Vansell, 2004) (Tóthová and Nagy, 2018).

Conclusion

Spontaneous feline hyperthyroidism is the most common endocrine disease in cats. It is seen in cats older than at least six years, and 90% of the cats with the disease present with a palpable cervical mass (goiter). The goitrous changes to the thyroid gland in FH have been deduced as benign adenomatous hyperplasia or the much rarer malignant carcinoma.

OFRs are all lipophilic and bio-accumulative chemicals that are structurally analogous to the THs, making them capable of acting as an agonist to TRs and TTR. Their potential to disrupt the HPT axis has been recorded in various animal models.

Since the ban of PBDEs in the 2000s, recent analytical and animal-trial studies have been focused on PFRs. PBDEs and other BFRs have been overrepresented in clinical studies the last three decades because they were the in heavy use at the time, however even in this group cats are greatly underrepresented as the species for animal-trials. The focus of most recent studies has now shifted towards inorganic flame retardants.

Studies have shown the increased exposure cats possess to these chemicals and their specific xenobiotic metabolism could further the bio-accumulative nature and enhance the adverse effects exhibited to OFRs.

The question of how chronic exposure to specific OFR metabolites in cats interacts with TRs, TTR and other sites of action of the THs leading to disruption of the HPT axis remains. This interaction precedes and is ongoing during FH. It would be necessary to evaluate this interaction for a model to clearly relate OFRs to the pathogenesis of FH. This model should consider the feline specific xenobiotic detoxification pathways and the feline-specific metabolites for the specific OFR studied.

A link between prolonged and repeated exposure to low concentration of OFRs and feline adenomatous hyperplasia would be necessary to prove the link between the two. OFRs could be the sole factor that leads to Feline Hyperthyroidism or one of many factors, the others possibly being as discussed in chapter one: BPA or Soy Isoflavones.

It is also important to hold preliminary tests to evaluate novel OFRs and to recommend safer FRs when banning incriminated OFRs, this would reduce the global industrial usage of unsafe chemicals. As is the current focus of the Stockholm Convention and other governmental bodies it is also important to phase out products which contain banned OFRs and to not use

their plastics and materials as recycling raw materials in other products as this will only increase the presence of indoor contaminants and increase their exposure to humans and animals alike.

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Figure 4: Own work

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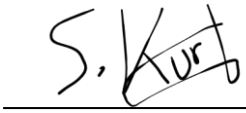

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- *increase awareness of Hungarian veterinary science not only in Hungary, but also internationally;*
- *increase citation numbers of publications authored by Hungarian veterinarians, thus improve the impact factor of Hungarian veterinary journals;*
- *present the knowledge base of the University of Veterinary Medicine Budapest and its partners in a focussed way in order to improve the prestige of the Hungarian veterinary profession, and the competitiveness of the organizations in question;*
- *facilitate professional relations and collaboration;*
- *support open access.*

I hereby confirm that I am familiar with the content of the thesis entitled „**Indoor cats' exposure to organic flame retardants and its association with the incidence of spontaneous feline hyperthyroidism**" written by **Kurt Scicluna** which I deem suitable for submission and defence.

Date: Budapest, 17th November, 2021

David Sandor Kiss

Supervisor name and signature

ÁLLATORVOSTUDOMÁNYI EGYETEM
Élettani és Biokémiai Tanszék
1787
1.
BUDAPEST

Department of Physiology and Biochemistry