Abiotic degradation of the brominated polymeric flame retardant “Polymeric FR” and ecotoxicity of generated decomposition products

Inaugural-Dissertation

zur

Erlangung des Doktorgrades

Dr. rer. nat.

Der Fakultät für

Biologie

an der

Universität Duisburg-Essen

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Angaben zur Prüfung

Die der vorliegenden Arbeit zugrundeliegenden Experimente wurden maßgeblich in der Abteilung für Aquatische Ökologie der Universität Duisburg-Essen durchgeführt.

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Tag der mündlichen Prüfung: 08.04.2019

DOI: 10.17185/duepublico/70085
URN: urn:nbn:de:hbz:464-20190415-112132-8

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Abbreviations

AOBr: Adsorbable organically bound bromine

BFR: Brominated flame retardant

BSA: 5-Bromosalicylic acid

DHA: 3,5-Dibromo-4-hydroxybencoic acid

EPS: Expanded polystyrene

EU: European Union

FiR: Fire retardant

FR: Flame retardant

HBCD: 1,2,4,6,9,10-Hexabromocyclododecane

PBB: Polybrominated biphenyl

PBDE: Polybrominated diphenyl ether

POP: Persistent organic pollutants

PS: Polystyrene

SBS: Styrene-butadiene-styrene

SC: Stockholm Convention on Persistent Organic Pollutants

TBBPA: Tetrabromobisphenol A

TBP: Tribromophenol

TeDB-DiPhOBz: Tetradecabromo-1,4-diphenoxybenzene

THA: 2,4,6-Tribromo-3-hydroxybenzoic acid

TOC: Total Organic Carbon

UV: Ultraviolet

XPS: Extruded polystyrene

Summary

Flame retardants are often incorporated in products such as electronical equipment, textiles, or thermal insulation to meet national fire safety requirements. For several years, hexabromocyclododecane has been the flame retardant of choice for building foam insulation until it was recently phased out due to a variety of environmental concerns. Thus, a new flame retardant was needed. Since 2014, “Polymeric FR” is used as a substitute on an industrial basis. The developer claims that its polymeric structure, persistence by design, and high molecular weight lead to a superior environmental profile.

The present thesis deals with the abiotic degradation of “Polymeric FR” and the ecotoxicity of potentially resulting monomeric decomposition products. The degradation is studied following exposure to UV radiation and heat at 60 °C. Both factors cause “Polymeric FR” to degrade, which is shown by multiple analytical methods, including among others, inductively coupled plasma mass spectrometry, determination of total organic carbon, and liquid chromatography mass spectrometry. The latter method was used to identify monomeric degradation products that could possibly pose a different toxic potential to the environment compared to the parent polymer. To assess their toxic potential, four chemicals were chosen based on their commercial availability and evaluated in three standardized tests with green algae and crustaceans. The test repertoire was accompanied by a trend analysis regarding the theoretical effect on fish to cover an additional tropic level.

The results show that “Polymeric FR” can degrade following UV irradiation and exposure to heat. While the environmental relevance of UV irradiation seems limited as long as this polymer is only used in building foam insulation, exposure to heat is a commonly encountered factor. However, significant degradation seems only possible under certain circumstances. The chance for these circumstances to occur, the presences of water and prolonged heat, is highest during the end-of-life – particularly if landfilling is applied for building foam insulation.

The tested degradation products seem to exhibit acute toxic effects only at comparable high concentrations, which are not to be expected following the degradation of “Polymeric FR”. Results obtained with an artificial mixture of those chemicals indicate however, that further research regarding negative effects following chronic exposure of native degradation mixtures should be performed.

Zusammenfassung

Nationale Brandschutzanforderungen bedingen häufig den Einsatz von Flammschutzmittel in Produkten wie Elektronikgeräten, Textilien oder thermischer Isolierung. Über viele Jahre hinweg war Hexabromcyclododecan das Flammschutzmittel der Wahl für Schaumdämmstoffe, bis es kürzlich zu einem stufenweisen Produktionsstopp aufgrund verschiedener negativer Auswirkungen auf die Umwelt kam. Daraus resultierte die Notwendigkeit ein neues Flammschutzmittel zu finden, welches mit dem seit 2014 industriell hergestelltem Substitutionsstoff „Polymeric FR“ gelang. Der Entwickler testiert, dass dieses Produkt aufgrund seiner polymeren Struktur, Persistenz und dem hohen Molekulargewicht ein verbessertes Umweltprofil aufweist.

Die vorliegende Arbeit beschäftigt sich mit dem abiotischen Abbau von „Polymeric FR“ und der Ökotoxizität möglicher monomerer Abbauprodukte. Dabei wurde speziell der Abbau durch UV-Strahlung und Hitzeeinwirkung bei 60 °C untersucht. Beide Faktoren führen zu einem Zerfall des Polymers, was anhand verschiedener analytischer Methoden, wie zum Beispiel der Massenspektrometrie mit induktiv gekoppeltem Plasma, der Bestimmung des Gesamtkohlenstoffes oder der Flüssigchromatographie mit Massenspektrometrie-Kopplung nachgewiesen wurde. Letztere Methode wurde hierbei genutzt, um monomere Abbauprodukte zu identifizieren, die sich in ihrem toxischen Potenzial der Umwelt gegenüber möglicherweise vom Ausgangs-Polymer unterscheiden. Um dieses toxische Potential zu überprüfen, wurden vier Abbauprodukte basierend auf ihrer kommerziellen Verfügbarkeit ausgewählt und in drei standardisierten Tests mit Grünalgen und Wasserflöhen evaluiert. Anhand einer computergestützten Trendanalyse wurde dieses Test-Repertoire mit Fischen um eine zusätzliche trophische Stufe erweitert.

Die Ergebnisse zeigen, dass „Polymeric FR“ durch UV-Einstrahlung und Hitzeexposition abgebaut wird. Während die Bedeutung von UV-Strahlung für die Umwelt als eher gering einzuschätzen ist – da dieses Flammschutzmittel nur in Schaumdämmstoffen eingesetzt wird – so ist Hitzeeinwirkung hingegen ein alltäglich auftretendes Phänomen. Ein signifikanter Abbau durch Hitze scheint jedoch nur unter bestimmten Umständen möglich. Die Chance, dass diese Umstände gegeben sind, also das Vorhandensein von Wasser und länger anhaltende Hitze, ist vermutlich am Lebensende der Dämmmaterialien – insbesondere während der Deponierung – am höchsten.

Die untersuchten Abbauprodukte bewirken negative akute Effekte nur bei vergleichsweise hohen Konzentrationen, welche voraussichtlich nicht beim Abbau von „Polymeric FR“ entstehen werden. Ergebnisse, die mit einer künstlichen Mischung dieser Chemikalien erzielt wurden, deuten jedoch darauf hin, dass weitere Forschung in Bezug auf negative Effekte durch chronische Exposition mit „natürlichen“ Abbaumischungen hilfreich wäre, um die Umweltrelevanz zu bewerten.

Introduction

The discovery of fire did not only enable mankind to cook food, have a source of heat during cold seasons, and bring light into darkness, but increased likewise the risk for uncontrolled fires. Until today, fires are still one of the major threats that our society has to deal with. Nevertheless, the cost of a fire is often hard to estimate, especially when not only loss of property, but fire fatalities and injuries occur.
Even though there is no standardized way for recording fire statistics, it can be stated that roughly 2 – 2.5 Mio. fires are registered every year in the European Union (CTIF, 2006, 2018; NIFV, 2009), including 20,000 – 25,000 fire deaths and 200,000 – 250,000 annual injuries. It was calculated that the total economic loss due to fires accounts for 1 % of the gross domestic product of most developed countries (CTIF, 2006). Statistics like this have led politicians to introduce fire safety requirements in different areas of application. Depending on the individual product, these requirements can often only be met by using fire retardants (FiR), which are generally utilized to stop, delay, or retard the burning process of a product (Chattopadhyay and Webster, 2009).

Similar to other chemicals such as biocides or medical drugs, the variety of FiRs is enormous. In an attempt to structure this variety, FiRs are typically classified according to a combination of three different characteristics. These commonly applied categories are “composition and structure”, “protection mechanism against fire”, and “bonding to the base material”.
The first category, structure and composition of FiRs, is divided into organic and inorganic substances. Examples for inorganic FiRs are ammonium sulfate or aluminium hydroxide, which are used among others in firefighting foam. Organic FiRs are typically further divided into nitrogenous, phosphorous, and halogenated chemicals.
Regarding the second category – the protection mechanism against fire – four main models can be defined. FiRs might either function in the vapour phase, thus the gas phase next to the product’s surface, or in the condensed phase, which describes the area directly above the product. Two mechanisms are known for each phase. On the one hand, the breakdown of FiRs in the condensed phase causes a layer of carbon (char) on the product’s surface. This layer acts as a thermal barrier and protects the product by exclusion of oxygen. Organic FiRs that function this way contain mostly nitrogen or phosphorous (Chattopadhyay and Webster, 2009). On the other hand, FiRs

might break down endogenous, meaning they “consume” energy and thus slow down the fire. Inorganic FiRs that work this way are for instance aluminium and magnesium hydroxide. Contrariwise, FiRs that function in the gas phase, typically reduce the concentration of flammable gases. Option one is to inhibit the radical chain reaction of the combustion process. Therefore, reactive radicals ($HO^{\cdot}$ and $H^{\cdot}$) are captured by halogenated radicals deriving from FiRs ($X^{\cdot}$). Thus, organic FiRs that work in the vapour phase are most often halogenated (Chattopadhyay and Webster, 2009). Exclusively this specific group of fire retardants is called flame retardants (FR). Contrary to organic FRs that inhibit the flaming process, inorganic FiRs often break down and “release” inert gases that dilute combustion gases to limit the available concentration of oxygen and therefore reduce the reaction rate.

The third category, bonding to the base material, is divided into two subcategories (Chattopadhyay and Webster, 2009). FiRs can either be strongly or loosely bonded to the product’s base material as for instance polystyrene. FiRs that are strongly bonded, are called “reactive FiRs”, because they form covalent bondings with the base material. The second group, the loosely bonded FiRs, are classified as “additive FiRs”, thus they do not react with the base material, but are simply added without covalent bondings. With polymeric FiRs becoming more prominent on the market, these FiRs are sometimes summarized in a third subcategory called “polymeric FiRs”, because even if still additive, their chemical structure can increase the integration with the matrix. In general, inorganic FiRs are typically only added to the base material, while organic FiRs can be strongly or loosely bonded.

Manufacturers tend to choose between these categories, depending on various factors such as the field of application, the production process, or the intended overall cost of a product. This led to four main groups of FiRs, which are used in large quantities today: inorganic salts, nitrogen based chemicals, phosphorous substances, and halogenated organic compounds (Fromme et al., 2016).

Brominated flame retardants (BFR) represent the biggest part of the latter group of FiRs, the halogenated organic compounds. More than 75 different chemicals were already recognized in 2001, with an annual production volume of more than 200,000 metric tons. Approximately 38 % of the worldwide bromine demand at this time was due to the production of BFRs (Birnbaum and Staskal, 2004). Only seven years later, the production volume had more than doubled, and accounted for roughly 21 % of the overall consumption of FRs (Covaci et al., 2011).

BFRs are applied in various products such as textiles, electronical equipment, furniture, or building materials (Covaci et al., 2011). Here, they are often necessary in order to comply with fire safety requirements (Babrauskas et al., 2012).
One of the first BFRs that were commercially incorporated were polybrominated biphenyls (PBB). These chemicals are not being manufactured anymore since a bag of PBB was mixed into animal feed in Michigan, USA in the early 1970s (de Wit, 2002). Over the last decades, polybrominated diphenyl ethers (PBDE), tetrabromobisphenol A (TBBPA), and hexabromocyclododecane (HBCD) were the BFRs of choice for the industry. Their chemical structures are shown in figure 1.

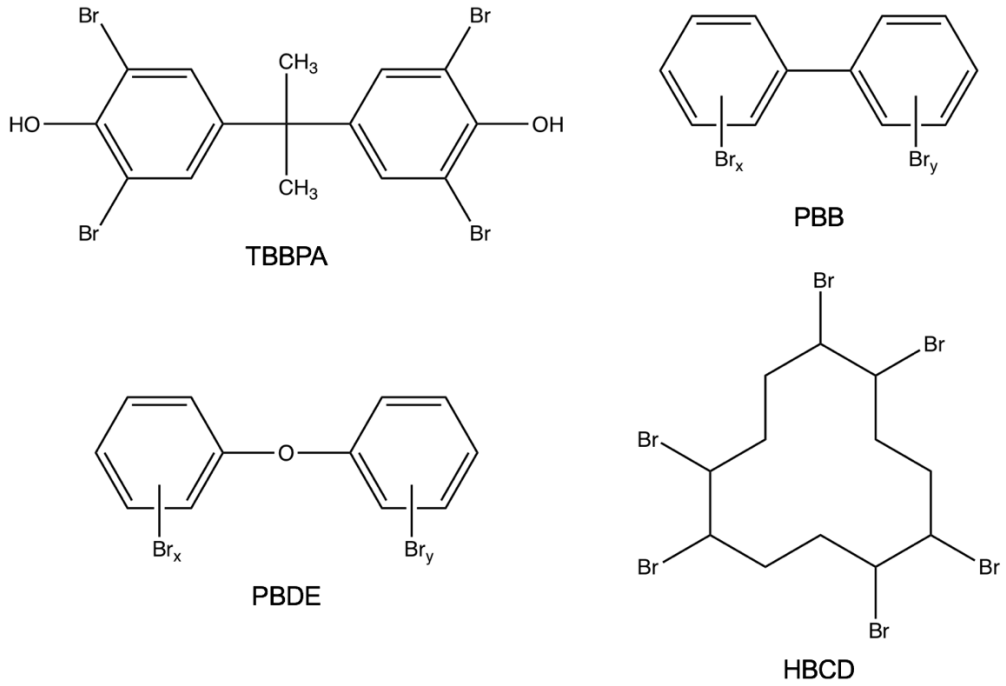

Figure 1: Chemical structure of tetrabromobisphenol A (TBBPA), polybrominated biphenyls (PBB), polybrominated diphenyl ethers (PBDE), and hexabromocyclododecane (HBCD).

Although the intention of supressing fires is generally favourable, the implementation of these BFRs started to raise environmental concerns. The first of this group to be detected in the environment were PBDEs in a Swedish river in 1981 (Andersson and Blomkvist, 1981). The collective name PBDE potentially includes 209 different congeners, however the bulk of these congeners are not used in industrial mixtures, due to their instability and tendency to debrominate (Birnbaum and Staskal, 2004). Following hundreds of studies, the commercial mixtures PentaBDE and OctaBDE were banned in the European Union (EU) in 2003. Three years later, DecaBDE was added.

Similarly, Penta- and OctaBDE were banned on an international level by inclusion in the Stockholm Convention (SC) on Persistent Organic Pollutants (POP) in 2009.
Since PBDEs were almost completely banned, TBBPA gained more attention in the EU. Different to PBDEs, TBBPA can also be used as a reactive FR by reaction of the phenolic hydroxy groups with the polymeric matrix (Birnbaum and Staskal, 2004), which in theory limits leaching to the environment. Nevertheless, studies have shown that additive- and reactive-treated products release TBBPA itself and in addition metabolites into the environment. However, TBBPA is generally not found in water samples, as it is the case for PBDEs (Birnbaum and Staskal, 2004). Instead, degradation is often observed. It was shown that TBBPA degrades both via biotic and abiotic factors. Biodegradation can occur under aerobic and anaerobic conditions (Hakk and Letcher, 2003), whereas abiotic decomposition mostly takes place in terms of photodegradation (Birnbaum and Staskal, 2004). It was reported that the generated decomposition products possess a different toxic potential compared to TBBPA (Chen et al., 2015).
While TBBPA is used in a variety of products, HBCD is mostly applied in textiles and foam insulation products such as expanded (EPS) and extruded polystyrene (XPS; figure 2) (Covaci et al., 2006). However, due to a variety of environmental concerns (Marvin et al., 2011), HBCD was included in the SC in 2013 and two years later banned in the EU (SC, 2013; REACH, 2016). Following this ban, a new FR was required to substitute HBCD mainly in foam insulation in order to remain compliant with fire safety requirements. Considering previous environmental hazards, the need for a safer alternative drove the development of potential substitutes forward. A comprehensive overview of environmental concentrations and the toxicology of HBCD is given in appendix I.

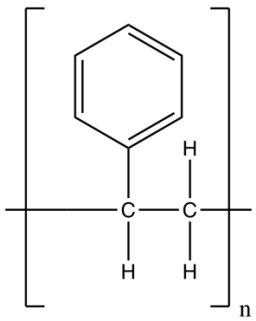

Figure 2: Chemical structure of polystyrene (PS).

Since 2013/14, a new BFR, called “Polymeric FR”, is commercially being used in building foam insulation (Jeannerat et al., 2016), where manufacturers previously had to rely on HBCD. According to the developer, significant efforts were made to ensure that “Polymeric FR” exhibits an improved environmental profile compared to HBCD (DOW, 2011a, 2011b). Different to this monomeric FR, “Polymeric FR” is a polymer, which was designed to be persistent, and to have no bioavailability due to a high molecular weight. “Polymeric FR” is synthesized by brominating a styrene-butadiene-styrene (SBS) triblock copolymer, which gives it a better compatibility when mixed with EPS or XPS (Beach et al., 2017). Its chemical structure is shown in figure 3. Due to copyright claims and different manufacturers, “Polymeric FR” is also known under varying names such as PolyFR, pFR, Emerald Innovation 3000, FR-122P, or GreenCrest (CAS No 1195978-93-8). According to the manufacturers, roughly 26,000 metric tons are produced every year (ICL, 2015; LANXESS, 2018). The only alternative to “Polymeric FR” is a monomeric TBBPA-like alternative which is currently only used in Japan (Kajiwara et al., 2017) called Milebrome B-972 (CAS No 97416-84-7).

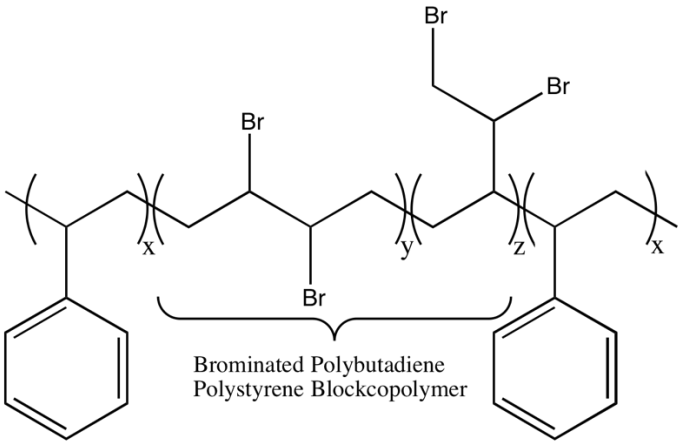

Figure 3: Chemical structure of “Polymeric FR”.

Claims of the developer regarding the superior environmental profile of “Polymeric FR” were mostly supported by initial evaluations of environmental protection agencies such as the US EPA (US EPA, 2014) or the Review Committee of the SC (SC, 2011). Nevertheless, questions were raised regarding the long-term behaviour of “Polymeric FR”. It was criticised that it is inherently persistent, and more data would be required to give a sound prediction on its fate in the environment, including potential degradation. However, no comprehensive research on these aspects has been published since the release of these initial reports.

Publications regarding the abiotic degradation of BFRs are rather scarce. It was however shown that photodegradation through ultraviolet (UV) or sunlight irradiation is a common and potent driver for decomposition (Eriksson et al., 2004b; Kajiwara et al., 2013; Santos et al., 2015). Even though some flame retarded products such as textiles are obviously exposed to sunlight, building insulation is unlikely to encounter such radiation during its use phase. These products – and thus the incorporated BFRs – are typically installed behind the building facade and therefore shielded against sunlight. Nevertheless, UV irradiation might be important before and after the use phase at point sources like manufacturing plants or construction and landfilling sites.

A second, but less studied factor for decomposition is thermal degradation (Barontini et al., 2004; Marsanich et al., 2004). Particularly during summer, high temperatures of up to 70 °C can be reached on a roof (FSEC, 2008), which does also affect the underlying insulation layer (Fraunhofer IBP, 2007). In addition, thermal degradation might occur during the end-of-life. Foam insulation products are either energetically recycled in municipal incinerators (Kajiwara et al., 2017; Mark et al., 2015) or brought to landfill (Weber et al., 2011). For the latter, comparably high temperatures were reported (UK Environment Agency, 2003; Yesiller et al., 2005).

The degradation studies above were most often performed with TBBPA. Albeit this substance is a monomer and one of the very few reactive BFRs on the market, it has considerable similarities with “Polymeric FR”: While being an additive FR, “Polymeric FR” is better integrated into the PS matrix than other additive FRs due to its polymeric structure which – when compared with TBBPA – indicates that this polymer might degrade as well.

Despite this knowledge, decomposition products are mostly left disregarded during the evaluation of a BFR, although it was shown that the toxic capability of generated degradation products can fundamentally differ from the one of the parent compound (Chen et al., 2015, 2013; Hill et al., 2018; Martin et al., 2017; Su et al., 2018). In consequence, the potential to degrade and the toxicity of possible degradation products should be part of a comprehensive evaluation of BFRs.

In order to fulfil such a requirement, more knowledge regarding the necessary analytical setup as well as appropriate methods is required. This includes, among others, suitable markers to quantify the degree of degradation, methods to monitor the quality of changes that the polymer undergoes, and techniques to identify possible degradation products. Such an analytical approach is developed in chapter I of this

thesis. Considering that “Polymeric FR” is the first polymeric BFR ever being commercially used and represents a prototype for polymers alike, this chapter covers a general approach that is also applicable for similar compounds that might be placed on the market in the future.

Following the introduction of such an analytical approach, these methods need to be applied to the specific case of “Polymeric FR”. This part is dealt with in chapter II of this thesis. This transfer includes with UV irradiation and heat two different abiotic degradation factors that are relevant throughout the life cycle of an insulation product containing “Polymeric FR”. In addition to comparing both scenarios by assessing different degradation markers, various decomposition products are suggested for further investigation regarding their environmental toxicity.

After gaining a better understanding of the different structural characteristics of degradation products, it is necessary to obtain first results regarding their effects on the environment. This part is covered in chapter III of this thesis. Considering the potential amount of different degradation products, it is beneficial to not only study individual compounds, but also recreate the native degradation mixture as accurate as possible while still being able to gain reproduceable results in such a complex situation. This is done for multiple trophic levels by conducting standardized OECD tests and performing different trend analyses based on structural characteristics.
By doing so, this thesis contributes substantially to the knowledge on how polymeric BFRs degrade and to which extent resulting monomers possess a different toxic potential compared to the original polymer.

Chapter I: Degradation of polymeric brominated flame retardants: Development of an analytical approach using PolyFR and UV irradiation

Within this chapter, the methodological approach on how to study the abiotic degradation of a brominated polymeric FR is covered. This approach is developed by using “Polymeric FR” and first results are evaluated.
Manuscript as published. The associated supporting information is given in appendix III.

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Degradation of Polymeric Brominated Flame Retardants: Development of an Analytical Approach Using PolyFR and UV Irradiation

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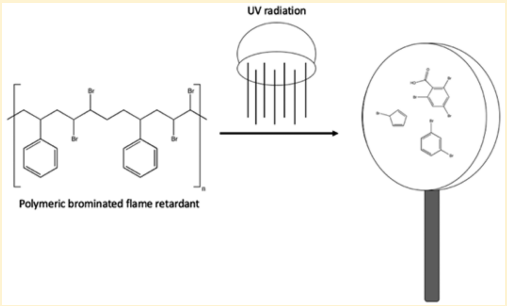
Supporting Information

ABSTRACT: Many well-established methods for studying the degradation of brominated flame retardants are not useful when working with polymeric and water insoluble species. An example for this specific class of flame retardants is PolyFR (polymeric flame retardant; CAS No 1195978−93−8), which is used as a substituent for hexabromocyclododecane. Although it has been on the market for two years now, almost no information is available about its long time behavior in the environment. Within this study, we focus on how to determine a possible degradation of both pure PolyFR as well as PolyFR in the final insulation product, expanded polystyrene foam. Therefore, we chose UV radiation followed by analyses of the total bromine content at different time points via ICP-MS and identified possible degradation products such as 2,4,6-tribromophenol through LC-MS. These results were then linked with measurements of the adsorbable organically bound bromine and total organic carbon in order to estimate their concentrations. With respect to the obtained ^{1}H NMR, GPC, and contact angle results, the possibility for further degradation was discussed, as UV irradiation can influence the decomposition of molecules in combination with other environmental factors like biodegradation.



1. INTRODUCTION

The anthropogenically induced climate change has been and will be one of the biggest challenges for mankind. The aim to hold the increase in the global average temperature below 2 °C above preindustrial levels has been emphasized with the adoption of the Paris Agreement on 12th of December 2015.[35] Within this agreement, even efforts to limit the temperature increase to 1.5 °C are demanded. One factor for achieving these goals will be an increased insulation efficiency for both old and new buildings. For this purpose, expanded (EPS) and extruded (XPS) polystyrene foams are used most often.

These foams almost always need to contain flame retardants in order to meet fire safety requirements for buildings.[1] A commonly used flame retardant during the last decades has been hexabromocyclododecane (HBCD). However, due to its bioaccumulative, persistent, and toxic characteristics, the manufacturing and use of HBCD has been prohibited under the Stockholm Convention on Persistent Organic Pollutants and since August 2015 (with granted authorization until 2017[36]) under the European Regulation (EC) No 1907/2006 (REACH) as well.[2] Therefore, new flame retardants (FR) are needed.

Since 2014, PolyFR (polymeric flame retardant; CAS No 1195978−93−8, Figure 1), a block copolymer of polystyrene and brominated polybutadiene (also known as polymeric FR, pFR, PolyBFR, Emerald Innovation 3000, FR-122P, and GreenCrest) is used as a substitute for HBCD on a commercial scale.[37,3,4] Different to HBCD, the bromine-containing polymer is chemically bonded to the base material and persistent by design. Additionally, because of its high molecular weight, no

Received: August 12, 2016
Revised: October 20, 2016
Accepted: November 2, 2016
Published: November 3, 2016

DOI: 10.1021/acs.est.6b04083
Environ. Sci. Technol. 2016, 50, 12912−12920

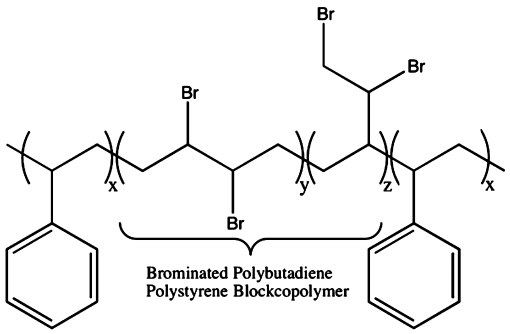


Figure 1. Chemical structure of PolyFR as described by the manufacturer.

bioavailability is expected. Thus, PolyFR is claimed to be more environmental friendly.[38] To our knowledge, all governmental risk evaluations until now focus on the polymer itself and leave potential degradation products mostly disregarded. The U.S. Environmental Protection Agency concluded 2014 that the "long-term behavior in the environment is currently not known".[38] Considering the long lifetime and various life cycle stages of EPS and XPS, abiotic and biotic environmental factors need to be taken into account. These factors may lead to a degradation of the commercial polymer and could result in smaller molecules with a different mobility and toxic potential. An environmental factor that is well-known for altering the structure of brominated flame retardants (BFR) is ultraviolet (UV) radiation.[5−8]

UV radiation is typically separated into three different categories: UV-A (with a wavelength of 315−400 nm), UV−B (280−315 nm), and UV−C (100−280 nm). UV−C radiation, however, is blocked by diatomic (100−200 nm) and triatomic oxygen (ozone; 200−280 nm) in the atmosphere. The irradiance of UV−B is also reduced, but not completely stopped, whereas crossing the earth's atmosphere. UV-A on the other hand, is affected to a much smaller extent by the atmosphere compared to UV−B. The UV radiation received at the earth's surface is strongly influenced by a variety of factors, like the solar elevation angle, clouds, aerosols or changes in the ozone density.[9,10]

The goal of this study was to establish a procedure to investigate a possible degradation of polymeric BFRs in general. The number of available studies in regard to polymeric BFRs is rather limited and only deals with aromatic brominated molecules, which is not the case for PolyFR.[11,12] To our knowledge, no information concerning possible reaction mechanisms during degradation of PolyFR is available as well as absorbance spectra for this polymer. UV radiation was chosen as a degradation factor because it is not as time-consuming as for example heat exposure. However, flame retardants used in insulation products are rather unlikely to be exposed to significant UV radiation during manufacturing or while attached to a building, though UV irradiation might appear when these products are not incinerated after their service life but for instance disposed on a landfill.

Due to its industrial relevance, PolyFR, pure, and incorporated in EPS, was chosen as an example of polymeric BFRs for this study. Besides the search for a suitable marker with regard to the level of decomposition, it was also of interest to determine the structure of water-soluble molecules that may derive from PolyFR during degradation. Due to its polymeric structure and the fact that PolyFR is insoluble in water, well-established methods that were applicable when working with BFRs like HBCD, had to be adapted in order to accomplish this goal.

2. MATERIALS AND METHODS

2.1. Experimental Design. In order to study possible degradation products of polymeric BFRs after exposure to UV radiation, PolyFR, and EPS were used. Bulk PolyFR with a bromine content of approximately 61 mass % (according to the manufacturer) was obtained from BASF SE (Ludwigshafen am Rhein, Germany). EPS insulation plates (1000 × 500 × 80 mm, 30 kg/m^3) that contained 1% per mass PolyFR (according to

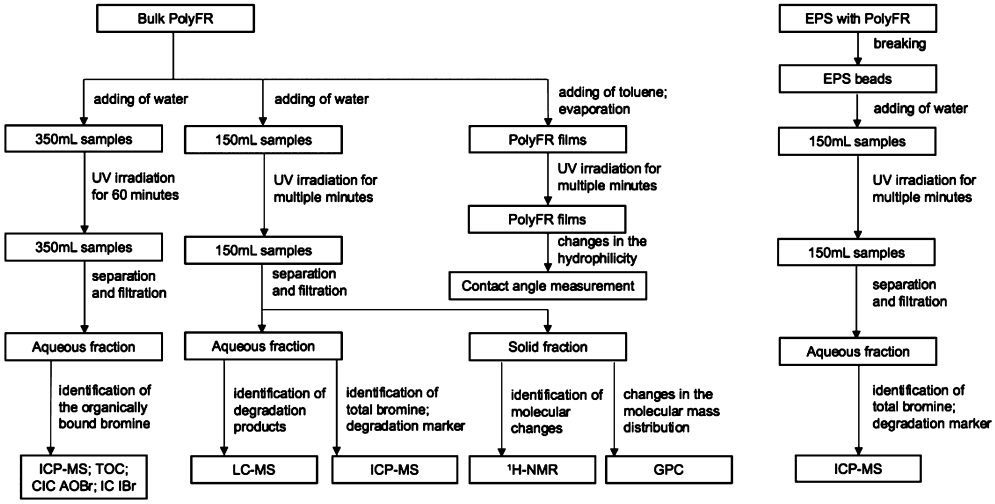


Figure 2. Overview of the applied methods and procedures.

DOI: 10.1021/acs.est.6b04083
Environ. Sci. Technol. 2016, 50, 12912−12920

the manufacturer) were bought from a local vendor in Germany.

An overview of the experimental procedure and the methods applied is given in Figure 2.

For the bromine measurements over time, approximately 0.5 g PolyFR (density of 2.55−2.65 g/cm^3; as a powder 0.5−0.7 g/cm^3; according to the manufacturer) was weighed (Libror AEG-220, Shimadzu, Kyoto, Japan) into an Erlenmeyer glass flask filled with 150 mL water (pH 5.43; prepared with a Milli-Q academic, Millipore, Billerica, MA) to obtain a concentration of 3.33 g/L. Additionally, EPS that contained PolyFR, was manually broken into small beads (except the outer layer of 1 cm thickness as this area might have already undergone UV irradiation), and prepared in the same way. To determine the concentration of total organic carbon, inorganic and adsorbable organically bound bromine, it was necessary to add approximately 1.17 g PolyFR to a bigger volume of 350 mL water (3.33 g/L). Only for the analyses of adsorbable organically bound bromine, the identical volume, but double concentration (6.67 g/L) was prepared as well. Although PolyFR is not water-soluble, concentrations are sometimes expressed in "g/L" to underline that the same concentration has been used for different volumes.

For the UV treatment a UV cube (UV-A cube 2000, Hoenle UV technology, Graefelfing, Germany) was used with an irradiance of approximately 500 W/m^2 (covers the UV-ABC and partly VIS spectrum, however, mainly between 290 and 450 nm; Supporting Information (SI) Figure SI-1). The irradiance decreased to approximately 150 W/m^2 on the ground of the Erlenmeyer flasks filled with 150 mL water. The irradiation was started approximately 20 min after water was added. Triplicates, each of 1 mL, were taken from every 150 mL preparation for each time point (after 0, 1, 6, 16, 26, 36, 46, and 60 min of irradiation) and stored in 1.5 mL Eppendorf tubes. The samples were manually shaken for 5 s before and after sampling. The 350 mL preparations were irradiated for 60 min without taking samples in between. All water samples were filtered (0.45 μm filter, Merck Millipore, Billerica), wrapped in aluminum foil and stored at −20 °C. The solid fraction was filtered off, freeze-dried and used for further analyses as well. Samples without PolyFR and EPS were treated in the same way and used as blanks. Furthermore, samples with and without PolyFR and EPS were not irradiated and used as a reference for free bromine. Triplicates were prepared for each time point. To account for possible effects due to the increasing water temperature during irradiation (increased from 22 to 61 °C), control samples were exposed to 90 °C for 60 min in a Thermo Scientific Heraeus Function Line oven (Thermo Fisher Scientific, Waltham, MA.).

For the contact angle measurements, 6 g bulk PolyFR were dissolved in 100 mL toluene (ACS reagent, ≥ 99.8%; Sigma-Aldrich, Munich, Germany). After placing 10 mL of this solution into eight glass Petri dishes, the toluene was evaporated at 22 °C within 3 days. The resulting PolyFR films were broken into ten samples per Petri dish and treated with UV radiation for 0, 1, 6, 16, 26, 36, 46, and 60 min as well.

The density of PolyFR was measured by weighting 1 mL of the powder without compressing it beforehand.

2.2. Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The concentrations of bromine in the samples were measured using a quadrupole ICP-MS system (Elan 6000, PerkinElmer) operating at 950 W plasma power and 0.95 L/min nebulizer gas flow. Instrument calibration was performed using series of dilutions (in range from 0.1 μg/L to 100 μg/L) prepared from standard solutions (Merk, Germany). Subsequently, the element concentrations were calculated in μg/L using the corresponding regression lines of the calibration (correlation factor ≥0.999).

Before measurements, each sample was diluted at least 10 times using 1% nitric acid (HNO_3, subboiled) with a concentration of yttrium (Y) of 10 μg/L as internal standard. In order to avoid contamination and memory effects, the wash time between measurements was set to at least 60 s (with 1% HNO_3, subboiled). The accuracy and stability of measurements was controlled additionally with a standard solution of bromine with a concentration of 10 μg/L (ICP multi element standard IV solution, Merck, Darmstadt, Germany), which was analyzed after every 10 samples.

2.3. Adsorbable Organically Bound Bromine (AOBr). Samples were treated according to DIN EN ISO 9562 (2005) using the batch method.[39] Eluent, calibration standards and other solutions were prepared with ultrapure water (18.1 MΩ·cm, TOC < 10 ppb; ELGA LabWater, Veolia Water Technologies Deutschland GmbH, Celle, Germany). For the 0.2 M nitric acid/nitrate stock solution sodium nitrate (pure; Riedel-de Haën, Honeywell, Seelze, Germany) and nitric acid (65%; Merck KGaA, Darmstadt, Germany) were used. From this a diluted 0.012 M nitric acid/nitrate washing solution was prepared.

For analysis of AOBr and blanks, 50 mg of powdered activated carbon (Pulsorb RD90, Chemviron Carbon GmbH, Beverungen, Germany) were weighed in 250 mL Erlenmeyer flasks. 100 mL of aqueous sample were filled into each flask and acidified with 5 mL of nitric acid/nitrate stock solution (final pH < 2). Samples and blanks were prepared as triplicates. The samples were shaken horizontally for 1 h in the Erlenmeyer flasks. Hereafter, the suspended samples were filtrated using polycarbonate membrane filters (pore size: 0.4 μm, diameter: 25 mm; Sartorius AG, Goettingen, Germany). The residual filter cake was washed with 25 mL of nitric acid/nitrate washing solution. Finally, the filter cake was transferred together with the membrane to a ceramic boat for analysis in the combustion ion chromatography system (CIC).

The combustion system consisted of the sample introduction device ABC-210, the combustion oven AQF-2100H, and the gas absorption unit GA-210 (all from Mitsubishi Chemical Analytech Co., Ltd., purchased from a1-envirosciences GmbH, Duesseldorf, Germany). The gas flow rates of the combustion oven were set to 200 mL min^{-1} for oxygen (4.8; Air Liquide, Duesseldorf, Germany) and 100 mL min^{-1} for argon (5.0; air liquid). The samples were combusted at 900−1000 °C. The combustion program is shown in SI Table SI-1. For every sample, a new ceramic boat was employed which was baked at 1000 °C in a stream of oxygen for 3 min to remove any contaminations before use.

The combustion gases were absorbed in 3.5 mL absorption solution. The absorption solution contained 33 mg L^{-1} H_2O_2 (p.a.; AppliChem, Darmstadt, Germany) and 1 mg L^{-1} P (*ortho*-phosphate, ≥ 99%, AppliChem). After combustion, the volume of the absorption solution was adjusted to 10 mL so that an enrichment factor of 10 had to be considered for the final result. Finally, 100 μL of the absorption solution were injected to the linked IC system.

The quantification of anions was performed with the ion chromatograph 881 Compact IC pro − Anion − MCS (Metrohm, Herisau, Switzerland) using the anion exchange

DOI: 10.1021/acs.est.6b04083
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column Metrosep A Supp 4 (250 × 4.0 mm with 9 μm particles). For matrix elimination prior to analysis, a Metrosep A PCC 1 HC/4.0 (6.1006.310) column with a capacity of 11.1 μmol (Cl^-) was used. Elution of ions was run isocratically at a flow rate of 1 mL min^{-1}. The eluent contained 1.8 mM Na_2CO_3 (ACS reagent, ≥ 99.5%; Sigma-Aldrich, Munich, Germany) and 1.7 mM $NaHCO_3$ (ACS reagent, ≥ 99.7%; Sigma-Aldrich).

For quantification of Br^- with IC, five standards were prepared from a 200 mg L^{-1} Br^- stock solution (sodium bromide, p.a., Fluka, Seelze, Germany). An aliquot of 100 μL of each standard was injected via an external sample injection port. The resulting concentrations were 1.0, 1.5, 2.0, 2.5, and 3.0 mg/L^{-1} Br^-. The limit of quantification was 0.15 mg L^{-1} Br^- and was determined according to the calibration method described in DIN 32645 (2008).[40]

2.4. Inorganic Bromine (IBr). IBr was determined using the ion chromatography set up described above for the AOBr measurements. An aliquot of 100 μL of the aqueous samples was injected via an external sample injection port. For quantification of IBr, five standards with the concentrations of 16, 20, 24, 28, and 32 mg L^{-1} Br^- (sodium bromide, p.a., Fluka) were used.

2.5. Total Organic Carbon (TOC). TOC was determined as nonpurgeable organic carbon (NPOC). The TOC-5000A-Total organic carbon analyzer was connected to an ASI-5000-autosampler (both from Shimadzu Deutschland GmbH, Duisburg, Germany). A total of 30 μL aqueous sample were injected for each measurement. Each determination was done in triplicate. Organic carbon standards were prepared from a 50 mg L^{-1} C caffeine stock solution (ReagentPlus, Sigma-Aldrich) with concentrations of 1, 2, 3, 4, and 5 mg L^{-1} C. The limit of quantification was 0.7 mg L^{-1} NPOC and was determined according to the calibration method described in DIN 32645 (2008).[40]

2.6. Liquid Chromatography−Mass Spectrometry (LC-MS). In order to determine possible degradation products, an Agilent 1290 Infinity liquid chromatography system was used, consisting of a 1290 Infinity binary pump (G4220A), a 1290 Infinity Flexible cube solvent management module (G4227A), a 1290 Infinity HiP sampler (G4226A), and a 1290 Infinity Thermostated Column compartment (G1316C). All solvents and mobile phases were used as LC-MS grade. Frozen samples were thawed, filtrated (0.2 μm syringe filter) and analyzed without further dilution.

Analyses were run on a 50 × 0.3 mm Kinetex C18 column with 2.6 μm particles (Phenomenex, Germany) with water (prepared with a purification system from Sartorius Stedim, Goettingen, Germany) containing 0.1% formic acid (Merck, Darmstadt, Germany) as eluent A and methanol (VWR, Leuven, Belgium) with 0.1% formic acid as eluent B with a flow of 500 μL/min. The linear gradient started with 20% B, increased to 80% B in 5 min and was held for 1 min. The column was reequilibrated for 3 min before the next injection. The injection volume was 20 μL.

Mass spectrometric measurements were performed with an Agilent 6560 IM-qTOF System, equipped with a Dual Agilent Jet Stream electrospray ionization (AJS ESI) Source run in qTOF-only mode. Instrument parameters are given in SI Table SI-2. The software Mass Hunter Qualitative Analysis Version B.07.00 (Agilent) was used.

2.7. Contact Angle Measurements. In order to determine the contact angle after UV treatment, drops of 5 μL purified water were given on each PolyFR film at a dosing rate of 1 μL/s via a Hamilton 500 μL Syringe (Hamilton, NV). After using the sessile drop technique on a DataPhysics contact angle system OCA (DataPhysics instruments GmbH, Filderstadt, Germany), calculations were performed with the SCA20 software by DataPhysics, applying the Laplace−Young fitting. Ten samples were analyzed per time point.

2.8. Gel Permeation Chromatography (GPC) or Size Exclusion Chromatography (SEC). GPC/SEC measurements were conducted to estimate molecular weight distribution of bulk PolyFR. The measurements were conducted at 60 °C, using DMAc 0.01 M LiBr as mobile eluent on a GPC system equipped with a dual-detector consisting of differential refractometer and viscometer (ETA-2020, WGE Dr. Bures, Germany). The mobile phase flows at 1.0 mL/min along with a polymer sample with an injected volume of 200 μL (and thus a concentration of approximately 4.0 mg/mL).

The stationary phase consisted of three stainless steel columns including precolumn filled with a cross-linked polyester gel. The columns (PSS Polymer Standard Service GmbH, Germany) are of the type GRAM analytical linear (8 × 300 mm) with a mean particle diameter of 10 μm and a separation range of 0.5−1000 kDa. A set of narrowly distributed standards, that is, poly(methyl methacrylate) (PMMA; PSS Polymer Standard Service GmbH, Germany) was applied to generate a conventional calibration for the GPC/SEC system. The universal calibration method is based on the principle that the hydrodynamic volume V_h or the product of intrinsic viscosity $[\eta]$ and molecular weight M is the same for every type of polymer at equal elution volume.[13] The Mark−Houwink equation $[\eta] = K M^a$ correlates the molecular weight M and intrinsic viscosity $[\eta]$ for a specific solvent and temperature. Generally, for broad-distributed copolymers such as PolyFR, there is great uncertainty in the measurement of intrinsic viscosities and application of universal calibration method.[14] Consequently, estimation of the number-average molecular weight (M_n) of PolyFr was based on the conventional calibration for the purpose of qualitative comparison. The acquisition and analysis of measurement data were performed using PSS WinGPC UniChrom Software (PSS Polymer Standard Service GmbH, Germany).

2.9. ^{1}H-Nuclear Magnetic Resonance (NMR) Spectroscopy. ^{1}H NMR was performed in order to determine changes in the molecular structure of PolyFR after UV irradiation. Therefore, ^{1}H NMR spectra were recorded on a Bruker DMX-300 (Billerica, 300 MHz) spectrometer. Deuterated chloroform was used as a solvent.

3. RESULTS

3.1. Total Bromine Concentration in Water after UV Irradiation. Measurements using ICP-MS showed that UV irradiation caused the aqueous bromine concentrations to increase over time in the 150 mL samples containing 3.33 g EPS with PolyFR per liter. After irradiating for 60 min, the bromine concentration raised to 69.2 μg/g EPS (Figure 3, standard deviation (SD) ± 1.0 μg/g) of which 2.5 μg/g were bromine that leaches out without UV treatment and additional 1.31 μg/g due to the increasing water temperature. Treating the 150 mL samples containing bulk PolyFR (3.33 g/L) caused an almost linear increase of the aqueous bromine concentration as well, but to a much higher extent. After 60 min, the concentration increased to 17 300 μg/g PolyFR (Figure 3, SD ± 3370 μg/g), including 57 μg/g that can be detected

DOI: 10.1021/acs.est.6b04083
Environ. Sci. Technol. 2016, 50, 12912−12920

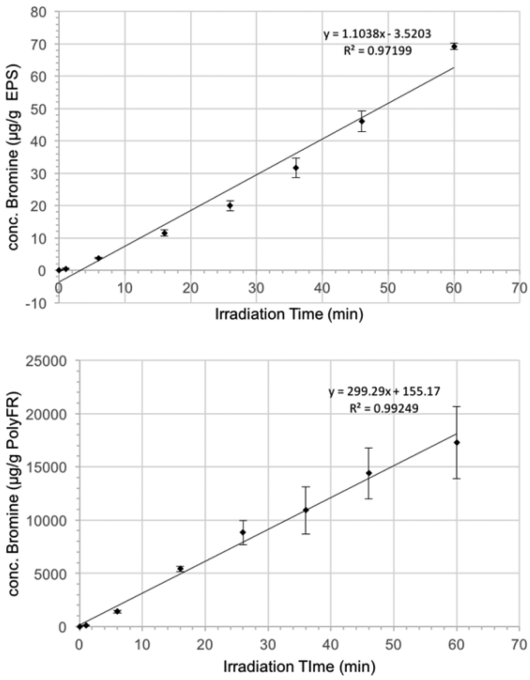

Figure 3. Concentration of aqueous bromine (μg bromine/g) following UV irradiation of 1 g EPS (containing PolyFR, top) and 1 g bulk PolyFR (bottom) per liter during time.

without previous UV treatment and additional 22 μg/g due to the increasing water temperature. When analyzing the 350 mL samples containing PolyFR (3.33 g/L), a lower concentration of bromine of 6110 μg/g PolyFR (SD $\pm$ 260 μg/g) was found after 60 min compared to the samples with the same PolyFR concentration, but a smaller volume. This effect was even stronger when increasing the concentration from 3.33 g PolyFR per liter to 6.67 g/L, but keeping the volume constant at 350 mL (3860 μg/g, SD $\pm$ 150 μg/g).

The pH of the solvent did not directly change after adding bulk PolyFR (5.4 with and without PolyFR), but decreased to 3.6 after UV treatment for 60 min.

3.2. Total Organic Carbon, Inorganic and Adsorbable Organically Bound Bromine in Water after UV Irradiation of Bulk PolyFR. Analyses of the adsorbable organically bound bromine in the 350 mL PolyFR samples via CIC, revealed comparably low concentrations of organically bound bromine in contrast to the total bromine concentrations. The concentration of organically bound bromine reached 60.8 μg/g PolyFR after irradiation for 60 min (3.33 g PolyFR/L). Irradiating a solution with a higher concentration of PolyFR (6.67 g/L) for the same period of time merely increased the level of organically bound bromine to 34.3 μg/g. Thus, the adsorbable organically bound bromine accounted on average for both concentrations only for 0.96% (SD $\pm$ 0.06%) of the total bromine measured with ICP-MS.

The concentration of IBr reached 6180 μg/g PolyFR (SD $\pm$ 567 μg/g) after irradiation for 60 min (3.33 g PolyFR/L). Thus, the amount of IBr determined by IC accounted for 101% of the total bromine measured with ICP-MS. The sum of IBr and AOBr accounted for 102% of the total bromine.

Analyses of TOC in the 350 mL samples with (3.33 g PolyFR/L) after an irradiation of 60 min revealed a concentration of 855 μg/g PolyFR (SD $\pm$ 76 μg/g).

3.3. Detection of Degradation Products after UV Irradiation. After irradiating the samples for 60 min, different degradation products were detectable via LC-MS. Their mass-to-charge ratio and corresponding possible molecular formula calculated from this are given in Table 1 (and SI Figure SI-2). For each molecular formula, one or two possible chemical structures are suggested.

Table 1. Information about Possible Degradation Products That Were Found via LC-MS After UV Treatment of Bulk PolyFR for 60 Min

Mass-to-Charge (m/z)	Name	Molecular Formula	Chemical Structure	Bromine Content (mass %)	Δ ppm
214.9348	5-bromo-2-hydroxybenzoic acid	$C_7H_5BrO_3$		36.82	0.5
292.8451	3,5-dibromo-4-hydroxybenzoic acid	$C_7H_4Br_2O_3$		54.00	1.0
306.8602	3,5-dibromo-2-hydroxy-4-methylbenzoic acid	$C_8H_6Br_2O_3$		51.56	2.9
	3,5-dibromo-2-methoxybenzoic acid				
326.7648	2,4,6-tribromophenol	$C_6H_3Br_3O$		72.46	4.0
344.7400	tribromofuran-2-carboxylic acid	$C_5HBr_3O_3$		68.73	0.9
370.7551	2,4,6-tribromo-3-hydroxybenzoic acid	$C_7H_3Br_3O_3$		63.95	2.4

DOI: 10.1021/acs.est.6b04083
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3.4. Concentration of Possible Degradation Products. In order to obtain an estimate of the overall concentration of possible degradation products after UV treatment at different time points, the total bromine concentration of each sample (determined via ICP-MS), the percentage of adsorbable organically bound bromine (determined via CIC), and the mean bromine content of the degradation products (Table 1) were used for the calculation as follows:

$$[\mathrm{CP}] = \frac{\mathrm{tBr} \times \mathrm{AOBr}}{\mathrm{dBr}} \quad (1)$$

with: [CP] = concentration of the sum of possible degradation products (μg/g); tBr = total bromine concentration of the sample (μg/g); AOBr = percentage of the adsorbable organically bound bromine (0.96%); dBr = mean bromine content of the detected degradation products (57,01%).

The results of these calculations are shown in Figure SI-3. When irradiating EPS, the cumulative concentration of degradation products raised to 1.17 μg/g EPS (SD ± 0.02 μg/g) after 60 min. A much higher concentration of 293 μg/g PolyFR (SD ± 57 μg/g) was calculated when irradiating bulk PolyFR for 60 min.

3.5. Changes to the solid PolyFR fraction after UV irradiation. A reduction of the mean water contact angle is observed upon increasing duration of UV irradiation (Figure 4). Without UV treatment, the mean contact angle value was found to be 79° (SD ± 4°). After irradiating for 60 min, the mean contact angle decreased to 22° (SD ± 3°).

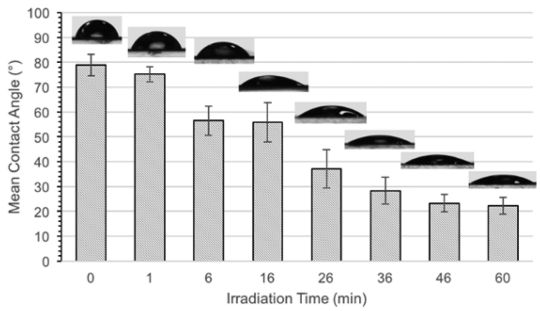

Figure 4. Mean water contact angle (deg) following UV irradiation for the given time.

After 60 min of UV treatment, the mean molecular weight M_n of the solid fraction, measured via GPC/SEC (SI Figure SI-5), remains approximately the same compared to untreated bulk PolyFR (untreated: 127 300 g/mol; treated: 125 900 g/mol). However, the molecular weight distribution broadens after UV irradiation for 60 min (untreated: approximately 78 500−189 400 g/mol; treated: 32 800−385 100 g/mol) and other polymer species are detectable in the chromatogram (SI Figure SI-4).

The density of PolyFR powder did not change after UV treatment (untreated: 0.58 g/cm^3 compared to treated: 0.59 g/cm^3).

When analyzing untreated bulk PolyFR with ^{1}H NMR, signals with the chemical shift in the range of δ = 6.24 to 7.25 ppm can be assigned to the aromatic groups in the polymer (Figure SI-6). Signals with the chemical shift in the range of 3.5 to 4.6 ppm belong to protons adjacent to brominated carbon atoms. After irradiating the samples for 60 min, changes can be observed in the solid fraction of the polymer, mainly within the signals with the chemical shift in the range of 0.5 to 2.5 ppm. These signals correspond to protons in the polymer main chain without adjacent bromine atoms and broaden after UV treatment for 60 min.

4. DISCUSSION

4.1. Aqueous Bromine Concentration As a Marker for Degradation. In order to evaluate a possible environmental risk that may arise when polymeric BFRs are exposed to UV radiation or other abiotic factors in future studies, it is necessary to establish an adequate analytical procedure that gives the opportunity to study the decomposition of these new compounds. Due to its commercial production and use since 2014, PolyFR was chosen as an example for this study. Taking into account the environmental problems of other BFRs[15−18] and particularly the previously used HBCD,[2,19,20] it is advantageous to obtain more data concerning PolyFR and its behavior in the environment.

The total bromine concentration has been chosen as a suitable marker for a possible degradation of PolyFR because it is time efficient and convenient to measure in contrast to AOBr concentrations. As shown in Figure 1, the aqueous bromine concentration raises to over 17 300 μg/g bulk PolyFR. This equates to 2.84% of the bromine content of 1 g bulk PolyFR (where the bromine content is 61 mass %). To account for possible free, thus unreacted bromine during industrial manufacturing, experiments were carried out without UV treatment. As a result of those, it was calculated that only 0.33% of these 17 300 μg leaches out of bulk PolyFR without UV irradiation. Similar experiments were used to account for bromine which leaches out of bulk PolyFR due the increasing water temperature during UV treatment (additional 0.13%) under this test procedure. It should also be mentioned that the possible formation of HBr was not studied.

While irradiating the samples, PolyFR powder was first floating on the water surface, where the irradiance was approximately 500 W/m^2. After 6 min, PolyFR began to sink to the bottom. Because of its constant density during irradiation, this process was not expected. Hence, contact angle measurements were conducted in order to check for changes in the hydrophilicity during UV irradiation. Sinking of PolyFR was almost completed after 26 min, which is congruent with the results of the contact angle measurements shown in Figure 4. The contact angle decreased by 28% after 6 min and 53% after 26 min. Sinking of PolyFR is important to consider when measuring the irradiance because it decreased to approximately 150 W/m^2 at the bottom of the Erlenmeyer flask. Interestingly, sinking of PolyFR does not seem to have an influence on the measured bromine concentration.

Another important factor is the thickness of the PolyFR layer on the water surface. Even though the same concentration of PolyFR was used for the AOBr experiments, the water surface in the bigger Erlenmeyer flask was comparably smaller and thus the PolyFR layer thicker, which led to a smaller irradiance at the bottom of the layer and consequently to less degradation (only 1.00% of the bromine content of 1 g bulk PolyFR leaches out compared to 2.84% when irradiating thinner layers). This effect should also be taken into account when interpreting the results of the UV treatment of EPS.

Experiments with the final insulation product represent a more realistic scenario, in which EPS contains around 1% per mass PolyFR. After UV treatment of EPS for 60 min, the

DOI: 10.1021/acs.est.6b04083
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bromine concentration rose to almost 70 μg/g EPS, which is much lower compared to bulk PolyFR. However, when taking the PolyFR content of 1% into account, the bromine concentration gets much closer (40%) to the observed bromine concentration in the bulk PolyFR experiments. The remaining difference might be due to the thicker layer in the EPS experiments (PolyFR is shielded against the UV irradiation by the surrounding matrix). However, it should also be considered that the underlying mechanisms might not be the same for EPS and bulk PolyFR, for example due to possible additional compounds incorporated into EPS like antioxidants.

In order to estimate the degree to which the obtained results reflect a realistic scenario, the applied irradiance needs to be evaluated. Therefore, the full irradiance of 500 W/m^2 for 60 min will be used (500 Wh/m^2), even though 150 W/m^2 would arguably be more precise after the 26 min mark. Under the extreme case that the applied dose consisted only of UV−B and assuming an annual average radiation intensity of 7 W/m^2 for UV−B, the applied dose of 500 Wh/m^2 equals approximately the irradiance of natural UV−B on the earth's surface of 3 days. The same consideration for UV-A with an annual average radiation intensity of 250 W/m^2 would mean that the applied dose equals approximately the irradiance of natural UV-A on the earth's surface of 2 h.[10]

4.2. Changes to the Polymer. Broadening of the molecular weight distribution of bulk PolyFR after UV treatment is detectable as well as other polymeric species, which are indicators of degradation of the polymer (SI Figures SI-4 and SI-5). It should be kept in mind that only the solid fraction, thus the part that does not contain the water-soluble fraction, is responsible for the molecular mass distribution pattern.

Although UV irradiation does not seem to change the density of the solid fraction of PolyFR, changes are observable via ^{1}H NMR (SI Figure SI-6). Broadening within the signals with the chemical shift in the range of 0.5−2.5 ppm possibly indicates a degradation of the main chain as well. No distinct peaks, but broadening of the signal can be observed in the spectrum because degradation probably occurs in every molecule and even every section of a single polymeric molecule to a different extent. Thus, the environment of each proton is changing and the spectrum only reflects an average of multiple peaks. Potential explanations for the broadening are changes in aromatic compounds or abstraction of bromine containing fragments, which would be in line with the increasing bromine concentration and the typical stepwise reductive debromination that BFRs undergo when exposed to UV radiation.[5,8] However, PolyFR does not have aromatic bounded bromine, which may cause different underlying mechanisms compared to BFRs with aromatic bounded bromine. Because the chain scission reactions take place in solid state, radical combinations leading to cross-linking can take place in parallel, thus explaining the increase in molecular weight which is seen (SI Figure SI-5). Overall, the changes observed by size exclusion chromatography analysis can be related to mechanisms of photodegradation of similar polymers well studied in literature.[21]

As mentioned previously, these changes are reflected in the alteration of the contact angle (Figure 4). The smaller the contact angle with water, the more hydrophilic a surface is.[22,23] Thus, PolyFR becomes more hydrophilic when exposed to UV radiation. This is especially important when considering other environmental factors such as biodegradation. It is commonly accepted that with increasing hydrophilicity of the surface, it can easier be colonized by microorganisms.[24,25] Thus, biodegradation, which can be enhanced by UV radiation,[26] may eventually lead to an increased water solubility/bioavailability of PolyFR and/or additional degradation products.

4.3. Possible Degradation Products and Assumptions about Their Concentration. As shown in Table 1, certain possible degradation products were found via LC-MS after UV treatment for 60 min. All of the detected degradation products were brominated. The fact that only brominated degradation products were detected is questionable when taking into account that only 0.01% of the bromine content of bulk PolyFR is organically bound in the water sample, but 0.24% of the carbon content of bulk PolyFR was detected. This difference between AOBr and TOC is either due to an incomplete detection of degradation products via LC-MS or the fact that not all brominated organic species were measured through CIC. The latter explanation seems rather implausible because the amount of IBr and AOBr equals approximately the amount of total bromine. As a result, it is more likely that various nonbrominated (and brominated) organic species were not detected via LC-MS, probably due to a concentration lower than the detection limit and less characteristic profiles (bromine isotopes can be assigned comparably easily). Interestingly, all the suggested degradation products contain bromine bound to aromatic rings, even though the precursor polymer only contains bromine bound to aliphatic carbon. The addition of bromine to the aromatic structure might either occur while still attached to the polymer itself or very rapidly in solution. More research is required in order to identify the underlying mechanisms that may lead to the suggested degradation products.

In order to estimate a possible concentration of (detected brominated) degradation products in the water samples after UV treatment, the total bromine concentration was used as a marker. However, only a small portion of 0.96% of the total bromine concentration is organically bound and can therefore be used when determining the concentration of degradation products. It should be kept in mind that this portion has not been measured for every individual time point and volume, thus it might vary. Also, the applied bromine content of the degradation products is only an average that is valid when assuming that all detected degradation products are present in equal proportions.

Cumulative concentrations of these products were calculated and are shown in SI Figure SI-3. As mentioned previously, concentration differences between bulk PolyFR and EPS are probably mainly due to the low concentration of PolyFR in EPS and the shielding of the surrounding matrix against UV irradiation. Hence, the calculations for the UV treatment of EPS are clearly more realistic and therefore more important because PolyFR will probably mainly enter the environment incorporated into EPS. However, calculations for bulk PolyFR should not be dismissed easily. High concentrations of BFRs are typically found near point sources like BFR or EPS manufacturing plants.[20,27,28] Consequently, there is also a risk for a contamination with bulk PolyFR. Thus, possible transformation products of the parent compound should also to be taken into consideration for future environmental risk assessments.[5]

The typical degradation pattern of a stepwise debromination generally follows pseudo-first-order kinetics,[29−31] which could not be validated here, probably because of a too short

DOI: 10.1021/acs.est.6b04083
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irradiation time. The degradation rate in general is greatly influenced by the solvent (or its absence, which typically lowers the rate).[8] For example, the hydrogen-donating ability of organic solvents (like Tetrahydrofuran) might impact the decomposition speed.[32] In this study, however, we have chosen to work with the most environmentally relevant solvent, water. When evaluating the decomposition rate in water, different parameters have to be assessed regardless. For instance, it was demonstrated that photochemical transformation of BFRs in aqueous solutions is influenced by the pH (in one study the decomposition rate was 6 times higher at a pH of 8 compared to a pH of 6).[33] Thus, even though we were working with bidistilled water (pH between 5.4 and 3.6), factors like the presence of humic acid[34] and other substances should be considered when transferring results from this study to situations in the field.

4.4. Future Experiments. This study gives a first indication of possible degradation products of the polymeric BFR PolyFR and presents a methodological approach for studying degradation products following exposure of PolyFR to UV radiation. Further studies should be conducted to use this approach following real environmental conditions or degradation scenarios. In principle, a possible degradation caused by UV irradiation is important to take into account when assessing the risk at all life cycle stages of bulk PolyFR and products that contain this BFR, for instance installation or disposal of EPS.

This study also underlines the general need for further research concerning the long-time behavior of polymeric BFRs. Most importantly, the exact composition and formation mechanisms of degradation products should be investigated, as well as their effects on the environment.

■ ASSOCIATED CONTENT

Supporting Information

Figure SI-1: Spectrum of the used UV lamp Figure SI-2: Exemplary LC-ESI-MS extracted ion chromatogram and mass spectrum Figure SI-3: Estimated cumulative concentration of degradation products Figure SI-4: Molecular weight (M_n) distribution of bulk PolyFR Figure SI-5: GPC/SEC results Figure SI-6: ^{1}H NMR spectra of bulk PolyFR Table SI-1: ABC combustion program Table SI-2: Instrument parameters of QTOF measurements The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b04083.

(PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Thanks are due to BASF for contributing the bulk PolyFR. We also want to thank John W. Davis (DOW) for his helpful comments.

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DOI: 10.1021/acs.est.6b04083
Environ. Sci. Technol. 2016, 50, 12912−12920

Chapter II: Degradation of the polymeric brominated flame retardant "Polymeric FR" by heat and UV exposure

In this chapter, the degradation of "Polymeric FR" is studied after UV irradiation and heat exposure. Both treatments are compared and individual degradation products are identified.

Manuscript as accepted for publication in Environmental Science & Technology. The associated supporting information is given in appendix IV.

Degradation of the polymeric brominated flame retardant "Polymeric FR" by heat and UV exposure

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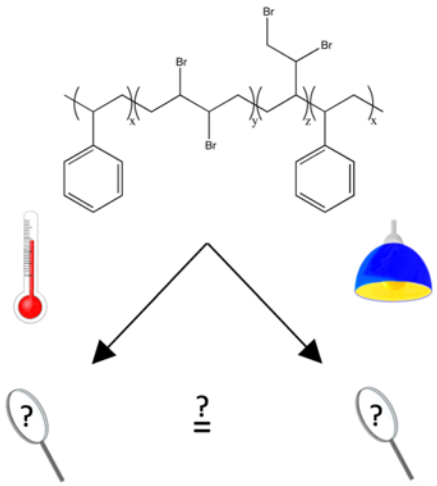
Abstract



Monomeric brominated flame retardants often pose risks to the environment. The new group of polymeric flame retardants is claimed to be a safer alternative due to their high molecular weight and persistence by design. Within this publication, the degradation of a commercially widely applied example of this group – the polymer "Polymeric FR" – was studied during UV irradiation and long-term exposure

to heat (60 °C) for up to 36 weeks. Both treatments led to a variety of degradation products, which might have potentially adverse environmental effects and an increased mobility compared to the mother polymer. Besides identifying some of the possible degradation products (including for instance 2,4,6-tribromo-3-hydroxybenzoic acid), the degradation via UV irradiation, which yields 75 different degradation products, and via heat, which led to significantly less products, was compared. In addition, further parameters like TOC and the concentration of free bromine were studied and it was demonstrated that the used type of water (distilled, reconstituted, and rain water) does not influence the outcome of the degradation experiments.

1. Introduction

Flame retardants (FR) are often necessary in order to comply with fire safety requirements[1] for different products like textiles, electronical equipment, furniture, or building materials[2]. A major group that is being extensively used are brominated FRs (BFR). However, several of these substances exhibit negative effects on biota when they enter the environment[2–4]. Their emission to the environment is often linked to their chemical structure, which is almost exclusively a monomeric one. A prominent example for such a chemical is hexabromocyclododecane (HBCD), which was mainly used in expanded (EPS) and extruded (XPS) polystyrene foams. However, due to its bioaccumulative, persistent, and toxic characteristics, the manufacturing and use of HBCD has been prohibited under the Stockholm Convention on Persistent Organic Pollutants and since August 2015 under the European Regulation (EC) No 1907/2006 (REACH) as well[5–7]. In order to continuously stay in conformity with fire safety requirement, alternative products were developed for foam insulation. The most common substitute for HBCD on a commercial scale is the additive FR “Polymeric FR” (CAS No. 1195978-93-8, Figure 1), a block copolymer of polystyrene and brominated polybutadiene[8–11]. According to the manufacturers, roughly 26,000 metric tons are produced every year[48,49]. This polymer – which is also known as PolyFR, pFR, Emerald Innovation 3000, FR-122P, and GreenCrest – was developed with the aim of having a superior environmental profile while still being suitable for the established technical process. Therefore, the bromine-containing polymer is persistent by design. Additionally, because of its high molecular weight, no bioavailability is expected. Thus, “Polymeric FR” is claimed to be more environmental friendly[10]. To the best of our knowledge, all governmental risk evaluations until now focussed on the polymer itself without considering possible degradation products. The U.S. Environmental Protection Agency concluded 2014 that the “long-term behavior in the environment is currently not known”[50].

Considering the long life time and various life cycle stages of EPS and XPS, abiotic and biotic environmental factors need to be taken into account. These factors may lead to a degradation of the commercial polymer and could result in smaller molecules with a different mobility and toxic potential.

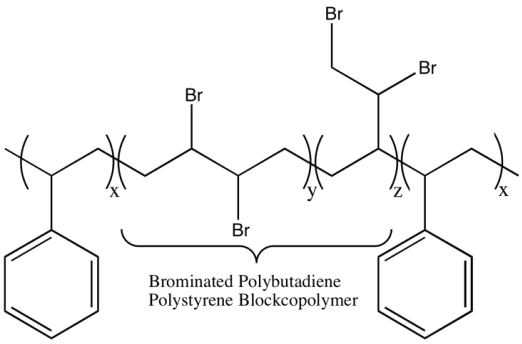


Figure 1: Chemical structure of "Polymeric FR" as described by the manufacturer.

In a recent study, degradation of "Polymeric FR" following ultraviolet (UV) irradiation was described within laboratory degradation experiments[12]. However, even though structural stability against UV irradiation certainly plays a role for ensuring the environmental safety of a brominated FR (BFR), other degradation factors may be more important in order to assess its long-term behaviour.

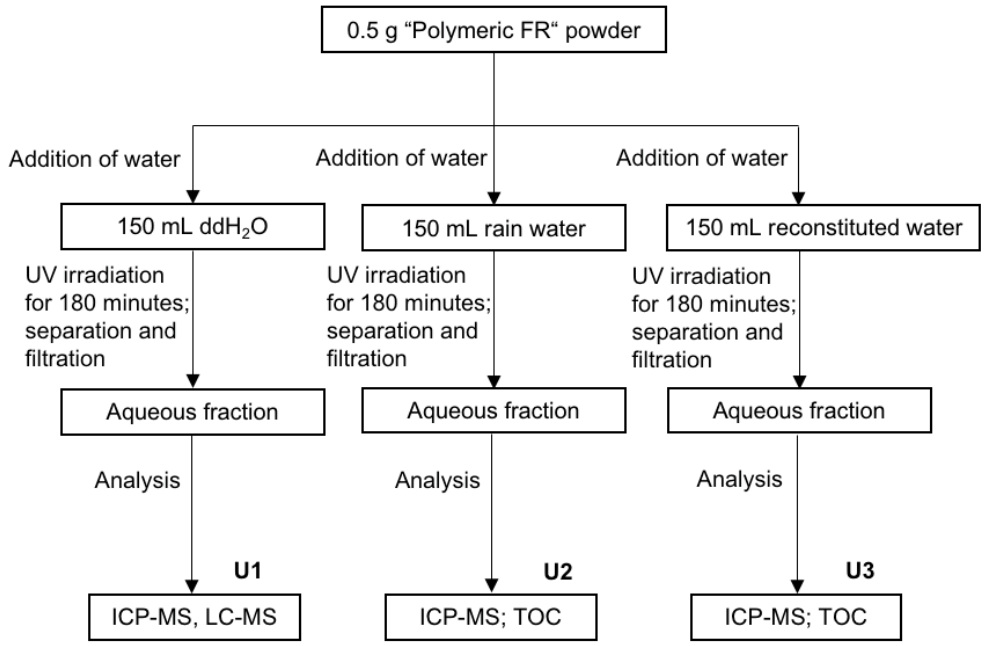
Considering the life cycle of a BFR used in insulation products, heat plays a significant role during almost all stages. Insulation products are often installed below a roof and in consequence face rather high temperatures during summer, which could lead to degradation of the incorporated BFR after a certain period. If these products are brought to landfill after their use as insulation material they might be exposed to heat again and in addition monomers deriving from the polymeric BFR could end up in the environment through water or attached to particles via air[13,14]. It should be noted that these possible ways of environmental contamination very much depend on national regulations and available technologies for recycling. Alternatively, incineration of foam based insulation products is regularly used[11,15]. Also, chemical recycling of EPS/XPS seems to be a possibility for the future[16]. However, as long as landfilling is still a common option for insulation products, heat exposure before and during landfilling might be an important way leading to degradation of BFRs. Therefore, possible degradation of "Polymeric FR" after heat treatment was studied and compared to UV degradation using a similar approach as described recently[12].

2. Material and methods

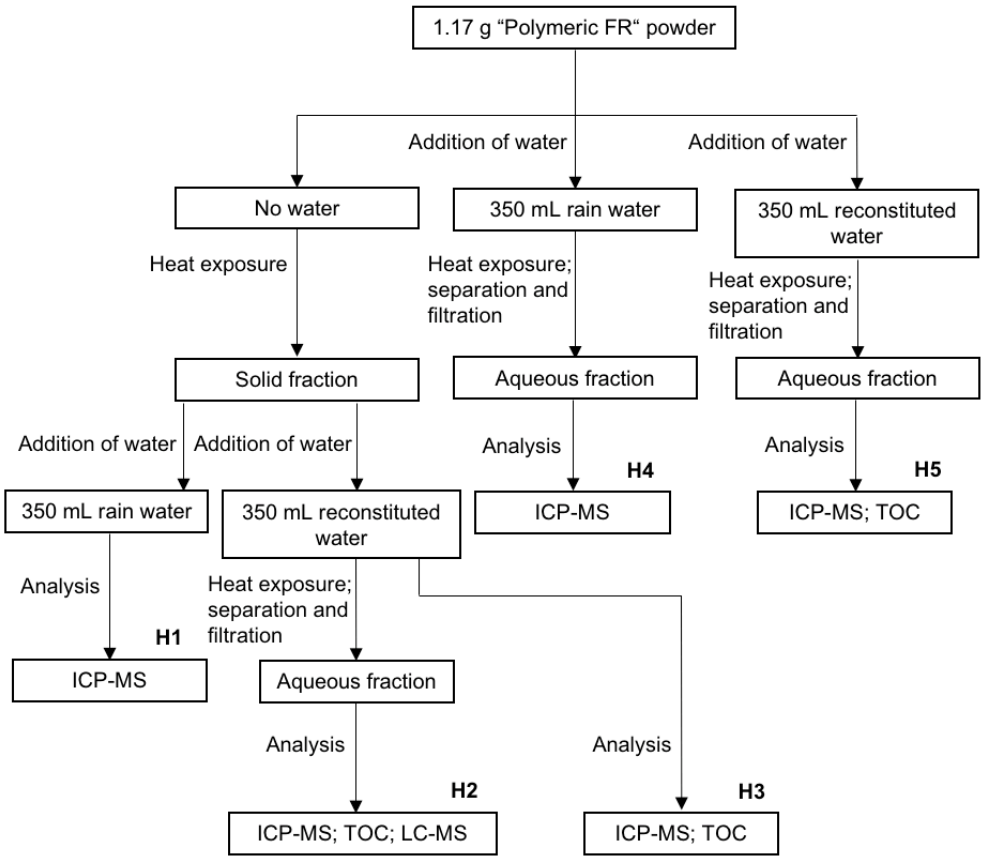
2.1 Experimental design

In order to compare the degree of degradation and the type of products which are possibly formed during the respective processes, pure “Polymeric FR” powder was exposed to two different treatments: UV radiation and heat. Therefore, “Polymeric FR” which has typically a bromine content of approximately 61 mass % (according to the manufacturer) was obtained from BASF SE (Ludwigshafen am Rhein, Germany). An overview of the experimental procedure and the methods applied is given in Figure 2.

a)



88 b)



89

90 Figure 2: Overview of the study design: a) Procedures applied to samples which were exposed to UV
91 radiation; b) Work flow which was applied for 60 °C heat exposed samples. U1 – 3 and H1 – 5 are used
92 to easier distinct between different treatments. Reconstituted water is commonly used during studies
93 focusing on ecotoxicological endpoints.

94

95 For the determination of total organic carbon (TOC) and the bromine measurements over time during
96 and after UV irradiation, approximately 0.5 g “Polymeric FR” was weighed (Libror AEG-220, Shimadzu,
97 Kyoto, Japan) into an Erlenmeyer glass flask filled with 150 mL ddH_2O (pH 5.43; prepared with a Milli-
98 Q academic, Millipore, Billerica, USA) to obtain a concentration of 3.33 g/L. Additionally, Erlenmeyer
99 glass flasks were prepared in the same way using reconstituted water (see supporting information (SI)
100 Table S1 for the applied recipe) and rain water, which was collected from a private rainwater re-use
101 system (see SI Table S2 for the analysed composition). Although “Polymeric FR” is not water soluble,

concentrations are expressed in “g/L” to underline that the same concentration within this study and compared to previous studies has been used.

A UV cube (UV-A cube 2000, Hoenle UV technology, Graefelfing, Germany) was used for the UV treatment from above (Figure 2 a) with an irradiance of approximately 500 W/m^2 (covers the UV-ABC and partly VIS spectrum, however, mainly between 290 and 450 nm; Figure S1). The irradiance decreased to approximately 150 W/m^2 on the ground of the Erlenmeyer flasks filled with 150 mL water. The irradiation was started approximately 20 minutes after water addition. Triplicates of 1 mL were taken from each of the 150 mL preparations every 15 minutes up to 180 minutes and stored in 1.5 mL Eppendorf tubes afterwards. The samples were manually shaken for 5 seconds before and after sampling. All water samples were filtered (0.45 µm filter, Merck Millipore, Billerica, USA), wrapped in aluminium foil and stored at -20 °C. Samples without “Polymeric FR” were treated in the same way and used as blanks. Furthermore, samples with and without “Polymeric FR” were not irradiated and used as a reference for free bromine. Triplicates were prepared for each time point. To account for possible effects due to the increasing water temperature during irradiation (increased from 22 °C to 63 °C), control samples were exposed to 60 °C for 180 minutes in a Thermo Scientific Heraeus Function Line oven (Thermo Fisher Scientific, Waltham, Massachusetts, USA.).

In addition to UV irradiation, samples of “Polymeric FR” were exposed to heat using the same Thermo Scientific Heraeus Function Line oven at 60 °C (Figure 2 b). Within this exposure scenario, 1.17 g “Polymeric FR” was either placed in Erlenmeyer flasks filled with 350 mL rain water or reconstituted water (same concentration of 3.33 g/L as before) or was placed without any kind of solvent in a closed glass petri dish. “Polymeric FR” in reconstituted water was exposed to heat for up to 36 weeks and in rain water for up to 12 weeks. Triplicates, each of 1 mL, were taken regularly (after 1, 2, 3, 4, 8, 12, 24, and 36 weeks), handled in the same way as UV irradiated samples, and stored at -20 °C. “Polymeric FR” samples (1.17 g) which were not placed in water, were heat exposed for up to 24 weeks (with sampling after 4, 8, 12, 16, 20, and 24 weeks). For sampling, the treated “Polymeric FR” powder was added to 350 mL rain water or reconstituted water, shaken for 1 hour, filtered, wrapped in aluminium foil, and stored at -20 °C. Furthermore, some powder samples were added to 350 mL reconstituted water after the 24 weeks period. These samples were then exposed to heat for 12 additional weeks (with sampling after 4, 8, and 12 weeks). Samples were taken as described above. To obtain blanks for all experiments, samples with and without “Polymeric FR” were also kept for the same duration at room

132 temperature (RT). In addition, water samples without “Polymeric FR” were exposed to heat as well. The
133 maximum exposure of “Polymeric FR” in ddH_2O was 2 years at RT.
134 UV irradiated ddH_2O samples, as well as samples that were heat exposed in reconstituted water, were
135 concentrated in an Eppendorf Concentrator plus (Eppendorf, Hamburg, Germany) at 60 °C and
136 analysed with liquid chromatography-mass spectrometry (LC-MS). The pH was determined using a
137 Mettler Toledo FiveEasy pH metre (Mettler Toledo, Ohio, USA).
138
139 2.2 Inductively coupled plasma mass spectrometry (ICP-MS)
140 The concentrations of total bromine in the samples were measured using a quadrupole ICP-MS system
141 (Elan 6000, Perkin Elmer, Waltham, Massachusetts, USA) operating at 950 W plasma power and
142 0.95 L/min nebuliser gas flow. Instrument calibration was performed using series of bromine dilutions
143 (in range from 0.1 µg/L to 100 µg/L) prepared from standard solutions (Merck, Darmstadt, Germany).
144 Subsequently, the element concentrations were calculated in µg/L using the corresponding regression
145 lines of the calibration (correlation factor ≥ 0.999). The limit of quantification was 2.3 µg/L.
146 Before measurements, each sample was diluted at least 10 times using 1 % nitric acid (HNO_3,
147 subboiled) with a concentration of yttrium (Y) of 10 µg/L as internal standard. In order to avoid
148 contamination and memory effects, the wash time between measurements was set to at least
149 60 seconds (with 1 % HNO_3, subboiled). The accuracy and stability of bromine measurements was
150 controlled additionally with a standard solution of bromine with a concentration of 10 µg/L (ICP multi
151 element standard IV solution, Merck, Darmstadt, Germany), which was analysed after every 10 samples.
152
153 2.3 Total organic carbon (TOC)
154 TOC was determined as non-purgeable organic carbon (NPOC). The TOC-5000A-Total organic carbon
155 analyzer was connected to an ASI-5000-autosampler (both from Shimadzu Deutschland GmbH,
156 Duisburg, Germany). Inorganic carbon was removed from the sample by the addition of 10 % v/v 1 mol/L
157 HCl and purging of the sample with synthetic air for 2 minutes. A total of 50 µL aqueous sample was
158 injected for each measurement. Each determination was done at least in triplicate. Organic carbon
159 standards were prepared from a 1000 mg/L C ± 10 mg/L C TOC reference solution (Sigma-Aldrich) with
160 final concentrations of 1, 2.5, 5, 10, 20, and 30 mg/L C. The limit of quantification was 0.8 mg/L C and
161 was determined according to the calibration method described in DIN 32645[51].
162

163 2.4 Liquid chromatography-mass spectrometry (LC-MS)

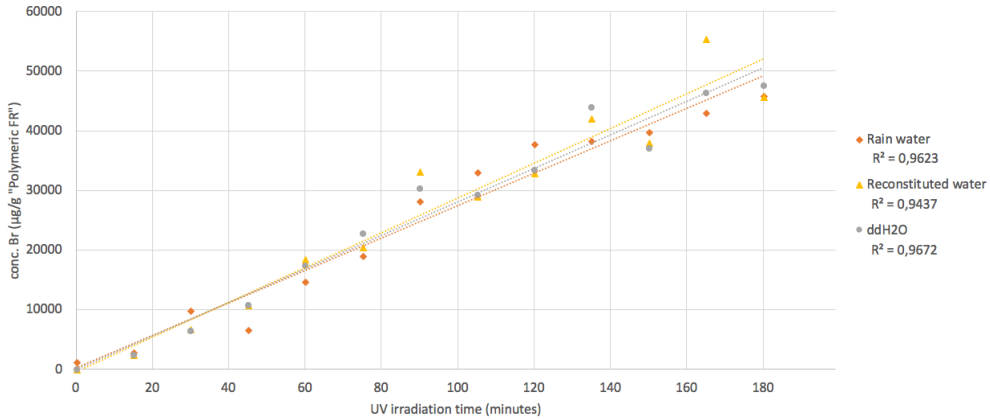
164 In order to determine possible degradation patterns and products, an Agilent 1290 Infinity liquid
165 chromatography system was used, consisting of a 1290 Infinity binary pump (G4220A), a 1290 Infinity
166 HiP sampler (G4226A), and a 1290 Infinity thermostatted column compartment (G1316C). All solvents
167 and mobile phases were used as LC-MS grade. Frozen samples were thawed, filtrated (0.2 µm syringe
168 filter) and analysed without further dilution.

169 Separations were performed using a 50x0.3 mm Kinetex C18 column with 2.6 µm particles
170 (Phenomenex, Germany) with water (prepared with a purification system from Sartorius Stedim,
171 Goettingen, Germany) containing 0.1 % formic acid (Merck, Darmstadt, Germany) as eluent A and
172 methanol (VWR, Leuven, Belgium) with 0.1 % formic acid as eluent B with a flow of 500 µL/min. The
173 linear gradient started at 20 % eluent B, increased to 80 % eluent B in 5 minutes and was held for
174 2.8 minutes, and back to initial conditions in 0.2 minutes. The column was re-equilibrated for 3 minutes
175 before the next injection. The injection volume was 20 µL.

176 Mass spectrometric measurements were performed in duplicates on an Agilent 6560 IM-qTOF System,
177 equipped with a Dual Agilent Jet Stream electrospray ionization (AJS ESI) Source run in qTOF-only
178 mode. Instrument parameters are given in SI Table S3. Based on previous results, analyses were only
179 run in ESI negative mode[12]. The software Mass Hunter Qualitative Analysis Version B.07.00 (Agilent)
180 was used to perform a feature analysis which was further specified with Mass Profiler Professional
181 (Agilent). During feature analysis sum formulas were calculated by the software according to accurate
182 masses and isotopic patterns. Bromine containing features were selected for further analysis. For
183 identification purpose, commercially available standards for previously suggested degradation
184 products[12] with the same sum formula were analyzed by LC-qTOF-MS; namely 5-bromosalicylic acid
185 (Sigma-Aldrich, purity 97 %; CAS No. 89-55-4), 2,4,6-tribromo-3-hydroxybenzoic acid (Sigma-Aldrich,
186 purity 97 %; CAS No. 14348-40-4), 3,5-dibromo-4-hydroxybenzoic acid (Alfa Aesar, purity 98 %;
187 CAS No. 3337-62-0), and 2,4,6-tribromophenol (Sigma-Aldrich, purity 99 %; CAS No. 118-79-6).

188 In addition, LC-MS/MS measurements were performed to obtain more information about the chemical
189 structure of possible bromine containing degradation products, especially for those, for which no
190 commercial standards were available. Therefore, the samples were analysed on the same Kinetex
191 column with identical eluents (flow of 300 µL/min). The linear gradient, starting at 5 % B, was held for
192 1 minute, then increased to 100 % B in 10 minutes and held for 4 minutes. The re-equilibration time of
193 the column was 10 minutes. The injection volume was 20 µL.

194 The MS/MS measurements were performed on an Agilent 6495B Triple Quadrupole LC/MS system with
195 iFunnel Technology, equipped with a Dual Agilent Jet Stream electrospray ionization (AJS ESI) source.
196 Instrument parameters are given in SI Table S4.
197
198 **3. Results**
199 3.1 Total bromine concentration and pH changes in ddH_2O, rain water and reconstituted water after UV
200 irradiation and heat exposure
201 Measurements using ICP-MS showed that UV irradiation caused the aqueous bromine concentrations
202 to increase linearly over time (Figure 3) in the 150 mL samples containing 0.5 g “Polymeric FR”
203 (3.33 g/L). After irradiating for 180 minutes, the bromine concentration raised to similar levels of around
204 46,000 µg/g “Polymeric FR” in ddH_2O (Figure 2 U1; 47,700 µg/g “Polymeric FR”; standard deviation
205 (SD) ± 5900 µg/g), rain water (U2; 45,900 µg/g “Polymeric FR”; SD ± 6700 µg/g), and reconstituted
206 water (U3; 45,800 µg/g “Polymeric FR”; SD ± 6000 µg/g) of which 82 µg/g were bromine that leaches
207 out without UV treatment and additional 71 µg/g due to the increasing water temperature.
208 The pH of the ddH_2O did not directly change after adding “Polymeric FR” (5.4 with and without
209 “Polymeric FR”), but decreased to 2.7 after UV treatment for 180 minutes. Presuming that this decrease
210 in pH is only due to originating HBr, (difference of 1.89 mmol Br^-/L ddH_2O), total Br as measured by ICP-
211 MS (1.99 mmol Br^-/L ddH_2O) would mostly (95 %) be dissociated as HBr. This elimination of HBr during
212 photodegradation is a known mechanism for various BFRs[6,17,18].
213



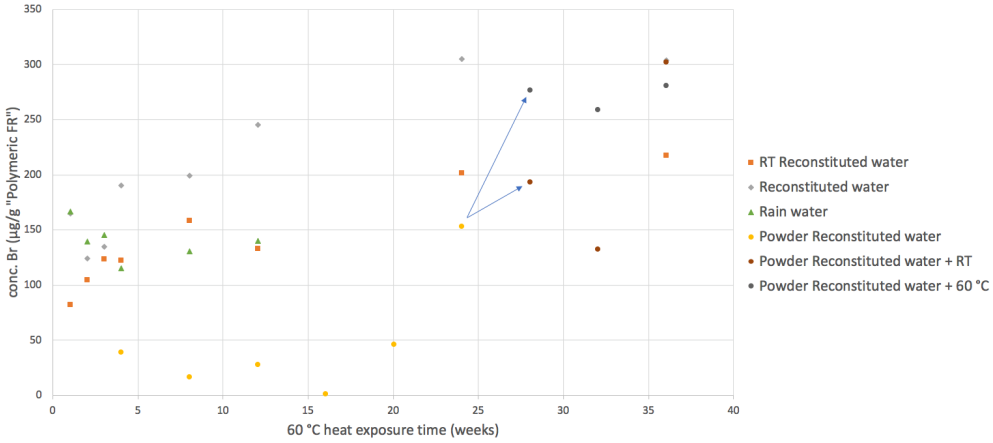
214

215 Figure 3: Concentration of aqueous bromine (µg Br/g "Polymeric FR") following UV irradiation of 0.5 g
216 "Polymeric FR" in 150 mL solvent (rain water in orange, reconstituted water in yellow, ddH_2O in grey)
217 over time.

218

219 Total bromine was likewise measured via ICP-MS in "Polymeric FR" samples which were exposed to
220 heat. The longest exposure of 36 weeks at 60 °C was done in reconstituted water (Figure 4). Here, the
221 bromine concentration raised to 300 µg/g "Polymeric FR" (Figure 2 H5; SD ± 41 µg/g). Without heat
222 exposure, the bromine concentration reached 220 µg/g "Polymeric FR" after 36 weeks in reconstituted
223 water (SD ± 23 µg/g). Especially in the first 4 weeks, the bromine concentrations of "Polymeric FR" in
224 reconstituted water (H5; ranges of 80 – 120 and 170 – 20 µg/g; at RT and at 60 °C respectively; SD of
225 max. ± 37 µg/g) and rain water (H4; ranges of 130 – 150 and 120 – 170 µg/g; SD of max. ± 19 µg/g)
226 were rather unstable and did not show a clear trend. Bromine concentrations in "Polymeric FR" powder
227 samples which were exposed to 60 °C without a solvent (but placed in rain water and reconstituted
228 water for 1 hour before measurement; H1 and H3) were below 50 µg/g "Polymeric FR" for the first
229 20 weeks (H3; or the maximum of 12 weeks for samples which were measured in rain water; H1). After
230 24 weeks of heat exposure and eventual measurement in reconstituted water, the bromine concentration
231 increased to 150 µg/g (H3; SD ± 17 µg/g). In addition, "Polymeric FR" powder samples which were
232 exposed to heat for 24 weeks, were placed in reconstituted water and further exposed to heat or kept
233 at RT to check if the previous heat treatment would influence consequential leaching. After 12 more
234 weeks of exposure (H2), the bromine concentration in these samples reached around 300 µg/g
235 "Polymeric FR" (at RT: 300 µg/g; SD ± 25 µg/g; and at 60 °C 280 µg/g; SD ± 17 µg/g). In one sample,

236 which was kept for 2 years in ddH_2O at RT, the bromine concentration reached 340 µg/g “Polymeric FR”
237 (SD ± 23 µg/g).
238 After 36 weeks, the pH changed from 7.0 to 5.2. Presuming that this decrease in pH is only due to
239 originating HBr (difference of 5.96 mmol Br^-/L reconstituted water), total Br as measured by ICP-MS
240 (6.40 mmol Br^-/L reconstituted water) would mostly (93 %) be dissociated as HBr, which has been
241 shown to be an important degradation mechanism for BFRs during heat exposure[19,20].
242



243
244 Figure 4: Selected concentrations of aqueous bromine (µg Br/g “Polymeric FR”) following heat exposure
245 of 1.17 g “Polymeric FR” in 350 mL solvent (reconstituted water at RT in orange, reconstituted water at
246 60 °C in grey, rain water at 60 °C in green, “Polymeric FR” powder without solvent at 60 °C (measured
247 in reconstituted water) in yellow, without solvent for 24 weeks exposed “Polymeric FR” powder added
248 to reconstituted water at RT in red, without solvent for 24 weeks exposed “Polymeric FR” powder added
249 to reconstituted water at 60 °C in black) over time. Blue arrows indicate which sample was used as basis
250 for the connected measurements.
251
252 3.2 Total organic carbon (TOC) in rain water and reconstituted water after UV irradiation and heat
253 exposure
254 Analyses of the TOC in the UV irradiated “Polymeric FR” samples revealed comparably low
255 concentrations in contrast to the total bromine concentrations (∼5 %) after UV irradiation (Figure 5).
256 The concentration of TOC after irradiation for 180 minutes reached 2600 µg C/g “Polymeric FR” in rain
257 water (Figure 2 U2; SD ± 73 µg/g) and 2200 µg C/g in reconstituted water (U3; SD ± 32 µg/g).
258

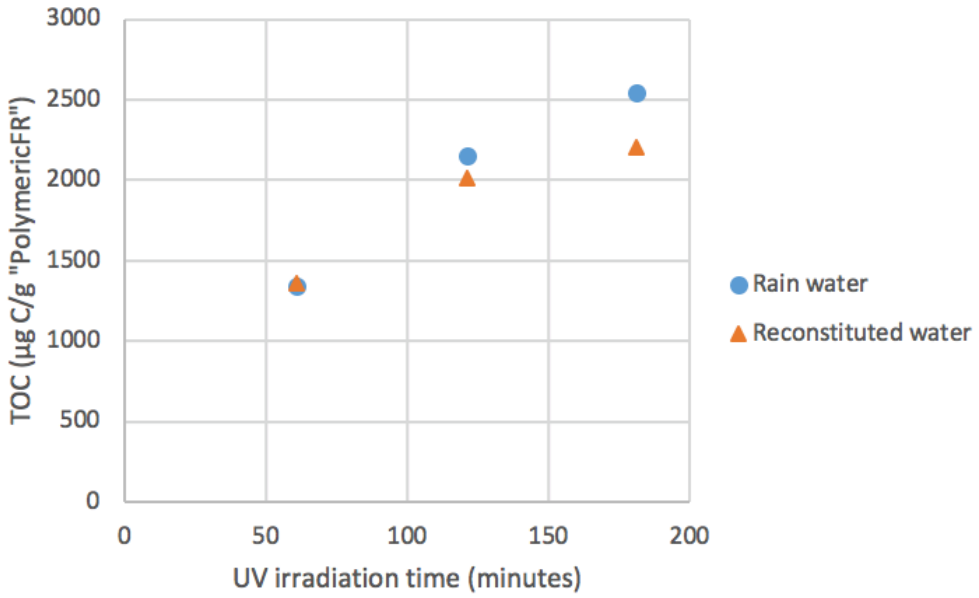


Figure 5: Concentration of TOC (µg C/g "Polymeric FR") following UV irradiation of 0.5 g "Polymeric FR" in 150 mL solvent (rain water in blue, reconstituted water in orange) over time.

A much higher ratio of total bromine to TOC was found after heat exposure at 60 °C for 36 weeks (~ 3000 %). Here, the concentration of TOC in reconstituted water increased to 9300 µg C/g "Polymeric FR" (SD ± 82 µg/g; Figure 6). Without heat exposure, the TOC concentration reached only 760 µg C/g "Polymeric FR" after 36 weeks in reconstituted water (SD ± 14 µg/g). Even lower concentrations were obtained when "Polymeric FR" powder was exposed to heat without a solvent (230 µg C/g "Polymeric FR" after 24 weeks; SD ± 7 µg/g). When keeping "Polymeric FR" powder which was heat treated for 24 weeks for additional 12 weeks at RT in reconstituted water, TOC concentrations stayed almost identically (230 µg C/g "Polymeric FR"; SD ± 2 µg/g). However, storing samples (in reconstituted water) at 60 °C instead of RT, caused the concentration to increase to 9500 µg C/g "Polymeric FR" (SD ± 109 µg/g). After 2 years in ddH_2O at RT, the TOC concentration reached 800 µg C/g "Polymeric FR" (SD ± 5 µg/g).

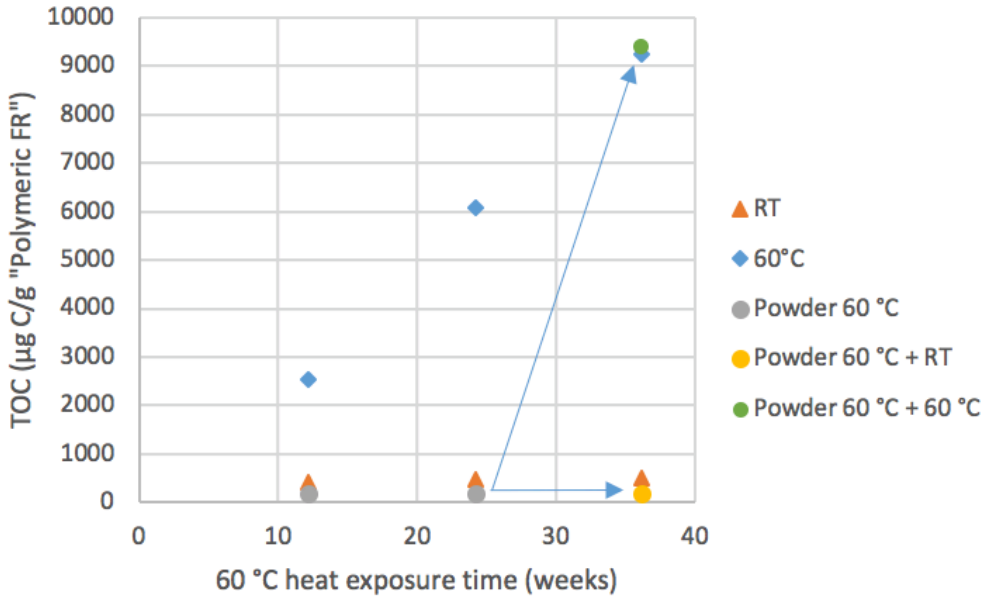


Figure 6: Concentration of TOC (µg C/g "Polymeric FR") following heat exposure of 1.17 g "Polymeric FR" in 350 mL reconstituted water (at RT in orange, at 60 °C in blue, "Polymeric FR" powder without solvent at 60 °C (measured in reconstituted water) in grey, without solvent for 24 weeks exposed "Polymeric FR" powder added to reconstituted water at RT in yellow, without solvent for 24 weeks exposed "Polymeric FR" powder added to reconstituted water at 60 °C in green) over time. Blue arrows indicate which sample was used as basis for the connected measurements.

3.3 Detection of degradation products via LC-MS

After treating ddH_2O samples for 180 minutes with UV radiation, LC-qTOF-MS was used to generate a feature list of possible degradation products. Only molecular formulas were taken into account which were identified in both measurements and had a score above 95. Based on this feature analysis, 75 molecular formulas were identified, including eight containing bromine. In addition, samples which were kept at 60 °C for 36 weeks were analysed as well to compare general degradation pattern. In this case, only seven molecular formulas were identified with a score above 95, including only one containing bromine ($C_7H_5BrO_3$).

The eight molecular formulas, which were detected in the UV irradiates samples (Figure 2 U1), were selected for further research to obtain more information regarding their molecular structure. Based on previous research[12] and the results of the heat treated samples, 5-Bromosalicylic acid ($C_7H_5BrO_3$) was included into the analysis as well (SI Table S5).

295 Spectra and retention time of the selected degradation products after LC-qTOF-MS measurements were
296 compared with the corresponding information obtained by analysing the four standards of degradation
297 products which were previously suggested[12]. This way, 5-Bromosalicylic acid, 2,4,6-tribromo-3-
298 hydroxybenzoic acid, and 3,5-dibromo-4-hydroxybenzoic acid were confirmed as degradation products
299 following UV irradiation. The forth structure – 2,4,6-tribromophenol – was not verified, although the
300 associated mass was detected. Contrary, no corresponding mass was found in the heat treated
301 duplicates. Similarly, no associated masses were found for 2,4,6-tribromo-3-hydroxybenzoic acid and
302 3,5-dibromo-4-hydroxybenzoic acid in these samples. Only 5-bromosalicylic acid was confirmed in heat
303 treated samples (Figure 2 H2).
304 The remaining five brominated degradation products – which were detected in the UV irradiated samples
305 and where no standards were commercially available – were further analysed using LC-MS/MS analysis.
306 During these measurements, partly different results were obtained by even the slightest changes in the
307 method and by varying the duration for which the samples were stored at -20 °C. As a consequence, a
308 reliable statement can only be made about the fact that some structural characteristics of degradation
309 products like carboxy groups, hydroxy groups, or aromatic rings were frequently observed.
310
311 **4. Discussion**
312 4.1 Degradation via UV radiation reflected in total bromine and TOC
313 "Polymeric FR" is currently solely applied as a FR in insulation products, which are typically installed
314 behind a roof or wall. Therefore, the question should be raised to which extent "Polymeric FR" will
315 actually face UV radiation. It could be argued that a lifespan of 50 years or more indicates that insulation
316 products are exposed to the sun only to a minor or even negligible degree. Even though this is certainly
317 a valid argument, the whole life cycle of a product should be considered. Depending on the end-of-life
318 scenario of EPS/XPS, which ranges from landfilling over incineration[15] to possibly recycling in the
319 future[16], a lifespan of 50 years might only be a fraction of the actual time (the persistent by design)
320 "Polymeric FR" will exist. The use of plastics is currently a topic of intensive discussions which shows
321 that lifetime of synthetic products ranges from several months to thousands of years[21] and that
322 environmental problems may manifest after its regular use. It has also been hypothesized that all
323 conventional plastic which has ever been produced and was introduced to the environment (and was
324 not incinerated) still remains as unmineralized particles or fragments[22]. Although prolonged exposure to
325 UV radiation is a common factor for the degradation of plastics[21], these products need to enter the

environment first. It has been shown multiple times that not only the end-of-life needs to be considered as a phase for potential contamination, but also the initial production plays an important role. BFRs have frequently been detected in high concentrations around point sources like EPS/XPS manufacturing plants[7,23,24]. Taking these facts into account, it should always be evaluated to which extent UV radiation is actually a relevant factor for degradation. Nevertheless, it is important to increase our knowledge regarding this scenario, especially for polymeric BFRs, where only little is known[12,25].

In this study, "Polymeric FR" samples were irradiated for 180 minutes and thus a cumulative dose of 1500 Wh/m^2 was applied. Under the extreme case that this dose consisted only of UV-B and assuming an annual average radiation intensity of 7 W/m^2 for UV-B, the dose equals approximately the irradiance of natural UV-B on the earth's surface of 9 days. The same consideration for UV-A with an annual average radiation intensity of 250 W/m^2 would mean that the applied dose equals approximately the irradiance of natural UV-A on the earth's surface of 6 hours[26].

Throughout the whole duration of the experiment, roughly 46,000 µg Br/g leaches out of "Polymeric FR" independent of the used solvent (reconstituted water, rain water, and ddH_2O; Figure 3). This is approximately 7.5 % of the bromine content of 1 g "Polymeric FR" and thus in the range of previous findings[12]. Interestingly, the degradation speed did not slow down over time, but remained linear in spite of the prolonged exposure compared to previous experiments[12]. Most of the detected bromine (95 %) seems to be dissociated to HBr, which was calculated based on changes of the pH. When comparing these results to previous measurements of inorganic bromine[12], it can be concluded that the pH might be applicable within a mass balance to predict the amount of organically bound bromine without using the time consuming analysis of adsorbable organically bound bromine (AOBr) via an combustion ion chromatography system (CIC). Applied to the scenario of degradation via UV irradiation, up to 5 % of the measured total bromine (and thus 0.4 % of the total bromine of 1 g "Polymeric FR") might be organically bound in water soluble degradation products. It should be noted that without actual measurements, this calculation can only be used in a predictive way. In addition, "Polymeric FR" is typically incorporated into a polystyrene matrix, which lowers the degradation speed[12].

To investigate whether the total bromine concentration can actually be used as a marker for degradation as previously suggested[12], the concentration of TOC was measured during different time points (Figure 5). At least during the first 120 minutes, both concentrations raise linear. After 180 minutes, release of TOC seems to decrease in reconstituted as well as rain water, which would limit the informative value of total bromine as a marker for the polymer degradation. To confirm this for UV

irradiation, the experiments would have to be carried out for longer periods, which was not possible in the current experiment due to an exponential loss of lifetime of the UV lamp with prolonged irradiation. The TOC concentration after 60 minutes in rain and reconstituted water is roughly twice as high as previously analysed in ddH_2O[12]. This can however be explained by the proportionally smaller surface which was irradiated previously and thus caused a lower degree of degradation. In consequence, the TOC leaching of roughly 2500 µg C/g "Polymeric FR" after 180 minutes might be more or less the same as stated previously (850 µg C/g * h), but this is mostly due to the decreasing slope of the curve and not based on a linear correlation. Therefore, even though the total bromine concentration might indicate roughly the degradation of "Polymeric FR" in terms of leaching of organic material, it does not seem to be suitable as a precise marker. In this study, it was found that almost 1 % of the carbon of "Polymeric FR" leaches into the solvent over the period of 6 hours to 9 days under natural sunlight. In reality, this is likely to be reduced – with the exception of point sources like manufacturing plants – via shielding of "Polymeric FR" by the surrounding polystyrene matrix.

4.2 Degradation via exposure to 60 °C

Even though UV radiation is an efficient way to study the degradation of a polymer, it can be discussed to which extent it affects the fate of foam-based insulation material. In contrast, temperature is a factor that all insulation material has to cope with. Especially during summer, temperatures on a roof can rise up to above 70 °C[52]. This does also affect the underlying insulation layer[53].In addition, it has been reported that products might face similar temperatures during their end-of-life at landfilling sites[27,54]. Therefore, the degradation of "Polymeric FR" was studied at 60 °C for a period of up to 36 weeks. It should be noted that 36 weeks (as well as 3 hours of irradiation) are only a fraction of the actual use phase (even excluding possible landfilling) of approximately 50 years.

Our experiments regarding leaching of bromine revealed no clear trend for at least the first 4 to 8 weeks (Figure 4). Without solvent, bromine leaching is lowest, whereas it is significantly higher independent of the applied temperature (RT or 60 °C) when using any of the solvents. After the initial 8 weeks, all concentrations seem to increase slightly, but to a considerably lower extent compared to the bromine leaching which was found for degradation via UV irradiation. Before considering a more detailed analysis of the obtained results, the measured TOC values should be taken into account (Figure 6). They reach 9300 µg C/g "Polymeric FR" after 36 weeks, which is much higher compared to the total bromine concentration of 300 µg Br/g "Polymeric FR". Because the ratio of bromine to carbon is roughly 2:1 in

388 terms of molecular weight in “Polymeric FR”, the bromine concentration could be expected to be around
389 20,000 µg Br/g “Polymeric FR”. Considering this and the rather incongruent results of bromine leaching,
390 it is likely that bromine slowly evaporates as HBr or even Br_2 during the period of 36 weeks. This would
391 also be consistent with the intended function of a BFR[28]. Nevertheless, total bromine can still be used
392 in combination with the pH to estimate the amount of organically bound bromine, which would be up to
393 7 % of the total bromine after 36 weeks of heat treatment.
394 In addition, the TOC results give a clear picture on the leaching of organic material. Neither the
395 temperature (60 °C or RT – particularly reflected in the long-term exposure at RT over 2 years) nor the
396 presence of the solvent is relevant for an increased leaching, but the combination of both. Only the heat
397 treated “Polymeric FR” which was kept in water showed an increasing concentration of TOC.
398 Interestingly, while “Polymeric FR” powder without a solvent did not degrade at 60 °C (in terms of
399 leaching of organic material), initial heat treatment seems to increase the degradation when
400 subsequently placed in a solvent at continuously 60 °C. In total, roughly 3 % of the carbon of “Polymeric
401 FR” was detected in water after the period of 36 weeks at 60 °C.
402 Based on our results, it seems that degradation without water as a solvent is negligible (at least when
403 solely considering water soluble compounds). Even so, it might be interesting to study the emission of
404 degradation products during heat exposure in the future, because it has been shown that foam insulation
405 can emit BFRs to the air, which would be enhanced at higher temperatures[29]. This would also be relevant
406 for landfilling as some studies have shown that the concentration of BFRs in the air around landfilling
407 sites can be elevated and sometimes even be seen as a point source[30,31]. Nevertheless, based on its
408 experimental design, the present study can only show that degradation is significantly enhanced when
409 “Polymeric FR” is heat treated and subsequently or at the same time in contact with water. This however
410 is important to consider, because it has been stated that leaching of BFRs from waste to groundwater
411 is a risk that is not yet well understood[30]. Nonetheless, it should be kept in mind that – in order to have
412 less confounding factors – the focus laid only on the degradation of pure “Polymeric FR”, which was not
413 incorporated into EPS or XPS products, but solely exposed to heat – which might have an impact on
414 the results observed. First results regarding the degradation of “Polymeric FR” incorporated in PS foam
415 have been reported previously[12].
416
417

418 4.3 Detected degradation products

419 Typically, the toxicity of a BFR is evaluated without considering degradation products, even though it
420 was shown that the degradation of a BFR can alter its toxic potential[32–36]. Especially for polymeric BFRs,
421 which are claimed to be more environmental friendly, degradation products should be considered. In a
422 previous study dealing with "Polymeric FR"[12], possible structures of such products were listed. Based
423 on these insights, UV radiation was used as a quick and effective tool for degradation to further improve
424 our knowledge regarding decomposition of polymeric BFRs. Among many substances, 75 molecular
425 formulas were identified in a feature analysis with a score above 95, including about 10 % brominated
426 compounds. This proves the previous prediction that only a minor fraction of the degradation products
427 is actually brominated[12]. Interestingly, some of the identified molecular formulas contain for instance
428 nitrogen, which is also used within the manufacturing process of "Polymeric FR"[10]. However, the focus
429 laid only on brominated degradation products, as these might be the toxicological most interesting
430 compounds.

431 With the aid of LC-qTOF-MS measurements and reference standards, it was possible to identify 5-
432 bromosalicylic acid, 2,4,6-tribromo-3-hydroxybenzoic acid, and 3,5-dibromo-4-hydroxybenzoic as
433 degradation products following UV irradiation. The standard of 2,4,6-tribromophenol showed a different
434 retention time compared to the detected compound with the same mass and spectrum, thus a different
435 isomer like 2,4,5-tribromophenol might derive from "Polymeric FR" when UV treated. Except
436 tribromophenol, which has been identified as a degradation product of other BFRs as well[20,37,38], not
437 much is known regarding these compounds. Especially – except tribromophenol – information
438 concerning their effects on biota is largely missing[39–41]. It was however shown that these or similar
439 substances are biodegradable[42–44]. No distinct chemical structure could be assigned to the remaining
440 five brominated masses without suitable standards available.

441 Comparing the detected brominated degradation products after UV irradiation and heat treatment, only
442 one chemical (5-bromosalicylic acid) was identified following both scenarios. Different explanations are
443 possible for the fact that almost none of the brominated chemicals was detected in the heat treated
444 samples. On the one hand, it is possible that the concentration of these degradation products is simply
445 too low to be detected and give distinct spectra. This explanation is supported by the rather low
446 concentration of total bromine in these samples as explained earlier. Taking into account that these
447 samples were exposed for 36 weeks and concentrated afterwards, it is questionable to which extent
448 degradation products would actually be relevant at such low concentrations. On the other hand, it is also

449 possible that the degradation mechanism following heat treatment is simply different to the one following
450 UV irradiation. This is supported by results obtained through the feature analysis performed on heat
451 treated samples, which showed a much lower amount of degradation products in general – and not just
452 brominated ones. However, taking the measurements of TOC in these samples into account, it is
453 surprising that less degradation products were found compared to UV irradiated samples, which showed
454 a lower TOC than the heat treated samples. In addition, it seems possible that the samples are not fully
455 stable – even at -20 °C. Results obtained during the LC-MS/MS and LC-qTOF-MS analysis indicate that
456 the longer a sample is stored at this temperature (or at RT), the less products can be identified. This
457 could also be seen as an explanation for why less degradation products were observed in the feature
458 analysis of heat treated samples. However, this requires more research, especially in the light of
459 comparably high TOC concentrations (which in contrary suggests more degradation products) in these
460 samples.
461 In conclusion, it seems rather unrealistic that the time efficient UV irradiation can be utilized to generate
462 similar brominated degradation products compared to the rather time-consuming heat exposure.
463 However, with some optimization, the feature list including 75 possible degradation products after UV
464 irradiation might be used as a kind of “fingerprint” in future studies to check if “Polymeric FR” contributes
465 in any regard to BFRs found in indoor dust in homes and offices[45–47] – which is probably not to be
466 expected, but is an important fact for evaluating the claimed superior environmental behaviour compared
467 to previous BFRs.
468
469 4.4 Consequences
470 Studies dealing with the environmental fate and toxicology of polymeric BFRs are still limited. Depending
471 on the long-term behaviour of “Polymeric FR”, this substance might be considered as a prototype for
472 future polymeric BFRs. Therefore, it is important to check if polymers are a more sustainable option
473 compared to previous monomeric FRs.
474 This study has focussed on degradation scenarios which are mostly relevant during the end-of-life of an
475 insulation product. It seems possible that the incorporated BFR breaks down and yields monomers
476 which could pose a toxic potential for the environment. Therefore, adequate handling during this
477 particular phase seems recommended to avoid any unwanted degradation of “Polymeric FR”.
478
479

480 **Supporting information**

481 Figure S1: Spectrum of the used UV lamp

482 Table S1: Applied recipe for the reconstituted water

483 Table S2: Analysed composition of the used rain water

484 Table S3: Instrument parameters of qTOF measurements

485 Table S4: Instrument parameters of MS/MS measurements

486 Table S5: Molecular formulas of possible brominated degradation products

487

488 **Acknowledgement**

489 Thanks are due to BASF for contributing the pure “Polymeric FR” powder. We also want to thank Mathias

490 Ulbricht for giving us access to the UV cube and ICL-IP for their useful comments.

491

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Chapter III: Evaluation of the toxicity of various degradation products of “Polymeric FR” in algae and daphnia OECD tests

Within this chapter, the toxicity of four possible degradation products of “Polymeric FR” is studied using three OECD tests and an *in silico* trend analysis.

Manuscript as accepted for publication in Science of the Total Environment. The associated supporting information is given in appendix V.

Ecotoxicological characterization of possible degradation products of the polymeric flame retardant "Polymeric FR" using algae and daphnia OECD tests

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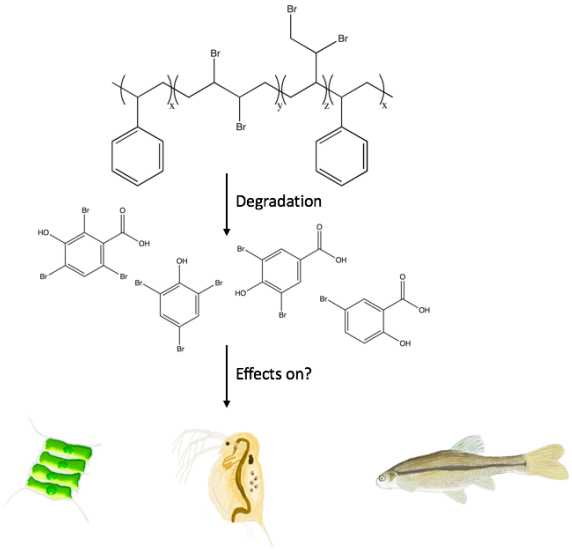
Abstract



History has shown that brominated flame retardants often pose risks to the environment. However, the new group of polymeric brominated flame retardants might be a safer alternative compared to previously used monomers due to their high molecular weight. An example for this new group is "Polymeric FR", which is persistent by design. Within this publication, we study the acute and chronic toxicity of possible degradation products that were previously described for this polymer following UV irradiation and heat exposure at 60 °C. We have applied the OECD tests No. 201 (Algae growth inhibition), 202 (*Daphnia* acute immobilisation), and 211 (*Daphnia* reproduction) to four individual substances, indicated to

originate as degradation products of “Polymeric FR” as well as a combination of these. In addition, we have used trend analysis to predict effects on fish as an additional trophic level. The results suggest that acute toxicity to the aquatic organisms chosen is rather limited or even not occurring. Chronic exposure, however, does exert effects that might be relevant from an environmental perspective.

Keywords

PolyFR, toxicity, decomposition, effect, mixture, brominated

1. Introduction

Flame retardants (FR) are being used in a wide variety of products like furniture, textiles, electronical equipment, and thermal insulation (Covaci et al., 2011). The latter product group has gained increasing interest from building owners over the last decades for energetical reasons, but also from governments all over the world in an attempt to slow down the anthropogenically induced climate change. Among others, expanded (EPS) and extruded (XPS) polystyrene foams are most often considered as the go-to type of insulation for this purpose. Nevertheless, due to the chemical composition of these products, fire safety requirements almost always force manufacturers to add FRs to the foam matrix (Babrauskas et al., 2012). For many years, the brominated flame retardant (BFR) of choice has been hexabromocyclododecane (HBCD). This has changed when HBCD was classified as bioaccumulative, persistent, and toxic (Covaci et al., 2006; Koch et al., 2015; Marvin et al., 2011). In consequence, new flame retardants were needed to stay in conformity with fire safety requirements. One of the possible substitutes is the additive FR “Polymeric FR” (CAS No. 1195978-93-8, Figure 1), a block copolymer of polystyrene and brominated polybutadiene, which is also known as PolyFR, pFR, Emerald Innovation 3000, FR-122P, and GreenCrest (Beach et al., 2017; Jeannerat et al., 2016; Kajiwara et al., 2017; Schlummer et al., 2015). According to the manufacturers, roughly 26,000 metric tons are produced every year (ICL, 2015; LANXESS, 2018).

Based on the aim to develop an environmentally more friendly product, the structure of “Polymeric FR” is significantly different to HBCD (Beach et al., 2017). Additionally, because of its high molecular weight, no bioavailability is expected. Therefore, “Polymeric FR” is assumed to be the most promising alternative to HBCD. Accordingly, it is already incorporated in EPX and XPS insulation products since 2014.

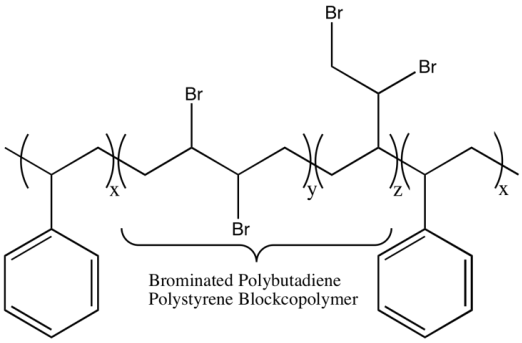


Figure 1: Chemical structure of “Polymeric FR” as described by the manufacturer.

To the best of our knowledge, all governmental risk evaluations focussed until now on “Polymeric FR” itself without considering possible degradation products. The U.S. Environmental Protection Agency concluded 2014 that its “long-term behavior in the environment is currently not known” (US EPA, 2014). Based on the long life time and various life cycle stages of EPS and XPS, degradation of the polymeric chain might occur. Within two recent studies, it was observed that degradation products such as 5-bromosalicylic acid can be produced while “Polymeric FR” is exposed to heat or ultraviolet (UV) radiation (Koch et al., 2018, 2016). Such abiotic factors can play an important role through all life cycle stages of this BFR. While UV radiation might mostly be encountered during the manufacturing phase and eventually at the end-of-life (Barnes et al., 2009; Chen et al., 2015), temperatures around 60 °C and even higher can be present throughout the actual use phase (FSEC, 2005) and also at the end-of-life – as long as products are brought to landfill (Stubbings and Harrad, 2014) and are not incinerated (Mark et al., 2015).

Even though our current knowledge regarding the degradation of BFRs in general is rather limited, it is known that abiotic and biotic degradation can produce more mobile compounds which in consequence might have adverse effects on the environment (Hill et al., 2018; Martin et al., 2017; Su et al., 2018, 2016, 2014). Aggravating this situation, nothing is known regarding the toxic potential of degradation products deriving from polymeric FRs. This is especially interesting, because this group is already being used on a commercial scale for some years and “Polymeric FR” can be seen as a prototype for future polymeric flame retardants. Therefore, we focus here on four possible degradation products that were previously predicted (Koch et al., 2016) and partly (three out of four) identified using experimental degradation approaches (Koch et al., 2018). We investigate their effects using different OECD tests, namely the Algae growth inhibition test (OECD 201, 2011), the *Daphnia* acute immobilisation test

(OECD 202, 2004), and the *Daphnia* reproduction test (OECD 211, 2012). These tests are typically part of the evaluation of potential environmental hazards according to the Globally Harmonized System (GHS, 2017) of the United Nations (UN). In addition, we have performed exposure tests with mixtures of the four chosen compounds 2,4,6-tribromophenol (2,4,6-TBP; CAS No. 118-79-6), 5-bromosalicylic acid (BSA; CAS No. 89-55-4), 2,4,6-tribromo-3-hydroxybenzoic acid (THA; CAS No. 14348-40-4), and 3,5-dibromo-4-hydroxybencoic acid (DHA; CAS No. 3337-62-0) to gain a better understanding of possible combined effects (see supporting information (SI) Figure S1 for the molecular structures). Finally, we have conducted a trend analysis to predict the acute toxicology of the individual substances for fish.

2. Material and methods

2.1 Experimental design

For every OECD test, stock solutions of the highest applied concentration were prepared from the acquired standards of 2,4,6-TBP (Sigma-Aldrich, purity 99 %), BSA (Sigma-Aldrich, purity 97 %), THA (Sigma-Aldrich, purity 97 %), and DHA (Alfa Aesar, purity 98 %). Therefore, 9 or 10 mg/L (which was the maximum water solubility for 2,4,6-TBP) was weighed (Libror AEG-220, Shimadzu, Kyoto, Japan) into an Erlenmeyer glass flask filled with reconstituted algae or daphnia water (see SI Table S1 and Table S2 for the applied recipes). In addition to this maximum concentration, dilutions were used according to Table 1, which gives a general overview of the different substances, concentrations, and test organisms that were applied during this study. For the analysis of possible combined effects, each of the four substances was used in the same concentrations in a mixture.

Table 1: Overview of the experimental design of this study

	OECD No. 201	OECD No. 202	OECD No. 211
Test organism	*Desmodesmus subspicatus*	*Daphnia magna*	*Daphnia magna*
Duration of exposure	72 hours	48 hours	21 days
Applied substances	2,4,6-Tribromophenol 5-Bromosalicylic acid 2,4,6-Tribromo-3-hydroxybenzoic acid	2,4,6-Tribromophenol 5-Bromosalicylic acid 2,4,6-Tribromo-3-hydroxybenzoic acid	Mixture of the four substances

	3,5-dibromo-4-hydroxybencoic acid Mixture of the four substances	3,5-dibromo-4-hydroxybencoic acid Mixture of the four substances	
Concentrations per substance (mg/L) (applied singly or as mixture)	1.25, 2.5, 5, 10	0.625, 1.25, 2.5, 5, 10	0.037, 0.111, 0.333, 1, 3

99

100 2.2 Algae growth inhibition test

101 The algae growth inhibition test was performed according to OECD No. 201 (2011) as described recently
102 (Ribeiro et al., 2018). The test organism used was *Desmodesmus subspicatus*, which was cultivated in
103 the department of biodiversity at the University Duisburg-Essen. Triplicates with an initial density of
104 3,000 cells/mL were exposed during their exponential phase for 72 hours at 20 °C to every concentration
105 displayed in Table 1 in 24-well-plates. The endpoint "biomass growth" was checked every 24 hours via
106 fluorescence spectroscopy (following initial counting to determine the conversion factor) using a Tecan
107 Infinite M200 plate reader (Maennedorf, Switzerland). A control group was kept as well and increased
108 its biomass – as necessary – by a factor higher than 16 (18.3; growth rate of 0.97 day^{-1}) during the
109 experimental period. As required, the coefficient of variation of average specific growth rates did – with
110 4.3 % – not exceed 7 % during the whole test period and stayed below 35 % for every section-by-section
111 specific growth rate. All water parameters remained in the ranges defined in the OECD guideline
112 throughout the whole experiment.

113

114 2.3 *Daphnia* acute immobilisation test

115 The *Daphnia* acute immobilisation test was performed according to OECD 202 (2004) as described
116 recently (Kresmann et al., 2018; Zimmermann et al., 2017). The applied test organism was
117 *Daphnia magna*, clone Elisa, which was provided by the department of animal ecology, evolution, and
118 biodiversity at the Ruhr University Bochum. This clone originates from a temporary pond at Mount Moses
119 in the Sinai region, Egypt. Four groups of five young daphnids each, aged less than 24 hours, were
120 exposed in 50 mL for 48 hours at 20 °C to every concentration listed in Table 1. The endpoints
121 "immobilisation" and "mortality" were checked after 24 and 48 hours according to the procedure
122 described in Zimmermann et al. (2017). A control group was kept as well and showed no immobilisation
123 during the test. All water parameters remained in the ranges defined in the OECD guideline throughout

124 the whole experiment. The dissolved oxygen concentration was determined using a Mettler Toledo
125 FiveEasy DO metre (Mettler Toledo, Ohio, USA) and stayed above the required 3 mg/L until the end of
126 the exposure.
127
128 2.4 *Daphnia* reproduction test
129 The *Daphnia* reproduction test was performed according to OECD No. 211 (2012) using *D. magna*,
130 clone Elisa. The experiment was carried out with a mixture of the four compounds only – no individual
131 substances were tested. Ten young daphnids, aged less than 24 hours, were exposed separately in
132 50 mL for 21 days at 20 °C for every concentration as displayed in Table 1. After renewal of the medium
133 (always based on freshly prepared stock solutions; three times per week), the animals were fed (0.2 mg
134 C/daphnia/day; with *D. subspicatus*) three times a week. The endpoints "number of live offspring" and
135 "mortality" were checked every day. A control group was kept to confirm reliability of the test procedure
136 (no negative impact on either of these endpoints). Dead parents were not classified as inadvertently
137 when their number exceeded 1 per concentration. The amount of live offspring in the control group was
138 above the required 60 per parent (mean of 90.4 individuals per parent). All water parameters remained
139 in the ranges defined in the OECD guideline throughout the whole experiment.
140
141 2.5 Inductively coupled plasma mass spectrometry (ICP-MS)
142 Despite knowing the nominal concentrations of the tested substances for every test, it is generally
143 important to be aware of the actual concentrations as well. Taking into account that the purpose of this
144 test is to evaluate possible effects of a mixture of degradation products, it is necessary to be aware of
145 possible depletion of the substances via vaporisation and adsorption to glass vessels, not further
146 degradation. Therefore, we measured the total bromine concentration at various time points of each
147 experiment. The bromine concentration is not expected to change if the applied chemicals degrade
148 further and/or release Bromine to the solvent. Thus, we are able to exclude major changes if the Br
149 concentration remains constant during the experiment.
150 Total bromine was measured using a quadrupole ICP-MS system (Elan 6000, Perkin Elmer, Waltham,
151 Massachusetts, USA) operating at 950 W plasma power and 0.95 L/min nebuliser gas flow. Instrument
152 calibration was performed using series of bromine dilutions (in range from 0.1 µg/L to 100 µg/L)
153 prepared from standard solutions (Merck, Darmstadt, Germany). Subsequently, the element
154 concentrations were calculated in µg/L using the corresponding regression lines of the calibration

(correlation factor ≥ 0.999). Before measurements, each sample was diluted at least 10 times using 1 % nitric acid (HNO_3, subboiled) with a concentration of yttrium (Y) of 10 µg/L as internal standard. In order to avoid contamination and memory effects, the wash time between measurements was set to at least 60 seconds (with 1 % HNO_3, subboiled). The accuracy and stability of bromine measurements was additionally monitored using a standard solution of bromine with a concentration of 10 µg/L (ICP multi element standard IV solution, Merck, Darmstadt, Germany), which was analysed after every 10 samples.

2.6 Statistical analysis

Evaluation of the effects following exposure of *D. subspicatus* or *D. magna* was performed using GraphPad Prism Version 5 (GraphPad Software, Inc., La Jolla, CA, USA). The Shapiro-Wilk test was applied to check for Gaussian distribution of the data sets, followed by a suitable variation of the *t*-test to determine significance levels. Effect values (after log-transformation) were plotted against the exposure concentrations.

2.7 Prediction of log K_{OW} values

The log K_{OW} was predicted for all four substances with ALOGPS 2.1 (VCCLAB), which uses the comparable accurate (Mannhold et al., 2009) ALOGPs method (Tetko et al., 2005). Additionally, 2,4,5-tribromophenol (2,4,5-TBP) – a structural isomer of 2,4,6-TBP, which was also found as a degradation product of BFRs – was included in the prediction as well.

2.8 Trend analysis to predict acute fish toxicity

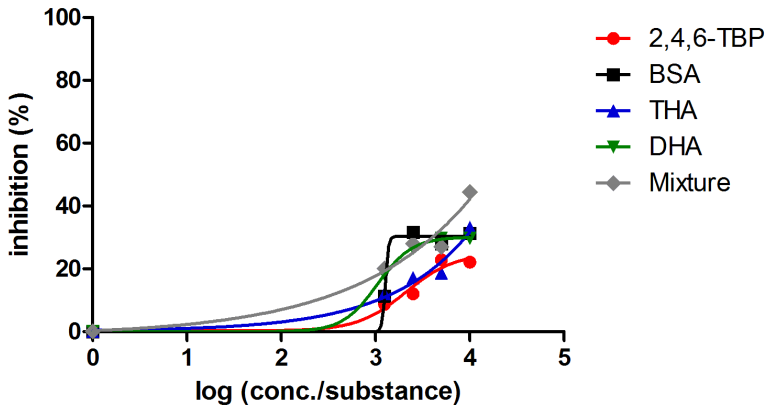
To add another trophic level while similarly avoiding vertebrate experiments, we used the QSAR Toolbox 4.2 (ECHA/OECD) to predict LC_{50} values for *Pimephales promelas* after 96 hours. For this purpose, a trend analysis was carried out for each individual product by applying the standardized workflow of the client (OECD, 2017). Aquatic ECETOC, Aquatic OASIS, ECHA CHEM, and ECOTOX were used as databases. Profilers were, among others, the US-EPA New Chemical Categories, and the Aquatic toxicity classification by ECOSAR.

184 **3. Results**

185 3.1 Algae growth inhibition test

186 After 72 hours, none of the tested substances has caused a growth inhibition of at least 50 % (Figure 2,
187 Figure S2). Not even a concentration of 10 mg/L 2,4,6-TBP did affect the growth significantly ($p > 0.05$;
188 standard deviation (SD) for the final cell number ± 26 %). In contrast, the same high concentrations of
189 BSA, THA, and DHA caused a significant inhibition ($p < 0.01$) of roughly 30 % for each individual
190 substance (Figure 2; SD for the final cell number ± 9, 23, and 8 %, respectively). The highest degree of
191 inhibition was detected when algae were exposed to a mixture of all four chemicals at the same
192 concentration per substance. In this case, the inhibition reached 44 % ($p < 0.001$; SD for the final cell
193 number ± 16 %). Plots for every individual substance are presented in the SI, Figure S2.

194



195

196 Figure 2: Growth inhibition of *D. subspicatus* exposed for 72 hours to 2,4,6-TBP, BSA, THA, DHA, and
197 a mixture of these four compounds. Concentrations per substance were ranging from 1.25 to 10 mg/L.

198

199 ICP-MS measurements showed that the initial bromine concentration was approximately 90 % of the
200 nominal value for every substance (including the mixture) which increased to 95 % after 72 hours of
201 exposure. These values are well within the limits defined in the OECD guideline No. 201.

202

203 3.2 *Daphnia* acute immobilisation test

204 The *Daphnia* acute immobilization test showed no effect for BSA, THA, and DHA after 24 and 48 hours
205 at all concentrations (Figure 3). When daphnids were exposed to 2,4,6-TBP, immobilisation occurred in
206 40 % of the test organisms after 24 hours and in 100 % after 48 hours at the highest test concentration

of 10 mg/L. Similar results were observed regarding mortality. Although no daphnids were dead after 24 hours, almost all (95 %) were classified as immobilized due to their death after 48 hours. At lower concentrations, no immobilization was noted. Thus, 5 mg/L can be seen as the no observed effect concentration (NOEC). Based on the limited amount of measurements within the relevant concentration range, calculating an EC_{50} would only be a rough estimation and is therefore not done. In addition to 2,4,6-TBP, the mixture of all four chemicals immobilized the daphnids as well, but at a lower concentration compared to 2,4,6-TBP alone. While the NOEC for 2,4,6-TBP individually was 5 mg/L, in presence of the three other substances (where no effect was measure before), the NOEC shifted to 2.5 mg/L for both endpoints. Plots for every individual substance are presented in the SI, as well as a more detailed overview for each replicate (Tables S3 and S4).

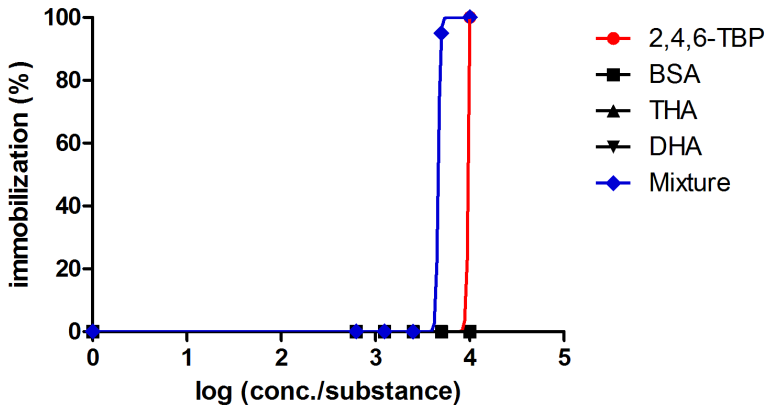


Figure 3: Immobilization of *D. magna* exposed for 48 hours to 2,4,6-TBP, BSA, THA, DHA, and a mixture of these four compounds. Concentrations per substance were ranging from 0.625 to 10 mg/L.

To ensure that the actual concentration of the tested substances stayed within the necessary limits, ICP-MS measurements were performed. The initial bromine concentration was approximately 90 % of the nominal value for every substance (including the mixture) at the beginning of the test. It remained within ± 2 % for 48 hours and is thus within the limits defined in the OECD guideline No. 201.

3.3 *Daphnia* reproduction test

Based on results obtained in the *Daphnia* acute immobilisation test, concentrations varying between 0.037 and 3 mg/L for each of the tested substances (2,4,6-TBP, BSA, DHA, and THA) were chosen for

the mixture (Table 1, Figure 4). A 50 % reduction of the number of live offsprings per parent occurred when a concentration of 187.0 µg/L per substance (and not in total) was used. The standard error of the mean (SEM) was always equal or below 2.76 %. All concentrations were significantly different ($p < 0.001$) compared to the control group, except the lowest concentration of 37 µg/L per substance ($p > 0.05$) – which could thus be considered as the NOEC concerning the endpoint "reproduction". Regarding "mortality", a NOEC of 1 mg/L per substance was determined. When increasing the concentration to 3 mg/L per substance a mortality of 100 % occurred after 14 days. Until death, the growth of all daphnids was extremely limited at this concentration. Compared to adults in the control group (3.71 mm; SD ± 0.32 mm; Figure S5), only 51 % of the normal length was reached (1.88 mm; SD ± 0.14 mm). This effect was observable during the whole life span of an animal in the highest concentration (up to 14 days). No similar growth inhibition was noted at lower concentrations. A more detailed overview for each replicate is presented in the SI, Table S5.

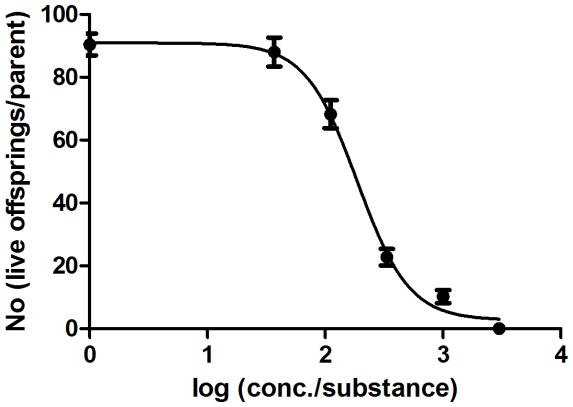


Figure 4: Fitted curve of the sum of live *D. magna* offspring per parent after exposure for 21 days to a mixture of 2,4,6-TBP, BSA, THA, and DHA. The SEM is indicated for each concentration. Concentrations per substance were ranging from 0.037 to 3 mg/L.

ICP-MS measurements have proven that the limits defined in the OECD guideline No. 201 regarding the nominal and actual concentration were met. The initial bromine concentration was approximately 85 % of the nominal value for each substance (including the mixture) at the beginning of the test and increased to 90 % after 72 hours.

253 3.4 Prediction of log K_{OW} and trend analysis for acute fish toxicity

254 The calculated log K_{OW} predictions are shown in Table 2. A measured log K_{OW} of 4.24 was only available
255 for 2,4,6-TBP (Kuramochi et al., 2004). In addition, LC_{50} values for *P. promelas* after 96 hours were
256 predicted using the QSAR Toolbox and are shown in Table 2 as well.

257

258 Table 2: Predicted log K_{OW} values and LC_{50} values for *P. promelas* after 96 hours

Substance	Predicted log K_{OW}	Predicted LC_{50} values
2,4,5-Tribromophenol	4.19	3.12 mg/L
2,4,6-Tribromophenol	4.20	3.02 mg/L
2,4,6-Tribromo-3-hydroxybenzoic acid	4.13	23.1 mg/L
3,5-Dibromo-4-hydroxybenzoic acid	3.58	13.2 mg/L
5-Bromosalicylic acid	2.68	10.3 mg/L

259

260 **4. Discussion**

261 Even though four individual substances and a mixture of these substances were used in this study, most
262 of the tested chemicals resulted in limited biological effects of a rather similar degree. Significant
263 differences between the treatments were only observed for 2,4,6-TBP and the mixture.

264 Results of the algae growth inhibition test indicate limited acute effects of THA, DHA, and BSA on the
265 growth of *D. subspicatus*. All three chemicals caused a significant inhibition of approximately 30 %, but
266 do not reach 50 % even at the highest concentration of 10 mg/L. Although no information has been
267 published regarding their presence in the environment, such high concentrations are rather unlikely to
268 occur, particularly because biodegradation of similar substances has been described (Häggblom, 1992;
269 Higson and Focht, 1990; Song et al., 2000). In general, only little is known about possible adverse
270 effects of THA, DHA, and BSA on the environment. No publications were found for THA and DHA; and
271 only one publication with a potential environmental relevance was identified for BSA (Kantouch et al.,
272 2013), in which antibacterial properties of derivatives of salicylic acid were determined. Within the
273 *Daphnia* acute immobilisation test, which similarly aims at acute effects, no immobilization was observed
274 after exposing *D. magna* for 48 hours to BSA, THA, and DHA, even at the highest concentration of
275 10 mg/L. In addition, the acute LC_{50} values that were predicted for fish are also all above 10 mg/L
276 (Table 2). Based on these results, it can be concluded that acute effects of BSA, THA, and DHA are
277 almost neglectable, especially when only considering the degradation of “Polymeric FR” as a potential

source. As almost no publications exist for these three chemicals, no other environmentally relevant sources are known.

Although still rather limited, more information has been published for 2,4,6-TBP. Within a recent publication, the currently available literature concerning its environmental toxicology was summarized (Koch and Sures, 2018). 2,4,6-TBP does not only enter the environment through anthropogenic sources (for instance through the application as a pesticide or the degradation of FRs), but also naturally by excretion of different organisms, such as algae, bryozoans, polychaetes, and hemichordates (Flodin and Whitfield, 1999; King, 1988; Koch et al., 2018; Offret et al., 2016; Whitfield et al., 1999). Interestingly, 2,4,6-TBP, which is classified as very toxic to aquatic life (ECHA, 2018), was the only substance that had no significant effect on the growth of *D. subspicatus* after the exposure period of 72 hours. This might be due to the fact that some species of algae synthesize 2,4,6-TBP naturally (Flodin and Whitfield, 1999) and are thus probably adapted to effects that 2,4,6-TBP might have on them. However, acute immobilisation due to death was observed after exposure of *D. magna* to 2,4,6-TBP at a concentration of 10 mg/L. Based on three experiments done in the 1970s, a median EC_{50} of 1.31 mg/L and a LC_{50} of 0.65 mg/L were derived for crustaceans after 48 hours of exposure (GESTIS, 2018). Our results indicate a higher EC_{50} and LC_{50}, ranging between 5 mg/L and 10 mg/L. This difference might be due to varying factors in the experimental setup. Nevertheless, acute effects occurred only at very high concentrations which are well below concentrations that have been reported in freshwater. Here, 2,4,6-TBP can be found in concentrations ranging from non-detectable to 21 ng/L (Blythe et al., 2006; Gustavsson et al., 2018; Polo et al., 2006; Reineke et al., 2006). Therefore, it can be assumed that concentrations of the determined acute LC_{50} for daphnids are non-relevant for the environment except of accidental spills. This conclusion is also valid for the predicted LC_{50} value for fish of around 3 mg/L (Table 2). It should be noted, that 2,4,6-TBP, which was used in this study, was not listed as a possible degradation product in the latest degradation study focusing on “Polymeric FR” anymore. Instead, an isomer of 2,4,6-TBP, presumably 2,4,5-TBP, was identified (Koch et al., 2018). Even though 2,4,6-TBP was used here based on previous findings (Koch et al., 2016), 2,4,5-TBP was included in all calculated predictions and showed equal characteristics to 2,4,6-TBP, due to its structural similarity.

In addition to estimating individual effects of THA, DHA, BSA, and 2,4,6-TBP, combined impacts were also investigated to simulate possible synergistic effects following degradation of “Polymeric FR”. In general, results for the artificial mixture used in this study should be interpreted carefully. Due to the limited literature available, it was not possible to compose the mixture according to the proportion of all

309 chemicals. Instead, a simple 1:1:1:1 (regarding their mass) mixture was used. Further, the chemicals
310 were chosen based on previous results (Koch et al., 2018, 2016) and commercial availability of
311 standards. Thus, they only reflect a small fraction of more than 75 previously described possible
312 degradation products (Koch et al., 2018), which are also likely to change depending on degradation
313 conditions – and might also not always contain the four chosen chemicals. Additionally, it should be kept
314 in mind that it is not possible to draw conclusions regarding the individual effect of one substance based
315 on the overall impact of the mixture. It should also be noted that with proper precautional care, such
316 products might never end up in the environment. Nevertheless, testing such a mixture, even though
317 artificially composed, might be advantageous for future research, for example when applying a native
318 degradation "cocktail". Concentrations of the mixture are expressed as concentration per substance,
319 which might not be ideal, however, other methods such as the total toxic unit approach (Lynch et al.,
320 2016; Zimmermann et al., 2017) were not applicable due to e.g. unknown modes of action and missing
321 LC_{50} values.
322 Within the algae growth inhibition test, the highest degree of inhibition was found after application of the
323 mixture of all four substances at concentrations of 10 mg/L each. This mixture also exhibited the highest
324 effect in the *Daphnia* acute immobilisation test, which is interesting, because no effect was caused by
325 the individual substances BSA, THA, and DHA, and in consequence indicates possible synergistic
326 effects among the tested substances. However, as mentioned before, concentrations are so high that
327 no acute adverse effects for *D. magna* and *D. subspicatus* are to be expected following the degradation
328 of "Polymeric FR" under environmentally relevant degradation scenarios. In consequence, chronic
329 exposure to the mixture was chosen as a kind of worst case scenario in order to test if – among almost
330 neglectable acute effects – chronic effects were also absent. This was done with the *Daphnia*
331 reproduction test.
332 Therefore, the test organisms were exposed to the mixture of BSA, THA, DHA, and 2,4,6-TBP for
333 21 days. When applying all four substances at a comparable low concentration of 187.0 µg/L each, a
334 50 % reduction of the amount of live offsprings per parent was observed. However, taking an
335 environmental concentration of 20 ng/L for 2,4,6-TBP into account (Gustavsson et al., 2018), the
336 concentration mentioned above is four magnitudes higher than currently detectable levels for this one
337 compound of the mixture. In addition to a decrease in the number of live offspring, growth inhibition was
338 observed at high concentrations. Thus, after observing chronic effects caused by an exposure to a
339 mixture of all four substances, it would be interesting to further test which substance is mainly driving

340 such effects. To our knowledge, only one publication deals with chronic exposure of one of the applied
341 substances. In this publication, Deng et al. have shown that 2,4,6-TBP might pose a risk to aquatic
342 organisms following chronic exposure to environmentally relevant concentrations (Deng et al., 2010).
343 Taking the predicted log K_{OW} values into account (Table 2), it seems possible for 2,4,5-TBP/2,4,6-TBP
344 and THA that bioaccumulation might take place. According to the Globally Harmonized System (GHS,
345 2017), a log K_{OW} > 4 is indicating a potential for bioaccumulation, which stresses precautional proper
346 handling of “Polymeric FR”.
347
348 **5. Conclusions**
349 Previous studies have demonstrated that the polymeric BFR “Polymeric FR” can degrade and yield
350 monomeric compounds which might get in contact with the environment under certain circumstances
351 for example if products containing such a FR are brought to landfill at the end of their life time. In order
352 to gain first data on possible effects of such degradation products for biota, we have performed the
353 present study using three different OECD tests. As discussed before, the acute toxicity seems to be
354 rather limited, which is also supported by our predictive trend analysis. If at all, effects can only be found
355 at extremely high concentrations, which are presumably not to be expected following degradation of
356 “Polymeric FR”. Nevertheless, data from chronic experiments indicate that the mixture of the four tested
357 degradation products had effects, but only at comparable high concentrations as well. However, it has
358 to be considered that the used mixture represents an artificial combination of a minor fraction of more
359 than 75 substances that were previously detected during degradation experiments. Therefore, tests
360 using a native degradation “cocktail” would be advantageous for a realistic risk assessment as well as
361 testing different groups of organisms.
362
363 **6. Supporting information**
364 Figure S1: Molecular structure of the tested substances
365 Figure S2: Inhibitory effect of every individual substance tested according to OECD No. 201
366 Figure S3: Immobilization due to every individual substance tested according to OECD No. 202
367 Figure S4: Mortality due to every individual substance tested according to OECD No. 202
368 Table S1: Applied recipe for the reconstituted algae water
369 Table S2: Applied recipe for the reconstituted daphnia water
370 Table S3: List of immobilized individuals per concentration according to OECD No. 202

371 Table S4: List of dead individuals per concentration according to OECD No. 202

372 Table S5: Effect on every replicate according to OECD No. 211

373

374 **7. Acknowledgement**

375 Thanks are due to BASF for contributing the pure “Polymeric FR” powder. We also want to thank Milen
376 Nachev for his support during the ICP-MS measurements and Marion Woermann for her support during
377 the daphnia tests.

378

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General discussion

With an annual production volume of roughly 26,000 metric tons (ICL, 2015; LANXESS, 2018), “Polymeric FR” is one of the major BFRs which are currently manufactured on an industrial basis. Although it is only applied within building foam insulation, it is seen as a prototype for future polymeric FRs that could be used in additional product categories such as textiles or electronical equipment. Furthermore, thermal insulation is continuously gaining importance as one measure of the overall attempt to limit the increase in the global average temperature below 2 °C above pre-industrial levels as recently emphasized with the Paris Agreement (UN, 2016). However, previous research has shown that the frequently incorporated FRs do not only possess beneficial properties, but are also often associated with drawbacks including human and environmental hazards (Birnbaum and Staskal, 2004; Covaci et al., 2011; de Wit, 2002; Fromme et al., 2016).

Even though “Polymeric FR” is one of the major BFRs currently produced, no studies have been published concerning its health properties. Especially its long-term behaviour in the environment is completely left disregarded. This lack of knowledge has been stressed multiple times by non-governmental organizations and environmental protection agencies like the US EPA (US EPA, 2014). However, no studies dealing with these aspects have been published so far. In this context, the present thesis draws first conclusions and builds the basis for future research – not only regarding “Polymeric FR”, but also for similar polymers that might be placed on the market.

The focus on potential degradation rather than on the original polymer in the context of a risk evaluation is based mainly on the likelihood that “Polymeric FR” itself will – even as an additive BFR – not be able to significantly leach out of the surrounding PS matrix. Provided that this would be the case, bioavailability can be expected to be rather limited due to its high molecular weight (Beach et al., 2017). Nonetheless, a potential toxicity of the polymer itself might be interesting for instance considering accidental spills with subsequent ingestion by biota. However, if degradation of the polymeric chain takes place, this would be a rather common process and consequently more relevant for the environment.

“Polymeric FR” combines the properties of polystyrene, which represents the majority of the chemical composition of a PS foam product, and a BFR with its ability to release

bromine radicals. Considering previous studies, it is a well-known fact that polymers in general, and specifically polystyrene can break down through photodegradation (Yousif and Haddad, 2013). Additionally, degradation of BFRs such as TBBPA or PBDEs is an often observed process as well (Chen et al., 2013; Eriksson et al., 2004b, 2004a; Kajiwara et al., 2013; Santos et al., 2015). Nevertheless, the degradation of a polymeric BFR has not been described yet.

While the development of an analytical approach to study the degradation of polymeric BFRs is summed up in the first chapter of this thesis, the application of this approach to “Polymeric FR” is described in chapter II. To start with, UV irradiation was chosen among different degradation factors like biodegradation and heat, because of its time efficiency. As explained in chapter I, depending on UV-A or UV-B, the dose applied during one hour of radiation equals approximately two hours or respectively three days of natural radiation on the earth’s surface (Bais et al., 2011). Thus, it is possible to obtain results comparably quickly. Moreover, degradation of the polymeric chain takes place even considerably earlier. The contact angle, which reflects the hydrophilicity in this study, decreased for instance from 80 ° to 40 ° within only half an hour – meaning the normally lipophilic “Polymeric FR” became more hydrophilic, which could for example raise the likelihood of biodegradation due to increased colonization by microorganisms (Donlan, 2002; Sudhakar et al., 2008; Wang et al., 2012). Although abstraction of bromine and shortening of the polymeric chain were measured, these observations alone do not necessarily decrease the molecular weight or increase the bioavailability of “Polymeric FR” decisively. Therefore, the search for distinct monomeric degradation products was also included in the analytical approach. While possible structures were only predicted based on detected masses in chapter I, the identification of substances is discussed in chapter II. Even though 75 distinct masses were detected following UV irradiation, only a minor percentage was identified. This is due to the fact that only roughly 10 % of the predicted molecular formulas contain bromine (which are the molecules of interest) and that suitable standards were rarely commercially available. Nevertheless, three compounds, namely 5-Bromosalicylic acid (BSA), 2,4,6-Tribromo-3-hydroxybenzoic acid (THA), and 3,5-dibromo-4-hydroxybencoic acid (DHA), were identified. A compound which was available as a standard, but not verified as a degradation product is 2,4,6-Tribromophenol (2,4,6-TBP). The fact that the recorded spectrum equals the one of 2,4,6-TBP, but only the retention time is significantly different, suggests that an isomer like for instance 2,4,5-TBP originated during the degradation process. To obtain more data regarding

potential degradation pathways of individual products, it might be advantageous to further investigate degradation mechanisms that were previously proposed for other BFRs, and particularly TBBPA. The aromatic rings of this BFR are – similarly to "Polymeric FR" and contrary to the ether group of PBDEs – connected only by carbon bonds (Eriksson et al., 2004b). Also identified pathways for PS via the production and reaction of polystyrene alkyl and peroxy radicals and subsequent chain scission (Yousif and Haddad, 2013) could be beneficial in this regard.

Considering that the detected degradation products of "Polymeric FR" are quite versatile, it is certainly also favourable to quantify the amount of organic material deriving from the polymer to get an overall picture of the quantity of degradation. Taking the concentration of total organic carbon (TOC) and adsorbable organically bound bromine (AOBr) into account, it can be calculated that only less than 1 % of the carbon content of "Polymeric FR" can be measured in the solvent after one hour of UV irradiation. Furthermore, only a small percentage of this number contains bromine as well. Thus, degradation seems rather limited. However, as mentioned above, one hour of artificial radiation might only equal two hours of natural radiation. In consequence, degradation might quickly amplify. Yet, it should be kept in mind that "Polymeric FR" will in theory only enter the environment incorporated into a PS matrix which will shield the BFR against UV irradiation. Thus, the environmental relevance of UV irradiation will probably be limited to potential point sources like EPS/XPS manufacturing plants (Covaci et al., 2006; Thomsen et al., 2007; Zhang et al., 2013) or construction and landfilling sites (Stubbings and Harrad, 2014; Weber et al., 2011).

Contrary, an omnipresent degradation factor for thermal insulation and thus BFRs, is heat. Starting with their use phase, insulation products can be exposed to temperatures of up to 70 °C (Fraunhofer IBP, 2007; FSEC, 2008) when installed below a roof. Similar temperatures can also occur at landfilling sites (UK Environmental Agency, 2003; Yesiller et al., 2005), which is a typical end-of-life scenario for foam insulation in addition to energetical recycling in municipal incinerators (Kajiwara et al., 2017; Mark et al., 2015).

The obtained results after up to 36 weeks of heat exposure at 60 °C indicate that "Polymeric FR" degrades considerably stronger when placed in a solvent (distilled, reconstituted, or rain water). Almost no degradation can be observed when solely exposed to heat without water. However, when in contact with water and exposed to heat – a scenario that might be encountered during the end-of-life – degradation takes place. In terms of TOC content, similar levels (of roughly 1 % of the carbon content of

“Polymeric FR”) were observed after three hours of irradiation and three months of heat treatment. Hence, heat seems to be a potent degradation factor when combined with a solvent. However, although the TOC content is on a similar level, the amount of detected degradation products is significantly smaller after heat treatment compared to UV irradiation. Only seven molecular formulas, including one containing bromine, were identified. The detected substance containing bromine was identified as BSA, which was already detected in UV treated samples. As discussed in chapter II, various explanations are possible for this observation, including a different degradation mechanism or low concentrations of originating products. In summary, degradation of “Polymeric FR” is definitely possible, but only under certain circumstances. It is unclear, if or how often the necessary conditions for degradation will be present over the whole life cycle of a foam insulation product containing “Polymeric FR”. Alternatives for landfilling like chemical recycling (Schlummer et al., 2017) are thus certainly worth pursuing, but it remains questionable if such options will be locally available wherever “Polymeric FR” is used. Considering this, it is important to have at least a basic understanding of the toxic potential deriving from a degradation of “Polymeric FR”.

As discussed in chapter III, based on measurements after UV treatment, four degradation products were chosen for this purpose. Three of which were clearly identified within this study, while 2,4,6-TBP was only predicted in chapter I but not detected in chapter II. 2,4,6-TBP was however used, because the results presented in chapter II were not finalized when the exposure period was started. Nevertheless, *in silico* modelling for 2,4,5-TBP, which was suggested as the possible chemical detected in chapter II, was performed where applicable. As discussed in the third chapter, the obtained results were very similar for 2,4,5- and 2,4,6-TBP, thus results might very well be transferrable. A comprehensive overview of TBP is given in appendix II of this thesis.

To generate first data regarding the acute toxicity of the four tested chemicals, two different OECD tests with *Daphnia magna* and *Desmodesmus subspicatus* were accompanied by one trend analysis for *Pimephales promelas*. As presented in chapter III, the acute toxicity can be considered rather low or almost neglectable. Very similar results were obtained for BSA, THA, and DHA. Different, but still low effects were measured following an exposure to 2,4,6-TBP. Only this substance exhibited an effect (at the highest dose of 10 mg/L) in the *D. magna* acute immobilization test. Contrary, TBP caused the lowest effect in the algae test, while BSA, THA, and DHA caused effects at lower concentrations, but still did not reach 50 % growth inhibition during the

test period. The relatively weak effect of 2,4,6-TBP within this test might be due to a natural excretion of TBP by some algae (Flodin and Whitfield, 1999) and there consequential adaption regarding this substance.

In addition to the four individual chemicals, a mixture of those was also used to mimic (in an artificial attempt) a product mixture which is generated during the degradation of "Polymeric FR". The mixture exhibited the strongest effects in both OECD tests, but still comparably weak and (aside accidental spills) non-relevant for the environment.

To extent the obtained results regarding the acute toxicity, a trend analysis was performed to predict the effects of the tested chemicals on a third trophic level, while similarly avoiding vertebrate experiments. With this analysis, fish were included in this project in addition to green algae and crustaceans. As discussed in chapter III, LC_{50} values for BSA, THA, and DHA are above 10 mg/L and around 3 mg/L for 2,4,5- and 2,4,6-TBP – thus at such a high level which is not to be expected following a degradation of "Polymeric FR".

However, previous research has shown that acute toxicity is rather uncommon for BFRs. Instead, these chemicals often possess the potential to be persistent and bioaccumulative, and exhibit toxic effects after chronic exposure (Bradshaw et al., 2015; Deng et al., 2010). Therefore, the scope of this thesis was broadened by a third OECD test to cover chronic exposure as well. Within this test, *D. magna* was exposed for 21 days to a mixture of the four chemicals listed above. This test was intended to gain a first impression if chronic exposure is environmentally relevant or not. As reported in chapter III, effects were observed at lower concentrations which could indeed be of environmental relevance. Nonetheless, further chronic exposure studies are certainly necessary to get a better understanding in this regard. Experiments focusing on a molecular and cellular level could be beneficial as well to be aware of potential sublethal effects that might not directly be observable on an organismic level. It was for instance shown that the degradation of high molecular weight BFRs – such as tetradecabromo-1,4-diphenoxybenzene (TeDB-DiPhOBz) and DecaBDE – can lead to cytotoxicity and dioxin-responsive mRNA expression in chicken embryonic hepatocytes (Su et al., 2018, 2014)

As touched upon before, despite the industrial relevance, comprehensive knowledge regarding the long-term behaviour of polymeric BFRs in the environment is currently still missing. This thesis builds the basis for future research and shows that the prototype of this group of chemicals, "Polymeric FR", can break down to monomeric

products under certain circumstances that might exhibit toxic effects after chronic exposure. There are however several aspects of this project that should be noted and considered during further research. The degradation scenarios were mostly performed with bulk “Polymeric FR” instead of the final foam product, which will most likely alter the degradation patterns. Additionally, the applied mixture in chapter III is very artificially composed due to the availability of substances. It includes only four chemicals, while the origination of 75 substances was indicated after UV irradiation. Not only the composition, but also the ratio between the individual test substances is artificial and does probably not reflect a realistic scenario. Furthermore, only two species were tested *in vivo* regarding ecotoxicological effects and only one test including chronic exposure was performed. Taking these aspects into account, more research is certainly required to evaluate the developer’s claim of a better environmental profile. Possible studies could for example include the analysis of indoor dust of houses where “Polymeric FR” is being used. Another possibility would be to predict the chemical structure of all detected molecular formulas and use those to perform trend analysis regarding chronic endpoints as far as possible. To work with fewer predictions, it could also be an opportunity to degrade “Polymeric FR” and use the native degradation mixture to perform chronic exposure experiments. Considering an occupational scenario, it would also be beneficial to gain more information about the pyrolysis of “Polymeric FR” when insulation boards are cut for instalment.

However, without further studies and solely based on results obtained during this thesis, it seems possible that “Polymeric FR” – under optimal circumstances – fulfils the claim of a better environmental profile compared to HBCD. Nevertheless, the proper handling of this BFR remains important; especially during its end-of-life. Considering that “Polymeric FR” is only applied in building foam insulation, but similar polymers might be used in other product categories as well, this thesis also indicates possible risks associated with new fields of application. Textiles for instance, were previously also frequently treated with HBCD, but might contain a polymeric BFR in the future. These products, in contrast to building foam insulation, encounter UV irradiation and washing frequently, which will probably lead to a higher magnitude of degradation of the polymeric BFR and should thus receive special attention. The theoretical feasibility of such an application was already shown some years ago with poly(pentabromobenzyl acrylate), which however – based on the scarce literature available – gained only little to no commercial and scientific attention (Borms and Georlette, 2004). This underlines the importance of further research regarding

polymeric BFRs. Especially, because regulations do normally not require manufacturers of polymers to test the polymer itself, check its degradability under different circumstances, evaluate possible degradation products, or to consider combinatory toxic effects of such degradation products.

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Appendix I: Review of hexabromocyclododecane (HBCD) with a focus on legislation and recent publications concerning toxicokinetics and -dynamics

This publication deals with HBCD, which was previously used in PS foam. It summarizes environmental concentrations of the chemical, but also highlights the toxicokinetics and -dynamics of HBCD, which is useful when assessing the developer's claim of a better environmental profile of "Polymeric FR".
Manuscript as published.

Environmental Pollution 199 (2015) 26–34

Contents lists available at ScienceDirect

Environmental Pollution

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

Review

Review of hexabromocyclododecane (HBCD) with a focus on legislation and recent publications concerning toxicokinetics and -dynamics

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ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form
7 January 2015
Accepted 8 January 2015
Available online 22 January 2015

Keywords:
HBCD
Legislation
Toxicokinetic
Toxicodynamic
Enantiomer

ABSTRACT

In this paper, we review recent publications regarding the toxicokinetics and -dynamics of the flame retardant Hexabromocyclododecane (HBCD). HBCD has recently been listed as a persistent organic pollutant, which therefore influenced the legislation concerning its manufacturing and formulation. However, under specific circumstances it may still be used until 2024. Early toxicity studies have only focussed on HBCD itself, which is a mixture of different isomers with different physical and toxicological characteristics. Here we take a more differentiated look at the three diastereomers α-, β- and γ-HBCD. We also address the different enantiomers to give an overview of the toxicity of HBCD to identify present gaps in our knowledge about this chemical, especially with respect to its possible formulation until 2024.

1. Introduction

Insulation of houses is an important measure in order to reduce CO_2 emissions with the goal to slow down the increasing trend of climate change. Accordingly, many older private houses particularly in temperate regions are part of renovation programs. For safety reasons, all thermal insulations have to be fire resistant. This resistance is currently often realized by the addition of 1,2,5,6,9,10-hexabromocyclododecane (HBCD) as a flame retardant in extruded (XPS) and expanded (EPS) polystyrene foams. Concentrations of this additive brominated flame retardant (BFR) in foams are typically lower than 3% w/w. Additionally, there are further fields of HBCD application such as its use in textiles, electronics and plastic materials.

HBCD has been manufactured since the 1960s and reached a global annual production of 16,700 t in 2001. Most of the consumption was in the EU with 9500 t. The global production of HBCD rose to 21,951 t in 2006 (BSEF, 2006). In 2007, the EU-wide consumption of 11,600 t still accounted for the biggest part of the global demand (ECHA, 2009). According to the European Chemicals Agency (ECHA), 3141 kg of HBCD is released into the environment (50% to waste water, 29% to surface water and 21% to air) on a European scale each year (ECHA, 2009).

Due to its wide use in many products and the fact that HBCD is a persistent compound, it occurs in different environmental matrices. As a result of its accumulation in the food web (Janak et al., 2008; Li et al., 2011; Leslie et al., 2011) and several toxic effects like the disruption of the thyroid homoeostasis (Chengelis, 1997), legislation measures are coming into force. The current review summarises the most obvious biological effects of HBCD and addresses aspects of future research after introducing the current status on legal aspects.

2. Legislation

Due to the environmental relevance of HBCD, the Conference of the Parties (COP) of the Stockholm Convention (SC) on persistent organic pollutants (POP) decided on 9th May 2013 to list HBCD in Annex A (POPRC8.3, 2013). The date of the communication of the adoption by the depository was 26th November 2013. This amendment to Annex A will come into force one year later, thus on

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http://dx.doi.org/10.1016/j.envpol.2015.01.011
0269-7491/

26th November of 2014. Until this date, the members have to implement the decision of the SC into their national laws. Within the EU, COP-decisions under the SC are implemented into EU-law by amendments to the EU-POP-Regulation (Regulation (EC) No 850/2004, 2004). However, the members of the SC (including the EU) may submit a notification of non-acceptance to ensure that they have enough time to harmonise their national law. This notification can be withdrawn at any time. The entry into Annex A will result in a short-term ban on manufacture and use of HBCD. However, a member of the SC can decide to register for an exemption for the production and use of HBCD in buildings in the form of EPS and XPS. This notification had to be submitted before 26th November 2014. The exemption can be valid for a period up to five years (up to November 2019) but may be extended by another period of up to five years (2024). These exemptions may be submitted in order to give the industry enough time to switch to alternatives.

Under REACH (Regulation (EC) No 1907/2006, 2006) HBCD is identified as a substance of very high concern (SVHC) and was included in Annex XIV to REACH in 2011. Accordingly, (i) the production and the use of these substances after 21st August 2015 (so-called sunset-date) are subject to the authorization, (ii) suppliers of EPS and XPS containing HBCD within the EU and the European Economic Area (EEA) have to provide sufficient information to their customers and on request also to consumers, and (iii) manufacturers are obliged to inform their customers that EPS/XPS insulation material contains HBCD (Article 33(1) REACH). Applications for an authorisation could only be submitted to the ECHA until 21st February 2014 (deadline). No authorization is possible for imported articles containing HBCD.

In February 2014, 13 European EPS producers have jointly applied for an authorisation for the formulation and manufacture of HBCD-containing EPS for use in building applications (ECHA, 2014a, 2014b). The authorisation shall be granted only if (i) socio-economic benefits outweigh the risk to human health or the environment arising from the use of the substance, and (ii) there are no suitable alternative substances or technologies (Article 60(4) REACH).

The decisions to add HBCD to Annex A of the POP and, under European law, to include it in Annex XIV as a SVHC were made due to its bioaccumulative, persistent and toxic (BPT) characteristics. Especially concentrations in the environment have been the topic of many studies on this BFR.

3. Environmental concentrations

With respect to the environmental relevance, Sellström et al. (1998) first detected HBCD in Swedish rivers in 1997. Since then it has been ubiquitously found in different environmental matrices (Law et al., 2006). HBCD has even been detected in the Arctic, indicating its high ability for long-range transport (LRT) via air (de Wit et al., 2010). The environmental levels usually described clearly reflect the commercial demand on a regional scale (Zhang et al., 2013a). Concentrations in sediments in Europe, where the biggest amount of HBCD is consumed (ECHA, 2009), range between concentrations below the detection level (ND) and 2660 ng/g dry weight (dw) (Guerra et al., 2008, 2010), which is much higher than concentrations in Southeast Asia (0.056–59 ng/g dw; see Minh et al., 2007; Ramu et al., 2010) or North America (ND-3.70 ng/g dw; see Marvin et al., 2006). Local concentrations clearly increase close to HBCD point emission sources like XPS- and EPS-producing plants (Zhang et al., 2013a). The technical HBCD (*t*HBCD), which is synthesized by bromine addition to 1,5,9-cyclododecatriene, consists mainly of three diastereomers: α-, β-, and γ-HBCD. The relative amount of these enantiomeric pairs varies between the different commercially available products, but tends to be around 1–12 % for α-, 10–13 % for β- and 75–89 % for γ- HBCD (Wu et al., 2012; Heeb et al., 2007). Two other diastereomers (δ and ε) are also found in *t*HBCD, but in much lower concentrations (Arsenault et al., 2007; Heeb et al., 2005). Even though δ-HBCD has also been found in abiotic (Li et al., 2012) and biotic matrices (Janak et al., 2005, 2008; Harrad et al., 2009a) most research has been conducted on α-, β- and γ-HBCD so far.

The composition of HBCD in abiotic matrices is usually similar to the commercial mixture, with γ-HBCD being the dominant diastereomer, although the relative importance of α-HBCD increases considerably. Zhang et al. (2013a) suggested using the ratio of γ- to α-HBCD in abiotic samples to detect recent contaminations with HBCD in the environment, because of the decreasing percentage of γ-HBCD with time. Other abiotic matrices such as soil (Li et al., 2011), air (Li et al., 2012) and water (Harrad et al., 2009a) show a similar composition to sediment, with γ-HBCD being the dominant diastereomer, although mostly in a decreased portion compared with *t*HBCD. However, some studies found α-HBCD to be the dominant diastereomer in abiotic matrices (Li et al., 2012; Meng et al., 2012). Law et al. (2014) have recently summarized several publications concerning the time trends of environmental HBCD concentrations. They found mixed temporal trends for HBCD, with both increasing and decreasing levels in different matrices. Concentrations of HBCD in air (indoors as well as outdoors) and dust are from particular interest for the human health and will be discussed below.

Only few studies have been performed on the HBCD concentration in plants until now. Li et al. (2011) found higher HBCD concentrations in root tissue in radish and cabbage than in shoot tissue with concentrations up to 70 ng/g in cabbage roots. In this laboratory study, the dominant diastereomer in root tissue was γ-HBCD, whereas α-HBCD dominated in shoot tissue most likely due to its higher water solubility (α: 48.8 μg/L, β: 14.7 μg/L and γ: 2.1 μg/L; see Hunziker et al., 2004) and bioisomerization (see below for details) compared to γ-HBCD (Li et al., 2011). On the other hand, Wu et al. (2012) found β-HBCD to be the dominant diastereomer in roots and shoots in maize, which is not congruent with the proportions found in abiotic matrices or animals. Additionally, no bioisomerization was described in maize after exposure for 120 h (Wu et al., 2012). Plants which are not raised in the laboratory, but are collected in the field, show the highest HBCD concentration in the leaves, which may be due to absorption from the air and could explain why γ-HBCD showed the highest concentration in this organ (Zhang et al., 2013b). However, more research is required concerning the effects of HBCD on plants.

In comparison to plants, there have been several publications concerning HBCD in animals, almost exclusively in birds (with concentrations in eggs up to 14,600 μg/kg lipid weight (lw), see Law et al., 2014) and aquatic organisms (low concentrations for example ranging from ND-0.19 μg/kg wet weight (ww) for fish collected in China, see Law et al., 2014) which show that α-HBCD is the dominant diastereomer. Its percentage usually increases in the food chain (Li et al., 2011) and sometimes ends up being around 100% in top predators like gannets and gulls (Janak et al., 2008; Leslie et al., 2011; Esslinger et al., 2011a; Haukas et al., 2009). However, trophic magnification factors (TMF) vary considerably. Tomy et al. (2004), for example, reported a TMF value of 6 for α-HBCD, which differs greatly from other, much lower TMF values (around 2, see Zhang et al., 2013b; Tomy et al., 2008; Wu et al., 2010).

4. Enantiomeric pattern

As stated before, *t*HBCD consists mainly of three diastereomers: α-, β- and γ-HBCD. Each of them represents a pair of two

enantiomers: the (−)- and the (+)-enantiomer. The ratio between these two enantiomers, the enantiomeric fraction (EF), gained attention recently (Li et al., 2012; Zhang et al., 2013a). Janak et al. (2005) were the first to report enantiomeric selection in marine fish in 2005. Although certain species (ranging from insects to birds) and abiotic matrices show a tendency to accumulate a specific enantiomer, no common trend has been found yet. The accumulation of (−)-α-HBCD can be observed more often in certain species of animals (e.g. birds, fish, shrimp and crabs) for example, although there are also reports about accumulation of (+)-α-HBCD or no specific accumulation pattern at all (Janak et al., 2008; Harrad et al., 2009a; Zhang et al., 2013b; Esslinger et al., 2011a, 2011b). It was hypothesized that the selective enrichment among different species might be due to an enantioselective metabolism (Wu et al., 2010; Köppen et al., 2010). Zhang et al. (2014) proposed that enantioselectivity might occur during bioisomerization. In conclusion, enantioselectivity seems to be very specific for certain animal species, even between species of the same taxonomic order or a predator and its prey (Janak et al., 2008). However, more research has to be conducted on this topic.

5. δ- and ε-HBCD

Concentrations of δ-HBCD of up to 15% have been reported in air samples from China, which exceed the concentrations found in *t*HBCD (Li et al., 2012). The δ-HBCD *meso* form has also been found in biota. Janak et al. (2005, 2008) reported δ-HBCD in samples from fish and birds. Harrad et al. (2009a) hypothesized that δ-HBCD may be a product of bioisomerization in fish, because it was not found in water or sediment samples in England. Abdallah et al. (2014) found δ-HBCD in trout, but not in rats after biotransformation. However, the source of δ-HBCD remains unclear. To the best of our knowledge, there has been no confirmation of ε-HBCD outside of *t*HBCD yet. The low number of records on δ- and ε-HBCD in environmental matrices is probably not due to its absence in the environment, but rather a consequence of the limited number of studies addressing this issue. Therefore, more research is required on these HBCD isomers.

6. HBCD in humans

HBCD was also found in human body fluids. Most studies paid particular attention to detect HBCD in breast milk and blood samples. Lower concentrations found in breast milk were for example 0.19 ng/g (Malarvannan et al., 2013a), 0.086 ng/g (Devanathan et al., 2012) or 0.39 ng/g lw (Fangström et al., 2008). Higher concentrations were 3.5 ng/g lw (Ryan et al., 2006), 4.0 ng/g (Kakimoto et al., 2008) or 5.9 ng/g (Abdallah and Harrad, 2011). The highest concentration found until now was 27 ng/g lw (Eljarrat et al., 2009) and the diastereomeric profile mainly showed a dominance of γ-HBCD. HBCD concentrations in blood serum were found to be 1.7 ng/g (Roosens et al., 2009), 1.1 ng/g (Meijer et al., 2008) or 0.46 ng/g lw (Weiss et al., 2006) with α-HBCD being the dominant diastereomer (almost 100%). Thomsen et al. (2007) described very high concentrations of HBCD ranging from 6 to 856 ng/g lw in blood serum from workers in an industrial EPS plant in Norway and found that α-HBCD only accounted for 60%, but γ-HBCD for 39% of the total HBCD, suggesting a recent contamination event. Furthermore, it appears that HBCD can be transferred from mothers to infants (Kim and Oh, 2014). Although most studies have been conducted on concentrations of HBCD in body fluids, HBCD has also been determined in the human body in different tissues, e.g. foetal liver, placenta and fat (Rawn et al., 2014; Malarvannan et al., 2013b).

There are two major ways of HBCD uptake for humans: via diet and via indoor dust. Schecter et al. (2012) collected 46 food samples from U.S. supermarkets in Dallas in 2009 and 2010. HBCD had been detected in 25 of these samples. 23 of them were dominated by α-HBCD. The samples from 2010 contained 0.114 ng/g ww HBCD on average. The highest concentrations in food are normally found in lipid-rich fish and meat (Dirtu and Covaci, 2010; Driffield et al., 2008; Fernandes et al., 2008; Knutsen et al., 2008; Nakagawa et al., 2010; Törnkvist et al., 2011; van Leeuwen and de Boer, 2008), however, foodstuff like chili with beans, peanut butter or nuts can also contain HBCD (Schecter et al., 2012; Driffield et al., 2008). The amount of HBCD in food can differ, even within the same type of food. This may be due to different ingredients, food sources and handling/processing of the food. Schecter et al. (2010) calculated a daily intake rate of HBCD for US Americans of 2.1 * 10^{-7} mg/kg-body weight (BW)/d. They stated that this value is much lower than 10 mg/kg-BW/d, which is the no observed adverse effect level (NOAEL) in rats after an exposure to *t*HBCD (Schecter et al., 2010; European Commission, 2008). In the European Union this level is used to estimate risks derived from HBCD (European Commission, 2008). However, as mentioned before, the daily intake of HBCD can vary considerably, i.e. for a 70 kg adult between 0.875 and 1.12 ng/d (Meng et al., 2012), to 200–500 ng/d (Fernandes et al., 2008; de Winter-Sorkina et al., 2003; U.K. Food Standards Agency, 2006) (Table 1). Small sampling size, eating habits and food contamination levels play an important role for the calculated intake values. There is evidence that the food contamination of brominated POPs, to which HBCD belongs, is reflected in human fluids. Pratt et al. (2013) for example, confirmed this relationship for Irish breast milk.

The second major route of uptake is via ingestion of house dust. It is important to note that dust ingestion plays a major role and not dust inhalation, with the latter contributing only little to the total HBCD uptake of humans. Indoor air concentrations of HBCD are much higher (250 pg/m^3) than outdoor concentrations (37 pg/m^3) (Abdallah et al., 2008b). Ni and Zeng (2013) calculated daily inhalation rates for adults at 93.2 pg/kg-BW of HBCD from which 61.9 pg/kg-BW are bound to particles with a diameter of 2.5–8.9 μm (PM_{10}) and are deposited in the upper part of the respiratory system. The other 31.3 pg/kg-BW are bound to particles with a diameter of 0.4–2.2 μm ($PM_{2.5}$). These particles can be inhaled deep into the lungs (Ni and Zeng, 2013).

There are many sources of HBCD in house dust such as electronic devices, thermal insulations and textiles (Kajiwara et al., 2009). Abdallah et al. (2008a) measured the HBCD concentration in dust from UK houses. They found an average concentration of 730 ng/g dw and measured a peak concentration of 140,000 ng/g dw in one of the houses, which is the highest concentration reported until now (Abdallah et al., 2008b). α-HBCD was reported to account for 32% of total HBCD in dust (Abdallah et al., 2008a). Roosens et al. (2009) also found α-HBCD to be the dominant diastereomer in their samples. This shows that α-HBCD contributes more to total HBCD in dust than it does in *t*HBCD. This seems to be due to two key processes. First, HBCD undergoes a thermal induced isomerization to α-HBCD at temperatures which are used while incorporating *t*HBCD into the final product (Peled et al., 1995; Heeb et al., 2008; Köppen et al., 2008). These temperatures are usually between 160 and 220 °C. The second process is also an isomerization, but photolytically mediated from γ-to α-HBCD (Harrad et al., 2009b). Harrad et al. (2009b) found that light-exposed dust samples show a faster decay in total HBCD concentration ($t_{1/2}$ = 12 weeks) than light-shielded samples ($t_{1/2}$ = 26 weeks). In this study, even a TV seems to influence the HBCD degradation. Calculated daily intake levels for HBCD via dust also vary substantially, especially between different intake scenarios. Calculated values of an average intake scenario (20 mg/d) for adults range between 3.2 ng/d (Roosens et al., 2009) and 145.5 ng/d (Ni and Zeng, 2013) (Table 1).

Table 1
Intake of HBCD from dust and food ingestion in adults (70 kg).

Country	Dust intake (ng/d)		Diet intake (ng/d)	Method	Reference
	Average	High			
United Kingdom	144.4	360.9		Mean	Abdallah and Harrad, 2009
United Kingdom	131.5	328.7		Mean	Abdallah et al., 2008b
United Kingdom	120	300		Mean	Abdallah et al., 2008a
United States	16	40		Mean	Abdallah et al., 2008a
Canada	13	33		Mean	Abdallah et al., 2008a
Belgium	3.2	8	7.2	Mean	Roosens et al., 2009
Romania	6	15	77	Median	Dirtu and Covaci. 2010
Japan			91–259	Median	Nakagawa et al., 2010
China			1.11/1.12	Median (f/m)	Meng et al., 2012
Sweden			133/150.5	Median (f/m)	Lind et al., 2002
United Kingdom			413	Upper bound (mean)	U.K. Food Standards Agency, 2006
United Kingdom			413–553	Upper bound (mean)	Fernandes et al., 2008
Norway			91	Upper bound (mean)	Knutsen et al., 2008
Netherlands			8.4	Medium bound (mean)	van Leeuwen and de Boer, 2008
Sweden			10.2	Mean	Törnkvist et al., 2011
United States			15.3	Mean	Schecter et al., 2012
Spain			177	Mean	Eljarrat et al., 2014
Japan			31.5–2380	Minimum and maximum	Ueno et al., 2010

Toddlers spend more time on the floor and put their fingers into their mouth more often than adults, which causes a higher HBCD intake per day under an average consumption scenario (50 mg/d). Abdallah et al. (2008b) calculated a daily intake of HBCD for toddlers of 403.8 ng/day (compared with 131 ng/d for adults). This difference is even more profound under a high dust intake scenario: 328.7 ng/d for adults (50 mg/d) and 1473.1 ng/d for toddlers (200 mg/d) (Abdallah et al., 2008b).

The daily intake of HBCD via dust (28%), diet (72%) and air (<1%) under a high intake scenario adds up to 1173 ng for adults, in which the dietary ingestion accounts for the biggest part. The daily intake of 1974.1 ng HBCD for toddlers is much higher and particularly based on their ingestion of dust (75%) compared with the intake via diet (25%) and air (<1%) (U.K. Food Standards Agency, 2006; Abdallah et al., 2008b) (Table 2).

7. Toxicokinetics of HBCD

The toxicokinetics of HBCD has recently gained more interest. Szabo et al. (2010; 2011a) and Sanders et al. (2013) performed studies on the individual diastereomers α-, β- and γ-HBCD in female mice. The absorption after an oral gavage compared with an intravenous injection was around 85–90 % of the total dose (Szabo et al., 2010, 2011a; Sanders et al., 2013). Tissue distribution differed between the diastereomers. α-HBCD was initially found in highly perfused organs such as the lungs, kidneys and particularly the liver (13% of the administered dose after an hour) followed by its distribution to the skin, muscle and adipose tissue, where α-HBCD showed the highest concentration after two days (5%) (3 mg/kg-BW). The sequestration of α-HBCD was dose-dependent at higher levels (10 and 30 mg/kg-BW), which means that its distribution to tissues increased with higher dose levels. In adipose tissue 3.7% of the total dose was found after 4 days following an administration of 3 mg/kg-BW α-HBCD, but 8.91% was found after the administration of 100 mg/kg-BW (Szabo et al., 2011a). The observed excretion reflects this tendency. Renal excretion is constant between 15 and 20% for different doses, whereas the excretion via faeces decreases with higher doses. Although 48% of the administered dose of 3 mg/kg-BW is excreted via faeces, only 33% is excreted after the administration of 100 mg/kg-BW α-HBCD (Szabo et al., 2011a).

The excretion of γ-HBCD is not dose-dependent with a 25–30 % elimination via urine and 45–50 % via faeces (Szabo et al., 2010). Its tissue distribution is markedly lower than that of α-HBCD. Only the liver showed (initially, <1 day) a concentration higher than 1% after the administered single dose of 3 mg/kg-BW. There is almost no effect of repeated doses on the concentration of γ-HBCD in blood and adipose tissue (Szabo et al., 2010). Few studies have been performed on the uptake kinetics of β-HBCD. The tissue distribution of β-HBCD is similar to α- and γ-HBCD. The concentration in liver tissue for example is initially higher than in all other tissues (Sanders et al., 2013). After a period of time (8–96 h), the adipose tissue shows the highest concentration. Concentrations of β-HBCD range between those of α- and γ-HBCD (Szabo et al., 2010, 2011a; Sanders et al., 2013).

Szabo et al. (2011b) performed a study on the toxicokinetics of α- and γ-HBCD in young mice by administering a single dose of 3 mg/kg-BW to mice at postnatal day 10 (PND 10). A similar distribution pattern to adult mice (PND 60) emerged, although concentrations of the individual diastereomers were higher in all organs. The body burden of γ-HBCD was 10 times higher in infantile mice than in adult mice. Likewise the body burden of α-HBCD increased up to 2.5 times more in young mice than in adult mice.

Table 2
Intake of HBCD via air, dust and food in adults and toddlers.

	Dust intake (ng/d)[a]								Diet intake (ng/d)[b]								Air intake (ng/d)[a]			
	Average				High				Average				High							
	α	β	γ	∑	α	β	γ	∑	α	β	γ	∑	α	β	γ	∑	α	β	γ	∑
Adult (70 kg)	46.6	15.3	69.6	131.5	116.4	38.3	174.0	328.7	203	105	112	413	385	269	217	840	1.2	0.6	3.2	5.0
Toddler (10 kg)	144.7	47.2	212.0	403.8	557.5	178.9	736.7	1473.1	120	57	67	240	240	110	140	500	0.2	0.1	0.6	1.0

[a] Abdallah et al. (2008b).
[b] U.K. Food Standards Agency (2006).

For example, in the liver 34% of the administered dose of α-HBCD was found after 3 h (Szabo et al., 2011b). Possible explanations for this were a greater pinocytic activity, higher stomach pH and differences in blood flow in the immature GI tract of young mice. The difference in the composition of tissues was also mentioned as a factor for the altered HBCD distribution (Szabo et al., 2011b).

As HBCD has toxic effects during development, higher body burden in early stages of development are of particular concern when assessing the toxicity of HBCD. This becomes even more important when taking into account that toddlers consume significant amounts of indoor dust, which contain a high quantity of HBCD. Thus, research should focus especially on this age group to evaluate the health risk of HBCD to humans.

One explanation for the high concentration of α-HBCD, especially over a long period of time in adipose tissue, is the bioisomerization of HBCD diastereomers, which was first reported in biota (rainbow trout) by Law et al. (2006), who did not only find the applied diastereomer, but also the other diastereomers in their test system. Especially bioisomerization from β- (Law et al., 2006) and γ-HBCD to α-HBCD (Szabo et al., 2010; Law et al., 2006; Fournier et al., 2012; Luo et al., 2013) is important for the enrichment of α-HBCD. Bioisomerization was also observed from β-to γ-HBCD (Sanders et al., 2013) and the other way round (Szabo et al., 2010; Law et al., 2006). However isomerization from α-HBCD to any other diastereomer has not been found *in vivo* until now (Szabo et al., 2011a) and is thermally less favoured (Heeb et al., 2008; Köppen et al., 2008). Fournier et al. (2012) suggested that isomerization from γ-HBCD to α-HBCD may take place in the liver. *In vivo* stereoisomerization of γ-HBCD to β- and α-HBCD in mice accounts for 11–15 % of the given dose. Thus, it appears to be unlikely that bioisomerization is the only factor responsible for the shift from a predominance of γ-HBCD in the technical product to α-HBCD in biota (Szabo et al., 2011a).

A second possible factor is the slower degradation of α-HBCD compared to β- and γ-HBCD (Szabo et al., 2010, 2011a; Sanders et al., 2013). Sanders et al. (2013) calculated similar half-lives for β-HBCD than Szabo et al. (2010) did for γ-HBCD, but significantly shorter than those for α-HBCD (Szabo et al., 2011a). For example, *in vivo* half-lives in adipose tissue of mice after a single dose of 3 mg/kg-BW are 2.5 d for β- (Sanders et al., 2013), 3.6 d for γ- (Szabo et al., 2010) and 17 d for α-HBCD.

Zegers et al. (2005) documented the *in vitro* degradation of the three diastereomers using rat and harbour seal liver microsomes, in combination with isolated diastereomers and an artificial 1:1:1 HBCD mixture. The obtained half-lives for γ- and β-HBCD were significantly shorter than for α-HBCD. Similar results were also obtained by Huhtala et al. (2006) and Abdallah et al. (2014). In contrast, Esslinger et al. (2011b) found shorter half-lives for β-HBCD compared to γ- and α-HBCD in their experiments using rat liver microsomes. Accordingly, these results do not suggest enrichment of α-HBCD due to its slower degradation during the phase I metabolism (Esslinger et al., 2011b).

The half-lives of the γ-HBCD enantiomers led to an enrichment of the (+)- (32.3 min) compared to the (−)-enantiomer (11.6 min), whereas no difference could be detected for (−)- and (+)-β-HBCD. Enantioselectivity was also observed for α-HBCD, leading to an enrichment of the (−)-enantiomer (24.4 min for (−)- and 14.1 min for (+)-α-HBCD) (Esslinger et al., 2011b). Abdallah et al. (2014) also found a significant enrichment of (−)-α-HBCD, but no preference for γ- and β-HBCD. The enrichment was more profound for (−)-α-HBCD in rat (EF of 0.321) than in trout (EF of 0.491 after 60 min) (Abdallah et al., 2014). Further research should be conducted on the degradation of the individual enantiomers in different species to unravel possible general patterns of microsomal degradation.

HBCD metabolism is mediated by enzymes of the cytochrome P450 system (CYP450) (Zegers et al., 2005) which are important during the stereoselective phase I oxidative metabolism of HBCD (Abdallah et al., 2014). Another important pathway of HBCD seems to be the sequential reductive debromination of HBCD to pentabromocyclododecane (PBCD) and tetrabromocyclododecane (TBCD), which is not catalysed by CYP450 (Abdallah et al., 2014). It was hypothesized that the deiodinase may be involved in the debromination of HBCD (Hakk et al., 2012) and that it is tightly coupled with the CYP450s and that they prefer γ-HBCD as a substrate compared to α-HBCD, because only debrominated and oxidized metabolites but no hexabrominated form of γ-HBCD were detected in tissues or faeces in mice (Hakk et al., 2012). However, more research is required about potential enzymes for HBCD degradation.

Hakk et al. (2012) performed a study with the three individual diastereomers and found different metabolic patterns for γ- and α-HBCD, but no metabolic products of β-HBCD. As biotransformation of HBCD seems to be very species-specific in terms of speed and different metabolites (Esslinger et al., 2011b; Abdallah et al., 2014; Brandsma et al., 2009), other test organisms may show metabolites of β-HBCD. This obvious species-specificity stresses the need for more basic research, but is similarly a warning sign that results from animal studies are not so easily transferred to humans.

A faster degeneration of γ-HBCD is congruent with the fact that its extent of metabolism is greater than the one of α-HBCD (Hakk et al., 2012) (Table 3). The metabolism of α-HBCD resulted in only monohydroxylated hexabrominated metabolites in liver, brain, adipose tissue and faeces, whereas metabolites in urine were glutathione conjugates of either tribromohexene or tetrabromohexene (Hakk et al., 2012). γ-HBCD on the other hand showed a greater variety of metabolites in mice. Many different hydroxylated metabolites were found in liver, adipose tissue and faeces. Hydroxylated metabolites in faeces did not only show hydroxylation but also dehydrogenation. γ-HBCD also underwent ring-opening and oxidation afterwards, which led to hexabromo dodecanedioic acid, a dicarboxylic acid. This acid was probably decarboxylated and entered β-oxidation (tribromo nonenoic acid) (Hakk et al., 2012). Hakk et al. identified a metabolite of γ-HBCD in the volatile fraction of urine as methyl mercapturate of tetrabromocyclododecadiene. They suggested that this metabolite was formed through the reductive debromination of monohydroxylated pentabromocyclododecene (OH-PBCDe) and its entry into the

Table 3
Metabolites of HBCD in mice (compare Hakk et al., 2012).

Name and molecular formula	Compartment	No. of isomers
Metabolites of α-HBCD		
OH-HBCD ($C_{12}H_{18}OBr_6$)	Liver, brain, adipose	1
	Feces	4
Glutathione of tribromohexene ($C_{16}H_{24}N_3O_6SBr_3$)	Urine	1
Glutathione of tetrabromohexene ($C_{16}H_{23}N_3O_6SBr_4$)	Urine	1
Metabolites of γ-HBCD		
OH-PBCD ($C_{12}H_{19}OBr_5$)	Liver, adipose	1
OH-PBCDe ($C_{12}H_{17}OBr_5$)	Feces	4
diOH-PBCDe ($C_{12}H_{17}O_2Br_5$)	Feces	3
diOH-PBCDee ($C_{12}H_{15}O_2Br_5$)	Feces	2
Hexabromo dodecanedioic acid ($C_{12}H_{16}O_4Br_6$)	Urine	3
Tribromo nonenoic acid ($C_9H_{13}O_2Br_3$)	Urine	1
Methylmercapturate of tribromo nonenoic acid ($C_{15}H_{22}NO_5SBr_3$)	Feces	1
Methylmercapturate of tetraBCDee ($C_{18}H_{25}NO_3SBr_4$)	Urine (volatile fraction)	1

mercapturic acid pathway (Hakk et al., 2012). Zhang et al. (2013) described that the dihydrodiol dehydrogenase was induced after exposure to HBCD, which suggests a participation of this enzyme in the metabolic pathway of HBCD.

It is important to note that metabolites like PBCD and TBCD are also present in *t*HBCD and abiotic matrices such as sediment and indoor dust (Harrad et al., 2009a; Abdallah et al., 2008c; Heeb et al., 2012). Hence, it may be difficult to distinguish between metabolites, which are produced in organisms and metabolites, which are already existent in the environment. However, according to Abdallah and Harrad (2009) the uptake of metabolites (1.4 ng/d for PBCD and 0.2 ng/d for TBCD) via dust intake is relatively small compared with the uptake of HBCD (48 ng/d).

8. Toxicodynamics of HBCD

Knowledge on the toxicodynamics of HBCD is still rather limited. Although most studies performed so far indicate a toxicological potential, adverse effects of HBCD on plants as well as a differentiation between individual isomers are poorly understood. Wu et al. (2010) have performed the only toxicity study of HBCD on plants up until now. Using maize, the authors demonstrated that α-HBCD inhibited root and shoot elongation, and biomass gain (Wu et al., 2010). Additionally, α-HBCD (conc.: 2 μg/L) also showed the strongest inhibition of seed germination after 96 h (29.44%) compared with β- (14.09%) and γ-HBCD (11.27%) and caused the strongest generation of endogenous reactive oxygen species (ROS). Histone H2AX phosphorylation was observed as well, indicating DNA damage through HBCD, and again particularly by α-HBCD (Wu et al., 2010).

Concerning the effects on animals, different species have been investigated so far. Zhang et al. (2013) for example found increased transcription of the catalase gene in the gills of clams after exposure to HBCD. Catalase is important because of its ability to protect cells against H_2O_2-mediated oxidative stress, which is a result of the increased ROS concentration following exposure to HBCD (Du et al., 2012). They also found two up-regulated genes, which include hemocyanin and C-type lectin. Both proteins are involved in the immune system of molluscs (Zhang et al., 2013). Koike et al. (2013) characterized the *in vitro* effects of BFRs on mouse immune cells. HBCD was the only BFR, which tended to decrease the percentage of macrophages and dendritic cells in splenocytes and thus may induce cell death in these cell types. Zhang et al. (2008) also observed cytotoxicity in human Hep G2 cells, where γ-HBCD showed stronger effects than β- and α-HBCD and all (+)-enantiomers were significantly more cytotoxic than the corresponding (−)-enantiomers. HBCD induced the expression of different cell surface molecules (TCR, MHC class II, CD11c, CD80 and CD86) and the production of cytokines (interleukin-4), hence HBCD may accelerate the immune system and may lead to allergic reactions (Koike et al., 2013). The maturation and function of bone marrow derived dendritic cells (BMDC), which show an increased expression of surface molecules after exposure to HBCD, are regulated by thyroid hormones. HBCD is well known for its ability to alter the composition of these hormones, which may lead to an accelerated maturation of BMDCs (Koike et al., 2013).

Most BFRs and also HBCD are disruptors of the thyroid homoeostasis. Effects of HBCD on the thyroid axis were first described by Chengelis (1997). The level of the circulating thyroid hormone (TH) thyroxine (T4) decreased in rats after exposure to 100 mg/kg-BW/d HBCD (Chengelis, 1997). Van der Ven et al. (2006) confirmed these findings and described an increased pituitary weight, an increased immunostaining of thyrotropin (TSH, thyroid-stimulating hormone) and an increased thyroid weight, but only in female rats. Increased liver weight and induction of UDP-glucuronic acid transferase (UGT) were observed, too (van der Ven et al., 2006). The conjugation to glucuronic acid is a major excretory pathway for T4. Palace et al. (2008) distinguished between the individual diastereomers and found an induction of T4-glucuronyl transferase for α- and β-HBCD. Although γ-HBCD lowered the concentration of circulating T4 and increased the concentration of triiodothyronine (T3), this effect was also seen when juvenile rainbow trout were exposed to α- and β-HBCD (Palace et al., 2008). Germer et al. (2006) showed that hepatic CYP2B and CYP3A were induced by HBCD especially in female rats. The pregnane-X receptor (PXR) and/or the constitutive androstane receptor (CAR) are responsible for this induction. Fery et al. (2010) identified HBCD as a common activator of the human PXR in Hep G2 cells. Phase II enzymes like UGT, the major enzyme for T4-conjungation, are also likely induced by HBCD through PXR and CAR (Palace et al., 2008). Palace et al. (2008) observed a lower T4 outer-ring deiodinase (T4ORD) activity, which normally converts T4 to T3. This fact may be due to a negative feedback because HBCD increases the concentration of free T3 (FT3) and therefore no more T4ORD is required (Palace et al., 2008). Lower concentrations of free T4 (FT4) may lead to an increased production of TSH as a feedback mechanism (Palace et al., 2008), which was already confirmed after exposure to HBCD (van der Ven et al., 2006). This may lead to higher concentrations of FT3 and thyroid gland hypertrophy. Palace et al. (2008) found that the height of the thyroid epithelial cells increased significantly, especially after the exposure to γ-HBCD, which showed the highest concentration of FT3.

Although HBCD seems to influence the thyroid axis, it also shows effects on other systems (e.g. a potentially higher risk for diet-induced obesity; see Yanagisawa et al., 2014). Saegusa et al. (2012) investigated the impact of HBCD on neuronal development in the hippocampus of mice and observed some abnormal alterations. The number of the "neuron-specific nuclear protein"-positive mature neurons was increased on PND 77 in the dentate hilus and apoptosis in the subgranular zone on PND 20 was enhanced (Saegusa et al., 2012). These results together with the high exposure of toddlers to HBCD and their higher body burden again underline the special need to consider younger age groups when assessing potential risks of HBCD. This is also highlighted by results of Ibhazehiebo et al. (2011a), who discovered that low-dose HBCD could potentially suppress the TH receptor-mediated transcription and impair the TH-induced dendrite arborisation of Purkinje cells, which is also important for the developing brain. This may be at least in part due to a disruption of the T3-stimulated increase in brain-derived neurotrophic factor (BDNF). However, an external administration of BDNF may counteract this disruption (Ibhazehiebo et al., 2011b). Concerning the thyroid homoeostasis in new-borns, Eggesbø et al. (2011) were interested in a potential association between HBCD in breast milk and TSH changes in neonates, but could not find any, which again stresses the need for further research on this particular population group.

Al-Mousa and Michelangeli (2012, 2014) assessed the acute neurotoxicity of various BFRs in human SH-SY5Y cells. HBCD was most potent in inducing cell death via apoptosis through the intrinsic pathway as determined by mitochondrial release of cytocrhome c, mitochondrial membrane depolarization and activation of caspases (Al-Mousa and Michelangeli, 2012). A major contributor to cell death was the inhibition of the sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase (SERCA). HBCD seems to affect SERCA through different mechanisms: altered ATP binding, phosphorylation and E2 to E1 transition step (Al-Mousa and Michelangeli, 2014).

Johnson et al. (2013) found an association between increased concentrations of HBCD in house dust and an increase of the free androgen index (and a decreased level of sex hormone-binding

globulin) in men. HBCD was also shown to display oestrogen-like effects on MCF-7 cells (Dorosh et al., 2011).

Fernie et al. (2011) observed reproductive changes in American kestrels, in relation to exposure to HBCD, in environmentally relevant concentrations. The exposed kestrels laid earlier, larger clutches of smaller eggs than the control group (Fernie et al., 2011). Marteinson et al. (2011) also worked with American kestrels and observed heavier testes and a trend towards seminiferous tubules containing elongated spermatids after exposure to HBCD. The plasma testosterone concentration was increased as well (Marteinson et al., 2011).

As stated above, individual diastereomers exhibit different effects on organisms. Hamers et al. (2006) observed the impact of HBCD on the estrogen receptor (ER) and found antagonistic responses for γ- ($IC_{50} = 4.9$ μM) and β-HBCD ($IC_{50} = 11.0$ μM), but no effects for α-HBCD. However, α-HBCD caused the strongest antagonistic responses on the dioxin receptor (DR) and androgen receptor (AR) (Hamers et al., 2006). Du et al. (2012) performed a study on zebrafish, where a diastereomer-specific toxicity of HBCD was observable, too. The heart rate of zebrafish embryos decreased at concentrations of 0.1 mg/L β- and γ-HBCD, but α-HBCD only exhibited a response after the concentration was raised to 1.0 mg/L. On the other side, α- and β-HBCD (1.0 mg/L) decreased the heart rate in zebrafish larvae (96 h after fertilization), but γ-HBCD increased the heart rate (Du et al., 2012).

Consequently, it is currently difficult to differentiate which diastereomer has the highest toxic potential or has to be considered as being most harmful to the environment, which again stresses the need for more research on the toxicity of HBCD.

9. Conclusion

HBCD is a persistent BFR, which will still be found in the environment after its production and use have been prohibited. Currently, it may be produced and used in buildings until 2024. Thus, it is important to continue the research on HBCD. Many studies have been performed on the bioaccumulation of HBCD in animals, especially birds and water organisms, however, only a few studies were conducted on plants. Due to their functional role as primary producers representing the basis of all food webs, more research should be conducted on plants. Although the kinetics and toxicology of HBCD gained more attention in recent years, not much is known about both fields, especially when it comes to distinguishing the different diastereomers from the individual enantiomers. It is also important to look for alternate flame retardants. Polymers may be one alternative because of their theoretically lower biological availability. In conclusion, it appears that HBCD still remains an important topic for future studies.

Acknowledgement

We thank Christian Selbach (University Duisburg-Essen) and Nikara Mahadeo for proofreading this manuscript.

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Appendix II: Environmental concentrations and toxicology of 2,4,6-tribromophenol

Within this publication, the currently available literature regarding environmental effects and concentrations of one of the possible degradation products of “Polymeric FR” – 2,4,6-tribromophenol – is summarized.
Manuscript as published.

Environmental Pollution 233 (2018) 706–713

Contents lists available at ScienceDirect

Environmental Pollution

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

Environmental concentrations and toxicology of 2,4,6-tribromophenol (TBP)☆

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ARTICLE INFO

Article history:
Received 7 September 2017
Received in revised form
29 October 2017
Accepted 29 October 2017
Available online 7 November 2017

Keywords:
Tribromophenol
Sources
Toxicology
Concentrations
Humans

ABSTRACT

2,4,6-Tribromophenol is the most widely produced brominated phenol. In the present review, we summarize studies dealing with this substance from an environmental point of view. We cover concentrations in the abiotic and biotic environment including humans, toxicokinetics as well as toxicodynamics, and show gaps of the current knowledge about this chemical.

2,4,6-Tribomophenol occurs as an intermediate during the synthesis of brominated flame retardants and it similarly represents a degradation product of these substances. Moreover, it is used as a pesticide but also occurs as a natural product of some aquatic organisms. Due to its many sources, 2,4,6-tribromophenol is ubiquitously found in the environment. Nevertheless, not much is known about its toxicokinetics and toxicodynamics. It is also unclear which role the structural isomer 2,4,5-tribromophenol and several degradation products such as 2,4-dibromophenol play in the environment. Due to new flame retardants that enter the market and can degrade to 2,4,6-tribromophenol, this compound will remain relevant in future years – not only in aquatic matrices, but also in house dust and foodstuff, which are an important exposure route for humans.

1. Introduction

Among the brominated phenols 2,4,6-tribromophenol (TBP; CAS No. 118-79-6; Fig. 1) is the most widely produced substance (WHO, 2005). It is typically produced in closed reactors by a non-aqueous process and discharged as a melt. This melt is then cooled and pelleted for easy handling (WHO, 2005). In addition to its industrial production, it is also known to be a naturally occurring molecule in different marine organisms, which produce it as a defense against predators and biofouling (Sheikh and Djerassi, 1975; Whitfield et al., 1999). However, in environmental research mainly anthropogenically produced and emitted TBP is studied, which derives as an intermediate during the synthesis of flame retardants (FR) (Ballesteros-Gómez et al., 2014; Ren et al., 2017) or is used as a fungicide and wood preservative (Nichkova et al., 2008; Offret et al., 2016; Savory et al., 1970). Its production volume was estimated in 2001 to account for 9100 t per year worldwide (OECD, 2004). In 2012, health concerns have led to an evaluation of TBP under REACH (European Regulation (EC) No 1907/2006; REACH, 2006). This ECHA (European Chemicals Agency) report focused on several endpoints, but contains no conclusion due to the limited amount of available studies (ECHA, 2016). With the current review, we summarize recent publications concerning the environmental presence and toxicology of TBP. An updated knowledge on TBP is particularly important as TBP has been identified as a possible degradation product of both legacy (Barontini et al., 2004a, 2004b) and novel FRs (Koch et al., 2016) and might therefore still be emitted to the environment via new routes – even if the aggregated tonnage of anthropogenically produced TBP in the European Union decreased from 10,000–100,000 t to 1–10 t between 2012 and 2016 (REACH, 2006). Additionally, it was recently stated that TBP concentrations determined in the field pose an ecotoxicological risk to the environment (Xiong et al., 2016).

2. Environmental concentrations and behavior

TBP ends up in the environment through different routes (Fig. 2). On the one hand, it can enter the environment unintentionally as a degradation product of brominated flame retardants

☆ This paper has been recommended for acceptance by Charles Wong.

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https://doi.org/10.1016/j.envpol.2017.10.127
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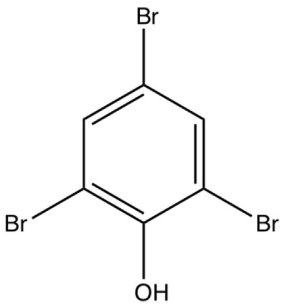

Fig. 1. Chemical structure of 2,4,6-tribromophenol (TBP).

(BFR) (WHO, 2005) or as an intermediate (Bergman, 1990) during the synthesis of these compounds (Ren et al., 2017). On the other hand, TBP is intentionally used as a pesticide and wood conservative in South America (WHO, 2005) due to its known biocidal effects (Nichkova et al., 2008; Offret et al., 2016). Additionally, TBP and other bromophenols are responsible for some of the typical flavors in marine seafood (Boyle et al., 1992). Therefore, TBP can also be part of the manufactured feed for cultivated shrimp that would otherwise not have access to this substance through their diet (Ma et al., 2005; Whitfield et al., 1997a) and can thus be considered as an indirect food additive. However, TBP is not only a molecule produced by humans, but appears naturally in several organisms as part of a defense mechanism against biofouling as well (Whitfield et al., 1999).

Despite a dispute about the role of TBP as an additive FR itself (Lee et al., 2016; Liu et al., 2011; Ríos et al., 2003), a clear focus is on its role as an intermediate (WHO, 1997) during the synthesis of other FRs like tetrabromobisphenol A (TBBPA) (Ren et al., 2017) or 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine (TTBP-TAZ) (Ballesteros-Gómez et al., 2014). In the 1980s, TBP was also detected in emissions from waste incinerators (Öberg et al., 1987) and in automotive emissions from leaded gasoline (Buser, 1986; Muller and Buser, 1986). Bromophenols were also shown to arise from the degradation of other environmental contaminants (Bergman, 1990) such as brominated benzenes and polybrominated diphenyl ethers (PBDE) (Wan et al., 2009). Particularly TBP was identified as a degradation product of BFRs such as TBBPA (Barontini et al., 2004a, 2004b; Eriksson et al., 2004) and possibly the new PolyFR (Koch et al., 2016), which is now commercially being used as a substitute for hexabromocyclododecane (HBCD) in polystyrene foam. TBP has also recently been detected in many TVs in the United States – which might be due to a degradation of TTBP-TAZ (Schreder, 2017). Additionally, the structural isomer of 2,4,6-TBP – namely 2,4,5-TBP (Fig. 3) – is often found exclusively as a degradation product of other FRs following biodegradation (Chen et al., 2006; Eriksson et al., 2004; Krieger et al., 2017, 2016; Stapleton et al., 2009; Zheng et al., 2015).

In addition to these unintended sources, 2,4,6-TBP is utilized as a fungicide and wood preservative as well due to its known biocidal effects (WHO, 2005) that are used by different marine organisms (Nichkova et al., 2008; Offret et al., 2016; Ríos et al., 2003; Savory et al., 1970). Since its detection in the marine environment in the 1970s (Sheikh and Djerassi, 1975), TBP was found in a huge variety of marine organisms similar to at least 50 other brominated phenols (Gribble, 2000). It was for instance detected in marine algae (e.g. *Ulva lacuta*; Flodin et al., 1999; Flodin and Whitfield, 1999a),

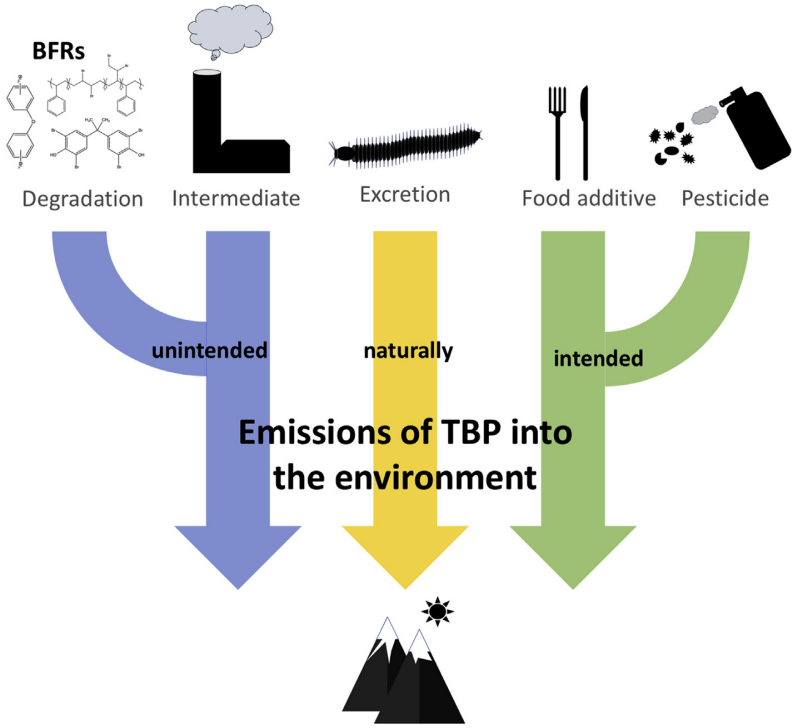

Fig. 2. TBP enters the environment either intentionally or unintentionally due to human activity and from natural sources.

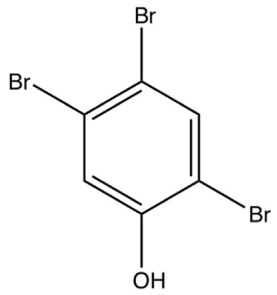

Fig. 3. Chemical structure of 2,4,5-tribromophenol (2,4,5-TBP).

sponges (Whitfield et al., 1997) (e.g. *Phorbas glaberrimus*; Vetter and Janussen, 2005), phoronids (e.g. *Phoronopsis viridis*; Sheikh and Djerassi, 1975), mollusks (e.g. *Tapes philippinarum* and *Ostrea rivularis*; Chung et al., 2003), crustaceans (e.g. *Metapenaeus ensis* and *Charybdis feriatus*; Chung et al., 2003; Whitfield et al., 1988), hemichordates (e.g. *Ptychodera flava* and *Balanaglossus carnosus*; Higa and Scheuer, 1980; Sheikh and Djerassi, 1975), and fish (e.g. *Siganus canaliculatus* and *Epinepheus areolatus*; (Chung et al., 2003; Whitfield et al., 1998). Interestingly, only some of these groups actually synthesize TBP. Algae, bryozoans, polychaetes (where the synthesis depends very much on the respective species; Whitfield et al., 1999), and hemichordates are for instance known to produce and excrete TBP (among other bromophenols) as a defense against predators and biofouling (Flodin and Whitfield, 1999b; Jensen et al., 1992; King, 1988, 1986; Sharief and Azariah, 1997; Sheikh and Djerassi, 1975; Whitfield et al., 1999; Yamada et al., 2008). Members of other taxonomic groups (such as fishes, shrimps, and crabs) take up and magnify TBP via ingestion through the food chain (Boyle et al., 1992; Whitfield et al., 1998, 1997a). The concentrations of TBP in some species show seasonal variations, which is congruent with seasonal growth cycles of TBP-synthesizing organisms such as the green marine alga *Ulva lactuca*, in which concentrations vary between summer and winter by a factor of 10–100 (Chung et al., 2003; Flodin et al., 1999). In contrast, the TBP concentrations in the marine polychaete *Lanice conchilega* are not varying with season (or sex and weight of the individual; Goerke and Weber, 1991), but are locality-dependent (Goerke and Weber, 1990). Exemplary concentrations of TBP in the marine environment are shown in Table 1.

Additionally, TBP has been detected in birds and mammals such as albatrosses, polar bears (Wan et al., 2009), seals (Vetter and Janussen, 2005), and cats (Engdahl et al., 2017; Nomiyama et al., 2017).

TBP has also ubiquitously been detected in abiotic matrices like soil (Ronen et al., 2005), sewage sludge (0.3–0.9 ng/g ww; Öberg et al., 2002), surface water (non-detected (ND)–21 ng/L; Blythe et al., 2006; Polo et al., 2006; Reineke et al., 2006), riverine systems (average of 0.66 ng/g dw; Sim et al., 2009), groundwater (Blythe et al., 2006), and sediments from freshwater (Rhône, France; 26–3690 ng/g dw; Tolosa et al., 1991) and marine habitats (Busan, South Korea; average of 3.02 ng/g dw; Sim et al., 2009) – but not in sediment extracts from the North Sea (Reineke et al., 2006). Concentrations of TBP in unrefined sea salt were measured to be 18.2 ng/g (Boyle et al., 1992). It was also detected in outdoor (49–73 pg/m^3; Takigami et al., 2009) and indoor air (220–690 pg/m^3; Takigami et al., 2009; Thomsen et al., 2001a), and house dust (15–620 ng/g; Suzuki et al., 2008; Takigami et al., 2009). Especially house dust is well known to be one of the major exposure pathways of BFRs for humans as described below (Abdallah et al., 2008; Harrad et al., 2010).

Within the ECHA report, it was stated that TBP fulfils the screening criteria for being potentially persistent (P) or very persistent (vP) (ECHA, 2016). Regarding the abiotic degradation of TBP, only a limited amount of data is published. It was predicted, that TBP is degraded in the air by photochemically produced hydroxyl radicals with a half-life of 34 days (Meylan and Howard, 1993). Because of its lack of hydrolysable functional groups, TBP is not expected to undergo hydrolysis in water (WHO, 2005), thus aquatic degradation is mainly due to biotic degradation, which will be covered later.

3. Concentrations in humans and food

TBP has also been found in human maternal (22 pg/g ww) and cord blood (37 pg/g ww; Kawashiro et al., 2008), as well as in blood samples of both sexes (81 pg/mL lw; Dufour et al., 2017; Thomsen et al., 2002a, 2001b). High levels of TBP were for instance detected in residents near the coastal area in India (360 pg/g ww; Eguchi et al., 2012). It was concluded that exposure to TBP is likely to be

Table 1
Concentrations of TBP in aquatic organisms based on wet (ww), dry (dw) or lipid weight (lw).

Species/Group	Location	Concentration (ng/g)	Comments	Reference
Algae	Australia	10–1600 (ww)		(Flodin et al., 1999)
		7–16 (ww)		(Flodin and Whitfield, 2000)
Bryozoa	Australia	17–1100 (ww)		(Whitfield et al., 1999)
Annelida (Polychaetes)	Australia	24–8,300,000 (ww)		(Whitfield et al., 1999)
	Germany	810 (ww)		(Goerke and Weber, 1990)
		40–50 (ww)		(Goerke and Weber, 1991)
	France	3220 (ww)		(Whitfield et al., 1999)
Hemichordata (Acorn worm)	Norway	500–7000 (ww)		(Jensen et al., 1992)
Arthropoda (Crustacean)	Australia	2.4–270 (ww)		(Whitfield et al., 1988)
		ND–170 (ww)	Wild-harvested	(Whitfield et al., 1997a)
		ND–0.53 (ww)	Cultivated	(Whitfield et al., 1997a)
	Hong Kong	0.32–2360 (dw)		(Chung et al., 2003)
	USA	ND–18.9 (dw)		(Boyle et al., 1992)
Mollusca	Hong Kong	1.37–198 (dw)		(Chung et al., 2003)
	USA	0.9–2.1 (dw)		(Boyle et al., 1992)
Chordata (Fish)	Australia	ND–6.1 (ww)	Pelagic carnivores	(Whitfield et al., 1998)
		ND–230 (ww)	Benthic carnivores	(Whitfield et al., 1998)
	Hong Kong	2.18–129 (dw)		(Chung et al., 2003)
	USA	3.7–33.2 (dw)		(Boyle et al., 1992)
		130 (lw)		(Jaffe and Hites, 1986)

more dependent on food ingestion than on the occupational setting, as no difference between blood concentration of different occupational groups was found (Thomsen et al., 2001b). However, a significant difference was found between residents near an e-waste recycling site (270 pg/g ww) and the reference site (220 pg/g ww) in Vietnam (Eguchi et al., 2015) – but not in India (110 pg/g and 360 pg/g ww respectively; Eguchi et al., 2012). In all these studies, the congener profile of analyzed bromophenols is dominated by 2,4,6-TBP (Ali et al., 2013; Dufour et al., 2017; Eguchi et al., 2015). Until now, neither an age-related trend nor increasing concentrations over time have been found for archived human blood samples collected between 1977 and 1999 (Thomsen et al., 2002b) or 1989 and 2010 (Fujii et al., 2014). TBP was also detected in human breast milk (up to 110 ng/g lw; Ohta et al., 2004), urine (calculated median of 200–520 ng/L; Feng et al., 2016; Nichkova and Marco, 2006), and placental tissue (1.31–316 ng/g lw with a geometric mean of 15.4 ng/g lw; Leonetti et al., 2016b), but not in adipose tissue (detection limit of 0.5 ng/g lw; Smeds and Saukko, 2003).

Besides house dust, the dietary uptake is one of the major exposure pathways of BFRs for humans (Knutsen et al., 2008; Sahlström et al., 2015). However, only a limited amount of studies has focused on TBP in terrestrial plants or food derived from plants. In one study, TBP was detected in the edible part of *Asparagus officinalis* (Mardones et al., 2003). TBP was also detected in pears (Wylie, 1997), sultanins (Whitfield et al., 1997b) and wine (Chatonnet et al., 2004), where it seems to play a role in the formation of a pungent musty odor. In this study, it was shown that this odor is strongest in barrels made out of wood that were previously impregnated with TBP (Chatonnet et al., 2004). Concentrations of up to 2000 μg/L have been reported for treated wood used in the food industry (Nichkova et al., 2008). This was also assumed to be the source of TBP in pears (Wylie, 1997). In a recent report focusing on BFRs in foodstuff in Belgium, 183 food samples were analyzed. TBP was detected most often among the selected BFRs (72% of all samples; Van Loco et al., 2017). Five years before the release of this report, the EFSA (European Food Safety Authority) had concluded that it was not possible to carry out a meaningful dietary exposure assessment for the general population. However, they have published a tentative dietary exposure scenario of 39 ng/kg body weight (bw) per day for high consumers of fish, mollusks and crustaceans (EFSA, 2012).

4. Biosynthesis

As described previously, TBP is produced naturally by different groups of organisms, such as bryozoans, algae or polychaetes (Flodin and Whitfield, 1999b; Whitfield et al., 1999). Although only a limited number of studies focused on the underlying mechanisms of the TBP biosynthesis, it appears that haloperoxidases are a main group of enzymes responsible for the production of TBP. It was shown that (vanadium-containing) bromoperoxidases (Itoh et al., 1986) brominate phenol and *o*-hydroxybenzyl alcohol in the presence of H_2O_2 (Collén et al., 1994) and bromine in coralline algae to yield TBP (Yamada et al., 1985). Bromoperoxidase was also identified as the enzyme brominating phenol, 4-hydroxybenzoic acid, and 4-hydroxybenzyl alcohol in the green marine alga *Ulva lactuca* (Flodin and Whitfield, 1999b). However, L-tyrosine and 4-hydroxybenzaldehyde, that were previously suggested as possible substrates (Higa and Scheuer, 1980; King et al., 1995), were not brominated to TBP under the applied circumstances. Interestingly, the bromoperoxidase activity showed seasonal variations. The enzyme was most active in summer and decreased drastically in winter. This seasonal variation was also reflected in the bromophenol concentration (Flodin et al., 1999).

A second group of haloperoxidases, a flavin-containing chloroperoxidase found in the polychaete *Notomastus lobatus*, was also shown to be able to brominate phenol in order to produce TBP (Chen et al., 1991). It was suggested that the production of bromophenols in general is a highly conserved mechanism among many marine organisms. Therefore, biomagnification and increased environmental concentration of TBP are not necessarily only due to anthropogenic emissions but may also be assigned to TBP synthesizing taxa.

5. Bioaccumulation

In general, information concerning the toxicokinetics of TBP is largely missing. Studies mentioning bioaccumulation of TBP are usually dealing with TBP-synthesizing taxa, for which it is not surprising that "accumulation" takes place (Goerke and Weber, 1990). In one study, pieces of *Lanice conchilega*, a TBP synthesizing polychaete, were fed for five weeks to three different groups of marine invertebrates (Goerke and Weber, 1991). A maximum of 3% of the TBP dose applied was detected after this period, which indicates that TBP is not accumulated in these groups.

According to Muir and Howard (Muir and Howard, 2006), a log $K_{ow} \geq 5$ is a common screening criterion for bioaccumulation in marine animals. In regard to bioconcentration (BCF) and bioaccumulation factors (BAF), a factor > 5,000 is often applied during screening. Based on a log K_{ow} of 4.24 for TBP (with a pK_a of 6.08; Kuramochi et al., 2004) and a BCF of 513 (Devillers et al., 1996) and 83 (Spehar et al., 1980) in zebrafish and fathead minnow, respectively, no or a rather low potential for bioaccumulation can be expected for TBP in marine animals. This is consistent with the very low level of bioaccumulation that was detected in another study with zebrafish (Haldén et al., 2010). Here, only 0.1% of the total oral dose of TBP was recovered in fish after six weeks of exposure. TBP was also detected in fish eggs after six weeks of exposure, however in even lower concentrations.

In pet cats, it was described that TBP concentrations were one magnitude higher in the bile than in blood, liver, and brain. The fact that TBP was detected in the brain indicates its ability to bypass the blood-brain barrier (Nomiyama et al., 2017). A correlation between serum levels of TBP and the TBP content in cats food was also detected (Engdahl et al., 2017).

After a single oral dose of 4–5.3 mg/kg bw, TBP was rapidly absorbed in rats. The TBP concentration in blood reached its maximum after 1 h and decreased already again after 2 h. Only 0.01% of the dose were left in the tissue after 48 h. The half life in blood was estimated at 2 h. TBP was mainly excreted via urine (50–91% after 48 h; WHO, 2005).

6. Biodegradation

The capability to degrade TBP has been described for different species, of which most were fungi and bacteria (Boyle et al., 1999; Donoso et al., 2008; Ronen et al., 2005, 2000; Yamada et al., 2008; Zu et al., 2012). Interestingly, these species naturally occur in a variety of habitats such as desert soil, forest, or estuarine sediment – and were thus not only found in marine matrices associated with TBP synthesizing species. It was shown that more than 90% of TBP was dehalogenated within two days in marine sediment slurry (King, 1988). Similar to this study, most studies found 2,4-dibromophenol (2,4-DBP) as an intermediate. In addition to 2,4-DBP, also 2,6-DBP has been identified as a degradation product (Abrahamsson and Klick, 1991). Steward et al. found that an anaerobic bacterium preferred to remove bromine in *ortho*-positions, which resulted in the transient intermediate 2,4-DBP and the eventual accumulation of 4-bromophenol (4-BP) (Steward et al., 1995).

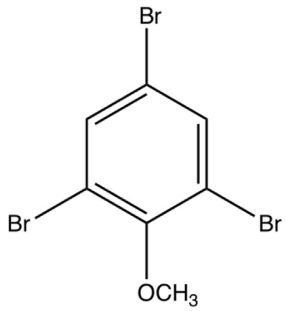

Fig. 4. Chemical structure of 2,4,6-tribromoanisole (TBA).

Another typical degradation product of TBP is 2,4,6-tribromoanisole (TBA; Fig. 4). The fungi *Agaricus augusus* is able to transform the fungicide TBP to TBA, possibly via methyltransferase-type enzymatic activity. Similar results were found for another fungi, *Trametes versicolor*, which was able to degrade TBP to TBA in the presence of an additional carbon source, while showing peroxidase and laccase activity (Donoso et al., 2008). In bacteria, it was observed that TBP is completely degraded via reductive debromination to phenol with 2,4-DBP, 2,6-DBP, 2-BP, and 4-BP being consecutive intermediates (Boyle et al., 1999; Yamada et al., 2008). Also, TBP was degraded to TBA through O-methylation (Allard et al., 1987). Biotransformation from TBP to TBA was observed in fish as well (Haldén et al., 2010). In human urine, 2,4,6-tribromophenyl glucuronide was detected, which could be a potential phase II metabolite of TBP (Ho et al., 2012). Interestingly, it has been suggested that TBP can be metabolized via bromoperoxidase by primary producers (algae e.g.) to environmentally relevant brominated dioxins like 1,3,6,8-TeBDD or 1,3,7-TrBDD, which have previously been detected in the abiotic as well as biotic environment (Arnoldsson et al., 2012).

7. Toxicodynamics

Compared to toxicokinetics, slightly more knowledge is available regarding interactions and effects of TBP in organisms. Several studies were performed using either human cell lines or mammals, which underlines the toxicological relevance to humans. This becomes even more important, when taking into account that TBP has been detected multiple times in human matrices such as blood (Thomsen et al., 2001b) or urine (Nichkova and Marco, 2006). Also, TBP can be found in indoor dust (Suzuki et al., 2008) and foodstuff (Van Loco et al., 2017), which reflect two major exposure routes of BFRs for humans (Abdallah et al., 2008; Harrad et al., 2010; Knutsen et al., 2008; Sahlström et al., 2015).

In one of the first studies focusing on the toxicity of TBP, Lyubimov et al. exposed female rats to different concentrations of TBP via inhalation during their entire pregnancy (Lyubimov et al., 1998). No immunotoxic effects were found, but developmental (NOEL <0.03 mg/m^3) and maternal (NOEL 0.3 mg/m^3) toxicity was noted. However, the applied concentrations are several magnitudes higher than levels of TBP in the outdoor air (49–73 pg/m^3; Takigami et al., 2009). By using human neuroblastoma cells, Ríos et al. have focused on the cellular effects of TBP (Ríos et al., 2003). Their findings show that TBP induces the differentiation of these cells by inhibiting cell growth and increasing the acetylcholinesterase activity at 0.1 μM. At higher concentrations, apoptosis was observed. It was concluded that the induction of cell differentiation and the sensitivity of the differentiated cells to TBP (three times higher compared to naïve cells) could explain some mechanisms involved in the embryotoxicity and foetotoxicity of TBP described by Lyubimov et al. (Lyubimov et al., 1998; Ríos et al., 2003). Similar to other BFRs (Koch et al., 2015), TBP seems to influence the thyroid system as well. It was shown that TBP exhibits an up to ten times higher affinity to human transthyretin (TTR; a thyroid hormone transport protein) than the natural ligand thyroxine (T4) itself (Hamers et al., 2006; Legler and Brouwer, 2003; Meerts et al., 2000). Within the TTR-TBP complex, both, the halogens and the hydroxyl group, are responsible for the strong binding (Ghosh et al., 2000). In experiments with house dust, which is an important exposure route for humans and pets (Nomiyama et al., 2017), it was indicated that TBP contributes largely to the TTR binding potency of indoor house dust (Suzuki et al., 2008). In another study, mice treated with high concentrations of TBP (40 and 250 mg/kg bw) had decreased expression levels of deiodinase 1 (*Dio1*) and thyroid hormone receptor β isoform 2 (*Thrβ2*) mRNA – both relevant for the thyroid homeostasis – in the pituitary gland, but not in the liver (Lee et al., 2016). Interestingly, it was shown that not only TBP concentrations in human placental tissue from male infants are twice as high compared to female infants (Leonetti et al., 2016a), but also other parts of the thyroid homeostasis (for instance levels of triiodothyronine (T3) and thyroxine 5-deiodinase (DIO3) activity) were different between both sexes (Leonetti et al., 2016a).

Another endocrine endpoint is the ability to inhibit the estradiol sulfotransferase (E2SULT). Here, TBP showed an IC_{50} of 0.27 μM, which is only slightly less effective compared to the well-known inhibitor pentachlorophenol (Hamers et al., 2006). Within the same study, a high antiestrogenic potency was also determined for TBP (Hamers et al., 2006). It was shown that TBP inhibits the estrogen sulfation (Hamers et al., 2006; Kester et al., 2000) and stimulates aromatase (key enzyme responsible for the synthesis of estrogens) gene expression and enzyme activity (Cantón et al., 2005; Ding et al., 2007). Hassenklöver et al. found that TBP disturbs cellular Ca^{2+} signaling in neuroendocrine cells (Hassenklöver et al., 2006). It was hypothesized that the increase of intracellular Ca^{2+} is linked to the endocrine effects of TBP. Different to 2,4-DBP, TBP only increase the Ca^{2+} entry via calcium channels and not the in- and outward current. The determined half-maximum concentration was 28 μM (Hassenklöver and Bickmeyer, 2006). Schäfer et al. found that the effect of TBP on Ca^{2+} signaling was strong enough to possibly inhibit the egg fertilization in sea urchins, by disturbing the Ca^{2+} wave, which is normally initiated after a certain sperm factor is introduced to the egg (Schäfer et al., 2009).

Deng et al. exposed zebrafish embryos chronically to environmental aqueous levels of 0.3 μg/L (and 3.0 μg/L) of TBP and evaluated the impact 120 days post fertilization (Deng et al., 2010). They found clear differences between both sexes: plasma levels of testosterone and estradiol increased in males and decreased in females. Also, the transcription of steroidogenic genes in the brain and testes of males was increased, but lowered in the brain and ovary of females. Interestingly, the sex ratio was altered as well, favoring males over females. Also, in the F_1 generation reduced survival, retarded growth, and increased malformation was observed – which was not the case for the F_0 generation. In a similar study, zebrafish were exposed to TBP orally via feed (doses of 33 to 3300 μg/g; Haldén et al., 2010). In this study, TBP reduced the fertilization success and disturbed the gonad morphology. Similar to the study by Deng et al. (2010). sex dependent effects were observed as well (Haldén et al., 2010). For example, TBP exposure decreased vitellogenesis (the process of yolk formation) in females, which is in line with the findings of Deng et al. where vitellogenin (VTG; essential for vitellogenesis) levels were down-regulated (– but upregulated in males) (Deng et al., 2010).

Li et al. examined the inhibitory effects of TBP on settlement and

survival of larvae of Japanese abalone (Li et al., 2009). No changes were observed at concentrations of 1 ppm, however at 10 ppm, the proportion of metamorphosed larvae was reduced to 53%. At 50 ppm, all larvae died. These findings underline the natural occurrence of TBP as an effective defense against competitors if concentrations are high enough.

Taking all these results into account, it should be noted that especially 2,4-DBP, which is a typical degradation product of TBP (Abrahamsson and Klick, 1991; King, 1988; Steward et al., 1995), seems to have a higher toxic potential than TBP itself (Hassenklöver et al., 2006; Hassenklöver and Bickmeyer, 2006; Li et al., 2009). This stresses the need to consider degradation products as an important part when assessing the hazard of certain chemicals.

8. Conclusion

TBP has gained increasing attention recently. This is due to its occurrence as a degradation product of BFRs and its detection in humans. Previously, studies have mostly focused on TBP in the marine environment where it is also naturally synthesized by some species. Thus, it is important to distinguish natural sources from anthropogenic emissions. Currently, it appears unclear under which circumstances TBP poses a risk to organisms and the environment. However, based on relatively high concentrations in some matrices – like indoor dust – TBP might be very problematic for certain population groups such as toddlers, because of their typically higher exposure to dust and their specific body composition. For future studies, it will be interesting to distinguish between 2,4,6-, its isomer 2,4,5-TBP, and possible degradation products like 2,4-DBP. In general, more information is required especially concerning the toxicokinetics of TBP. It would be helpful to gain more information about the proportion of naturally and anthropogenically produced TBP, but also of TBP intake via diet and dust. Moreover, it appears to be important to continue studying the environmental behavior and possible adverse effects of TBP as long as new BFRs, which can degrade to TBP, are commercially manufactured.

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Appendix III: Supporting information for chapter I

The following pages contain the supporting information for chapter I of this thesis as published online.

Supporting Information for: Degradation of polymeric brominated flame retardants: Development of an analytical approach using PolyFR and UV irradiation

Christoph Koch[1,2,3,*], Alexander Dundua[4], Jackelyn A. Gomez[2,4], Milen Nachev[1,2], Susanne Stephan[2,5], Sarah Willach[2,6], Mathias Ulbricht[2,4], Oliver J. Schmitz[2,5], Torsten C. Schmidt[2,6], Bernd Sures[1,2]

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8 pages

6 figures

2 tables

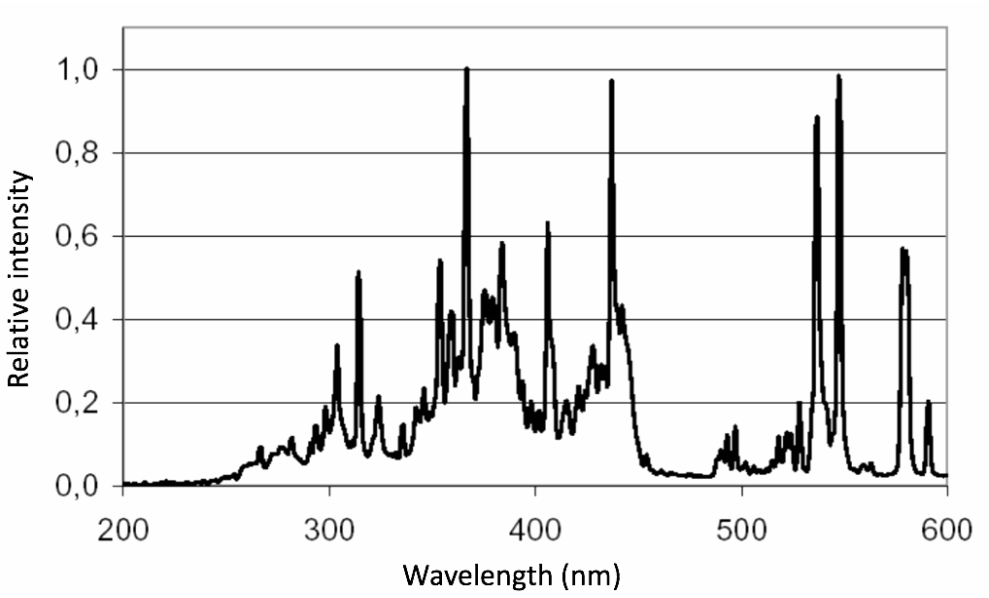


Figure SI-1: Spectrum of the used UV lamp. Radiation below 254 nm has been blocked by internal filters. Spectrum provided by the manufacturer.

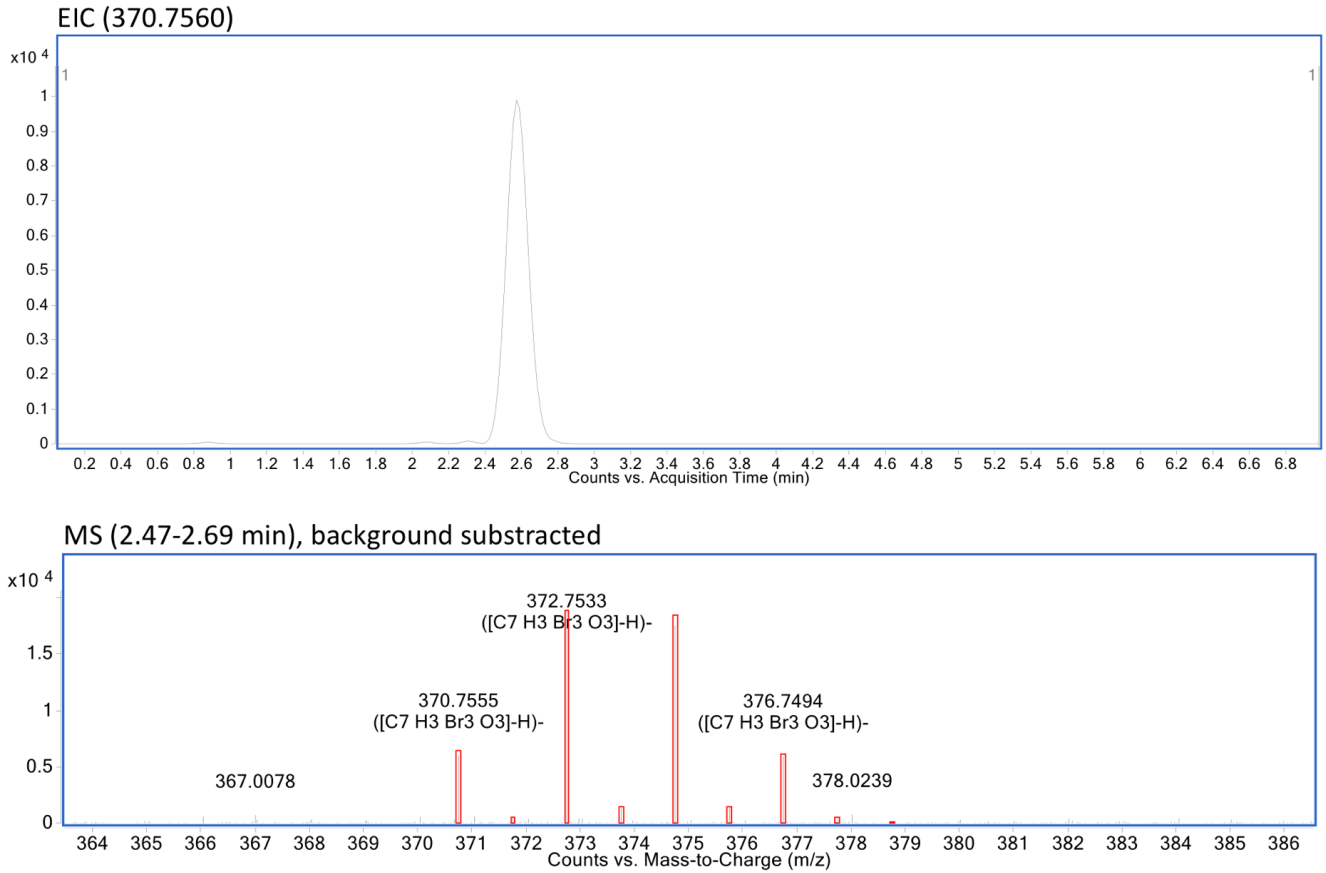


Figure SI-2: Exemplary LC-ESI-MS extracted ion chromatogram and mass spectrum of a possible degradation product ($C_7H_3Br_3O_3$) of bulk PolyFR after UV irradiation for 60 minutes. Red boxes show the theoretical calculated isotopic pattern.

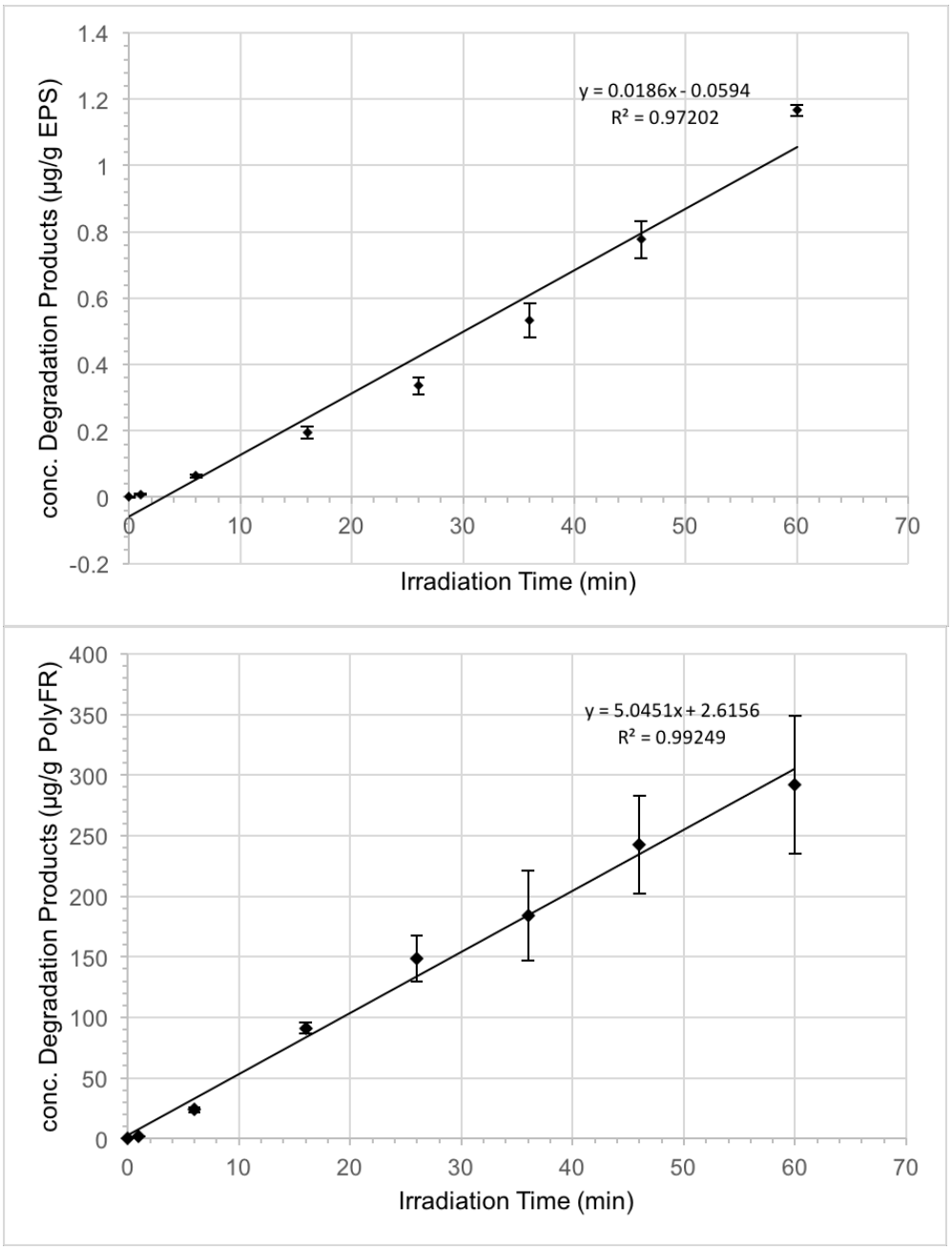
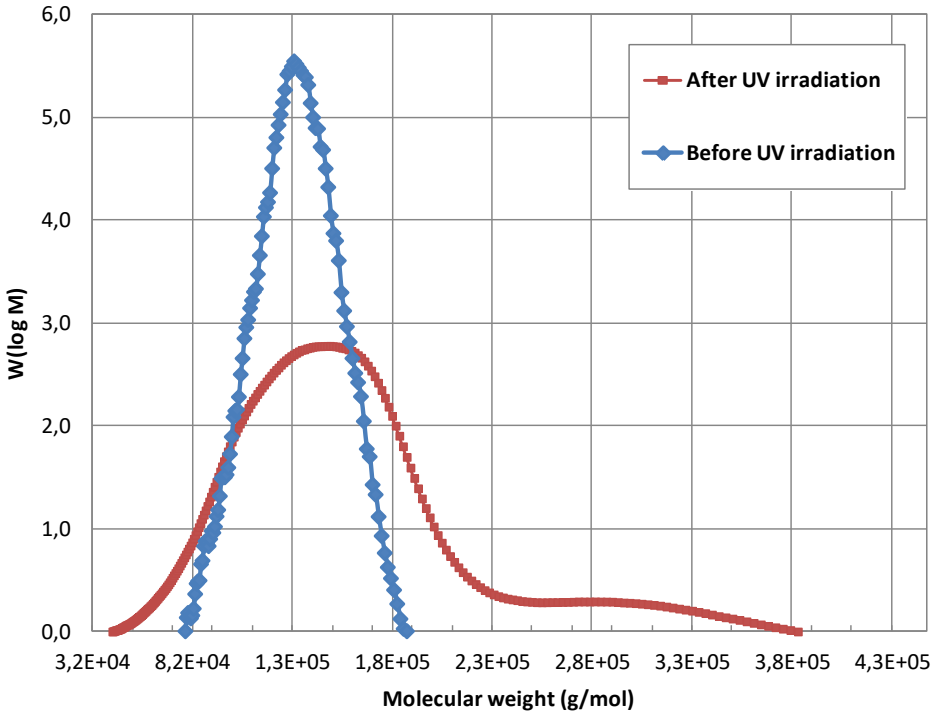


Figure SI-3: Estimated cumulative concentration of degradation products (µg/g) following UV irradiation of 1 g EPS (top) and 1 g bulk PolyFR (bottom) per litre during time.



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34 Figure SI-4: Molecular weight (M_n) distribution of bulk PolyFR before and after UV irradiation for

35 60 minutes obtained by GPC/SEC based on calibration with PMMA standards.

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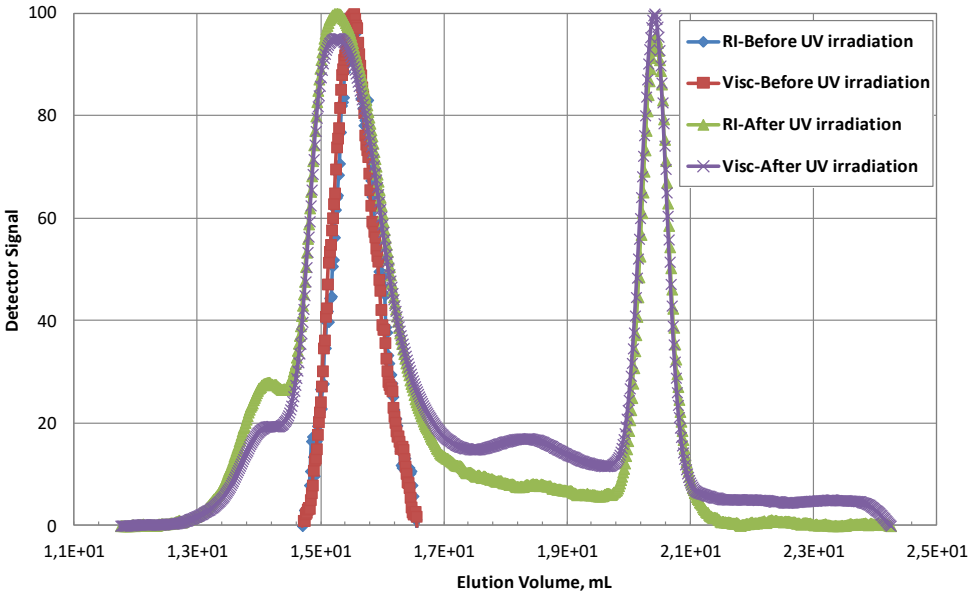
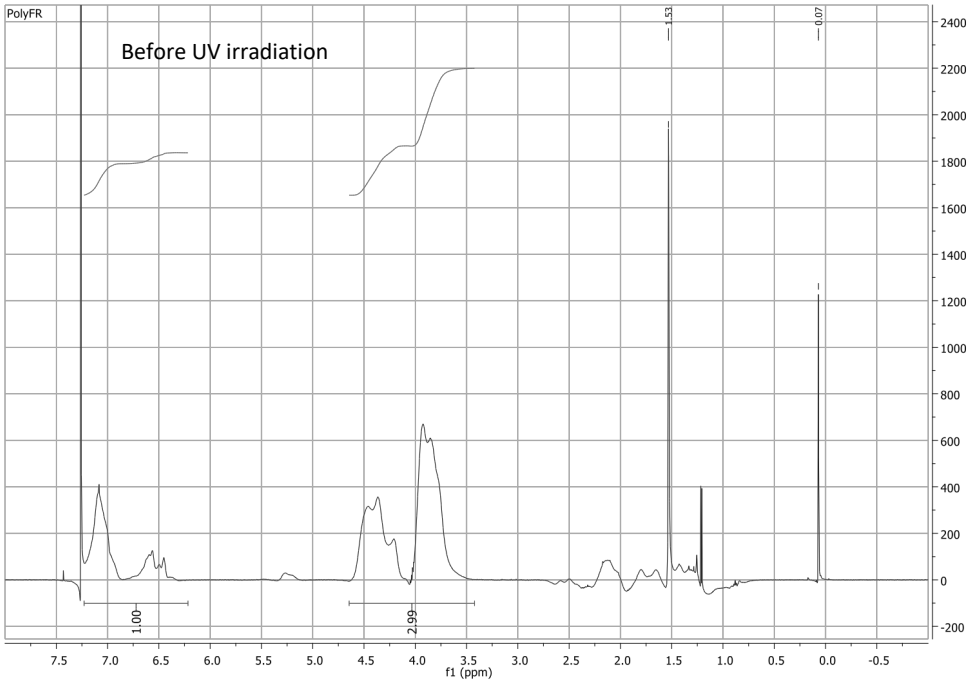


Figure SI-5: GPC/SEC results: Elution volume of bulk PolyFR before and after UV irradiation for 60 minutes by double detection RI (refractive index) and Visc (viscometer).



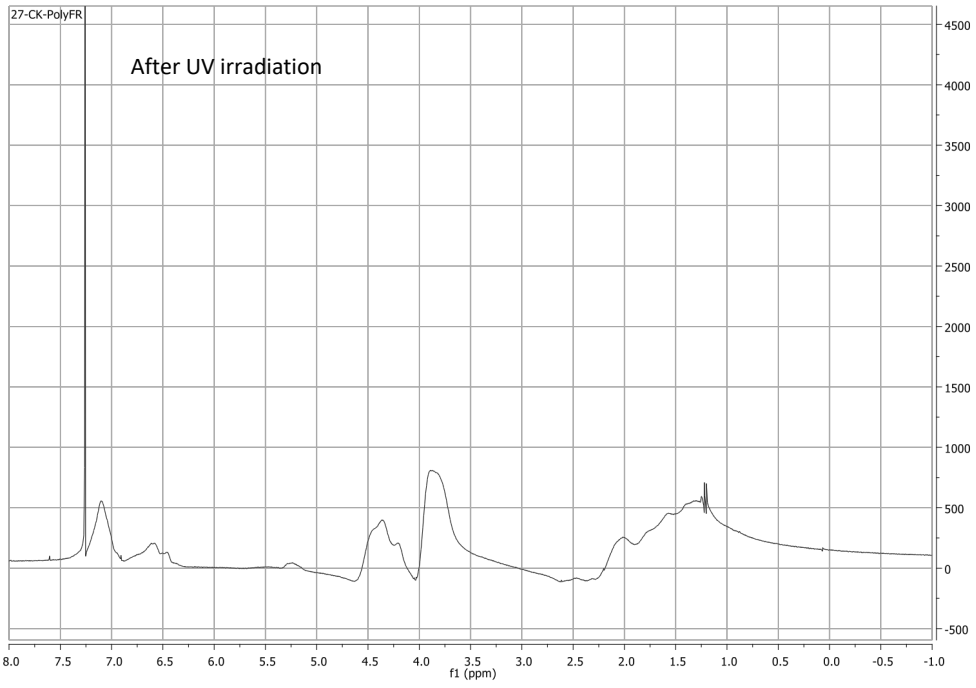


Figure SI-6: ^{1}H-NMR spectra of bulk PolyFR before (top) and after (bottom) UV irradiation for 60 minutes.

Table SI-1: ABC combustion program: The 1st-4th stop position describe the distance [mm] of the ceramic boat to the home position. The according time is given in seconds.

ABC-Parameter											Gas	
1st		2nd		3rd		4th		End	Cool	Home	Ar	O_2
Pos.	Time	Pos.	Time	Pos.	Time	Pos.	Time	time	time	time	time	time
130	130	140	120	150	95	175	60	100	60	120	30	600

Table SI-2: Instrument parameters of QTOF measurements.

Mode	ESI negative
Gas temp.	300 °C
Gas flow	5 L/min
Nebulizer	20 psig
Sheath gas temp.	300 °C
Sheath gas flow	8 L/min
VCap	5000 V
Nozzle Voltage	2000 V
m/z range	80-1700
Acquisition	1 spectra/s

Appendix IV: Supporting information for chapter II

The following pages contain the supporting information for chapter II of this thesis as accepted for publication.

Supporting Information for: Degradation of the polymeric brominated flame retardant "Polymeric FR" by heat and UV exposure

Christoph Koch[1,2,3,*], Milen Nachev[1,2], Julia Klein[2,4], Daniel Köster[5], Oliver J. Schmitz[2,4], Torsten C. Schmidt[2,5], Bernd Sures[1,2]

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[2]: Centre for Water and Environmental Research (ZWU), University Duisburg-Essen, 45141 Essen, Germany

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4 pages

1 figure

5 tables

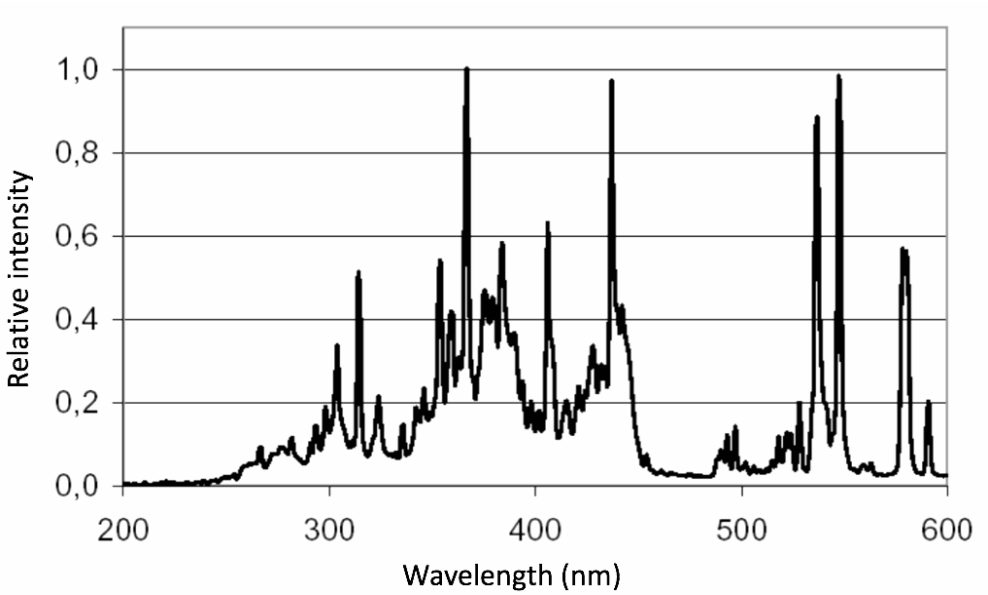

Figure S1: Spectrum of the used UV lamp. Radiation below 254 nm has been blocked by internal filters. Spectrum provided by the manufacturer.

Table S1: Applied recipe for 1 L reconstituted water (mg/L) according to ISO 6341.

$MgSO_4$	30.095
$CaCl_2$	110.96
$NaHCO_3$	32.375
KCl	2.8750

28 Table S2: Composition (µg/L) of the rain water as revealed by ICP-MS analysis.

Na	504
K	264
Ca	396
Mn	8
Fe	62
Co	0
Ni	0
Cu	11
Zn	1
As	0
Se	0
Mo	0
Ag	0
Cd	0
Sn	0
Sb	0
Pt	1

29

30

31 Table S3: Instrumental parameters of qTOF measurements.

Mode	ESI negative
Gas temp.	300 °C
Gas flow	5 L/min
Nebulizer	20 psig
Sheath gas temp.	300 °C
Sheath gas flow	8 L/min
VCap	5000 V
Nozzle Voltage	2000 V
m/z range	80-1700

32

Table S4: Instrumental parameters of MS/MS measurements.

Mode	ESI negative
Gas temp.	250 °C
Gas flow	17.5 L/min
Nebulizer	20 psig
Sheath gas temp.	250 °C
Sheath gas flow	10 L/min
VCap	3500 V
Nozzle Voltage	1500 V
Scan Type	Product ion Scan
Scan Time	100 ms
Collision Energy	30

Table S5: Information about possible brominated degradation products that were found via LC-qTOF-MS measurements after degradation of "Polymeric FR" by UV treatment for 180 minutes and consequently used to compare to heat treated samples at 60 °C for 36 weeks.

Mass-to-Charge (m/z) of [M-H]	**Suggested Molecular Formula**
214.9349	$C_7H_5BrO_3$
250.8542	$C_6H_4Br_2O$
258.9248	$C_8H_5BrO_5$
294.8440	$C_7H_4Br_2O_3$
322.8390	$C_8H_4Br_2O_4$
328.7647	$C_6H_3Br_3O$
338.8339	$C_8H_4Br_2O_5$
346.7389	$C_5HBr_3O_3$
372.7546	$C_7H_3Br_3O_3$

Appendix V: Supporting information for chapter III

The following pages contain the supporting information for chapter III of this thesis as accepted for publication.

Supporting Information for: Ecotoxicological characterization of possible degradation products of the polymeric flame retardant "Polymeric FR" using algae and daphnia OECD tests

Christoph Koch[1,2,*], Bernd Sures[1]

[1]: Aquatic Ecology and Centre for Water and Environmental Research (ZWU), University Duisburg-Essen, 45141 Essen, Germany

[2]: Deutsche Rockwool GmbH & Co. KG, 45966 Gladbeck, Germany

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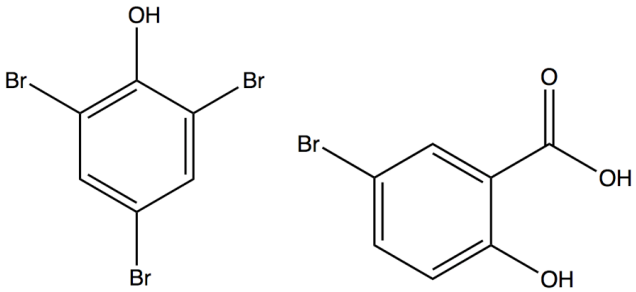
5 tables

18



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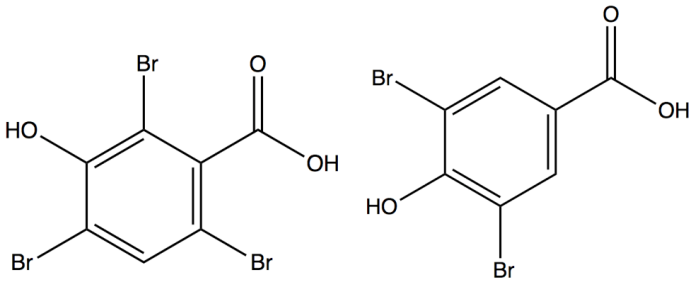

20 Figure S1: Molecular structure of the tested substances. From top left to bottom right: 2,4,6-
21 tribromophenol (2,4,6-TBP), 5-bromosalicylic acid (BSA), 2,4,6-Tribromo-3-hydroxybenzoic acid
22 (THA), 3,5-Dibromo-4-hydroxybenzoic acid (DHA)

23

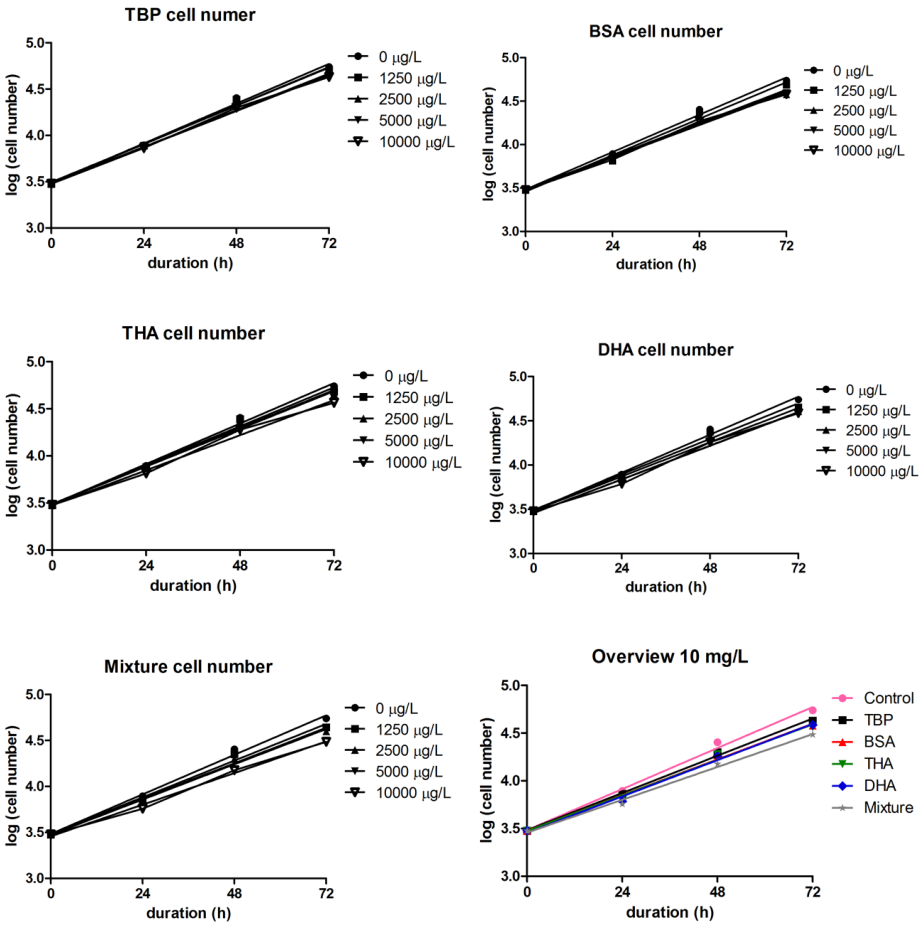

Figure S2: Inhibitory effect on the growth of *D. subspicatus* of every individual substance tested according to OECD No. 201. From top left to bottom right: Effect of 2,4,6-TBP, BSA, THA, DHA, the mixture (concentration per substance), and an overview of all substances at their highest concentration of 10 mg/L.

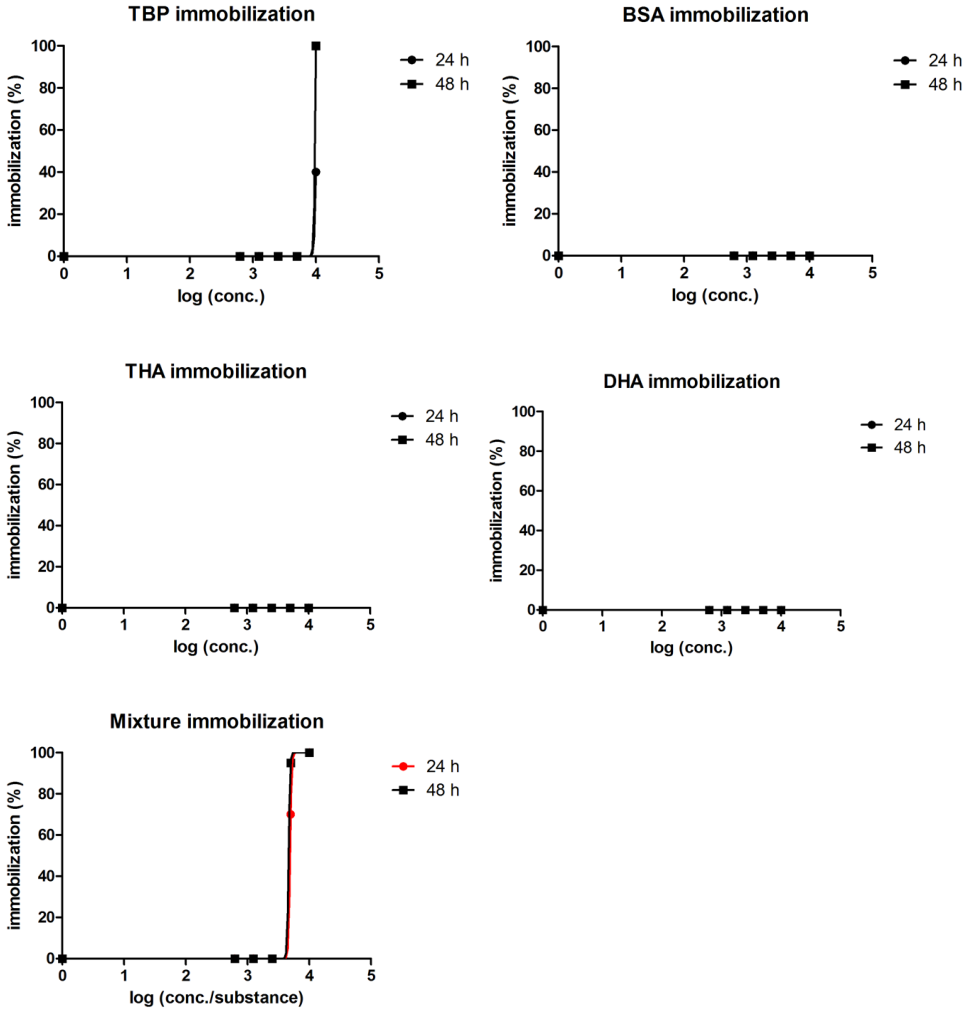

Figure S3: Immobilization of *D. magna* due to every individual substance tested according to OECD No. 202. From top left to bottom right: Effect of 2,4,6-TBP, BSA, THA, DHA, and the mixture (concentration per substance).

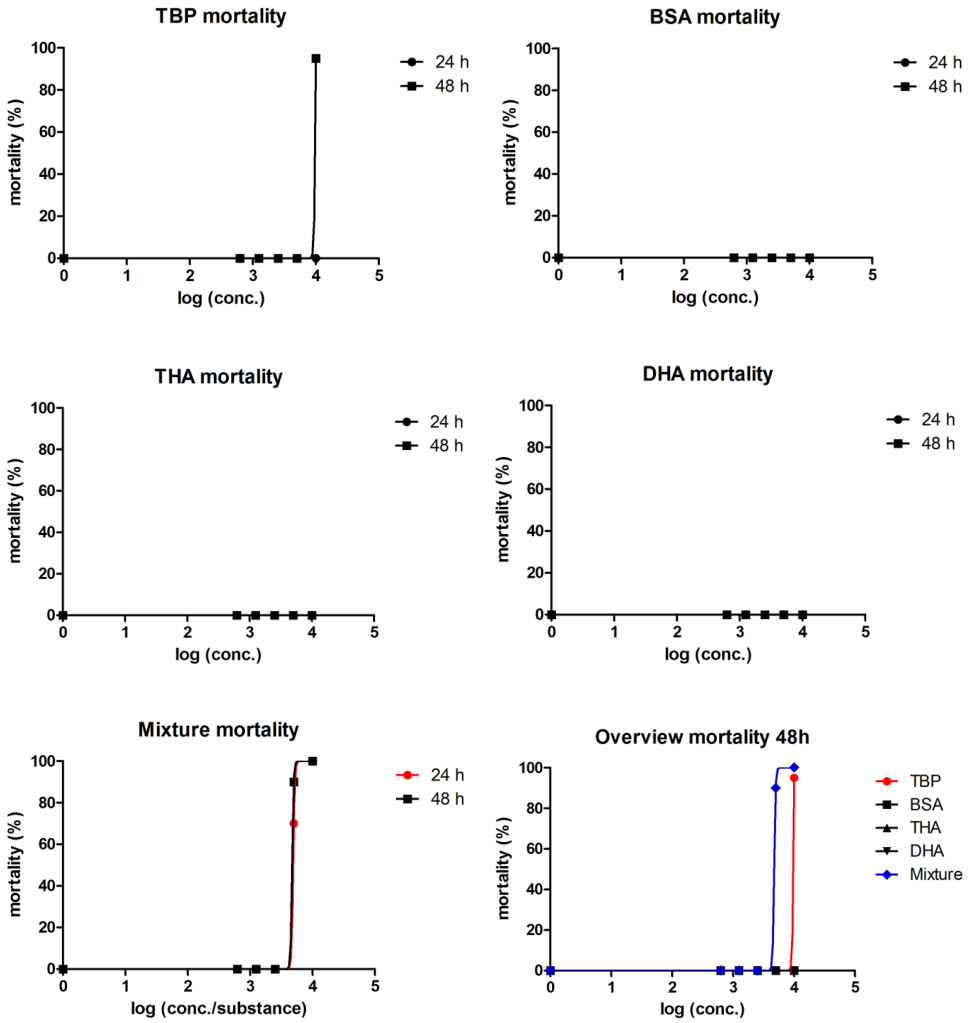

Figure S4: Mortality of *D. magna* due to every individual substance tested according to OECD No. 202. From top left to bottom right: Effect of 2,4,6-TBP, BSA, THA, DHA, the mixture (concentration per substance), and an overview of all substances after 48 hours.

Table S1: Applied recipe for 1 L reconstituted algae water (µg/L).

$Ca(NO_3)_2$*4 H_2O	14.75
$NaNO_3$	116.8
K_2HPO_4*3 H_2O	10.25
$MgSO_4$*7 H_2O	6.250
$NaHCO_3$	168.0
NaEDTA	11.45
$FeSO_4$*7$H_2$0	3.000
H_3BO_3	0.248
$MnSO_4$* H_2O	0.135
$(NH_4)_6Mo_7O_{24}$*4 H_2O	0.007
$ZnSO_4$*7 H_2O	0.023
$Co(NO_3)_2$*6 H_2O	0.012
$CuSO_4$*5 H_2O	0.010

Table S2: Applied recipe for 1 L reconstituted daphnia water (µg/L).

Sea salt	333.0
$CaCl_2$*2 H_2O	2705
$NaHCO_3$	554.0
SeO_2	0.070

51 Table S3: List of immobilized individuals per concentration according to OECD No. 202.

Concentration per substance (mg/L)	2,4,6-TBP		BSA		THA		DHA		Mixture	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
0	0	0	0	0	0	0	0	0	0	0
0.625	0	0	0	0	0	0	0	0	0	0
1.25	0	0	0	0	0	0	0	0	0	0
2.5	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	70	95
10	40	100	0	0	0	0	0	0	100	100

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53

54 Table S4: List of dead individuals per concentration according to OECD No. 202.

Concentration per substance (mg/L)	2,4,6-TBP		BSA		THA		DHA		Mixture	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
0	0	0	0	0	0	0	0	0	0	0
0.625	0	0	0	0	0	0	0	0	0	0
1.25	0	0	0	0	0	0	0	0	0	0
2.5	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	70	90
10	0	95	0	0	0	0	0	0	100	100

55

Table S5: Effect on every replicate according to OECD No. 211 for the tested concentrations per substance.

Control	**Day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Sum**
Vessel / total		0	0	0	0	0	0	0	0	112	24	0	147	19	44	155	21	48	137	15	62	120	904
Sum live parents		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
Avg live offspring		0	0	0	0	0	0	0	0	11,2	2,4	0	14,7	1,9	4,4	15,5	2,1	4,8	13,7	1,5	6,2	12	
Sum at each day		0	0	0	0	0	0	0	0	11,2	13,6	13,6	28,3	30,2	34,6	50,1	52,2	57	70,7	72,2	78,4	90,4	

3000 µg/L	**Day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Sum**
Vessel / total		0	0	0	0	0	0																0
Sum live parents		10	10	10	8	8	8	3	3	1	1	1	1	1	1	0	0	0	0	0	0	0	
Avg live offspring		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Sum at each day		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

1000 µg/L	**Day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Sum**
Vessel / total		0	0	0	0	0	0	0	0	0	0	6	13	29	8	8	0	12	12	0	2	2	92
Sum live parents		10	10	10	10	10	10	10	10	9	9	9	9	9	9	9	9	9	9	9	9	9	
Avg live offspring		0	0	0	0	0	0	0	0	0	0	0,67	1,44	3,22	0,89	0,89	0	1,33	1,33	0	0,22	0,22	
Sum at each day		0	0	0	0	0	0	0	0	0	0	0,67	2,11	5,33	6,22	7,11	7,11	8,44	9,78	9,78	10	10,2	

333 µg/L	**Day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Sum**
Vessel / total		0	0	0	0	0	0	0	0	50	0	0	27	6	19	3	15	17	12	7	17	32	205
Sum live parents		10	10	10	10	10	10	10	10	9	9	9	9	9	9	9	9	9	9	9	9	9	
Avg live offspring		0	0	0	0	0	0	0	0	5,56	0	0	3	0,67	2,11	0,33	1,67	1,89	1,33	0,78	1,89	3,56	
Sum at each day		0	0	0	0	0	0	0	0	5,56	5,56	5,56	8,56	9,22	11,3	11,7	13,3	15,2	16,6	17,3	19,2	22,8	

111 µg/L	**Day**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Sum**
Vessel / total		0	0	0	0	0	0	0	0	0	34	28	0	106	47	0	97	86	19	96	79	22	614
Sum live parents		10	10	10	10	10	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
Avg live offspring		0	0	0	0	0	0	0	0	0	3,78	3,11	0	11,8	5,22	0	10,8	9,56	2,11	10,7	8,78	2,44	
Sum at each day		0	0	0	0	0	0	0	0	0	3,78	6,89	6,89	18,7	23,9	23,9	34,7	44,2	46,3	57	65,8	68,2	

37 µg/L Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Sum
Vessel / total	0	0	0	0	0	0	0	0	0	71	34	9	124	54	0	53	171	25	65	162	24	792
Sum live parents	10	10	10	10	10	10	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
Avg live offspring	0	0	0	0	0	0	0	0	0	7,89	3,78	1	13,8	6	0	5,89	19	2,78	7,22	18	2,67	
Sum at each day	0	0	0	0	0	0	0	0	0	7,89	11,7	12,7	26,4	32,4	32,4	38,3	57,3	60,1	67,3	85,3	88	

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List of publications and declaration of contribution as a co-author

- Koch, C.; Dundua, A.; Aragon-Gomez, J.; Nachev, M.; Stephan, S.; Willach, S.; Ulbricht, M.; Schmitz, O.J.; Schmidt, T.C.; Sures, B. Degradation of polymeric brominated flame retardants: Development of an analytical approach using PolyFR and UV irradiation. *Environmental Science & Technology* **2016**, 50, 12912-12920

  Personal contribution within this publication: I prepared and degraded the samples (100%). JAG and AD performed the NMR and GPC measurements, including analysis (10%). SS did the LC-MS analytics (10%). I conducted the Contact Angle measurements (100%) and performed with MN the ICP analytics (90%). SW did the TOC determination (0%) and together with me the AOBr measurements (50%). I analysed the Contact Angle and ICP measurements (100%). In addition, I drafted the manuscript including all further calculations like assumption of degradation products (90%). MU, OJS, TCS, and BS commented on the draft afterwards. BS oversaw the study. I handled the submission (100%).

- Koch, C.; Nachev, M.; Klein, J.; Köster, D.; Schmitz, O.J.; Schmidt, T.C.; Sures, B. Degradation of polymeric brominated flame retardant “Polymeric FR” by heat and UV exposure. Accepted for publication in *Environmental Science & Technology*

  Personal contribution within this publication: I prepared and degraded the samples (100%). JK did the LC-MS analytics (10%). MN and I performed the ICP analytics (90%). DK did the TOC determination (0%). I analysed the data and drafted the manuscript (90%). OJS, TCS, and BS commented on the draft afterwards. BS oversaw the study. I handled the submission (100%).

- Koch, C.; Sures, B. Evaluation of the toxicity of various degradation products of “Polymeric FR” in algae and daphnia OECD tests. Accepted for publication in *Science of the Total Environment*

  Personal contribution within this publication: I performed the experiments (100%), analysed the data (100%), performed the *in silico* predictions and drafted the

manuscript (100%). BS commented on the draft afterwards and oversaw the study. I handled the submission (100%).

- Koch, C.; Schmidt-Kötters, T.; Rupp, R.; Sures, B. Review of hexabromocyclododecane (HBCD) with a focus on legislation and recent publications concerning toxicokinetics and -dynamics. *Environmental Pollution* **2015**, 199, 26-34

  Personal contribution within this publication: I did the literature research (100%) and drafted the manuscript (80%). TSK contributed the legislative part. RR helped with organizational aspects. BS helped to draft the manuscript. I handled the submission (100%).

- Koch, C.; Sures, B. Environmental Concentrations and Toxicology of 2,4,6-Tribromophenol. *Environmental Pollution* **2018**, 233, 706-713

  Personal contribution within this publication: I did the literature research and drafted the manuscript (90%). BS helped to draft the manuscript. I handled the submission (100%).

Acknowledgement

Not only one person, but many are responsible for the eventual completion of this thesis:

First of all, I want to thank my supervisor, Prof. Dr. Bernd Sures, who supported me throughout this whole project. He was continuously able to involve helpful colleagues that contributed to this project, mainly to the analytical part. Thanks are also due to him because of his advice over the last years. Not only scientifically, but also personally.

I also want to thank Dr. Roman Rupp and Dr. Claus Garn, who gave me the opportunity to continue to realize my thesis while already working fulltime. Without them, I would not have been able to attend all the conference and meet so many incredible people along the way.

In addition, I want to thank Prof. Dr. Oliver Schmitz and Prof. Dr. Torsten Schmidt and their (sometimes former) PhD students Susanne Stephan, Sarah Willach, Julia Klein, and Daniel Köster for all their analytical assistance.

Also for their analytical help, I would like to thank Prof. Dr. Matthias Ulbricht, Dr. Alexander Dundua, and Dr. Jackelyn Aragon-Gomez.

Within the “Aquatische Ökologie” I would like to thank particularly Dr. Milen Nachev for his always rather spontaneous help. In addition, I would like to thank Katrin Honka, Mark Schumann, Thomas Knura, Marion Woermann, Gerhard Schertzinger, and Dr. Daniel Grabner.

I would also like to thank Prof. Dr. Adrian Covaci, Prof. Dr. Robert Letcher, and Dr. Arlene Blum for their helpful advice and introducing me into scientific community of flame retardants. Special thanks are also due to Sharlye Patton and Erika Schreder for all the interesting conversation and opportunities.

Furthermore, I want to thank especially my parents, my brother, and my friends for their love and accompany during this time and already long before.

Thank you.

Curriculum Vitae

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