Norwegian University
of Life Sciences

Analytical challenges in the determination of legacy and novel Brominated Flame Retardants: A method development study

Lovise Skogeng Pedersen

Chemistry

Acknowledgements

Abstract

Legacy brominated flame-retardants (BFRs) such as polybrominated diphenyl ethers (PBDE) have been widely used in technical flame-retardant mixtures, as they inhibit the combustion processes and secures fire resistance in different materials. However, evidence of persistency, toxicity and the ability to bioaccumulate led to restrictions in production and use through the Stockholm Convention (SC), creating a market for novel brominated flame-retardants (n-BFR), compounds BDE-like properties but with largely unknown implications on environment and health.

BFRs can be monitored in the atmosphere by passive air sampling (PAS), a logistically easy and cost-effective sampling method. PAS can be implemented in many different ways, one of which is by a "flying saucer" passive air sampler. Polyurethane foam (PUF) disks are used as the sampling material, a material with large surface areas and good affinity with non-polar substances such as BFRs.

As n-BFRs are a new topic in sense of environmental chemistry, the chemical group lacks satisfying laboratory procedures that secure accuracy and reproducibility. One such procedure, is to satisfyingly clean the PUF disks post extraction, removing PUF materials from the extract and securing a good recovery of n-BFRs. Many "traditional" clean-up methods involve acidified absorbents or treatment with acid, but many n-BFRs are acid labile and will decompose of the treatment.

A newly developed multi-layer solid phase extraction (SPE) method (method C) was validated and tested together with a traditional reference method involving acid (method A) and a comparison SPE method (method B). The methods were validated by preparing a set of parallel PUF samples and looking at accuracy (recovery) and reproducibility (relative standard deviation, RSD). PUF matrix effects were assessed by GCxGC and GC-MS lock-mass.

Accuracy expressed as recovery (%) was calculated for PBDEs to 92-130 %, 80-120 and 41-119 % for methods A, B and C, respectively. For n-BFRs, the accuracy was only obtained for method A and B due to insufficient clean-up with method C (54-99 % and 49-104 %, respectively).

Exposed passive air samples were also cleaned according to the different methods (A, B and C). Method detection limits (MDL) were established from blank samples.

Norsk sammendrag

Blandinger av tradisjonelle bromerte flammehemmere (BFR) slik som polybromerte difenyl etere (PBDE) har blitt brukt som flammehemmere for bruk i ulike materialer, da de inhiberer forbrenning av gasser og hemmer brann. Men, PBDE har blitt identifisert som persistente, toksiske og med egenskaper til å bioakkumulere, noe som ledet til restriksjoner i produksjon og bruk av stoffene gjennom Stockholmkonvensjonen (SC). Dette har skapt et marked for nye bromerte flammehemmere (n-BFR), stoffer med BDE-like egenskaper men med ukjente påvirkninger på helse og miljø.

Prøvetaking av bromerte flammehemmere i atmosfæren kan gjøres med passiv luftprøvetakere, som er både kostnadseffektivt og enkelt sammenlignet med aktiv luftprøvetakere. Felles for aktiv og passiv luftprøvetaking er absorbenten som er polyuretan-skum, da det er et materiale med stor overflate og god affinitet til u-polare forbindelser slik som bromerte flammehemmere.

Siden n-BFR er et nytt tema innenfor miljøkjemi mangler stoffgruppen metoder for prøveopparbeidelse med tilfredsstillende nøyaktighet og reproduserbarhet. En type prøveopparbeidelse er rensing av passive prøver etter ekstraksjon for å fjerne PUF-rester fra ekstraktet samtidig som en tilfredsstillende gjenvinning av n-BFR-komponenter er sikret. Tradisjonelle opprensingsmetoder involverer gjerne syre, men mange n-BFRer er syrelabile og brytes ned av behandlingen.

En god opprensningsmetode som gir tilfredsstillende gjenvinning av både tradisjonelle og nye BFR trengs derfor. Nylig ble en multi-lags fastfase-ekstraksjonsmetode (C) utviklet ved Norsk Institutt for Luftforskning. Denne ble testet og validert sammen med en tradisjonell referansemetode med svovelsyre (A) og en sammenligningsmetode (B). Metodene ble validerte ved å klargjøre et sett med PUF prøver for hver metode, undersøke nøyaktighet (gjenvinning) og reproduserbarhet (relativt standardavvik). Rester av PUF materiale i prøven ble undersøkt med GCxGC og GC-MS.

Nøyaktigheten for PBDE var 92-130 %, 80-120 og 41-119 % hhv. for metode A, B og C. For n-BFR gjorde utilstrekkelig opprensning fra metode C at det kun beregnet nøyaktighet for metode A og B, hhv. 54-99 % og 49-104 %.

Passive luftprøver ble også analysert i prosjektet, også disse etter opprensning med metode A, B og C. Deteksjonsgrenser for metodene ble beregnet ut fra blindverdier.

Abbreviations

POPs	persistent organic pollutant
LRT	long range transport
LRAT	long range atmospheric transport
SC	Stockholm Convention
PBDE	polybrominted diphenyl ethers
BDE	brominated diphenyl ethers
BFR	brominated flame retardants
n-BFR	novel brominated flame retardants
GAPS	Global Atmospheric Passive Sampling network
EMEP	the European Monitoring and Evaluation Programme
AMAP	The Arctic Monitoring and Assessment Programme
EU	European Union
ATE, TBP-AE	Allyl-2,4,6- tribromophenyl ether
a-TBECH	a-tetrabromoethylcyclohexane
b-TBECH	b- tetrabromoethylcyclohexane
g/d-TBECH	g/d- tetrabromoethylcyclohexane
BATE, TBP-BAE	2-bromoallyl- 2,4,6 tribromophenyl ether
PBT	Pentabromotoluene
PBEB	pentabromoethylbenzene
HBB, HBBz	hexabromobenzene
DPTE, TBP-DBPE	2,3-dibromopropyl- 2,4,6-tribromophenyl ether
EHTBB, EH-TBB	2-ethylhexyl- 2,3,4,5-tetrabromobenzoate
BTBPE	1,2-bis(2,4,6- tribromophenoxy)ethane
BEHTBP, BEH-TEBP	Bis(2-ethylhexyl)tetrabromophthalate
DBDPE	Decabromodiphenylethane
PAS	passive air samplers
PUF	polyurethanefoam
SPE	solid phase extraction
RSD	relative standard deviation

HSE	environment health and safety
GC	gas chromatography
GC-GC	two-dimentional gas-spectrometry
GFF	glass fibre filter
ACN	acetonitrile
MS	mass spectrometry
GC-MS	gas chromatography spectrometry
PFK	perfluorokerosene
ISTD	internal standard
RSTD	recovery standard
MDL	method detection limit
LOD	limit of detection
CanPUF	Canadian PUF type
TCN	tetrachloronaphtalene

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1. Introduction

An increasing human population and technological progressions create new challenges. Through history, humans have developed techniques and products to increase the productivity and the overall way of life. Unfortunately, some of these innovations have shown to harm the environment by polluting the air, water and soil with harmful chemical components. One chemical group of urgent concern, are *persistent organic pollutants* (POPs), which are components sharing the three criteria: they degrade slowly in nature (are *persistent*), and are *toxic* to humans and animals. Additionally, they *bioaccumulate* in tissues of both humans and wildlife, and can cause harm such as inhibiting development, affecting the endocrine system and harming reproduction (Li et al., 2017; Qi et al., 2014; Wilson et al., 2016; Yadav, Devi, Li, & Zhang, 2017).

The properties of persistency, toxicity and bioaccumulation (PBT) together make of the criteria for chemical components to be classified as *persistent organic pollutants* (POPs).

A wide range of POPs are intentionally and unintentionally produced and emitted all over the world, the majority from highly populated and industrialized areas. POPs are also generally able to undergo long-range transport (LRT), which means that the pollutants can be spread out, transported and deposited in vast distances from their original source. With LRT as the main delivery route of contaminant, relatively high concentrations can be found of POPs never used in the area. For example are POPs detected in pristine areas in the Arctic, far away from their original source (Hung et al., 2016). LRT was discovered as early as 1974, when researchers suggested that chemicals migrated through the atmosphere in the form of gases and aerosols, and were deposited in polar regions in the north and in the south (Hung et al., 2016; Wania & Mackay, 1996).

The LRT process happens in the atmosphere, through ocean currents or with a biological vector, such as migratory species. However, the fastest LRT is the atmospheric one, or long-range *atmospheric* transport (LRAT). Compounds can be detected several latitudes away from their original sources as fast as a couple of hours after being released to the environment. In contrast, it usually takes months or years before pollutants are detected after transport through the ocean currents. As compounds are released in temperate areas, volatile compounds are vaporized and transported north- and southward through the atmosphere. As the most populated and industrialised cities are found in the northern hemisphere, the Arctic has through history received the largest pollution load and is therefore given a high priority in environmental analysis. As the air masses cool down further north, gas phase pollutants condensate and are deposited to surfaces such as vegetation, soil or snow. This type of air-surface exchange can happen several times during the pollutants journey, in a process called *the grasshopper effect* (Figure 1)*.* The effect has been investigated for POPs by looking

at daily and seasonal changes in concentrations in air and soil. In addition to enhancing deposition, the colder temperatures also slow down breakdown of chemicals, thus favouring their persistency in the environment (Gouin, MacKay, Jones, Harner, & Meijer, 2004; Hung et al., 2016; Wania & Mackay, 1996).
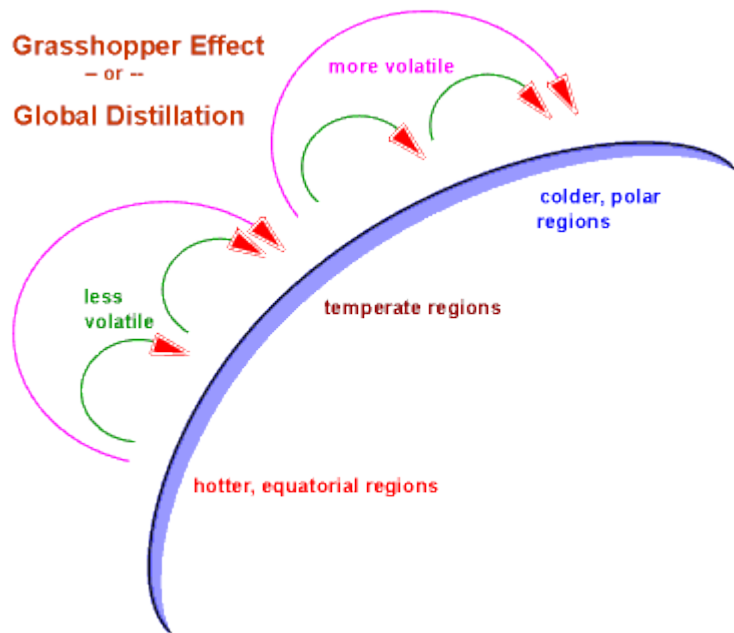
Figure 1: The grasshopper effect, or global distillation, where contaminant are vaporized and transported via the atmosphere to colder regions where they condensate (collected at http://www.arctic.uoguelph.ca/cpe/arcticnews/articles/Grasshopper/Grasshopper.htm, 2018)

If POPs are deposited to terrestrial or aquatic surfaces, they are taken up by organisms on the lowest trophic levels. Through the food-chain, they are bio-accumulated in fat, brain and liver of organisms. *Bio-magnification* (further enrichment through the food-chain) may also occur, resulting in the highest concentrations being reached in top predators at the highest trophic levels. There, the toxicity of the pollutants can be expressed through harmful effects, including some cancers, birth defects, neurotoxicity, and negative implications on reproduction and the immune system. As POPs undergo LRAT, single countries are not able to protect their inhabitants and wildlife against the health implications caused by the pollutants. Global collaboration between countries and continents is thus necessary. In the late 1990's, the *Stockholm Convention* (SC) was proposed, an international treaty which suggested ban or restriction on production and use of several POPs with known negative health effects. The SC was building on the already existing *Aarhus protocol*, which restricted some environmentally harmful compounds. The SC was adopted and signed by several countries, and entered into force in May 2004, aiming to reduce emission and exposure of harmful pollutants to the environment worldwide. The "dirty dozen", twelve pollutants recognized as causing negative

effects on human and environmental health, were the first compounds implemented into the convention. As of March 2018, there are more than 30 different chemical groups listed as POPs and 179 participating countries in the SC, and the list is constantly expanding. The convention aims to identify new POPs, as monitoring networks and new knowledge proposes chemicals as pollutants. (Hung et al., 2016; The Stockholm Convention (UNEP), 2008).

Whether or not treaties such as SC have an actual effect on the concentrations of harmful pollutants in the environment, is investigated by monitoring the occurrence of the compounds and establishing time trends. Over time, it is then possible to conclude whether or not the convention has had an effect on levels in the environment. Moreover, the detection of compounds in polar areas far away from the source is an evidence of persistency and LRAT, important criteria for characterizing them as POPs. Monitoring will then contribute to the development of the treaty, through banning more pollutants and keeping it updated. The half-life and persistency of the compounds is also investigated, an important factor to include when evaluate the harmfulness of the compounds (Hung et al., 2016; Kallenborn, Hung, & Harner, 2016).

There are three main global monitoring programmes:

- The Global Atmospheric Passive Sampling network (GAPS), which is a Canadian monitoring programme aiming to establish long-term trends of legacy and novel POPs in the atmosphere using passive air samplers (further revised in CHAPTER ABOUT PAS). The GAPS network has more than 50 sampling sites globally.
- The European Monitoring and Evaluation Programme (EMEP), which is a European network governed by the European Union (EU). It aims to secure a broad network of emissions data to model and assess transport and deposition of air pollution. NILU is a contributor to the EMEP network.
- The Arctic Monitoring and Assessment Programme (AMAP), which is aiming to monitor trends and effects of contaminants and climate change in the Arctic.

1.1. Brominated flame retardants

One group of POPs, and the one chosen to focus on in this study, are *brominated flame retardants* (BFRs). BFRs are compounds produced to inhibit combustion by reacting with flammable gases, thus reducing the flammability of materials. Demands for inflammability of different products for the protection of the consumer, has created a market for BFRs to be added to in plastics, such as building materials (paint, covers and spray foam insulation); electrical equipment (wires and covers); furniture (sofas, mattresses, wall-to-wall carpets and curtains); and fabric (underwear, sports- and

workwear, tents and sleeping bags). Together with other halogenated flame retardants (chlorinated, fluorinated, iodinated), BFRs represent about 25 % of the global production of flame retardants, woth a growth of about 5 % annually (M Harju, E S. Heimstad, D Herzke T Sandanger, S Posner, 2009).

Flame-retardants are either of *reactive* or *additive* nature, meaning they are incorporated into the plastic polymer, or are added during the polymerization process of plastics. Reactive flame-retardants are added during the polymerization process of plastics and become an integral part of the material, which keeps the compounds chemically trapped in the material and prevents them from leaking out into the environment. Most *inorganic* flame-retardants are reactive, and do not pose a significant threat to environment and health. Additive flame-retardants on the other hand, are incorporated into the material before, during or after polymerization. The compounds then act either as an integrated element of the polymerization process, either way not being chemically bound to the material. This means that the compounds can leak out into the environment over time, through wear and tear or vaporization. There are both reactive and additive BFRs on the market today (Kurt-Karakus et al., 2017; M Harju, E S. Heimstad, D Herzke T Sandanger, S Posner, 2009; The Stockholm Convention (UNEP), 2008).
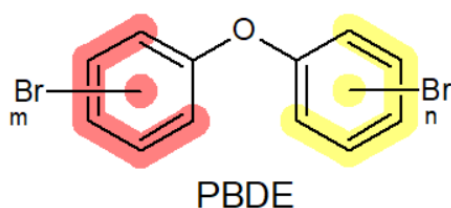
Figure 2: The general structure of polybrominated diphenyl ether (PBDE), where one or both of the phenyl rings are substituted with 1-10 bromine atoms in total.

One subgroup of brominated flame retardants, are the legacy BFRs *polybrominated diphenyl ethers* (PBDEs) which have historically been the most widely used and BFRs. PBDEs consists of two phenyl rings connected by an ether functional group, and substituted with 1-10 bromine atoms (Figure 2), resulting in a total of 209 different possible congeners. The PBDEs are sorted in homologous groups by the degree of bromination from *mono-* to *deca-*BDE, and the different congeners within each homologous group is listed in Table 1 (Kurt-Karakus et al., 2017; Roscales et al., 2018).

Table 1: There are 209 possible structures of polybrominated diphenyl ethers (PBDEs), which are divided into homologous groups based on the number of bromine atoms. Listed are 23 of them, which are analyzed at the Norwegian Institute of Air Research (NILU). PBDE congeners that are incorporated into PBDE technical mixtures are marked by *.

Homologous group	PBDE congener
Tri-BDE	BDE-17
	BDE-28
Tetra-BDE	BDE-47*
	BDE-49
	BDE-66
	BDE-71
	BDE-77
Penta-BDE	BDE-85
	BDE-99*
	BDE-100*
	BDE-119
	BDE-126
Hexa-BDE	BDE-138
	BDE-153*
	BDE-154*
	BDE-156
Hepta-BDE	BDE-183*
	BDE-184
Octa-BDE	BDE-196*
	BDE-197*
Nona-BDE	BDE-206
	BDE-207
Deca-BDE	BDE-209*

Commercial PBDE technical mixtures consist of three major groups: penta-, octa- and deca-BDE, where PBDE-47, PBDE-183 and PBDE-209 are the main congener in each mixture, respectively. The technical mixtures and their simplified content is listed in Table 2. The commercial mixtures are named after which PBDE-congener is the most abundant one in the mixture, even though they

contain several different PBDE congeners (marked by bold **X** in the table). Annual market demands of deca-, penta- and octa PBDE were at 56 100, 1700 and 3790 metric tons in 2001, respectively. However, the evidence of PBT characteristics of PBDE since the 1990s has led to restrictions in production and use of PBDE congeners: Penta- and octa-BDE mixtures were listed as a POPs under the SC in 2009, while deca-BDE achieved a similar status in the SC in May 2017. The restrictions for deca-BDE have some exceptions, as it is permitted for use in aircrafts and vehicles until March 2027 and 2019, respectively (Kurt-Karakus et al., 2017; Roscales et al., 2018; The Stockholm Convention (UNEP), 2008).

*Table 2: A simplified content list of the three major commercial PBDE technical mixtures. The mixtures are named by the most abundant homologous group, marked by a bold **X**. The annual market demands for the technical mixtures are as reported by* (Kurt-Karakus et al., 2017)*.*

Technical mixture	Homologous groups						Market demands metric tons (2011)
	Tetra-BDE	Penta-BDE	Hexa-BDE	Hepta-BDE	Octa-BDE	Deca-BDE	
Penta-BDE:	X	**X**	X				1700
Octa-BDE:			X	X	**X**		3790
Deca-BDE:						**X**	56 100

1.2. The need for a successor: n-BFRs

Restrictions in production and use of legacy BFRs such as PBDEs, accompanied by an ever-increasing demand for fire safety for products, has made a market for the development of new BFRs. These *novel brominated flame retardants* (n-BFRs) are now found in a wide range of products and materials, such as high-impact and insulating plastic materials, textiles, rubbers, wood products, paper and neoprene, where they are both implemented as additive and reactive component. Commercial presence includes use in electrical equipment, in furniture, toys, building materials, coatings and insulation (M Harju, E S. Heimstad, D Herzke T Sandanger, S Posner, 2009).

The n-BFRs that have been included in this study are listed in TABLE below.

*Table 3: The novel brominated flame retardants (n-BFRs) included in this study. Many of the n-BFRs are known by several names and abbreviations. Listed are the names used at the Norwegian Institute of Air Research. Where several are available, the abbreviation used further in the thesis is listed in **bold**.*

Name (used at NILU)	CAS	Abbreviation(s)
Allyl-2,4,6-tribromophenyl ether	3278-89-5	**ATE**, TBP-AE
a-tetrabromoethylcyclohexane	3322-93-8	a-TBECH
b- tetrabromoethylcyclohexane	-	b-TBECH
g/d- tetrabromoethylcyclohexane	-	g/d-TBECH
2-bromoallyl- 2,4,6 tribromophenyl ether	99717-56-3	**BATE**, TBP-BAE
Pentabromotoluene	87-83-2	PBT
pentabromoethylbenzene	85-22-3	PBEB
hexabromobenzene	87-82-1	**HBB**, HBBz
2,3-dibromopropyl- 2,4,6-tribromophenyl ether	-	**DPTE**, TBP-DBPE
2-ethylhexyl- 2,3,4,5-tetrabromobenzoate	183658-27-7	**EHTBB**, EH-TBB
1,2-bis(2,4,6- tribromophenoxy)ethane	37853-59-1	BTBPE
Bis(2-ethylhexyl)tetrabromophthalate	26040-51-7	**BEHTBP**, BEH-TEBP, TBPH
Decabromodiphenylethane	84852-53-9	DBDPE

n-BFRs have relatively recently caught the attention of the environmental chemists, as elevated levels of congeners have been detected in the Arctic (e.g. has ATE been detected in the blubber and brain of harp seals, indicating an ability to cross the blood-brain barrier). BTBPE, DBDPE, PBEB, PBT and TBECH has been detected in higher trophic levels in the Arctic, suggesting that they are able to bio-accumulate. The presence in pristine environments also indicate the possibility of LRAT. As many of these compounds a used as replacements for legacy-BFRs which have been restricted in use and production (PBDE), the presence in the environment could be a warning sign of increasing concentrations in the environment over time (Covaci et al., 2011; de Wit, Herzke, & Vorkamp, 2010; M Harju, E S. Heimstad, D Herzke T Sandanger, S Posner, 2009).

Several n-BFRs have been detected in atmospheric samples. In Nepal, the authors Yadav et al. (2017) concluded with n-BFRs being the most abundant of all detected halogenated flame retardants (including PBDEs) from indoor air in Nepalese cities. As humans spend most of their time indoors, the indoor air is an important exposure pathway for assessing risk to human health. However, little is known on possible health impacts of n-BFRs (Yadav et al., 2017).

1.2.1. Monitoring BFRs in the atmosphere

1.2.1.1. Sampling of airborne PBDEs and n-BFRs

To measure semi-volatile trace contaminants such as PBDEs and n-BFRs in the air, a large volume of air needs to be extracted. Unlike for other sample matrices (e.g. soil, vegetation, biota), the volume required for air is too large to directly collect a sufficient amount. Therefore, the target compounds present in the air must be trapped to concentrate them compared to their concentrations in the air. This can be done either by active or passive sampling. Active sampling of PBDEs and n-BFRs requires the use of a pump to draw known volumes of air through filters and sorbents. This is the most accurate method for monitoring airborne concentrations but is usually not feasible be conducted at a large number of sites simultaneously, because of the high cost and logistical limitations. To overcome this, passive sampling strategies can be used. Their basic principle is to trap compounds that have reached the sampling medium passively, i.e. by advection and diffusion. A number of different passive air samplers (PAS) have been developed and tested, but because of the low cost and ease of shipping and deployment, polyurethane foam (PUF)-based passive air samplers have become very popular and have been used successfully in many studies (Roscales et al., 2018; Tuduri, Harner, & Hung, 2006). In these samplers, a PUF disk is housed in a chamber made from two stainless steel bowls (Figure 3). The chamber shields the PUF disk from being directly exposed to the wind, as this would result in a high variation of uptake rates depending on the sampling location. It also protects the PUF from direct sunlight and deposition of coarse particulate matter such as soil.
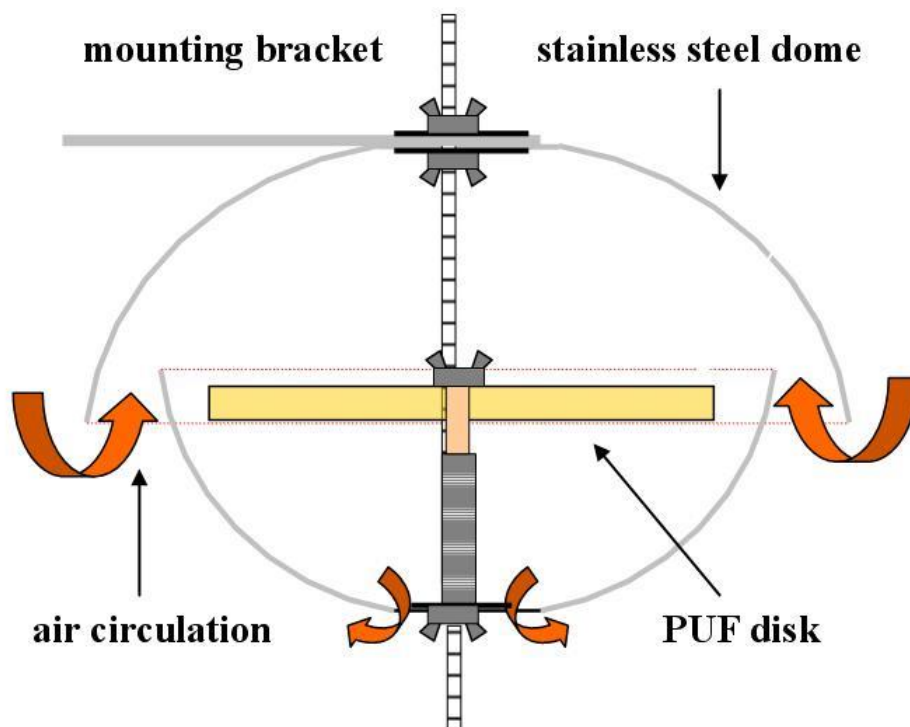
Figure 3 Schematic drawing of the "flying saucer" passive air sampler (collected at http://www.monairnet.eu/index-en.php?pg=methods--passive-air-sampling (2018))

PBDEs and n-BFRs, like many other non-polar compounds that are present in the gas phase of the air, have a high affinity to polymers including polyurethane, as those are relatively non-polar materials, compared to many inorganic matrices. PU can therefore accumulate large amounts of PBDEs and n-BFRs. In PU-foam, the polyurethane has been given a large surface (because of the many pores), enhancing the uptake of compounds from the air compared to solid sheets of plastic of the same mass (Rauert & Harner, 2016; Roscales et al., 2018; Tuduri et al., 2006).

PUF-passive air samplers do also trap particle-bound compounds to some extent, but this is less efficient than for gas phase compounds and the uptake rates are more variable and therefore more difficult to relate to concentrations in the air.

After deployment any air samples collected using filters or sorbents need to be extracted. For PBDEs and n-BFRs this requires fairly strong solvents because of the compounds' high affinity to the PUF material. Unfortunately, some of the most effective solvents such as acetone or dichloromethane do also dissolve substantial amounts of the PUF matrix. This is problematic for the instrumental analysis where it can alter the behaviour of the analytes in the instrument or cover, suppress or enhance the signal generated by the compounds of interest. It is therefore necessary to remove as much as possible of this PUF matrix while losing as little as possible of the analytes – an ongoing analytical challenge.

1.2.1.2.   Instrumental analysis of PBDEs and n-BFRs

For the analysis of trace amount of PBDEs and n-BFRs in extracts of environmental samples including air, highly sensitive instruments are required. The most common systems used for this purpose are gas chromatographs coupled a mass spectrometer as the detector, often referred to as GC-MS. The gas chromatograph separates the target compounds from each other and from other components present in the extracts, while the detector produces a measurable signal that depends on the concentration of the target compounds. The signal can therefore later be used to quantify the target compounds. The principle of both components of the GC-MS system will be explained briefly.

Gas chromatography (GC)

Since environmental samples contain a large number of components, both target compounds and other substances, it is necessary to separate them before the detector can be used to measure them. This is achieved chromatographically by injecting the sample into the GC where it is first vaporised under high temperatures. The vaporised sample will then be carried through GC column by an inert gas – the mobile phase. With MS detectors the gas used as mobile phase is usually helium. The GC column is a long (often 15 to 100 m) glass capillary that is coated on the inside with a polymer which acts as the stationary phase. Depending on their physicochemical properties, analytes and other components present in the extract (including the solvent) interact with the stationary phase. The stronger the interaction, the slower the compounds will be carried through the column by the mobile phase. How strongly they are slowed down depends on both their physicochemical properties (mainly vapour pressure and polarity) and on the temperature in the GC. The most volatile compounds will be carried all the way through the column to the detector at fairly low temperatures while less volatile compounds will require higher temperatures. Therefore the GC uses a temperature program, starting at temperatures that only allow the solvent to elute, followed by a steady or step-wise rise of the temperature to eventually elute all analytes. To separate depending on polarity, GC columns with a number of different stationary phases – from polar to highly unpolar – are available.

Mass spectrometer (MS)

Once the compounds reach the end of the GC column they are transferred into the detector. In a mass spectrometer (MS) they have to be ionised first because ions can be guided through the parts of the detector by applying suitable voltages. With the GC-MS instrument used in this project the ionisation occurs in the ion source of the MS by bombarding the often uncharged analyte molecules with electrons. This leads to an electron of the molecule being expelled, producing positive ions. As

the electrons have a high energy they can also fragment the molecules during this process, resulting in fragment ions. Under specific ionisation conditions, the mass and relative abundance of these ions are characteristic for each compound and its molecular structure. The ions are usually distinguished by their mass-to-charge ratios (m/z), in our application only ions of a charge of +1 are used. The ions are then guided through the detector by applying different electrical or magnetic fields. Only ions of a certain (chosen) m/z ratio are able to pass the detector on a stable path and be counted in the end, while other ions are lost. By changing the electric or magnetic field, different ions can be counted, either by scanning a wider m/z range or by selecting specific masses.

GC-MS is a highly sensitive technique, but for this reason it also requires relatively clean sample extracts. GC-MS cannot cope with large amounts of matrix components as they can often not be vaporised and will therefore foul the injector or if they reach the detector they can foul this part of the instrument, suppress the signal gained from analytes or produce a signal that cannot be distinguished from that of the analytes. Therefore a suitable clean-up is crucial for successful analysis of PBDEs and n-BFRs in environmental samples.

1.3. Analytical clean-up procedures for BFR analysis

Traditional clean-up methods for POPs very often use a step where concentrated sulphuric acid is used to oxydise and ultimately remove matrix components while not affecting the target compounds. At NILU for instance, an clean-up method involving sulphuric acid is used for clean-up of passive and active air samples for the analysis of PBDEs. The same methods, relying on sulphuric acid, have also been applied to n-BFRs (Table 4).

Table 4: Clean-up methods for (n-)BFR analysis, reported by different authors (extracted from (Covaci et al., 2011; Papachlimitzou, Barber, Losada, Bersuder, & Law, 2012)

n-BFR congners	Sample matrix	Method	Author
BTBPE, DBDPE, PBDE	Air, dust	Mixed silica column (KOH + sulphuric acid treated silica) then GPC	Pettersson-Julander et al. (2004)
HBB, PBDE-47, PBEB, PBT	Air	(6% water) neutral deactivated alumina column	Gouteux et al. (2008)
BTBPE, PBDE, phosphate ester FRs	Air	Silica/sulphuric acid column	Sjödin et al. (2001)
BTBPE, DBDPE, PBDE	Air, dust	KOH and sulphuric acid treated silica column	Karlsson et al. (2006 a,b)
DBDPE, PBDE	Indoor air and dust	Multilayer silica gel; silica, 2% KOH silica, silica, 44% sulphuric acid silica, 22 % sulphuric acid silica, silica	Takigami et al. (2009 a,b)
BTBPE, PBEB	Air	1% water deactivated silica, fractioned with hexane, 3:2 hexane-DCM, and DCM *(BTBPE in 2ⁿᵈ fraction)*	Hoh et al. (2005)
BTBPE, DBDPE, TBBPA-DBDPE + PBDEs	Air, dust	Silica/alumina column eluted with 30 ml hexane and 60 ml hexane:DCM (1:1)	Shi et al. (2009)
DBDPE	Air, dust	Concentrated sulphuric acid, Florisil column (1 g, eluted with 20 ml hexane)	Muenhor et al. (2010)
BTBPE, DBDPE, PBDEs	Air	3,5% w/w water deactivated silica gel, eluted with 25 ml hexane (F1) and 25 ml DCM (F2)	Venier et al. (2008)
HCDBCO	Air	Pipette w/glass wool and anhydrous sodium sulphate. Frozen at -20 °C overnight to remove excess water	Zhu et al. (2008)
-	Air	multilayer silica gel/alumina column followed by anhydrous sodium sulfate. The column was eluted with around 40 mL mixture of DCM/hexane (1:1 vol), and the eluent was concentrated to 0.2 mL under gentle N2 stream	Yadav et al. (2017)

However, many n-BFRs such as TBECH, EHTBB and BEHTBP are acid-labile (Geens, Ali, Roosens, Neels, & Covaci, 2010; Sahlström, Sellström, & De Wit, 2012), meaning they decompose when treated with acidic adsorption medias or acid. Therefore, when using sulphuric acid treatment, at least a fraction of those compounds can be expected to be destroyed during the clean-up. The extent of this loss will depend on the reaction time given (which in turn will depend on the amount of matrix to be removed) but also on the amount of matrix present and its nature. Since all these parameters can vary from sample to sample the loss is likely to be variable between samples, possibly resulting in a low accuracy and repeatability. n-BFRs are fairly new in an analytical aspect, thus robust multi component clean-up methods for n-BFRs are still lacking.

A number of different methods have been successfully applied to PUF-based air samples (see Table 4), including some methods that do not require the use of sulphuric acid, but often acid-labile compounds had not been studied.

Therefore, our aim was to develop and validate a method that allows to clean PUF-based passive air sample extracts in a way that produces extracts which are clean enough to be analysed on the highly sensitive instruments required for the analysis of n-BFRs at trace levels.

1.3.1. Solid phase extraction (SPE)

Solid phase extraction (SPE) is a promising technique that may allow to remove the PUF matrix from the extracts without the use of sulphuric acid.

SPE is a chromatographic method where the sample is applied to a sorbent or resin that was packed into a column and the target compounds are eluted using a suitable solvent. In order to clean extracts using SPE, the technique requires the target compounds and the interfering substances that need to be removed to have different affinities to the sorbent used. If the interfering compounds bind more strongly to the sorbents than the analytes then the analytes can be eluted from the SPE column with a solvent that is strong enough to wash them off the sorbent but does not elute the matrix compounds. If the interfering compounds bind less strongly to the sorbent than the analytes then a weak solvent can be used to first wash out the matrix compound. This first fraction will be discarded and the analytes can then be eluted with a stronger solvent.

SPE has become a widely used technique to clean sample extracts for the analysis of various organic compounds and there are many different sorbents and resins available to suit different analytes and sample matrices (Andrade-Eiroa, Canle, Leroy-Cancellieri, & Cerdà, 2016). Many of those sorbents and resins can also be purchased as ready-packed cartridges, potentially saving the user time.

In this study, two SPE techniques have been tested: Supelclean ENVI Florisil (from the manufacturer Supelco) and a modified version of the commercially available Supelclean EZ-POP (originally also manufactured by Supelco). Both methods are described in detail under 2.1. The performance of both methods regarding their suitability for n-BFR and PBDE analysis was compared to the original NILU method for PBDEs in PUF-based air samples which requires the use of sulphuric acid (described under 2.1.).

1.4. Method validation

The methods will thus be tested and validated based on a set of criteria:

The accuracy of a clean-up method is its ability to remove matrix effectively from the sample while simultaneously retaining a high percentage of compounds of interest. The accuracy is defined by the recovery (in %) of said components. Normally, to monitor the performance of the clean-up procedure for each sample analysed, a set of ^{13}C-labelled analogues (internal standards) of some of the target compounds would be added to each sample prior to the extraction. The recovery of the internal standard can then be determined. However, the PBDE internal standard at NILU only contains one congener from each homologous group, and for n-BFRs there are ^{13}C-labelled standards available for only some of the compounds. Therefore, to assess the method performance for all target compounds, known amounts of native ^{12}C congeners were used to determine the recovery.

The second validation parameter is repeatability, which is the ability of a method to produce similar results from multiple samples with similar concentrations. The repeatability is defined by the relative standard deviation (RSD in %), which is the deviation of the data from the mean value in the data set. The smaller the RSD, i.e. the less the data is deviating from each other, the better.

The third validation parameter was the suitability for real samples. This was assessed by analysing samples that had been exposed to ambient air outside NILU for three months.

1.5. Motivation and goals

As many n-BFRs are acid-labile and decompose when treated with acidic adsorbents or acid, an effective multi component clean-up method is lacking for the component group.

The HSE aspect is also important, as laboratory work with concentrated sulphuric acid requires stringent health and safety measures.

The main aim of this master's project was therefore to test and validate methods that were believed to be potentially suitable for cleaning PUF-based passive air sample extracts for the analysis of both n-BFRs and PBDEs, without the use of sulphuric acid.

During the project, it became clear that the performance of the methods tested can vary largely between different PUF samples, presumably depending on the behavior of co-extracted PUF matrix. As a result, a large part of the project was devoted to different ways of assessing the extent of matrix effects, i.e. effects caused by matrix components that interfere with the analysis of the target compounds. This enabled us to gain a clearer picture of the problems caused by the PUF matrix that

could not be removed successfully. In future, this knowledge will help with the search for a more suitable clean-up method.

2. Study design

To meet the goals set for this master thesis, the following study design was followed.

2.1. Comparison of clean-up methods

The effectiveness of the clean-up procedures were investigated by looking at different aspects: the clean-up method capacity in removing co-extracted polyurethane foam (PUF) material from the sample, and the methods ability to secure a satisfying recovery of the compounds of interest, in this case selected polybrominated diphenylether (PBDE) and newly brominated flame-retardants (n-BFR) congeners.

A thorough description of the clean-up methods, evaluating the properties of materials and solvents used, in an analytical aspect is given in Section 2.1.1.

The comparison was carried out by preparing parallel sets of samples, which were cleaned according to three different methods chosen for comparison (section 2.1.2). A selection of samples from each clean-up was analysed using two-dimensional gas chromatography (GCxGC), which provides a simplified image of the impurities caused by PUF matrix in the sample. This together with the use of chromatograms for the same reason is described in Section 2.1.3.

Additionally, validation parameters such as relative standard deviation (RSD) and percentage of recovery, were used for validating the clean-up methods, described in Section 2.1.4.

2.1.1. Methods description: Properties of materials in an analytical aspect

For simplicity reasons, the three clean-up methods used in the comparison is hereby named method A, B and C:

A) The reference method: Sulphuric acid treatment of the sample followed by elution through a column with activated silica.

B) The comparison method: SPE using Supelco Supelclean ENVI Florisil columns

C) The developed method: SPE with Zirconia-coated silica included as a sorbent, along with the more conventional Florisil and C18 sorbents. A manipulated form of the commercially available Supelclean EZ-POP SPE columns (produced by Supelco/Merck).

Following is a description of each methods. A full description of procedures in the lab is found in Appendix B.

2.1.1.1. Method A) Sulphuric acid/silica

In addition to be widely used for samples of other nature (biota, sediments etc.), this method has been found to be very efficient in terms of removing co-extracted substances from PUF-based air

samples, and is the preferred laboratory analytical method of participating institutions and laboratories in the EMEP. As the Chemical Coordinating Centre of EMEP, NILU has accredited procedures for this clean-up of actively collected air samples. In a survey of European background air conducted by NILU, the clean-up procedure was used for air samples collected passively, similar to the samples in this study.

Acidified aluminium/silica/florisil are also commonly used when analyzing novel brominated flame retardants (n-BFRs) (Covaci et al., 2011). However, it is known that some brominated flame-retardants degrade when exposed to acid; they are acid labile (Geens et al., 2010). This was the very reason the method for validation was developed, as a good clean-up method for analysis of brominated flame-retardants is needed. Additionally, the Health, Safety and Environment aspect is an important factor, as sulphuric acid is not a pleasant chemical to work with in the lab.

Method description:

The clean-up was done by "washing" the sample (in hexane) with concentrated sulphuric acid twice, before eluting it through a column with activated silica (activated by removing water traces at 450 °C for 8 hours).

When adding sulphuric acid to the sample, it oxidizes organic matrix materials, such as PUF. The acid and the non-polar solvent (n-hexane) are immiscible and forms two layers in the sample tube. Once a clear separation is obtained, the hexane phase (still containing the organic components of interest) can be carefully transferred to a new sample tube, leaving behind the acid phase containing matrix impurities. Depending on how matrix affected the sample is, the treatment can be repeated several times, each time oxidizing more of the matrix material.

To remove remaining co-extracted substances from the sample, it run through a column of activated silica (). Silica has a big external surface area, which makes it suitable as an absorbent of especially polar chemicals. The silica also secures a "pure" hexane phase without traces of acid, which could damage the instruments.

2.1.1.2. Method B) ENVI-Florisil SPE

The department for Environment and Climate Change by the Government of Canada governs the chemical analysis of passive air samples through the Global Atmospheric Passive (GAPS) network. This method is only based on passing the extracts through an anhydrous sodium sulphate column (Lee et al., 2016) without removing any unwanted matrix. Recently however, they have reported by e-mail a more thoroughly clean-up of passive air samples by using Florisil Typically, sorbents like Florisil retain co-extracted matrix components more strongly than the analytes of interest, allowing

for their removal from the extracts. The method is considered a good alternative to the developed method C, and was thus decided to be used for comparison in the project.

Method description:

The sample (in isooctane) was cleaned by eluting it with n-hexane through Supelco Superclean ENVI-Florisil SPE tubes (500 mg, 3 ml), purchased from Sigma. Florisil consists of magnesium silicate, a highly polar sorbent that interacts with polar functional groups, hence removing compounds with a polar character in the PUF matrix. Because our PUF matrix has shown to consist of a large variety of compounds with polar and nonpolar character, and given the limited capacity of the small tubes, sufficient clean-up is expected to be challenging.

However, given that the original GAPS analytical method is only based on sodium sulphate, we question that there are differences in the PUF material that give rise to more co-extracted compounds in our PUF extracts. Another issue of concern, is that the columns are packed in plastic tubes, which may give rise to elevated concentrations due to additives in the plastic materials.

2.1.1.3. Method C) Modified EZ-POP

As described in the Introduction chapter about method development, the manipulated form of EZ-POP showed promising results in meeting the wanted requirements for a clean-up method. The column is originally pre-packed in plastic tubes and only available with 2.5 grams packing material. In order to manually prepare SPE glass columns, the size was increased due to differences in diameter of the plastic- and glass columns. Further increase was introduced to maximize the capacity and clean-up efficiency. Initially, the packing material was therefore doubled to 5 g. However, in this project, it was decided that the size was impractical as there was very little room on top of the column to perform a controlled elution. Therefore, the manipulated EZ-POP column was reduced back to 4 g.

Method description:

The modified EZ-POP column was packed with two layers of sorbents, separated by a glass fibre filter (GFF) frit. The top layer consists of 2 g of conventional Florisil sorbent (Supelclean LC-Florisil from Supelco/Merck. The bottom layer consists of a 1:1 mixture of conventional C18 sorbent (Discovery DCS-18) and Zirconia-coated silica (Z-Sep). While the Florisil retains polar functional groups and the C18 retain non-polar functional groups, Zirconium is capable to act as a Lewis acid (i.e. electron pair acceptor) and to interact with Lewis bases (i.e. electron pair donors), hence removing interfering compounds through Lewis acid-base interactions. PUR, possible degradation products and additives that co-extract with the substances of interest may have electron pairs available for donation.

The clean-up was carried out by adding the sample (in Acetonitrile) to the manually packed EZ-POP column, which had been washed with acetone, dried by vacuum and conditioned with acetonitrile (ACN). Cleaning the column with acetone removes possible water traces and other unwanted substances from the materials. By adding 1-2 times the volume of the column with acetonitrile, it is made sure of that all acetone and impurities are removed. Additionally, the column is prepared for the samples, which secures consistent interaction and maximizes retention of impurities in the sample.

The sample was eluted with ACN.

2.1.2. Sample description

The following set of samples were prepared for each round of clean-up:

- Three parallels of blank PUF samples spiked with native PBDE and n-BFR congeners to meet the validation criteria as described in Section 2.1.4.
- Lab blanks to establish method detection limits and
- Samples (PUF-disk) that had been deployed in "flying saucer" passive air samplers next to each other on a fence at NILU, Kjeller for three months in (February to May 2016).

Additionally, a second set of PUF disks for spiking, lab and solvent blanks were prepared for clean-up with method B, as it was believed that the clean-up could be affected by differences in the PUF matrix.

2.1.3. Visualizing matrix effects: GCxGC and GC-MS lockmass

Plots obtained from two-dimensional gas chromatography (GCxGC), in addition to assessments of the lock-mass intensities from gas chromatography-mass spectrometry (GC-MS) was used to visualize the presence of matrix in the samples.

The GCxGC method uses two subsequent gas chromatography columns so separate the samples, which gives a two-dimensional chromatogram where the sample is separated by boiling point in the x direction and by polarity in the y direction.

GCxGC is a useful and quite complicated tool for analysing a wide range of samples. In this thesis however, the GCxGC method was only used for obtaining a good visualization of matrix in comparable samples.

A simplified schematic "map" on how to read the GCxGC plots was obtained from Röhler et al. (2014). The map is shown in Figure 4.
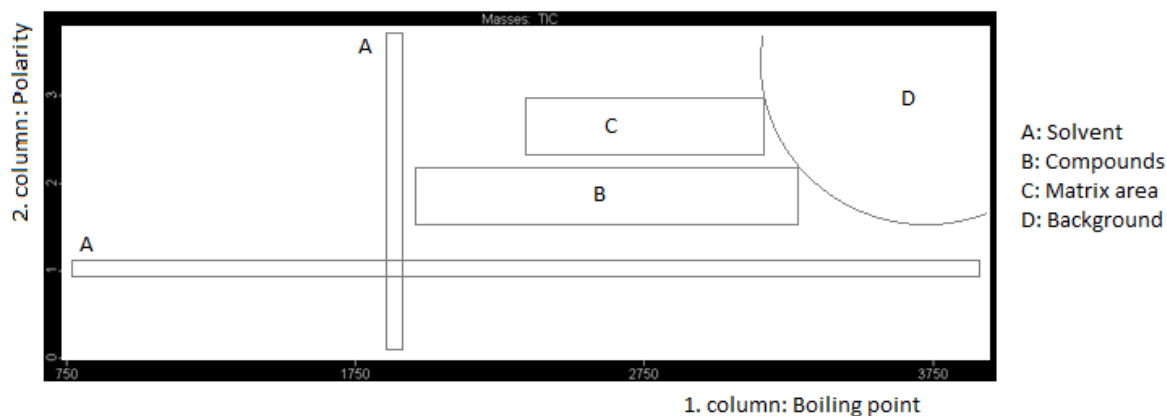
Figure 4: Simplified and schematic «map» on how to read two-dimensional plots (chromatograms) obtained from two-dimensional gas chromatography (GCxGC). For the purpose in this thesis, area C was the most interesting one, as a presence of signals in the form of green-to-red colour (weaker-to-stronger signal) implied a presence of matrix from the sampling material polyurethane foam (PUF). The x-axis shows the first column, which separated the sample based on boiling point. The y-axis shows the second column, which separates the sample from polar to non-polar.

GC-MS is a two-instrument method for separating and quantitatively analysing a sample. The principle of GC-MS is that gas chromatography is used to separate the sample through a capillary column with coated with stationary phase, which makes the compounds in the sample elute at their respective retention time based on chemical and physical interaction with the stationary phase. Second, the mass-spectrometer captures each "pulse" of compounds from the GC, ionizes them, filters out the ions selected by the uses and detects them. Organic compounds tend to fragment during ionization, with the masses of the fragments and their relative abundance depending on the molecular structure. When using specific ionisation conditions the resulting mass spectrum (i.e. the relative abundance of each fragment ion) is compound specific and can be used to identify chemicals. To improve sensitivity in the routine analysis of samples with low analyte concentrations, only a few of the most abundant fragments are monitored for each compound instead of scanning a wide mass range

The GC-MS used at NILU is a high-resolution instrument that is very precise in terms of masses, meaning ions with even very small differences in mass can be distinguished from each other. For high-resolution instrumentation to work, it has to be constantly corrected for small changes in mass-reading caused by the samples. This is done by using a MS lockmass standard (PFK-perfluorokerosene) that fragments with exact known masses which is being constantly injected at the same time as the sample, giving the instrument a constantly high signal throughout the sample series. In each retention time window, one of those fragments is chosen as the *lock-mass*. The instrument then cycles through all the ions in the sample, including the fragment ions that is the lock-mass, and corrects everything to make the lock-mass match the known mass of which it is supposed to be.

Ideally, a constantly high and horizontal lock-mass signal should be obtained for each time-window. However, when matrix from the samples gets into the detector, the sensitivity of the instrument drops, making the lock-mass signal go down in intensity (or opposite, which is also possible if there are co-eluting substances). This happens temporarily, making the lock-mass signal drop for a little while until the disturbing compounds are out of the detector, before it again reaches a high and stable signal. It can also happen permanently if the detector becomes too spoiled by impurities from the sample.

Just by looking at the lock-mass in the retention time window of where the compounds of interest would be eluted, the effect of matrix in the sample can thus be seen. An example of this is shown in Figure 5, which shows a "perfect" lock-mass signal in the top chromatogram, and one that has been affected by matrix from a dirty sample in the bottom chromatogram.
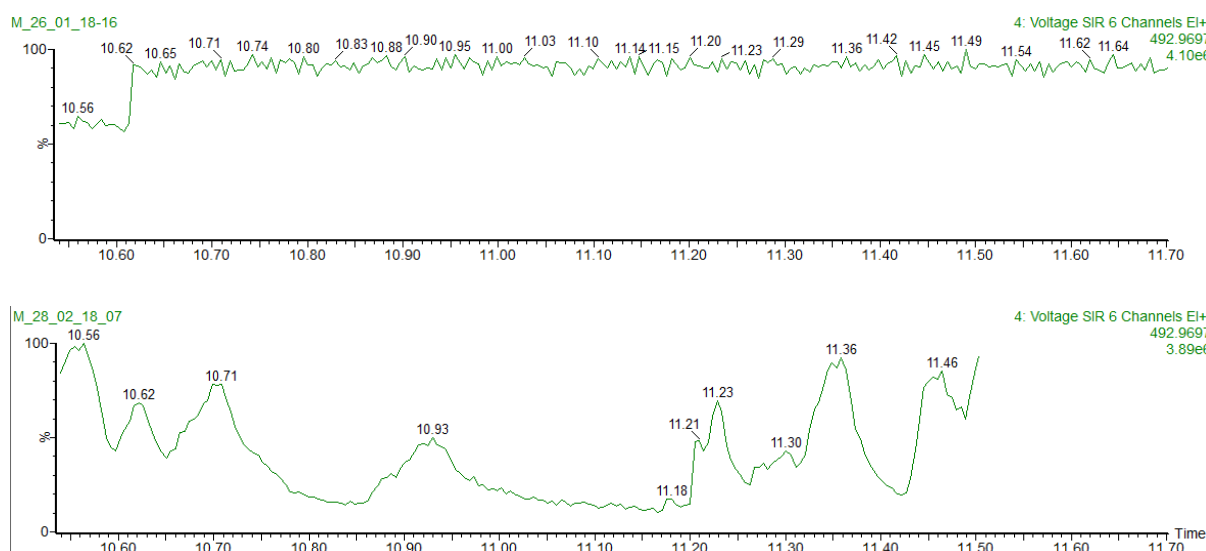
Figure 5: An example of a GC-MS lock-mass signal that have been affected by impurities in the injected sample is shown in the bottom chromatogram. The top chromatogram is an example of how the lock-mass "should" look like: A high and stable signal of high intensity throughout the retention time window.

The risk of a drop in lock-mass intensity is when the drop happens at the same retention time as a compound of interest is eluted; The signal from the compound may then be interfered by the same signals as the lock-mass, and be suppressed by it. A change in signal leads to a lower detected concentration of the compound in the sample. The opposite can also happen if the lock-mass experiences a "hop" in signal intensity just as the compound in eluted. Then the sample signal is magnified by the lock-mass signal, leading to a higher detected concentration than what is true.

Therefore, it is important to check the lock-mass signal intensity in the time window to evaluate the uncertainty in the data.

2.1.4. Accuracy and repeatability

The compared clean-up methods in the project are validated based on two parameters: accuracy and repeatability.

The accuracy of a clean-up method is its ability to remove matrix effectively from the sample while simultaneously retaining a high percentage of compounds of interest. The accuracy is defined by the recovery (in %) of said components. Normally, to monitor the performance of the clean-up procedure for each sample analysed, a set of ^{13}C-labelled analogues (internal standards) of some of the target compounds would be added to each sample prior to the extraction. The recovery of the internal standard can then be determined. However, the PBDE internal standard at NILU only contains one congener from each homologous group, and for n-BFRs there are ^{13}C-labelled standards available for only some of the compounds. Therefore, to assess the method performance for all target compounds, known amounts of native ^{12}C congeners were used to determine the recovery.

The recovery is retrieved by calculating the percentage that the measured amount of a compound is compared to the known added amount. According to NILU's quality manual for accredited organic analysis, the recovery should lie somewhere between 40 and 130 % (from here on referred to as the quality interval).

The second validation parameter is repeatability, which is the ability of a method to produce similar results from multiple samples with similar concentrations. The repeatability is defined by the relative standard deviation (RSD in %), which is the deviation of the data from the mean value in the data set. The smaller the RSD, the less the data is deviating from each other, the better. A complete table for all RSD values from the project can be found in Appendix A.

2.2. Matrix effects

There are several things that may have an influence on the matrix caused by PUFs in a passive air samples, such as the environment in which they have been deployed (e.g. growth of mould or fungi, insects or dust), the cleaning procedure of PUFs before use, etc. This subject could make up a whole project by itself, so only two aspects of PUF matrix were selected for investigation in this thesis: The difference of PUF disks that have been exposed to the natural environment vs. PUF disks that are new, and difference between PUF types from suppliers.

These parameters were investigating by comparing GCxGC plots and GC-MS chromatograms of exposed samples and lab blanks, as explained in Section 2.1.3

2.2.1. PUFs

During previous work in the lab, a sort of "maturing" of the PUF disks that had been deployed in a "flying saucer" passive air sampler for three months was observed. To different extents, exposed PUFs seemed to endure rougher treatment than new PUFs, as they did not break as easily during removal from the Soxhlet post extraction. Additionally, more PUF material was observed as particles in extracts from blank samples compared to exposed samples. It was believed that this would have an impact of the detection limits when working with larger datasets of exposed samples, as the clean-up of blank samples would be more affected by PUF matrix than the exposed PUFs.

2.2.2. PUF suppliers

During the course of the project, NILU's supplier of polyurethane foam (Sunde Søm og Skumfabrik AS) changed their supplier of materials. Even though the supplier reports the same PUF material as before, there might still be structural differences between the PUF "types".

This meant that the samples that had been exposed for three months was of the old type of PUF, while all other samples were of the new kind. However, any difference in PUF matrix between the new and the old type could not be investigated as no blanks from the old PUF type nor any exposed samples from the new PUF type was available. Still, it is an aspect to keep in mind when comparing the clean-up methods for the exposed samples.

Disregarding the possible difference in matrix between the old and the new type of PUF, the matrix effect from exposed and new PUFs were investigated using GCxGC and GC-MS lockmass for each of the three clean-up methods A, B and C.

2.3. Suitability of ISTDs for n-BFR quantification

Internal standards (ISTDs) are compounds used to quantify target compounds in samples by adding a known amount of the ISTD to the sample. By using the knowledge regarding how much ISTDs were added and the area of the ISTD to mention a few parameters, one is able to calculate the amount of target compounds in the sample. Additionally, in combination with a recovery standard (RSTD), the amount of internal standard can be calculated (using the RSTD as an "ISTD" for the actual ISTD). There are a few things to consider when choosing an internal standard for a compound. First, the compound must be absent in the sample. Secondly, it must behave in the same manner as the target compound in terms of ionization. Moreover, it should have similar structure and molecular weight as the target compound, and behave in a similar way during sample preparations (extraction, clean-up etc.).

The best and most fitting ISTDs are molecules of the target compound that has been labelled with one or several ^{13}C isotopes. However, as n-BFRs are relatively new to both the marked and in the

interest of analytical chemists, most of them lack these ^{13}C isotope-labelled ISTDs. For their analysis, ISTDs have been selected based on similarities in fragment ion masses, and are listed in Table 5.

Table 5: The n-BFRs analyzed at NILU with their associated ISTDs. Most n-BFRs lack their "own" ^{13}C-isotope labelled ISTD. For these, other labelled compounds have been chosen, e.g. ^{13}C-PBDEs.

ISTD	n-BFR
[13]C-BDE-28	ATE (TBP-AE)
	a-TBECH
	b-TBECH
	g/d-TBECH
	BATE
	PBT
	PBEB
[13]C-HBB	HBB
[13]C-BDE-47	DPTE
[13]C-EHTBB	EHTBB
[13]C-BTBPE	BTBPE
	BEHTBP
[13]C-DBDPE	DBDPE

However, these does not always work very well, as the compounds behaves differently both during clean-up and in the instrument during analysis. They thus often gives an under- or over-estimation of the target compound concentration.

To disregard effects caused by matrix from the PUFs, results for n-BFRs from control standards (no PUF material or clean-up procedure involved) were quantified manually disregarding their internal standards (full procedure described in 6.5.2). How well the ISTDs suited their assigned n-BFR was evaluated by comparing the manual quantification to the quantification done the more adapted way using MassLynx as the quantification software and their assigned ISTDs.

2.4. Detection limits

For quantitative analysis, the limit of detection (LOD) is a measure of the lowest possible value that is detectable in the sample. It is obtained by the signal-to-noise ratio times three, and is given by MassLynx.

The method detection limit (MDL) is the detection limit of each compound within a method, given by the detected amount of a compound in a blank sample which is multiplied by three. For compounds that are not detected in the blank sample, the MDL equals the LOD.

3. Results and discussion

3.1. Comparison of clean-up methods

Three clean-up methods called A, B and C (as described in 2.1.1), were compared and validated. In this chapter the different methods are compared and validated regarding the performance aspects described under Section 2.1

3.1.1. Visualizing matrix effects using GCxGC and GC-MS lockmass

Two dimensional gas chromatography (GCxGC) was used to get an overview of matrix effects on lab blanks prepared with the three clean-up methods A, B and C (as explained in Section 2.1.3) (Figure 6).

Using the simple approach described in Section 2.1.3, GCxGC plots were interpreted. Background signal is indicated by blue colour, while a stronger signal goes from green to yellow to red, and indicate matrix. A lack of signals in the "matrix area" for the lab blanks prepared with clean-up method A (top picture) is less matrix affected than lab blanks from method B and C (Figure 6).
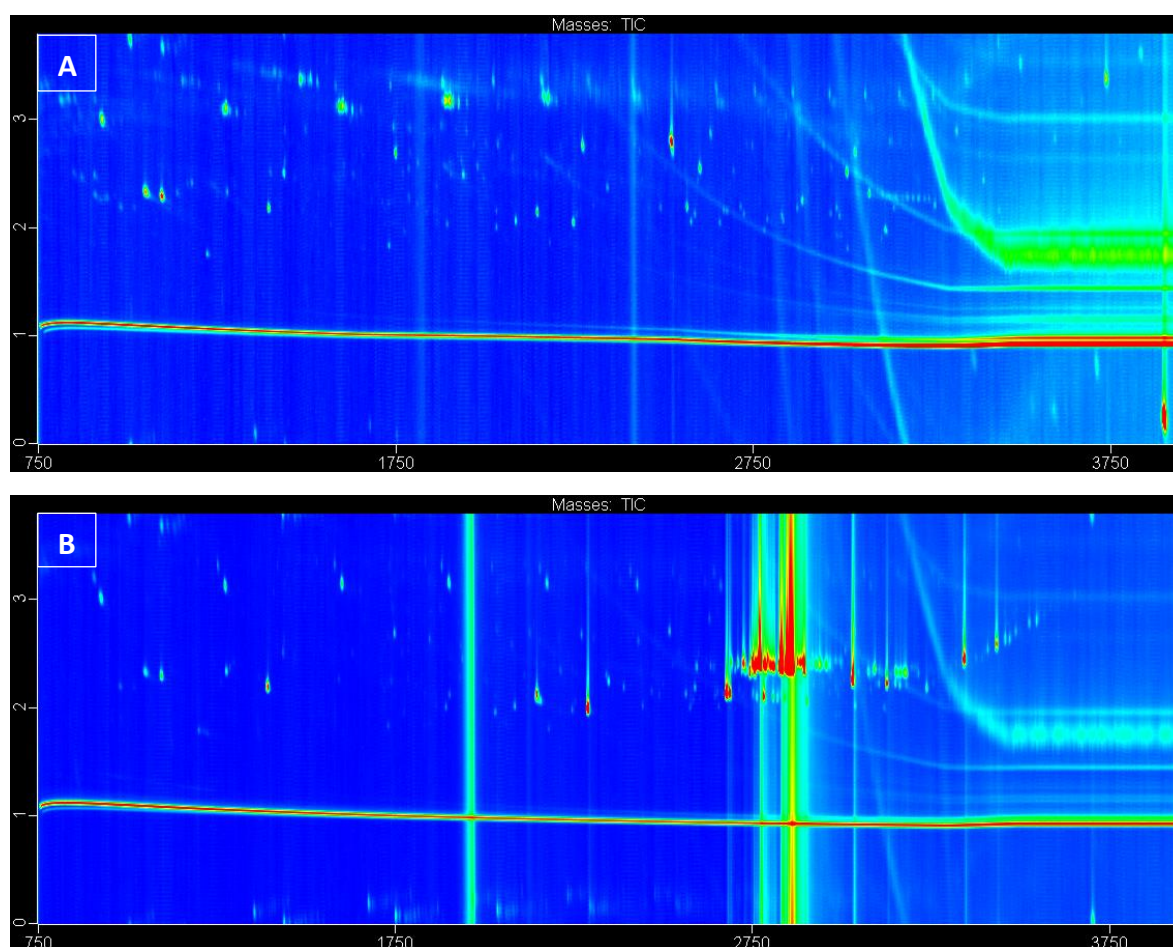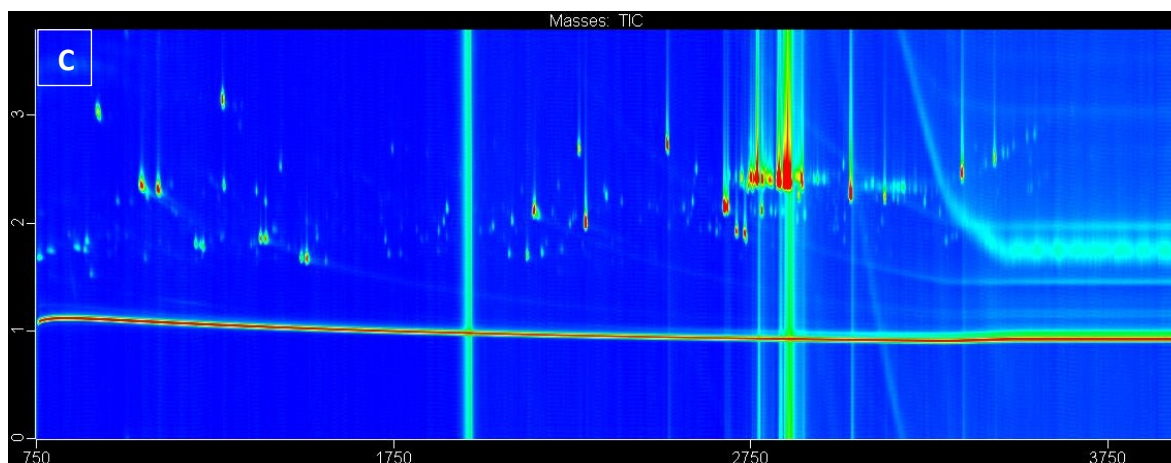
Figure 6: GCxGC plots of laboratory blank samples cleaned with three consecutive clean-up methods: Method A) (top) sulphuric acid treatment followed by silica clean-up; method B) (middle) SPE w/ 300 mg ENVI-Florisil columns, eluted with hexane; and method C) (bottom) SPE w/ 4 g EZ-POP columns eluted with acetonitrile.

The matrix effects were also assessed by comparing the lock-mass signal intensities between both lab and solvent blanks cleaned with methods A, B or C. The lock-mass signal is the signal from a MS lock-mass standard (Perfluorokerosene (PFK)) that has a high intensity through the entire retention time window, and is expected to be stable; a temporary drop in the intensity indicates suppression by interfering substances (as further described in section 2.1.3). Since this will also affect other masses, including those of the compound fragment ions, it gives rise to a higher uncertainty of the results.

The lock-mass signal in every retention time window was assessed (see Appendix B) and the most severe drops in their intensity were observed in function 4 (retention time window 10:55-11:70 min). In the PBDE method, this is the retention time window where tetra-BDEs elute, with 13C-BDE-47 used as the internal standard (ISTD). These were also the analytes that tended to show the worst peak shape (i.e. shoulders and double peaks), confirming that this retention time window is suitable for comparing matrix effects based on the lock-mass signal. The lock-mass used in this function is 492.9697 m/z.

The n-BFR instrument method uses the same GC oven program as the PBDE instrument method, with nearly identical retention time windows but the lock-mass in function 4 is differently (430.9728 m/z). The n-BFR compounds HBB and DPTE elute during this window and also 13C-HBB and 13C-BDE-47, which are used as ISTD for HBB and DPTE, respectively. Even if the masses are differently, the effect on lock mass is comparable. Therefore, the lock-mass for PBDE in function 4 is used for interpretation of matrix effects for both PBDEs and n-BFRs, and are given in figure 8.
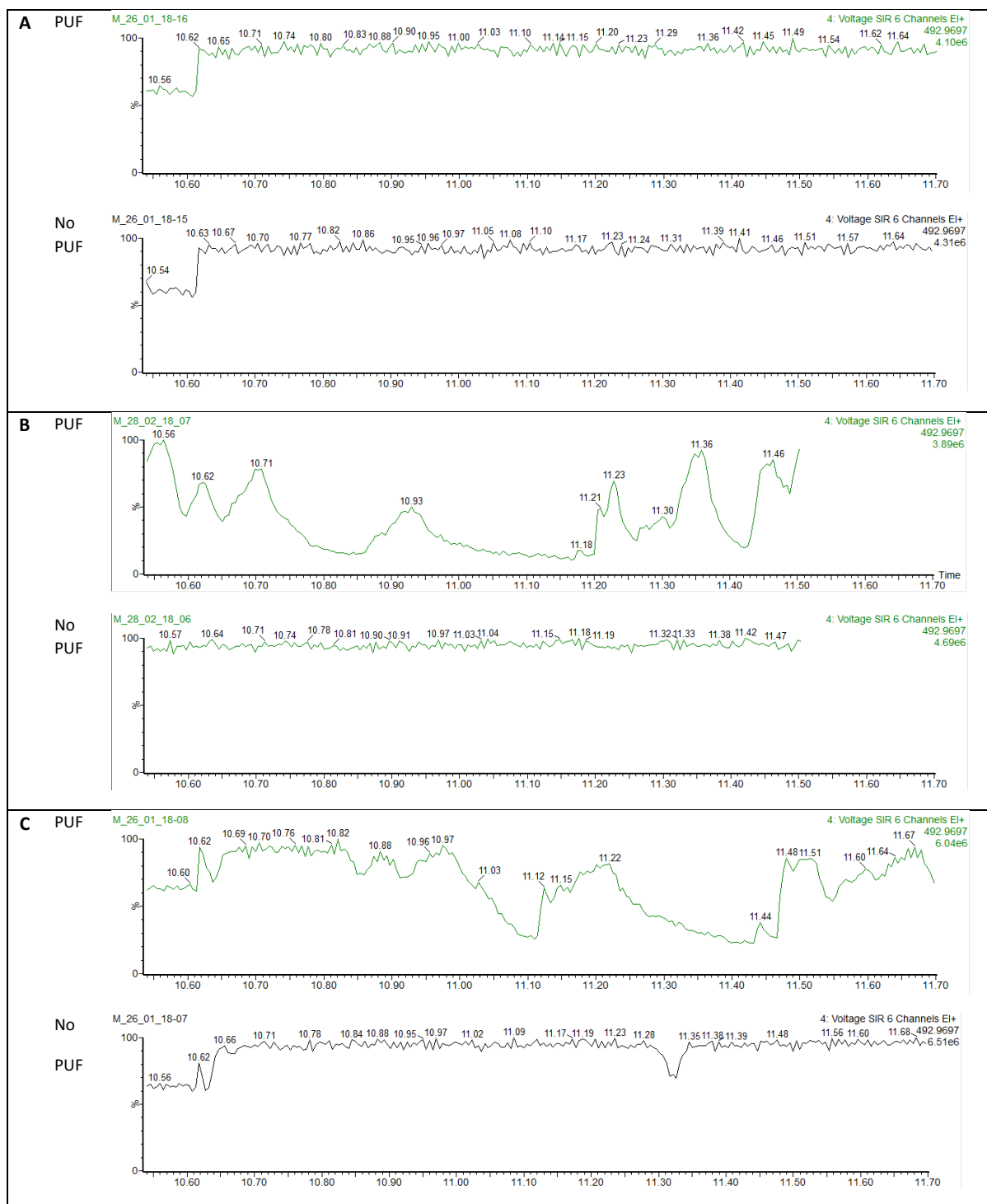
Figure 7: Plots of the Intensity of the lock-mass signal for PUF (lab blank) and noPUF (solvent blank) samples cleaned with method A, B and C prior to PBDE analysis. Matrix effects are seen as "dips" in the lock-mass line, which should be a stable signal at a high intensity (as seen for noPUF sample cleaned with method A (top)). Collected from MassLynx, function 4 (retention time window 10:55-11:70), selected ion 492.96 m/z (channel 4).

For method A, the intensity of the lock-mass signal was constant for both the PUF sample (laboratory blank) and the noPUF sample (solvent blank). This shows that clean-up method A is successful for removing PUF matrix. This result is expected, as method A is the reference method used in the accredited NILU laboratory for sample clean-up.

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A PUF extract cleaned according to method B on the other hand, resulted in a substantial dip of lock-mass signal intensity, while the lock-mass signal for the noPUF extract did not show any sign of being affected by this problem. This indicates that clean-up method B is not successful in removing PUF matrix from the sample, and that compounds eluting at the same retention time during GC analysis can experience suppression of signal. The same is seen for method C, where analysis of the PUF sample also resulted in a loss in lock-mass signal intensity.

3.1.2. Method validation: Accuracy and repeatability

In order to evaluate the clean-up methods, the recovery of 12C-PBDEs and 12C-BFRS after clean-up with methods A, B and C where monitored (see Figure 4 and Figure 5, respectively). Further, to evaluate the spread of the data as a vital parameter to assess accuracy and repeatability, relative standard deviation (RSD) was calculated as the ratio between the standard deviation and the average recovery of the three parallels in each method. Selected RSD values are shown for PBDE and n-BFR analysis in Table 1 and Table 2, respectively, and are used for the discussion of recoveries. A complete RSD table for all PBDE and n-BFR congeners can be found in Appendix A.

3.1.2.1. PBDEs

Chromatograms showing all PBDE congeners analysed are given in Appendix B.

When evaluating the recovery of native PBDE congeners (^{12}C) after clean up with method A, B and C, some choices were made. The medians for method A and B was used, as this provides statistically relevant information.

Second, method C proved to deliver rather unreliable data, as one parallel of the three showed too much matrix influence to be quantified successfully. The recovery data of this parallel were discarded, as they cannot provide credible results.

For the other two parallels from method C, the PUF matrix affected the instrument sensitivity during different retention time periods. Based on the highest reliability of the ISTDs (indicated by the most stable lock-mass signal as discussed in Section 2.1.3, one parallel was chosen as the most useful for recovery of congeners belonging to the ISTDs from ^{13}C-PBDE-28 to -99, and the other parallel for congeners belonging to the ISTDs from ^{13}C-PBDE-153 to -209.

Additionally, recovery of standard controls (3 parallels) containing the same amounts of ISTDs and native components as the spiked PUF samples, were used to assess the influence of PUF matrix on the recovery, as these samples did not have any PUF matrix nor had been treated with any clean up methods. The standard controls thus were good indicators of where the recovery of the samples

"should" lie, if they were not influenced by matrix or sample preparations (extraction, clean-up, etc.).

Figure 9 shows the median of the three parallels for method A and B plotted together with the chosen PBDE recoveries from method C. In addition, the median values from the control standard parallels are included.
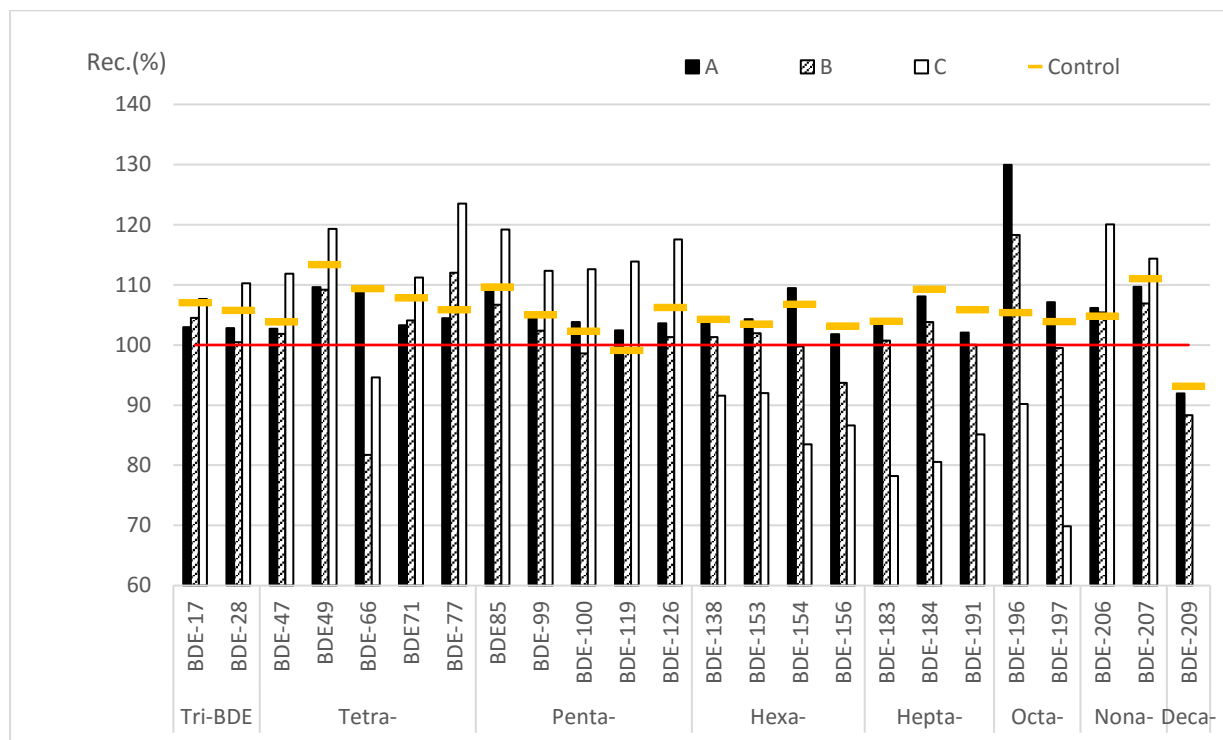
Figure 8: Recovery of PBDE congeners after clean-up of PUF disks with three different methods: A) Sulphuric acid/silica (black column), B) SPE w/ENVI-Florisil (patterned column) and C) SPE with 4 g EZ-POP (white column). Recovery of standard control samples with the exact same concentrations of compounds but without PUF matrix is also shown (yellow line). Prior to sample preparation, the samples had been spiked with ^{12}C-PBDE congeners and ISTDs. The bars for method A and B and the standard control represents the median of three parallel samples, while the bars for method C are picked out of the dataset of two parallels (further described in Section 3.1.2.1)..

Evaluating the recovery of PBDEs shown in Figure 89, we see that method A generally yields a good recovery of PBDE congeners of around 100 %. Only BDE-196 had a slightly higher than expected recovery of 130%.

Method B generally follows method A, but with a slightly lower recovery of all PBDEs (10-20 pp. lower). The exception are BDE-17, BDE-71 and -77, which have a higher recovery for method B than for method A (2, 1 and 7 pp., respectively). BDE-66 on the other hand is significantly lower in recovery, with a recovery of about 25 pp. lower than for method A.

Method C has high recoveries between BDE-77 to BDE-126 (more than 100 %, highest value 124 %) and lower recoveries for BDE-138 to BDE-197 (less than 100 %, lowest value 70 %). These data have been selected out of the data sets of two parallels cleaned with method C, separated by the same

PBDE homologous groups (Tri-, tetra-, penta-, etc.-BDE). Checking the "ignored" data (the second half of PBDE congeners from two of the parallels that were picked out to complete the data set, as previously explained) for the parallels, the same pattern with high recoveries for lighter PBDEs than for heavier ones was found. This was most likely a result of PUF matrix that had not been sufficiently removed, as the matrix compounds may hinder the transfer of PBDE congeners from the liner to the column. This effect is generally stronger in more highly brominated PBDE congeners as they are more "sticky", i.e. less volatile. It may also lead to degradation of compounds in the injector – for PBDEs this effect is also usually stronger with the more highly brominated congeners.

The lock-mass signal intensity can once again be used for determining the impact of matrix on the response of analytes: Interferences may suppress the signal obtained from both analytes and MS reference standard. A drop in lock-mass signal indicates reduced sensitivity, which will also affect the signal of the target compounds during the corresponding retention time.

An example of this effect is observed for BDE-66 for method B: When comparing lock-mass and chromatograms for PBDE congeners eluted in function 4 (time window 10:55-11:70) (Figure 9), drops in lock-mass intensity in two of the three parallels were observed at the retention time of BDE-66 (approximately at 11:30, marked by a red square in Figure 9). For the same two parallels, a lower amount of BDE-66 was recorded (respectively 82 and 47 % recovery compared to 113 % for the third parallel).
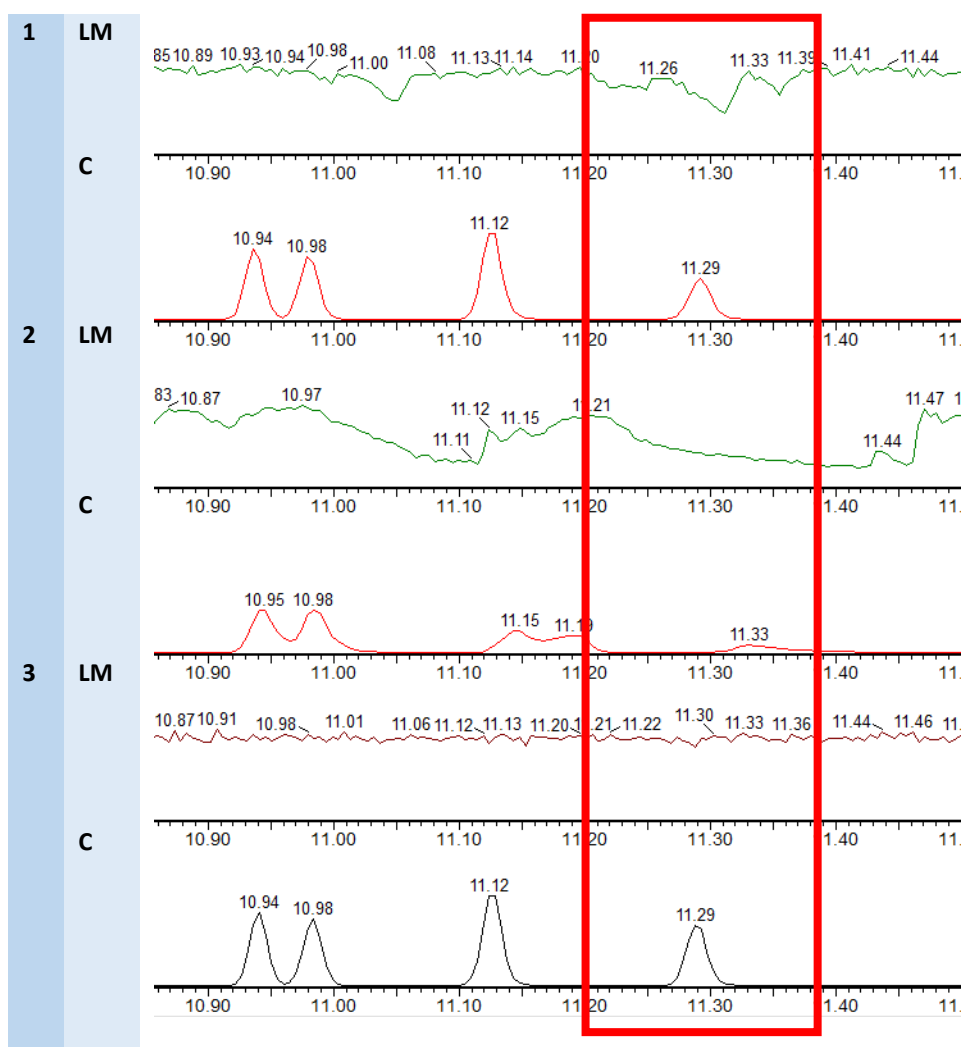
Figure 9: Lock-mass (LM) and chromatograms (C) for the three parallels (1-3) cleaned with method B. This is an example of the suppression effect that a drop in lock-mass intensity has on the signal of the analyte: Parallel 1 and 2 has a drop in lock-mass intensity at the retention time of BDE-66 (11:30), which reflect on the intensity of the peak at that retention time.

This again affected the RSD, as it is a measure of spread in the data: Compared to clean-up method A which had a high and stable lock-mass intensity throughout the time window (10:55-11:70) and a low RSD of 1.1 %, clean-up method B had a high RSD value of 40.5 % (marked red in Table 6).

Table 6: Relative standard deviation (RSD) for the clean-up methods A, B and C, in addition to the standard (std.) control for PBDE congeners eluted in the same time window (10:55-11:70, Function 4 in MassLynx). The RSD values for BDE-66 are discussed in Section 3.1.2.1, and is therefore marked in red. RSD (%) is a measure of repeatability, and is calculated from the ratio of the standard deviation and the average value (n=3). The selected PBDE congeners shown were discussed, the complete table for all PBDEs and n-BFRs can be found in Appendix A.

	A (%)	B (%)	C (%)	Std.control
BDE-49	2.4	8.2	10.6	1.4
BDE-71	2.6	19.0	34.0	1.2
BDE-47	0.7	1.1	3.7	0.4
BDE-66	1.1	40.5	92.4	1.1
BDE-77	1.0	14.9	173.2	1.3

3.1.2.2.   n-BFR

Chromatograms showing all n-BFRs analysed are given in Appendix B.

Results from the n-BFRs analysis of the blank samples that had been cleaned according to method C showed that the matrix levels were high. The matrix interferences caused a substantial loss in lock-mass signal and fouled the instrument so badly that it required instrument maintenance (e.g. cleaning liner and cutting of GC-column)before any analysis could continue. The three parallels prepared with method C were therefore not analysed for n-BFRs.

However, later on in the project a single 12C-spiked PUF sample cleaned with method C was prepared, together with a solvent blank. This was done as part of a follow-up project to this thesis. The recovery for the single PUF sample was calculated, and plotted together with the median of the recoveries for the three parallels from method A and B in Figure 5.
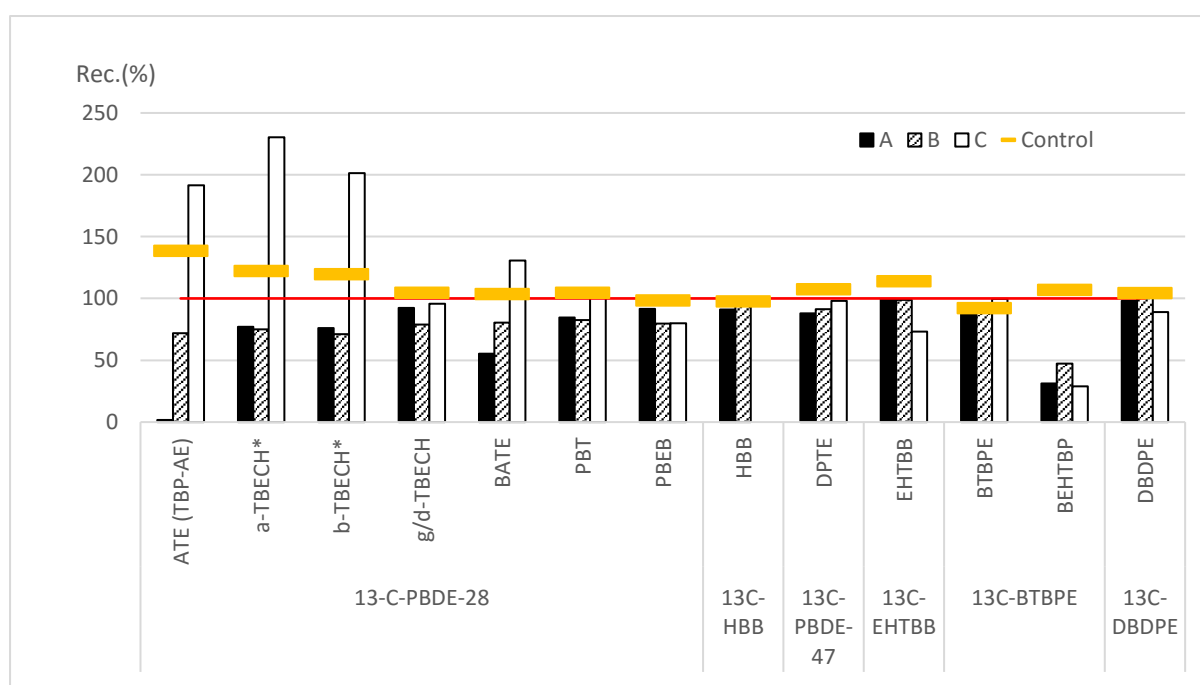
Figure 10: Recovery (%) of n-BFR congeners after clean-up of passive air samples with three different methods: A) Sulphuric acid/silica (black column); B) SPE w/ 300 mg ENVI-Florisil (Sigma) (patterned column); and C) SPE w/ 4 g EZ-POP (white column). Recovery of standard control samples spiked with the same concentrations of native compounds but without PUF matrix or clean-up is also shown (yellow lines). The bars for method A and B and the standard control represent the median of three parallels for each method, while the bars for method C represent recovery of a single sample. The ISTDs for the n-BFRs are also shown on the x-axis.

From Figure 5 we see that method A has low recovery of some n-BFR congeners (between 3 and 70 pp. lower than for method B). A low recovery of compounds in samples cleaned with this method can have three explanations. Either a reduction in sensitivity at the retention time of the compound (which would also cause a drop in lock-mass signal intensity, as discussed for PBDE in Section 3.1.2.1); the ISTD is not suitable for the compound (discussed later in Section 3.3); or the n-BFR compound is acid labile and breaks down when treated with sulphuric acid.

Especially prominent is the recovery of allyl-2,4,6-tribromophenyl (ATE), with a recovery of only 1.7 % for method A. Method B however, yielded a satisfying recovery of 73 % for ATE. This suggests that the compound is acid-labile and is degraded when treated with sulphuric acid, which is also in agreement with the ATE properties as reported by the Authors Geens, Ali, Roosens, Neels, & Covaci (2010). The Authors also report acid lability for BATE, which is yielded lower recovery for method A compared to methods B and C (55, 80 and 130 % recovery for methods A, B and C, respectively).

The recovery of BEHTBP is also rather low (39.4 %) for method A. Decomposition of the component during clean-up could be one explanation to the low recovery. However, the recovery of BEHTBP is also rather low for method B and C (49 and 29 %). The standard control has a close to 100 % recovery of BEHTBP (see chapter 3.3.), which suggests that the low recovery is not due to differences to the ISTD (^{13}C-BTBPE).

Looking at the RSD valued for BEHTBP in Table 7 (marked in red), a high RSD is seen for the compound after clean-up with method A (46.3 %). Checking the data, the three parallels for method A range from 27 to 60 % recovery. However, there are no drop in lock-mass at corresponding retention times for BTBPE and BEHTBP, which indicates that there are no matrix effects in the MS-detector causing the large variations. The explanation could therefore be that the component is stuck at the front end of the instrument (injector/beginning of column), as described for the heavier PBDEs.

The RSD value for BEHTBP method B is at 10.3 %, and the parallels are ranging from 45 to 55 % recovery. This spread in data is not as big, but the average recovery is still low (at 49 %).

Table 7: Relative standard deviations (RSD) for n-BFRs after clean-up of three parallels per method (A and B) is shown. Three parallels for a standard (Std.) control without clean-up or PUF matrix is also displayed. The RSD is calculated by the ratio between the standard deviation and the average value within the data set of each method. Low RSD suggests low spread in data between the three parallels.

n-BFR:	A (%)	B (%)	Std.control (%)
ATE	20.8	12.3	7.4
a-TBECH	2.9	5.6	7.3
b-TBECH	5.4	7.0	7.5
g/d-TBECH	1.2	3.2	8.3
BATE	7.0	4.2	7.4
PBT	1.7	3.9	2.2
PBEB	2.0	15.1	2.8
HBB	1.0	5.2	1.2
DPTE	3.8	2.8	3.1
EHTBB	1.8	6.0	0.5
BTBPE	2.8	4.0	0.4
BEHTBP	46.3	10.3	0.8
DBDPE	2.3	4.2	1.1

3.2. Matrix effects

3.2.1. Exposed PUFs vs. new NILU PUFs

During laboratory work, a difference in behaviour of exposed and new PUFs was observed. After extraction, the PUF disks were removed from the Soxhlet body using metal tweezers, and new PUFs were nearly impossible to remove without tearing them. Additionally, new PUFs release more visible PUF material during extraction, and blank samples that were volume-reduced often had a thick or sticky layer of material in the bottom of the TurboVap evaporation glass. In comparison, it seemed as if the exposed PUFs were "aged" in the field and did not decompose as easily.

To visualize this effect, lock mass and GC chromatograms of exposed samples were compared with non-exposed laboratory blanks for each of the methods A, B and C as describes in section 3.1.1 (Figure 1112-13). It is important to keep in mind that the PUF disks used for the exposed samples are not of the same type as PUF disks used for the lab blanks (further explained in Section 2.2), as the supplier of the PUFs (Sunde Søm og Skumfabrikk AS) got a new supplier of material during the course of the project.
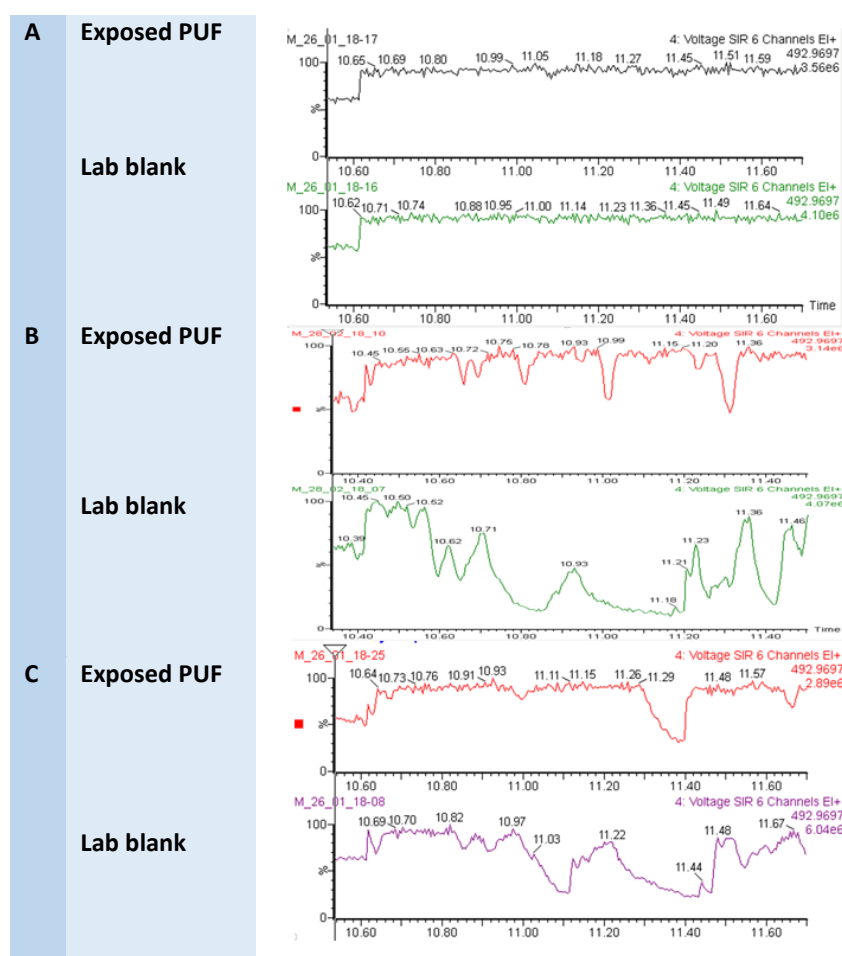
Figure 11: Plots of the Intensity of the lock-mass signal for exposed PUF and laboratory blanks cleaned with method A, B and C prior to PBDE analysis. Matrix effects are seen as "dips" in the lock-mass line, which should be a stable signal at a high intensity (as seen for method A (top)). Collected from MassLynx, function 4 (retention time window 10:55-11:70), selected ion 492.96 m/z (channel 4).
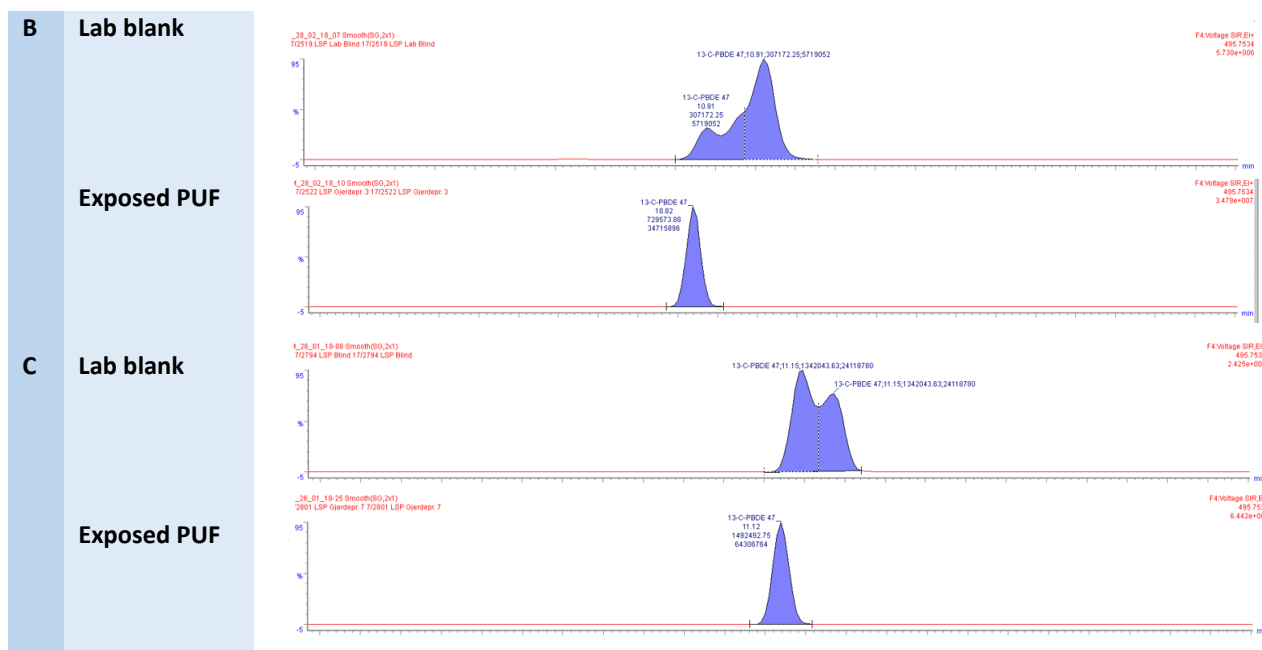
F*igure 123: GC chromatograms of exposed PUF and laboratory blanks cleaned with method B and C prior to PBDE analysis. Matrix effects are seen as bad peak shape of the ISTD of PBDE47.*

The large differences in lock masses of exposed PUFs and laboratory blanks (figure 12), suggest that lab blanks seem to be more matrix affected than exposed samples for method B and C. The GC chromatograms of these two methods are given in figure 13. The bad peak shape indicates that the matrix in blank samples also affect column chromatography.

3.2.2. Comparison of NILU PUF and Canadian PUF

It was decided to expand the test of method B, since the method originated from the Canadian Government who uses a different PUF type than NILU. The results clearly indicated that method B do not remove PUF matrix sufficiently. However, the question is if the clean-up method would be sufficient if the Canadian PUF type (hereby called "CanPUF") was analysed?

Three parallels of CanPUFs were spiked with ^{12}C PBDE and n-BFR components in addition to ISTDs and extracted according to the NILU PUF samples, followed by clean-up with method B. In addition, one laboratory blank (with CanPUF) and a solvent blank was prepared in the same manner.

A comparison of GCxGC plots of PUFs and CanPUFs is shown in Figure 13Figure 13, below.
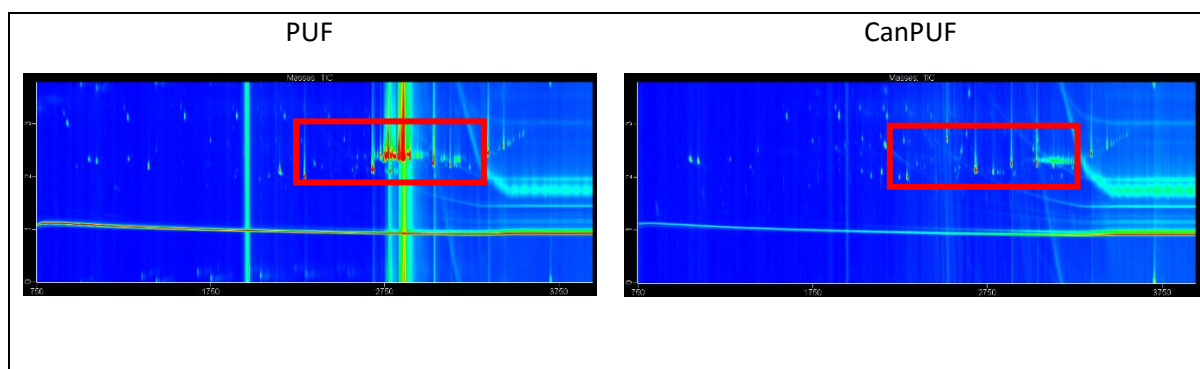
Figure 13: Comparison of two dimensional gas chromatography (GCxGC) plots for laboratory blanks with the PUF type used at NILU (PUF, left) and the PUF type used at the Canadian Governments department of Environment and Climate Change (CanPUF, right), to see the difference in matrix effects of two different PUF types. The PUF and the CanPUF are both cleaned with method B (SPE w/ 300 mg ENVI-Florisil columns (Sigma)).

From the GCxGC plots, we see that the Canadian PUF type is less affected by matrix than the NILU PUF type, as there are strong signals in the "matrix area" for the NILU PUF (marked by red squares in Figure 13).

Additionally, the lock-mass signals obtained when analyzing NILU PUF and CanPUF laboratory blanks are a good visualization of the same effect of matrix from the two PUF types (Figure 14), as lock-mass signal intensity has several big dips for the NILU PUF while the CanPUF has a high and stable lock-mass signal through the entire retention time window (Figure 14).
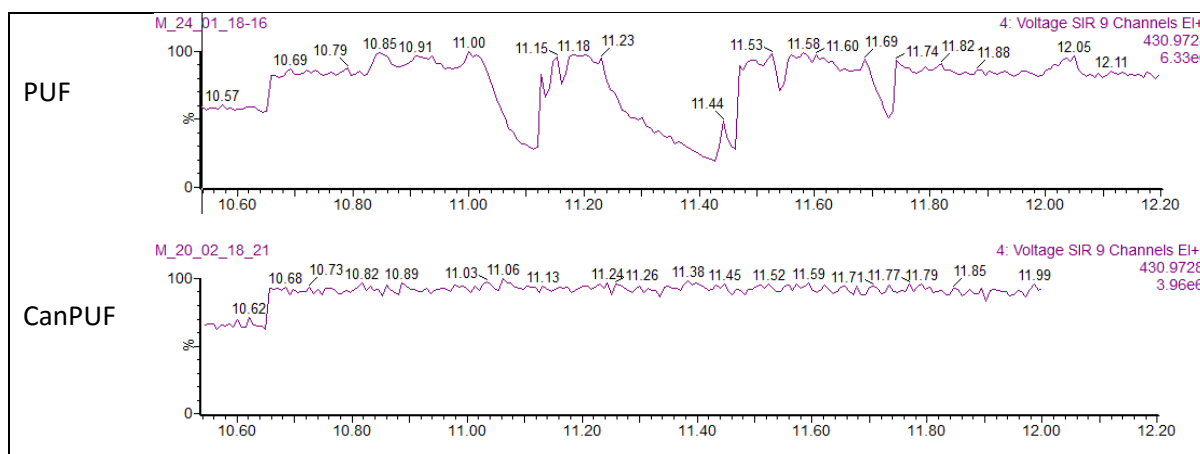
Figure 14: Lockmass screenshots for laboratory blanks of the PUF type used at NILU (PUF, top), and of the Canadian PUF type (CanPUF, bottom), prepared with the same clean-up method C.

It might therefore seem as if clean-up method B is more effective in removing PUF matrix from Canadian PUFs than from NILU PUF, as the PUF material is different.

3.3.   Evaluation of internal standards for n-BFRs

Standard control samples were quantified with and without respect to their ISTDs as described in Section 2.3. The average recovery (%) of the three parallels after quantification with and without ISTDs are plotted in Figure 15.
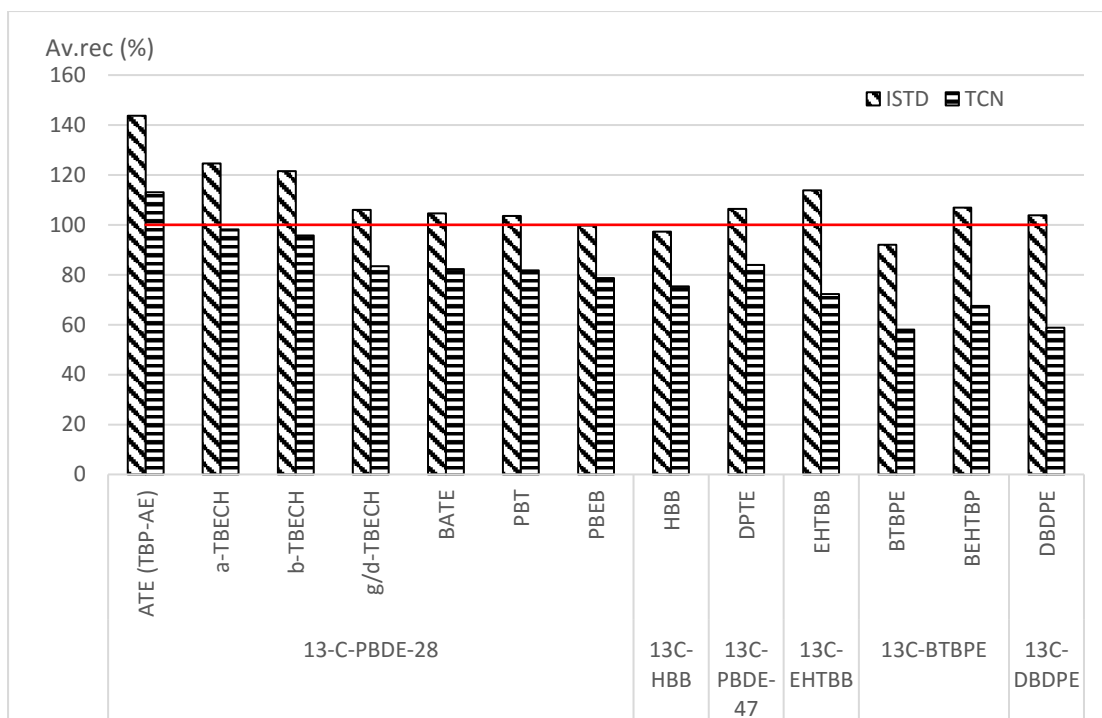
Figure 15: Recovery of n-BFR congeners in standard control sample, quantified with respect to ISTDs listed in bottom column and with respect to TCN as an internal standard for all. This was done to observe the how the ISTDs affect the quantification and to see whether or not the ISTDs for n-BFR compounds that are lacking ^{13}C-isotope labelled ISTDs of their own, are suitable.

From the average recovery of n-BFRs in Figure 6, we see that all n-BFRs have a lower recovery when quantified with TCN as the ISTD instead of the ISTDs listed in the bottom column. TCN elutes a lot earlier than the n-BFR congeners of interest. For routine analysis, it is used as recovery standard and is added to the sample in the very end of the sample preparations, which gives it a recovery of 100 %. TCN is used as an instrument performance standard, it only corrects for differences between samples that affect all compounds to the same extend (e.g. small differences in injection volume, differences in final extract volume, differences in the instrument sensitivity that are constant over the time it takes to analyse one sample). However, here TCN results have been used in the same way as ISTDs to calculate target compound concentrations. This was done to see if any ISTD appears to be an unsuitable choice, i.e. behaves differently in the instrument from the compounds that are usually corrected with this ISTD (without the presence of matrix). Internal standards that correct well for any differences the standard controls may experience but do not correct well for differences when analysing PUF extracts must be caused by target compounds and ISTDs behaving differently during the cleanup or reacting differently to matrix being present. The latter is most likely to happen in the injector and the beginning of the column where matrix may cause some compounds to "get stuck", i.e. not be released further into the column, or in the detector where co-eluting matrix can temporarily reduce the sensitivity. If the sensitivity is lower while the ISTD elutes than when an analyte elutes then the analyte concentration will be overestimated. Likewise, if the sensitivity is

higher while the ISTD elutes than when an analyte elutes the analyte concentration will be underestimated. Signal suppression and resulting loss in sensitivity can be confirmed by a loss in lock-mass signal intensity. It is important to note that the lock-mass signal will not react to matrix effects in the GC.

Table 8: Relative standard deviations (RSD) for three parallels of a standard control sample, quantified with respect to their original ISTD (column "ISTD") and with respect to TCN, ignoring the original ISTD (column "TCN"). The RSD (%) is calculated from the ratio between the standard deviation and the average recovery, and is a measure of the spread in the data: A smaller RSD shows a smaller spread in the data.

Original ISTD	n-BFR	ISTD	TCN
^{13}C-BDE-28	ATE	7,4	1,2
	a-TBECH	7,3	1,2
	b-TBECH	7,5	1,7
	g/d-TBECH	8,2	2,5
	BATE	7,4	1,9
	PBT	2,2	5,4
	PBEB	2,8	7,4
^{13}C-HBB	HBB	7,9	7,9
^{13}C-BDE-47	DPTE	1,2	7,7
^{13}C-EHTBB	EHTBB	10,1	10,1
^{13}C-BTBPE	BTBPE	3,1	7,7
	BEHTBP	9,4	9,4
^{13}C-DBDPE	DBDPE	0,5	8,9

From Table 8, we see that n-BFRs that have their "own" ^{13}C isotope labelled ISTD (such as DBDPE) have the lowest RSD values for the recoveries calculated with respect to the ISTD. For compounds eluting later than BATE (in Table 3 below BATE) the RSDs for the recoveries calculated when taking the assigned ISTD into account are slightly higher than for compounds with an "own" ISTD but are still lower than when ignoring the ISTD. Only BATE and the n-BFRs eluting before BATE show lower RSD values for their recoveries when TCN is used instead of the assigned internal standard (^{13}C-BDE-28)

This shows that n-BFRs that have their own ISTD have a lower spread in data, meaning that the repeatability is satisfying compared to when ignoring the ISTD, and that the ISTD is suitable for the compound. Only 13C-BDE-28 may not be the most suitable ISTD for the compounds eluting earliest. to correct for variations during instrumental analysis, even without matrix being present.

This could imply that the ISTD is not very suitable for those compounds and that using it could give an over-estimation of recovered concentrations in the sample (as seen in Figure 6).

3.4. Levels of PBDEs and n-BFRs in passive air samples

Eight PUFs were deployed in "flying saucer" passive air samplers and hung from February to May 2016 on the fence outside the NILU building. At the end of the sampling period, the PUF disks were collected and properly stored in a freezer until sample preparations began in fall 2017.

The eight samples were extracted according to the rest of the project and divided between the three clean-up methods: 2 samples for method A, 3 samples for method B and 3 samples for method C. Additionally, a set of PUF lab blanks and solvent blanks were prepared in the same way. However, it is important to remember that the exposed samples and the lab blanks were extracted from different PUF materials with possible differences in properties, as described in Section 44, as the supplier of PUF to NILU changed their material supplier during the course of the project. After sample preparations (as described in Appendix B), the samples were analysed for PBDEs and n-BFRs.

From the levels of the lab blanks, method detection limits (MDL) were calculated as described in Section 2.4, and used to assess the validity of the data.

3.4.1. PBDEs

The MDL is calculated by multiplying the detected amount in the laboratory blank by 3. In blank samples where the compound is not detected, the MDL equals the LOD (given by the quantification).

MDL for the three methods A, B and C for the detection of PBDEs in lab blanks are shown in Table 9.

Table 9: Method detection limits (MDL) given in amount per sample (pg/s) for PBDE congeners after sample preparations with the clean-up methods A, B and C. The MDL is calculated from 3 times the detected amount in the laboratory blank. Where no amount is detected, the MDL equals the instrumental limit of detection (LOD), which is obtained from the quantification. Where the MDL is equal to the LOD is marked by *. For the reference method A, the LOD is used for MDLs all over, as this method has well established LODs from the accredited work at NILU.

ISTD	PBDE	A MDL* (pg/s)	B MDL (pg/s)	C MDL (pg/s)
Tri-BDE	BDE-17	1.96	8.11	1.37*
	BDE-28	8.12	9.66	3.49*
	BDE-47	95.64	46.48	1.01*
Tetra-	BDE-49	4.94	11.57	1.52*
	BDE-66	9.18	61.36	36.08*
	BDE-71	2.47	11.24	1.51*
	BDE-77	1.78	2.11.*	0.97*
	BDE-85	2.44	0.53*	0.28*
Penta-	BDE-99	23.70	14.78	5.83*
	BDE-100	5.35	0.32*	0.75*
	BDE-119	1.60	0.47*	1.56*
	BDE-126	1.21	0.37*	0.19*
	BDE-138	5.45	5.82	0.71*
Hexa-	BDE-153	5.36	10.87	5.23*
	BDE-154	2.88	5.44	0.43*
	BDE-156	5.88	6.19	1.14*
Hepta-	BDE-183	5.93	15.70	5.46*
	BDE-184	2.21	5.03	0.41*
	BDE-191	5.33	1.26*	0.87*
Octa-	BDE-196	11.18	8.13	1.78.*
	BDE-197	11.05	8.40	1.37*
Nona-	BDE-206	54.78	39.69	35.22
	BDE-207	22.98	41.53	23.26
Deca-	BDE-209	215.08	188.38	153.17

The levels of PBDE congeners in the passive air samples are shown in **Error! Reference source not ound.**, and results from lab and solvent blank samples prepared with the same clean-up methods are shown in **Error! Reference source not found.**. High amounts of BDE-47 and BDE-209 made the y-xis too large to properly view the results all together, so BDE-47 and BDE-209 levels are shown in separate figures (Figure 17 and Figure 18), respectively. The PBDE amounts of lab and solvent blanks for the congeners are shown in the same figure.
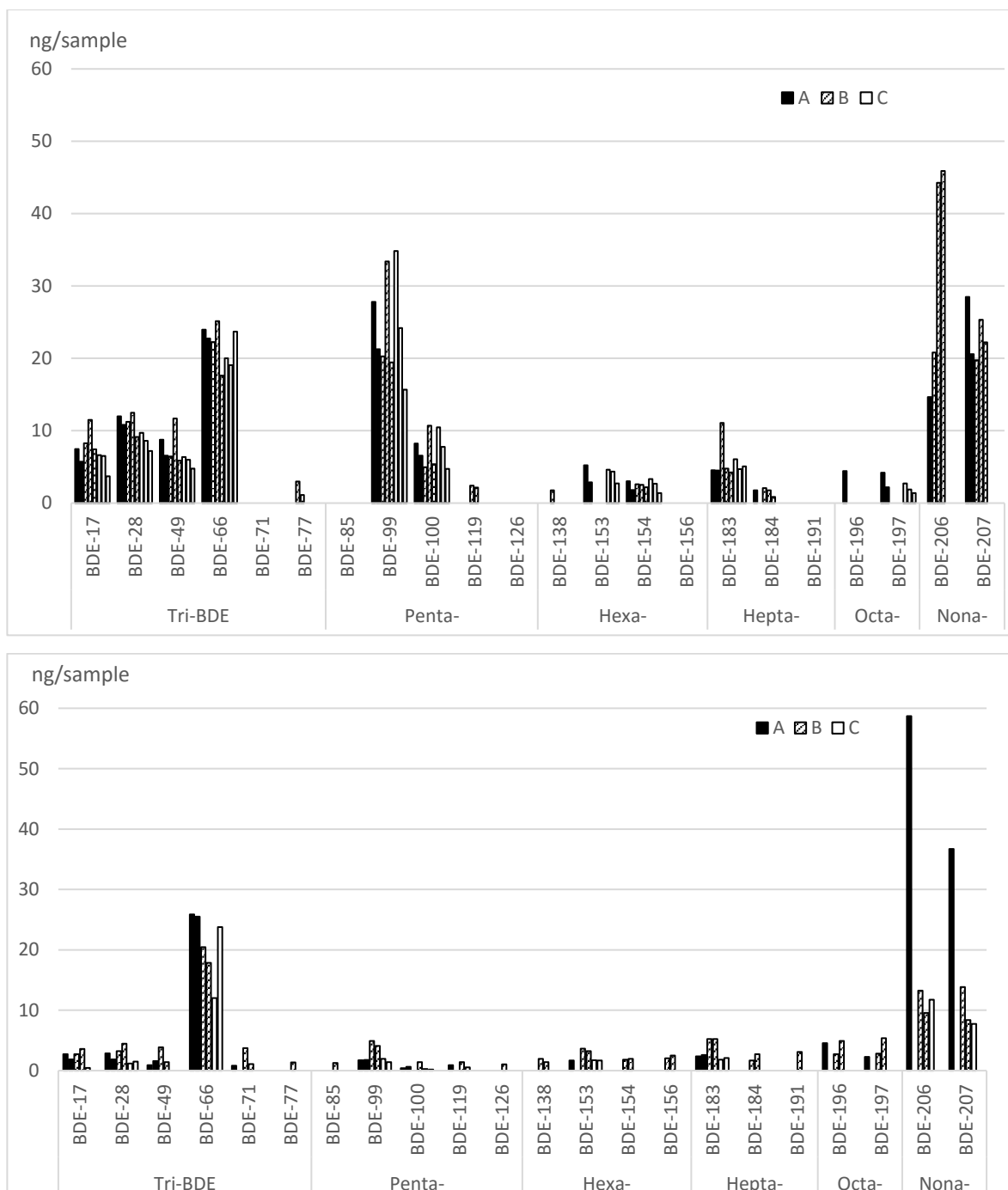
Figure 16: Levels of PBDE congeners in passive air samples from Kjeller, Norway (top) and in blank samples (bottom) for samples prepared with each of the clean-up methods A, B and C. For the passive air samples, eight samples were deployed and divided between the three clean up methods (A, B and C). For the blank samples, the lab blank (with PUF) is represented by the left column, and the solvent blank (without PUF) is represented by the right column. The PBDE congeners are divided into homologous groups (from tri- to nona-PBDE).

The passive air samples (top, Figure 16) shows higher levels of BDE-66, -99, -206 and -207, than for the other congeners. BDE-66 is equally high in both passive samples and in lab- and solvent blank (bottom, Figure 16), from 22 to 25 pg/sample. The same is seen for method B and C, but with bigger variation between the samples (RSD of 17.5 and 11.7 %, respectively (Table 10)). This implies that the samples have been exposed to BDE-66 during sample preparations rather than in the field.

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For BDE-99 on the other hand, high levels in the exposed samples (19-34 pg/sample) are seen for all exposed samples with some variations within the data sets (RSD of 18.9, 32.2 and 38.6 % for methods A, B and C, respectively). This congener is found in very small amounts in the blank samples, with the highest value found for method B (4 pg/sample). This implies that congener existed in the outside environment.

The same pattern in seen for BDE-47 (Figure 17), as even higher levels (51.1-89.5 pg/sample) where detected in the exposed samples (the second most abundant congener detected in the exposed samples). In the blank samples, the congener was detected at a maximum of 15.5 pg/sample (in the lab blank for method B).

This could be explained by the fact that BDE-99 is one main congener in the penta-BDE technical mixture and that BDE-47 is the main congener in the Tetra-BDE technical mixture (Table 2), which have been widely used flame retardant products.
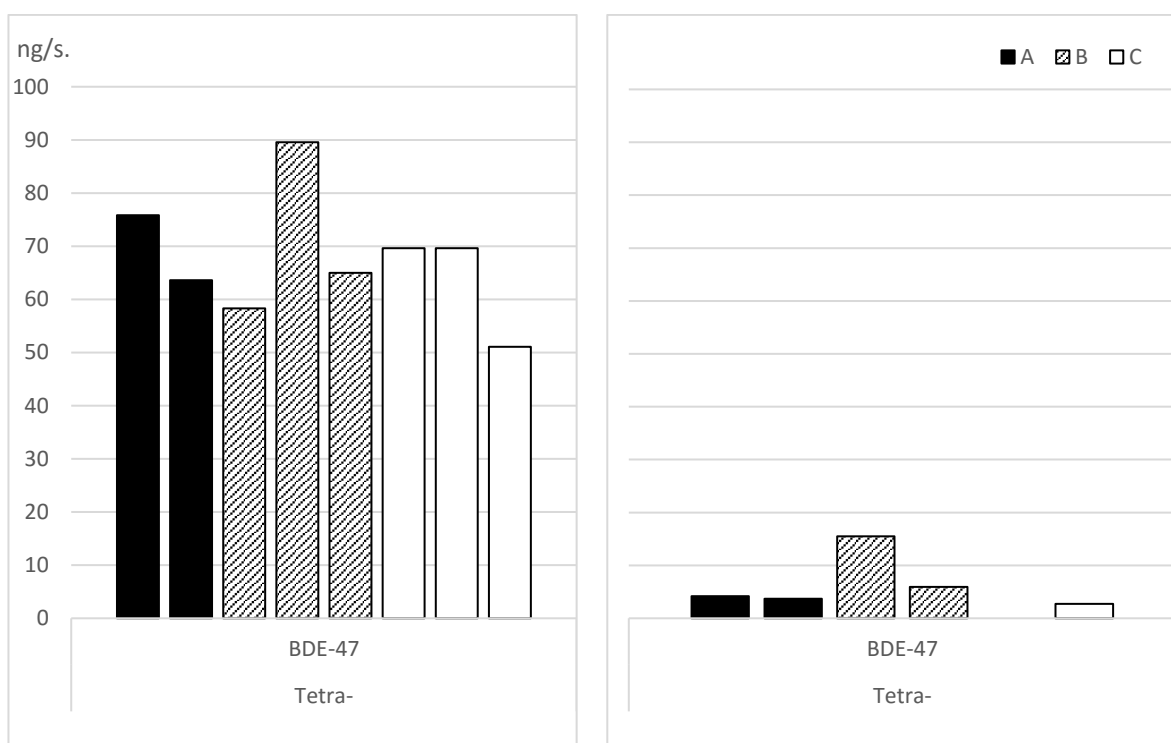
Figure 17: The levels of BDE-47 in passive air samples (right) and in laboratory and solvent blanks (left) after clean-up with method A, B and C. The amounts are given in pg/sample.
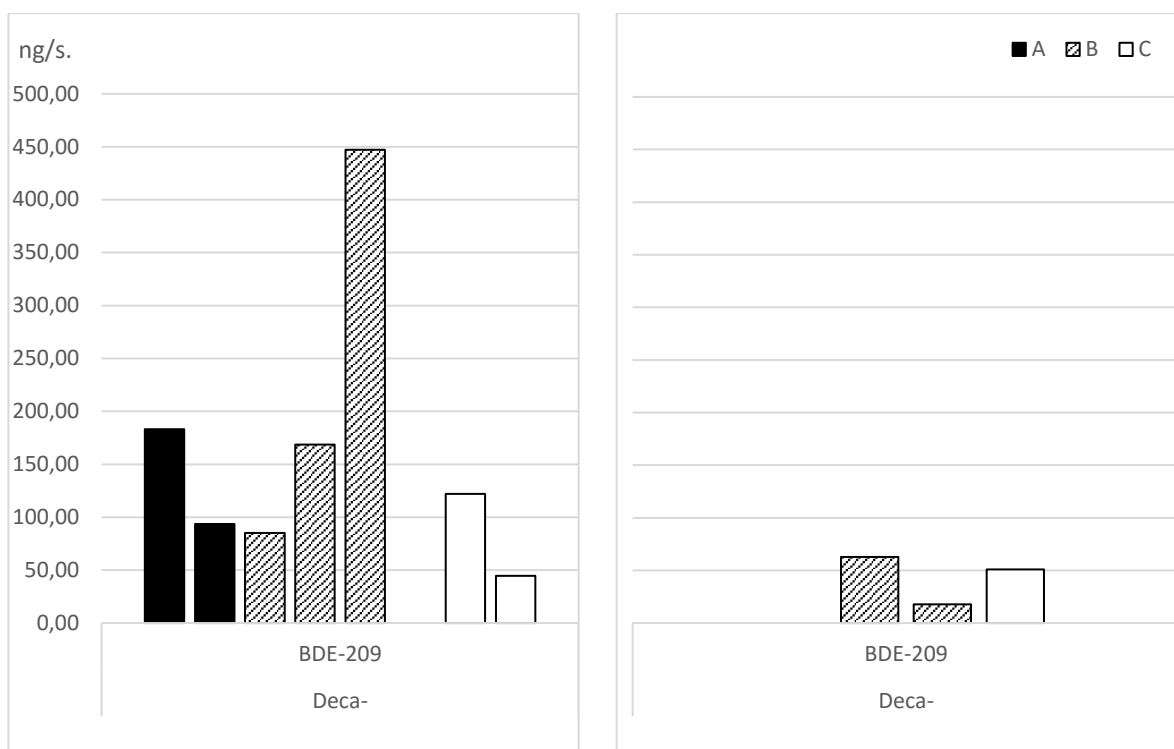
Figure 18: The levels of BDE-209 in passive air samples (right) and in laboratory and solvent blanks (left) after clean-up with method A, B and C. The amounts are given in pg/sample (pg/s.).

The highest most abundant PBDE congener detected in the exposed samples was BDE-209 (left, Figure 18). For method A, the detected amounts ranged from 93.7 pg/sample to 183.2 pg/sample. For method B, the amount of BDE-209 ranged from 85.1 pg/sample to 447.2 pg/sample, yielding a higher RSD value. The lowest amount of BDE-209 was found in one of the samples cleaned with method C (44.7 pg/sample), but it was also not detected for one of the samples from that method. This could mean that the method is less effective in securing a satisfying recovery of the higher brominated PBDE.

In the blank samples (right, Figure 18), BDE-209 was only detected in two lab blanks (method B and C) and one solvent blank (method C). The detected blank levels were quite high at 17.8-62.8 pg/sample.

Table 10 shows the relative standard deviations for the exposed samples after clean-up with methods A, B and C. This is a measure of the repeatability of real samples. The large numbers for some PBDE congeners indicate that LOD-values are included in the calculation of RSDs. No large differences between the methods were observed when the concentrations are more than 10 x blank levels.

Table 10: Relative standard deviations (RSD) values for PBDE congeners in passive air samples after clean-up with methods A, B and C. RSD is a measure of spread in the data, and is calculated from ratio of the standard deviation and the average amount for within each method.

Homologous group	Congener	A (%)	B (%)	C (%)
Tri-BDE	BDE-17	18.7	23.7	29.4
	BDE-28	7.2	15.4	14.7
Tetra-	BDE-47	12.4	23.2	16.9
	BDE-49	20.1	40.0	14.6
	BDE-66	3.7	17.5	11.7
	BDE-71	-	-	-
	BDE-77	-	109.9	-
Penta-	BDE-85	-	-	-
	BDE-99	18.9	32.2	38.6
	BDE-100	15.8	45.4	37.6
	BDE-119	-	87.0	-
	BDE-126	-	-	-
Hexa-	BDE-138	-	173.2	-
	BDE-153	41.3	-	26.5
	BDE-154	36.1	9.3	40.3
	BDE-156	-	-	-
Hepta-	BDE-183	1.1	56.5	13.4
	BDE-184	141.4	40.4	-
	BDE-191	-	-	-
Octa-	BDE-196	141.4	-	-
	BDE-197	44.7	-	33.4
Nona-	BDE-206	141.4	38.0	-
	BDE-207	22.7	12.5	-
Deca-	BDE-209	45.7	81.2	111.1

3.4.2. n-BFRs

As explained in earlier chapters, clean-up method C yielded samples that were not analyzed due to high levels of matrix. Detection limits and levels in exposed samples are thus only discussed for methods A and B.

Method detection limits for method A and B is shown in Table 11.

Table 11: Method detection limits (MDL) are the lowest possible detectable amount for the method, and is here shown for methods A and B. The MDL is calculated by multiplying the detected amount of the n-BFR compound by three. For compounds that are not detected in the lab blank, the MDL is the same as the limit of detection (LOD). Compounds of which this applies are marked by *.

ISTD	n-BFR	A MDL (pg/s.)	B MDL (pg/s.)
^{13}C-BDE-28	ATE (TBP-AE)	1.7*	1.4*
	a-TBECH	1.4*	6.5*
	b-TBECH	1.0*	4.7*
	g/d-TBECH	0.7*	3.2*
	BATE	0.5*	1.0*
	PBT	12.0*	16.3
	PBEB	4.4*	2.9
^{13}C-HBB	HBB	92.2	35.1
^{13}C-BDE-47	DPTE	34.8	2.7*
^{13}C-EHTBB	EHTBB	18.6	5.2
^{13}C-BTBPE	BTBPE	21.	48.4
^{13}C-DBDPE	BEHTBP	8.3*	12.8*
	DBDPE	80.3*	6832

The levels of n-BFRs in exposed samples with corresponding lab- and solvent blank samples are shown in Figure 19. a- and b-TBECH and DBDPE are displayed in separate figures (Figure 20and Figure 21, respectively), as their levels greatly exceeded the other congeners, making the y-axis too big.
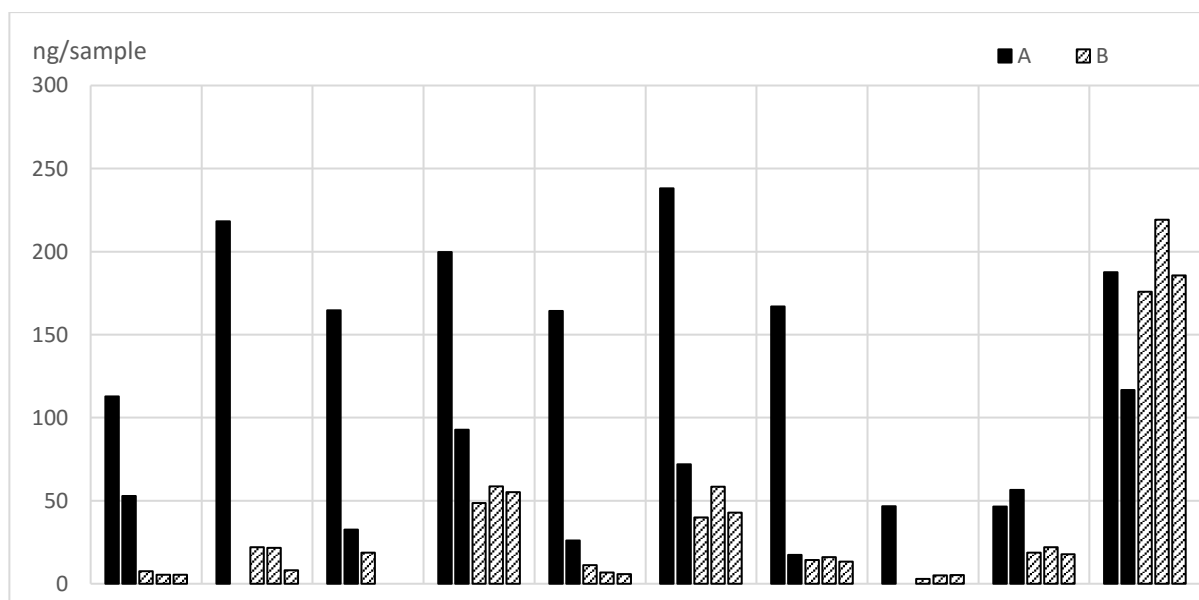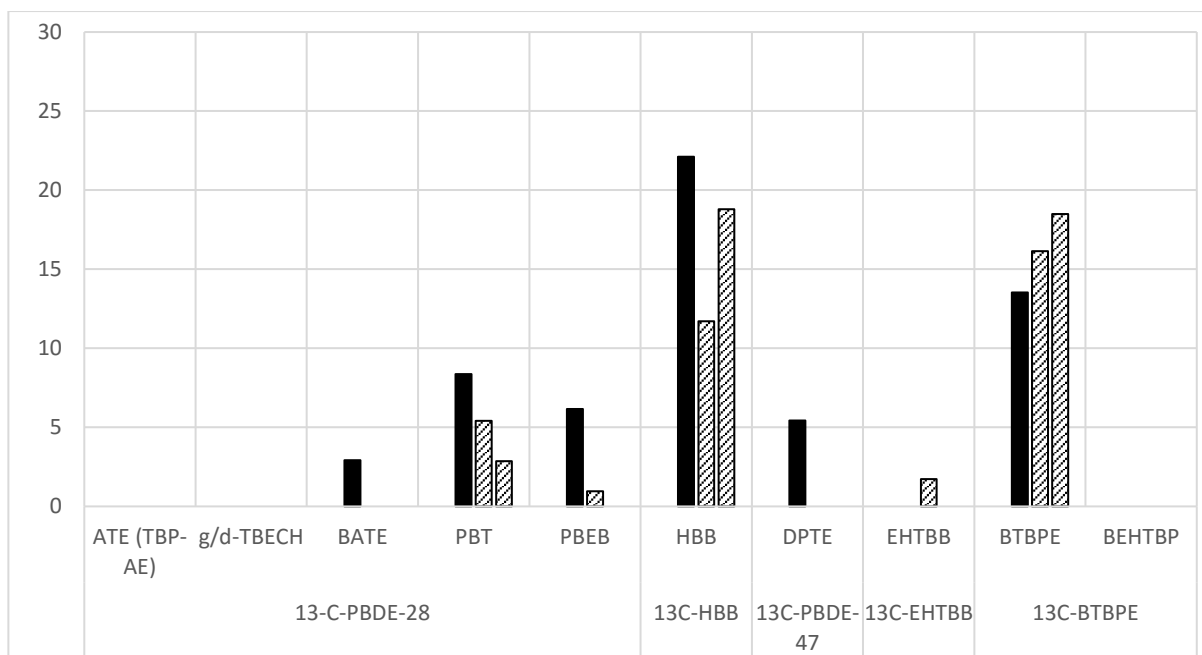
Figure 19: n-BFR detected in passive air samples from Kjeller (top) and in laboratory and solvent blanks (bottom), prepared with the clean-up methods A and B. Note that the y-axis for the exposed samples (top) are 10 times higher than the one for the blank samples (bottom), which implies very low blank values detected for the n-BFRs.
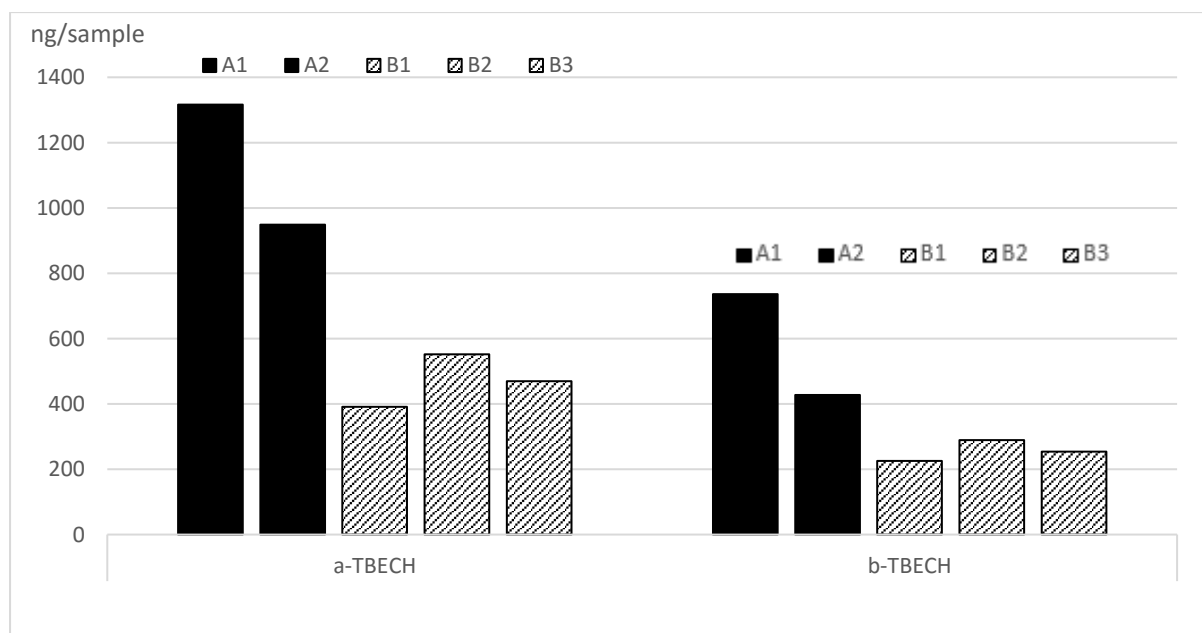
Figure 20: The levels of a- and b-TBECH in passive air samples from Kjeller after clean-up with method A and B. These congeners were the second most abundant n-BFRs found in the exposed samples. No lab or solvent blank values were detected for either method. The labels over each column shows the parallel samples (1-3) belonging to each clean up method (A and B).
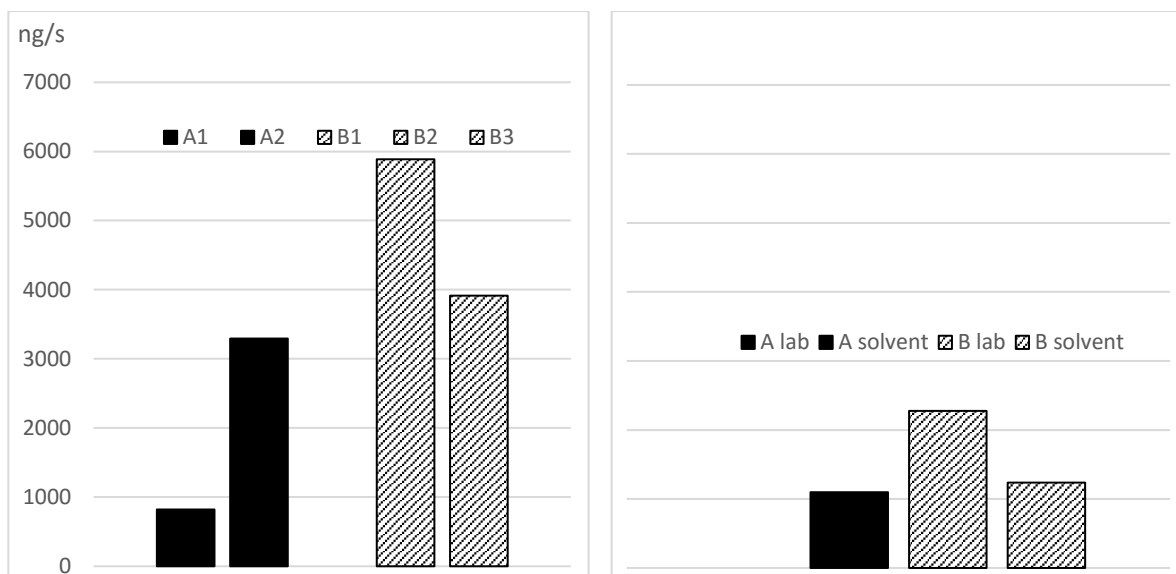
Figure 21:The levels of DBDPE in passive air samples from Kjeller (left) and in lab and solvent blank samples (right) after clean-up with method A and B. This congener was the most abundant n-BFR detected in the exposed samples. The labels above the columns indicate the different samples belonging to each method (A and B), showing indicating samples where DBDPE was not detected (one parallel for method B (B1) and the lab blank for method A (A lab)).

For all n-BFRs listed in Figure 19, low blank values (less than 1:10 of the measured air levels) were detected (bottom). This implies low contamination from the lab, solvents or equipment, and that the congeners comes from the outside environment. All listed n-BFRs were detected after clean-up with method A, with the highest amount found for HBB (238 pg/sample). Method B yielded lower values of all n-BFRs (0-58.6 pg/sample), except for BEHTBP (175-219 pg/sample).

a- and b-TBECh was not detected in any blank samples, but was the second most abundant n-BFRs detected in the exposed samples (390-1316 pg/sample and 225-376 pg/sample, respectively).

DBDPE were the most abundant n-BFR congener detected in the exposed samples, with a maximum value at 5888 pg/sample (method B). However, the congener was not detected in all parallel samples and had showed large deviations between parallels (0, 3916 and 5888 pg/sample for parallels cleaned with method B). Additionally, the blank levels for the congener was also high; DBDPE was detected in solvent blanks for method A and B at 1095 and 1235 pg/sample respectively. The lab blank for method B had as high as 2277 pg/sample (LOD 6832 pg/sample), while none was detected in the lab blank for method A. This could mean that the samples were exposed to DBDPE somewhere during sample procedures.

3.5.   Summary: Evaluation of clean-up methods

A summary of the validation parameters and matrix effects obtained for the different methods when analyzed for PBDEs and n-BFRs is found in Table 12. Congeners that did not fit into the recovery interval set by NILU (40-130 %) were ignored, and accuracy, repeatability and MDL for these were excluded from the summary.

Relative standard deviations for PBDEs and n-BFRs between all methods were assessed by calculating the average and standard deviation of all recoveries for each components group. This RSD value describes the overall reproducibility for the PBDE and n-BFR.

The matrix is assessed by looking at the lock-mass signal (LM) for the lab blank in the retention time window of which compounds of interest and matrix is eluted (10:55-11:70, Function 4 (F4)). The matrix is also assessed by looking at GCxGC plots for lab blanks and answering whether or not signals are present in the matrix area (sorted under PBDEs in the table but applies for n-BFR too).

Table 12: A summary of validation parameters and matrix effects found for the three clean-up methods A, B and C analyzed for legacy and novel brominated flame retardants (PBDEs and n-BFRs).

	PBDE			n-BFR		
	A	B	C	A	B	C
Ignored congeners*	BDE-196	-	BDE-49, -71, -154, -184, -207	ATE, BEHTBP	-	-
Accuracy (Rec. %)	92.2-130	80.6-122	41.2-119	54.1-99	49.3-104	-
Repeatability (RSD %)	0.1-4.6	0.4-40.5	0.7-173	1.2-7.0	2.8-15	-
Reproducibility (RSD %)	2.2-52			3.8-115		
Applicability (RSD % of exposed PAS)	0.1-4.6	0.4-41	0.7-173	1.0-46	2.8-15	
MDL (pg/sample)	1.2-215	0.3-188	0.2-153.2	0.5-92.2	1.0-6832	-
Matrix effects (LM at F4)	No	Yes	Yes	No	Yes	-
Matrix effect (GCxGC)	No	Yes	Yes			

*Rec. outside the quality interval (40-130 %)

4. Conclusions and further prospects

The goal of this thesis was to compare and validate three different clean-up methods for different types of PUFs: New type of PUF, old type of PUF and a PUF type sent from Canada.

Results show that there were large differents between the PUFs. The GCxGC analysis showed that the different PUF types were affected by various type of matrix. Furthermore, evaluating the lock-mass intensity, the difference between the clean-up methods were assessed. The result from this showed that the accreditated method A (using sulphuric acid) was the most efficient method for removing matrix from the PUF, while method C was most suitable for "old" PUFs.

As many n-BFRs lack their own isotope labelled ISTD, PBDE ISTDs were used as substitutes for those n-BFRs. Which lack suitable isotope labelled standards. Our results shows that inclusion of more labelled n-BFR standard would make the method more robust.

The results for the real samples exposed to ambient air, show that the method give adequate results for most of the analytes monitored. The results revealed that the congener pattern was similar to other studies.

Our studies show that there many challenges remaining:

- The difference in PUF matrix should be further assessed. Prior to use, new PUFs should be tested for matrix.
- More effort should be put into the development of the clean-up methods.
- A larger data set of exposed samples should be prepared in order to further assess the clean-up method.

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6. APPENDIX A: Chemical analysis

6.1. Materials and Equipment

All solvents used in the sample preparations and handling was of analytical grade. Materials and equipment utilized during sampling, sample preparations and chemical analysis, their units and suppliers are listed in **Error! Reference source not found.** .

Concentrations of the recovery standard (RSTD) tetrachloronaphtalene (TCN) is listed in **Error! eference source not found.**. Content and concentrations of ^{13}C internal standard (ISTD) mixtures for PBDE and n-BFR congeners are listed in Table 15 and Table 16, respectively. The Equivalent for ^{12}C-mixtures of PBDE and n-BFR congeners are listed in Table 17 and Table 18. RSTD, ISTDs and ^{12}C mixtures were mixed in Isooctane, except from one of the RSTDs which was kept in Acetonitrile.

Table 13: Materials and equipment in the laboratory and analytical procedures

Name	Supplier	Size	Purity	Description/use
Acetone	VWR Chemicals	2,5 L	≥ 99.7 % Pestinorm	Cleaning of equipment, extraction, clean-up of samples
Acetonitrile	VWR Chemicals	2,5 L	≥ 99,9 % LiChrosolv	Cleaning of equipment, clean-up of samples
Agilent MassHunter qual B 07.00	Agilent (Santa Clara, USA)			Inspection of chromatogramps, MS spectra
Agilent MassHunter qual B 06.00	Agilent (Santa Clara, USA)			Quantification
Aluminium foil	Caterwrap	450m x 150mm		Cover and protection of equipment
Aluminium foil sheets	Korff/VWR	0,02 x 100 x 100 mm		Various laboratory work
Auto sampler Agilent 7693	Agilent (Santa Clara, USA)			Analytical procedure (GC)
Brown glass vials with screw top	Supelco/Merck (Darmstadt, D)	15 mL, 22 mL		Containing sample after SPE
Brown glass vials with screw top	Supelco/Merck (Darmstadt, D)	2 mL		Containing sample after Soxhlet extraction and volume reduction
Caps for GC vials w/septum	Teknolab (NO)	11 mm		Analytical procedure (GC)
GC-column RTX-1614 (5% diphenyl, 95% dimethyl polysiloxane)	Restek (Bellefonte, USA)	15m x 0,25 mm x 0,10 μm		Analytical procedure (GC)
Cotton wool	Vernon Carus			For silica clean-up
Diethyl ether SupraSolv	Merck (Darmstadt, D)	1 L		For silica clean-up
Discovery DSC-18 SPE Bulk Packing	Supelco/Merck (Darmstadt, D)	100 g		EZ-POP clean-up
Extran MA01	VWR/Merck (Darmstadt, D)	2,5 L		Soap for cleaning of equipment (Extran:water 1:100)
Fume hood cover Versi-dry	Thermo Scientific	508x91500 mm		Plastic backed paper cover for fume hood

GC-Agilent 6890	Agilent (Santa Clara, USA)			Separation (GC)
GC vials (brown)	Chromacol	300 µL		Analytical procedure (GC)
Glass centrifuge tubes w/glass stopper	Schott Duran (D)	10 mL		Acid clean-up
Glass columns	Schott Duran (D)	15 mm diam.		Silica-clean up
Glass columns	Matriks / LC Tech (Obertaufkirchen, D)	105 mm, diameter 17 mm		EZ-POP clean-up
Glass fiber filter frit	Matriks / LC Tech (Obertaufkirchen, D)	15 mm diam.		EZ-POP clean-up
Glass vial (pointed bottom)	Chromacol (USA)	1 mL		Volume reduction (N_2) and storage of sample
Glass water cooled condensation tubes	Schott Duran (D)			Extraction
Glassware (Erlenmeyer flasks, beakers, measuring cylinders etc.)	Schott Duran (D)			General laboratory work
Heat mantles	VWR			Extraction
Helium gas (He)	Paraxair (NO)		5.0	Analytical procedure (GC/MS)
Hypodermic needles Microlance)	Becton Dickinson Medical			Volume reduction (N_2)
Isooctane	Merck (Darmstadt, D)	1 L	≥ 99.5 % Emsure	Clean-up and analysis of samples
KNF Laboport Mini pump	Merck (Darmstadt, D)			SPE clean-up
Latex tops for Pasteur pipettes	Svenska Latex AB (SE)			General laboratory work
MassLynx V4.2	Waters (Milford, USA)			Quantification
Metal tweezers				Extraction
Methanol				
Micropipettes	Blaubrand (D)	20, 50 and 100 µL		Transfer of standards and samples
MS office excel	Microsoft (USA)			Final data processing
Nitrogen gas (N_2)	Paraxair (NO)		5.0	Volume reduction of samples
n-hexane	VWR Chemicals	2,5 L	≥ 95 %, Pestinorm	Cleaning of equipment, extraction, clean-up of samples
Nitrile gloves	Ansell			General laboratory work
Ovens				Cleaning of glassware, heating of silica, sodium sulphate and Florisil
Pasteur pipettes	Scherf pazision GMBH			General laboratory work
PTV inlet	Agilent (Santa Clara, USA)			Sample introduction (GC)
PUF disks Richfoam (Polyether)	Sunde Søm og Skumplast A/S (NO)	14x1,35 mm		Sampling material
PUF disks TE-1014	Tisch Environmental (USA)	5,5'' x 0,5''		Sampling material

Round bottom flask	Schott Duran (D)	500 mL		Extraction
Silica gel 60 Å	Merck (Darmstadt, D)	1 kg		Clean-up of samples
Sodium sulphate (Na_2SO_4)	Merck (Darmstadt, D)	1 kg	Emsure	Remove water from samples
Soxhlet extractor	Schott Duran (D)	28 x 120 mm, 100 mL		Extraction
Sulphuric acid (H_2SO_4)	Merck (Darmstadt, D)	1 L	95-97 %, Emsure	Clean-up of samples
Supelclean LC-Florisil SPE Bulk Packing	Supelco/Merck (Darmstadt, D)	100 g		EZ-POP clean-up
Supelclean ENVI-Florisil SPE Tubes	Supelco/Merck (Darmstadt, D)	500 mg, 3 mL		Florisil clean-up
Supel QuE Z-sep SPE Bulk Packing	Supelco/Merck (Darmstadt, D)	20 g		EZ-POP clean-up
TurboVap 500	Zymark			Volume reduction
TurboVap glasses	Biotage	200 mL		Volume reduction with the TurboVap system
Ultrasonic bath	VWR			Cleaning equipment
Vial caps w/ Teflon liner	Supelco			Storing samples
Ultima AutoSpec Micromass	Waters (Herts, UK)			Analytical procedure (MS)
Visiprep-DL SPE Vacuum manifold	Supelco/Merck (Darmstadt, D)	12-port		SPE clean-up
Ziplock bags	Polynova			Storing PUF disks

Table 14: Concentrations (pg/ul) of tetrachloronaphtalene (TCN) in the recovery standards (RSTD) used in the project. Two RSTDs were used (in acetonitrile and in isooctane), noted by their date (dilution.week.year).

Component	(2.11.16) pg/µl in ACN	(2.23.17) pg/µl in isooctane
TCN	96,2	98,8

Table 15: Concentrations of ^{13}C-PBDE congeners in the PBDE ISTD mixture used in this project. The ISTD is in isooctane.

Component	pg/ul
^{13}C-PBDE-28	260
^{13}C-PBDE-47	263
^{13}C-PBDE-99	263
^{13}C-PBDE-153	265
^{13}C-PBDE-183	265
^{13}C-PBDE-197	261
^{13}C-PBDE-206	262
^{13}C-PBDE-209	589

Table 16: Concentrations of ^{13}C-labelled compounds used as ISTDs for n-BFRs. Two mixtures were used, noted by their date (week.year). Both ISTDs are in isooctane.

Component	(35.17) pg/µl	(46.17) pg/µl
^{13}C-BTBPE	1019	986

[13]C-HBB	1023	986
[13]C-d17 EHTBB	994	1022
[13]C-DBDPE	1010	965
[13]C-PBBZ	1024	1000

Table 17: Concentrations of ^{12}C-PBDE congeners in mixtures used in the project. Two mixtures were used, noted by their date (week.year). Both are in isooctane.

Component	(05.16) pg/µl	(49.17) pg/µl
[12]C-PBDE-3	52,6	50
[12]C-PBDE-7	52,6	50
[12]C-PBDE-15	52,6	50
[12]C-PBDE-17	52,6	50
[12]C-PBDE28	52,6	50
[12]C-PBDE-47	52,6	50
[12]C-PBDE-49	52,6	50
[12]C-PBDE-66	52,6	50
[12]C-PBDE-71	52,6	50
[12]C-PBDE-77	52,6	50
[12]C-PBDE 85	52,6	50
[12]C-PBDE 99	52,6	50
[12]C-PBDE 100	52,6	50
[12]C-PBDE 119	52,6	50
[12]C-PBDE-126	52,6	50
[12]C-PBDE-138	105	100
[12]C-PBDE-153	105	100
[12]C-PBDE-154	105	100
[12]C-PBDE-156	105	100
[12]C-PBDE-183	105	100
[12]C-PBDE-184	105	100
[12]C-PBDE-191	105	100
[12]C-PBDE-196	105	100
[12]C-PBDE-197	105	100
[12]C-PBDE-206	263	250
[12]C-PBDE-207	263	250
[12]C-PBDE-209	263	250

Table 18: Concentrations of ^{12}C-n-BFR congeners in mixtures used in the project. Three mixtures were used, noted by their date (week.year).

Component	(16.17) pg/µl	(01.16.17) pg/µl	(46.17) pg/µl
[12]C-HBB	2422	478	2425
[12]C-TBP-AE	2455	485	2476
[12]C-DPTE	2483	490	2485
[12]C-BTBPE	2526	499	2502

12C-BATE	2506	495	2566
12C-BEHTBP	4972	982	4978
12C-EHTBB	2478	489	2470
12C-PBT	2500	494	2491
12C-PBEB	2455	485	2499
12C-a/b TBECH	2511	496	2430
12C-g/d TBECH	2427	479	2433
12C-PBBZ	2478	489	2465
12C-HCDBCO	2500	494	2447
12C-DBDPE	2455	485	2570

6.2. Quality control in accordance to accredited routines

All solvents in the project were approved for analytical use according to the accredited routines at The Norwegian Institute of Air Research (NILU). Standard solutions were prepared and their accuracy and performance is continually controlled at NILU according to the accredited routines.

PUFs were cleaned prior to use in accordance with NILU's accredited routines: Soxhlet extraction with toluene (24 h) followed by acetone (8 h) and cyclohexane (8 h), and then dried in a vacuum chamber. Clean PUFs were kept wrapped in aluminium foil and zip-lock bags and stored in an airtight plastic box prior to usage.

All glassware was soaked overnight in water and Extran soap, thoroughly rinsed with water and baked at 450 °C for 8 hours. Prior to usage, all glassware was rinsed with acetone and hexane or other suitable solvent involved in the clean-up procedures.

Silica and sodium sulphate were baked at 550 °C for 14 h, Florisil was baked at 450 °C for 9.2 hours, and given expiry date of one month after the heat-treatment.

After deployment the PUF disks were wrapped in two layers of aluminium foil, followed by two plastic bags, and stored at -20 °C until further treatment.

Blank samples (solvent and un-exposed PUF-disk), processed in the same way as exposed samples, were used to investigate blank levels from the PUF material itself and from the laboratory procedure.

6.3. Methods description: Laboratory procedures

6.3.1. Sample description

For each of the three clean-up methods, three parallels of PUF disks were prepared for spiking with 12C components. Additionally, each round of clean-up included a solvent blank (without PUF) and a

lab blank (with PUF). Eight passive air samples (PUF-disk) from Kjeller (Norway) (sampled 29.02.-02.05.16) were divided between the three clean-up methods. Furthermore, a second set of samples was prepared for clean-up method C, using PUFs sent from the Institute for Environment and Climate Change Canada, of the type Tisch Environmental. A summary of the samples is shown in Table 19.

Table 19: Number of samples prepared for the project. The purpose of the ^{12}C spiked samples was to establish the accuracy of the clean-up methods. To investigate matrix effects from the PUFs, a second set of samples was prepared for method B, which is a method used at the Institute for Environmental and Climate Change, Canada. These samples were prepared from PUF of the type Tisch Environmental, and are marked with *.

Clean-up method	^{12}C spiked (PUF)	Lab blank (PUF)	Solvent blank	Passive air sample (PUF)	Number of samples
A	3	1	1	2	7
B	3	1	1	3	8
	3*	1*	1	-	5
C	3	1	1	3	8
				Total:	31

Additionally, a set of three standard controls was also prepared, in order to monitor the instrumental conditions (neither extracted nor cleaned).

6.3.2. Extraction

Prior to extraction, all glassware and cooling devices were rinsed with acetone/n-hexane (1:1) and covered in aluminium foil to limit potential contamination, e.g. from dust.

The PUFs were unwrapped and rolled into a sausage (using metal tweezers cleaned with acetone and hexane) and folded to fit the Soxhlet extraction apparatus, lab gloves were changed between the individual samples. The Soxhlet extraction setup is described in graphic form in **Error! Reference**
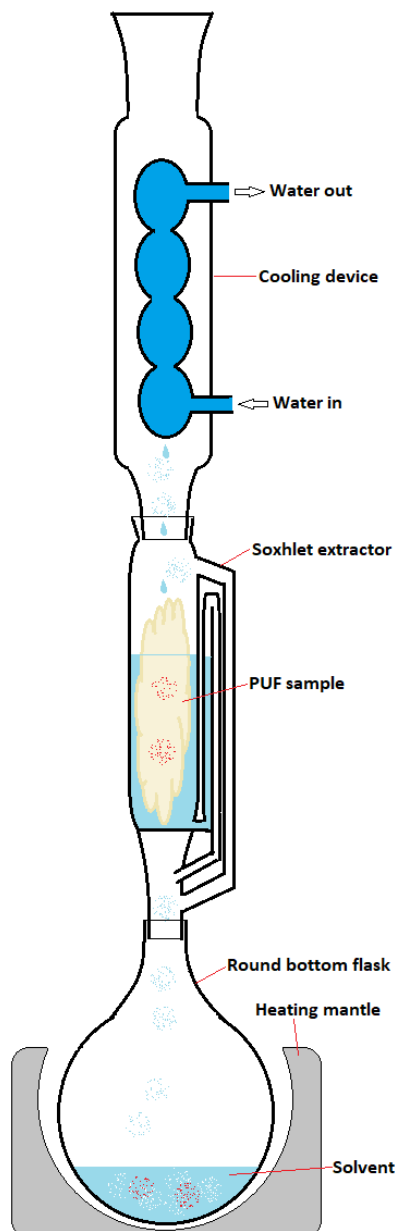
Figur 1: The Soxhlet extraction setup: Solvent in the round bottom flask (acetone/n-hexane (1:1)) is heated by the heating mantle and evaporated up through the outer tube of the Soxhlet. As the vapor is cooled down by the cooling device, it condenses and drips down into the Soxhlet chamber, soaking the polyurethane foam (PUF) disk in solvent. As the inner tube of the Soxhlet reaches its critical point, the solvent and dissolved component and material is drained from the chamber back into the round bottom flask. This cycling process was allowed to continue for 8 hours.

ource not found..

Solutions of internal standard (^{13}C-ISTD) and native components (^{12}C-NC) according to the concentrations listed in Table 15 to Table 18 were mixed in a clean glass vial with approximately 0.5-1 ml acetone. Accurate volumes were obtained using capillary micropipettes. A capillary pipette

controller was used to suck solvent up and down three times before emptying the pipette into the glass vial. Each ISTD/NC solution was applied directly to its respective PUF disk using a Pasteur pipette, and the ISTD solution glass vial was rinsed twice with acetone/n-hexane (1:1), adding the solvent to the PUF disk.

The round bottom flask was filled with 250-300 ml acetone/n-hexane (1:1), and the apparatus was mounted. The upper half of the round bottom flask was covered in aluminium foil to preserve heat and protect against UV-radiation. The adapter between round bottom flask and the Soxhlet was also covered in aluminium foil to preserve heat and to prevent condensation of solvents in this region. The samples were extracted for 8 hours.

Post extraction, the extract was cooled down to room temperature and capped with a clean glass stopper before volume reduction. If the volume reduction was not done the same day, the extract in the round bottom flask was covered with aluminium foil and stored dark.

6.3.3. Volume reduction

The extracts were volume-reduced using a TurboVap 500 from Zymark. Prior to the reduction, the cooling system was cleaned twice with 10-20 ml acetone by evaporating first without cooling, followed by evaporation of the same volume of acetone with cooling. This was repeated once in-between samples to prevent cross-contamination of the extracts.

The sample was transferred to a TurboVap glass (hereby referred to as a TV glass), and the round bottom flask was rinsed out once with 2-3 pipette volumes of acetone/n-hexane (1:1). The Sensor endpoint setting was used to stop the evaporation at 0.5 ml, and fan speed was set to "B".

The sample was reduced to 0.5 mL and solvent exchanged twice into the solvent needed for the series' respective clean-up method (n-hexane, acetonitrile or isooctane).

During solvent exchanging from acetone/n-hexane to acetonitrile, approximately 1 ml ethyl acetate was added to the sample as keeper to prevent loss of compounds in the evaporation process and to avoid phase separations, as acetonitrile is immiscible with n-hexane.

After volume reduction, the sample was transferred to a clean glass vial with screw top and the TurboVap glass was rinsed twice with the exchanged-to solvent, during which the volume increased to approximately 1.5 ml. The sample was then further reduced to 1 ml using a gentle stream of N_2 gas.

The samples were then stored in a fridge (4 °C) until clean-up procedures were carried out.

6.3.4. Clean-up methods for comparison

6.3.4.1. Method A: Sulphuric acid/silica

The sample in n-hexane (1 ml) was transferred to a clean centrifuge glass and the sample vial was rinsed twice with n-hexane. The volume was adjusted to approximately 2 ml with n-hexane, and 3 ml concentrated sulphuric acid was added. The sample was vigorously vortexed for 10 seconds using a whirl-mixer, capped and then stored overnight. The hexane-phase was transferred to another clean centrifuge glass and the process repeated.

The hexane phase was transferred to a TurboVap evaporation glass and reduced to 0.5 ml with TurboVap.

Possible acid residues were removed by solid phase extraction (SPE): 4 g activated silica was packed in a glass column (inner diameter 15 mm) with a glass stopcock and clean cotton wool in the bottom. A 1 cm layer of sodium sulphate (Na_2SO_4) was placed on top of the silica. The silica was then cleaned and conditioned by eluting 30 ml ether/n-hexane (1:10), without letting the column run dry.

The sample was applied to the column and the sample vial was rinsed twice with 1 ml ether/n-hexane (1:10), and eluted through the column with 30 ml ether/n-hexane (1:10). Removing the stopcock, the sample was eluted into a pre-cleaned TV-glass. The eluate was collected directly from the column into a clean TurboVap glass and approximately 1 ml isooctane was added as keeper. The volume was reduced to 0.5 ml on the TurboVap system and solvent exchange to isooctane.

The sample was then transferred to a pointed-bottom glass vial and further reduced to 250 μl using a gentle stream of N_2 gas.

6.3.4.2. Method B: SPE w/ 300 mg ENVI-Florisil

Method B was carried out using ENVI-Florisil columns of 500 mg/3ml size, mounted to a vacuum chamber. The columns were rinsed and conditioned with 8 ml methanol, followed by 4 ml n-hexane without letting the columns run dry.

The sample in isooctane (1 ml) was added to the column, and the sample glass was rinsed twice with 1 ml n-hexane. The sample was drawn through the column using a vacuum chamber, and eluted with 5 ml n-hexane (1 drop/sec). An 8 ml fraction was collected in a brown glass tube, which was then kept at 4 °C awaiting volume reduction.

The sample was transferred to a TV glass, volume reduced to 0.5 ml and solvent-exchanged into isooctane by TurboVap.

The samples were added to a pointed-bottom glass vial and further reduced to 250 μl using a gentle stream of N_2-gas. Samples containing ^{12}C components were added to pointed-bottom glass vials that had been volume-controlled for exactly 250 μl.

6.3.4.3. Method C) SPE w/ 4 g EZ-POP

The 4 g EZ-POP method is a modification of an established method using pre-packed EZ-POP columns (2.5 g) from Sigma (further explained in Section 2.1.1).

Glass columns (105 mm, diameter 17 mm) were packed with a glass fibre filter frit at the bottom, followed by 2 g of a mixture of Discovery DSC-18 and Supel QuE Z-Sep sorbents (1:1). The sorbent was compressed by gently knocking the column to the counter a couple of times, before a second frit was placed on top of the sorbents. Then, 2 g LC-Florisil were added to the column and compressed, before a third frit was placed on top. Approximately 0.5 g of Sodium Sulphate (Na_2SO_4) were added to the top, and the column was wrapped in aluminium foil awaiting use.

The vacuum chamber was rinsed on the inside with acetone, and the SPE manifold was mounted on top. Plastic tubes were placed through the valves on the manifold, and plastic adapters were mounted, before the tubes and adapters were rinsed with acetone. The 4 g EZ-POP columns were then placed on the adapters.

The column was washed with 20 ml acetone, dried for about 20 min using a vacuum chamber and conditioned with 8 ml ACN (without letting the column run dry). The sample in ACN (1 ml) was added to the column and the glass was rinsed twice with ACN. Fraction 1 was eluted with 16 ml ACN (1 drop/sec) into a brown glass vial (20 ml), without letting the column run dry. Fraction 2 was eluted with 8 ml ACN (1 drop/sec) until semi-dry into a brown glass vial (15 ml).

6.3.5. Preparation of samples for GCxGC and GC-MS analysis

Prior to analysis 20 μl recovery standard (TCN) were added to the sample (concentrations in **Error! eference source not found.**), using a capillary glass pipette. The sample was then capped and homogenized on a whirl-mixer for 10 seconds.

20 μl of the sample were transferred to a brown GC vial and capped using an automatic capper. The vials were then kept at 4 °C until instrumental analysis.

6.4. Instrumental analysis

6.4.1. Two dimensional gas chromatography (GCxGC)

The GCxGC/ToF-MS system consisted of a Pegasus® 4D (LECO, St. Joseph, MI) system equipped with a Restek (Bellefonte,PA, USA) Siltek Guard column 1,97 m, 0.25mm) and a SGE (SGE Internat. Pty Ltd., Australia) BPX-50 (26,58 m, 0.25 mm x 0.25 μm) as the first

dimension column and an Agilent J&W (Agilent J&W GC columns) VF-1ms (1.5 m, 0.15 mm x 0.15 μm) as the second dimension column. Helium (purity 99.9990%, Hydro Gas and Chemicals, Oslo, Norway) was used as carrier gas with a constant flow of 1 mL/ min. 1 μL of each extract were injected in PTV solvent vent mode

with 20 sec solvent vent time, 20 mL/ min solvent vent flow at 1 psi, with a Gerstel PTV injector. Initial inlet temperature was 50°C with a duration of 0.55 min, ramped with 200°C/ min to 280°C with a duration of 6 min and ramped with 100°C/ min to 320°C with a duration of 2 min.

The primary GC column was programmed as follows: 45 °C (hold time 0.55 min), ramped with 50 °C/min to 80 °C (hold time 1.5 min) and ramped with 4°C/ min to 300°C (hold time 8 min). The secondary oven temperature was programmed 105 °C (hold time 2.25 min) and ramped at 4 °C/min to 315 °C (hold time 10.5 min). Modulation period was set to 3.8 s with 0.46 s hot pulse time and 19 °C modulator temperature offset relative to the primary oven temperature. Liquid N_2 was used as the coolant of the GCxGC modulator. The ion source and the transfer line temperatures were set to 200 °C and 300 °C, respectively. The electron energy was 70 eV and the detector voltage was 1600 V. A data acquisition rate of 100 spectra/ s was used in combination with an acquired mass range of 33 – 1000 u. Autotuning was performed by using the *m/z* 219 perfluorotibutylamine (PFTBA) ion instead of the default *m/z* 69 ion. In order to avoid system contamination, solvent (3 times Toluene) was injected after each sample run.

Data treatment:

Leco ChromaTOF V4.60.8 was used for data processing with NIST 2014 and inhouse library.

6.4.2. Gas chromatography-coupled mass spectrometry (GC-MS)

Table 20: GC instrumental parameters

GC:	Agilent 6890 GC
Column:	RTX-1614
Dimension:	15 m x 0,25 mm x 0.10 μm
Injection:	Programmable-temperature vaporizer (PTV) (ref. Table 21)
Mode:	Solvent vent (ref.Table 22)
GC temperature program:	Ref. table Table 23
Helium flow:	1,7 mL/min

Table 21: Temperature program for the injection

Rate (°C/min.)	Temperature (°C)	Hold time (min.)
	50	0,55
200	300	15
10	50	

Table 22: Solvent ventilation program for the injection

Time (min.)	Helium vent. flow (mL/min.)
0,5	45
2,15	50

Table 23: GC column temperature program

Rate (°C/min.)	Temperature (°C)	Hold time (min.)
	45	2,50
22	220	0
7	280	0
40	300	9

Table 24: MS instrumental parameters

MS:	Ultima Autospec Micromass
MS mode:	Electon Impact Ionization (EI)
Interface temperature:	280 °C
Ion source temperature:	280 °C
Dwell time:	25-60 ms
Acceleration voltage:	7500 V
Detector voltage:	385 V
MS lockmass standard:	Perfluorokerosene (PFK)

6.5. Data processing

6.5.1. Quantification

MassLynx V4.2 quantification tool (Waters) were used to quantify the samples after GC-MS. The TargetLynx processing method automatically integrates the signal from the compound, and calculates the analyte amounts while taking into account the signal of the ISTDs. The integrations were looked over and manually modified when needed. From this, the amount and the calculations area of the compounds in the sample was obtained, and the data were copied to an Excel file.

For samples where ^{12}C compounds were added prior to sample preparations, the recovery of the compounds were calculated from Equation I.

$$Rec\% = \frac{m_{measured}}{m_{theoretical}} \cdot 100\% \qquad \textbf{Eq. I}$$

Where m is the measured amount from TargetLynx and the known added amount calculated from the concentrations in Table 17 (PBDE) and Table 18 (n-BFR).

For the three parallels in each clean-up round, the average recovery was calculated and plotted together with the average recoveries from the other clean-up round. That way, the effectiveness of the method was investigated, as the recovery should be as close to 100% as possible.

The standard deviation (st.dev) was calculated between the three parallels (1-3) for each compound (formula "=stdeva(Rec1;Rec2;Rec3)" in Excel) and used to calculate the relative standard deviation (RSD), Equation II.

$$RSD = \frac{st.dev}{Rec\%_{average}} \cdot 100\% \qquad\qquad \textbf{Eq. II}$$

6.5.2. Manual data processing

To determine differences in instrumental (rather than clean-up method-related) recovery when ignoring the selected ISTD for n-BFR during quantification, the quantification of n-BFRs from standard control samples (samples that did not contain any PUF matrix) was done "manually" in Microsoft Excel using areas obtained from MassLynx.

The first step was to calculate the relative response factor (RFF) from the quantification standards (QS) that were run together with the samples, using Equation III.

$$RRF_{QS} = \frac{m_{TCN} \cdot A_{QS}}{m_{QS} \cdot A_{TCN}} \qquad\qquad \textbf{Eq. III}$$

Where m is the amount of TCN and an n-BFR compound (pg), and A is the integrated area of the signal from TCN and an n-BFR compound.

The RRF was calculated for each compound x in the QS, meaning that one RRF was calculated for HBB, one for PBEB, and so on (RRF_x). Two QSs were run in the same sequence as the standard control samples, so that there were two RRF values calculated for each n-BFR compound. The average RRF value was then calculated for each compound, $RRFx_{av.}$

TCN in the standard control was then used as the ISTD instead of the selected ISTDs. The amount of TCN added to the standard control sample was calculated from the concentrations in **Error! eference source not found.**. The measured amount of the compound x in the standard control sample was then calculated using Equation IV.

$$m_x = \frac{m_{TCN} \cdot A_x}{RRFx_{av} \cdot A_{TCN}} \qquad\qquad \textbf{Eq. IV}$$

Where m is the amount of compound x and TCN (pg), and A is the area of compound x and TCN.

The recovery (Rec%) of compound x was calculated from Equation V.

$$\text{Rec}_x\% = \frac{m_x(calculated)}{m_x(added)} \cdot 100\% \qquad\qquad \textbf{Eq. V}$$

Where m_x is the measured amount of compound x (Equation 2) and the known amount of compound x calculated from the concentrations in Table 18.

The recoveries of the n-BFR compounds, calculated with and without considering the selected ISTDs, were plotted together to show the difference. This was then used to evaluate the suitability of the assigned ISTD.

7. APPENDIX B: Raw data

7.1. Raw data

7.1.1. Chromatograms of all PBDe congeners monitored (from quantification standard)

Identification of all PBDEs in the GC chromatograms of the M-1 peak with the function numbers indicated.
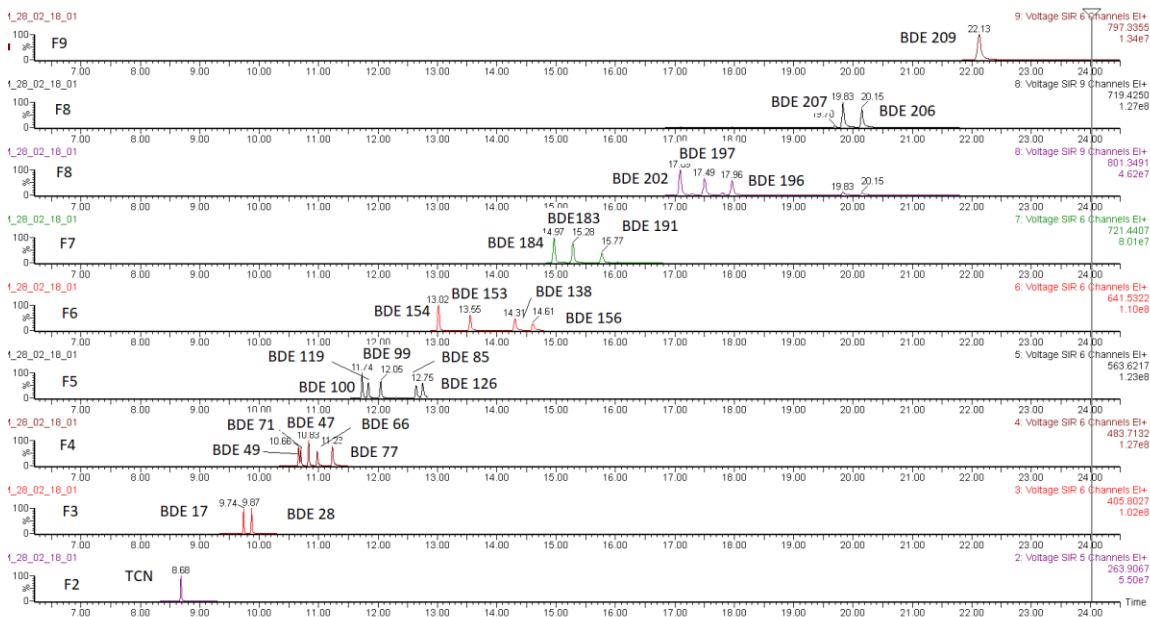
7.1.2. Chromatograms of all n-BFRs monitored (from quantification standard)

Identification of all n-BFRs in the GC chromatograms of the M-1 peak of the novel brominated flame retardants with the function numbers indicated.

7.1.3. PBDE) ^{12}C spiked samples for method validation (Method A)

A) NILU-02		Sample:	17/2662	12C-spike 4			
			A) Calculated from ISTD		B) Calculated from response (TCN as ISTD)		
Component	RT	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	8,92	1924			2378490		
TBA	7,23						
13-C-PBDE 28	10,02	5191,7	3586,3	69,1	1707612,9	3586,3	69,07749
PBDE-17	9,89	5260,0	5417,0	103,0	1913366,5	3725,7	70,83105
PBDE-28	10,03	5260,0	5404,5	102,7	2060954,8	3717,1	70,66815
13-C-PBDE 47	11,04	5269,8	4467,4	84,8	1879848,9	4467,4	84,77382
PBDE-49	10,83	5260,0	5765,3	109,6	1531357,6	4859,4	92,38379

Component	RT	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
PBDE-71	10,88	5260,0	5432,9	103,3	1449812,2	4579,3	87,05821
PBDE-47	11,02	5260,0	5397,2	102,6	2142674,6	4549,1	86,48528
PBDE-66	11,19	5260,0	5725,7	108,9	1388895,3	4826,0	91,74952
PBDE-77	11,46	5260,0	5521,4	105,0	2297914,1	4653,8	88,47515
13-C-PBDE 99	12,34	5258,7	4559,7	86,7	1398893,4	4559,7	86,70864
PBDE-100	11,98	5260,0	5459,4	103,8	1872166,1	4654,3	88,48447
PBDE-119	12,09	5260,0	5395,2	102,6	1212985,4	4599,5	87,44322
PBDE-99	12,31	5260,0	5510,8	104,8	1500338,3	4698,1	89,31777
PBDE-85	12,96	5260,0	5869,5	111,6	1189234,6	5003,9	95,13093
PBDE-126	13,07	5260,0	5674,0	107,9	1688545,8	4837,3	91,96303
13-C-PBDE 153	13,91	5295,0	4586,3	86,6	798635,4	4586,3	86,61639
PBDE-154	13,33	10500,0	11508,6	109,6	2346263,6	9653,4	91,93688
PBDE-153	13,89	10500,0	10824,1	103,1	1629910,8	9079,2	86,46887
PBDE-138	14,68	10500,0	11122,8	105,9	1383937,0	9329,8	88,85519
PBDE-156	15	10500,0	11023,0	105,0	854980,0	9246,0	88,05754
13-C-PBDE 183	15,69	5297,7	4607,1	87,0	582188,3	4607,1	86,96348
PBDE-184	15,36	10500,0	11190,2	106,6	1484520,6	9233,6	87,93889
PBDE-183	15,68	10500,0	10934,4	104,1	1218897,4	9022,5	85,92864
PBDE-191	16,18	10500	11060,6846	105,3399	684614,032	9126,711	86,92105
13-C-PBDE-197	17,97	5221,9128	4889,9581	93,64304	342572,063	4889,954	93,64297
PBDE-202	17,54						
PBDE-197	17,95	10500	11294,4357	107,5661	844200,156	9269,965	88,28538
PBDE-196	18,38	10500,0	13567,6	129,2	778854,0	11135,7	106,1
13-C-PBDE-206	20,46	5230,8	3542,3	67,7	92350,7	3542,3	67,7
PBDE-207	20,14	26300,0	28846,1	109,7	762313,4	20367,3	77,4
PBDE-206	20,49	26300,0	26319,8	100,1	441559,2	18583,5	70,7
13C-PBDE-209	22,67	11773,8	6872,8	58,4	23510,7	6872,8	58,4
PBDE-209	23,05	26300,0	24053,5	91,5	54374,7	14687,2	55,8

		Sample:	17/2663	12C-spike 5			
A) NILU-02			A) Calculated from ISTD		B) Calculated from response		
Component	RT	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	8,92	1924,0			2540354,6		

TBA	7,23					
13-C-PBDE 28	10,02	5191,7	4225,5	81,4	2148918,1	4225,5	81,4
PBDE-17	9,89	5260,0	5389,1	102,5	2395466,6	4367,3	83,0
PBDE-28	10,03	5260,0	5408,0	102,8	2595259,4	4382,6	83,3
13-C-PBDE 47	11,04	5269,8	5088,0	96,5	2286664,0	5088,0	96,5
PBDE-49	10,83	5260,0	5577,9	106,0	1802205,8	5354,5	101,8
PBDE-71	10,88	5260,0	5402,9	102,7	1753814,2	5186,5	98,6
PBDE-47	11,02	5260,0	5401,0	102,7	2608234,9	5184,7	98,6
PBDE-66	11,19	5260,0	5658,4	107,6	1669593,3	5431,7	103,3
PBDE-77	11,46	5260,0	5494,1	104,5	2781424,4	5274,1	100,3
13-C-PBDE 99	12,34	5258,7	5110,5	97,2	1674574,7	5110,5	97,2
PBDE-100	11,98	5260,0	5473,5	104,1	2246912,3	5230,0	99,4
PBDE-119	12,09	5260,0	5387,0	102,4	1449841,4	5147,4	97,9
PBDE-99	12,31	5260,0	5548,2	105,5	1808200,9	5301,4	100,8
PBDE-85	12,96	5260	5665,2988	107,7053	1374076,88	5413,249	102,9135
PBDE-126	13,07	5260	5428,8797	103,2106	1933980,88	5187,347	98,61877
13-C-PBDE 153	13,91	5294,9558	4913,0586	92,78753	913756,938	4913,054	92,78744
PBDE-154	13,33	10500	11367,3997	108,2609	2651540	10214,27	97,27878
PBDE-153	13,89	10500	10950,5866	104,2913	1886650,25	9839,74	93,71181
PBDE-138	14,68	10500	10892,8635	103,7416	1550692,31	9787,874	93,21785
PBDE-156	15	10500	10689,0271	101,8003	948587,719	9604,712	91,47345
13-C-PBDE 183	15,69	5297,747	4858,3332	91,70565	655715,188	4858,328	91,70555
PBDE-184	15,36	10500	11346,0561	108,0577	1695293,31	9872,699	94,0257
PBDE-183	15,68	10500	10845,9702	103,295	1361734,56	9437,556	89,88149
PBDE-191	16,18	10500	10718,8816	102,0846	747248,407	9326,968	88,82826
13-C-PBDE-197	17,97	5221,9128	5461,0837	104,5801	408619,094	5461,079	104,5801
PBDE-202	17,54						
PBDE-197	17,95	10500	11250,0303	107,1431	1003000,84	10311,95	98,20908
PBDE-196	18,38	10500	13646,0897	129,9628	934386,531	12508,22	119,1259
13-C-PBDE-206	20,46	5230,7576	4435,3809	84,79424	123502,051	4435,377	84,79415
PBDE-207	20,14	26300	29082,9831	110,5817	1027824	25711,33	97,76172
PBDE-206	20,49	26300	27918,9797	106,1558	626382,125	24682,28	93,84895

Component	RT	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
13C-PBDE-209	22,67	11773,8	10662,872	90,56441	38958,043	10662,86	90,56431
PBDE-209	23,05	26300	24175,0162	91,92021	90555,821	22901,64	87,07848

		Sample:	17/2664	12C-spike 6			

A) NILU-02			A) Calculated from ISTD		B) Calculated from response		
Component	RT	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	8,92	1924			1649042,94		
TBA	7,23						
13-C-PBDE 28	10,02	5191,6804	3951,1725	76,10585	1304370,56	3951,167	76,10575
PBDE-17	9,89	5260	5471,2527	104,0162	1476177,25	4145,903	78,81945
PBDE-28	10,03	5260	5416,4317	102,974	1577739,31	4104,362	78,02969
13-C-PBDE 47	11,04	5269,8347	4697,0132	89,13018	1370301,63	4697,008	89,13009
PBDE-49	10,83	5260	5839,4775	111,0167	1130639,16	5174,856	98,3813
PBDE-71	10,88	5260	5666,5088	107,7283	1102266,72	5021,573	95,46717
PBDE-47	11,02	5260	5459,6669	103,7959	1579970,81	4838,271	91,98233
PBDE-66	11,19	5260	5778,6155	109,8596	1021782,53	5120,919	97,35587
PBDE-77	11,46	5260	5418,5231	103,0137	1643851,56	4801,813	91,28921
13-C-PBDE 99	12,34	5258,6698	4780,5092	90,90719	1016835,75	4780,505	90,90712
PBDE-100	11,98	5260	5329,5274	101,3218	1328478,44	4763,567	90,56211
PBDE-119	12,09	5260	5366,9453	102,0332	877091,781	4797,011	91,19792
PBDE-99	12,31	5260	5494,4683	104,4576	1087340,75	4910,994	93,36491
PBDE-85	12,96	5260	5774,8143	109,7873	850496,469	5161,57	98,12871
PBDE-126	13,07	5260	5448,5563	103,5847	1178608,81	4869,958	92,58476
13-C-PBDE 153	13,91	5294,9558	4617,7414	87,2102	557501,407	4617,737	87,21011
PBDE-154	13,33	10500	11494,8694	109,4749	1635898,56	9707,96	92,45676
PBDE-153	13,89	10500	11080,5862	105,5294	1164748,16	9358,078	89,12455
PBDE-138	14,68	10500	10961,4988	104,3952	952069,813	9257,504	88,16671
PBDE-156	15	10500	10607,8915	101,0275	574359,313	8958,863	85,32251
13-C-PBDE 183	15,69	5297,747	4608,602	86,99173	403770,672	4608,597	86,99164
PBDE-184	15,36	10500	11387,3896	108,4513	1047716,13	9399,333	89,51746
PBDE-183	15,68	10500	10698,8185	101,8935	827140,719	8830,979	84,10456
PBDE-191	16,18	10500	10487,7068	99,88292	450210,469	8656,721	82,44497
13-C-PBDE-197	17,97	5221,9128	4886,7676	93,58195	237355,407	4886,764	93,58187

PBDE-202	17,54						
PBDE-197	17,95	10500	10918,915	103,9897	565467,39	8955,907	85,29435
PBDE-196	18,38	10500	13875,6951	132,1495	551891,328	11381,12	108,3916
13-C-PBDE-206	20,46	5230,7576	4551,0116	87,00483	82260,02	4551,007	87,00474
PBDE-207	20,14	26300	27989,0668	106,4223	658844,406	25389,32	96,53734
PBDE-206	20,49	26300	28824,7808	109,5999	430745,219	26147,41	99,41981
13C-PBDE-209	22,67	11773,8	11282,4812	95,82702	26758,71	11282,47	95,82692
PBDE-209	23,05	26300	24514,7278	93,21189	63073,178	24572,95	93,43328

7.1.4. PBDE) ^{12}C spiked samples for method validation (Method B)

B) CANADA			17/2523	12C-spike 1			
			A) Calculated from ISTD		B) Calculated from response (TCN as ISTD)		
Component	RT	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	8,92	1924	9,025	0,469075	3105069,5		
TBA	7,23						
13-C-PBDE 28	10,02	5191,7	4766,3	91,8	2962746,4	4766,3	91,80612
PBDE-17	9,89	5260,0	5496,1	104,5	3368189,4	5023,9	95,5107
PBDE-28	10,03	5260,0	5283,6	100,4	3495769,8	4829,6	91,81806
13-C-PBDE 47	11,04	5269,8	5325,8	101,1	2925627,0	5325,8	101,062
PBDE-49	10,83	5260,0	5665,0	107,7	2341819,1	5692,3	108,2187
PBDE-71	10,88	5260,0	5475,3	104,1	2273967,8	5501,7	104,5953
PBDE-47	11,02	5260,0	5356,7	101,8	3309669,8	5382,5	102,3295
PBDE-66	11,19	5260,0	5930,9	112,8	2239024,8	5959,5	113,2982
PBDE-77	11,46	5260,0	5641,3	107,2	3653928,6	5668,4	107,765
13-C-PBDE 99	12,34	5258,7	5596,4	106,4	2241421,4	5596,4	106,422
PBDE-100	11,98	5260,0	5281,8	100,4	2902146,4	5526,6	105,0684
PBDE-119	12,09	5260,0	5216,8	99,2	1879306,9	5458,6	103,7763
PBDE-99	12,31	5260,0	5386,3	102,4	2349641,8	5635,9	107,1471
PBDE-85	12,96	5260,0	5611,7	106,7	1821792,3	5871,8	111,6305
PBDE-126	13,07	5260,0	5388,2	102,4	2569237,1	5637,9	107,1852
13-C-PBDE 153	13,91	5295,0	5558,4	105,0	1263591,5	5558,4	104,9756
PBDE-154	13,33	10500,0	10421,0	99,2	3361427,8	10593,9	100,8943

Component	RT	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
PBDE-153	13,89	10500,0	10707,3	102,0	2550987,5	10884,9	103,6655
PBDE-138	14,68	10500,0	10637,6	101,3	2094134,9	10814,1	102,9914
PBDE-156	15	10500,0	10072,5	95,9	1236099,3	10239,6	97,52005
13-C-PBDE 183	15,69	5297,7	5375,9	101,5	886859,5	5375,9	101,4748
PBDE-184	15,36	10500,0	10803,3	102,9	2183205,0	10401,8	99,06478
PBDE-183	15,68	10500,0	10688,2	101,8	1814958,3	10291,0	98,00933
PBDE-191	16,18	10500	10673,2162	101,6497	1006353,16	10276,59	97,87226
13-C-PBDE-197	17,97	5221,9128	5828,1381	111,6093	533023,844	5828,134	111,6092
PBDE-202	17,54						
PBDE-197	17,95	10500	10631,7821	101,2551	1236464,5	10400,26	99,05012
PBDE-196	18,38	10500,0	12419,5	118,3	1109307,0	12149,1	115,7
13-C-PBDE-206	20,46	5230,8	5549,2	106,1	188865,3	5549,2	106,1
PBDE-207	20,14	26300,0	28298,9	107,6	1529422,6	31300,9	119,0
PBDE-206	20,49	26300,0	27725,2	105,4	951246,4	30666,3	116,6
13C-PBDE-209	22,67	11773,8	14831,0	126,0	66232,5	14831,0	126,0
PBDE-209	23,05	26300,0	23223,3	88,3	147893,3	30600,0	116,3

| B) CANADA | | | 17/2524 | 12C-spike 2 | | | |
| | | | A) Calculated from ISTD | | B) Calculated from response | | |
Component	RT	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	8,92	1924,0	697796,0	36268,0	2539460,4		
TBA	7,23						
13-C-PBDE 28	10,02	5191,7	3954,8	76,2	2010507,1	3954,8	76,2
PBDE-17	9,89	5260,0	5458,1	103,8	2269855,1	4139,7	78,7
PBDE-28	10,03	5260,0	5139,5	97,7	2307509,4	3898,0	74,1
13-C-PBDE 47	11,04	5269,8	3306,2	62,7	1485384,8	3306,2	62,7
PBDE-49	10,83	5260,0	6556,4	124,6	1376065,3	4089,8	77,8
PBDE-71	10,88	5260,0	7465,2	141,9	1574115,8	4656,7	88,5
PBDE-47	11,02	5260,0	5262,8	100,1	1650913,4	3282,9	62,4
PBDE-66	11,19	5260,0	2494,3	47,4	478093,8	1555,9	29,6
PBDE-77	11,46	5260,0	7379,0	140,3	2426623,3	4602,9	87,5
13-C-PBDE 99	12,34	5258,7	4749,3	90,3	1555661,4	4749,3	90,3
PBDE-100	11,98	5260,0	4831,7	91,9	1842606,2	4290,4	81,6

Component	RT	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
PBDE-119	12,09	5260,0	4901,3	93,2	1225454,0	4352,2	82,7
PBDE-99	12,31	5260,0	5291,6	100,6	1602116,0	4698,8	89,3
PBDE-85	12,96	5260	5343,3835	101,5852	1203968,44	4744,768	90,20471
PBDE-126	13,07	5260	4996,3954	94,98851	1653519,44	4436,652	84,347
13-C-PBDE 153	13,91	5294,9558	4386,9777	82,85202	815626,376	4386,973	82,85194
PBDE-154	13,33	10500	10474,5921	99,75802	2180894,94	8404,21	80,04009
PBDE-153	13,89	10500	10627,6909	101,2161	1634381,75	8527,047	81,20997
PBDE-138	14,68	10500	10201,6372	97,15845	1296325,38	8185,207	77,95435
PBDE-156	15	10500	9376,4791	89,2998	742744,906	7523,145	71,649
13-C-PBDE 183	15,69	5297,747	4059,5561	76,62797	547713,688	4059,552	76,62789
PBDE-184	15,36	10500	10898,0174	103,7906	1360146,75	7923,731	75,4641
PBDE-183	15,68	10500	10325,777	98,34073	1082892	7507,669	71,50161
PBDE-191	16,18	10500	10367,5595	98,73866	603712,844	7538,046	71,79092
13-C-PBDE-197	17,97	5221,9128	4469,2902	85,58722	334291,61	4469,287	85,58716
PBDE-202	17,54						
PBDE-197	17,95	10500	10090,0147	96,09538	735946,469	7569,004	72,08576
PBDE-196	18,38	10500	13863,5913	132,0342	776606,312	10399,75	99,0452
13-C-PBDE-206	20,46	5230,7576	4234,2565	80,9492	117860,289	4234,252	80,94912
PBDE-207	20,14	26300	28074,5697	106,7474	946861,031	23694,36	90,09262
PBDE-206	20,49	26300	27032,0197	102,7833	578777,5	22814,47	86,74703
13C-PBDE-209	22,67	11773,8	10155,5446	86,25545	37091,402	10155,53	86,25536
PBDE-209	23,05	26300	21439,8246	81,52025	76462,227	19344,17	73,55197

			17/2525	12C-spike 3			
B) CANADA			A) Calculated from ISTD		B) Calculated from response		
Component	RT	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	8,92	1924	298556	15517,46	2157810,88		
TBA	7,23						
13-C-PBDE 28	10,02	5191,6804	4134,1516	79,63032	1785841,06	4134,146	79,63022
PBDE-17	9,89	5260	5511,936	104,7897	2036093,5	4370,156	83,08281
PBDE-28	10,03	5260	5308,4964	100,922	2117070,5	4208,858	80,01632
13-C-PBDE 47	11,04	5269,8347	4661,2558	88,45165	1779421,13	4661,251	88,45156
PBDE-49	10,83	5260	5743,9593	109,2007	1444188,75	5051,459	96,03534

PBDE-71	10,88	5260	5421,9553	103,079	1369587	4768,275	90,65162
PBDE-47	11,02	5260	5368,4383	102,0616	2017406,69	4721,208	89,7568
PBDE-66	11,19	5260	4298,2188	81,71519	986928,657	3780,017	71,86344
PBDE-77	11,46	5260	5890,6772	111,9901	2320649	5180,488	98,48836
13-C-PBDE 99	12,34	5258,6698	4963,271	94,38263	1381421	4963,267	94,38256
PBDE-100	11,98	5260	5185,2496	98,57889	1755944,25	4811,795	91,47898
PBDE-119	12,09	5260	5251,8036	99,84417	1166008,19	4873,555	92,65313
PBDE-99	12,31	5260	5515,2446	104,8526	1482791,25	5118,025	97,30085
PBDE-85	12,96	5260	5612,3682	106,699	1122938,31	5208,154	99,01433
PBDE-126	13,07	5260	5327,839	101,2897	1565721,75	4944,117	93,99461
13-C-PBDE 153	13,91	5294,9558	4713,871	89,02569	744689,907	4713,866	89,02561
PBDE-154	13,33	10500	10932,0732	104,115	2078185,75	9424,852	89,7605
PBDE-153	13,89	10500	10861,3745	103,4417	1525048,25	9363,9	89,18
PBDE-138	14,68	10500	10659,6564	101,5205	1236720,5	9189,995	87,52376
PBDE-156	15	10500	9838,3701	93,69876	711553,001	8481,938	80,78036
13-C-PBDE 183	15,69	5297,747	4442,157	83,84993	509261,547	4442,152	83,84984
PBDE-184	15,36	10500	10960,1021	104,3819	1271862,5	8719,913	83,04679
PBDE-183	15,68	10500	10576,7376	100,7308	1031338,97	8414,91	80,142
PBDE-191	16,18	10500	10502,1067	100,0201	568614,062	8355,531	79,57648
13-C-PBDE-197	17,97	5221,9128	4733,1396	90,63996	300821,016	4733,136	90,63989
PBDE-202	17,54						
PBDE-197	17,95	10500	10447,9663	99,50444	685754,844	8300,218	79,04969
PBDE-196	18,38	10500	12037,4894	114,6428	606797,5	9562,989	91,07609
13-C-PBDE-206	20,46	5230,7576	4223,687	80,74714	99897,359	4223,683	80,74706
PBDE-207	20,14	26300	28119,5677	106,9185	803837,532	23673,1	90,01178
PBDE-206	20,49	26300	28208,861	107,258	511923,657	23748,27	90,29761
13C-PBDE-209	22,67	11773,8	12574,2493	106,7986	39023,304	12574,24	106,7985
PBDE-209	23,05	26300	23605,2784	89,75391	88569,793	26370,41	100,2677

7.1.5. PBDE) ^{12}C spiked samples for method validation (Method C)

7.1.6. n-BFR) ^{12}C spiked samples for method validation (Method A)

A) NILU-O2 Component	m STD (pg)	17/2662 12C-spike 4 A) Calculated from ISTD		B) Calculated from response (TCN as ISTD)		
		m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	1924,0	6,4	0,3	635019,0		
13-C-PBDE 28	5191,7	3145,9	60,6	1055257,4	3145,9	60,6
ATE (TBP-AE)	49100,0	853,8	1,7	4473,1	518,0	1,1
a-TBECH	50220,0	37400,7	74,5	747355,0	22693,4	45,2
b-TBECH	50220,0	37338,0	74,3	1121526,8	22655,3	45,1
g/d-TBECH	48540,0	44426,6	91,5	3477431,5	26956,4	55,5
BATE	50120,0	24973,2	49,8	2865792,4	15152,8	30,2
PBT	50000,0	42324,6	84,6	6053346,0	25681,0	51,4
PBEB	49100,0	44970,2	91,6	5593516,5	27286,3	55,6
13C_HBB	20456,7	11224,7	54,9	1316711,8	11224,7	54,9
HBB	48440,0	44503,2	91,9	3525313,4	25115,9	51,8
13-C-PBDE 47	5269,8	3846,2	73,0	798313,1	3846,2	73,0
DPTE	49660,0	43694,2	88,0	5716090,8	31785,6	64,0
13-C-EHTBB	19882,4	8415,4	42,3	486189,4	8415,4	42,3
EHTBB	49560,0	49862,0	100,6	1742843,6	20948,9	42,3
13-C-BTBPE	20387,8	12746,4	62,5	2463963,9	12746,4	62,5
BTBPE	50520,0	45318,1	89,7	5674230,5	29324,8	58,0
BEHTBP	99440,0	31147,5	31,3	612939,3	20155,2	20,3
13-C-DBDPE	20209,8	10135,0	50,1	297272,3	10135,0	50,1
DBDPE	49100,0	46830,9	95,4	811691,3	23352,1	47,6

A) NILU-O2 Component	m STD (pg)	17/2663 12C-spike 5 A) Calculated from ISTD		B) Calculated from response		
		m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	1924,0	9,1	0,5	897788,1		
13-C-PBDE 28	5191,7	3545,6	68,3	1681479,2	3545,6	68,3
ATE (TBP-AE)	49100,0	658,7	1,3	5499,3	450,5	0,9
a-TBECH	50220,0	39660,4	79,0	1262809,4	27122,1	54,0
b-TBECH	50220,0	41275,7	82,2	1975544,6	28226,7	56,2
g/d-TBECH	48540,0	45451,8	93,6	5668921,0	31082,6	64,0

Component	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
BATE	50120,0	28602,9	57,1	5230160,5	19560,4	39,0
PBT	50000,0	43460,1	86,9	9904368,5	29720,5	59,4
PBEB	49100,0	46386,6	94,5	9193603,0	31721,9	64,6
13C_HBB	20456,7	12622,0	61,7	2093298,6	12622,0	61,7
HBB	48440,0	44036,6	90,9	5545754,6	27946,4	57,7
13-C-PBDE 47	5269,8	4401,5	83,5	1291583,9	4401,5	83,5
DPTE	49660,0	41417,0	83,4	8766051,5	34478,5	69,4
13-C-EHTBB	19882,4	10227,0	51,4	835348,9	10227,0	51,4
EHTBB	49560,0	49322,7	99,5	2962086,9	25183,4	50,8
13-C-BTBPE	20387,8	14180,3	69,6	3875412,5	14180,3	69,6
BTBPE	50520,0	45071,7	89,2	8876107,5	32446,2	64,2
BEHTBP	99440,0	60031,3	60,4	1858044,2	43215,3	43,5
13-C-DBDPE	20209,8	11216,4	55,5	465128,6	11216,4	55,5
DBDPE	49100,0	49059,6	99,9	1330455,6	27073,8	55,1

A) NILU-O2		17/2664 12C-spike 6 A) Calculated from ISTD		B) Calculated from response		
Component	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	1924,0	6,4	0,3	632459,6		
13-C-PBDE 28	5191,7	3413,4	65,7	1140377,3	3413,4	65,7
ATE (TBP-AE)	49100,0	1008,0	2,1	5707,0	663,6	1,4
a-TBECH	50220,0	38693,6	77,0	835556,8	25474,3	50,7
b-TBECH	50220,0	38129,5	75,9	1237684,3	25102,9	50,0
g/d-TBECH	48540,0	44823,0	92,3	3791464,0	29509,7	60,8
BATE	50120,0	27722,0	55,3	3437841,9	18251,1	36,4
PBT	50000,0	42060,8	84,1	6500855,5	27691,2	55,4
PBEB	49100,0	44700,2	91,0	6008399,5	29428,8	59,9
13C_HBB	20456,7	12096,9	59,1	1413300,0	12096,9	59,1
HBB	48440,0	43592,6	90,0	3706492,6	26513,6	54,7
13-C-PBDE 47	5269,8	4104,9	77,9	848562,8	4104,9	77,9
DPTE	49660,0	44639,9	89,9	6207402,0	34657,3	69,8
13-C-EHTBB	19882,4	10221,6	51,4	588163,7	10221,6	51,4
EHTBB	49560,0	48125,3	97,1	2034956,1	24559,1	49,6

13-C-BTBPE	20387,8	14050,8	68,9	2705153,1	14050,8	68,9
BTBPE	50520,0	43025,3	85,2	5914476,8	30690,1	60,7
BEHTBP	99440,0	26496,4	26,6	572451,5	18900,0	19,0
13-C-DBDPE	20209,8	10241,9	50,7	299195,6	10241,8	50,7
DBDPE	49100,0	48081,2	97,9	838753,1	24228,3	49,3

7.1.7. n-BFR) ^{12}C spiked samples for method validation (Method B)

B) CANADA		17/2523	12C-spike 1			
		A) Calculated from ISTD		B) Calculated from response (TCN as ISTD)		
Component	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	1924,0	1541507,0	80119,9	2091142,1		
13-C-PBDE 28	5191,7	4577,2	88,2	1560672,1	4577,2	88,2
ATE (TBP-AE)	48500,0	34892,3	71,9	1368728,6	30653,8	63,2
a-TBECH	49600,0	37142,5	74,9	3893889,6	32630,7	65,8
b-TBECH	49600,0	35246,8	71,1	5162826,8	30965,2	62,4
g/d-TBECH	47900,0	37819,2	79,0	8090597,8	33225,1	69,4
BATE	49500,0	39754,9	80,3	7316827,0	34925,7	70,6
PBT	49400,0	40774,1	82,5	10894083,0	35821,1	72,5
PBEB	48500,0	45506,6	93,8	9365926,0	39978,7	82,4
13C_HBB	20456,7	15351,2	75,0	444919,1	15351,2	75,0
HBB	47800,0	45493,9	95,2	1281362,7	34926,5	73,1
13-C-PBDE 47	5269,8	5013,7	95,1	433336,3	5013,7	95,1
DPTE	49000,0	45034,9	91,9	10534138,5	42655,1	87,1
13-C-EHTBB	19882,4	17550,4	88,3	1125144,3	17550,4	88,3
EHTBB	48900,0	49671,3	101,6	3781905,6	43202,7	88,3
13-C-BTBPE	20387,8	17864,0	87,6	4315335,3	17864,0	87,6
BTBPE	49900,0	44367,9	88,9	9884928,5	40162,5	80,5
BEHTBP	98200,0	44707,5	45,5	1002128,1	40469,9	41,2
13-C-DBDPE	20209,8	20971,9	103,8	86809,7	20971,9	103,8
DBDPE	48500,0	51428,0	106,0	242726,2	50397,2	103,9
B) CANADA		17/2524	12C-spike 2			
		A) Calculated from ISTD		B) Calculated from response		

Component	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	1924,0	2147872,0	111635,8	2686159,1		
13-C-PBDE 28	5191,7	4034,0	77,7	1766849,5	4034,0	77,7
ATE (TBP-AE)	48500,0	40221,7	82,9	1786221,4	31142,5	64,2
a-TBECH	49600,0	40814,9	82,3	4844160,3	31601,9	63,7
b-TBECH	49600,0	39667,9	80,0	6578002,8	30713,8	61,9
g/d-TBECH	47900,0	38128,1	79,6	9234244,8	29521,6	61,6
BATE	49500,0	40922,0	82,7	8526615,5	31684,8	64,0
PBT	49400,0	43319,1	87,7	13103059,5	33540,8	67,9
PBEB	48500,0	33719,2	69,5	7856724,8	26107,9	53,8
13C_HBB	20456,7	14547,6	71,1	541598,5	14547,6	71,1
HBB	47800,0	47067,8	98,5	1613762,6	34243,2	71,6
13-C-PBDE 47	5269,8	4523,3	85,8	502188,3	4523,3	85,8
DPTE	49000,0	44797,0	91,4	12143410,5	38279,3	78,1
13-C-EHTBB	19882,4	19286,6	97,0	1588277,9	19286,6	97,0
EHTBB	48900,0	48251,6	98,7	5186040,5	46119,9	94,3
13-C-BTBPE	20387,8	16358,9	80,2	5076183,8	16358,9	80,2
BTBPE	49900,0	44975,3	90,1	11786941,5	37282,1	74,7
BEHTBP	98200,0	46501,2	47,4	1226111,7	38547,0	39,3
13-C-DBDPE	20209,8	13012,6	64,4	69190,2	13012,6	64,4
DBDPE	48500,0	52208,7	107,6	196397,6	31745,1	65,5

		17/2525	12C-spike 3			
B) CANADA		A) Calculated from ISTD		B) Calculated from response		
Component	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	1924,0	1165672,0	60585,9	2062865,1		
13-C-PBDE 28	5191,7	4135,7	79,7	1391065,8	4135,7	79,7
ATE (TBP-AE)	48500,0	31569,8	65,1	1103811,6	25059,6	51,7
a-TBECH	49600,0	37075,0	74,7	3464408,9	29429,6	59,3
b-TBECH	49600,0	35212,2	71,0	4597226,4	27950,9	56,4
g/d-TBECH	47900,0	35897,9	74,9	6844993,0	28495,2	59,5
BATE	49500,0	37670,6	76,1	6179734,8	29902,3	60,4
PBT	49400,0	40307,7	81,6	9599083,0	31995,7	64,8

PBEB	48500,0	38647,2	79,7	7089729,8	30677,6	63,3
13C_HBB	20456,7	13078,9	63,9	373936,1	13078,9	63,9
HBB	47800,0	42436,2	88,8	1004551,0	27756,7	58,1
13-C-PBDE 47	5269,8	4458,7	84,6	380153,7	4458,7	84,6
DPTE	49000,0	42805,4	87,4	8783797,3	36055,1	73,6
13-C-EHTBB	19882,4	18627,5	93,7	1178047,3	18627,5	93,7
EHTBB	48900,0	44228,5	90,4	3525839,6	40829,6	83,5
13-C-BTBPE	20387,8	16194,3	79,4	3859097,1	16194,3	79,4
BTBPE	49900,0	41661,1	83,5	8300550,5	34187,5	68,5
BEHTBP	98200,0	54107,6	55,1	1084606,7	44401,1	45,2
13-C-DBDPE	20209,8	14934,8	73,9	60984,3	14934,8	73,9
DBDPE	48500,0	48206,0	99,4	159833,4	33641,1	69,4

7.1.8. n-BFR) ^{12}C spiked samples for standard control

Standardkontroll		18/0161 Std.kontroll1				
		A) Calculated from ISTD		B) Calculated from response (TCN as ISTD)		
Component	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	1924,0	918465,0	47737,3	716248,5		
13-C-PBDE 28	5191,7	3844,6	74,1	1342157,0	3844,6	74,1
ATE (TBP-AE)	49520,0	77209,9	155,9	587412,1	56677,6	114,5
a-TBECH	48600,0	65520,2	134,8	1805255,4	48096,4	99,0
b-TBECH	48600,0	63933,8	131,6	2585431,3	46931,9	96,6
g/d-TBECH	48660,0	56130,1	115,4	6929221,5	41203,5	84,7
BATE	51320,0	57887,6	112,8	8686292,5	42493,6	82,8
PBT	49820,0	52071,2	104,5	9811022,5	38223,9	76,7
PBEB	49980,0	49161,1	98,4	8366684,0	36087,7	72,2
13C_HBB	20456,7	14202,1	69,4	1244144,6	14202,1	69,4
HBB	48500,0	47246,5	97,4	3724077,5	33323,4	68,7
13-C-PBDE 47	5269,8	3748,6	71,1	743000,9	3748,6	71,1
DPTE	49700,0	54225,3	109,1	8470891,5	38000,6	76,5
13-C-EHTBB	19882,4	11394,9	57,3	719090,9	11394,9	57,3
EHTBB	49400,0	56495,2	114,4	2915770,5	32062,2	64,9

Component	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
13-C-BTBPE	20387,8	11149,3	54,7	2449672,9	11149,3	54,7
BTBPE	50040,0	46220,8	92,4	5881987,5	26137,1	52,2
BEHTBP	99560,0	105430,4	105,9	2012970,4	59619,2	59,9
13-C-DBDPE	20209,8	10478,5	51,8	302750,7	10478,5	51,8
DBDPE	51400,0	53598,7	104,3	910557,1	27411,7	53,3

Standardkontroll		18/0162	Std.kontroll2			
		A) Calculated from ISTD		B) Calculated from response		
Component	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	1924,0	1276372,0	66339,5	647821,1		
13-C-PBDE 28	5191,7	4234,4	81,6	1336987,0	4234,4	81,6
ATE (TBP-AE)	49520,0	68492,0	138,3	519079,4	55374,6	111,8
a-TBECH	48600,0	59283,0	122,0	1627111,3	47929,2	98,6
b-TBECH	48600,0	58111,0	119,6	2340909,6	46981,7	96,7
g/d-TBECH	48660,0	50910,3	104,6	6260633,8	41160,1	84,6
BATE	51320,0	53034,4	103,3	7927397,0	42877,4	83,5
PBT	49820,0	52375,0	105,1	9830264,5	42344,3	85,0
PBEB	49980,0	51440,6	102,9	8720913,0	41588,8	83,2
13C_HBB	20456,7	16103,6	78,7	1275939,6	16103,6	78,7
HBB	48500,0	47701,8	98,4	3856049,0	38148,9	78,7
13-C-PBDE 47	5269,8	4358,5	82,7	781346,5	4358,5	82,7
DPTE	49700,0	53334,7	107,3	8761766,5	43457,2	87,4
13-C-EHTBB	19882,4	13330,4	67,0	760864,1	13330,4	67,0
EHTBB	49400,0	56191,4	113,7	3068565,8	37306,5	75,5
13-C-BTBPE	20387,8	13107,9	64,3	2604858,5	13107,9	64,3
BTBPE	50040,0	46083,4	92,1	6236020,0	30637,3	61,2
BEHTBP	99560,0	106487,3	107,0	2161947,7	70795,0	71,1
13-C-DBDPE	20209,8	11876,6	58,8	310362,3	11876,6	58,8
DBDPE	51400,0	53785,2	104,6	936698,8	31177,2	60,7

Standardkontroll		18/0163	Std.kontroll3			
		A) Calculated from ISTD		B) Calculated from response		
Component	m STD (pg)	m 12C (pg)	Rec (%)	A	m x (pg)	Rec(%)
TCN	1924,0	2953169,0	153491,1	606781,9		

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13-C-PBDE 28	5191,7	4328,0	83,4	1279991,8	4328,0	83,4
ATE (TBP-AE)	49520,0	67707,8	136,7	491261,3	55951,5	113,0
a-TBECH	48600,0	56955,1	117,2	1496579,5	47065,8	96,8
b-TBECH	48600,0	55214,5	113,6	2129410,5	45627,4	93,9
g/d-TBECH	48660,0	47699,4	98,0	5615720,3	39417,2	81,0
BATE	51320,0	50010,0	97,4	7156650,8	41326,6	80,5
PBT	49820,0	50287,5	100,9	9036093,5	41555,9	83,4
PBEB	49980,0	48876,7	97,8	7933013,8	40390,1	80,8
13C_HBB	20456,7	16502,1	80,7	1224683,1	16502,1	80,7
HBB	48500,0	46602,7	96,1	3615867,1	38192,2	78,7
13-C-PBDE 47	5269,8	4574,0	86,8	768034,5	4574,0	86,8
DPTE	49700,0	51079,3	102,8	8248279,0	43677,3	87,9
13-C-EHTBB	19882,4	13580,5	68,3	726036,2	13580,5	68,3
EHTBB	49400,0	55919,9	113,2	2913954,9	37822,8	76,6
13-C-BTBPE	20387,8	13097,8	64,2	2437964,6	13097,8	64,2
BTBPE	50040,0	45819,7	91,6	5803072,0	30438,5	60,8
BEHTBP	99560,0	107211,0	107,7	2037182,1	71221,3	71,5
13-C-DBDPE	20209,8	12454,8	61,6	304853,1	12454,8	61,6
DBDPE	51400,0	52715,6	102,6	901774,5	32044,8	62,3

ISTD	Congener	A Rec. 1 (%)	Rec. 2 (%)	Rec. 3 (%)	Av. Rec (%)	St.dev.	RSD (%)	B Rec. 1 (%)	Rec. 2 (%)	Rec. 3 (%)	Av. Rec (%)	St.dev.	RSD (%)
(Tri-) 13C-BDE-28	BDE-17	103,0	102,5	104,0	103,2	0,8	0,8	104,5	103,8	104,8	104,3	0,5	0,5
	BDE-28	102,7	102,8	103,0	102,8	0,1	0,1	100,4	97,7	100,9	99,7	1,7	1,7
	BDE-47	102,6	102,7	103,8	103,0	0,7	0,6	101,8	100,1	102,1	101,3	1,1	1,1
	BDE-49	109,6	106,0	111,0	108,9	2,6	2,4	107,7	124,6	109,2	113,8	9,4	8,2
	BDE-66	108,9	107,6	109,9	108,8	1,1	1,1	112,8	47,4	81,7	80,6	32,7	40,5
	BDE-71	103,3	102,7	107,7	104,6	2,7	2,6	104,1	141,9	103,1	116,4	22,1	19,0
(Tetra-) 13C-BDE-47	BDE-77	105,0	104,5	103,0	104,1	1,0	1,0	107,2	140,3	112,0	119,8	17,9	14,9
	BDE-85	111,6	107,7	109,8	109,7	1,9	1,8	106,7	101,6	106,7	105,0	2,9	2,8
	BDE-99	104,8	105,5	104,5	104,9	0,5	0,5	102,4	100,6	104,9	102,6	2,1	2,1
	BDE-100	103,8	104,1	101,3	103,1	1,5	1,5	100,4	91,9	98,6	97,0	4,5	4,6
	BDE-119	102,6	102,4	102,0	102,3	0,3	0,3	99,2	93,2	99,8	97,4	3,7	3,8
(Penta-) 13C-BDE-99	BDE-126	107,9	103,2	103,6	104,9	2,6	2,5	102,4	95,0	101,3	99,6	4,0	4,0
	BDE-138	105,9	103,7	104,4	104,7	1,1	1,1	101,3	97,2	101,5	100,0	2,5	2,5
	BDE-153	103,1	104,3	105,5	104,3	1,2	1,2	102,0	101,2	103,4	102,2	1,1	1,1
	BDE-154	109,6	108,3	109,5	109,1	0,7	0,7	99,2	99,8	104,1	101,0	2,7	2,6
(Hexa-) 13C-BDE-138	BDE-156	105,0	101,8	101,0	102,6	2,1	2,0	95,9	89,3	93,7	93,0	3,4	3,6
	BDE-183	104,1	103,3	101,9	103,1	1,1	1,1	101,8	98,3	100,7	100,3	1,8	1,8
	BDE-184	106,6	108,1	108,5	107,7	1,0	0,9	102,9	103,8	104,4	103,7	0,8	0,7
(Hepta-) 13C-BDE-183	BDE-191	105,3	102,1	99,9	102,4	2,7	2,7	101,6	98,7	100,0	100,1	1,5	1,5
	BDE-196	129,2	130,0	132,1	130,4	1,5	1,2	118,3	132,0	114,6	121,7	9,2	7,5
(Octa-) 13C-BDE-197	BDE-197	107,6	107,1	104,0	106,2	2,0	1,8	101,3	96,1	99,5	99,0	2,6	2,7
	BDE-206	100,1	106,2	109,6	105,3	4,8	4,6	105,4	102,8	107,3	105,2	2,2	2,1
(Nona-) 13C-BDE-206	BDE-207	109,7	110,6	106,4	108,9	2,2	2,0	107,6	106,7	106,9	107,1	0,5	0,4
(Deca-) 13C-BDE-209	BDE-209	91,5	91,9	93,2	92,2	0,9	1,0	88,3	81,5	89,8	86,5	4,4	5,1
	ATE (TBP-AE)	1,7	1,3	2,1	1,7	0,4	20,8	71,9	82,9	65,1	73,3	9,0	12,3
13-C-PBDE-28	a-TBECH	74,5	79,0	77,0	76,8	2,3	2,9	74,9	82,3	74,7	77,3	4,3	5,6
	b-TBECH	74,3	82,2	75,9	77,5	4,1	5,4	71,1	80,0	71,0	74,0	5,2	7,0
	g/d-TBECH	91,5	93,6	92,3	92,5	1,1	1,2	79,0	79,6	74,9	77,8	2,5	3,2
	BATE	49,8	57,1	55,3	54,1	3,8	7,0	80,3	82,7	76,1	79,7	3,3	4,2
	PBT	84,6	86,9	84,1	85,2	1,5	1,7	82,5	87,7	81,6	83,9	3,3	3,9
13C-HBB	PBEB	91,6	94,5	91,0	92,4	1,8	2,0	93,8	69,5	79,7	81,0	12,2	15,1
	HBB	91,9	90,9	90,0	90,9	0,9	1,0	95,2	98,5	88,8	94,1	4,9	5,2
13C-PBDE-47	DPTE	88,0	83,4	89,9	87,1	3,3	3,8	91,9	91,4	87,4	90,2	2,5	2,8
13C-EHTBB	EHTBB	100,6	99,5	97,1	99,1	1,8	1,8	101,6	98,7	90,4	96,9	5,8	6,0
13C-BTBPE	BTBPE	89,7	89,2	85,2	88,0	2,5	2,8	88,9	90,1	83,5	87,5	3,5	4,0
13C-DBDPE	BEHTBP	31,3	60,4	26,6	39,4	18,3	46,3	45,5	47,4	55,1	49,3	5,1	10,3
	DBDPE	95,4	99,9	97,9	97,7	2,3	2,3	106,0	107,6	99,4	104,4	4,4	4,2

ISTD	Congener	C						Standard control					
		Rec. 1 (%)	Rec. 2 (%)	Rec. 3 (%)	Av. Rec (%)	St.dev.	RSD (%)	Rec. 1 (%)	Rec. 2 (%)	Rec. 3 (%)	Av. Rec (%)	St.dev.	RSD (%)
(Tri-) 13C-BDE-28	BDE-17	107,8	109,2	107,6	108,2	0,9	0,8	109,2	107,0	106,0	107,4	1,7	1,5
	BDE-28	109,1	108,9	110,2	109,4	0,7	0,7	107,1	105,7	104,0	105,6	1,5	1,5
(Tetra-) 13C-BDE-47	BDE-47	109,0	103,9	111,9	108,3	4,0	3,7	104,0	103,8	103,3	103,7	0,4	0,4
	BDE-49	133,6	147,6	119,3	133,5	14,2	10,6	112,0	115,2	113,4	113,5	1,6	1,4
	BDE-66	25,0	17,9	94,6	45,9	42,4	92,4	109,4	111,3	109,1	109,9	1,2	1,1
	BDE-71	213,2	223,8	111,2	182,7	62,1	34,0	109,9	107,8	107,6	108,4	1,3	1,2
	BDE-77	0,0	0,0	123,5	41,2	71,3	173,2	105,8	106,0	103,6	105,2	1,3	1,3
	BDE-85	123,6	108,0	119,2	116,9	8,0	6,9	109,6	109,9	108,8	109,4	0,6	0,5
	BDE-99	111,7	110,9	112,3	111,6	0,7	0,7	105,1	105,0	103,6	104,6	0,9	0,8
	BDE-100	105,2	109,2	112,6	109,0	3,7	3,4	102,7	102,3	100,2	101,7	1,3	1,3
	BDE-119	109,0	110,6	113,9	111,2	2,5	2,2	100,4	99,1	98,7	99,4	0,9	0,9
(Penta-) 13C-BDE-99	BDE-126	116,4	100,1	117,6	111,4	9,8	8,8	106,8	106,2	105,0	106,0	0,9	0,9
	BDE-138	111,5	89,3	155,7	118,8	33,8	28,5	104,7	104,3	102,7	103,9	1,1	1,0
	BDE-153	112,0	109,0	110,3	110,5	1,5	1,4	104,2	103,5	103,4	103,7	0,5	0,4
	BDE-154	101,7	113,8	296,6	170,7	109,2	64,0	106,8	106,7	106,4	106,6	0,2	0,2
(Hexa-) 13C-BDE-138	BDE-156	105,5	68,0	115,2	96,2	24,9	25,9	103,7	103,1	100,6	102,5	1,6	1,6
	BDE-183	113,1	108,4	109,1	110,2	2,5	2,3	104,6	103,8	103,9	104,1	0,4	0,4
	BDE-184	116,5	134,1	142,9	131,2	13,4	10,2	109,2	109,9	108,5	109,2	0,7	0,6
(Hepta-) 13C-BDE-183	BDE-191	123,1	96,5	90,9	103,5	17,2	16,6	105,8	107,3	105,1	106,1	1,1	1,1
	BDE-196	142,5	135,1	61,2	112,9	45,0	39,8	107,2	105,3	102,7	105,1	2,2	2,1
(Octa-) 13C-BDE-197	BDE-197	110,4	111,3	112,9	111,6	1,3	1,1	103,9	105,0	102,0	103,6	1,5	1,4
	BDE-206	107,3	97,9	90,5	98,6	8,4	8,5	103,5	106,5	104,8	104,9	1,5	1,4
(Nona-) 13C-BDE-206	BDE-207	102,2	162,3	221,7	162,1	59,8	36,9	110,9	112,1	111,0	111,3	0,7	0,6
(Deca-) 13C-BDE-209	BDE-209	96,6	96,0	0,0	64,2	55,6	86,6	93,1	94,3	93,1	93,5	0,7	0,8
13-C-PBDE-28	ATE (TBP-AE)	191,4	-	-	-	-	-	155,9	138,3	136,7	143,7	10,7	7,4
	a-TBECH	230,2	-	-	-	-	-	134,8	122,0	117,2	124,7	9,1	7,3
	b-TBECH	201,3	-	-	-	-	-	131,6	119,6	113,6	121,6	9,1	7,5
	g/d-TBECH	95,6	-	-	-	-	-	115,4	104,6	98,0	106,0	8,7	8,2
	BATE	130,8	-	-	-	-	-	112,8	103,3	97,4	104,5	7,7	7,4
	PBT	102,8	-	-	-	-	-	104,5	105,1	100,9	103,5	2,3	2,2
	PBEB	79,9	-	-	-	-	-	98,4	102,9	97,8	99,7	2,8	2,8
13C-HBB	HBB	98,0	-	-	-	-	-	97,4	98,4	96,1	97,3	1,1	1,2
13C-PBDE-47	DPTE	73,2	-	-	-	-	-	109,1	107,3	102,8	106,4	3,3	3,1
13C-EHTBB	EHTBB	99,5	-	-	-	-	-	114,4	113,7	113,2	113,8	0,6	0,5
13C-BTBPE	BTBPE	97,2	-	-	-	-	-	92,4	92,1	91,6	92,0	0,4	0,4
	BEHTBP	88,9	-	-	-	-	-	105,9	107,0	107,7	106,8	0,9	0,8
13C-DBDPE	DBDPE	0,0	-	-	-	-	-	104,3	104,6	102,6	103,8	1,1	1,1

7.3.   Exposed samples

ISTD	Congener	Exposed 1	Exposed 2	Blank(PUF)	Blank	Average	St.dev.	RSD
Tri-BDE	BDE-17	7,5	5,7	2,7	1,9	6,6	1,2	18,7
	BDE-28	12,0	10,8	2,8	1,9	11,4	0,8	7,2
Tetra-	BDE-47	75,9	63,6	4,2	3,7	69,7	8,7	12,4
	BDE-49	8,8	6,6	0,9	1,6	7,7	1,5	20,1
	BDE-66	24,0	22,8	25,9	25,5	23,4	0,9	3,7
	BDE-71	0,0	0,0	0,8	0,0	0,0	0,0	
	BDE-77	0,0	0,0	0,0	0,0	0,0	0,0	
Penta-	BDE-85	0,0	0,0	0,0	0,0	0,0	0,0	
	BDE-99	27,8	21,2	1,7	1,8	24,5	4,6	18,9
	BDE-100	8,2	6,6	0,4	0,6	7,4	1,2	15,8
	BDE-119	0,0	0,0	0,0	0,9	0,0	0,0	
	BDE-126	0,0	0,0	0,0	0,0	0,0	0,0	
Hexa-	BDE-138	0,0	0,0	0,0	0,0	0,0	0,0	
	BDE-153	5,2	2,9	1,7	0,0	4,1	1,7	41,3
	BDE-154	3,0	1,8	0,0	0,0	2,4	0,9	36,1
	BDE-156	0,0	0,0	0,0	0,0	0,0	0,0	
Hepta-	BDE-183	4,6	4,5	2,3	2,6	4,5	0,1	1,1
	BDE-184	1,8	0,0	0,0	0,0	0,9	1,2	141,4
	BDE-191	0,0	0,0	0,0	0,0	0,0	0,0	
Octa-	BDE-196	0,0	4,4	4,5	0,0	2,2	3,1	141,4
	BDE-197	4,2	2,2	2,3	0,0	3,2	1,4	44,7
Nona-	BDE-206	0,0	14,6	58,7	0,0	7,3	10,4	141,4
	BDE-207	28,5	20,6	36,7	0,0	24,5	5,6	22,7
Deca-	BDE-209	183,2	93,7	0,0	0,0	138,4	63,3	45,7

ISTD	Congener	B									
		Exposed 3	Exposed 4	Exposed 5	Blank(PUF)	Blank	Average	St.dev.	RSD	LOD	MDL
Tri-BDE	BDE-17	8,3	11,5	7,4	2,7	3,6	9,1	2,1	23,7	0,3	8,1
	BDE-28	11,2	12,5	9,1	3,2	4,5	11,0	1,7	15,4	0,3	9,7
Tetra-	BDE-47	58,3	89,5	65,0	15,5	5,9	70,9	16,4	23,2	2,2	46,5
	BDE-49	6,5	11,7	5,9	3,9	1,4	8,0	3,2	40,0	3,4	11,6
	BDE-66	22,3	25,1	17,6	20,5	17,9	21,7	3,8	17,5	3,7	61,4
	BDE-71	0,0	0,0	0,0	3,7	1,1	0,0	0,0		3,3	11,2
	BDE-77	3,0	1,1	0,0	0,0	1,4	1,4	1,5	109,9	2,1	2,1
Penta-	BDE-85	0,0	0,0	0,0	0,0	1,3	0,0	0,0		0,5	0,5
	BDE-99	20,3	33,4	19,4	4,9	4,1	24,4	7,8	32,2	0,4	14,8
	BDE-100	5,0	10,7	5,4	0,0	1,4	7,0	3,2	45,4	0,3	0,3
	BDE-119	0,0	2,4	2,2	0,0	1,4	1,5	1,3	87,0	0,5	0,5
	BDE-126	0,0	0,0	0,0	0,0	1,1	0,0	0,0		0,4	0,4
Hexa-	BDE-138	1,8	0,0	0,0	1,9	1,4	0,6	1,0	173,2	1,2	5,8
	BDE-153	0,0	0,0	0,0	3,6	3,2	0,0	0,0		1,0	10,9
	BDE-154	2,6	2,5	2,2	1,8	2,0	2,4	0,2	9,3	0,6	5,4
	BDE-156	0,0	0,0	0,0	2,1	2,5	0,0	0,0		1,9	6,2
Hepta-	BDE-183	11,1	4,8	4,3	5,2	5,2	6,7	3,8	56,5	0,7	15,7
	BDE-184	2,1	1,8	0,9	1,7	2,7	1,6	0,6	40,4	0,6	5,0
	BDE-191	0,0	0,0	0,0	0,0	3,1	0,0	0,0		1,3	1,3
Octa-	BDE-196	0,0	0,0	0,0	2,7	4,9	0,0	0,0		1,7	8,1
	BDE-197	0,0	0,0	0,0	2,8	5,4	0,0	0,0		1,4	8,4
Nona-	BDE-206	20,8	44,3	45,9	13,2	9,6	37,0	14,0	38,0	1,8	39,7
	BDE-207	19,8	25,4	22,2	13,8	8,4	22,4	2,8	12,5	2,1	41,5
Deca-	BDE-209	85,1	168,5	447,2	62,8	17,8	233,6	189,6	81,2	2,4	188,4

ISTD	Congener	C									
		Exposed 6	Exposed 7	Exposed 8	Blank(PUF)	Blank	Average	St.dev.	RSD	LOD	MDL
Tri-BDE	BDE-17	6,6	6,5	3,7	0,5	0,0	5,6	1,7	29,4	0,3	1,4
	BDE-28	9,7	8,6	7,2	1,2	1,5	8,5	1,3	14,7	0,3	3,5
Tetra-	BDE-47	69,6	69,6	51,1	0,0	2,7	63,4	10,7	16,9	1,0	1,0
	BDE-49	6,4	6,0	4,8	0,0	0,0	5,7	0,8	14,6	1,5	1,5
	BDE-66	20,0	19,0	23,7	12,0	23,8	20,9	2,4	11,7	1,7	36,1
	BDE-71	0,0	0,0	0,0	0,0	0,0	0,0	0,0		1,5	1,5
	BDE-77	0,0	0,0	0,0	0,0	0,0	0,0	0,0		1,0	1,0
Penta-	BDE-85	0,0	0,0	0,0	0,0	0,0	0,0	0,0		0,3	0,3
	BDE-99	34,8	24,2	15,7	1,9	1,4	24,9	9,6	38,6	0,2	5,8
	BDE-100	10,5	7,8	4,7	0,2	0,2	7,6	2,9	37,6	0,2	0,7
	BDE-119	0,0	0,0	0,0	0,5	0,0	0,0	0,0		0,2	1,6
	BDE-126	0,0	0,0	0,0	0,0	0,0	0,0	0,0		0,2	0,2
Hexa-	BDE-138	0,0	0,0	0,0	0,0	0,0	0,0	0,0		0,7	0,7
	BDE-153	4,6	4,4	2,7	1,7	1,7	3,9	1,0	26,5	0,6	5,2
	BDE-154	3,3	2,7	1,4	0,0	0,0	2,5	1,0	40,3	0,4	0,4
	BDE-156	0,0	0,0	0,0	0,0	0,0	0,0	0,0		1,1	1,1
Hepta-	BDE-183	6,1	4,7	5,1	1,8	2,1	5,3	0,7	13,4	0,5	5,5
	BDE-184	0,0	0,0	0,0	0,0	0,0	0,0	0,0		0,4	0,4
	BDE-191	0,0	0,0	0,0	0,0	0,0	0,0	0,0		0,9	0,9
Octa-	BDE-196	0,0	0,0	0,0	0,0	0,0	0,0	0,0		1,8	1,8
	BDE-197	2,7	1,9	1,4	0,0	0,0	2,0	0,7	33,4	1,4	1,4
Nona-	BDE-206	0,0	0,0	0,0	11,7	0,0	0,0	0,0		3,9	35,2
	BDE-207	0,0	0,0	0,0	7,8	0,0	0,0	0,0		2,5	23,3
Deca-	BDE-209	0,0	122,1	44,7	51,1	0,0	55,6	61,7	111,1	10,0	153,2